

**Body composition, cardiovascular and nutritional risk
of 5–10-year-old children consuming
vegetarian, vegan or omnivore diets.**

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Doctor of Philosophy in Nutrition, Epidemiology and Child Health.

UCL

DECLARATION

'I, Małgorzata, Agnieszka Desmond, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.'

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ABSTRACT

Interest in vegetarian and vegan diets has increased rapidly in many regions. There are three reasons for this: planetary sustainability, concern for animal welfare and prevention of chronic disease. These issues primarily concern adults, who may then act on them when selecting diets for their offspring. In adults, the health effects of plant-based diets have been evaluated, and some benefits, mainly cardiometabolic, established. Equivalent evidence in children is scant. Atherosclerosis originates in childhood, and relates to classical cardiovascular risk factors which, along with dietary habits, track to adulthood. Therefore plant-based diets in childhood might positively affect adult cardiometabolic health. However, vegetarians and vegans restrict intake of whole food groups, which might affect children's growth and development. This thesis investigates potential health benefits and risks of vegetarianism and veganism in children. It tests four hypotheses. Plant-based diets in childhood: favourably affect cardiovascular risk; negatively affect growth; increase the risk of nutritional deficiencies; and increase risk of inadequate nutrient intakes. The following data were collected from 187 children aged 5–10 years, including 63 vegetarian, 52 vegan and 72 omnivore: body composition, blood markers of cardiovascular risk and nutritional status; bone mineral content (BMC) and dietary intake. Vegetarians and vegans were shorter than omnivores, with lower adiposity but similar lean mass. The body composition differences were more pronounced in vegans, who also had BMC deficits. Vegans had lower

cholesterol status than omnivores and evidence of nutritional deficiencies. Vegetarians were at risk of B3, folate, zinc and calcium inadequacy, while vegans at risk of vitamin A, B2, B3, B12, calcium, phosphorus, iron and zinc inadequacy. In conclusion, evidence of cardiometabolic benefits, but also nutritional harm, was found in vegan children. The magnitude of harm in vegetarians was intermediate, while the benefits were smaller. Restriction of animal-origin foods in childhood could constrain height and BMC, and cause nutritional deficiencies.

IMPACT STATEMENT

Plant-based diets are currently recommended for human and planetary health by numerous international and national health organisations. These two issues, along with heightened consideration for animal welfare, are the main three reasons for the increasing popularity of vegan and vegetarian ways of eating in industrialized countries. Adults concerned with these issues might be influenced to adopt these diets both for themselves and their offspring. In adults, the health effects and safety of appropriately planned vegetarian and vegan diets have been evaluated, and some benefits, mainly cardio-metabolic, established. However, equivalent evidence in children is sparse, and relates mainly to anthropometric outcomes and nutrient intakes in vegetarians. There are no comprehensive data regarding the health outcomes in vegan paediatric population. Of concern, these diets restrict intake of whole food groups, which may be of particular importance early in life when nutrient and energy needs are higher relative to body weight. This is the reason why some organisations, including the Polish Consultant for Paediatrics and Academy of Sciences, the Belgian Royal Academy of Medicine, and the German Nutrition Association do not approve veganism for children. This position is contrary to the American Academy of Pediatrics, the British Dietetic Association and US Academy of Nutrition and Dietetics, who – based on the same limited data – endorse it. Therefore, more evidence on the health status of vegetarian and vegan children is urgently needed.

The results of this thesis provide substantial new evidence on this issue. They constitute the first detailed and systematic health assessment in both paediatric vegans and vegetarians in comparison to otherwise similar omnivores. They cover a broad range of outcomes, assessing the prevalence of cardiovascular (CVD) risk, body composition and growth, micronutrient status along with dietary intakes. In vegans, they demonstrate evidence of superior CVD profile but also some nutritional harm. In vegetarians, the data indicate an intermediate magnitude of harm, limited CVD benefits accompanied by some undesirable outcomes such as less healthy body composition and CVD risk profile. The results demonstrate that the restriction of animal-origin foods in childhood leads to nutritional deficiencies and provide preliminary evidence (to be confirmed in longitudinal research) that it could constrain height and bone mineral.

These findings will benefit medical and public health professionals along with members of the general public. They will provide physicians and dietitians with evidence, based on which they can educate patients on both the benefits and risks of vegetarian and vegan diets in children. They will also help parents in decisions to adopt vegetarianism or veganism for their children. And finally, these results should inform current public health debates and the position statements of expert organisations on plant-based diets.

The benefits to academia include providing a rationale to conduct intervention trials and longitudinal observational research. Such efforts could elucidate, among other issues, the mechanisms through which these diets restrict stature and bone mineral accretion, and the effects of nutrient supplementation. They could also investigate the risks and benefits among children of different ages, especially infants.

EXECUTIVE SUMMARY

This thesis explores a range of potential health benefits and nutritional risks of vegetarian and vegan diets in children, based on a cross-sectional observational study of 187 healthy children aged 5–10 years, including 63 vegetarian, 52 vegan and 72 otherwise similar omnivores. The results of this study are put in the context of existing evidence in this area and the current public health debate promoting dietary patterns minimising animal products for the whole population in order to promote human and planetary health. Below is a summary of the thesis' contents.

Chapter I, Introduction

This chapter sets the background for undertaking this study and presents the definitions of vegetarian and vegan diets. It describes recent global trends of increasing interest in plant-based diets and the reasons for their rising popularity, which include planetary sustainability, heightened concern for animal welfare along with human health concerns relating to prevention of non-communicable diseases. It outlines existing, though still informal, evidence suggesting that the number of adult vegetarians and vegans is growing and discusses its likely consequence – increased numbers of children consuming vegetarian or vegan diets. Health effects of these diets in adults, mainly relating to lower cardiometabolic risk, are discussed along with the potential of lowering the adult

cardiovascular disease burden via consumption of plant-based diets from childhood. At the same time, this chapter emphasizes the paucity of evidence on health outcomes of these diets in vegetarian children, and the lack of almost any current evidence in vegan children. It finishes with the recognition that in light of growing global campaigns to encourage plant-based diets, reliable evidence is urgently needed, so that plant-based diets can help decrease ecological damage while also promoting health in both adults and children.

Chapter II, Literature review: plant-based diets and health

This chapter summarizes relevant literature in order to demonstrate the need for conducting this study. It also provides the rationale for choosing the health parameters to evaluate potential differences between vegetarian or vegan children in relation to children following traditional diets. It starts summarizing the existing evidence on the health effects of vegetarian and vegan diets in the context of cardiovascular disease and children's health. At the beginning, it introduces CVD, recent trends in CVD-associated mortality and the importance of lifestyle, in particularly diet, for lowering CVD risk factors and the associated disease burden. Next, it examines the substantial effects of plant-based diets on CVD risk in adult vegetarians and vegans. Subsequently, it discusses the links of atherosclerosis origins with childhood CVD risk factor profile. Following this, it reviews evidence on the potential of lowering the adult burden of CVD, placing emphasis on preventive efforts that reduce CVD risk factors via plant-based dietary patterns from childhood. The second part of the chapter is devoted to the available evidence on the health of vegetarian and vegan children. This section summarises the existing, albeit limited, data on the dietary intakes, body composition, micronutrient status, CVD risk factor profile and bone status of

children on meatfree diets. Based on the reviewed evidence, this chapter provides the rationale for the choice of health parameters selected to study in this research project to assess both the CVD-related outcomes and the safety of vegetarian and vegan diets in children.

Chapter III, Literature review: dietary assessment methods

Since the methodology of dietary assessment in observational studies is a complex subject, it is discussed in a separate part of the dissertation. In this chapter, approaches applied in epidemiological research to capture and analyse dietary intakes, the main exposure in this study, are reviewed, and the rationale for the selection of dietary assessment methods is provided. The first part describes the methods utilised in the collection of dietary data, the second part illustrates the problems linked to the analysis of dietary data, when the aim is to evaluate the proportion of individuals with inadequate intakes of nutrients, one of the main correlates of the exposure in this study. Procedures utilized to calculate percentages of the population with inadequate nutrient intakes, along with the concept of usual dietary intakes, are discussed, as these are utilised in this study to assess the dietary risks of plant-based diets in children. The selected methods of dietary data collection and analysis are discussed at the end of this chapter.

Chapter IV, Research aims, questions and hypotheses

This chapter presents the research aims and questions, and puts forward four main study hypotheses.

Chapter V, Methods

This chapter describes the remaining methods used to test the study hypotheses. Hence it outlines the methods used to measure all outcomes and

physical activity. Additionally, it discusses study design, recruitment, sampling, ascertainment of data on background characteristics, ethical considerations and explains the selection criteria of the dietary groups and the operational details of capturing dietary intakes with food records. Next, the chapter elaborates on the statistical models applied, and the rationale behind the choice of confounders and mediators of the association between dietary exposure and health outcomes. Lastly, the chapter summarizes all the methods used in this study to measure background characteristics, exposure and outcomes.

Chapter VI, Results

In this chapter the study results are presented. It starts with the results of the recruitment and data collection, then it proceeds to describe the baseline characteristics of the study participants, placing them in the context of the Polish, or, in the absence of relevant data, international population. Next, it presents the results of dietary analysis, correlates of the main exposure in this study. These are discussed in the context of the existing evidence. The last section presents the health outcome results, i.e. anthropometry and body composition, bone status, CVD risk, and micronutrient status analyses. The health outcome results are discussed in relation to the dietary findings as well as in the context of existing evidence. The discussion is continued, and in the case of outcomes like bone mineral content and stature, in more detail in the following chapter.

Chapter VII, Discussion

This chapter summarizes the principal findings, separately for vegan and vegetarian children, and compares them with existing studies. Next, it discusses the implications of this study in light of its results showing both benefits and

risks. The benefits are discussed in the context of CVD risk prevention; the risks in the context of the findings of lower height, lower bone mineral content and micronutrient deficiencies. Two hypotheses are put forward as to why vegetarian and vegan diets constrain height in children. Next, unanswered questions are identified and discussed in the context of potential future research projects. Following, clinical significance of the findings is presented for both health professionals and medical and nutritional organisations. And lastly, this chapter concludes that this study generated new important data on the range of both benefits and risks of vegetarian and vegan diets in children that add to current political, public health and medical debates on planetary and human health.

Chapter VIII, Supplementary material

This chapter includes complete case analyses along with several documents used to inform parents and children about this study and to obtain their consent to participate: information sheets for parents and children, parent and guardian consent forms, study advertisement content.

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LIST OF ABBREVIATIONS

24HR	24-hour recalls
25 (OH) D	25-hydroxy Vitamin D
95%CI	95% Confidence interval
AHA	American Heart Association
ASF	animal-source foods
BA	bone area
BALP	bone alkaline phosphatase
BMC	bone mineral content
BMD	bone mineral density
BMAD	bone mineral apparent density
BMI	body mass index
CAD	coronary artery disease
CBC	complete blood count
CC	complete case
cIMT	carotid intima media thickness
CRP	c-reactive protein
CTX	collagen type I terminal telopeptide
CVD	cardiovascular disease
DBP	diastolic blood pressures
DHA	docosahexaenoic acid
Dkk-1	Dickkopf-related protein 1
DXA	dual x-ray absorptiometry
EPA	eicosapentaenoic acid
FMI	fat mass index
FFQ	food frequency questionnaires
HDL-C	HDL cholesterol
Hgb	haemoglobin

HOMA-IR	homeostatic model assessment of insulin resistance
hs-CRP	high sensitivity CRP
Ht	haematocrit
IGF-1	insulin-like growth factor 1
IGFBP	IGF-binding protein
IMT	intima media thickness
IR	insulin resistance
L2-L4	lumbar spine vertebrae 2-4
LDL-C	LDL cholesterol
MCV	mean corpuscular volume
MI	multiple imputation
MUFA	monounsaturated fatty
MVPA	moderate and vigorous physical activity
NCD	non-communicable disease
PDAY	Pathobiological Determinants of Atherosclerosis in Youth Study
PUFA	polyunsaturated fatty acids
RAE	retinol equivalents
RBC	red blood cells
RR	relative risk
SBP	systolic blood pressure
SD	standard deviation
SDA	Seven Day Adventist
SE	standard error
SES	socioeconomic
SFA	saturated fatty acids
SGA	small for gestational age
sTfR	soluble transferrin receptor;
TBLH BMC	total body less head bone mineral content
TG	triglycerides
VLDL-C	VLDL-C

I. INTRODUCTION

Recently, interest in plant-based diets has rapidly increased in many global regions. Though formal estimates are lacking, numerous sources indicate that more people are adopting meat-free diets in industrialised countries (The Vegan Society 2016; Roy Morgan Research n.d.; CNBC.COM 2019; Marketingchina.com n.d.). Broadly, vegetarian diets (sometimes termed lacto-ovo-vegetarian) exclude meat, fish and products made from these, while vegan diets (sometimes termed strictly vegetarian), eliminate all products of animal origin, including dairy and eggs (Fraser 2009). There are three main reasons for their rising popularity. First, minimizing meat and dairy products is considered critical for planetary sustainability. According to recent analysis, producing meat, aquaculture, eggs and dairy for human consumption uses 83% of the world's farmland, produces 56–58% of food's different greenhouse gas emissions and yet contributes only 37% of protein and 18% of energy consumption (Poore and Nemecek 2018). Evidence on the environmental unsustainability of current animal-product-rich diets has penetrated healthy eating recommendations, including the 2015 Dietary Guidelines for Americans (U.S. Department of Health and Human Services and U.S. Department of Agriculture 2015) and the EAT-Lancet Commission (Willett et al. 2019), both of which advocate more plant-based diets to improve human and planetary health. Second, vegetarian and vegan diets are chosen for ethical reasons of heightened concern for animal welfare (The Vegetarian Resource Group n.d.; The Sentience Institute 2017). In particular, this seems the major

reason for adopting veganism (Wrenn, Wrenn, and Lee 2017). Third, plant-based diets are supported by the World Health Organisation (WHO 2019a) and World Cancer Research Fund (WCRF 2019a) to counter non-communicable disease (NCD) risk. Messages from these organisations are then reinforced by animal-welfare groups that extoll the human health benefits (PCRM n.d.). These issues primarily concern adults, who may then act on them when selecting diets for their offspring.

In adults, the health effects of vegetarianism and veganism have been evaluated and some benefits and risks established. The benefits include lower risk of cardiometabolic risk factors and diseases along with possible lower cancer risk (Dinu et al. 2017; Desmond et al. 2018). At the same time, these diets, if not planned well in adults, increase risks of several micronutrient deficiencies (Pawlak, Lester, and Babatunde 2014; Kristensen et al. 2015) along with fracture risk in vegans with low dietary calcium content (Appleby et al. 2007). Equivalent evidence in children is sparse. Atherosclerosis originates in childhood, and relates to classical cardiovascular risk factors which, along with dietary habits, track to adulthood (Desmond et al. 2018). Therefore plant-based diets in childhood might positively affect subsequent cardiometabolic health in adulthood, however any such benefits must be considered in light of safety in the paediatric population.

Vegetarians and vegans restrict intake of whole food groups (Melina, Craig, and Levin 2016a). This is of particular importance in children, whose nutrient and energy needs are higher relative to body weight and whose growth might be programmed by nutrient deficiencies at several sensitive periods of development (Lucas 1998). Previous studies have noted a reduced intake of

several nutrients in this population (Schürmann, Kersting, and Alexy 2017), which may show more severe consequences at later ages (Martins et al. 2011). The data on the health and growth of vegetarian children comes from studies of very heterogeneous design, various definitions of vegetarianism and selection criteria, and different, usually small, sample sizes. There are no informative studies on vegan children. Therefore, the available data are insufficient to thoroughly assess the health effects of vegetarian or vegan diets in children in developed countries (Schürmann, Kersting, and Alexy 2017). The sparsity of evidence contributes to inconsistencies between medical and nutrition organisations' statements regarding the safety of meat-free diets in childhood. While the American Academy of Pediatrics (Kleinman and American Academy of Pediatrics Committee on Nutrition 2009), British Dietetic Association (BDA 2017a) and Academy of Nutrition and Dietetics (Melina, Craig, and Levin 2016a) all assert that vegetarian and vegan diets, if planned appropriately, can be healthy and nutritionally adequate at all ages; the Polish Consultant for Paediatrics (Reguła 2013), the Committee of Human Nutrition Science of Polish Academy of Sciences (Wądołowska 2019), the Belgian Royal Academy of Medicine (de Medécine de Belgique 2019), German Nutrition Association (Die Deutsche Gesellschaft für Ernährung n.d.) and ESPGHAN (M. Fewtrell et al. 2017) do not recommend vegan diets in children, due to the higher risk of nutrient deficiencies. Moreover, these endorsement statements have been issued despite a lack of substantial research on health in vegan children.

Given growing global campaigns to encourage plant-based diets, reliable evidence is urgently needed, so that plant-based diets can help decrease ecological damage while also promoting health in both adults and children. This

thesis investigates a range of potential health benefits and risks of vegetarian and vegan diets in children. The first aim is to quantify prevalence differences in several indicators of health, including body composition, CVD risk and micronutrient status, along with estimating the prevalence of inadequate serum micronutrient and abnormal cholesterol status in either vegetarian or vegan children relative to a reference group of omnivore children. The second aim is to assess the prevalence of inadequate nutrient intakes and to calculate median intakes of nutrients.

II. LITERATURE REVIEW: PLANT BASED DIETS AND HEALTH

This chapter summarizes the existing evidence on the health effects of vegetarian and vegan diets in the context of cardiovascular disease prevention and children's health.

At the beginning, it introduces CVD, recent trends in CVD-associated mortality and the importance of lifestyle, in particularly diet, for lowering CVD risk factors and the associated disease burden. Next, it examines the substantial effects of plant-based diets on CVD risk in adult vegetarians and vegans. Subsequently, it discusses the links of atherosclerosis origins with childhood CVD risk factor profile. Following this, it reviews evidence on the potential of lowering the adult burden of CVD, placing emphasis on preventive efforts that reduce CVD risk factors via plant-based dietary patterns from childhood. The second part of the chapter is devoted to the available evidence on the health of vegetarian and vegan children. This section summarises the existing, albeit limited, data on the dietary intakes, body composition, micronutrient status, CVD risk factor profile and bone status of children on meatless diets.

Based on the reviewed evidence, in the last section this chapter provides rationale for the choice of health parameters chosen to study in this research project to assess both the CVD-related outcomes and the safety of vegetarian and vegan diets in children.

1. Cardiovascular disease

Cardiovascular disease remains the number one cause of premature mortality in the world (WHO 2011; WHO 2018b). In high-income countries it also contributes to the highest percentage of ill-health in adults (WHO 2019b). The main types of CVD responsible for these trends are coronary artery disease (CAD) and stroke (WHO WHF WSO2011). Both of them are linked to the underlying disease process of atherosclerosis, which involves a build-up of fatty material and calcium, otherwise called plaque, in the lumen of blood vessels. This makes their inner surface irregular, their lumen narrower, and their walls less pliable, which impedes normal blood flow. The rupturing of plaque can lead to blood clot formation, resulting in heart attacks if the clot is located in the coronary artery; or stroke if the same event happens in the brain. The process of atherosclerosis is linked to modifiable lifestyle factors, of which diet and exercise play the fundamental role (WHO WHF WSO 2011).

In most industrialized countries, CVD death rates have been declining since the late 1970s (Bertuccio et al. 2011). However, two factors threaten to reverse this trend today: aging of the population (i.e. increased absolute numbers of those aged >70years), which increases the lifetime exposure to relevant risk factors; and global increases in the prevalence of obesity and diabetes (Allender et al. 2008; Thorolfsdottir et al. 2014; Smith et al. 2015). These trends may be beginning to cancel out the health gains linked to declines in other risk factors and better care and treatment achieved in recent decades as recently shown for younger adults in the European Union, United States, and Australia (Ford and Capewell 2007; Allender et al. 2008; Martin O'Flaherty et al. 2012; Lewiecki et

al. 2008; Nichols et al. 2013). The consequence of these antagonistic trends might be an increase in the actual burden of CVD (Allender et al. 2008).

It has been postulated that the only strategy capable of substantially reducing the CVD burden in the current scenario is to focus on population-wide reduction of major CVD risk factors (Emberson et al. 2004; Guzman-Castillo et al. 2015). Based on estimations for the UK population, a 10% reduction in population-wide blood pressure and cholesterol levels has been estimated to bring about a 45% reduction in cardiac events. If we compare this to the pharmacological treatments, 26% of the population at high risk would have to be treated, to achieve a 34% reduction in these outcomes (Emberson et al. 2004).

2. Plant based diets and adult cardiometabolic risk

Diet is a major modifiable direct risk factor for CVD. It also influences other components of risk, including: obesity, dyslipidaemia, hypertension, insulin resistance, increased serum glucose, and even physical activity level. The foods of plant origin have the most established protective effect (Mozaffarian 2016).

In adults, dietary patterns emphasizing intake of unprocessed plant-based foods seem to lower cardiovascular disease and its risk factors. Consumption of fruit and vegetables, whole grains, legumes and nuts is inversely linked with CVD risk in a dose-dependent fashion (Mozaffarian 2016). Adult vegetarians and vegans have a more favourable profile of CVD risk factors (Wang et al. 2015), which also translates to lower prevalence of ischemic heart disease (IHD; the major form of CVD in Western populations) risk (Key et al. 1999; Crowe et al.

2013a) than otherwise comparable nonvegetarians. This difference is primarily attributed to diet (Fraser 2009). However, the evidence is inconsistent regarding whether the rate of mortality from IHD differs between vegetarians/vegans and omnivores. The rate ranges from 26%–34% reductions in risk for different vegetarian diets (Key et al. 1999), to none (Appleby et al. 2016). Additionally, vegetarians/vegans do not have lower rates of cerebrovascular disease (Key et al. 1999; Appleby et al. 2016; Dinu et al. 2017). It needs to be emphasized, that all these results come from observational studies. Analysis of cardiovascular risk profile of vegetarians may shed light on the mechanisms through which plant-based diets exert their beneficial effects.

The following sections constitute a narrative review of the literature on cardiovascular risk factors among adult vegans and vegetarians. It is based on the most up-to-date systematic reviews (body fatness, blood lipids, blood pressure, insulin resistance) and the only existing data on the CRP-levels and cIMT in adult vegetarians coming from four observational studies and one clinical trial. The search terms used in the pursuit of this review were: “vegan” and/or “vegetarian”, and the keywords for respective risk factors in the advanced PubMed search and Google Scholar in English. In the case of numerous publications on one particular risk factor, only the most recent systematic reviews were taken into account.

2.1. Body fatness

Vegetarians and vegans have lower levels of body fat, mainly measured by body mass index (BMI). A recent systematic review incorporating meta-analysis of 71 cross-sectional studies including participants on a vegetarian diet and 19

cross-sectional studies examining subjects on a vegan diet showed that these diets are associated with 1.49 kg/m² (95% confidence interval (95% CI) -1.72, -1.25) and 1.72 kg/m² (95% CI -2.21, -1.22) lower BMI, respectively (Dinu et al. 2017). These lower BMI values are usually attributed to the lower energy density of diets high in vegetables and fresh fruit, and the satiating effect of increased fibre intakes (Sabaté and Wien 2010). Overweight and obesity is a direct cause of cardiometabolic disease (Cercato and Fonseca 2019). Excess adipose tissue, particularly its visceral form, induces a state of chronic low-grade inflammation which leads to atherosclerosis (Van Gaal, Mertens, and De Block 2006). In a recent study which examined data from 10 large US prospective cohorts of 190,672 people, obesity was associated with shorter longevity and significantly increased risk of cardiovascular morbidity and mortality compared with normal BMI (Khan et al. 2018). The results challenged the so called 'obesity-paradox', in which overweight and obese people tend to live longer after the diagnosis of CVD compared to people with healthy weight (Chang et al. 2017). Recently provided explanations (Chang et al. 2017; Khan et al. 2018) to refute the paradox include the so called *lead-time bias* (i.e. early diagnosis of a disease gives the indication of prolonged survival with that disease), and lack of adjustment for disease-related weight loss, as no evidence for an obesity paradox is found when the normal-weight reference category includes only those who have always been normal weight (Stokes and Preston 2015).

Excess body weight additionally increases the risk of CVD by adversely affecting its risk factor profile, leading to dyslipidaemia, insulin resistance,

diabetes type 2, increased blood pressure (Klein et al. 2004). Vegetarians and vegans have a better profile of all these parameters than omnivores.

2.2. Blood lipid profile

Vegetarians, especially vegans, have lower levels of total and non-HDL cholesterol. A recent systematic review with meta-analysis of observational studies showed estimates of effect size ranging from 0.72 mmol/L (95% CI -0.8, -0.64) reductions in total cholesterol and 0.55 mmol/L (95% CI -0.62, -0.47) in LDL cholesterol (LDL-C) associated with vegetarian diets compared to omnivore diets (based on 64 and 46 cross sectional studies respectively), and 0.80 mmol/L (95% CI -0.90, -0.70) reductions in total cholesterol and 0.59 mmol/L (95% CI -0.77, -0.40) in LDL-C associated with vegan diets (based on 19 and 13 cross-sectional studies respectively) (Dinu et al. 2017). Both total and non-HDL cholesterol concentrations tend to be highest in meat eaters and lowest in vegans, with vegetarians having intermediate values (Bradbury et al. 2014). In another systematic review and meta-analysis of 11 randomised clinical trials (7 included a vegan diet, 2 included a lacto-ovo vegetarian diet, i.e. containing dairy and eggs, 2 included a lacto-vegetarian diet, i.e. containing dairy without eggs), Wang et al. 2015, showed that vegetarian diets were associated with significantly lower total cholesterol, LDL-C and non-HDL cholesterol with pooled estimated effects of -0.36 mmol/L (95% CI -0.55, -0.17), -0.34 mmol/L (95% CI -0.57, -0.11), and -0.30 mmol/L (95% CI -0.50, -0.10) respectively. A 1-mmol/L reduction in TC and LDL-C levels results in a 26.6% to 29.5% decrease for any cardiovascular disease-related event (Schwingshackl and Hoffmann 2013). Therefore, the average reductions of TC and LDL-C concentrations following a vegetarian diet would correspond to a

decrease in CVD risk of about 9.0% to 10.6% (Wang et al. 2015). The mechanisms through which vegetarian diets may reduce blood cholesterol concentrations are considered to include their lower content of saturated, total fat and cholesterol (Li 2011) and their higher amount of dietary fibre and numerous phytochemicals, all of which have been shown to have lipid lowering properties (Craig 2010).

2.3. Blood pressure

Adults on plant-based diets have lower systolic (SBP) and diastolic blood pressures (DBP), and lower risk of hypertension, compared to meat eaters. In the cross-sectional analysis of a sub-set of 592 black women and men enrolled in the Adventist Health Study-2 (25% vegetarian and vegan; 75% non-vegetarian), the risk of hypertension differed depending on the dietary group and was lowest for vegans and highest for omnivores. The relative risk (RR) in comparison to omnivores was 0.37 (95% CI 0.19, 0.74) and 0.57 (95% CI 0.36, 0.92) for vegans and vegetarians respectively in an analysis adjusted for, age, sex and physical activity (Orlich and Fraser 2014). In a matched cohort study of 4,109 non-smoking Taiwanese, where each person on a vegetarian diet was matched with five non-vegetarians by age, sex, and study location, vegetarians had 28% lower risk (RR 0.72; 95% CI 0.55, 0.86) for hypertension adjusting for age, sex, C-reactive protein, waist circumference, and fasting glucose (Chuang et al. 2016). Likewise, a systematic review and meta-analysis of controlled clinical trials and observational studies showed a reduction in mean SBP (-4.8 mm Hg; 95% CI -6.6, -3.1) and DBP (-2.2 mm Hg; 95% CI -3.5, -1.0) after adopting a vegetarian diet compared with the consumption of omnivorous diets (7 controlled trials, including 311 participants; mean age, 44.5 years) and

lower mean SBP (−6.9 mm Hg; CI, −9.1, −4.7) and DBP (−4.7 mm Hg;95% CI, −6.3, −3.1) associated with consumption of vegetarian compared with omnivorous diets (32 observational studies; a total of 21,604 participants; mean age 46.6 years) (Yokoyama et al. 2014). Mechanistically, several dietary factors in plant-based diets, other than those affecting BMI, may account for their effects on blood pressure. Vegetarians have higher fibre intakes (Sobiecki et al. 2016), which have been shown to exert a blood pressure lowering effect (Evans et al. 2015). Other factors which may beneficially affect blood pressure regulation are higher plant protein (He et al. 2005), and potassium intake (Haddad and Tanzman 2003; Aburto et al. 2013), and higher non-haeme iron intake (Galan et al. 2010). The potential mechanisms exerted by these plant-based dietary components include improved baroreceptor sensitivity, direct vasodilatory effects, and changes in catecholamine and renin–angiotensin–aldosterone metabolism (Sciarrone et al. 1993), along with changes in blood viscosity (Ernst et al. 1986). Every 10 mm Hg reduction in SBP significantly reduces the risk of major cardiovascular disease events (RR 0.80, 95% CI 0.77, 0.83), coronary heart disease (RR 0.83, 95% CI 0.78, 0.88), and stroke (RR 0.73, 95% CI 0.68, 0.77) (Ettehad et al. 2016).

2.4. Insulin resistance and diabetes

Plant-based diets are associated with lower serum glucose level along with decreased insulin resistance and diabetes risk (Tonstad et al. 2009; Snowdon and Phillips 1985). In a systematic review and meta-analysis of observational studies, including 27 studies with 2256 vegetarian and 2192 non-vegetarian participants, and 4 studies of 83 vegans and 125 omnivores, plant-based diets were significantly associated with lower blood glucose levels

(vegetarians -5.08 mg/dL, 95% CI -5.98 , -4.19 ; vegans -6.39 mg/dL, 95% CI -12.35 , -0.41) (Dinu et al. 2017). Observational studies further show that the prevalence of type 2 diabetes up to 2 times lower in vegetarians compared to omnivores, even after controlling for body weight (Kahleova and Pelikanova 2015). Likewise, dietary interventions trials in subjects with type 2 diabetes have demonstrated that implementing a vegetarian diet leads to a greater reduction in fasting plasma glucose, HbA1c, and hypoglycaemic medications compared to a conventional hypocaloric diet (Kahleova et al. 2011; Barnard et al. 2006). These results confirm substantial protective effects of the portfolio of foods found in plant-based diet (Fung et al. 2004; Sievenpiper et al. 2009; Aune et al. 2013; Cooper et al. 2012), along with the avoidance of meat (Pan et al. 2011) on glycaemic control and risk of diabetes. Mechanistically, plant-based diets may deliver protective effects on diabetes risk through caloric restriction, reduced intake of saturated fatty acids (SFA), high intake of polyunsaturated (PUFA) and monounsaturated fatty acids (MUFA), low glycaemic index (only if based on unprocessed foods), increased fibre intake, higher intake of non-haem iron and reduction in iron stores, increased intake of antioxidants, vitamins and several micronutrients, high intake of vegetable instead of animal protein, and high intake of plant sterols and prebiotics. All of these have been shown to have a positive effect on diabetes prevention (Kahleova et al. 2011). Diabetes increases the risk for CVD two-fold, independently from other conventional risk factors. In people without diabetes, fasting blood glucose concentration is modestly and non-linearly associated with risk of vascular disease (The Emerging Risk Factors Collaboration et al. 2010).

2.5. Other risk CVD factors

Lower levels of C-reactive protein (CRP), a marker of inflammation, have been reported in adult vegetarians (0.77 mg/L; standard error (SE) 1.29) for vegetarians; 1.30 mg/L; (SE 1.38) for matched omnivores, $P < 0.01$) (Szeto, Kwok, and Benzie 2004), and a decrease in CRP was observed in adults implementing vegan diets (-28.2% ; (SE 10.8%), $P = .02$) (Jenkins et al. 2003). Some studies have shown that healthy adult vegetarians have significantly lower carotid intima media thickness (cIMT) (Acosta-Navarro et al. 2017) and the advantage was related to the duration of consuming the vegetarian diet (Yang et al. 2011), but one study showed no such difference (Su et al. 2006).

Altogether, a lower prevalence of cardio-metabolic risk factors among adults following plant-based diets is likely to be the primary reason why they have ~25% lower risk of developing ischemic heart disease (Dinu et al. 2017; Crowe et al. 2013b). The overall pattern in which vegetarian or vegan diets affect IHD risk factors and incidence is illustrated in Figure 1. The effects of meat-free diets on cardiovascular risk factors are summarised in Table 1.

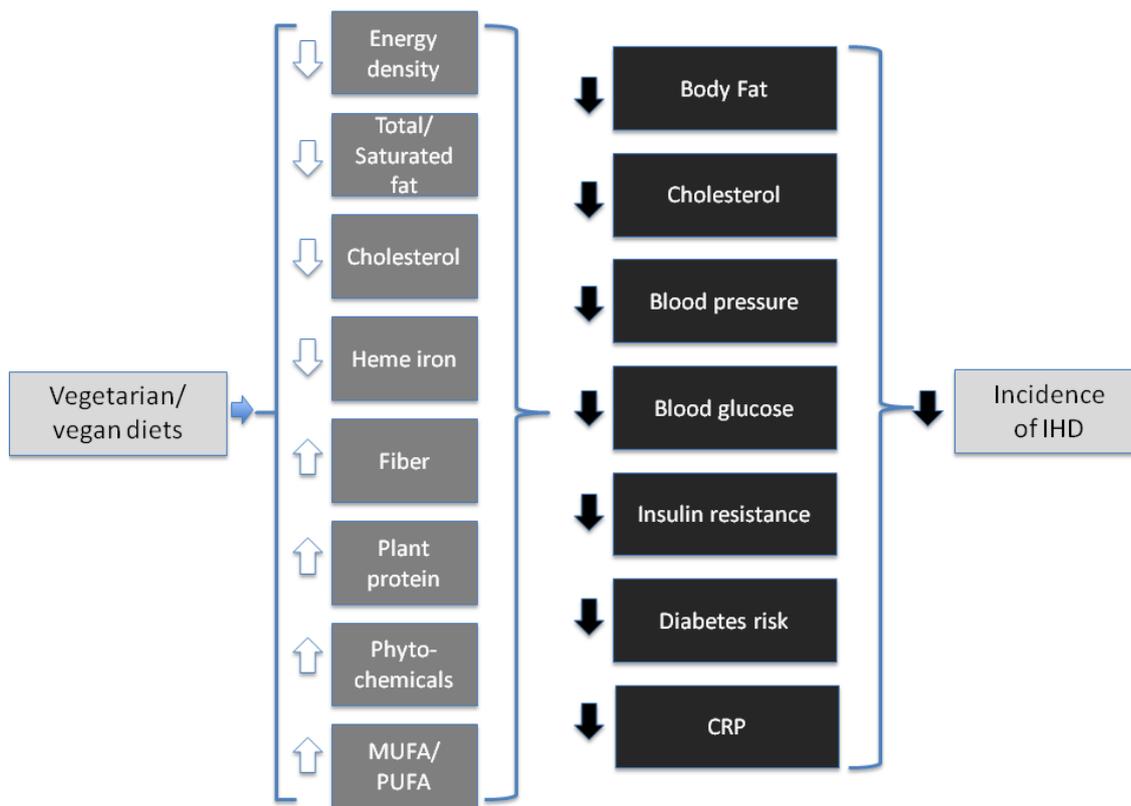
2.6. Possibility of confounding

Although the methodology used in more recent epidemiological studies on plant-based diets has become more sophisticated in measuring and adjusting for a variety of confounders, residual confounding is always a concern in observational research. Choosing a vegetarian or a vegan diet may be associated with a range of other health behaviours, personality traits or could be a response to a previous illness. In a large study of Dutch adults aged 55-69 years, being a vegetarian was associated with female gender, higher education,

lower probability of being a smoker and with having fewer children (Gilsing et al. 2013). In the same study, following a vegetarian diet was more likely among cancer patients than in cancer-free individuals. Similarly, more recent cross-sectional data from the US suggested that vegetarians and vegans were more likely to be female, not Hispanic, from the Western US region, with high-school or higher education, physically active and, at the same time, chronically ill (Cramer et al. 2017). Some data have suggested an association between personality traits linked to dieting (and hence choosing low - calorie or healthy foods) and following plant-based diet {Formatting Citation}.

Hence a variety of other factors associated with vegetarianism, some difficult to measure, can influence the results of observational studies in this area and caution in the interpretation of results is warranted. Much less is known about this situation for vegan diets, but the same issues remain relevant.

Figure 1. The overall pattern by which vegetarian/vegan diets affect ischaemic heart disease risk factors and incidence (Desmond et al. 2018).



Abbreviations: CRP, C-reactive protein; IHD, ischaemic heart disease; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 1. Effects of vegetarian diets on cardiovascular risk factors (Desmond et al. 2018).

Study design	Populations	CVD Risk Factor	Key Findings	Reference group	Ref
Systematic review and meta-analysis of cross-sectional studies	71 studies for vegetarian and 19 studies for vegan diet	BMI	-1.49 kg/m ² (95% CI -1.72, -1.25) for vegetarians; -1.72 kg/m ² (95% CI -2.21, -1.22) for vegans	Various otherwise similar non-vegetarian populations	(Dinu et al. 2017)
Systematic review and meta-analysis of cross-sectional studies	64 studies for vegetarian and 19 studies for vegan diet	Total cholesterol	-0.72 mmol/L (95% CI -0.8, -0.64) for vegetarians; -0.80 mmol/L (95% CI -0.90, -0.70) for vegans	Various otherwise similar non-vegetarian populations	(Dinu et al. 2017)
Systematic review and meta-analysis of cross-sectional studies	46 studies for vegetarian and 13 studies for vegan diet	LDL cholesterol	-0.55 mmol/L (95% CI -0.62, -0.47) for vegetarians -0.59 mmol/L (95% CI -0.77, -0.40) for vegans	Various otherwise similar non-vegetarian populations	(Dinu et al. 2017)
Systematic review and meta-analysis of randomised clinical trials	7 trials of intervention with vegan diet and 4 trials of intervention with vegetarian diet	Total cholesterol	-0.36 mmol/L (95% CI 0.55, -0.17) with intervention with vegetarian or vegan diet (pooled estimates)	Trial control period	(F. Wang et al. 2015)
Systematic review and meta-analysis of 11 randomised clinical trials	7 trials of intervention with vegan diet and 4 trials of intervention with vegetarian diet	LDL cholesterol	-0.34 mmol/L (95% CI -0.57, -0.11) with intervention with vegetarian or vegan diet (pooled estimates)	Trial control period	(F. Wang et al. 2015)

Study design	Populations	CVD Risk Factor	Key Findings	Reference group	Ref
Systematic review and meta-analysis of 11	7 trials of intervention with vegan diet and 4 trials of intervention with vegetarian diet	non-HDL cholesterol	-0.30 mmol/L (95% CI -0.50, -0.10) with intervention with vegetarian or vegan diet (pooled estimates)	Trial control period	(F. Wang et al. 2015)
Adventist Health Study 2, a cohort study	96,000 participants (7% vegan, 29.2% vegetarian)	Risk of hypertension	RR 0.86 (95% CI 0.51, 1.45) for vegetarians RR 0.53 (95% CI 0.25, 1.11) for vegans	Non-vegetarian Adventists	(Orlich and Fraser 2014)
Matched cohort study	4109 Taiwanese non-smokers	Risk of hypertension	RR 0.72 (95% CI 0.55-0.86) for vegetarians	Five omnivores matched to one vegetarian by age, sex, and study site,	(Chuang et al. 2016)
A systematic review and meta-analysis of controlled clinical trials and observational studies	Seven trials, a total of 311 participants, mean age 44.5 years; 32 observational studies, a total of 21,604 participants, mean age, 46.6 years	Systolic blood pressure	a reduction in mean systolic blood pressure (-4.8 mm Hg; 95% CI -6.6, -3.1) for vegetarian diet	Trial control period	(Yokoyama et al. 2014)
			lower mean systolic blood pressure (-6.9 mm; 95% CI, -9.1 to -4.7) for vegetarian diet	Various otherwise similar non-vegetarian populations	
A systematic review and meta-analysis of controlled clinical trials and observational studies	Seven trials, a total of 311 participants, mean age 44.5 years; 32 observational studies, a total of 21,604 participants, mean	Diastolic blood pressure	a reduction in mean diastolic blood pressure (-2.2 mm Hg; 95% CI -3.5, -1.0) for vegetarian diet	Trial control period	(Yokoyama et al. 2014)

Study design	Populations	CVD Risk Factor	Key Findings	Reference group	Ref
	age, 46.6 years		lower mean systolic BP (-4.7 mm Hg; 95% CI, -6.3, -3.1) for vegetarian diet	Various otherwise similar non-vegetarian populations	
Two prospective cohort studies	Adventist Health Study 1 (25,698 participants; ca. 50% vegetarians) and 2 (60 903 participants; 52% vegetarians)	Prevalence of diabetes	1.5 to 2 times lower	Non-vegetarian Adventists	(Snowdon and Phillips 1985; Tonstad et al. 2009)
Systematic review and meta-analysis of observational studies	Twenty-seven studies with 2256 vegetarian and 2192 non-vegetarian participants; 4 studies of 83 vegans and 125 omnivores	Blood glucose levels	-5.08 mg/dL (95% CI -5.98, -4.19) for vegetarians -6.39 mg/dL (95% CI -12.35, -0.41) for vegans	Various otherwise similar non-vegetarian populations	(Dinu et al. 2017)
Review	Two randomized clinical trials of interventions with vegetarian diet in diabetes including 43 and 74 participants with diabetes	Effect on various diabetes markers	a greater reduction various measures of diabetes, including body weight, fasting plasma glucose, HbA1c, and hypoglycaemic medication and greater increase in insulin sensitivity with vegetarian diet	Hypocaloric diet or a diet recommended by American Diabetes Association	(Kahleova and Pelikanova 2015)
Cross – sectional study	Thirty long term (≥5 years) vegetarians and 30 age- matched omnivores	C reactive protein (CRP) levels	Lower CRP levels in vegetarians (0.77 mg/L; (1.29), P<0.01) than in omnivores (1.30 mg/L; (1.38))	Matched omnivores	(Szeto, Kwok, and Benzie 2004)

Study design	Populations	CVD Risk Factor	Key Findings	Reference group	Ref
Randomized control trial	Forty-six healthy, hyperlipidaemic adults randomised to a diet low in saturated fat and a vegetarian diet high in plant sterols	Effect on CRP levels	Vegetarian diet intervention reduced CRP levels by 28.2% (10.8%) ($p = .02$), whereas control diet by 10% (8.6%) ($p = .27$)	Control arm of the clinical trial	(Jenkins et al. 2003)

However, while the main burden of CVD morbidity and mortality occurs in adults, there is compelling evidence that CVD risk is strongly shaped by experience at earlier ages.

3. Atherosclerosis starts in childhood

The following sections (3.-6.) constitute a narrative literature review based on the paper “Plant based diets for children as means of improving adult cardiometabolic health” co-authored by the PhD candidate and published in the vol. 76(4) of Nutrition Reviews (Desmond et al. 2018).

Atherosclerosis comprises arterial lesions that are a fundamental causative component of adult CVD. These lesions develop over time, starting with the harmless accumulation of lipid-filled macrophages, called fatty streaks, and progress to more advanced stages where the streaks are raised and vulnerable to rupture, manifesting as fibrous and calcified plaques (McGill et al. 2000). Histological studies indicate that clinically significant lesions develop from these initially harmless changes in symptomatic individuals (Stary 2000).

It was early autopsy studies that first suggested that atherosclerosis begins early in life. Enos et al. 1953, showed evidence of substantial coronary atherosclerosis among autopsies of 77.3% US soldiers, average age 22 years, killed in the Korean war. Around the same time, Holman et al. 1958, demonstrated the existence of fatty streaks in the aortas of children as young as 3 years. Subsequently, McNamara et al.1971, reported atherosclerosis in 45% and advanced coronary atherosclerosis in 5% of 105 autopsies of US soldiers killed in Vietnam. More recently, the Pathobiological Determinants of

Atherosclerosis in Youth (PDAY) study described the emergence of clinically significant atherosclerotic lesions in a large sample of autopsied persons aged 15–34 years who died in accidents (McGill et al. 2000). Other studies reported some degree of fatty streaks in the aorta in all 12–15 year olds (Stary 1989), and fatty streaks in the coronary arteries in ~30% of children aged 8–11 years and 69% of 12–15 year olds (Stary 2000). The clinical significance of these lesions depends on their anatomical site. Epidemiological studies have not directly confirmed the link between the early presence fatty streaks in the aorta and the occurrence of clinically significant atherosclerotic plaques in later life (Stehbens 1990; Newman et al. 1995). However, there is a relationship between the location of fatty streaks in the coronary arteries in children and atherosclerotic lesions in the same site later in life (Montenegro and Eggen 1968). In non-black populations, the extent of involvement of coronary artery with fatty streaks in youth predicts the extent of its involvement with raised lesions in older persons. These predictions were calculated based on the comparisons of the lesion localizations in people of different ages (McGill 1968). In an autopsy study of coronary arteries of 565 persons aged 0 to 29 years, the progressive transformation of fatty streaks in children's coronary arteries to a well-advanced fibrous plaque in young adulthood was observed (Stary 1989). In this study, by puberty, about 8%–10% of children had evidence of more advanced lesions and about 30% of the young adults in their twenties had well-developed raised lesions with large extracellular lipid cores and thick fibromuscular caps (Stary 1989). Overall, the evidence indicates that the atherosclerotic process starts in the early years, and this emphasises the importance of understanding the factors contributing to its variability between individuals during childhood.

4. Childhood precursors of adult atherosclerosis and clinical CVD

4.1. Direct evidence from genetic disorders altering lipoprotein metabolism and family history of CVD

The only direct evidence linking cardio-metabolic risk factors other than BMI in youth (Twig et al. 2016) with overt clinical disease in adulthood comes from genetic disorders related to high cholesterol. In homozygous familial hypercholesterolaemia, a genetic disease whereby LDL-C clearance is impaired, levels of this lipid fraction exceed 20.68 mmol/L already in infants. In affected subjects, CVD events begin in the first decade of life and life span is reduced (Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents and National Heart, Lung, and Blood Institute 2011). In heterozygous hypercholesterolaemia, in which LDL-C levels usually exceed 5.17 mmol/L and total cholesterol levels exceed 6.5 mmol/L beginning in infancy, 50% of men and 25% of women experience clinical coronary events by the age of 50 (Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents and National Heart, Lung, and Blood Institute 2011). Furthermore, in familial hypertriglyceridaemia, another genetic disorder resulting in excess triglyceride levels, childhood triglycerides (TG) independently predict CVD in the 4th–5th decade of life (Morrison, Glueck, and Wang 2012).

The substantial genetic component of CVD risk can also be seen among children of patients suffering from premature myocardial infarction (<55 years), who have higher levels of total cholesterol, LDL-C, TG and lower levels of HDL cholesterol (HDL-C) than control (Kelishadi et al. 2002). Another piece of

evidence linking childhood blood lipids with CVD risk in adulthood in the context of genetic predisposition stems from Mendelian randomization studies. Meta-analysis of such studies found a 54.5% (95% CI 48.8%–59.5%) reduction in the risk of IHD per each mmol/l reduction in LDL-C (effect size 3-fold greater than that achieved via treatment with statins in later life) due to genetic polymorphism, and thus relating to lifetime exposure (Ference et al. 2012).

4.2. Indirect evidence from autopsy and imaging studies

Most evidence suggesting that childhood cardiometabolic risk affects adult arterial pathology is indirect and comes from autopsy and imaging studies linking childhood risk factors with atherosclerosis and its surrogate markers. The extent to which the artery surface is involved in individual children with lesions varies; however, it is influenced by the same classical risk factors that predict adult coronary heart disease (Stary 2000; Newman et al. 1986).

The Bogalusa Heart Study has demonstrated a strong association of BMI, SBP, DBP and serum concentrations of total cholesterol, TG, LDL-C, and HDL-C with vascular lesions in children and young adults on autopsy (Berenson et al. 1998). The PDAY autopsy study showed robust relationships between atherosclerotic severity and its extent with age, non-HDL cholesterol, HDL-C, hypertension (determined by renal artery thickness), tobacco use (thiocyanate concentration), diabetes mellitus (glycohaemoglobin), and (in men) obesity. It also showed that a 30 mg/dL incremental increase in non-HDL cholesterol was equivalent to 2 years of 'vascular aging' (McGill et al. 2000). The severity and extent of the lesions were positively associated with age, and increased in association with the number of risk factors. At the same time, an absence of risk

factors was associated with a virtual absence of advanced atherosclerotic lesions, even in the oldest subjects in the study.

Analyses from four longitudinal cohorts (Cardiovascular Risk in Young Finns Study, Childhood Determinants of Adult Health study, Bogalusa Heart Study, and Muscatine Study) showed that risk factors measured at age 9 years or after (total cholesterol, TG, blood pressure, and BMI) were predictive of elevated cIMT in adulthood (Juonala et al. 2010), recognized as a predictive measure of clinical coronary events in middle-aged and elderly populations (Hodis et al. 1998). Similarly, SBP, DBP, total cholesterol, LDL-C, HDL-C and smoking status were linked to intima media thickness of the femoral artery, a surrogate measure of coronary and peripheral atherosclerosis, in asymptomatic young individuals in the Bogalusa Heart Study (Paul et al. 2005). Additionally, in the Cardiovascular Risk in Young Finns Study, childhood LDL-C ($\geq 80^{\text{th}}$ percentile), elevated blood pressure, skin fold thickness, low HDL-C ($\leq 20^{\text{th}}$ percentile), and smoking were inversely associated with artery elasticity in adulthood (Juonala et al. 2005), a marker of path physiological changes in the arteries relevant to the development of atherosclerosis later in life (Oliver and Webb 2003). Increased body size, increased blood pressure and decreased HDL-C were associated with coronary artery calcification in young adults in the Muscatine Study (Mahoney et al. 1996b).

Other studies have examined the relationship of isolated childhood risk factors to various measures of atherosclerosis and CVD risk. Dietary fat quality reflected in the serum cholesterol ester fraction in childhood was associated with cIMT in adult women (Kaikkonen et al. 2013). Children with hypercholesterolaemia and diabetes showed increased cIMTs compared with

healthy controls (Järvisalo et al. 2001) and cumulative exposure to hyperlipidaemia in young adulthood increased subsequent risk of coronary heart disease in a dose-dependent fashion (Navar-Boggan et al. 2015).

Other, non-diet-related risk factors like smoking (Priest et al. 2014) and physical activity (Ried-Larsen et al. 2015) in childhood were also associated with adult atherosclerosis in a pattern similar to that in adulthood.

Collectively, all these studies indicate that modifiable phenotypic traits in childhood, including diet and body fatness and their correlates, are associated with the early emergence of atherosclerotic progression to calcified plaque, manifesting as overt cardiovascular disease in adult life. Children with fewer cardio-metabolic risk factors show lower prevalence of atherosclerotic risk in later life. Dietary-influenced risk factors in childhood and their relation to atherosclerosis are summarised in Table 2.

Table 2. Dietary-influenced risk factors in childhood and their relation to atherosclerosis (Desmond et al. 2018).

Study	Population	Findings	Locations of the lesions measured	Additional information	Reference
The Bogalusa Heart Study	Autopsies on 204 young subjects 2 to 39 years	A strong association of BMI, SBP, DBP and serum concentrations of total cholesterol, TG, LDL-C, and HDL-C with vascular lesions in children and young adults on autopsy	Coronary arteries, aorta	The association between less advanced lesions (fatty streaks) and more advanced ones (fibrous plaques) was much stronger in the coronary arteries than in the aorta	(Berenson et al. 1998)
The Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study	>3000 autopsies of persons 15 to 34 years	Strong relationships between atherosclerotic severity/extent with age, non-HDL cholesterol, HDL-C, hypertension, tobacco use, diabetes mellitus, and (in men) obesity on autopsy	Left anterior descending coronary artery, right coronary artery, and abdominal aorta	Severity and extent of lesions positively associated with age and with number of risk factors.	(R. M. Lauer, Burns, and Daniels 2006)
4 longitudinal cohorts (Cardiovascular Risk in Young Finns Study, Childhood Determinants of Adult Health study, Bogalusa Heart Study, and Muscatine Study)	4380 members of 4 prospective cohorts with cardiovascular risk factor data from childhood (3 to 18 years) and intima media thickness (IMT) in adulthood (20 to 45 years)	Risk factors at 9+ years (total cholesterol, TG, blood pressure, and BMI) were predictive of elevated carotid IMT in adulthood	Carotid IMT	The associations with risk factors measured at age 3 years and 6 years were weaker and nonsignificant.	(Juonala et al. 2010)
The Bogalusa Heart Study	1080 black and white subjects (24–43 years; 71% white, 43% male); individuals in the top (n=54) versus bottom fifth (n=54) percentiles distribution of femoral IMT were compared for traditional cardiovascular risk factors profile	SBP, DBP, total cholesterol, LDL-C, HDL-C and smoking status were linked to IMT of the femoral artery	Femoral artery IMT		(Paul et al. 2005)

Study	Population	Findings	Locations of the lesions measured	Additional information	Reference
The Cardiovascular Risk in Young Finns Study	2255 healthy white adults aged 24 to 39 years who had risk factor data available since childhood	Childhood LDL cholesterol (≥ 80 th percentile), elevated blood pressure, skinfold thickness, low HDL cholesterol (≤ 20 th percentile), and smoking were inversely associated with artery elasticity in adulthood	Carotid artery elasticity comprising carotid artery compliance, Young's elastic modulus, and stiffness index	Associations remained highly significant after adjustment for the number of risk factors identified in adulthood	(Juonala et al. 2005)
The Muscatine Study	384 subjects (197 men, 187 women) who had coronary risk factors measured in childhood (mean age 15 years) and twice during young adult life (mean ages 27 and 33 years)	Increased body size, increased blood pressure and decreased HDL-C were associated with coronary artery calcification in young adults			(Mahoney et al. 1996b)
Cross-sectional study	88 children (aged 11 ± 2 years)	children with hypercholesterolaemia and diabetes showed increased IMTs compared with healthy controls	Aortic and carotid IMT		(Järvisalo et al. 2001)

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglycerides; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol

5. Tracking of childhood CVD risk factors and their determinants into adulthood

Cardio-metabolic risk factors in childhood deserve attention not only because of their association with atherosclerosis in adulthood, but also because there is evidence that they themselves track (i.e. persist) into adult life, therefore generating a cumulative impact on the disease process. Evidence for tracking is strongest for obesity, with childhood BMI levels predictive of adult obesity (Srinivasan et al. 1996). A systematic review of 13 prospective or retrospective longitudinal studies published after 2001 showed that the risk of an overweight child becoming an overweight adult is at least twice as high compared with normal-weight children and it is even higher for obese children (Singh et al. 2008). This could be due both to the direct tracking of body composition, and also due to the tracking of obesity-related behaviours – such as physical inactivity and unhealthy diets – between childhood and adulthood (Craigie et al. 2011).

Correlation coefficients for cholesterol tracking are in the range of 0.4 and have been reported consistently in numerous studies examining children as young as 5 to 10 years of age and their lipid levels 20 to 30 years later (Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents and National Heart, Lung, and Blood Institute 2011). In the Muscatine Study, 75% of children aged 5–10 years who had total cholesterol concentrations greater than the 90th percentile at baseline had total cholesterol concentrations of >200 mg/dL in their early 20s (Lauer, Lee, and Clarke 1988; Lauer and Clarke 1990). In the Bogalusa Heart Study, approximately 50% of those children who had total cholesterol levels or LDL-C levels above the 75th

percentile at baseline remained elevated 12 years later (Webber et al. 1991). In the same study, adverse glucose levels in childhood not only persisted into adulthood but also predicted adult pre-diabetes and type 2 diabetes (Nguyen and Berenson 2010). In a retrospective cohort study of 1058 normoglycaemic, 37 pre-diabetic, and 25 type-2 diabetic adults aged 19–39 years followed on average for 17 years since childhood, at least 50% of the individuals who ranked in the top childhood quintile for glucose, insulin, and homeostatic model of assessment (HOMA) of insulin resistance maintained their high rank by being above the 60th percentile in adulthood (Nguyen and Berenson 2010). Elevated blood pressure in youth predicts adult hypertension, and a systematic review and meta-analysis of 50 cohort studies reported a degree of tracking, with correlation coefficients of 0.38 for SBP and 0.28 for DBP (Chen and Wang 2008). Significant tracking of CRP levels was observed between childhood and adulthood in a cohort of 1,617 subjects, aged 3 to 18 years at baseline and re-examined at 24 to 39 years. The age-and sex-specific correlations were the highest in the group aged 18 years at baseline ($r=0.47$ in females, $r=0.32$ in males) (Juonala et al. 2006).

Of particular relevance to this thesis, diet itself, one of the strongest correlates of cardiometabolic risk, also tracks from childhood into adulthood. The Cardiovascular Risk in Young Finns Study, a prospective cohort study with 21-year follow-up, reported some level of tracking of dietary patterns between childhood until adulthood (Mikkilä et al. 2005). The tracking was highest for the lowest and highest quintile of the dietary pattern score and for older children (adolescents), and thus 30–42% and 27–41% of subjects originally belonging to the extreme quintile of the energy-adjusted pattern scores persisted in the same

quintile 6 and 21 years later, respectively. Similarly, a review of studies published between 2003 and 2013 reported moderate level of tracking for a range of eating behaviours (e.g. food preferences, dietary variety, dietary intake, eating habits) measured before 10 years of age and reassessed in adulthood (Nicklaus and Remy 2013).

6. The significance of primordial prevention

The considerable gains made in reducing CVD mortality rates since the 1970s through risk factor reductions and better treatment of the disease are increasingly challenged by adverse trends in obesity and diabetes (IOM, Fuster, and Kelly 2010; Capewell and Buchan 2012). Better treatment strategies are not expected to offset these adverse trends (Emberson et al. 2004; Capewell et al. 2010), and would increase exponentially the medical costs, given the ageing of most populations. Moreover, they only reduce, but do not eliminate, the risk of CVD (Bibbins-Domingo et al. 2016; Gosmanova and Kovesdy 2015).

Therefore, risk factor reduction strategies are critical for reducing the CVD burden. Numerous modelling studies have estimated that population-wide risk factor reductions can bring substantial decreases of CVD burden (O'Flaherty et al. 2016; Hughes et al. 2015; Scholes et al. 2013; Huffman et al. 2013), even taking into account current trends of obesity and diabetes. It has also been shown that mortality trends respond very rapidly to changes in risk factors at the population level (Capewell and O'Flaherty 2011). Given compelling evidence that the atherosclerotic process starts in childhood, and is linked to well-defined, modifiable risk factors that track into adulthood, there is increasing recognition that primordial prevention, i.e. avoiding the development of risk factors before

the disease onset, should be embraced as a major component of global CVD prevention policies (Tanrikulu, Agirbasli, and Berenson 2016). This is an approach through which favorable patterns for all lifestyle and all major lifestyle-related risk factors can be promoted from conception throughout childhood. Those patterns can then potentially be maintained into adulthood (Lauer, Burns, and Daniels 2006). The concept of primordial prevention is strongly backed by data. Two prospective studies from the late 1960s and 1970s – the Chicago Heart Association Detection Project in Industry Study (Stamler et al. 1993) (n = 8,816), and the Multiple Risk Factor Intervention Trial Study (“Multiple Risk Factor Intervention Trial. Risk Factor Changes and Mortality Results. Multiple Risk Factor Intervention Trial Research Group.” 1982) (n = 12,866) – showed that favorable levels of all readily-measured major CVD risk factors in young adulthood (TC <5.17 mmol/L; SBP ≤ 120, DBP ≤ 80 and BMI < 25.0 kg/m²) lead to considerably reduced CVD mortality rates (76–89% for men and 60–67% lower in women) and substantial increases in life expectancy (8–12 years longer).

So far, CVD prevention strategies targeting early life have primarily been focused on tackling childhood obesity, though success rates are poor (Colquitt et al. 2016; Christie et al. 2017). Interest has also focused on factors like birth weight (Wang et al. 2014) and early catch-up growth (Jain and Singhal 2012), though some of these traits are difficult to target through interventions, due to the need to change maternal physiology. We know, however, from the Cardiovascular Risk in Young Finns Study, Childhood Determinants of Adult Health study, Bogalusa Heart Study, and Muscatine Study, that other classical CVD risk factors relate to adult atherosclerosis independently from BMI (Juonala et al. 2010), and would therefore benefit from additional interventions.

For example, recent evidence from the US suggests that up to 1/3 of pre-pubertal children with normal weight have abnormal lipid levels (Seery et al. 2014), and that the prevalence of hypertension in the paediatric population has been increasing (Din-Dzietham et al. 2007). Therefore, interventions effectively targeting all classical risk factors in young people could potentially play a key role in preventive strategies.

If one was to link the two components of this literature review – that adults consuming plant-based diets have lower CVD risk, and that cardio-metabolic risk tracks from childhood into adulthood – a new testable hypothesis can be put forward: namely that plant-based diet in childhood could promote cardio-metabolic health in adults, and thereby reduce CVD and promote longevity and health.

7. Plant based diets in children and cardio-metabolic health

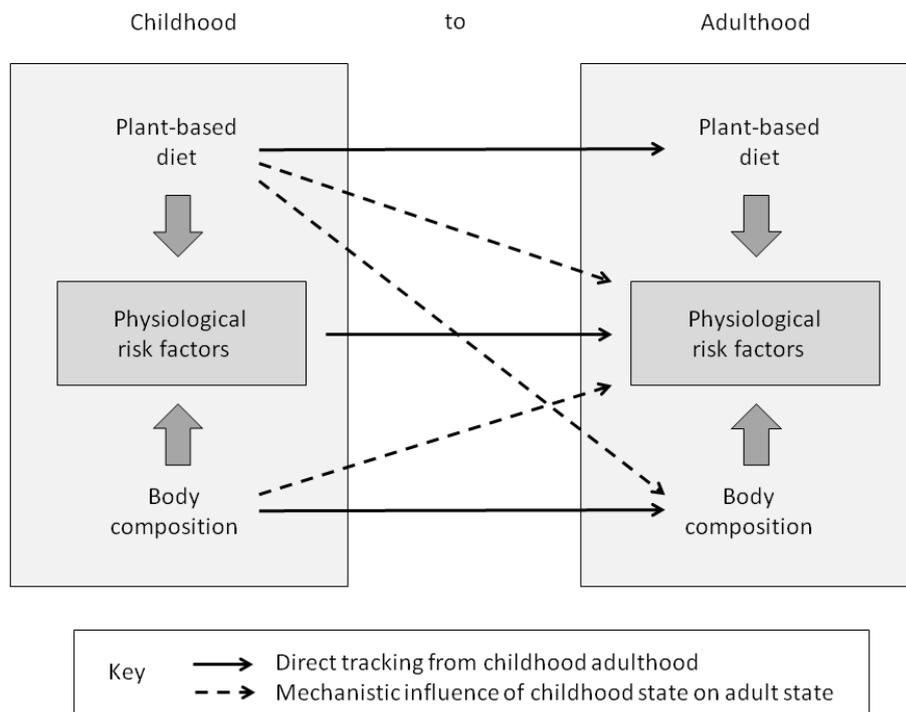
Diet in children appears to be one of the strongest determinants of their CVD risk factors (Laitinen et al. 2013a). The few available studies in this area suggest that healthy childhood dietary patterns are also associated with lower adulthood CVD risk and vascular markers of subclinical atherosclerosis (Kaikkonen, Mikkilä, and Raitakari 2014; Laitinen et al. 2013b). This could be due to the fact that childhood diets show some degree of tracking into adulthood (Mikkilä et al. 2005; Nicklaus and Remy 2013).

Intakes particularly of plant foods (vegetables, fruits and fibre) and polyunsaturated fatty acids have shown protective effects (Kaikkonen, Mikkilä,

and Raitakari 2014; Dahm et al. 2016). Vegan and vegetarian children have lower rates of overweight and obesity (Sabaté and Wien 2010) than their omnivorous peers. Preliminary evidence suggests that they have lower cholesterol levels (Ruys and Hickie 1976; Yen et al. 2008; Schürmann, Kersting, and Alexy 2017) and higher antioxidant status in the blood (Krajcovicová-Kudlácková et al. 1997). They consume more fruits and vegetables than their omnivore counterparts (Nathan, Hackett, and Kirby 1996a; al-Dlaigan, Shaw, and Smith 2001). Moreover, a recent trial showed that an intervention with low-fat vegan diet was more effective at reducing CVD risk factors in obese and hypercholesterolaemic children aged 9 to 18 years old than with the American Heart Association (AHA) recommended diet. Children assigned to the vegan intervention had more significant reductions in CVD risk factors from baseline: BMI z-score (-0.14), systolic SBP (-6.43 mm Hg), total cholesterol (-22.5 mg/dL), LDL-C (-13.14 mg/dL), high sensitivity CRP (-2.09 mg/L), insulin (-5.42uU/ml), myeloperoxidase (-75.34 pmol/L), mid-arm circumference (-2.02 cm), weight (-3.05 kg); whereas the significant reductions in the AHA group were noted only for the last 3 risk factors (69.23 pmol/L, -1.55 cm, -1.14 kg respectively) and waist circumference (-2.96 cm) (Macknin et al. 2015).

Therefore, vegetarian and vegan children might have a better CVD risk profile than omnivore children, and if the diet and risk profile were to track into adulthood, plant-based diets in paediatric populations, through their effects on blood biochemistry, other cardiovascular risk factors and establishing healthy eating patterns, could offer an effective strategy of CVD primordial prevention. This would present a novel opportunity to promote a healthy childhood trajectory towards cardiovascular health (see Figure2) (Desmond et al. 2018).

Figure 2. Potential direct and indirect mechanisms through which plant-based diets in children could benefit cardiometabolic health in adults (Desmond et al. 2018).



8. The rationale for the choice of CVD risk factors assessed in this study

Previous sections of this chapter reviewed substantial evidence linking plant-based diets and better cardiometabolic health profile. Based on the reviewed literature, I hypothesize that the diet composition of vegetarian, and especially vegan children, is likely to affect numerous parameters of CVD risk differently from that of omnivore children. However, the corresponding evidence, as reviewed in subsequent sections, is sparse. Therefore, the CVD risk profile of this group merits comprehensive scrutiny. This section outlines the rationale for the choice of the parameters used in this study to assess the potential differences in the CVD risk factor profile in the studied children. Additionally, it discusses the importance of childhood physical activity in improving cardiometabolic health, as well as other aspects of body composition, which

needs to be measured along with other markers of CVD risk in order to understand the effects of the diet alone.

8.1. Body fatness

Body composition, which entails information on lean mass and body fatness, is a fundamental index of health in children. It is strictly linked to dietary practices and predicts numerous health outcomes, foremostly cardiovascular disease risk. Body mass index is the most commonly studied measure of body fatness, including among the paediatric population (Weber, Leonard, and Zemel 2012). Elevated BMI is a well-established risk factor for adverse cardiovascular risk profile in children. A systematic review and meta-analysis of 63 studies including 49,220 children found that BMI-categorized overweight, and especially obesity, is linked to increased blood pressure, dyslipidaemia, hyperinsulinaemia, insulin resistance and larger left ventricular mass in school aged children (Friedemann et al. 2012). In the Avon Longitudinal Study of Parents and Children, a one standard deviation (SD) greater BMI at age 9–12 years was associated with higher SBP, higher concentration of triglycerides, LDL cholesterol, low concentrations of HDL cholesterol, and higher concentration of insulin at age 15–16 years (The Emerging Risk Factors Collaboration et al. 2010). The associations of cardiovascular risk factors with body fatness measured with techniques other than BMI – waist circumference, and fat mass (measured by DXA) – were of comparable strength, and all the body fatness measures were strongly correlated with each other. In obese children aged 7–11 years, visceral adipose tissue mass measured with magnetic resonance imaging, explained a significant proportion of the variance in lipid abnormalities (Owens et al. 1998). In a cross-sectional study of 1,432 children aged 12 years, both high BMI and large waist circumference

were associated with higher blood pressure levels and adverse cholesterol concentrations (Bekkers et al. 2012).

Associations of increased body fatness in childhood and adverse cardiovascular risk profile in adulthood are less clear. A systematic review and meta-analysis of, respectively, 21 and 23 longitudinal studies, that collected childhood exposure and adult outcomes within individuals over time, suggested that childhood obesity is positively associated with adult SBP and DBP, triglyceride levels, and negatively associated with adult HDL cholesterol. However, these associations were nullified in studies that adjusted for adult BMI, suggesting that adult BMI may be a key potential mediator. The authors concluded that childhood obesity may increase the risk of adverse adult CVD risk factor profile, although higher-quality studies with the aim to isolate the effect of childhood obesity independent of adult BMI, are needed to confirm this assertion (Umer et al. 2017). However, higher BMI in childhood, even when well within the range currently considered normal, might increase adult coronary artery disease risk, independent of adult BMI (Tirosh et al. 2011). This suggests that the adverse processes leading to cardiovascular disease related to excess body fat may originate in childhood.

8.2. Blood lipids

Autopsy studies discussed in the previous sections established that the extent of plaques in the aorta and coronary arteries correlated positively with total cholesterol, LDL cholesterol and non-HDL cholesterol, and negatively with HDL cholesterol levels (McGill et al. 2000) in children and young adults. Elevated childhood LDL cholesterol and decreased HDL cholesterol predict cIMT and

adult coronary artery calcification, surrogate measures of atherosclerosis (Davis et al. 2001; Mahoney et al. 1996b, 1996a). Diet has a profound effect on blood lipids levels (Grundy and Denke 1990), even in genetic conditions affecting their metabolism (Connor and Connor 1993).

8.3. Inflammation (CRP)

CRP is an acute-phase protein and non-specific marker of inflammation, produced predominantly in hepatocytes. Elevated high sensitivity C-reactive protein (hs-CRP) is an independent predictor of subsequent cardiovascular disease and myocardial infarction in adults (A. Wang et al. 2017). Hs-CRP is significantly elevated in children and adolescents with excess weight as compared to normal-weight individuals (Kitsios et al. 2013). Children with numerous CVD risk factors (hypercholesterolaemia, hypertension, obesity, low HDL cholesterol and familial history of coronary artery disease (CAD)) had significantly higher serum levels of hs-CRP compared to the control group (Guran et al. 2007). A study of 70 healthy children found a significant association between hs-CRP levels and cIMT as well as impaired brachial artery flow-mediated dilatation, a marker of disturbed endothelial function (Järvisalo et al. 2002). This suggests a role of elevated CRP in early atherosclerosis.

Foods can have anti- and pro-inflammatory potential and thus modulate low-grade inflammation affecting inflammatory markers levels, including CRP. A high score of the dietary inflammation index was positively associated with hs-CRP levels in 9–10 year old obese children (Lazarou et al. 2010). In another study, among adolescents with obesity-induced higher hs-CRP levels, a higher

frequency of vegetable/legume intake, foods with anti-inflammatory potential, was inversely related to this marker (Cabral et al. 2018).

8.4. Insulin resistance

In insulin resistance (IR) a given concentration of insulin produces an inadequate (less-than-expected) biological effect. This results in increased insulin secretion to maintain normal glucose and lipid homeostasis. The consequences of this pathological state include hyperglycaemia, hypertension, dyslipidaemia, visceral adiposity, hyperuricemia, elevated inflammatory markers, endothelial dysfunction, and a prothrombic state. In adults, IR can lead to a development of cardiovascular disease, as metabolic changes induced by IR lead to atherosclerotic plaque formation (Ormazabal et al. 2018). IR in children is associated with obesity and correlates with prevalence of the components of the metabolic syndrome (increased blood pressure, increased waist circumference, increased triglyceride levels, low levels HDL-C). Therefore a higher cardiovascular risk is predicted among children with IR (Júrez-López et al. 2010). Childhood IR and hyperinsulinaemia are positively associated with blood pressure (BP), independent of adiposity and age (Arslanian and Suprasongsin 1996), and predict future BP in adolescents (Taittonen et al. 1996). IR is closely linked to dietary habits. According to the consensus of all the major scientific societies in paediatric endocrinology, IR results from obesity induced by increased energy intake (Levy-Marchal et al. 2010). Alternative explanations exist and suggest that IR results from excessive sugar intake which leads to metabolic modifications that induce chronic weight gain and depress physical activity. Therefore, obesity is, in this scenario, the consequence rather than the cause of IR (Lustig 2008; Wells and Siervo 2011).

Nonetheless, in both explanations the dietary component plays a central role in the IR aetiology. This is confirmed by the preliminary data on paediatric IR – cross-sectional studies in children suggest that other than excess calorie supply, selected dietary characteristics, like high saturated fat and sweetened beverages intake, may be associated with alterations in insulin sensitivity and secretion (Weigensberg et al. 2005).

8.5. Insulin-like growth factor 1

Insulin-like growth factor 1 (IGF-1) is a nutritionally regulated peptide and serum levels are positively affected by animal protein intake (especially dairy protein) (Crowe et al. 2009; Hoppe et al. 2004). The IGF-1 system has been implicated in cardiovascular risk (Laughlin et al. 2004; Higashi et al. 2019). The biological effects of IGF-1 are modulated by IGF-binding proteins (IGFBPs 1-6) (Higashi et al. 2019). The ratio of IGF-1 to IGFBP-3 is considered to reflect the bioavailability of IGF-1 for target tissues, and the higher it is, the greater are its effects (Le Roith 1997). Low levels of IGF-1 in adults are linked to greater risk of cardiovascular morbidity (Juul et al. 2002) and mortality (Laughlin et al. 2004). Less understood is the relationship between childhood IGF-1 and adult CVD risk. Higher childhood IGF-1 is linked to earlier menarche, which may increase future risk of obesity (Juonala et al. 2010; Laitinen, Power, and Järvelin 2001; Pierce and Leon 2005) diabetes (Lakshman et al. 2008), and metabolic syndrome (Frontini, Srinivasan, and Berenson 2003). On the other hand, studies have shown that childhood IGF-1 is positively associated with stature (Alberti et al. 2011). Findings from the Boyd-Orr cohort (n = 2642) suggest that childhood stature is inversely associated with CVD morbidity and premature mortality in adulthood (Whitley et al. 2009), which is consistent with similar

findings on adults (McCarron et al. 2002; Rosenbush and Parker 2014). Childhood diets might program IGF-1 levels long-term. Observational studies (Larnkjaer et al. 2009; Larnkjær, Mølgaard, and Michaelsen 2012), along with a randomized control trial (Ben-Shlomo et al. 2005), suggest that early childhood milk intake can increase IGF-1 in the short term, with a contrasting, lowering effect in adulthood. In this case, early accelerated growth would be associated with lower life course IGF levels and this may be one of the biological pathways linking early growth with CVD, as suggested by Lucas (Singhal and Lucas 2004).

8.6. Carotid intima media thickness

Thickening of the artery wall is a hallmark of atherosclerosis (Øyegarden 2017). In adults, ultrasound measurement of carotid cIMT is an accepted, validated, non-invasive method used to assess the extent of atherosclerosis and CAD risk (Touboul 2014). Changes in cIMT are known to parallel atherosclerotic changes in coronary arteries. Increased cIMT is significantly related to other adult cardiovascular risk factors. CIMT is also an accepted method for assessment of vascular atherosclerotic alterations and surrogate marker for cardiovascular risk in children (Doyon, Kracht, Bayazit, Deveci, Duzova, Krmar, Litwin, Niemirska, Oguz, Schmidt, Sözeri, Querfeld, Melk, Schaefer, Wühl, and Consortium 2013; Dalla Pozza et al. 2015). Higher cIMT has been observed in children with hypercholesterolaemia, insulin resistance, hypertension, type 1 diabetes and metabolic syndrome along with obesity (Urbina et al. 2009), however a recent systematic review found the association with obesity to be significant only in adolescents (Park et al. 2015).

8.7. Physical activity

Inactivity and different levels of physical activity intensity have been recognised to exert their health effects independently and are now usually measured separately in studies (Talarico and Janssen 2018).

Regular physical activity during childhood decreases the risk of overweight and obesity (Janz et al. 2009; Hills, Andersen, and Byrne 2011; Hong et al. 2016), promotes muscular fitness, bone health and improves cardiometabolic health biomarkers (Janssen and LeBlanc 2010). Sedentary behaviours, usually measured as screen-time, are linked to increased adiposity, unhealthy food intake along with other health harms (Stiglic and Viner 2019). Sedentary behaviours track in childhood (Janz, Burns, and Levy 2005), physical activity tracts from childhood to adulthood (Raitakan et al. 1994; Telama 2009).

Both moderate and vigorous activity as well as inactivity impact on overweight and obesity risk in children (Janssen and LeBlanc 2010; Chaput et al. 2018). Sedentary behaviour might induce overweight and obesity independently of the effects of more vigorous activity (Tremblay et al. 2011), through its association with other behaviours that promote excess weight gain, rather than merely through a reduction in energy expenditure (Wolf et al. 1993; Stiglic and Viner 2019).

Systematic review of the health benefits of physical activity in childhood indicated that as little as 10 minutes of moderate-to-high impact activity performed on as few as 2 or 3 days a week can have a modest effect on bone mineral density when combined with more general weight bearing aerobic activities like jogging or play (Janssen and LeBlanc 2010). Inactivity in children is also linked to worse

CVD risk factor profile (Tremblay et al. 2011) and physical activity is linked to a better profile in childhood (Proudfoot et al. 2019; Andersen et al. 2006) and young adulthood (Raitakan et al. 1994). The CVD risk factor improving effects of physical activity are primarily linked to its capacity to improve the regulation of glucose and fat oxidation (Kuhl et al. 2006; Kiens 2006). Its impairment, otherwise called metabolic inflexibility, might lead to insulin resistance through excess lipid accumulation in non-adipose tissues like skeletal muscle, which impair insulin signalling (Corpeleijn, Saris, and Blaak 2009).

All the above discussed health parameters influenced in children by activity levels, namely – body composition, bone strength (as discussed in the following section) and cardiovascular risk profile, might be affected in children both by exercise and also by plant-based diets. Vegetarians and vegans may differ from the omnivores in their exercise habits (Key et al. 1999), hence their activity levels need thorough examination.

9. Safety of vegetarian diets in childhood

The discussion of potential benefits of plant-based diets in childhood would be incomplete without considering their potential risks. Vegetarian and especially vegan diets restrict whole food groups, and therefore lead to reduced intake of several nutrients along with energy (Schürmann, Kersting, and Alexy 2017). This is of particular importance in children, whose nutrient and energy needs are higher in relation to body weight than in adults. Deficiency of energy and micronutrients during growth and development might exert negative impact on health that can last into adulthood (Sawaya et al. 2005; Martins et al. 2011).

Despite growing interest in vegetarian diets, comprehensive data on their health effects in children are still lacking. The existing studies are very heterogeneous in terms of design and other aspects of methodology, with predominantly small sample sizes, different definitions of vegetarianism and lack of control groups. There are no informative studies on vegan children. Therefore, the current state of knowledge is insufficient to thoroughly assess the safety of vegetarian or vegan diets in children in developed countries.

The sparsity of evidence contributes to inconsistencies between medical and nutrition organizations' statements regarding the safety of meat-free diets in childhood. While the American Academy of Pediatrics (Kleinman and American Academy of Pediatrics Committee on Nutrition 2009), British Dietetic Association (BDA 2017a) and the US Academy of Nutrition and Dietetics (Melina, Craig, and Levin 2016b) all assert that vegetarian and vegan diets, if planned appropriately, can be healthy and nutritionally adequate at all ages, the Polish Consultant for Paediatrics (Reguła 2013), the Belgian Royal Academy of Medicine (Belgique 2019), the Committee of Human Nutrition Science of Polish Academy of Sciences (Wądołowska 2019) and the German Nutrition Association (Die Deutsche Gesellschaft für Ernährung n.d.) do not recommend vegan diets in children, due to the higher risk of nutrient deficiencies. The endorsement statements exist despite a sparsity of data on vegan children. This chapter summarizes all the available data from industrialised countries.

The following sections constitute a review of the literature based on the selection criteria used by the recent systematic review of Schürmann et al. 2017. I used the MEDLINE database to search for relevant studies in English and German until 6th November 2014 from industrialized countries only. The

keywords used were as follows: vegetarian OR vegan AND infant OR infancy OR child OR adolescent OR pregnant OR pregnancy OR breastfeeding OR breastfed. Since then, I identified 5 additional studies using the same search criteria excluding keywords relating to pregnancy and included them in this review.

9.1. Characteristics of published studies so far

In 2017, a systematic review (Schürmann, Kersting, and Alexy 2017) of studies on vegetarian and vegan children was published. It included 24 observational studies of healthy children from industrialized countries, and excluded studies on macrobiotic diets, as these constitute a special kind of pescatarian diet (including fish) (Soare et al. 2016).

The studies from the review date from 1981 to 2013. Since then, 5 studies have been published in industrialized countries, so this chapter will summarize the methodologies and findings from the total 29 studies published between 1981 and September 2019.

The study design was mainly cross-sectional, with 8 prospective studies (Sanders 1988; Sievers et al. 1991; Nathan, Hackett, and Kirby 1996b, 1997a; Taylor, Redworth, and Morgan 2004; Kissinger and Sanchez 1987; Sabaté et al. 1991; Harris et al. 1981). The majority of research on vegetarian children has been conducted in Central Europe ($n = 16$) (Laskowska-Klita et al. 2011; Sievers et al. 1991; Krajcovicova-Kudlackova et al. 1997; Hebbelinck, Clarys, and De Malsche 1999; Ambroszkiewicz, Laskowska-Klita, and Klemarczyk 2003; Ambroszkiewicz et al. 2006, 2007; Jadwiga Ambroszkiewicz et al. 2011; Krajcovicová-Kudlácková et al. 1997; Gorczyca et al. 2011; Gorczyca et al.

2013; Ambroszkiewicz et al. 2017; Ambroszkiewicz, Chełchowska, Szamotulska, et al. 2018a; Ambroszkiewicz, Chełchowska, Rowicka, et al. 2018; Weder et al. 2019a; Ambroszkiewicz et al. 2019); 4 in the UK (Sanders 1988; Nathan, Hackett, and Kirby 1996b, 1997b; Taylor, Redworth, and Morgan 2004), the majority in Poland (11).

Eight studies were undertaken in the USA (Harris et al. 1981; Kissinger and Sanchez 1987; O'Connell et al. 1989; Lombard et al. 1989; Sabaté et al. 1991; J Sabaté, Llorca, and Sánchez 1992; Persky et al. 1992; Matthews, Wien, and Sabaté 2011) and most of them, apart from the study of O'Connell, were conducted among the Seventh-day Adventist (SDA). SDAs are a Christian religious denomination, in which numerous members follow a vegetarian diet and general health principles (e.g. abstinence from alcohol, tobacco, and caffeinated beverages), as advocated by the church. Fourteen of the articles were published after 2000 – 11 Polish, 1 American, 1 British, and 1 German study. Ten articles were published in the 1990s (1 German, 2 British, 2 Slovak, 1 Belgian and 4 US studies), and the remaining five in the 1980s (4 US studies, 1 British). The age range of children spanned from 0 to 18 years; three studies (Sievers et al. 1991; Taylor, Redworth, and Morgan 2004; Weder et al. 2019a) included only infants and/or toddlers.

Participants were recruited either from previous studies, or from vegetarian institutions and websites devoted to vegetarianism or veganism, or through advertisements in health food stores, vegetarian communities, media or social media. Most Polish studies selected their participants from a group of consecutive patients seeking dietary counselling in the Department of Nutrition at the Institute of Mother and Child in Warsaw (Poland), where the studies took

place (Ambroszkiewicz et al. 2017). Study samples of vegetarian or vegan children were usually relatively small, with fewer than 100 children (Table 4), though four studies examined 250–400 participants (O’Connell et al. 1989; Sabaté et al. 1991; Weder et al. 2019a; Matthews, Wien, and Sabaté 2011; Joan Sabaté et al. 1990; Harris et al. 1981). In five studies that provided information on family characteristics, socioeconomic status (SES) and/or educational attainment were described as high, although exact data were not given (Sanders 1988; Nathan, Hackett, and Kirby 1996b, 1997a; Taylor, Redworth, and Morgan 2004; O’Connell et al. 1989; Sabaté et al. 1990). In the latest German study (Weder et al. 2019a), the family characteristics were measured by an index combining three scores of social status (education, profession, total net household income). The majority (over 70%) of study participants had the highest combined score.

Dietary assessment methods included food frequency questionnaires (FFQ) (11 studies), food records (10 studies), weighed records (3), or interviews (24-hour recalls (24HR)) (3). Only three studies focused on vegan diets (Sanders 1988; O’Connell et al. 1989; Weder et al. 2019a). The vegetarian and/or vegan status of participants was categorised in various ways. Some studies included no information on how they categorised children, for example it is not known how many vegetarians were included in the study of Harris et al (Harris et al. 1981), or how many vegans were studied in one of the very few, but widely cited, studies on vegan children – the Farm Study (O’Connell et al. 1989). Some studies classified their participants in dietary groups based on several 24HRs administered over a long period (Kissinger and Sanchez 1987), others collected single food records with no long-term dietary habits ascertainment (Sanders

1988). In a few studies details on the dietary assessment method, on which the categorisation was based, were entirely lacking (Krajcovicova-Kudlackova et al. 1997; Ambroszkiewicz et al. 2006). Few publications provided very detailed information on both short and long – term dietary assessment methods (Ambroszkiewicz et al. 2017). Some studies classified as vegetarians or vegans children who followed the diet for at least 1 year (Gorczyca et al. 2011), others only accepted children who had followed this diet for ≥ 3 years (Hebbelinck, Clarys, and De Malsche 1999). Nathan et al. 1996 defined vegetarian diet as one including fish, and therefore some of the ‘vegetarian’ children in this study were eating on average 5 g of fish a day. The details of dietary categorisation criteria of vegans and vegetarians in the published studies are described below, in Table 3.

Table 3. Dietary categorisation criteria of vegans and vegetarians and ascertainment of dietary data in the published studies

Study	Study objective	Dietary categorisation criteria and ascertainment of dietary data
(Harris et al. 1981)	To establish the distribution of Blood Pressure Levels of SDA and Non-SDA Children.	No details given on the percentage of vegetarians among the participants of the study.
(Kissinger and Sanchez 1987)	To establish the relationship between dietary factors and the age of menarche.	24HRs were administered at school to girls at 2–4 unannounced times during the school year to establish vegetarian status, those who reported no meat intake, were categorised as vegetarians.
(Sanders 1988)	To study growth and development of children born of vegan mothers and reared on a vegan diet, studied longitudinally.	7-day weighed food diary was collected, no information on ascertainment of long – term vegan status other than declaration.
(Lombard et al. 1989)	To measure plasma carnitine concentrations and urinary carnitine excretion in adults and children consuming vegan, vegetarian or mixed diet.	FFQ was administered to study participants. Vegans – those who did not consume meat and consumed dairy < 1 a month; vegetarians – those consuming meat less than once per month but consuming dairy products at least twice per week; omnivores-those consuming meat at least twice per week with no restriction on dairy. Subjects with diets that did not conform to one of these categories were excluded.
(O’Connell et al. 1989)	To examine the effects of a vegetarian diet on child growth in height and weight of 404vegetarian (mainly vegan) children who lived in a collective community.	Dietary data in the form of modified food frequencies was provided in health surveys (collected twice in three years before the study), and one dietary history administered just before the study. The children were classified as omnivore (nonvegetarian), lacto-ovo-vegetarian, or vegan for each year based on the amount of eggs, dairy products, and meats reported in their diets. Some members of the vegan community introduced dairy and eggs into their diet before the study took place, however no exact details on the numbers were provided.
(Sabaté et al. 1990)	To analyse height and weight data obtained from a 2-year longitudinal survey of 2272 children aged 6 through 18 years who were attending public schools or SDA schools in southern California.	No data on the number of vegetarians, instead frequency data of meat consumption per month were provided.

Study	Study objective	Dietary categorisation criteria and ascertainment of dietary data
(Sabaté et al. 1991)	To examine attained height of vegetarian children and adolescents.	Non-quantitative FFQ was used to collect the frequency of 6 principal food groups. This was compared to data from one 24HR and based on this a child consuming meat less than once per week was considered vegetarian.
(Sabaté, Llorca, and Sánchez 1992)	To examine the association between vegetarian life-styles and height of children during preadolescence– age 11 to 12 years.	Methodology the same as in (Sabaté et al. 1991).
(Sievers et al. 1991)	To examine iron status in vegetarian infants.	Article in German, I was not able to retrieve full text and hence detailed information on methodology.
(Persky et al. 1992)	To explore hormonal differences among teenage girls whose nutritional intakes indicate varying risk of breast cancer, specifically girls who consume vegetarian or nonvegetarian diets.	The participants filled in a health questionnaire, including a question on vegetarian status. They also completed a 3-day food record.
(Nathan, Hackett, and Kirby 1996b)	To assess dietary intake of vegetarian children.	Criteria for inclusion: the child had to follow a vegetarian diet for at least 3 months. A vegetarian diet was defined as one that may include dairy products, eggs and fish, but not meat or meat products; diet was assessed using a 3-day dietary diary and interview. Children were asked to fill in a pocket-sized diary on three occasions at 6-month intervals. 23 out of 50 studied vegetarian children regularly consumed fish, on average 5 g a day.
(Nathan, Hackett, and Kirby 1997b)	To assess the ability of a meat free diet to support normal growth of children.	Methodology the same as in (Nathan, Hackett, and Kirby 1996a).
(Krajcovicova-Kudlackova et al. 1997)	To examine plasma fatty acid profile of healthy vegetarians.	The span of duration of vegetarian diet in studied children was 1–7 years, on average – 3.85 years. Dietary status was ascertained by means of dietetic questionnaires (no further details were provided).

Study	Study objective	Dietary categorisation criteria and ascertainment of dietary data
(Krajcovicová-Kudláčková et al. 1997)	To evaluate the health and nutritional status of children with two different nutritional habits (vegetarian and omnivore).	Article in German, I was not able to retrieve full text and hence detailed information on methodology. Information from the abstract: an average period of vegetarianism duration – 2.8 years.
(Hebbelinck, Clarys, and De Malsche 1999)	To assess average daily dietary intakes of energy in vegetarian children and to determine their height and weight; triceps, suprailiac, and calf skinfold thicknesses; puberty ratings; and physical fitness.	The children had to follow a vegetarian diet for ≥ 3 y. Dietary information was obtained using a 7-day FFQ.
(Ambroszkiewicz, Laskowska-Klita, and Klemarczyk 2003)	To investigate serum concentration of osteocalcin and leptin in prepubertal children with two different nutritional habits: vegetarian and omnivorous diet.	Article in Polish, I was not able to retrieve full text and hence detailed information on methodology. Information from the abstract: the children were divided into vegetarian and omnivores groups. Requested full text.
(Taylor, Redworth, and Morgan 2004)	To determine whether iron and micronutrient status is improved with an increased amount of meat in the diet among infants.	7-day weighed food intake diaries were recorded when the infants were aged 4, 8, 12, 16, 20, and 24 months. No meat consumption was equivalent to vegetarian status classification.
(Ambroszkiewicz et al. 2006)	To assess the effect of vegetarian diets on serum concentrations of homocysteine, folate, vitamin B12 and total antioxidant status in children.	In the studied group there were: <ul style="list-style-type: none"> – lacto-ovo-vegetarians (n=21), who did not consume meat, poultry or fish, but ate eggs and dairy products, – lacto-vegetarians (n=1), who excluded eggs, – ovo-vegetarians (n=5), who ate eggs, but excluded milk products, – vegans (n=5), who excluded all foods of animal origin. No information was provided how this data was ascertained.
(Ambroszkiewicz et al. 2007)	To investigate the serum concentrations of biochemical bone turnover markers in prepubertal vegetarian children.	In the studied group there were 28 lacto-ovo-vegetarians (did not consume meat, poultry, fish, but ate eggs and dairy products), 4 lacto-vegetarians (excluded eggs), 5 ovo-vegetarians (excluded milk products, but ate eggs) and 13 vegans (excluded all foods of animal origin). Dietary data were assessed by questionnaire. No further details were provided.

Study	Study objective	Dietary categorisation criteria and ascertainment of dietary data
(Ambroszkiewicz et al. 2011)	To investigate associations between serum adipocytokines status and anthropometric parameters as well as total energy and macronutrient intake in vegetarian, normal weight omnivorous and obese omnivorous children.	In the studied group, there were 15 lacto-ovo-vegetarians (did not consume meat, poultry, fish, but ate eggs and dairy products), 2 lacto-vegetarians (excluded eggs, but ate milk products), 9 ovo-vegetarians (excluded milk products, but ate eggs) and 4 vegans (excluded all foods of animal origin). Dietary constituents were assessed by questionnaire (nutrient intake from a 3-day period: 2 weekdays and 1 weekend day).
(Laskowska-Klita et al. 2011)	To assess the intake and serum status of vitamin B12, folate, vitamins A, E and D, as well as concentrations of homocysteine, total antioxidant status and iron balance in Polish vegetarian children.	In this group there were: <ul style="list-style-type: none"> – lacto-ovo-vegetarians (n=21), who did not consume meat, poultry or fish, but had no restrictions to eggs and dairy products consumption, – lacto-vegetarians (n=1), who also excluded eggs, – ovo-vegetarians (n=5), who had no restrictions to eggs, but excluded milk products, – vegans (n=5), who excluded all foods of animal origin.
(Gorczyca et al. 2011)	This study aimed to study an effect of vegetarian diet on lipid profile in serum in a group of Polish children in Poland and to investigate lipid parameters in healthy vegetarian children and in omnivorous children with diagnosed atopic disease.	The inclusion criteria of vegetarian group were: the child had followed a vegetarian diet for a minimum of 1 year. The classification of diet group and duration of adherence to vegetarian diet types was based on the qualifying questions from an FFQ. Subjects who reported that they ate meat were classified as omnivores. Subjects who reported that they did not eat meat but ate fish were classified as semi-vegetarians. Children who reported that they did not eat meat or fish but ate dairy products were classified as lacto-vegetarians, those eating eggs were classified as ovo-vegetarians, and those eating dairy products and eggs as lacto-ovo-vegetarians. Those who reported that they did not eat any food of animal origin were classified as vegans.
(V. Matthews, Wien, and Sabaté 2011)	To investigate the association between the risk of overweight and the consumption of food groups in children and adolescents.	Same methodology as in (Sabaté et al. 1991).
(Gorczyca et al. 2013)	To examine the effect of iron intake on iron status in vegetarian children.	Same methodology as in (Gorczyca et al. 2011).

Study	Study objective	Dietary categorisation criteria and ascertainment of dietary data
(Ambroszkiewicz et al. 2017)	To assess the effect of a vegetarian diet on iron metabolism parameters.	Dietary constituent data were assessed by questionnaire and a 10-day food diary. They had been previously been advised by the nutritionist on how to do it correctly. The diary was checked by the nutritionist in the presence of the child and parent. When necessary, the food diary was corrected by the nutritionist during the visit. Out of the 10 days in the record, three consecutive days (two weekdays and one weekend day) were selected for analysis of nutritional intake. No further details provided.
(Ambroszkiewicz, Chełchowska, Rowicka, et al. 2018)	The aim of the study was to assess serum adipokine profile in prepubertal vegetarian and omnivorous children.	The same methodology as in (Ambroszkiewicz et al. 2017).
(Ambroszkiewicz, Chełchowska, Szamotulska, et al. 2018b)	The aim was to evaluate serum levels of bone metabolism markers and to analyse the relationships between biochemical bone markers and anthropometric parameters in children consuming vegetarian and omnivorous diets.	The same methodology as in (Ambroszkiewicz et al. 2017).
(Ambroszkiewicz et al. 2019)	To assess body composition, bone mineral density, bone turnover markers and adipokine levels in relation to vegetarian and omnivorous diets.	The same methodology as in (Ambroszkiewicz et al. 2017).
(Weder et al. 2019b)	The VeChi Diet Study examined the energy and macronutrient intake as well as the anthropometrics of vegetarian, vegan and omnivorous children in Germany, 1–3 years old.	A 3-day weighed dietary record assessed dietary intake, and an online questionnaire assessed lifestyle, body weight (BW), and height. Vegetarians ate no meat, sausage, fish, but ate dairy products and/or eggs; vegans ate no meat, sausage, fish, dairy products, or eggs; omnivorous ate meat and/or sausage and/or fish.

Abbreviations: SDA, Seventh Day Adventist; FFQ, Food Frequency Questionnaire; 24HR: 24-hour- recall.

Below is the summary of the findings divided into different health parameters.

9.2. Dietary data

Most studies of vegetarian and vegan children assessed their dietary intake. Sanders et al. 1988, collected dietary data by 7-day weighed dietary record from 39 vegans (1–7 years). Their total energy intake was up to 300 kcal/d below the national reference value. Protein and fat amounted to 10% and 30% of energy intake, respectively. The intake of calcium was 52%, iron was 142%, vitamin B12 including supplementation constituted 280% of the reference (although those not supplementing had lower intakes). Vitamin D3 intake was below the reference, but no further details were provided. The studies conducted in the 1990s, examined the diets of vegetarians only. Sabaté et al. 1991, assessed the diets of SDA 283 vegetarians (7–18 years) by food frequency questionnaire, and found this group to be consuming more fruit, vegetables, starchy foods and vegetable protein products and less 'junk food'. In a study by Sievers E, Dörner K, Hamm E, Hanisch C 1991, vegetarian infants were consuming vegetarian baby food fortified with whey protein in jars. Persky et al. 1992, measured dietary intake of 35 SDA girls and 40 omnivores (aged 15–17 years) by 3-day food records. In comparison to omnivores, vegetarians stated consuming less fat (33.7 versus 39.7% of total energy), saturated fat (12.3 versus 15.5%), and protein (13.0 versus 15.1%), cholesterol (203.8 versus 302.0 mg), sucrose, caffeine and more unsaturated fat (13.6 versus 10.9 g), thiamine, riboflavin, starch, iron, and fibre. Three-day dietary records administered by Nathan et al. 1996, to 50 vegetarians (7–11 years) and 50 omnivores, revealed higher consumption of vegetarian ready-meals, cereals rich in dietary fibre, milk products and less soft drinks by vegetarians than

omnivores. The same study reported similar fruits and vegetable intake among the two dietary groups. In terms of nutrients, the omnivores consumed significantly more protein, but both groups met the recommendations. Vegetarians had a similar carbohydrate intake but ingested less sugar, saturated and monounsaturated fat and more polyunsaturated fat and fibre. There was no difference between groups in the overall fat intake, although the omnivores in the study consumed less fat than the national average. The calcium intake of the vegetarians was significantly higher than that of the omnivores, but both groups met the national recommendations. There was no difference in iron intake, but zinc intake of the vegetarians was lower than that of the omnivores and both groups had average intakes below recommendations. The consumption of total retinol equivalents between the two groups was similar and both met the recommendations. The vegetarians, however, consumed more carotene. Their diets contained higher amounts of vitamins E, D, folic acid and thiamine, but there was no difference in the mean vitamin C intakes. Krajcovicova-Kudlackova et al. 1997, reported lower total fat and cholesterol consumption and increased content of vegetable oils in the diet of 32 vegetarians (age 11–15 years) compared to 19 omnivores. Based on a 7-day food-frequency questionnaire, Hebbelinck, Clarys, and De Malsche 1999, concluded that energy intake was considerably lower than the mean reference data in all vegetarian subjects studied (n=38, age 6–17 years). Ambroszkiewicz, Laskowska-Klita, and Klemarczyk 2003, reported similar total energy, protein and carbohydrate intake of vegetarians (n=23, 2–10 years) in comparison to 26 omnivores, but lower intake of fat and higher intake of fibre and polyunsaturated fatty acids. Based on a 7-day food diary, Taylor, Redworth, and Morgan 2004, found significantly higher iron intakes in vegetarian infants than in omnivores. In

2006, Ambroszkiewicz et al., assessed dietary intakes of 32 vegetarian children (age 2–10, including 5 vegans), and concluded that their total energy intake, protein, carbohydrate, fat and vitamin B12 were within recommendations, apart from 9 subjects (including all the vegans) who did not meet vitamin B12 intake guidelines. A year later, the same group of researchers assessed nutrient intake of 50 vegetarians (aged 2–10 years) and 50 omnivores (Ambroszkiewicz et al. 2007) with a dietary questionnaire. Mean total energy intake and the percentage of energy from protein, fat and from carbohydrates were similar in both groups and met the recommendations. The vegetarians' intakes of calcium were below the recommended range and adequate in omnivores; vitamin D intake was about two-fold lower than in omnivores. In a study by Ambroszkiewicz et al. 2011, the nutritional intake was assessed by a 3-day food record among 30 vegetarians (4–10 years) and 60 omnivores. The vegetarian diet did not differ in terms of dietary energy, carbohydrates and fat intake in comparison to that of the omnivorous children. Similar findings regarding macronutrients were reported by Laskowska-Klita et al. 2011, among 32 vegetarians and 18 omnivores (2–10 years), apart from that of carbohydrates which exceeded, and fat, that was below the recommendations. In this study vegetarians' mean intake of vitamin B12 was in the reference range, however in 28% of studied group it was inadequate. Moreover, vitamin A and folate intake were 200% and 160% of the reference values respectively. Vitamin E intake was at the lowest recommended level and vitamin D3 was three times lower than the reference values.

Gorczyca et al. 2013, analysed 7-day food records of 24 vegetarians (2–18 years) and 16 omnivores. Intakes of energy, protein and iron were similar in

both groups, whereas dietary vitamin C levels were higher among vegetarians. In the four recent studies by Ambroszkiewicz et al. (Ambroszkiewicz et al. 2017; Ambroszkiewicz, Chelchowska, Szamotulska, et al. 2018b; Ambroszkiewicz, Chelchowska, Rowicka, et al. 2018; Ambroszkiewicz et al. 2019) the diet of the studied vegetarians was analysed by a 3-day food record. There were no differences in daily energy intake and macronutrient intakes were within recommended daily intake. However, the percentage of energy from protein was significantly lower and from carbohydrates higher in vegetarians than in omnivores. The vegetarians ingested similar amounts of iron and significantly more vitamin C than the omnivores. Additionally, the dietary intake of fibre and magnesium were higher in vegetarians compared with omnivores. Additionally, in (Ambroszkiewicz, Chelchowska, Szamotulska, et al. 2018b) only 55% of vegetarians and 64% of omnivores met the recommended daily intakes for calcium and 38% of vegetarians and 45% of omnivores for vitamin D. Furthermore, the vegetarian children had significantly lower intakes of vitamin B12 compared with omnivores. In a recent German study of 1–3-year-old vegetarian, vegan and omnivore toddlers (Weder et al. 2019b), diet was assessed by a 3-day weighed dietary record. After adjustment for confounders (age, sex, breast-milk intake, socioeconomic status, weight-for-height z-score, energy intake, paternal BMI, and season) omnivore children had the highest intake of total protein, total fat and added sugars. In contrast, vegan children consumed more carbohydrates and fibre.

These published studies broadly reveal the following patterns of nutrient intake of vegetarians in comparison to omnivores: lower (however normal) protein, lower vitamin B12 (if unsupplemented), vitamin D, zinc, fat (including saturated fat), cholesterol and sugar intake; lower or similar energy and calcium intakes;

higher intakes of carbohydrates, iron, polyunsaturated fatty acids (PUFA), fibre, vitamin C, magnesium, folate, and beta carotene. Vegetarians seem to consume more fruit, vegetables, starchy foods, plant oils and less junk foods. Nearly half of the publications including nutrient intakes of vegetarian and vegan children come from 1980-ties and 1990-ties, when children's dietary patterns might have differed. Yet, food and nutrient intakes seem similar in studies published in the previous decades and the most recent studies. There is not enough data to conclude on the consumption patterns of vegans.

9.3. Body composition

The body composition techniques used in the studies on vegetarian and vegan children were simple anthropometric measurements apart from the recent Polish studies, which additionally used DXA (Ambroszkiewicz et al. 2011; Ambroszkiewicz, Chełchowska, Szamotulska, et al. 2018b; Ambroszkiewicz, Chełchowska, Rowicka, et al. 2018; Ambroszkiewicz et al. 2019). In the two studies assessing the health of vegan children, 1–10 years old, by (Sanders 1988, 39 vegans, UK) and (O'Connell et al. 1989, 404 vegetarians including undefined number of vegans, USA), heights, weights (UK study), weight for age and height for age (US study) were within the normal range, although they tended to be below the 50th percentile. These two studies did not include an omnivore reference group. In a recent study of German omnivore (n=164), vegetarian (n=127) and vegan (n=139) toddlers, 1–3 years old (Weder et al. 2019a), median weight-for-height, height-for-age, and weight-for-age z-scores did not differ significantly between the groups, however a higher proportion of vegan children (3.6%), compared to vegetarians (2.4%) and omnivores (0%)

were categorised as stunted according to the WHO growth standards. The corresponding figures for wasting were 3.6%, 0%, 0.6%.

In the early 1990s three studies were conducted among Californian Seven Day Adventist in the US. The first one by Sabaté et al. 1991, looked at attained height of vegetarian children and adolescents (n=283) attending Californian SDA schools and their non-vegetarian counterparts from public schools (n= 895). It found that all diet groups were on average above the 50th percentile of the National Center for Health Statistics growth references. Additionally, the SDA 7–18-year-old vegetarian children were taller than controls (1.8 cm and 1.9 cm taller for boys and girls respectively). In a sub-analysis from the same cohort of schoolgirls aged 11–12 years (n =95) , Sabaté, Llorca, and Sánchez 1992 reported that mean height was 2.5 cm lower for vegetarians compared to controls. The authors attributed this difference to a different pattern of maturation of vegetarians, rather than stunting. Similarly, in a prospective study of 35 US SDA vegetarian girls and 40 omnivore counterparts aged 15–17 years by Persky et al. 1992, vegetarians were on average 5 cm shorter and 2.3 kg lighter than nonvegetarians. In the studies conducted in the late 1990ties in Slovakia, the UK and Belgium (Krajcovicová-Kudlácková et al. 1997, Nathan et al. 1997, Hebbelinck, Clarys, and De Malsche 1999) vegetarians were on average lighter and/or leaner. The height was similar or lower than that of the omnivores, although in the UK study that defined vegetarians as those who also consume fish, both vegetarians and omnivores lay close to the 50th percentiles for both height and weight.

In the Polish studies by Ambroszkiewicz et al., and Gorczyca et al. 2011 conducted between 2003 and 2019 on prepubertal vegetarians, no significant

differences in BMI or BMI z-scores, body weight, height, were reported, however vegetarians tended to have lower fat mass and lower fat/lean ratio measured by DXA.

The results of all studies are summarised in Table 4.

Table 4 Body composition of vegetarian and vegan children based on the available data

Study	Study design; objective	Population	Control Group	Findings
(Sanders 1988), UK.	Longitudinal; growth and development of vegan children.	39 life-long vegan children, no information on gender, aged 1–7.	none	Heights, weights, head and chest circumference measurements within normal range for the majority of children; girls tended to be below the 50th percentile for weight, the boys, for both height and weight.
(O’Connell et al. 1989), USA.	Cross–sectional; the effects of a vegetarian diet on child growth, height and weight data.	404 children, a high proportion of vegans (no further details), 4 months to 10 years old, mean age ca. 6 years, living in a commune ‘The Farm’.	none	The height-for-age and weight-for-age data were less than the median of the reference population for most ages. The differences in height-for-age z-score between The Farm and the reference population were significant for ages 5 years and younger; the greatest differences were seen at ages 1 to 3 years; the height- for-age differences not significant for ages ≥ 5 years. The Farm weight- for-age data were slightly less than those of the reference population for most ages. The differences between The Farm and the reference population were greater for the height-for-age indicator than for the weight-for-age indicator.
(Sabaté et al. 1990), USA.	Longitudinal; to obtain height and weight data from children of different diets and lifestyles.	1090SDA children from SDA schools, aged 6–18years with lower (varied) meat consumption than the control group; 542 boys.	1182 public school children.	The mean height and weight were at or above national reference values in each school. SDA school boys were 1.6 cm taller than public schoolboys; there were no significant differences in height for girls. After controlling for height, boys and girls in the SDA schools were found to be leaner by, 1.27 and 1.16 kg respectively.
(Sabaté et al. 1991), USA.	Cross-sectional; to examine the relationship between diet and attained height.	870 SDA children, including 283 vegetarians, aged 7–18; 427 boys.	1765 children, from public schools.	All school and diet groups were above the 50 th percentile of the national reference. On average, the SDA vegetarian children were taller than controls:1.8 and 1.9 cm taller, boys and girls respectively.

Study	Study design; objective	Population	Control Group	Findings
(Sievers et al. 1991), Germany.	Longitudinal; to examine iron status in infants on vegetarian diets.	13 vegetarian infants, 4–12 months, 7 boys, 6 girls.	14 omnivores	Vegetarian weight and height within the reference range and similar to omnivores.
(Sabaté, Llorca, and Sánchez 1992), USA.	Cross-sectional; to report the association between vegetarian life-styles and height of children during preadolescence.	Subset A: 95 vegetarians (or children eating meat less than once a day) 11–12years attending SDA schools; 49 boys; Subset B: 27SDA children, 14 boys.	Subset A:107 children attending public schools. Subset B: 101 omnivore children from public schools; 46 boys.	SDA girls had 2.5 cm lower mean height than that of their public-school counterparts (Sample A). Sample B: mean height of SDA girls was 3 cm lower.
(Persky et al. 1992), USA.	Longitudinal; to explore hormonal differences among teenage girls on vegetarian or nonvegetarian diets.	35 SDA vegetarians, 15–17 years.	40 nonvegetarians	Vegetarian girls were an average of 2 inches shorter and 5 lb lighter than nonvegetarian girls.
(Nathan, Hackett, and Kirby 1997b), UK.	Longitudinal; to assess the ability of a meat free diet to support normal growth of children.	50 vegetarians (including 23 consuming fish on a regular basis), 7–11 years, 21 boys.	50 omnivores matched for age, sex.	A tendency for the vegetarians to be leaner than the omnivores (not significant at the 5% level);both the vegetarian and omnivorous groups lay close to the 50th percentiles for both height and weight at the base and follow-up measurement. On average, all measurements (initial and final) of upper mid-arm circumference and mid-arm muscle circumference, BMI and skinfolds of the vegetarians were slightly lower than those of the omnivores. The vegetarian children grew in height slightly more than the omnivorous children.

Study	Study design; objective	Population	Control Group	Findings
(Krajcovicová-Kudlácková et al. 1997), Slovakia.	Cross-sectional; to evaluate the health and nutritional status of children with two different nutritional habits.	26 vegetarians, age 11–14, 11 boys.	32 omnivores	Vegetarians' weight on average 4 kg lower, height similar in both groups.
(Krajcovicova-Kudlackova et al. 1997), Slovakia.	Cross-sectional; to examine plasma profile of fatty acids.	32 vegetarians (including 7 vegans), 11–15 years old, 15 boys.	19 omnivores	The weights and heights were comparable; however, vegans were lighter and shorter.
(Hebbelinck, Clarys, and De Malsche 1999), Belgium.	Cross-sectional; to assess average daily dietary intakes of energy and to determine height and weight; triceps, suprailiac, and calf skinfold thicknesses; puberty ratings; and physical fitness among children on vegetarian diets.	38 vegetarians, group A: 6–9-year-old girls (n=9); 6–11-year-old boys (n=9); adolescents (group B: 10–15-year-old girls (n=10), and 12–17-year-old boys, (n=10).	Reference data from a recent representative study- stratified random sample of 2837, of 6–18-year-old Flemish youth.	The vegetarian adolescents (group B), had significantly lower body weights and BMIs than the reference means; adolescent boys were 8.5 cm smaller than the reference mean. There were no differences in children (group A) with regard to height and weight. The skinfold thickness at all sites were lower in all age groups.
(Ambroszkiewicz, Laskowska-Klita, and Klemarczyk 2003), Poland.	Cross-sectional; examination of osteocalcin and leptin blood concentrations in vegetarian children.	23 vegetarian children (including 8 vegans), 2–10 years, 12 boys.	26 omnivores.	BMI of vegetarians similar to omnivores.
(Ambroszkiewicz et al. 2007), Poland.	Cross-sectional; the aim of this study was to investigate the serum concentrations of biochemical bone turnover markers in prepubertal vegetarian children.	50 vegetarians, aged 2–10 years, 27 boys.	50 omnivorous children, 25 boys.	Vegetarian had similar average BMI ($15.6 \pm 1.4 \text{ kg/m}^2$) as omnivores ($16.0 \pm 1.3 \text{ kg/m}^2$).

Study	Study design; objective	Population	Control Group	Findings
(Ambroszkiewicz et al. 2011), Poland.	Cross-sectional; to investigate associations between serum adipocytokines status and anthropometric parameters as well as total energy and macronutrient intake in vegetarian, normal weight omnivorous and obese omnivorous children.	30 vegetarian children (including 4 vegans), 4–10 years, 12 boys.	30 normal-weight omnivores	There were no significant differences in body weight, height, BMI and lean mass values (the latter measured by DXA) between vegetarians and normal-weight omnivores. Children on vegetarian diet had lower fat mass (measured by DXA) and fat mass/lean mass ratio than normal-weight omnivores.
(Gorczyca et al. 2011), Poland.	Cross-sectional; to study an effect of vegetarian diet on lipid profile in serum in children in Poland and to investigate lipid parameters in healthy vegetarian children and in omnivorous children with diagnosed atopic disease.	24 children of both sexes, aged from 2 to 18 years, 6 boys.	18 omnivores	Vegetarians' height and weight similar to omnivores.
(Matthews, Wien, and Sabaté 2011), USA.	Cross-sectional; to examine the association between the risk for overweight and the frequency of consumption of 7 food groups in children and adolescents.	870 SDA children with lower but variable levels of meat intake from SDA schools, 894 omnivore children, 6–18 years old, 879 boys.	none	Specific plant-based food groups were inversely related to overweight among children and adolescents whereas dairy intake was associated with an increased risk of overweight.
(Ambroszkiewicz et al. 2017), Poland.	Cross-sectional; to assess the effect of vegetarian diet on iron metabolism parameters.	43 vegetarians, age range 4.5–9.0 years	46 omnivores	No significant differences in weight, height, and BMI between vegetarian and omnivorous children.

Study	Study design; objective	Population	Control Group	Findings
(Ambroszkiewicz, Chelchowska, Rowicka, et al. 2018), Poland.	Cross-sectional; to assess serum adipokine profile in prepubertal vegetarian and omnivorous children.	62 vegetarians, 5 to 10 years, 54.8% male	55 omnivores, aged 5 to 10 years, were studied, 49.1% male	Both groups were similar in weight, height, BMI, lean and fat mass (measured by DXA) The mean values of LMI were similar in both, but FMI was significantly lower in the vegetarians.
(Ambroszkiewicz, Chelchowska, Szamotulska, et al. 2018a), Poland.	Cross-sectional; to evaluate serum levels of bone metabolism markers and to analyze the relationships between biochemical bone markers and anthropometric parameters in children on vegetarian and omnivorous diets.	70 vegetarians, age range 5–10 years.	60 omnivores, age range 5–10 years.	Body composition, BMC, BMD were assessed by DXA. Both groups of children were similar weight, height, BMI. Mean values of fat mass, lean mass, the ratio of fat/lean mass and total and spine BMC as well and total and lumbar spine BMD were comparable in vegetarians and omnivores.
(Ambroszkiewicz et al. 2019), Poland.	Cross-sectional; to assess body composition, bone mineral density, bone turnover markers and adipokine levels in relation to vegetarian and omnivorous diets.	53 vegetarians, age 5–10 years.	53 omnivores	The growth and development of all children was normal; there were no significant differences in weight, height, BMI and BMI z-score. However, vegetarians had lower percentage of fat mass and ratio of fat to lean mass (assessed by DXA).
(Weder et al. 2019a), Germany	Cross-sectional; to examine the energy and macronutrient intake as well as the anthropometrics of 430 vegan, vegetarian and omnivorous children, 1–3 years.	127 vegetarians, 139 vegans	164 omnivores	There were no significant differences in median weight-for-height, height-for-age, and weight-for-age z-scores between the groups. A slightly higher percentage of vegans (3.6%), compared with vegetarians (2.4%) and omnivores (0%) were classified as stunted, whereas 3.6% of vegans and 0.6% of omnivores were classified as wasted (0% vegetarians). In contrast, a higher percentage of omnivores (23.2%) than vegetarians (18.1%) and vegans (18.0%) were classified as overweight or at possible risk of overweight.

Abbreviations: SDA, Seventh Day Adventist; BMI, body mass index; BMD, bone mineral density; BMC, bone mineral content; LMI, lean mass index; FMI, fat mass index.

Overall, children following meat-free diets were either similar, or somewhat below, the national or omnivore group reference in terms of height, weight, BMI, fat mass, lean mass and skinfold measures.

9.4. Bone status

Bone status of vegetarian children in industrialized countries has only been studied by the group of Polish researchers from the Institute of Mother and Child in Warsaw. In 2003, Ambroszkiewicz et al., examined serum osteocalcin and leptin concentrations of 23 vegetarian children and 26 omnivores, aged 2–10 years. The group included 8 vegan subjects. Osteocalcin and leptin concentrations were 12% and 45% lower in vegetarians. Osteocalcin is a protein produced by bone-forming cell osteoblasts. It is regarded as a marker of bone formation, involved more in the process of mineralization rather than bone matrix creation. Concentrations correlate well with histological measures of bone formation rates (Cundy, Reid, and Grey 2014). Leptin, a fat-cell adipokine, has been previously regarded as mainly responsible for energy balance and appetite. However, its effects on bone metabolism have recently been increasingly researched (Upadhyay, Farr, and Mantzoros 2015). The leptin receptor is found in osteoblasts and chondrocytes, which suggests direct effects of leptin on bone growth and metabolism (Driessler and Baldock 2010). Studies have also shown that leptin may impact bone growth indirectly, through the activation of numerous hormones and growth factors, including osteocalcin (Tsuji et al. 2010; Ferron and Lacombe 2014). The total effect of leptin on bone might be dependent on its serum concentrations (Cirmanová et al. 2008). In humans, states of caloric restriction and resulting low serum leptin, have been associated with low bone mass (Hamrick 2007). Leptin therapy has a normalizing effect on bone density of

hypoleptinaemic subjects (Foo, Hamnvik, and Mantzoros 2012). At the same time, a Mendelian randomization study, using SNPs strongly associated with leptin levels, found that higher levels were associated with lower lumbar spine bone mineral density (BMD) (Meng et al. 2019).

In 2007, Ambroszkiewicz et al., examined additional blood markers of bone formation in 50 vegetarian children (age 2–10, including 13 vegans) and compared them to 50 healthy omnivores in the same age range. The markers studied were osteocalcin, bone alkaline phosphatase (BALP) – markers of bone formation; and products of osteoclast activity – collagen type I terminal telopeptide (CTX) – as a marker of bone resorption. These are considered useful in the clinical investigation of bone turnover in children (Szulc, Seeman, and Delmas 2000). Vegetarians had lower serum concentrations of osteocalcin, BALP and CTX by about 20%, 10% and 15%, respectively. The authors concluded that inadequate intake of calcium and vitamin D3 (also investigated in this study, discussed below) may retard relevant bone growth and development. In 2018 (Ambroszkiewicz, Chelchowska, Szamotulska, et al. 2018a), assessed bone mineral content (BMC), and BMD by dual-energy X-ray absorptiometry, along with BALP, CTX and additional cytokines influencing the balance between osteoblast and osteoclast activity (osteoprotegerin, nuclear factor κ B ligand (RANKL), sclerostin, and Dickkopf-related protein 1 (Dkk-1)) among 70 prepubertal children on a lacto-ovo-vegetarian diet and 60 omnivorous controls. Vegetarians had significantly higher levels of BALP and CTX-I, and slightly lower spine BMC and BMD, whereas the cytokine concentrations were comparable among groups. The authors concluded that vegetarians had a higher rate of bone turnover and subtle changes in bone

regulatory markers (CTX), which may protect vegetarians from bone abnormalities. In 2019, the same group of researchers (Ambroszkiewicz et al. 2019) examined 53 vegetarian and 53 omnivorous prepubertal healthy children matched for age and sex (median age 7.0 years). They measured BMD by DXA, along with 25-hydroxy vitamin D, parathormone levels and carboxy-terminal propeptide of type I collagen (marker of bone formation), total osteocalcin (and its carboxylated (c-OC) and undercarboxylated (uc-OC) forms), CTX and leptin. Total BMD and lumbar spine BMD z-score were lower in vegetarians compared with omnivores, along with serum leptin level which was about 2 times lower (1.39 vs. 2.94 ng/mL, $p < 0.001$). Vegetarians had similar 25-hydroxy vitamin D, but higher parathormone concentrations. The rest of the studied bone metabolism markers did not differ between the groups.

The existing data suggest altered bone metabolism and lower bone mass in vegetarian than in omnivore children. However, none of these studies adjusted for bone size, which is recommended when using DXA among children with potentially different growth and maturation patterns and thus bone size (Fewtrell 2003). This concept is expanded on in the next section.

9.5. CVD risk factors

Harris et al. 1981, detected no difference in blood pressure between two large groups of children, 3159 from SDA schools and 4681 from non-SDA schools. Although the SDA group had lower meat intake, no information on the exact number of vegetarians were given. Nathan et al. 1996, reported similar mean cholesterol levels of studied 50 vegetarians (7–11 years old) and 50 omnivores. Twenty-three of the children classified as vegetarian in this study habitually

consumed fish. In a study by Krajcovicová-Kudlácková et al. 1997, total and LDL cholesterol was lower in vegetarians (n=26, 11–14 years) than in omnivores (n=32), whereas the HDL cholesterol and triglycerides concentrations were comparable. Ambroszkiewicz et al. 2006, reported total, LDL-, HDL cholesterol and triglycerides within the healthy range in a group of 32 vegetarians (including 5 vegans) aged 2–10 years old. This study did not involve an omnivore reference group. In another study by Ambroszkiewicz et al. 2011, vegetarian children (n=30, age 4–10 years) had significantly lower total, LDL cholesterol and triglycerides than omnivores (n=60). There was no difference in HDL cholesterol between dietary groups. In 2017, Ambroszkiewicz et al., reported lower level of CRP in vegetarians (n = 43, age 4.5–9 years) than omnivores (n=46).

The available data suggest either comparable or more favourable lipid profiles in vegetarian children than in their omnivore counterparts. No representative data exist in this area in vegans.

9.6. Micronutrient status

Only about 2/3 of the available studies (n=18) measured blood biomarkers, and only 13 assessed those related to nutrition status.

Lombard et al. 1989, studied 32 vegetarians and 25 vegans (age 3–17 years) and found no significant differences in protein nutritional status as judged by serum concentrations of total protein and albumin. Sievers et al. 1991, found blood iron status measured by serum haemoglobin (Hgb), haematocrit (Ht), red blood cell count and ferritin to be similar in 13 vegetarian infants and 14 in the omnivore control group. Nathan, Hackett, and Kirby 1996, found that haemoglobin concentrations were significantly lower in 50, 7–11-year-old

vegetarians (mean 118.6 mg/L) than in 50 omnivores (mean 124.1 mg/L). Krajcovicová-Kudlácková et al. 1997, reported significantly lower erythrocyte number as well as reduced levels of haemoglobin (135.4 g/L in vegetarians, 142.0 g/L in omnivores), and serum iron in vegetarians aged 11–14 years (n=26) compared to omnivores (n=32). The average level of iron in vegetarians did not reach the lower limit of the physiological range and hyposiderinaemia was found in 58% of vegetarians vs 9% of omnivores (mean serum iron 16.0 $\mu\text{mol/L}$ in vegetarians, 22.5 $\mu\text{mol/L}$ in omnivores). The incidence of hypoalbuminaemia and hypoproteinaemia in vegetarian children was 38% and 12%, respectively, compared to 0% in the omnivore reference group.

Taylor, Redworth, and Morgan 2004, studied 20 vegetarian infants and toddlers (0–24 months) and 178 omnivore counterparts. Meat consumption was not associated with markers of iron status except at 12 months of age, when the percentage of infants with low serum iron was inversely related to dietary meat intake. Ambroszkiewicz et al. 2006, reported normal serum homocysteine, folate, vitamin B12 levels in 32 vegetarian children (including 5 vegans), aged 2–10 years old. This study did not include an omnivore reference group. In 2007, Ambroszkiewicz et al., analysed the sera of another sample of 50 children on vegetarian diets aged 2–10 years. The concentration of 25-hydroxyvitamin D was nearly 2-fold lower compared with a group of 50 omnivores. Laskowska – Klita et al. 2011, reported physiological serum concentrations of vitamin B12 (548 pg/ml), folate (12.8 ng/ml), vitamin A (1.2 $\mu\text{mol/L}$) and vitamin E (15.6 $\mu\text{mol/l}$) among 32 vegetarians (aged 6.5 ± 4.2 years). However, their 25-hydroxy vitamin D (13.7 $\mu\text{g/L}$) was only half of the lowest limit of the reference value. Similar homocysteine levels (6.13 $\mu\text{mol/L}$ vs 5.45 $\mu\text{mol/L}$) and vitamin A (1,17 $\mu\text{mol/L}$ vs

1.32 $\mu\text{mol/L}$) were observed in comparison to 18 omnivores (aged 7.9 ± 2.7 years), however the levels of vitamin E were lower. Concentrations of iron, ferritin, transferrin and total iron-binding capacity in vegetarians were in the physiological range. Gorczyca et al. 2013, studied 22 vegetarians aged from 2 to 18 years and 18 omnivores. The prevalence of iron deficiency measured by serum ferritin levels was higher in the vegetarian group. Mean corpuscular volume in the vegetarians was also found to be lower than in the omnivores.

Ambroszkiewicz et al. 2017, measured serum iron, ferritin, transferrin, and hepcidin and soluble transferrin receptor (sTfR) in 43 prepubertal vegetarian children (age range 4.5–9.0 years) and 46 omnivores. Hepcidin is a small peptide involved in iron homeostasis. Lower levels of hepcidin allow increased iron absorption from diet and its mobilization from bodily stores, while higher levels inhibit this pathway (Ganz 2015). sTfR is a marker of iron status. Its concentrations are negatively correlated with intracellular iron levels and they are less affected by inflammation, and more sensitive to erythropoietic activity. All parameters were within the reference range in both groups of children, however, ferritin and hepcidin concentrations were markedly lower in vegetarians, whereas sTfR concentrations were significantly higher compared with omnivorous children. The authors concluded elevated sTfR concentration coexisting with decreased hepcidin levels is a consequence of increased erythropoiesis in vegetarian children, resulting from a subclinical iron deficiency.

Overall, in comparison to the omnivore group or physiological references, these studies suggest either normal micronutrient status or increased risk of iron and vitamin D3 deficiency. There are no studies on blood micronutrient status in vegans. The results are summarised in Table 5.

Table 5. Blood micronutrient status of vegetarian and vegan children in the existing studies

Study	Study objective	Population	Control Group	Findings
(Lombard et al. 1989), USA.	To evaluate carnitine status in vegetarians and vegans.	SDA: 32 vegetarians, 24 vegans; 3–17 years.	29 omnivores	No difference in serum concentrations of total protein and albumin; lower plasma and urinary carnitine concentration in vegetarians, lowest in vegans.
(Sievers et al. 1991), Germany.	To examine iron status in infants on vegetarian diets.	13 vegetarian infants, 4–12 months.	14 omnivores	Blood iron status (Hgb, Ht, RBC, erythrocyte volume, ferritin) similar to omnivores, serum iron higher and transferrin lower in vegetarians with 12 months than in omnivores.
(Nathan, Hackett, and Kirby 1996b), UK.	To assess the ability of a meat free diet to support normal growth of children.	50 vegetarians (including 23 habitually consuming fish) 7–11 years.	50 omnivores matched for age, sex and ethnic group.	Hgb concentrations significantly lower in vegetarians (mean 118.6 mg/L) than in omnivores (mean 124/1 mg/L).
(Krajcovicová-Kudláčková et al. 1997), Slovakia.	To evaluate the health and nutritional status of children with two different nutritional habits.	26 vegetarians, 11–14 years.	32 individuals on mixed diet.	Vegetarians had significantly lower erythrocyte number, reduced levels of Hgb, (135.4 g/L in vegetarians, 142.0 g/L in OM), lower serum iron compared to omnivores. Hyposiderinaemia was found in 58% of vegetarians vs 9% of omnivores; hypoalbuminemia prevalence – 38% in vegetarians compared to 0% in omnivores.
(Krajcovicova-Kudlackova et al. 1997), Slovakia.	To examine plasma profile of fatty acids	7 vegans, 15 vegetarians, 10 semi-vegetarians, 11–15 years old.	19 omnivores	Vegetarians had lower blood concentrations of saturated SFA; MUFA, similar in both groups; EPA, DHA, total Omega 3 lower in vegans.
(Taylor, Redworth, and Morgan 2004), UK.	To determine whether iron and micronutrient status is improved with an increased amount of meat in the diet.	20 non-meat-eating infants, 4 months old and followed up until 24 month of age.	178 omnivorous infants	Meat consumption was not associated with dietary iron intake nor with the haematological and other markers of iron status except at 12 months of age, when the proportion of infants with low serum iron was inversely related to meat in the diet.

Study	Study objective	Population	Control Group	Findings
(Ambroszkiewicz et al. 2006), Poland.	To assess the effect of vegetarian diets on serum concentrations of homocysteine, folate, vitamin B12 in children.	32 vegetarians (including 5 vegans), age 2–10 years.	none	Serum concentrations of homocysteine, folate, vitamin B12 in vegetarian children within the physiological range.
(Ambroszkiewicz et al. 2007), Poland.	To investigate the serum concentrations of biochemical bone turnover markers in vegetarian children.	50 vegetarian children, aged 2–10 years.	50 omnivores	The serum level of 25 (OH) D in vegetarians was nearly 2-fold lower compared with omnivores.
(Laskowska-Klita et al. 2011), Poland.	To assess the intake and serum status of selected nutrients as well as concentrations of homocysteine and iron balance in Polish vegetarian children.	32 were vegetarians, aged 6.5±4.2 years.	18 omnivores, aged 7.9±2.7 years.	Vegetarians' serum concentrations of vitamin B12 (548 pg/ml), folate (12.8ng/ml), vitamin A (1.2 µmol/L), vitamin E (15.6 µmol/l), homocysteine (6.13 µmol/L), and iron markers within physiological range; concentrations of 25 (OH) D (13.7 µg/L) half of the lowest limit of the reference value.
(Gorczyca et al. 2013), Poland.	To examine the effect of iron intake on iron status in vegetarian children.	22 vegetarians, aged from 2 to 18 years.	18 omnivores	The prevalence of iron deficiency was higher in the vegetarian group; serum ferritin and MCV in vegetarians were lower.
(Ambroszkiewicz et al. 2017), Poland.	To assess the effect of vegetarian diet on iron metabolism parameters.	43 vegetarians, age range 4.5–9.0.	46 omnivore children	Serum transferrin levels were similar in all subjects; ferritin concentrations significantly lower in vegetarians, median hepcidin levels were lower but sTfR higher.
(Ambroszkiewicz et al. 2019), Poland.	To assess body composition, BMD, bone turnover markers and adipokine levels in relation to vegetarian and omnivorous diets.	53 vegetarians	53 omnivorous matched for age and sex (median age 7.0 years).	Vegetarians had similar concentration of 25 (OH) D.

Abbreviations: SDA, Seven Day Adventist; Hgb, haemoglobin; Ht, haematocrit; RBC, red blood cells; SFA, saturated fatty acids; MUFA monounsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; 25 (OH) D, 25-hydroxy vitamin D; MCV, mean corpuscular volume; sTfR, soluble transferrin receptor; BMD, bone mineral density.

10. Rationale for the choice of parameters assessing risks of vegetarian and vegan diets

The previous section summarized the available evidence on the health of children on vegetarian and vegan diets. Studies have shown that these diets increase the risk of inadequate intakes of several nutrients, which – as the preliminary available evidence shows – can affect children’s physiology. This was apparent across several studied health parameters, including body composition, growth, bone status, cardiovascular risk profile and micronutrient status. Moreover, the results of studies where blood micronutrients were assessed suggest that plant-based sources of iron, even if consumed in recommended amounts, might not always be adequate to sustain normal haematopoiesis. There are inadequate data on the intakes and micronutrient status of vegans, however since they exclude additional two food groups, their risks might be even greater.

This section presents the rationale for the choice of parameters to assess the safety of vegetarian and vegan diets in children. I decided to thoroughly assess the children’s’ dietary intakes, along with the blood parameters of status of selected micronutrients, most likely to be consumed in inadequate amounts and/or deficient based on the available data. These include iron, vitamin B12, and vitamin D3. The blood assays to evaluate their physiological adequacy are discussed in the following sections. Additionally, I chose to measure bone status, which was not assessed in vegan children so far, and measured in few studies of vegetarians, however without adjusting for body and bone size. The importance of this adjustment is discussed below.

Dietary intake methodology is discussed separately in the next chapter, as its complexity deserves a separate, longer section.

10.1. Blood markers of iron status

Iron deficiency is defined as a state of inadequate iron stores in which supply of iron to tissues, including the erythron, is compromised (WHO 2001). This condition of deficiency can take different forms, from mild to severe. Iron deficiency anaemia is the severe manifestation of iron deficiency, whereby indicators like haemoglobin and haematocrit fall below the reference ranges. These two parameters are most commonly used for screening individuals or populations for iron deficiency. However, there are two limitations of this approach.

First, low Hgb and Ht are not specific for iron deficiency, and other conditions can lead to their suboptimal serum concentrations. These include malaria, several parasitic infections, and other nutrient deficiencies that affect haematopoiesis (including protein, B12, folic acid and vitamin C) (WHO 2001).

Second, in mild-to-moderate forms of iron deficiency, although anaemia is absent, tissues are still functionally impaired. Therefore, it is recommended to add more sensitive parameters, like serum ferritin or transferrin saturation (WHO 2001), that will help exclude other potential causes of anaemia. In this study complete blood count, including Hgb, Ht, along with serum ferritin levels were chosen to assess iron status of vegetarian and vegan children.

10.2. Blood markers of vitamin B12 status

Dairy, eggs and especially meat and fish are the only reliable dietary sources of vitamin B12, otherwise called cobalamin. Therefore vegetarians, and especially vegans, unless they supplement or consume products fortified with this vitamin, do consume lower amounts (vegetarians) or nearly none (vegans) of this nutrient. A review of studies published in 2014, documented relatively high prevalence of cobalamin deficiency among adult vegetarians. Vegans who do not ingest vitamin B12 supplements were found to be at particularly high risk (Pawlak, Lester, and Babatunde 2014). Data in children are very limited.

There is no gold standard test to assess cobalamin deficiency (Devalia, Hamilton, and Molloy 2014). The classic manifestation is megaloblastic anaemia (characterised by decreased Hgb, red blood cells (RBCs), increased mean corpuscular volume (MCV)), accompanied by low serum vitamin B12 levels (so called first-line test). The clinical picture is, however, not always unequivocal. Neurological manifestations of severe deficiency, often irreversible, can occur without haematological changes and with serum cobalamin levels within the reference range (Devalia, Hamilton, and Molloy 2014). Therefore, second-line tests are recommended to clarify cobalamins status. They include plasma methylmalonic acid (MMA) and plasma homocysteine (Devalia, Hamilton, and Molloy 2014), with negative associations between low plasma B12 levels and high both MMA and homocysteine levels. Although plasma MMA is more specific, it is also significantly more expensive than serum homocysteine. The latter parameter is also involved in CVD risk (Ganguly and Alam 2015), therefore it was chosen, along with serum vitamin B12, to assess cobalamin status of children in this study.

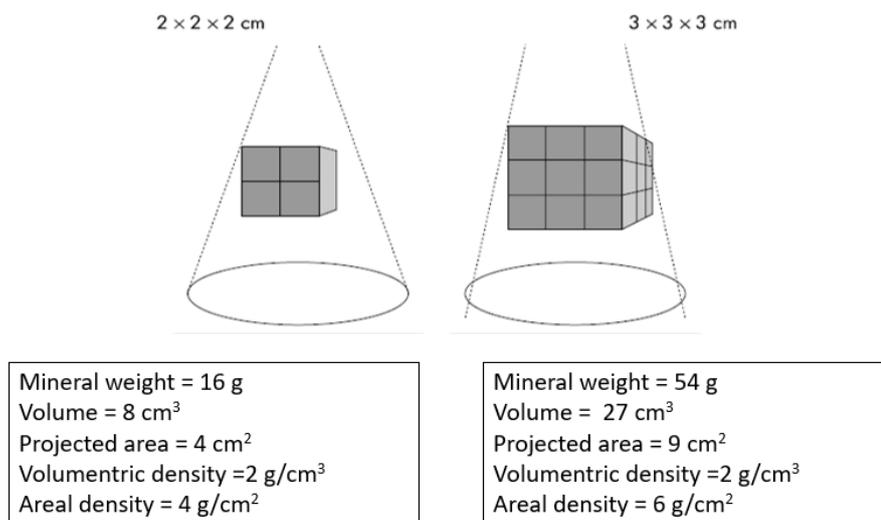
10.3. Bone status

Childhood bone acquisition is closely linked to the intake of calcium (Bonjour et al. 1997; Chevalley et al. 2005; Ondrak and Morgan 2007), circulation of the 25 (OH) D levels (Bachrach 2001), physical activity (Ondrak and Morgan 2007), as well as IGF-1 levels (Locatelli and Bianchi 2014; Kanbur, Derman, and Kinik 2005). Preliminary data from animal studies suggest that vitamin B12 status might affect bone mass (Clemens 2014). All of those parameters can be influenced by vegetarian, especially vegan diets (along with physical activity, discussed above).

There are no studies assessing bone status in vegan children, and the available data in vegetarian children suggest suboptimal bone health. However, in none of these studies was BMD measured by DXA adjusted for the size of the bones.

In DXA, BMD is measured as the ratio of the amount of bone and the area scanned. This size correction assumes that BMC and bone area (BA) are directly proportional to one another, such that a 1% change in BA is matched by a 1% change in BMC. This assumption rarely holds (Prentice, Parsons, and Cole 1994). Since DXA BMD measurement relies on the two-dimensional areal projection of a three-dimensional object (bone), it is inherently related to the size of the bone. Bone size, in turn, is related to the size of the child. Therefore, in smaller children, it tends to underestimate their actual bone mineral status, due to the fact that their bones are smaller, even though their volumetric density might be normal (Fewtrell 2003; Lewiecki et al. 2008). This is illustrated in Figure 3.

Figure 3. Graphical presentation of the underestimation of areal BMD in smaller bones when measured by DXA (Carter, Bouxsein, and Marcus 1992; redrawn from original and typographical error corrected).



Failure to account for this is a common cause of misinterpretation of paediatric DXA results (Leonard and Zemel 2002; Gafni and Baron 2004). Data suggest that vegetarian, and especially vegan, children are shorter, therefore correction for size is of particular importance in this population.

There is no consensus as to the most appropriate way to correct for size DXA BMD results in children, however several approaches have been proposed (Fewtrell 2003). The use of multiple regression analysis to adjust BMC simultaneously for BA, weight, height, and other relevant factors such as age, pubertal status, and calcium intake (Prentice, Parsons, and Cole 1994) and the use of calculated volumetric bone density (bone mineral apparent density, BMAD), in which BMC is adjusted for calculated bone volume rather than bone area (Carter, Bouxsein, and Marcus 1992), are two major approaches, and are utilised in this study.

III. LITERATURE REVIEW: DIETARY ASSESSMENT METHODS

This chapter outlines the techniques that can be used to assess dietary intakes, the main exposure in this study. In the first part, it describes the methods utilised in the collection of dietary data. In the second part it illustrates the problems linked to the analysis of dietary data, when the aim is to evaluate the proportion of individuals with inadequate intakes of nutrients. Methods utilized to calculate percentages of the population with inadequate nutrient intakes along with the concept of usual dietary intakes are outlined, as these are utilised in this study to assess the dietary risks of plant-based diets in children. The literature review in this chapter is based on the most up-to-date publications on the discussed issues from authoritative sources of knowledge in nutritional epidemiology: National Cancer Institute, US Institute of Medicine, US National Research Council, European Food Safety Authority, and UK Medical Research Council.

The purpose of this chapter is to provide background information and thus rationale for the choice of the methods assessing the main exposure. The selected methods are discussed at the end of this chapter.

1. Dietary assessment methods: collection of dietary data

Contrary to other lifestyle factors, diet is very difficult to measure. The difficulty lies not only in the complexity of capturing total intake of foods, dishes and beverages. Additionally, accurate measurement relies on memory, and the disclosure of food habits is also subject to social expectations. Methods range from subjective reporting, objective observation to biomarker ascertainment. Each method has its strengths and limitations. This chapter focuses on subjective methods, as these are most often used in epidemiological studies and are utilised in this study.

1.1. 24-hour dietary recalls

This method involves a specially trained interviewer who contacts study participants on a random day and asks them about the details of their diet (and possibly supplement consumption) over the previous 24 hours. All the details, including all foods and drinks consumed, food preparation methods, ingredients used in prepared dishes, and the brand names of store-bought products are the subject of the interview (Shim, Oh, and Kim 2014). During the interview, the amounts of consumed foods are compared to habitually used volume quantities like spoons, bowls, cups, etc. to increase accuracy of reporting. Additionally, food photographs of different portion sizes are also utilised. 24HR employs a so called multiple pass approach (National Cancer Institute 2018), whereby questions are not asked in chronological order, but rather in stages tailored to human cognition. Thus, at first, a simple list of foods and drink is ascertained. After that additional questions on potentially forgotten foods, meal time and occasion, details on dish ingredients are asked. Finally the approach finishes

with probing questions as to whether anything else was consumed (MRC n.d.). The interview can be conducted in person or over the telephone, it can be administered via computer or with pen and paper. The newest advances in this method include menu-driven standardized 24HR software (called the EPIC-Soft) in the European Prospective Investigation into Cancer and Nutrition study (Slimani et al. 2011) enabling interviewers to collect information in a standardized manner, therefore improving the accuracy of the collected data, and its self-application via automated computer systems (National Cancer Institute 2019).

The strengths of this method include unlimited choice of foods, quantification of the intake, details on eating frequency and cooking habits, lower sample selection bias (literacy is not required, which might be relevant in some research settings), and low respondent burden (if administered by an interviewer). Most importantly, this method is not subject to reactivity, i.e. it does not risk changing the eating behaviour during the period being assessed (National Cancer Institute 2018). Potential limitations can stem from imperfect memory and knowledge of the details of consumed foods and dishes. Additionally, highly trained interviewers are required and multiple interviews are necessary to establish usual intake. Furthermore, decline in reporting accuracy with multiple administrations has been observed along with underreporting, the universal problem in all dietary assessment methods. Beyond any underreporting, the major source of error in this method is random error (National Cancer Institute 2018a).

1.2. Food frequency questionnaires

Food frequency questionnaire constitutes a closed list of foods and dishes with their predefined quantity categories. Their purpose is to assess the type, frequency and – in most cases – the amount of foods and nutrients consumed over longer periods (National Cancer Institute 2018b). The responder chooses consumed foods and dishes from a multiple choice of consumption frequency and quantity options. The list comprises usually between 100 to 200 food or dish choices, takes 30–60 minutes to finish, and can be self-administered or completed via an interview (National Cancer Institute 2018b). The choice of foods and dishes is based on open-ended surveys that were first conducted in the population of interest (Shim, Oh, and Kim 2014). Based on these surveys, foods are grouped in a closed-ended format by nutritional content or cooking methods. The main factor determining the FFQ content is the extent to which a given food contributes to the population's specific nutrients of interest, or explains between-person variation in intake (Kim et al. 2003).

FFQs can vary in format, ranging from a simple version that determines the frequency of consumption only (Craigie et al. 2011), to quantitative versions that additionally collect data on the amount of consumption (Naska, Lagiou, and Lagiou 2017), to the most widely used semi-quantitative FFQs, that collect data on average portion sizes in a closed-ended format (Mulligan et al. 2014; Michels and Willett 2009). Strengths of the FFQ include the ability to capture long-term, episodic and even usual dietary intake in a relatively simple, time-effective manner. They are characterised by low respondent burden, do not affect eating behaviour and are the only practical dietary assessment method in retrospective case-control studies (National Cancer Institute 2018b). The weaknesses include

closed and thus limited list of food choices, which is particularly relevant when collecting data on vegetarian subjects; and the need to be developed specifically for different age and ethnic groups. They do not capture detailed information about food preparation, brands, or contextual information about intake. Their completion involves complex cognitive tasks, relies on generic memory, and requires literacy. They are prone to large systematic measurement error (Day et al. 2001) and this is the reason why they are not recommended for use in cross-sectional studies (National Cancer Institute 2018b).

1.3. Food records

Food records (FR), also called food diaries, involve capturing all the foods, dishes, beverages eaten and possibly supplements consumed over one or several days by an individual (National Cancer Institute 2018c). Usually the respondents are asked to record all the items as they are consumed throughout the day. There are two types of food records -weighed FR and estimated FR. In the former, participants need to weigh every item consumed, whereas in the latter, common household measures (e.g. spoons, cups, etc.) and food pictures and other visual aids are utilized to help define the quantity eaten. This method can be used to assess a population's nutritional intake in cross-sectional studies (National Cancer Institute 2018c). FR can be used to examine relationships between diet and health, where diet is the independent variable, however they should not be used to evaluate dietary intake as a result of an intervention, due to the fact that their use can cause changes in eating behaviour as a result of keeping a record, phenomenon called reactivity. The strength of the method is that FRs reveal detailed information on meal and snack patterns, eating out, or – in the case of children – foods eaten at school or nursery (if cooperation from

the staff is achieved). They are also completed in real time; hence they do not rely on memory. They are inexpensive to collect.

The major weakness is their potential to change eating behaviour and be affected by reactivity. They only capture short-term, as opposed to long-term intakes. Weighed records can cause a very high respondent burden, while estimated records can produce bias in reported quantities. The quality of data may decline as the number of recorded intake days increases, and two or more non-consecutive administrations are required to estimate usual dietary intake distributions. In data from non-consecutive multiple administrations of food records, the major type of error is random measurement error, however systematic error can also occur, especially across consecutive administrations. Underreporting can happen in FR, but this is a feature of all the above-mentioned methods (National Cancer Institute 2018c).

2. Dietary assessment methods: analysis of dietary data

2.1. Assessment of the prevalence of inadequate intakes

Once the dietary data have been collected with one of the discussed methods, they can be analysed to serve different purposes. One of them is the assessment of the percentage of the group or population that is at risk of nutrient inadequacy.

Although commonly applied, computing the proportion of individuals whose intakes are below the Recommended Daily Allowance (RDA) (US), (US Institute of Medicine 2000a), otherwise known in the EU as Population Reference Intakes (PRI) (EFSA 2010), is likely to overestimate the true prevalence of

inadequacy. This is because the RDAs (PRIs) establish nutrient intake recommendations that exceed the requirement of 97.5% of the population (US Institute of Medicine 2000b). Instead, the fraction of individuals with inadequate nutrient intakes can be estimated with the so-called probability approach or the Estimated Average Requirement (EAR) cut-point method. These methods calculate the proportion of population or group whose usual intakes are below the EAR (US) (US Institute of Medicine 2000a), which in Europe is known as Average Requirement, (AR) (EFSA 2010). These terms describe nutrient intake that meets the daily needs of 50% of the population.

Although in the case of most nutrients average intakes exceed average requirements, there is more variability in the daily nutrient intakes than in their requirements. Some people in the population will have intakes below or above the EAR. Therefore, comparing the averages of intakes to requirement does not answer the question about the percentage of the population with intakes below EAR. Additionally, individual requirements are usually not known.

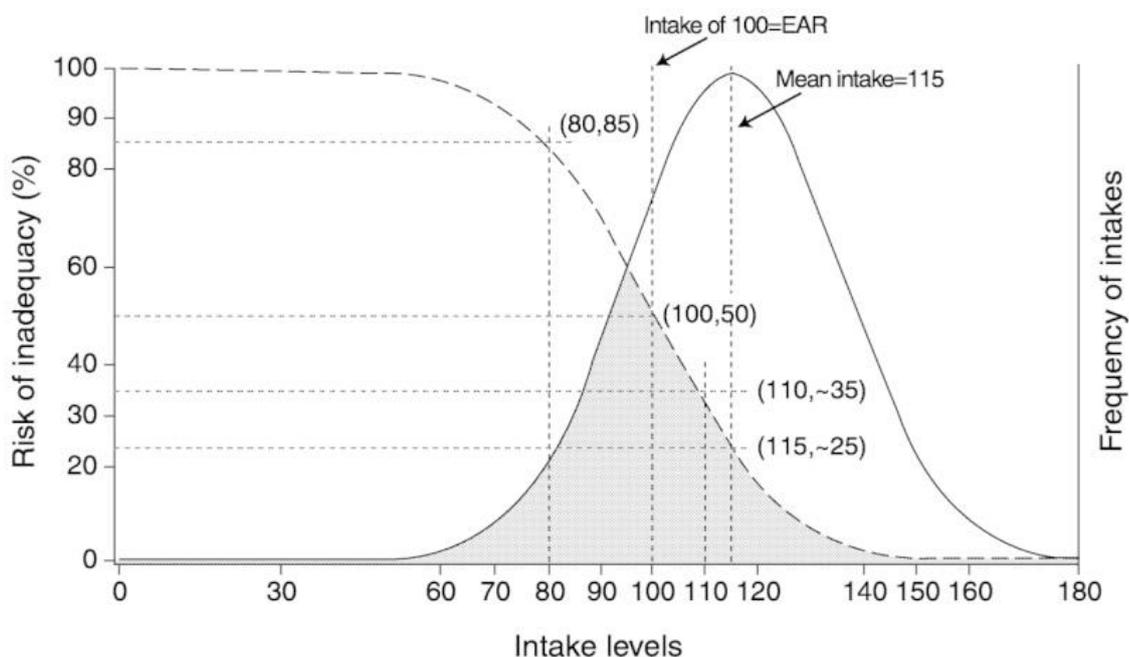
These problems are addressed by the two following statistical approaches: the probability approach (NRC 1986) and the EAR cut point method (Beaton 1994), (Carriquiry 1999), both of which estimate the prevalence of inadequate intakes.

2.1.1. The probability approach

The probability approach combines the information collected on the nutrient intake distribution of the group in question with the nutrient requirement distribution of a comparable group and combines these into a risk-probability curve (Carriquiry 1999; US Institute of Medicine 2000b). The curve specifies the probability that any given intake is inadequate for a given individual. Following the

definition of the EAR, there is a 50% probability of inadequacy at an intake at the level of the average requirement, for all nutrients whose requirements follow a normal distribution (NRC 1986). This risk increases or decreases accordingly as the intakes decrease or increase, and information on the mean and variance of the requirement distribution is needed to calculate the exact risk values. Then the risk-curve is compared to the distribution of usual intakes for the population and the proportion of the population with an inadequate intake is thus determined. Figure 4 (US Institute of Medicine 2000b) depicts a usual intake distribution with mean intake of 115 units, which is slightly higher than the EAR (100 units). The percentage of individuals with the risk of inadequacy at the level of consumption of 100 units is 50, and at the mean intake of 115 it is 25%.

Figure 4. Usual intake distribution of a hypothetical nutrient with mean intake of 115 units, and EAR of 100 units. At 100 units consumption level, 50% of population are at risk of inadequacy, the corresponding number for mean consumption level of 115 units is 25% (US Institute of Medicine 2000b).



The probability approach operates under 2 assumptions:

- intakes and requirements are independent,

- the distribution of requirements (mean, variance) is known,
- the form of the distribution is known (normal, log normal, etc).

However, the details of the distribution of requirements is not always known, therefore the next section describes a second method, that can be utilised without that knowledge, and hence it is more widely applied.

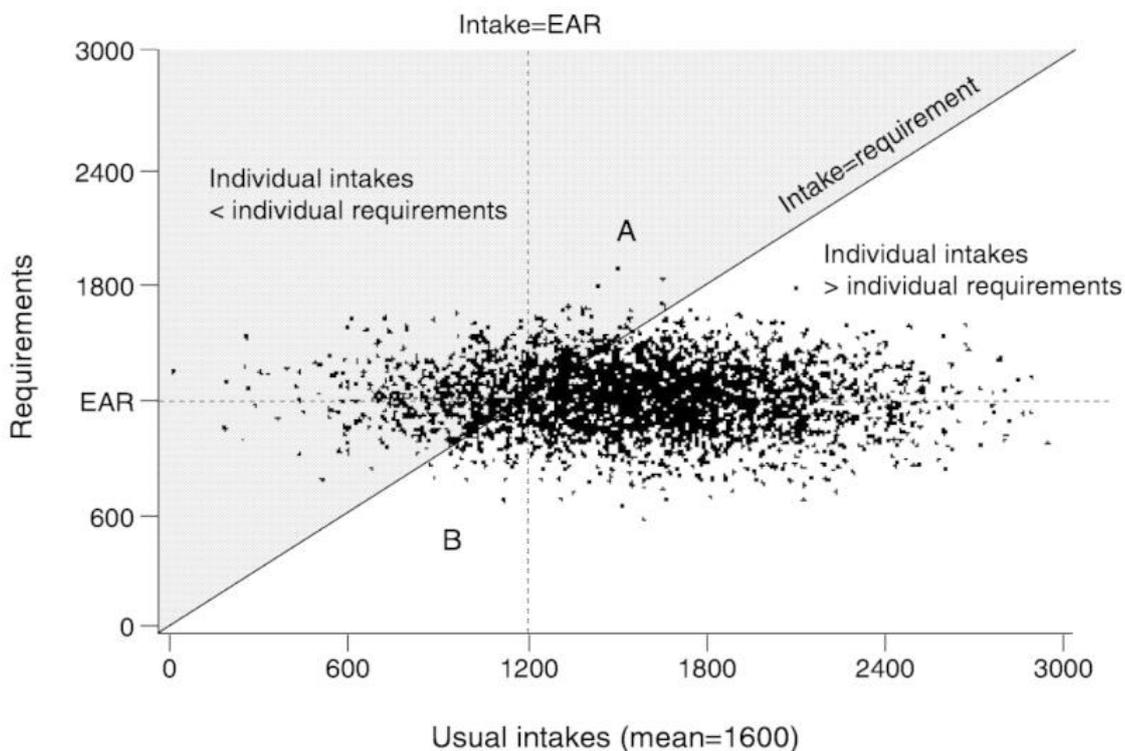
2.1.2. The Estimated Average Requirement (EAR) cut-point method

The EAR cut-point method, first described by Beaton (Beaton 1994), estimates the proportion of individuals with inadequate intakes. This method is based on the probability approach, but it is simpler. The data on the exact variance of the requirement distribution is not needed, instead the method requires this distribution to be symmetrical with its variance smaller than the variance of the intake distributions. Additionally, it utilises information on the EAR and the distribution of usual intakes in the group of interest. Based on this data, the proportion of individuals with inadequate intakes can be appraised as the proportion of the group with intakes below the EAR. The reasoning behind this estimation is shown in the Figure 5 below (US Institute of Medicine 2000b).

Figure 5 represents the combined distributions of intakes and requirements of a hypothetical nutrient with mean intakes 1600 and EAR of 1200 units. The 45° line represents those individuals whose nutrient intake equals their requirements. It is possible to estimate the number of individuals who have usual intakes less than the EAR (left to the vertical line), as both the EAR and the intake values are known. They consist of individuals whose intakes are below the requirements (the grey area) and individuals whose intakes are greater than their individual requirement (but still below the EAR), in the figure –

the triangle B. However, we are interested in the number of individuals whose intakes are below their requirements (the whole grey area). The graphic suggests that the number of people in the triangle B approximates the number of people in the triangle A – which are those, whose intakes are higher than the EAR but still lower than their individual requirement. As the individual requirement cannot be estimated, the EAR cut-point method replaces the individuals from triangle B with those in triangle A, and thus estimates the number of individuals in the population whose intakes are lower than their requirements. Using this reasoning, data on individual requirements, which are impossible to establish in most research settings, are not needed.

Figure 5. Combined distribution of intakes and requirements of a hypothetical nutrient with mean intakes 1600 units and EAR of 1200 units (US Institute of Medicine 2000b).



In order for this method to produce unbiased results, the following assumptions must be met.

- intakes need to be measured correctly,
- the prevalence of inadequacy in the population cannot be very low (> 8–10%) or very high (< 90–92%),
- the estimated usual intakes of individuals are independent of each individual's requirement,
- the distribution of requirements is roughly symmetrical,
- the variance in intakes of individuals in the group is greater than the variance in requirements of the individuals (US Institute of Medicine 2000b).

The EAR cut-point method is robust to moderate departures from almost all of the above-mentioned assumptions. The only exception arises when the intakes are correlated with requirements (as is the case with dietary energy). In that case the requirement distribution will be highly skewed rather than symmetrical and the method will produce biased estimates. However, if the prevalence of inadequacy in the group is about 50 percent and thus the mean intake is approximately equal to the EAR, virtually unbiased estimates of prevalence of inadequacy can be obtained even if intakes and requirements are correlated and the variance of requirements is larger than the variance in intake. The latter situation can arise in case of institutionalised populations, such as prisoners or residents of long-term care facilities.

If the true prevalence of inadequacy in the group is higher or lower than 50%, then the estimates produced by the EAR cut-point method are slightly biased and the bias increases in proportion with the difference between the prevalence of inadequacy and the EAR for that particular nutrient. However, the bias is negligible if the prevalence of inadequacy in the group is moderate (> 10 and < 90%) (US Institute of Medicine 2000b; Haubrock et al. 2011). The application

of the EAR cut point methods requires data on the EAR of a nutrient and usual intake distributions, which is discussed next.

2.1.3. Usual intake distributions of foods and nutrients

Usual intake of a nutrient is the individual's average intake of that nutrient over a longer period of time. Usual (as opposed to daily) intakes of a nutrient or a food are of interest to researchers or policy makers (Institute of Medicine (US) Subcommittee on Interpretation and Uses of Dietary Reference Intakes; Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. 2003; Carriquiry 2003). Researchers or government bodies use data on the usual intakes of groups or populations to monitor their nutritional status, to establish guidelines for a healthy diet, to implement food assistance programs and for other purposes.

The distribution of usual intakes should be distinguished from the distribution of observed intakes. The National Research Council (National Research Council 1986) has proposed a model of measurement error, that defines the daily intake as a deviation from usual intake. This deviation is equal to a measurement error.

$$Y_{ij}=y_i+e_{ij}$$

In this equation, Y_{ij} is the observed intake for individual i on day j , y_i is their usual intake with mean μ_y and variance σ_y^2 ; e_{ij} is the deviation from the usual intake for that individual on day j , with mean 0 and variance σ_e^2 . The variance σ_e^2 signifies the day-to-day intra-personal variance and σ_y^2 embodies the inter-personal variance in population intakes, i.e. the variance of usual intakes. As

per the equation, the variance of daily intake has two components: the intra-individual day-to-day variability and the inter-individual variability in intakes.

In order to estimate the usual nutrient intake in a population, we need to eliminate or minimise the intra-individual component of the variance, or otherwise predict the distribution of y_i from the observed Y_{ij} (US Institute of Medicine 2000b).

The observed intake should not be used as an estimate of the usual intake distribution without statistical corrections, not only due to the large within intrapersonal variance of intakes. All the reasons are listed in detail below.

2.1.3.1. Large within – person variance of intakes

For some nutrients the individual variation in intake from day to day can be greater than the variation in intake between individuals. This is the case for example for vitamin A, where within-person variability in intakes can be as much as six times greater than the between-person variability (Basiotis et al. 1987). For some other dietary components, however, such as energy or protein, the individual day-to-day variability in intakes is similar to the between-person variability (Basiotis et al. 1987). The mean of the distribution of the observed intakes is an unbiased estimate of the mean of the usual intakes, especially if the intakes are collected over numerous days. As the number of days with collected dietary information increases (> 10), the variance of the mean of those days approaches the inter-personal variance, even for episodically consumed foods and nutrients (Institute of Medicine (US) Subcommittee on Interpretation and Uses of Dietary Reference Intakes; Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. 2003;

Carrquiry 2003). However, collecting numerous days of dietary intake is not feasible in most research settings.

2.1.3.2. Heterogeneous within-person variation in intakes

Observed intakes should not be analysed to assess usual diets, as individuals not only vary in the intakes of foods and nutrients they consume from day to day, but the degree of this variability is not constant across population. People with higher usual intakes vary more in their day-to day intake fluctuations than people with lower usual intakes (Nusser et al. 1996).

2.1.3.3. Correlation in intake data collected over consecutive days

Consumption of foods on one day may affect their consumption on the next. This is especially the case for episodically-consumed foods (e.g. liver), which are unlikely to be eaten on consecutive days. However, at the same time, it is possible that people may consume leftovers from the previous day. In both cases the data from consecutive days cannot be assumed to be independent. The assumption of independence for intra-person observations can hold if the dietary data is collected several days apart, though the necessary interval depends on the nutrient. The necessary time gap is smaller for energy (1–2 days) but greater for e.g. vitamin A (3–4 days) (US Institute of Medicine 2000b).

2.1.3.4. Distribution of intakes of episodically consumed foods spikes at zero

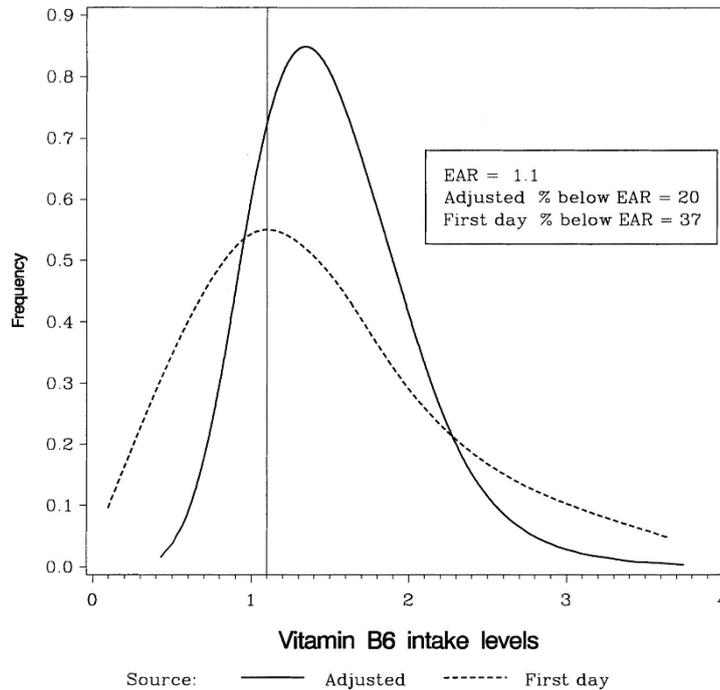
Unless dietary data are collected on numerous days, which is not viable in most research settings, intakes of episodically eaten foods (e.g. fish, liver) and their corresponding nutrients are not likely to be captured. Zero values will capture

not only individuals who never consume the food, but also individuals who sometimes do, but who did not consume them over the days on which the dietary data was collected (Carrquiry 2003).

Therefore, statistical procedures need to be applied to remove or minimise the intra-individual part of the variance and account for all or at least some of the above – mentioned aspects of observed dietary data, i.e. heterogenous within-person variation in intakes, episodically consumed foods, and correlation of intake data collected on consecutive days (NRC 1986; Nusser et al. 1996), to estimate the distribution of the usual intakes rather than that of the observed ones. Failure to apply these procedures, can lead to inflated variance in the observed intake distribution, and to over-estimates of the prevalence of inadequacy or excess, regardless of the method chosen (probability approach or EAR cut-point method) or to outright bias in estimates of nutrient or food intakes.

Figure 6 (Carrquiry 2003) below represents the first scenario graphically. The two distributions have different spreads and different degrees of right-skewedness. The lower one with a larger spread represents data from one 24HR, with estimated prevalence of nutrient inadequacy of 37%. The higher distribution with smaller spread represents data from two 24HRs. The estimated prevalence of inadequacy in this case is ~20%. This almost two-fold difference can lead to different conclusions and policies

Figure 6. Distributions of nutrient intake of vitamin B6. The dashed line represents data from one 24-hour recall (24HR), the solid line represents data from two 24HR. The vertical line represents the EAR for vitamin B6 (1.1 units). Based on data from one 24HR, 37% of population is at risk of inadequacy, whereas the corresponding figure for data from two 24HR is 20% (Carriquiry 2003).



2.2. Methods to analyse usual intakes of foods and nutrients

The application of any statistical method aiming at reducing the intrapersonal variance in nutrient intake requires that at least two measurements are present for at least some individuals in the group. The number for a 24-hour recall, or a non-consecutively recorded diet record, is estimated to be at least two, or at least three when data are collected over consecutive days (US Institute of Medicine 2000b).

The replicate measurements contain the data required for approximating the day-to-day individual variance portion in intakes, which is then used in the statistical adjustment. Additionally, the distributions of most observed and also usual nutrient intakes have a right-skew. This is exacerbated in the case of

supplement consumption. For example, the mean calcium intakes in the US range from 871 to 1,266 mg/day for males, and from 748 to 968 mg/day for females depending on the life stage (Bailey et al. 2010). Clearly, it is not possible to consume more than 750 mg below the mean on a natural diet, but it is possible to consume 750 mg above the mean from diet and/or supplements. Therefore, statistical procedures that assume normality cannot be applied to nutrient intake data

The following methods are applied to establish unbiased estimates for the usual dietary intake distribution for most nutrients, with the exception of phytochemicals like lycopene, β -cryptoxanthin and α -carotene, or foods such as green leafy vegetables, shellfish and vitamin C-containing fruits and supplements. These nutrients and foods are not consumed daily and require more than the standard statistical procedures used for this purpose (Carriquiry 2003).

2.2.1. NRC Method

The National Research Council (National Research Council 1986) method, otherwise called a measurement error method, accounts for the day-to-day variability of individual intakes as well as the non-normal distribution of nutrient intakes. The measurement error model (mentioned in the previous section) is applied on power-transformed data (which reduces skewedness), according to the following equation:

$$(transformed) \text{ observed intake} = \text{usual intake} + \text{deviation from usual intake.}$$

The random deviation from the usual intake (the measurement error) has a mean of zero, and its variance reflects the day-to-day variability in intakes.

Upon transforming the data to achieve normality, the adjusted usual intakes are calculated as follows: the mean intake of the group is deducted from the observed intake, and the resulting figure is then multiplied by the ratio of individual-to-individual variance to the total variance of daily intake. The result is then added back to the mean intake of the group. These values are then back-transformed to the original scale.

The following relationships emerge from the model: the smaller the intra-personal variability, the closer the resultant usual intake distribution estimation will be to the distribution of individual means; on the other hand, the larger the day-to-day variance in intakes, the closer the estimated usual intake distribution will be to the general group mean intake.

The weakness of this method lies in its assumption that the variance of the measurement error is constant across individuals, in other words that their variability in intakes is homogenous. As elaborated in the previous section, this is not the case, especially for individuals with higher nutrient intakes. Additionally, the mean of a nonlinearly transformed variable is usually not equal to the transformed mean of the same variable. Therefore, using the same transformation and its inverse on means can result in biased estimates when transformed back to the original scale (Carriquiry 2003).

2.2.2. Iowa State University method

The Iowa State University (ISU) method (Nusser et al. 1996; US Institute of Medicine 2000b) is very similar to the NRC method. However, it employs several statistical improvements. Firstly, at the back-transformation step, the intakes are mapped back onto the original scale through a bias-adjusted back

transformation. Secondly, it is designed to allow for other factors such as day of week, season, and physical activity effects (apparent in patterns of reported intake in relation to the sequence of observations) that may introduce systematic error in the observed distribution of intakes. Additionally, it can take into account correlations between observations on consecutive days and heterogeneous within-person variances. The ISU method is not particularly suitable for smaller samples, as its complexity requires a higher number of observations to make sure that different adjustment procedures retain adequate reliability (Institute of Medicine (US) Subcommittee on Interpretation and Uses of Dietary Reference Intakes; Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. 2003).

2.2.3. The MSM method

The MSM method (Haubrock et al. 2011; EFCOVAL 2011), is the newest method. It can be applied for estimation of the usual intake distribution of foods, nutrients and episodically consumed foods. The advantage of this method lies in the fact that it can combine information from 24h recalls or food records with data from food frequency or propensity questionnaires which provide information on consumption frequency and thus enable the incorporation of data on periodically consumed items. Therefore, it is possible to distinguish between real non-consumers and occasional consumers of a food or nutrient. The method works if at least two repeated measurements for at least some participants are available. The MSM method proceeds in three statistical steps that estimate the usual dietary intakes.

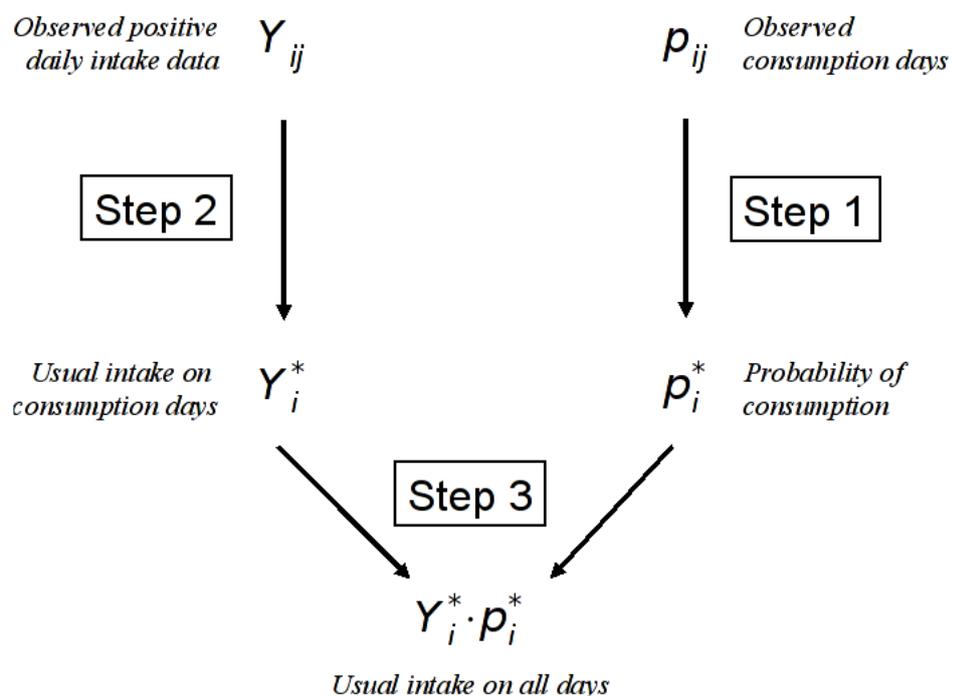
Firstly, it evaluates the probability of eating a certain food on a random day. For this step, information from FFQs or propensity questionnaires is utilized. If there is no reported frequency of consumption, the method treats this individual as a habitual non-consumer, whereas if the frequency of use is reported, then the individual is treated as a habitual consumer. The default setting of the method (in the absence of questionnaire data) is that all individuals are habitual consumers. The estimation of that probability is achieved by applying logistic regression that utilizes the data on consumption frequency (if available) and may include covariates like gender, season, etc. The regression residuals are converted into actual numbers and multiplied by the quotient of inter-individual by intra-individual variance, which are both calculated at this stage as well. Residuals are then converted back to the original scale resulting in the estimation of the probability of consumption by an individual on a random day.

Secondly, the usual amount consumed is estimated with linear regression. The observed food or nutrient intake is a function of independent variables that are assumed to be predictive for the dietary intake, e.g. gender, age and, if available, consumption frequency. Similarly, to the previous step, the corresponding residuals of the linear regression model are transformed to normality (symmetry) by a two-parameter Box-Cox transformation. The transformed residuals are then utilised to estimate inter- and intra-individual variances, which are then used, similarly to the other methods discussed above, to shrink the individual mean food intake to a group mean. The quantities calculated in this process for each individual are converted back to the original scale and added to the linear regression estimates, giving an estimate of the usual intake of an individual on the measured day.

Lastly, in step 3 the probability of consumption on a random day and the usual intake of food or nutrient on the measured consumption day is multiplied to estimate the daily usual intake of food or nutrient for an individual. This information is then used to derive the usual nutrient distribution for the study group.

The three steps are depicted in Figure 7 below (EFCOVAL 2011).

Figure 7. Three statistical steps of the MSM method estimating usual intake distributions (EFCOVAL 2011).



3. The choice of the dietary assessment methods for this study

The previous section presented a literature review on the methods used to capture and analyse dietary intakes. The following sections present the methods chosen for this study and discuss the rationale for this choice.

3.1. Collection of dietary data

The estimated food record was chosen as a method to assess dietary intake of the study participants, as this type of FR poses less burden on participants than the weighed FR. I considered the burden as high, given the fact that parents/guardians were not recording their own intakes, and that both children's home intake as well as school intake were expected to be captured. The major limiting factor that determined my decision not to use FFQ was its closed-ended format, which would increase the risk of inadequately capturing the range of foods and dishes consumed by the vegetarians and vegans, and the lack of validated FFQs for the paediatric population in Poland. The major drawback of the 24HR method for this study was its inability to adequately assess children's intake of foods outside of the home, as the interviewee (usually parent or guardian) would have limited knowledge of the child's diet at school or in kindergarten. Moreover, the 24HR interview is conducted without notice, therefore parents acting as proxies for their children would not be able to accurately recall their food intake without prior preparation.

3.2. Analysis of dietary data

The MSM method was used to obtain usual dietary intakes. The MSM program, developed by the former Department of Epidemiology of the German Institute of Human Nutrition Potsdam-Rehbrücke, available online ("MSM" n.d.), derived the usual dietary intakes adjusting the observed intakes collected with the food record with the MSM method. The following independent variables were entered in the MSM regression model of daily nutritional intake data: age, sex, dietary group, season and age*sex interaction (to allow the effects of age to differ

between categories of sex), as these are predictive for the usual dietary intake and recommended by the MSM method user guide (EFCOVAL 2011). The reasons for choosing this method for this study are as follows. It can be applied to both foods and nutrients and can assess the intake of episodically consumed foods by combining the information from food records data with data from screener questionnaires. This gives me the opportunity to assess the intake of episodically consumed foods and dietary patterns of children in my study. These tasks are outside the scope of this PhD but can be applied in future analyses that will further my academic development. For the purpose of this study, the usual dietary intakes were used to estimate the percentage of inadequate nutrient intakes with the EAR cut point method, along with calculating medians of usual intakes.

IV. RESEARCH AIMS, HYPOTHESES AND QUESTIONS

1. Research aims

As outlined in chapter III, plant-based diets are associated with better CVD risk factor profile and lower CVD prevalence and mortality in adults. They are also considered to be safe for adults, when appropriately planned (Melina, Craig, and Levin 2016a). At the same time, comprehensive data on health outcomes in children on vegetarian, and especially vegan diets, are lacking (Schürmann, Kersting, and Alexy 2017). The aim of this study was to fill this gap in knowledge and to quantify prevalence of differences in several indicators of health, including body composition, CVD risk and micronutrient status, along with estimating the prevalence of inadequate micronutrient and abnormal cholesterol status in either vegetarian or vegan children relative to a reference group of omnivore children. The second aim was to thoroughly assess dietary intake, and in particular, the prevalence of inadequate nutrient intakes and to calculate median usual intakes of nutrients adjusted by relevant confounders in the same group of children.

2. Hypotheses

I aimed to test four main hypotheses in this research project. I formed these based on the existing data from adult vegetarian and vegan subjects and the limited evidence available in children.

The first hypothesis is that in comparison to omnivorous diets, plant-based diets in childhood are associated with more favourable CVD risk profile including:

- healthier blood lipids, lower insulin resistance, lower chronic subclinical inflammation measured as hs-CRP levels,
- lower body fatness,
- lower cIMT,

but unfavourably with IGF-1 and homocysteine levels.

My second hypothesis is that in comparison to omnivorous diets, plant-based diets in childhood are associated with less favourable patterns of growth and selected aspects of body composition:

- shorter height,
- lower lean mass,
- lower bone mineral content.

Thirdly, I hypothesised that plant-based diets in childhood are associated with increased risk of nutritional deficiencies especially that of iron, vitamin B12 and vitamin D. And lastly, I hypothesised that vegetarian and vegan children have higher prevalence of inadequate intakes along with lower intakes of several nutrients, in particular, vitamin B12, iron and vitamin D3.

3. Research questions

In order to meet the aims of this research, I developed a set of research questions.

The primary research question for the first research aim is:

- Do vegetarian and vegan children differ in relation to selected parameters of health in relation to omnivorous children?

In particular:

- Are there differences in body composition between children on vegetarian or vegan diets in comparison to omnivorous children?
- Are there differences in height between children on vegetarian or vegan diets in comparison to omnivorous children?
- Are there differences in bone mineral content between vegetarian or vegan children in relation to omnivore children?
- Do vegetarian and vegan children have a better CVD risk profile than omnivorous children?
- Is there evidence of nutritional deficiencies among vegetarian and vegan children?

The primary question for the second research aim was:

- Do dietary intakes of vegetarian and vegan children differ from these of the omnivores?

In particular:

- What is the proportion of vegetarian and vegan children with inadequate nutrient intakes in comparison to the omnivore children?
- What are the usual median nutrient intakes of vegetarian and vegan children and do they differ in relation to their omnivore counterparts?

V. METHODS

In chapter II, a literature review was presented to outline the reasons for conducting this study and for choosing the health parameters to evaluate potential differences between vegetarian or vegan children in relation to children following traditional diets. Chapter III summarized the methods applied in epidemiological research to capture and analyse dietary intakes and justified the choice of these methods for this study. Since the methodology of dietary assessment in observational studies is complex, it was discussed in a separate part of the thesis. This chapter describes the remaining methods used to test my hypotheses. In particular, it elaborates on the study design, recruitment, sampling, ascertainment of data on background characteristics, methods to measure physical activity and outcomes; along with providing information on ethical considerations. It defines the criteria for allocating children to the different dietary groups and describes operational details of capturing dietary intakes with food records in this study. Next, the statistical models applied and the rationale behind the choice of confounders and mediators of the association between dietary exposure and health outcomes are discussed. Lastly, the chapter summarizes all the methods used in this study to measure background characteristics, exposure and outcomes.

1. Study type

A cross-sectional observational methodology was chosen for this study. The main characteristic of this study design is that the exposure and outcome are measured at the same time. This is also considered its major limitation as it

leads to *antecedent-consequent bias* (Andersen 2000), which occurs due to lack of temporality sequence between exposure and outcome, as both are measured simultaneously. Therefore, cross-sectional research can measure and compare the prevalence of disease between exposed and non-exposed, however its findings do not allow us to derive definitive causal relationships (Andersen 2000). Nevertheless, some authors have argued that cross-sectional designs can establish or uncover preliminary evidence for a causal relationship, especially if the temporal directionality from the exposure to the outcome is sustainable (Reichenheim and Coutinho 2010, Kesmodel 2018). Although longitudinal study designs or intervention trials are more suitable to achieve these aims, cross-sectional studies have often been used instead for pragmatic reasons such as cost and duration, or ethical constraints. It is unethical and unfeasible to randomise and subject healthy children to different dietary regimens of unknown health effects for periods long enough to elicit effects on growth, body composition or selected cardiovascular risk factors. Therefore, a cross-sectional design is the only viable option for this study, and its results can provide preliminary evidence that can be further investigated in future cohort studies. Furthermore, in this study, exposure tracks back into the past, i.e. the children recruited to the study had to have followed their respective diets for at least one year and their diet was measured two weeks before the outcome data collection took place. Consequently, the measured outcomes have followed the dietary exposure and hence it could be argued that the preliminary evidence it produces might be considered, albeit with great caution, as a causal relationship.

2. Study subjects

2.1. Study eligibility

The study included healthy Polish children (age 5–10 years), all of Caucasian ethnicity. Vegetarian and vegans had to have followed their diet for ≥ 1 year prior to recruitment to the study. Those receiving any treatment other than bronchodilators and/or steroid inhalers for asthma were excluded from participation. Other exclusion factors were conditions adversely affecting growth and development. The latter included obesity and wasting defined using age-specific paediatric international BMI cut-offs, corresponding to 30 kg/m^2 at age 18 years and -2 z-scores respectively (T. J. Cole et al. 2007), or short stature, lower than the 5th percentile for Polish sex-specific growth curves (Kułaga Z, Rózdżyńska-Świątkowska, Grajda A 2015) due to a diagnosed growth disorder. Eligibility was established via electronic questionnaires sent to parents before the study and confirmed when the study began.

2.2. Recruitment

Vegan and vegetarian children were recruited by advertisements using internet portals and social media, targeting issues of vegetarianism and veganism. Hence, a convenience-type sampling rather than population-based sampling was chosen. Although population-based sampling is preferable, as it reduces bias, it involves recruiting from a subset of subjects that is representative of the entire population (Martínez-Mesa et al. 2016). Vegan and vegetarian children can be described as a rare population (Lavrakas 2012). Additionally, based on my experience from the pilot project, some vegan and vegetarian parents are reluctant to consult with conventional medicine centres regarding the health of

their children, or disclose the information of the nature of their children's diets, anticipating criticism from doctors or other health - professionals. Hence, recruiting this group from the subsample representative for the whole population would prevent me from obtaining adequate numbers of participants within the time-frame available for this study.

Omnivores were recruited by asking vegan and vegetarian children to bring a friend of similar age and sex. Additionally, advertisements were placed in Warsaw health-food stores, and on internet portals devoted to healthy eating, from which the remaining omnivores were matched to vegetarians and vegans by sex, age (within ± 1 year), maternal education (higher, secondary, primary), and place of residence (urban vs. rural). This recruitment approach aimed to reduce selection bias, i.e. to minimise the differences in background traits in three dietary groups (Pandis 2014).

2.3. Sampling

The sample size per group was calculated using data for blood lipids (total and LDL cholesterol) from the pilot study investigating (among other health parameters) blood lipid levels in healthy Polish prepubertal children on vegan diet (n=46), vegetarian diet (n=29) in comparison with age- and sex-matched omnivores (n=61). I conducted this study in 2010 as part of my thesis for the Masters in Dietetics degree at the Warsaw Medical University. I detected a mean difference of 33 mg/dL between vegan and omnivore children in total cholesterol levels, along with a mean difference of 28 mg/dL in LDL cholesterol levels between these two dietary groups, which was equivalent to -0.65 standard deviation scores in both of these outcomes. The crude mean

differences between blood lipid levels from the pilot project are displayed in Table 6 and 7 below. On this basis, I used the following formula to calculate the sample size required for my study: $n \text{ per group} = 16 * (SD^2/D^2)$, where D is the difference to be detected with 80% power, $p < 0.05$, and SD is the standard deviation of the trait. I aimed to detect, with a statistical power of 80%, differences of ≥ 0.5 z-score between omnivore and either vegan or vegetarian groups in each outcome, thus requiring 64 children per group. Anticipating occasional missing data, I intended to recruit 66 children per group. I specified age groups for recruitment taking into account both the scarcity of vegan children in Poland, and the aim of achieving similar age distributions across dietary groups. I aimed to recruit 7 of each sex-diet combination at 5 years, and 13 in the 6–7 and 8–10-year age-groups (total 198).

Table 6. Total cholesterol levels in prepuberal children on different diets from the pilot project (unpublished data)

			Total cholesterol levels (in mg/dL)					
Diet type	n	Mean	Median	Min.	Max.	SD	p value	
Omnivore	61	164.2	157.0	105.0	233.0	26.8	p <0.001	
Vegetarian	29	154.7	159.0	101.0	232.0	30.8		
Vegan	46	130.6	134.0	90.0	194.0	22.4		

Table 7. LDL cholesterol levels in prepuberal children on different diets from the pilot project (unpublished data)

			LDL cholesterol levels (in mg/dL)					
Diet type	n	Mean	Median	Min.	Max.	SD	p value	
Omnivore	61	99.7	98.6	54.2	160.0	23.0	p <0.001	
Vegetarian	29	88.9	85.6	51.2	154.4	25.9		
Vegan	46	71.6	69.3	38.4	136.2	20.4		

2.4. Consent and ethical considerations

The study was approved by Ethical Committees of University College London (UCL), and the Children's Memorial Health Institute in Warsaw, Poland, where the study took place. I prepared two separate study description documents, one designed for parents, the second written in appropriate language for children. The children's version was accompanied by pictures of the different procedures involved in this study (DXA, cIMT assessment), that the children were going to undertake. Parents gave written informed consent and children orally assented to participate, after they became familiar with the study description.

After all the assessments were finalised, each family was offered a nutritional consultation by myself or a fellow dietician. Each consultation was based on the results of the analysis of child's food record with a nutritional software program, conducted before they arrived at the clinic (as explained below, parents had to fill in the food record prior to arriving for the examination day). Most families agreed to the consultation, which explained the principles of healthy eating for all children. Additionally, for vegetarians and vegans, detailed instructions were provided (orally and in the form of a printed booklet) on how to compose their respective diets to provide adequate amounts of all nutrients. Parents of vegetarians and vegans were informed of the need to supplement their children's diets with vitamin B12 and, depending of the season, vitamin D3. All the test results were sent to the parents once they became available, with the exception of those with no informative value to the individual due to lack of reference ranges (cIMT for all participants, bone density results for vegetarians and vegans only). Blood tests results were accompanied by appropriate commentary, and in the case of abnormalities detected, additionally with the

recommendation to consult their doctor. In the case of results suggesting megaloblastic anaemia (low serum vitamin B12, and/or high homocysteine, and/or abnormal complete blood count (CBC) count results), separate phone calls were made to parents emphasizing the need to supplement vitamin B12 in higher amounts for 2 months and to conduct follow-up blood tests to verify if the supplementation was effective. Almost all parents of affected children agreed to that, with the exception of one family who refused to supplement their child's diet with B12 despite being given extensive information about its necessity.

2.5. Background characteristics

The following background data were collected before enrolment via an electronic questionnaire: child's date of birth, parent-reported weight and height of the child, current health status, medications, information on parental smoking and educational attainment, family size, family history of non-communicable diseases (NCD), (parental/grandparental hypertension, obesity, diabetes or coronary artery disease or myocardial infarction before age 55 years for men and 65 for women), religion, breastfeeding/formula feeding practices. Upon recruitment, additional questionnaires in the clinic ascertained the child's birth order, fracture history, lactose intolerance status, birth weight, APGAR score, gestational age, self-reported parental height, maternal pre-pregnancy nutritional status (weight, nutritional supplementation practices, dietary practices), and if the child had been on holiday with significant sun exposure recently.

3. Primary exposure assessment – diet

Food record forms were emailed to the parents/guardians of eligible participants of the study. Each food diary included example pages to show how to complete the diary and how much detail to include. Separate diaries with different examples (including meat and dairy) were emailed to omnivore children and vegans/vegetarians (including plant – based alternatives). A parent/guardian was asked to complete a diary with the help from the child as appropriate. Participants were instructed to record food and drinks consumed both at home and away from home and were therefore asked to take the diary with them when away from home. If insufficient details were obtained by parents on the composition of meals eaten outside of the home, school and kindergarten staff and restaurants were contacted directly. During the recording period, two phone calls were made to the parents. The first call at the beginning aimed to explain the method, the significance of the research and detailed recording, as well as the importance of not changing any dietary habits during the recording period. The participants were guided through the different sections including the instruction page, informed how to describe details of foods and drinks and their portion sizes, and an example day was discussed together. The second call was made during the recording period to check for compliance, answer questions or deal with problems, and review the diary to identify and edit possible omissions and missing details. At the end of the recording period, parents emailed the diary back and sometimes a third phone call was made to correct omissions or clarify inaccuracies. In case of the parent/guardian responsible for the child's feeding being temporarily absent, the child's data collection date was postponed until their return.

Participants were asked to keep a record of everything eaten or drunk over four consecutive days including two weekend days. This number was chosen to minimise the correlation of intake data (and thus systematic error) due to lack of intervals between data collection days. The decision to choose two weekend days rather than one was made in order to minimize the FR inaccuracies inevitably linked to kindergarten/school consumption data collection. Existing data suggests that dietary patterns in children track between weekdays and weekends, apart from children following 'processed type' dietary pattern (Rothausen et al. 2013). This was considered unlikely among vegetarian and vegans, who generally have high health-consciousness (Hoek et al. 2004), and their omnivore study counterparts, who were specifically recruited to represent that trait.

The recording was conducted on paper and then transferred into electronic form by scanning or hand-copying. Participants were asked to record portion sizes in household measures (e.g. one tablespoon of baked beans, one Kit Kat (two fingers)), or for packaged foods to note the weight indicated on the packet. Pictures of household measures of the most commonly consumed products (milk, flour, butter, etc) were attached to the diary. Leftovers were not recorded separately, instead participants were asked to take into account leftovers when recording how much they consumed. As a prompt for this, a question at the end of each diary day asked participants whether they had finished all the food and drink they recorded for that day.

Participants recorded brand names for foods wherever possible and were asked to collect the food label information/wrappers for any unusual foods and ready meals consumed to help coders identify or clarify items. For homemade dishes,

participants were asked to record the individual ingredients and quantities for the whole dish along with a brief description of the cooking method and how much of the dish they had consumed. For meals consumed at school or nursery school, the recipes were obtained by contacting the institutions. Parents were also asked to record the information on packed lunches and instructed to ask the teachers if the child had eaten anything else apart from the food offered at kindergarten or school and if so, how much of it. After each day, participants recorded if their intake was typical for that day (and if not, the reason why) and details of any dietary supplements taken. The diary also contained a series of questions about usual eating habits (for example, type of milk or fat spread usually consumed) and supplements taken, to facilitate coding in cases where details were omitted in the diet record.

Prior to recruitment, parents were asked to fill in a screener questionnaire to qualify the child's history of monthly consumption frequency of meat, fish, dairy products and eggs. This was used to preliminarily classify children as omnivore (non-vegetarian), lacto-ovo-vegetarian or vegan at the recruitment stage and to assess the frequency of animal product consumption from birth. Final categorisation into dietary groups was performed after taking into account both screener questionnaire and food records. Participants were classified as vegan if they consumed no flesh foods (meat and fish), or other animal-based products (eggs, dairy), for at least the previous year, or if they consumed no flesh foods (meat and fish), and nearly no other animal-based products (eggs, dairy), over the last year, with minor exceptions that amounted to < 5% of reported dietary energy from eggs and dairy based on the FR. Vegetarians were classified as those consuming eggs and dairy ≥ 1 per month, but red meat, poultry and fish

< 1 per month, for at least the previous year. For clear distinction of dietary patterns, the study did not accept pesco-vegetarians (those who consumed red meat and poultry < 1 per month, and fish \geq 1 per month), and semi-vegetarians (who consumed red meat, poultry, fish 1 per month to 1 per week, and eggs or dairy at any level), and defined as omnivores those who eat meat, poultry, fish > 1 per week, and eggs or dairy at any level (Tantamango-Bartley et al. 2013). The food records were obtained within two weeks prior to health outcome data collection, as most of the measured blood variables in this project respond to diet changes within that time (Bonanome and Grundy 1988; Hermansen et al. 2001; Rasmussen et al. 1993). Food record data were entered into nutritional analysis software, Esha Food Processor, version 10.14. Polish food composition tables (Kunachowicz, Przygoda, and Iwanow 2005) linked to the software were used as the primary reference for calculating nutrient intakes. Nutrient contents of foods not available in the Polish tables, e.g. vegetarian-specific foods, were obtained from the database of the US Department of Agriculture (USDA n.d.).

4. Secondary exposure assessment – physical activity

The preferred methods for monitoring physical activity performed by young children are direct observation or accelerometers (Oliver, Schofield, and Kolt 2007; Pate, O'Neill, and Mitchell 2010) Both accelerometers and direct observation are appropriate for this particular age group and allow the detection of short spurts of activity that are characteristic of young children (De Vries et al. 2009). Direct observation of physical activity can provide detailed contextual information on pre-schoolers' physical activity, but is subjective and impractical

for understanding regular physical activity patterns (Oliver, Schofield, and Kolt 2007). The advantage of accelerometers is that they enable objective quantification of the frequency, intensity, and duration of physical activity during waking hours over a longer period. Moreover, the relatively low researcher and participant burden associated with accelerometers, and their lower cost as compared with direct observation, makes them particularly attractive (Oliver, Schofield, and Kolt 2007; Reilly et al. 2008; Cliff, Reilly, and Okely 2009). Nevertheless, this method involves some limitations. Accelerometers primarily measure locomotor activity, and do not register upper body movement. Further, they cannot register if a person is carrying any weight (which expends more energy than movement without weight) nor do they provide information on body posture (sitting versus standing still) (Lee and Shiroma 2014). However, these do not seem to be major limitations for paediatric populations, where standing still or carrying weights is unlikely to contribute much to overall PA patterns. Due to its numerous advantages described above, accelerometry was chosen as the physical activity assessment method in this study and was applied using Actigraph GT1M devices. Children were asked to wear an accelerometer on the right hip during waking hours for 4 days. A minimum of 2 days with ≥ 8 hours of activity recordings was deemed valid (Corder et al. 2008; Reilly et al. 2008). Average counts per minute (CPM) were used as an indicator of overall physical activity (number of registered movement counts divided by wear time (counts/minutes)). Additionally, time spent in sedentary, moderate and vigorous physical activity was extracted to compare time spent at different PA intensity levels between dietary groups.

5. Outcome assessment – body composition

The gold standard of body composition assessment is cadaver dissection, and no in-vivo technique can approximate its accuracy (Wells and Fewtrell 2006). This is because all other methods rely on assumptions when converting raw measurements to body components and thus introduce error. The simplest and most traditionally used method of body composition assessment is anthropometry. Anthropometric techniques seek to obtain markers of fatness (e.g. BMI, skinfolds) or of lean mass (e.g. calf, arm and chest girth). Most other methods divide body weight into two major compartments (so called two-component (2C) models) (Lee and Gallagher 2008), namely fat mass (FM) and fat free mass (FFM) so that total body mass equals FM + FFM. These include techniques like bioelectrical impedance analysis, densitometry methods (including hydrostatic underwater weighing and air displacement plethysmography) and hydrometry (deuterium dilution) (NIHRa n.d.). More body components can be measured to obtain more valid estimates of body composition and thus three (3C), four (4C), five (5C) and six (6C) component models exist (Wells et al. 1999; Wang et al. 2005). They divide body weight into components such as water, fat, protein, bone mineral, non-osseous mineral (soft tissue minerals) and glycogen, and use more than one mode of measurement to obtain data on multiple body components. An example of a 3C model is dual x ray absorptiometry (DXA), that divides body into fat, soft and osseous tissue. The 5C and 6C models are rarely used and the 4C model is regarded as the gold standard reference method for in-vivo assessment of overall body composition (van der Ploeg, Withers, and Laforgia 2003). Body composition can also be measured by newer technologically advanced

methods, including quantitative computed tomography (QCT), MRI, and magnetic resonance spectroscopy and quantitative magnetic resonance (Lee and Gallagher 2008). Although these provide some advantages over more traditional approaches (e.g. three-dimensional tissue volume quantification, quantification of visceral fat, ability to provide regional and whole-body measurements), they are also expensive, not commonly available, and in case of QCT, expose subjects to ionising radiation. Another disadvantage of imaging methods is that they produce volumes rather than masses, which poses interpretation difficulties, especially in case of adipose tissue (Institute of Medicine (U.S.). Committee on Military Nutrition Research., Newberry, and Costello 1997).

For this particular study three methods were chosen: anthropometry, DXA, and deuterium dilution. This decision was made based on the availability of equipment in the Children's Memorial Health Institute in Warsaw, and the suitability of methods for younger children and their accuracy (Wells and Fewtrell 2006).

Multiple methods were chosen to increase the internal validity of the study. The assumption of 2C models that FM and FFM have constant physical or chemical properties introduces error in each of the 2C methods. Although, this is a realistic assumption for fat mass, it does not always hold for FFM. Fat free mass is a complex component of weight, incorporating skeletal muscle, vital organs, bone, and supporting tissues, and fluids including the non-lipid content of adipose tissue (Weber, Leonard, and Zemel 2012). Its components and thus its density vary in childhood due to ongoing physical maturation (Fomon et al. 1982), and the inter-individual variability in healthy children is also high (Wells et

al. 1999). Measured values of FM and FFM vary according to the method used (Wells et al. 1999). Therefore, the use of more than one 2C technique and inclusion of one 3C method aimed to improve reliability of the findings.

5.1. Simple measurements – anthropometry

Anthropometry involves direct measurements of body dimensions such as weight, height, skinfold thickness and waist, hip and thigh circumferences.

5.1.1. Body mass index

Body mass index (BMI) is the ratio of weight to height squared (calculated as $\text{weight}/\text{height}^2$). Paradoxically, though not a direct measure of body fatness, it is the most widely applied approach used to index this outcome. Strictly speaking, it provides a measure of relative weight, often expressed as SDS to account for the effects of age and sex (Cole, Freeman, and Preece 1995). BMI values among adults correlate with percentage of body fat (Gallagher et al. 1996; Deurenberg, Yap, and van Staveren 1998; Jackson et al. 2002), however this is due to a wide range of both measures, and at any given BMI, fat percentage can vary significantly. In children the associations have been even more variable and rather weak (Daniels, Khoury, and Morrison 1997; Schaefer et al. 1998). This can be the result of the unsystematic changes occurring in the levels of fat mass and fat-free mass during growth (Freedman et al. 2005). Most importantly, BMI cannot distinguish fat and lean mass (Weber et al. 2014). This is especially significant in childhood, during which the contributions of body fat and lean mass to body weight depend on sex, age, ethnic background (Wells 2000; Maynard et al. 2001; Weber et al. 2013). Therefore children's body

composition can vary widely at any given BMI (Borga et al. 2018) and optimally this measure should be accompanied by other techniques in order to distinguish the FM and FFM.

5.1.2. Skinfolds

Measuring skinfold thicknesses is one of the oldest approaches for predicting body fat content. It is a quick, inexpensive, non-invasive and simple method to assess the thickness of subcutaneous fat at various body sites (Tanner and Whitehouse 1975). Usually, intra-observer and inter-observer error are lower than the between-subject variability (Wells and Fewtrell 2006). This approach can be used to rank the individuals in terms of their peripheral fat content. Raw skinfold values can be converted into standard deviation scores to allow for comparison between different groups of interest. Skinfolds, however, do not convey information on lean mass. For assessment of total fat mass, predictive equations are unsuitable, as they can confound accurate raw values with predictive error (standard error of the estimate). Moreover, the theoretical assumptions through which these values are converted into final body composition data may often only be valid in the populations from which they were derived (Wells and Fewtrell 2006).

5.1.3. Waist circumference

Waist circumference (WC) is another simple, inexpensive and non-invasive predictive measure of central body fat. It is regarded as the best anthropometric obesity index (Bosy-Westphal et al. 2010). It estimates central (visceral) fatness, relevant in CVD risk assessment. It has a low inter-observer and intra-observer error, and when adjusted for clothing, good accuracy (McCarthy,

Jarrett, and Crawley 2001). Waist circumference has been found to be more reproducible than skinfold measurements (Maffeis et al. 2001). Measures of WC in children are more complicated to interpret than in adults, because waist circumference changes with growth and development and it does comprise both fat and lean tissue element. However, they do correlate well with fat deposits measured by abdominal computed tomography (Spolidoro et al. 2013), and MRI (Bosy-Westphal et al. 2010), although more with subcutaneous fat than with visceral fat.

5.1.4. Hip and thigh circumference

Hip and thigh circumference provide measures of peripheral gluteofemoral fat. In adults, this fat depot, unlike abdominal fat, is associated with more favourable glucose and insulin levels (in men) and lipid levels (in both sexes) (Snijder et al. 2005). Larger hip and thigh circumferences confer lower risk of type 2 diabetes, independently of waist circumference, BMI, age (Snijder et al. 2003). Although data in children are lacking, it is likely that these relationships will be similar.

5.2. Anthropometry measurements used in this study

Weight and height; mid-thigh, waist and hip girths; biceps, triceps, subscapular and suprailiac skinfolds, were all measured by two trained raters according to the standard operating procedures of UCL Great Ormond Street Institute of Child Health. The digital scales (Seca 86l) were regularly calibrated. Height was measured with a portable stadiometer (Seca 213), skinfolds with callipers (Harpenden), and girths with a non-stretchable tape.

Anthropometry is a simple technique but is limited in that it tends to provide information about fat mass but not lean mass, crucial for this study. Therefore, additional measurements were undertaken to estimate FFM.

5.3. Methods distinguishing fat and fat free mass

Two-component methods divide body into fat mass (FM) and fat free mass (FFM), DXA additionally assesses osseous tissues. By addressing these components of weight, prediction of estimates from regional proxies is not necessary. However, the theoretical assumption of constancy of the composition of FFM still remains. Between birth and adulthood, proportions of water, protein, and mineral in FFM, change with age and pubertal status. These changes are addressed by assuming constant lean mass characteristics for a given age and sex. Such assumptions may be problematic in various disease states; however, they are largely accurate for healthy subjects (Wells and Fewtrell 2006).

5.3.1. Dual energy x ray absorptiometry

Dual energy x ray absorptiometry was originally developed as a method for bone mass composition assessment. Bone mass is calculated from low-dose X-rays of two different energies that are sent by the machine and absorbed differently by bones and soft tissue. What is not absorbed is detected on the other side of the body. The higher the mineral content of bones, the more energy is absorbed, and the less energy detected on the other side of the body. DXA is considered a gold standard in bone mineral density assessment (Webber 2012). It has been shown to be both accurate and precise when used

for this purpose. Additionally, the different energy levels allow a separate estimation for bone and soft tissue absorption (Berger 2002). Therefore, apart from bone density, values for fat and fat free mass can be calculated based on specific algorithms. The advantage of DXA body composition assessment is its good reproducibility (coefficient of variation for body fat is in the range 1–2%, lean tissue in the range of 0.5–2%) (Borga et al. 2018). Due to its high availability, acceptability by children, quick duration of the measurement and ability to measure regional fat and FFM, DXA is widely used to measure body composition in paediatric population (Albanese, Diessel, and Genant 2003; Verduin et al. 2016; Karlsson et al. 2013). However, caution is needed in interpreting body composition data, as it gives inaccurate, consistently biased results in healthy persons and patients when compared with the gold-standard reference, the 4-C model (Wong et al. 2002; J. Williams et al. 2006). The bias results from the fact that only in pixels that do not contain bone can soft tissue composition (fat versus lean) be assessed. In the trunk, the bones of the pelvis, spine and ribs obscure substantial numbers of pixels and therefore soft tissue composition can only be estimated, but not measured (Wells 2009). The bias varies according to subject, age, sex, instrumentation, disease state (Williams et al. 2006) and therefore cannot be adjusted by a single correction factor. Therefore, body composition assessment by DXA should not be used as a reference method (Wells and Fewtrell 2006). Some researchers call it a 2-C technique (Wells and Fewtrell 2006), rather than 3-C technique, as it separates bone and non-bone tissue well, but its differentiation of FFM versus fat is an estimate (incorporating assumptions), not a measurement. This method exposes the patient and operator to ionizing radiation, but the dose is very small. The effective radiation dose from a single whole body DXA (< 10 micro

Sieverts) is similar to the normal background radiation received over one day at sea level (Shepherd et al. 2017).

5.3.2. Deuterium dilution

The deuterium dilution method, otherwise called hydrometry, is based on the principle that water is present in all body compartments apart from fat. Water is only found in fat free tissues in humans. This method enables the estimation of FFM by measuring total body water (TBW) and adjusting for hydration. Hydrometry enables measurement of TBW with an error of < 1% when correctly applied (NIHR n.d.). For any age and sex, lean mass has an approximately constant water content (Wells et al. 2010). Between-individual variability in children is low (Wells et al. 1999) and published reference values exist for different ages (Wells et al. 2010). Hence fat-free mass can be derived from TBW, and fat mass then calculated from the difference between fat-free mass and body weight. The method involves the oral administration of deuterium oxide, a naturally occurring, non-toxic isotope of water present in all humans. The technique presents no practical difficulties. From simple dilution principles, TBW can be calculated by the change in isotope enrichment following the drink. The method is considered acceptable by the general population, and has been applied in epidemiological research (Wells et al. 2005; Liu et al. 2011). The limitations of this method include the cost of the equipment involved in the sample analysis and the assumption that FFM has constant hydration values for each sex at any given age.

5.4. Total Body Water and DXA measurements in this study

Saliva samples were collected prior to a drink containing 0.05 g of deuterium oxide per kg body weight using cotton wool swabs. After four hours a second saliva sample was collected. The concentration of deuterium was measured by isotope-ratio mass-spectrometry using facilities (Gasbench-Delta XP system, ThermoFisher) at UCL Great Ormond Street Institute of Child Health. Fat-free mass was calculated from TBW using published hydration coefficients and fat mass as the difference between body weight and lean mass (Wells et al. 2012).

DXA measurement was conducted by an experienced technician on a Lunar Prodigy Advanced machine. The subject was asked to lie on a machine bed and wore light indoor clothing. Calibration of the densitometer, a quality control procedure, was performed daily. Additionally, an anthropometric spine phantom was scanned at least twice weekly. The technician was blind to participants' dietary exposure. Bone mineral content values for the body minus the head (TBLH BMC) and the L2-L4 region (L2-L4 BMC) were extracted along with the corresponding bone areas in order to correct results for bone size in regression analysis. As an additional method correcting the BMC results for bone size, bone mineral apparent density was calculated using the Carter method, which adjusts BMC for calculated bone volume rather than bone area, utilizing data for age, sex, BMC and bone area for L2-L4 (Carter, Bouxsein, and Marcus 1992). UK reference data to obtain BMAD z-scores (Crabtree and et al. 2004) and DXA fat and lean mass z-scores were used (Wells et al. 2012).

6. Outcome assessment – blood markers or cardiovascular risk and nutrition status

Fasting blood (15 ml) was drawn between 8 and 10am. Total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol (VLDL-C), and triglycerides were analysed on an A15 Biochemistry Analyser (Biosystems) using agarose gel electrophoresis, a method commonly applied in the paediatric population (Kuromori et al. 2006; Huffman et al. 2013; Magnussen et al. 2008). The complete blood count was determined on a Coulter LH 750 analyser, by the impedance method. This is a traditional method for counting cells, also known as the Coulter Principle, and is used in almost every haematology analyser (Scoffin 2014).

Fasting glucose was analysed on an A15 Biochemistry Analyser by an enzymatic spectrophotometric method, widely used in clinical diagnostic laboratories (Buzanovskii 2015). Plasma vitamin B12 and homocysteine were determined by Chemiluminescent Microparticle Immunoassay (CMIA) using commercial kits on an Architect i1000SR Analyzer (Abbott). This technology is approved by the National Institute of Health and Care Excellence for diagnosing vitamin B12 deficiency (NICE 2015). Insulin was determined by Immunoradiometric Assay (IRMA) with KIP1251 kit, (DiaSource). IGF-1 was determined by Radioimmunoassay (RIA), with KIP1589 kit, (DiaSource), using the Automatic Gamma counter1470 Wizard (Perkin Elmer). Immunoassays are the standard method used in insulin and IGF-1 assessment (Roberts 2012; Ketha and Singh 2015). IGFBP-3 was determined by sandwich enzyme-linked immunosorbent assay (ELISA) method (E03A kit, BioVendor) on an ELISA Plate Reader (PowerWave XS Bio-TEK). Sandwich ELISA method is

commonly applied in measurement of human IGFBP (R&D Systems n.d.; ThermoFisher n.d.). The IGF-1/IGFBP-3 molar ratio was calculated according to the formula: 1 ng/mL IGF-1 = 0.130 nmol IGF-1 and 1 ng/mL IGFBP-3 = 0.036 nmol IGFBP-3 (Naspi et al. 2014). 25-hydroxyvitamin D was measured on the IDS iSYS Analyser, by chemiluminescent immunoassay (CLIA) method, routinely used for this purpose (Gallelli et al. 2019). Ferritin was ascertained by immunochemiluminescence, and hs-CRP by immunoturbidimetry on a Cobas 600 analyser. Both methods are commonly used for these purposes (Maldonado-Araque et al. 2018; Escobar-Morreale et al. 2005; Rothkrantz-Kos et al. 2002). Hs-CRP and ferritin were analysed from frozen 3 ml samples remaining 3 years after the original data collection started. Both ferritin and hs-CRP are considered stable after being stored for, accordingly, 3–5 years and up to 11 years, at temperature of – 80°C (Sacri et al. 2017; Doumatey et al. 2014).

7. Outcome assessment – insulin resistance

Insulin resistance cannot be measured directly. It can be clinically suspected by assessing the units of insulin needed to achieve normoglycaemia (in diabetes), or by measuring fasting plasma insulin and interpreting it in conjunction with fasting glucose levels. In the latter clinical scenario, one needs to take into account a considerable overlap between fasting insulin levels between healthy and diabetic subjects (Wallace and Matthews 2002). Quantitative estimates of insulin resistance assessment are used in research studies and can be categorised into those that estimate basal insulin resistance (Homeostatic Assessment Model, HOMA-IR) and those that assess stimulated IR (Continuous Infusion of Glucose

with Model Assessment (CIGMA), Frequently Sampled Intravenous Glucose Tolerance Tests (FSIGTT), or Hyperinsulinaemic Euglycaemic Clamp (HEC)) (Borai et al. 2011). For the purpose of the study of healthy children, the only feasible option is HOMA-IR. In contrast to other methods, HOMA-IR is simple, non-invasive, i.e. does not involve application of intravenous glucose or insulin and is easy to compute, as the equation requires only fasting glucose and insulin values. At the same time, its estimates correlate well with the gold standard HEC method of IR assessment in non-diabetic patients (Matthews et al. 1985). HOMA-IR allows quantification of insulin sensitivity and β -cell function in percentage values. For optimal results, the calculation should include the mean of three fasting insulin and glucose results at 5-min intervals (0, 5, and 10-min samples), because insulin secretion is pulsatile (Wallace and Matthews 2002). However, single basal samples are generally used for epidemiological studies. HOMA-IR is calculated as $\text{fasting insulin (microU/L)} \times \text{fasting glucose (nmol/L)} / 22.5$ (Matthews et al. 1985).

8. Outcome assessment – carotid intima media thickness

High-resolution vascular ultrasound is a reliable method to assess increased carotid intima media thickness (cIMT) (Ludwig et al. 2003; Doyon, Kracht, Bayazit, Deveci, Duzova, Krmar, Litwin, Niemirska, Oguz, Schmidt, Sözeri, Querfeld, Melk, Schaefer, Wühl, and 4C Study Consortium 2013). The ultrasound measurement was performed by one experienced examiner blinded to the dietary exposure using a high-resolution ultrasound Hitachi AlokaProsound Alpha 6 and a 5.5 to 12.5-MHz probe. cIMT was measured bilaterally on the common carotid arteries (CCA) using a linear array transducer

set to 12.5–13 MHz according to the methodology described by Jourdan et al. (2005). The child was in a supine position, following at least 10 min rest. The CCAs were assessed 1–2 cm proximal of the bifurcation over a range of 1 cm of the posterior wall. cIMT was defined as the distance between the leading edges of the lumen-intima interface and the media-adventitia interface of the far wall (Ludwig et al. 2003) and was measured manually with the caliper method.

9. Statistical analyses and the choice of confounders

9.1. Statistical analyses

To describe the background characteristics of the diet groups, means or medians and SDs were calculated and to test the null hypothesis of no difference between the groups, chi-square, ANOVA or Kruskal-Wallis tests, were applied. For anthropometric outcomes ascertained by two raters, inter-rater reliability (interclass correlation coefficient) was computed and differences between raters' means were tested using paired t-tests. To compare differences in the main outcomes across diet groups, linear regression models were used, with vegetarians or vegans categorised using dummy variables in order to compare them to the reference group of omnivores. Cluster-robust standard errors were used to calculate 95% confidence intervals (CIs) to account for clustering of siblings (Rogers 1994). Outcomes that were not symmetrically distributed (HOMA-IR, VLDL cholesterol, triglycerides, hs-CRP, TBLH BMC, L2-L4 BMC, ferritin and homocysteine) were successfully natural log-transformed and differences between diet groups were expressed as percentages (Cole and Altman 2017). Two physiologically implausible values (insulin: 29.2 μ UI/mL; hs-CRP: 15.79 mg/dL) were excluded. The lowest

detectable level of two variables, vitamin B12 and 25 (OH) D, that had values < 93 pg/mL and < 7 ng/mL respectively, were divided in two, to address truncation due to limits of detection of the instrument.

Nutrient intakes, one of the main correlates of the exposure in this study, of the dietary groups were expressed as medians. As mentioned in chapter III, section 2.2, the distributions of nutrient intakes have a right-skewedness. Therefore, medians are better measures of central tendency with data of this kind. When crude medians are compared across groups, confounding can be an issue (McGreevy et al. 2009), so adjusted medians were chosen in an attempt to represent the casual effects of vegetarian or vegan diets on nutrient intakes (the adjustment sets are discussed in the next section). Hence median regression was used, with vegetarians or vegans categorised using dummy variables, in order to compare their nutrient intakes to the reference group of omnivores. The same approach was used to account for clustering of siblings, as in the case of linear regression comparing differences in the health outcomes. However, unlike in the case of the rest of the outcomes where crude and adjusted mean differences were calculated, marginal predictions of median nutrient intakes were obtained. I considered this way of reporting of nutrient intakes to have more informative value, as it would allow the comparison with other studies and international nutrient recommendations.

The MSM method, described in chapter III, section 2.2.3 above, was used to obtain usual dietary intakes. Usual intakes were used to calculate the median nutrient intakes and percentages of inadequate nutrient intakes according to the (EAR) cut-point method using the following equation: number of children with intakes below EAR/total number of children * 100% (US Institute of Medicine

2000b). The EARs for iron and zinc for both vegetarians and vegans were increased 1.8 times, to account for lower bioavailability of these minerals from plant -based diets (US Institute of Medicine 2001).

Directed acyclic graphs (DAGs) (explained below) were used to state my assumptions about the inter-relationships of numerous variables, including background characteristics of dietary groups, associated with the exposure and each set of outcomes (namely, anthropometry and body composition; bone; CVD risk; iron and vitamin B12; vitamin 25 (OH) D) and nutritional intake) and exposure correlates. DAGs helped me identify a minimum set of confounders to control for (Williams et al. 2018). Linear and median regression models were then fitted for each set of outcomes on diet group that controlled for the relevant (often different) potential confounders. The simplest models included diet group (the exposure) and – if relevant for the outcome – age and sex (models 1). These are presented to appreciate the effect of confounding present in the data. A more complex model (models 2) included further confounders identified by the relevant DAG. Additional models were fitted for some outcomes where mediators (i.e. variables assumed to be on the causal pathway from exposure to outcome, explained below) were also controlled for to examine possible pathways of association, assuming that no additional confounders may be at play (models 3). Confounders which had biologically plausible non-linear relationships with the outcomes (birth weight, gestational age, maternal pre-pregnancy BMI) were categorised into fifths and used in the analysis as categorical variables. In the analyses of serum parameters of vitamin B12 and 25 (OH) D status, dietary groups were further separated into whether or not the child took vitamin supplementation or used fortification. Seasonality in

concentrations of vitamin 25 (OH) D was adjusted for by including sine and cosine functions of the day of blood draw in models with this outcome (Fanidi et al. 2016; Degerud et al. 2016).

Multiple imputation using multivariate normal regression (Lee and Carlin 2010) was used to deal with missing values that affected some of the outcomes used as explanatory variables (fat and lean mass index), and explanatory variables (birth weight, gestational age, maternal pre-pregnancy BMI, average CPM, paternal education and height, religion), under the assumption of missing at random (Little and Rubin 2002). For all other outcomes that were affected by missingness only marginally ($\leq 2.1\%$), records with missing values were excluded. Continuous variables with skewness parameter ≥ 1 were natural log-transformed prior to multiple imputation in order to satisfy the assumption of multivariate normal distribution. Variables used in the analysis as fifths were imputed as continuous variables, and then categorised into fifths in each imputed dataset.

Separate to the above, in secondary analyses ordinal logistic regression was used to compute marginal predictions of the prevalence of several categories of inadequate status of vitamin B12, iron and cholesterol in the three diet groups. Pairwise comparisons of the marginal predictions were used. The ordinal logistic models included the indicators of diet group, and confounders identified by the respective DAGs for the corresponding continuous outcomes. Probable and possible vitamin B12 deficiency were defined as $<200\text{pg/mL}$ and 201 to 350pg/mL , respectively (Devalia, Hamilton, and Molloy 2014). Iron deficiency anaemia was defined, following WHO (WHO 2018a), as mild (Hb 11.0 – 11.4 g/dL), moderate (haemoglobin (Hb) 8.00 – 10.9 g/dL) or severe (Hb <8 g/dL).

Paediatric LDL-C values were classified, following the Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents (Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents and National Heart, Lung, and Blood Institute 2011), as high (≥ 130 mg/dL), borderline (110–129 mg/dL) or acceptable (< 110 mg/dL); and HDL-C as low (< 40 mg/dL), borderline (40–45 mg/dL) or acceptable (> 45 mg/dL). The results of complete case (CC) and multiple imputation (MI) analyses were compared. All statistical analyses were performed in Stata release 13.1 (Stata-Corp, College Station, Texas, USA).

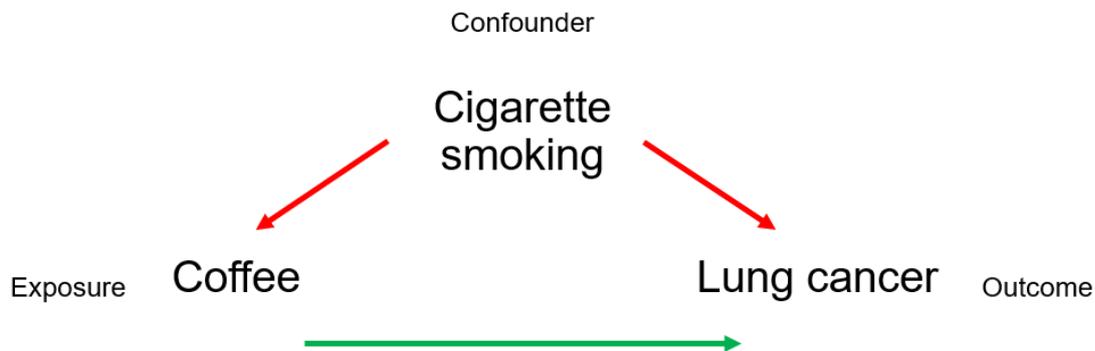
This is the first investigation of its kind and it has an exploratory nature. Hence, corrections for multiple testing were not carried out. Another reason is that this study aimed to assess the safety of plant-based diets in children, which was more important than detecting differences in their CVD benefits, and correction for multiple testing could have obscured the evidence of harm. However, the percentage of false positive results is likely to be lower than that expected from the number of tests in this study, as several health outcomes were tested with more than one method, and in those cases, are affected by a single biological relationship. Additionally, in the analysis of median nutrient intakes, the main correlate of the exposure, pairwise comparisons were undertaken without correction for multiple testing. Nutrient intake profiles of children aimed to help understand causes of differences of health outcomes between diet groups and were not treated as a main outcome of interest. This rationale should be taken into account when interpreting the results.

9.2. Directed Acyclic Graphs, confounding and mediators

The identification of confounders and mediators for each outcome was carried out with the Directed Acyclic Graphs (DAG) method, put forward by the most recent theoretical and methodological developments in casual inference (Van der Weele 2019). DAGs provide a simple graphical way of displaying exposure-outcome relationships and thus identify confounders and other potential sources of bias (Williams et al. 2018). Bias is a systematic, erroneous, incorrect interpretation of the exposure-outcome relationship. As opposed to random error, it cannot be eliminated by increasing sample size or replicating the study. Bias may prevent researchers from finding a true effect or it may lead to an underestimation or overestimation of the true association between exposure and an outcome (Skelly, Dettori, and Brodt 2012). Confounding is one type of bias. It is also called “mixing of effects” (Weiss 2006), where the effects of the exposure on a given outcome are mixed with some other effect that is associated both with the exposure and outcome. This results in various distortions of the true relationship. Confounding may mask a true association, under- or overestimate it, or falsely demonstrate one that does not exist. The existence of confounding makes it difficult to establish a true casual effect of the exposure on the outcome, as confounders compete with the exposure in explaining variability in the outcome. Therefore, the effect of confounding needs to be addressed. One of the ways to achieve this is to identify confounding and adjust for it in the analysis. By displaying assumptions about the relationship between variables, DAGs are a helpful tool to recognise confounders even in situations where the outcome is influenced by numerous other variables other than the exposure. They depict variables as points or edges connected by arrows or directed paths, in logical, causal sequences. An arrow from edge A

points to some direction (e.g. B), meaning that A causes B. There is no path that can form a closed loop, i.e. a variable cannot cause itself (therefore the graphs are called acyclic). In Figure 8, an arrow from smoking points to coffee drinking, meaning that both habits are associated with each other. This relationship can cause a spurious association between coffee drinking and lung cancer. The presence of a common cause in a DAG is correspondent to the presence of confounding. The DAG in Figure 8 indicates two paths from coffee to lung cancer. One path leads directly from coffee to cancer, representing the effect of coffee on cancer. There is, however, another path from coffee to cancer, via their common cause, smoking. In DAG terminology, this path is called a *backdoor path* because it can lead from coffee via smoking to lung cancer and starts with an arrowhead towards the exposure. The presence of a common cause or backdoor path in a DAG identifies the presence of confounding. Conditioning on the confounder (graphically represented in a DAG by putting a circle around it) blocks the backdoor path and thus eliminates confounding (Suttorp et al. 2015).

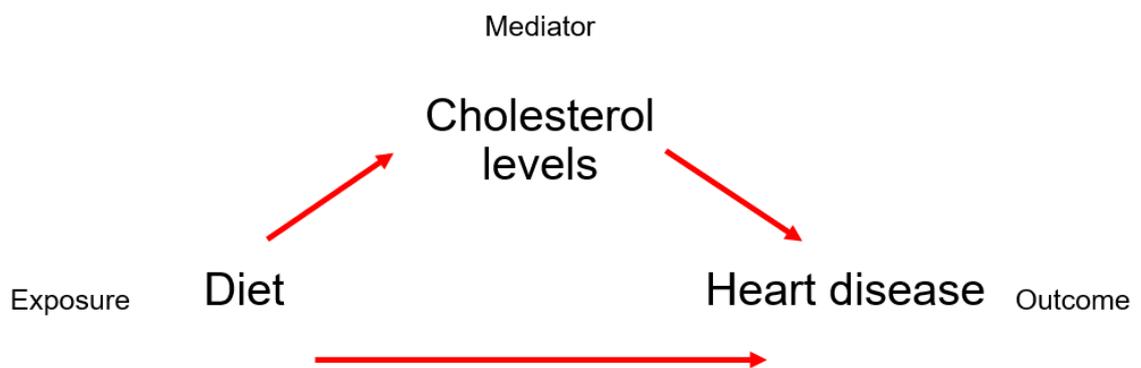
Figure 8 shows a simple DAG depicting basic confounding concept of the relationship between coffee drinking and lung cancer that is confounded by cigarette smoking. Smoking is casually associated both with coffee drinking and lung cancer (red lines) and if, not accounted for, may cause a spurious association (green line) between coffee drinking and lung cancer.



Not every variable that is associated with both the exposure and the outcome is a confounder. Such a variable can instead be a mediator. This is another concept that can be well displayed with the use of DAGs. Apart from being associated with both the independent and dependent variables, a mediator is part of the causal chain between the independent and dependent variables (Williams et al. 2018). Adjusting for mediators is considered an epidemiological error, or an over adjustment, as it eliminates one of the causal paths between exposure and outcome and distorts the overall relationship between them. One of the commonly quoted examples is the relationship between diet and heart disease, partly mediated by cholesterol levels. If we adjust the analysis for cholesterol levels, we remove the part of the association between diet mediated through cholesterol levels which attenuates the true causal effect of the diet, or in some cases may even reverse it, leading to counterintuitive results (Williams et al. 2018; Schisterman, Cole, and Platt 2009).

In Figure 9, the DAG informs us that diet affects both cholesterol levels and heart disease through independent pathways, and that cholesterol levels are also associated with heart disease. This means that part of the effect of diet on heart disease is explained by a dietary influence on blood lipids. There is no *backdoor path* here, as the arrowhead does not point towards the exposure (Suttorp et al. 2015).

Figure 9 shows a DAG depicting mediation concept. Diet affects heart disease risk partly through its effect on cholesterol levels. The path from the diet to heart disease via mediator (cholesterol) is not a backdoor path, because it does not start with an arrowhead towards the exposure.

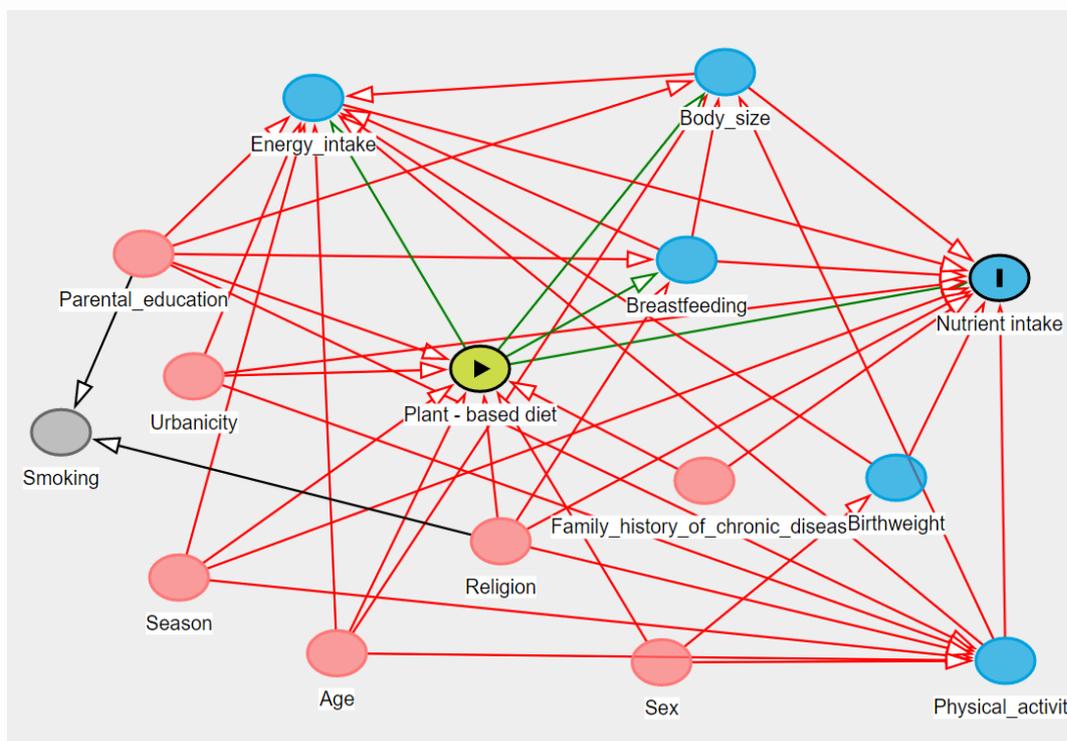


9.3. Confounders and mediators in this study

In order to identify confounders and mediators for this study, I used DAGitty (Textor et al. 2017), a popular web application for drawing and analysing DAGs. For each outcome I created a separate DAG including all the measured background variables with the potential to confound and mediate the association between the dietary group and that particular outcome. Based on this information, DAGitty automatically created two exposure-outcome adjustment sets, one group for the total effect (without adjusting for mediators)

and one for the direct effect (eliminating the casual pathway created by a mediator), in order to assess potential mechanisms of the exposure-outcome association, based on the assumption that no other confounders are at play (Textor 2015). Below is the example of DAG drawn for the set of nutrition intake, the main correlate of the exposure (Figure 10).

Figure 10 shows a DAG created by DAG-itty software illustrating relationships between the exposure (vegan or vegetarian diet) and outcome (nutrient intake) and all other measured potential confounding or mediating variables (www.dagitty.net).



Based on the DAG for nutrient intakes, the adjustment set for total effect was age, sex, religion, urbanicity, seasonality, family history of obesity, parental education. The suggested variables in the direct effect set were age, sex, religion, urbanicity, seasonality, family history of obesity, parental education, breastfeeding, body composition, birthweight, physical activity and caloric intake. The adjustment sets for all the outcomes used in regression models are displayed in Table 8.

Table 8. Adjustment sets for regression models estimating the difference in vegetarian or vegan children in relation to omnivore reference group for different outcomes, including the correlates of exposure. Adjustment sets for total effect were used in model 2, for direct effect in model 3 of the regression.

Outcome	Adjustment set for total effect	Adjustment sets for direct effect
Anthropometry and body composition	age, sex, maternal height, paternal height, birthweight, gestational age, maternal pre-pregnancy BMI, physical activity, breastfeeding duration, maternal education, paternal education, urbanicity	-
Cardiovascular risk factors	age, sex, birthweight quintile, gestational age quintile, maternal pre-pregnancy BMI quintile, breastfeeding at 6, 6–12 and over 12 months, maternal education, paternal education, religion, urban	age, sex, birthweight quintile, gestational age quintile, maternal pre-pregnancy BMI quintile, breastfeeding at 6, 6–12 and over 12 months, maternal education, paternal education, religion, urban, height z-score (UK), fat mass z-score (DXA), lean mass z-score (DXA).
Bone mineral content	age, sex, maternal education, religion, urban	age, sex, maternal education, religion, urban, height z-score (UK), weight z-score (UK), bone area
Indicators of iron status	age, sex, maternal education, urban, maternal smoking	-
Indicators of B12 status	maternal education, religion	-
Vitamin 25 (OH) D	age, sex, season, maternal education	-
Nutrient intake	age, sex, family history of chronic disease, parental education, urbanicity, religion, season	age, sex, family history of chronic disease, parental education, urbanicity, religion, season, energy intake, body size (i.e. body fatness), breastfeeding, birthweight and physical activity

10. Summary of the methods used in this study

The methods used in this study to measure exposure and outcomes are summarised in table 9.

Table 9. Methods used to measure background characteristics, exposures and outcomes

Type of variable	Method	Comments
Background characteristics		
Perinatal, socioeconomic and family characteristics	Questionnaires	
Dietary exposure correlates		
Time on meatless diet	Screening questionnaire	
Supplementation and fortification practices	Food record	
The prevalence of inadequate nutrient intake	The EAR cut point method	Based on usual dietary intakes calculated from observed intakes collected with the food record and adjusted with the MSM method.
Medians of usual intakes of nutrients in dietary groups	Food record data adjusted into usual intakes with the MSM method	
Secondary exposure		
Physical Activity	Accelerometry	
Health outcomes		
Body composition	Anthropometry (weight, height, skinfolds, girths) DXA (fat and lean mass, BMC) deuterium dilution (fat-free mass)	Isotopic analyses conducted by researcher blinded to dietary exposure
Fasting glucose	Enzymatic spectrophotometry	
Fasting insulin	Immunoradiometric Assay (IRMA)	
Insulin resistance	HOMA-IR	
Serum lipid levels	Agarose gel electrophoresis	
hs-CRP	Immunoturbidimetry	
cIMT	High resolution vascular ultrasound Hitachi AlokaProsound Alpha 6	Conducted by an experienced examiner, blinded to the dietary exposure, according to the methodology described by Jourdan et al. 2005.

Type of variable	Method	Comments
IGF-1	Radioimmunoassay (RIA)	
IGFBP3	Sandwich enzyme-linked immunosorbent assay (ELISA)	
IGF-1/ IGFBP3 molar ratio	1 ng/mL IGF-1 =0.130 nmol IGF-1 and 1 ng/mL IGFBP-3 =0.036 nmol IGFBP-3.	
BMC	DXA	
RBC, Hgb, Ht	Impedance method	
Ferritin	Immunochemiluminescence	
Serum vitamin B12	Chemiluminescent Microparticle Immunoassay (CMIA)	
Homocysteine	Chemiluminescent Microparticle Immunoassay	
25 (OH) D	Chemiluminescent Immunoassay (CLIA)	

Abbreviations: hs-CRP, high sensitivity CRP; cIMT, carotid intima media thickness; IGF-1, insulin growth factor 1; IGFBP3, insulin growth factor binding protein 3; BMC, bone mineral content; RBC, red blood cells; Hgb, haemoglobin, Ht, haematocrit; 25 (OH) D, 25-Hydroxy Vitamin D.

VI. RESULTS

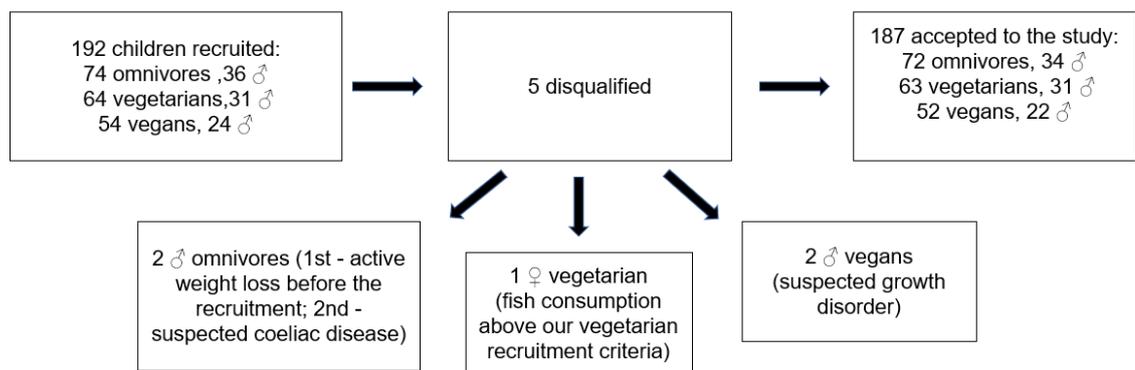
In this chapter I present the results of this study. First, I introduce the results of the recruitment and data collection, then I proceed to describe the baseline characteristics of the study participants placing them in the context of the Polish, or, in the absence of relevant data, international population. This section includes information on age, sex, perinatal and socioeconomic features of the study participants, as well as their family history of disease and physical activity levels. Next, I present the results of dietary analysis, correlates of the main exposure in this study. I start from supplemental practices and the lifetime exposure to vegetarian or vegan diet of the study participants. Following this, the percentages of children with inadequate nutrient intakes in dietary groups will be discussed and lastly, adjusted usual median intakes of nutrients will be presented and compared between the groups. The results of exposure assessments are discussed in the context of the existing evidence from other studies. The last section of this chapter shows health outcome results. Here, the results of anthropometry and body composition, bone status, CVD risk, and micronutrient status analyses will be presented.

The health outcome results are discussed in relation to the dietary findings as well as in the context of existing evidence. The discussion is continued, and in the case of outcomes like bone mineral content and stature, in more detail in the next chapter.

1. Recruitment results

I managed to recruit 192 children, 74 omnivores (36 boys), 64 vegetarians (31 boys) and 54 vegans (24 boys). Five of these children were disqualified for not fulfilling inclusion criteria. The reasons included suspected coeliac disease and recent active weight loss (2 omnivore boys), consuming fish > once a month (1 girl from the vegetarian group), and suspected growth disorder due to abnormal IGF-1 and growth hormone levels (2 vegan boys, adopted brothers). This left 187 children in the analysis, 72 omnivores (34 boys), 63 vegetarians (31 boys) and 52 vegans (22 boys). The recruitment took place between August 2014 and July 2016; the data collection took place between September 2014 and July 2016. The recruitment results are presented in Figure 11.

Figure 11. Study recruitment flow chart



2. Background characteristics

2.1. Baseline characteristics

Table 10a summarizes baseline characteristics by diet group. Mean age of the studied children did not differ between groups and was 7.6 years. The percentages of boys and girls in three dietary groups were not significantly different and amounted to 47.2% and 52.8% in the omnivore, 49.2% and 50.8%

in the vegetarian and 42.3% and 57.7% in the vegan group respectively. Although not statistically different to the other groups, the vegans diverted most from the almost equal distribution of sexes observed in the other two groups with notably more girls than boys.

Table 10a. Background characteristics by diet groups- baseline characteristics

	Omnivore (n = 72)	Vegetarian (n = 63)	Vegan (n = 52)	p value*
Age (years)				0.85
Mean (SD)	7.7 (1.7)	7.6 (1.6)	7.6 (1.8)	
Sex				0.75
Boys	34 (47.2%)	31 (49.2%)	22 (42.3%)	
Girls	38 (52.8%)	32 (50.8%)	30 (57.7%)	
*ANOVA (means) and chi-square test (percentages) were used to test the null hypothesis of no difference between the groups.				

2.2. Perinatal characteristics

Table 10b summarizes perinatal characteristics by diet group. There were no differences in gestational age between dietary groups. On average, children from all three dietary groups were born at term (39 weeks for omnivores, 39.2 weeks for vegetarians and 38.8 weeks for vegans). However, vegan children were lighter at birth with birthweight of 3233 g vs. 3414 g in omnivores and 3355 g in vegetarians. None of these values were, however, significantly different from each other. The average birthweight in Poland for male neonates born in 39th week of gestation is 3534 g (SD 409), for females – 3376 g (SD 387) (Pawlus et al. 2017). These figures come from data on 27000 neonates born between 2011 and 2016 in a single centre in Warsaw to mothers living in the Mazovia Province, located in central Poland. The values for omnivore, vegetarian and vegan boys in this study (not shown in the table) were: 3426 g

(SD 449), 3415 g (SD 581), 3498 g (SD 423); for omnivore, vegetarian and vegan girls: 3404 g (SD 466), 3270 g (SD 594), and 3041 g (SD 545), respectively. Thus, vegetarian and vegan boys had comparable birthweight values to both the general Polish population and the reference omnivore group, while vegetarian, and especially vegan girls had lower birthweights. The average height of mothers in omnivore, vegetarian and vegan group of 167.2 cm, 167.1 cm and 168.2 cm respectively, was above the Polish average of 165.1 cm (Kułaga et al. 2011). The paternal heights of 181 cm, 180 cm and 182 cm in omnivores, vegetarians and vegans respectively, were similarly not significantly different from each other, and above the Polish average of 178.7 cm (Kułaga et al. 2011). There were no significant differences between the heights of mothers and fathers between the girls and boys (data not shown in the table). Only 1 omnivore child (1.5%) came from a pregnancy where the mother had consumed a vegetarian diet. Nearly 52% of vegetarians, and 40% of vegans came from pregnancies where the mother had consumed a vegetarian or vegan diet. Among vegetarians, 14 (45%) boys vs. 17 (53%) girls came from 'vegetarian' or 'vegan' pregnancies. The figures for vegans were 7 (31%) boys and 13 (43%) girls (data not shown in the table).

The median duration of breastfeeding was highest in vegans (18 months) and lowest in omnivores (12 months). This difference was borderline statistically significant. The percentage of children breastfed until the 6th month was high in all groups – 85%, 86%, and 88% in omnivores, vegetarians and vegans respectively, and these figures did not significantly differ between the groups. Overall, breastfeeding duration in this study compares favourably to the data from two recent studies in Polish women, where 56.01% and 32.49%

(Romaszko et al. 2013) and 38% and 17% (Królak-Olejnik, Błasiak, and Szczygieł 2017) of women breastfed up to the 6th and 12th month, respectively.

Table 10b. Background characteristics by diet groups- perinatal characteristics

	Omnivore (n = 72)	Vegetarian (n = 63)	Vegan (n = 52)	p value*
Gestation age (weeks)				0.57
Mean (SD)	39.0 (1.5)	39.2 (1.9)	38.8 (1.9)	
Birth weight (g.)				0.18
Mean (SD)	3415 (455)	3355 (582)	3233 (545)	
Maternal height, self-reported (cm)				0.55
Mean (SD)	167.2 (6.2)	167.1 (6.0)	168.2 (6.4)	
Paternal height, self-reported (cm)				0.27
Mean (SD)	181.0 (7.1)	180.0 (6.1)	182.0 (7.3)	
Breastfeeding (months)				0.06
Median (Q1, Q3)	12.0 (8.0, 16.5)	13.0 (7.0, 18.0)	18.0 (9.0, 24.0)	
Breastfed until 6mths				0.83
Yes	61 (84.7%)	54 (85.7%)	46 (88.5%)	
Formula introduction timing				< 0.001
Never formula-fed	24 (33.8%)	28 (44.4%)	31 (60.8%)	
1–5 months	15 (21.1%)	21 (33.3%)	12 (23.5%)	
>= 6 months	32 (45.1%)	14 (22.2%)	8 (15.7%)	
Maternal pre-pregnancy BMI				0.16
Mean (SD)	22.5 (3.4)	21.2 (2.5)	21.9 (5.4)	
Maternal diet in pregnancy				< 0.001
Meat-eater	64 (97.0%)	18 (30.0%)	21 (42.0%)	
Vegetarian	1 (1.5%)	29 (48.3%)	15 (30.0%)	
Vegan	0 (0.0%)	2 (3.3%)	5 (10.0%)	
Fish-eater	1 (1.5%)	11 (18.3%)	9 (18.0%)	
*ANOVA (means), Kruskal-Wallis test (median) and chi-square test (percentages) were used to test the null hypothesis of no difference between the groups.				

Vegans were more likely than the other groups to have never been formula-fed (61%) in comparison to 34% omnivores and 44% vegetarians. In the study of 1679 mothers who gave birth to healthy infants at term (Królak-Olejnik, Błasiak, and Szczygieł 2017), more than half of the new-borns were supplementary bottle-fed, with formula, during hospitalisation at birth, and 28.9% of infants were exclusively breastfed by the 4th month, which means that the rest (~70%) were given formula. In another study by Bernatowicz-Łojko et al. 2012, 57% of infants at 4 months of age were exclusively breastfed, i.e. 43% were given formula at that life stage. The maternal pre-pregnancy BMIs were in the normal range, similar and amounted to 22.5 (SD 3.4); 21.2 (SD 2.5), 21.9 (5.4) in omnivores, vegetarians and vegans respectively. This again compares favourably to Poland's overweight and obesity prevalence, which in the population over > 20 years old for women is 54.7% and 26.7%, respectively (WHO 2013).

The finding of a tendency to lower birthweight in vegetarian and vegan children in comparison to omnivores is of interest. The evidence on the influence of vegetarian and vegan diets in pregnancy on infant birthweight is sparse, however preliminary data, along with studies on nutrient deficiencies in pregnancy, suggest an association between plant-based diets and birthweight. A systematic narrative review published in 2015 (Piccoli et al. 2015), identified 7 studies that reported birthweight from healthy 'vegan' or 'vegetarian' pregnancies. Five of them reported lower birthweight than controls, the other two higher birthweights. However, the data came from heterogenous ethnic populations and lacked homogenous control groups, therefore definite conclusions could not be derived. More recently, in four multi-ethnic birth cohorts in Canada, involving 3997 pairs of full-term infants and mothers in whom dietary patterns were assessed by FFQ,

among white Europeans a plant-based dietary pattern was negatively associated with birth weight and positively with increased risk of small for gestational age (SGA) (Zulyniak et al. 2017). “Nutrient dense” and “protein-rich” dietary patterns were positively associated with birthweight, whereas “vegetarian” patterns – negatively in a review published in 2014 (Kjøllestad and Holmboe-Ottesen 2014). Both iron and vitamin B12 deficiency, critical nutrients on plant-based diets, have been linked to low birth weight (Molloy et al. 2008; Haider et al. 2013). The reason for lower birthweights in vegetarian and vegan girls in relation to the omnivore group is difficult to explain, however it be attributed to the fact that more girls than boys came from vegetarian or vegan pregnancies. This seems more likely, given the fact that there were no differences in the average self-reported parental height between dietary groups.

2.3. Socioeconomic status characteristics

Table 10c summarizes socioeconomic characteristics by diet group. The majority of children were from towns and cities with more than 70% in each group, and this SES trait did not differ significantly between the groups, although slightly more vegans tended to live in villages. Vegans were more likely to have non-smoking parents (no vegan child had a parent that smoked), however the prevalence of smoking in the other dietary groups ($\leq 7\%$ in parents of omnivores and $< 13\%$ in parents of vegetarians) was below the standardized estimate of smoking prevalence in Poland that amounted to 27.9% of men and 18.9% for women in 2013 (WHO 2015). The majority of children in all dietary groups had mothers with higher education, however that number was significantly lower among vegans (81% in vegans vs 84% in vegetarians and 94% in omnivores). The percentages of fathers with higher education were

lower than those among mothers and amounted to 78% in omnivores, 66% in vegetarians and 73% in vegans. These differences were not significant. According to governmental, statistics, 32% young Polish adults received higher education in 2008, 10 years later it was 44% (OECD 2019).

Lastly, vegetarian and vegan children were more likely to have atheist parents (60% and 55% respectively vs. 12.5% in omnivores). In 2015 92.8% of the Polish population aged over 16 years declared that they belonged to the Roman Catholic Church (GUS 2015).

Table 10c. Background characteristics by diet groups- socioeconomic characteristics

	Omnivore (n = 72)	Vegetarian (n = 63)	Vegan (n = 52)	p value*
Urbanicity				0.69
City	55 (76.4%)	49 (77.8%)	37 (71.2%)	
Village	17 (23.6%)	14 (22.2%)	15 (28.8%)	
Maternal smoking				0.02
Yes	4 (5.6%)	8 (12.7%)	0 (0.0%)	
Paternal smoking				0.13
Yes	5 (7.0%)	5 (7.9%)	0 (0.0%)	
Maternal education				0.05
Secondary	4 (5.6%)	10 (15.9%)	10 (19.2%)	
Tertiary	68 (94.4%)	53 (84.1%)	42 (80.8%)	
Paternal education				0.33
Secondary	16 (22.2%)	20 (33.9%)	14 (26.9%)	
Tertiary	56 (77.8%)	39 (66.1%)	38 (73.1%)	
Religion				<0.001
None	9 (12.5%)	37 (59.7%)	28 (54.9%)	
Christian	63 (87.5%)	22 (35.5%)	12 (23.5%)	
Other	0 (0.0%)	3 (4.8%)	11 (21.6%)	
ANOVA (means) and chi-square test (percentages) were used to test the null hypothesis of no difference between the groups.				

2.4. Family history of disease

The percentages of families with history of hypertension was broadly similar across groups, ranging from 61% in vegetarians to 77.5% in omnivores. The dietary groups did not significantly differ in terms of their family history of diabetes type 2, which ranged from 25% in vegans to 32.4% in omnivores. However, vegans and vegetarians were more likely than omnivores to have a family history of coronary heart disease, the figures being 21% in vegans, 27% in vegetarians and 8% in omnivores (Table 10d). This difference was significant.

Table 10d. Background characteristics by diet groups- family history of disease

	Omnivore (n = 72)	Vegetarian (n = 63)	Vegan (n = 52)	p value*
Family history of hypertension				0.12
Yes	55 (77.5%)	36 (61.0%)	30 (66.7%)	
Family history of T2 diabetes				0.57
Yes	22 (32.4%)	14 (25.0%)	13 (25.0%)	
Family history of coronary heart disease				0.02
Yes	5 (7.7%)	16 (27.1%)	10 (20.8%)	
* Chi-square test was used to test the null hypothesis of no difference between the groups				

2.5. Physical activity

Table 10e. summarizes physical activity levels and intensities in children of this study according to their dietary group. Overall, study groups did not differ in relation to physical activity. All groups had similar average movement count per minute. The figures were 8.9 for omnivores, 9.2 for vegetarians and 9.8 for vegans. Additionally, the groups had similar average times spent in physical activities of different intensity. On average, the omnivores spent 358 minutes a day as sedentary, the figures for vegetarians and vegans were 332 and 335

minutes, respectively. The average times of daily light activity were 396 minutes for omnivores, 403 minutes for vegetarians and 402 minutes for vegans. Respective times for moderate activity were 33 minutes, 32 minutes and 35 minutes, and for vigorous – 9 minutes, 19 minutes and 11 minutes. None of these numbers differed significantly from the others.

Table 10e. Background characteristics by diet groups – physical activity

	Omnivore (n = 72)	Vegetarian (n = 63)	Vegan (n = 52)	p value*
Average movement count per minute				0.17
Mean (SD)	8.9 (2.4)	9.2 (2.2)	9.8 (2.6)	
Sedentary activity (min/day)				0.18
Mean (SD)	357.7 (81.7)	331.8 (76.0)	335.2 (85.6)	
Light activity (min./day)				0.84
Mean (SD)	396.4 (61.2)	403.5 (71.5)	401.6 (67.0)	
Moderate activity (min/day)				0.56
Mean (SD)	33.1 (16.4)	31.7 (13.9)	35.0 (14.7)	
Vigorous activity (min/day)				0.40
Mean (SD)	9.0 (8.1)	18.8 (69.7)	10.7 (7.5)	
MVPA of ≥ 60 min/day				0.49
Yes	10 (16%)	12 (23.5%)	11 (24%)	
*ANOVA (means) and chi-square test (percentages) were used to test the null hypothesis of no difference between the groups				

Abbreviations: MVPA, moderate and vigorous physical activity; min, minutes.

To my knowledge, there are no published data on physical activity intensities of healthy Polish preschool and elementary school children measured by accelerometry. Therefore, to compare these results to those of children living in the same European region and in the similar period, recent data from 215 normal weight pre-schoolers aged 6 to 7 years from Estonia, are cited. The

average times spent in sedentary, light, moderate and vigorous activity measured by Actigraph GT3X accelerometer for these children were: 409 min, 305 min, 48 min, 21min (Riso et al. 2019). However, these children were, on average, younger than this study's sample and therefore these comparisons should be interpreted with caution.

The World Health Organization recommends at least 60 minutes of moderate to vigorous physical activity a day for children (Guthold et al. 2019). According to a recently published report on global children's physical activity, the prevalence of insufficient physical activity among Polish children in 2016 was 74% in boys and 84% in girls (data from a school-based survey of at least 100 children 11–17 year old) (Guthold et al. 2019). Although these figures come from adolescents, they are presented for comparison, as data on children aged 5–10 years are lacking. The percentages in our study for boys were: 69% for omnivores, 74% for vegetarians and 72% for vegans; for girls: 97%, 79% and 79% (data not shown in the table). The difference in girls bordered on statistical significance.

Overall, when put in the context of the contemporary Polish society, the children from all dietary groups came mainly from high-educated and health conscious families and most of them lived in cities or towns. Vegans were more likely than the other groups to have been breastfed for longer and to have never been formula-fed, and to have non-smoking parents. Vegans and vegetarians were more likely than omnivores to have a family history of coronary heart disease, and to have atheist and less educated parents. The groups did not differ with regards to the remaining perinatal and socioeconomic characteristics or physical activity. The children in this study had similar prevalence of insufficient

physical activity compared to the national average and were comparable in their intensity of physical activity to children living in the same European region.

3. Missing data

Although great care was taken at the stage of data collection, some missing data occurred. Most missing values were of the background characteristics – confounders and mediators. The most affected were: physical activity – 21 (11.2%) in average movement per minute and 27 (14.4%) in PA intensities; gestational age – 11 (5.9%); birthweight – 9 (4.8%). All the others had complete datasets or were affected by missingness of ≤ 5 ($\leq 2.1\%$). The outcomes were less affected by missingness. Other than parameters for lean and fat mass calculated by deuterium dilution method, where 13 (6.9%) missing values occurred, all other outcomes had all data points or missing values of less than 5 ($\leq 2.1\%$). Table 11 summarizes the numbers of missing data for outcomes and independent variables. All the other variables had no missing data. The methods used to deal with missing data are discussed in chapter V, section 9.1.

Table 11. Number and percentage of missing data for outcomes and independent variables

Outcome	Missing (n)	Missing (%)	Independent variable	Missing (n)	Missing (%)
Lean mass by D ₂ O	13	6.9	Physical activity intensities	28	15%
Fat mass by D ₂ O	13	6.9	Average movement count	21	11.2
Biceps skinfold	4	2.1	Gestational age	11	5.9
Subscapular skinfold	4	2.1	Maternal diet in pregnancy	11	5.9
hs-CRP	4	2.1	Birthweight	9	4.8
cIMT	3	1.6	Father's height (SF)	8	4.3
25 (OH) D	3	1.6	Mother's height (SF)	4	2.1
Suprailiac skinfold	3	1.6	History of diabetes type 2	2	1.1
Homocysteine	2	1.1	Paternal education	2	1.1
Vitamin B12	2	1.1	Religion	2	1.1
Insulin	2	1.1	Paternal smoking	1	0.5
LDL-C	2	1.1	History of CHD	1	0.5
VLDL-C	2	1.1			
HDL-C	2	1.1			
TCH-C	2	1.1			
Triglycerides	2	1.1			
Thigh girth	2	1.1			
BMAD	2	1.1			
IGFBP-3	1	0.5			
IGF-1	1	0.5			
Fasting glucose	1	0.5			
RBC	1	0.5			
HGB	1	0.5			
HT	1	0.5			
MCV	1	0.5			
Triceps skinfold	1	0.5			
Ferritin	1	0.5			

Abbreviations: D₂O, deuterium dilution; hs-CRP, high sensitivity C-reactive protein; cIMT, carotid intima media thickness; SF, self-reported; 25 (OH) D, 25-hydroxy vitamin D; LDL-C, LDL cholesterol; VLDL-C, VLDL cholesterol; HDL-C, HDL cholesterol; TCH-C, total cholesterol; BMAD, bone apparent mineral density; IGFBP-3, insulin growth factor binding protein 3; IGF-1, insulin growth factor 1; RBC, red blood cells; HGB, haemoglobin; HT, haematocrit; MCV mean corpuscular volume.

4. Outcomes of the exposure assessment

This section outlines the results of the exposure assessment. It starts with presenting the average duration of meatless diet in dietary groups, then in presents the vitamin B12 and vitamin D3 supplementation and fortification practices of vegetarians and vegans. The last two sections are devoted to presenting the percentages of children with inadequate nutrient intakes and the medians of usual nutrient intakes in all dietary groups.

4.1. Lifetime exposure to vegetarian diet

The average time on a meat-free diet was close to the average age of the study participants. As presented in Table 12a, on average, vegetarians avoided meat for 5.9 years and vegans for 5.3 years. The average age for both dietary groups was 7.6. This means that the majority of the vegetarians and vegans had followed at least vegetarian diet for most of their lives. Other studies reported various lengths of adherence to meatless diet prior to the study commencement. The average span of duration of vegetarian diet in the study of 32 Slovak vegetarians (including 7 vegans) aged 11–15 years was 3.4 years (Krajcovicová-Kudlácková et al. 1997). Gorczyca et al. 2013 reported a median duration of adherence to vegetarian diet of 3.4 years among 22 Polish vegetarians aged from 2 to 18 years. The longest duration of adherence was

reported in the studies of Ambroszkiewicz et al from 2011, 2018 and 2019, where all the vegetarian subjects (average n=68; aged 2–10 years) reported following their diets from birth, as well as in The Farm Study of mostly vegan children (n=404), where 73% of participants with mean age of 6 years were vegan from birth (O’Connell et al. 1989).

Table 12a. Time on meatless diet

	Omnivore (n = 72)	Vegetarian (n = 63)	Vegan (n = 52)	p value*
Time on meatless diet (years of lifetime)				<0.001
Mean (SD)	0.1 (0.3)	5.9 (2.0)	5.3 (2.4)	
*ANOVA was used to test the null hypothesis of no difference between the groups.				

4.2. Supplementation and fortification practices

The supplementation and fortification practices of children in this study are presented in Table 12b. Around 45% of vegans supplemented their diet with B12 vitamins and 65.4% used B12 fortified products. However, 29% did not use any supplementation or fortification. The respective figures for vegetarians were 35%, 60% and 27%. Thus, nearly a third of children on diets without meat or fish, the main dietary sources of vitamin B12, did not provide this nutrient from other sources, as recommended by dietetic authorities (BDA 2017a; Melina, Craig, and Levin 2016). To my knowledge, this is the first study to estimate the differences in supplementation and fortification practices of vitamin B12 among contemporary vegan and vegetarian children. The only other available evidence in children comes from a study of 23 vegan subjects (Sanders 1988), where, as the author noted, ‘most parents were aware of the need to supplement the diet with vitamin B-12’, and from The Farm study of 404 vegetarian children (O’Connell et al. 1989), where 76% of children used supplements, however the

supplemented nutrients were not specified. There were no differences in the use of vitamin D3 supplements in dietary groups, the figures ranged from 33% in vegans to 37.5% in omnivores. In a study of 53 vegetarian children aged 5–10 years by Ambroszkiewicz et al. 2019, ~80% of omnivorous and vegetarian children took vitamin D3 supplements.

Table 12b. Supplementation and fortification practices

	Omnivore (n = 72)	Vegetarian (n = 63)	Vegan (n = 52)	p value*
Vit. B12 supplement use				<0.001
Yes	5 (6.9%)	22 (34.9%)	23 (44.2%)	
Vit. B12 fortified products use				<0.001
Yes	17 (23.6%)	38 (60.3%)	34 (65.4%)	
No Vit. B12 supplement and no B12 fortification use				<0.001
Yes	52 (72.2%)	17 (27%)	15 (29%)	
Vit. D supplement use				0.82
Yes	27 (37.5%)	21 (33.3%)	17 (32.7%)	
* Chi-square test was used to test the null hypothesis of no difference between the groups.				

4.3. Prevalence of inadequate nutrient intakes

This section discusses the prevalence of inadequate nutrient intakes in all three dietary groups according to the EAR cut point method. These figures are based on observed data provided in the food records, which were converted to usual intakes with the MSM method. They include intakes from diet, fortification and supplementation. The supplementation values come almost entirely from vitamin B12 supplements (in vegetarians and vegans), and vitamin D3 supplements, in all dietary groups. As explained in chapter III section 2.1, the prevalence of inadequate nutrient intakes can only be calculated for nutrients

with existing EARs. For reference purposes, the EARs established by the Polish National Food and Nutrition Institute are presented first in Table 12c.

Table 12c. Estimated average requirements of nutrients by the Polish National Food and Nutrition Institute

Nutrient	EAR children aged 4–6	EAR children aged 7–9	EAR boys/girls aged 10–12
Protein (g)	0.84 g/kg body weight,	0.84 g/kg body weight	0.84 g/kg body weight
Vitamin A (mcg RAE)	300	350	450/430
Vitamin B1 (mg)	0.5	0.7	0.9/0.8
Vitamin B2 (mg)	0.5	0.8	0.9/0.8
Vitamin B3 (mg)	6	9	9
Vitamin B6 (mg)	0.5	0.8	1.0
Vitamin B12 (mcg)	1.0	1.5	1.5
Folate (mcg)	160	250	250
Vitamin C (mg)	40	40	40
Calcium (mg)	800	800	1100
Phosphorus (mg)	410	500	1050
Magnesium (mg)	110	110	200
Iron (mg)	4	4	7
Iron vegetarians (mg)*	7.2	7.2	12.6
Zinc (mg)	4	4	7
Zinc vegetarians (mg) *	7.2	7.2	12.6
Copper (mg)	0.3	0.5	0.5
* Higher requirement recommended by the US Institute of Medicine due to lower bioavailability of this mineral from plant sources (US Institute of Medicine 2001).			

Abbreviations: g, grams; mg, milligrams; RAE, retinol activity equivalents

The percentages of children with inadequate nutrient intakes in dietary groups according to the EAR cut point method are presented in Table 12d. All dietary groups had adequate protein, vitamin B6 and copper intake. The intakes of vitamin B1, vitamin C, magnesium, were adequate in nearly all children, with the exception of vitamin B1 and magnesium which were inadequate among 3% of omnivores and vitamin C, which did not reach adequacy among 1% of omnivores. The prevalence of inadequacy of the rest of the nutrients with established EAR was higher in at least one dietary group. Vitamin A intake was inadequate among 3% of omnivores, 2% of vegetarians and 17 % vegans. The corresponding figures for vitamin B2 were: 0%, 2%, 6%; for vitamin B3: 4%, 27%, 8%; vitamin B12: 0%, 0%, 8%; folate: 38%, 8%, 4%; calcium: 90%, 92%, 94%; phosphorus: 8%, 5%, 10%; iron: 1%, 5%, 6%; and zinc: 4%, 29%, 8%.

This is the first study quantifying inadequate nutrient intakes in vegetarian and vegan children. It is also the first study in this group analysing nutrient intake inadequacy based on usual rather than observed intake patterns, which is more informative, as usual intakes are meant to represent long-term dietary habits. All the previous studies that calculated nutrient intakes compared them to the RDAs (Sanders 1988; Nathan, Hackett, and Kirby 1996a; Gorczyca et al. 2013), which, as described in chapter III, section 2.1, is likely to overestimate the true prevalence of inadequacy. This is because the RDAs establish nutrient intake recommendations that exceed the requirement of 97.5% of the population (US Institute of Medicine 2000b). Polish studies comparing omnivore children nutrient intakes to the EARs exist, however they use observed rather than usual dietary intakes. In a recent such study of 122 Polish omnivore children aged 4–6 years, randomly selected from two preschools in the north-western part of

Poland, nutrient intakes calculated on the basis of a 7-day weighed food record (Merkiel-Pawłowska and Chalcarz 2017) were compared to the Polish EARs. The results were broadly consistent with the nutrition outcomes of omnivores in this study. The percentages of inadequate intakes were: vitamin A (Retinal Activity Equivalents (RAE) mcg) – 0.8 %, folate – 44.3%, vitamin B3 – 5.7%, vitamin C – 12.3%, calcium – 85.2%, phosphorus – 0.8%, magnesium – 2.5%, iron – 2.5%, zinc – 6.6%. These children had adequate intakes of protein, B2, B6, B12 and copper.

Table 12d. The percentage of inadequate nutrient intakes in dietary groups according to the EAR cut point method

Nutrient	Omnivore (n)	Omnivore (%)	Vegetarian (n)	Vegetarian (%)	Vegan (n)	Vegan (%)
Protein	0	0	0	0	0	0
Vitamin A	2	3	1	2	9	17
Vitamin B1	2	3	0	0	0	0
Vitamin B2	0	0	1	2	3	6
Vitamin B3	3	4	17	27	4	8
Vitamin B6	0	0	0	0	0	0
Vitamin B12	0	0	0	0	4	8
Folate	27	38	5	8	2	4
Vitamin C	1	1	0	0	0	0
Calcium	65	90	58	92	49	94
Phosphorus	6	8	3	5	5	10
Magnesium	2	3	0	0	0	0
Iron	1	1	0	0	0	0
Iron vegetarians	1	1	3	5	3	6
Zinc	3	4	0	0	1	2
Zinc vegetarians	3	4	18	29	4	8
Copper	0	0	0	0	0	0

4.4. Median intakes of nutrients

Table 12e. presents adjusted medians of usual nutrient intakes and pairwise comparisons between the dietary groups. The table includes minimally adjusted (age and sex) Model 1 and multivariable-adjusted multiply imputed models (model 2 and 3). Model 1 is presented to aid evaluation of any confounding. model 2, additionally adjusted for presumed confounders (family history of obesity, religion, urbanicity, season and parental education), aims to represent the total casual effect (the limitations of cross-sectional study notwithstanding) of respective diet on nutrient intakes. Model 3, additionally to model 2, adjusts for presumed mediators of that effect (breastfeeding duration, body composition, birthweight, physical activity internal z-score, and usual caloric intake), thus eliminating the casual pathway created by a mediator (Textor 2015). Tables with complete case analyses are included in the supplementary material.

4.4.1. Median intakes of nutrients that were not affected by confounding or mediation

This section discusses usual median intakes of nutrients for which there was no evidence of confounding or mediation. This means their levels did not meaningfully change across models 1, 2, 3. Hence, only the numbers from fully adjusted model 2 are presented.

4.4.1.1. Nutrients with no significant difference of intakes between dietary groups

The median intake of energy (in kcal) did not significantly differ between dietary groups and was around 1600 kcal. The figures were 1608 kcal for omnivores,

1587 kcal for vegetarians and 1609 kcal for vegans. Other median nutrient intakes that did not differ significantly between the dietary groups were vitamin A, Omega 3 PUFA and phosphorus. The levels for vitamin A in the omnivore group were 618 mcg RAE, 583 mcg RAE in vegetarians and 535 mcg RAE in vegans. The levels for Omega 3 PUFA in omnivores were 1.67 g, 1.96 g in vegetarians and 2.26 g in vegans; for phosphorus they amounted to 936 mg for omnivores, 989 mg for vegetarians and 1042 mg for vegans.

4.4.1.2. Nutrients with significant difference in intakes between dietary groups

The following nutrient intakes were not meaningfully affected by confounding or mediation, but differed between vegetarians and omnivores or vegans and omnivores: protein, carbohydrates, sucrose, starch, fibre, total fat, saturated fat, MUFA, PUFA, cholesterol, vitamin B1, vitamin B2, vitamin B3, vitamin B6, folate, vitamin C, vitamin E calcium, iron, magnesium, copper, manganese, potassium, zinc, and Omega 6 PUFA.

In comparison to omnivores, vegetarians consumed significantly less: protein (46.5 g vs. 56.8 g), total fat (55.8 g vs. 65.1 g), saturated fat (18.3 g vs. 24.7 g), MUFA (19.8 g vs. 25.1 g), cholesterol (160 mg vs. 253 mg.), vitamin B3 (9.8 mg vs. 12.8 mg), and significantly more carbohydrates (245 g vs. 213 g), fibre (24.2 g vs. 16.5 g), total PUFA (11.7 g vs 9.0 g), vitamin B1 (1.24 mg vs. 0.95 mg), folate (306 mcg vs. 236 mcg), vitamin C (139 mg vs.108 mg), vitamin E (11 mg vs. 8.7 mg) iron (11.5mg vs.8.7 mg), magnesium (301 mg vs. 218 mg), copper (1.5 mg vs. 0.94 mg), manganese (4.6 mg vs.2.8 mg). The intake differences of the following nutrients between vegetarians and omnivores did not reach statistical significance: sucrose (46.2 g vs. 53.5 g), vitamin B6 (1.61 mg vs.

1.53mg), calcium (591 mg vs. 541 mg), potassium (2542 mg vs. 2232 mg), zinc (8.2 mg vs. 7.45 mg). The intake of vitamin B2 was comparable (1.30 mg vs. 1.35 mg).

In comparison to omnivores, vegans consumed significantly less: protein (43.7 g vs. 56.8 g), sucrose (37.7 g vs. 53.5 g), total fat (54.4 g vs. 65.1 g), saturated fat (12.2 g vs. 24.7 g), MUFA (20.0 g vs. 25.1 g), cholesterol (34 mg vs. 253 mg), calcium (419 mg vs. 541 mg) and significantly more: carbohydrates (273 g vs. 213 g), starch (120 g vs. 94 g), fibre (33.4 g vs. 16.5 g), PUFA (16.4 g vs. 9.0 g), vitamin B1 (1.53 mg vs. 0.96 mg), vitamin B6 (2.16 mg vs. 1.53 mg), folate (396 mcg vs. 236 mcg), vitamin C (199 mg vs. 108 mg), vitamin E (15 mg vs. 8.7 mg), iron (13.7 mg vs. 8.7 mg), magnesium (422 mg vs. 218 mg), copper (2.08 mg vs. 0.94 mg), manganese (6.66 mg vs. 2.85 mg), potassium (3310 mg vs. 2232 mg), zinc (9.36 mg vs. 7.45 mg), and Omega 6 PUFA (13.7 mg vs. 7.0 mg). The intake difference (1.24 mg vs. 1.35 mg) of vitamin B2 between vegans and omnivores did not reach statistical significance. The median intake of vitamin B3 was comparable (12.6 mg vs. 12.8 mg).

4.4.2. Median intakes of nutrients that were affected by confounding or mediation.

This section presents usual adjusted median intakes of nutrients for which there was some evidence of confounding by socioeconomic traits, family history, season or of mediation by presumed mediators (body composition, caloric intake, PA, perinatal traits). This means their levels did meaningfully change across models 1, 2 or 3. These were beta-carotene, B12, and vitamin D3. These changes were, however, minor.

Intake of beta-carotene equivalents (mcg) changed in omnivores from 3199 in model 1, to 3276 in model 2 and in vegetarians from 3984 to 3792 from model 1 to 2. This could be explained by seasonal variation of fruit and vegetable intake, which could have confounded the unadjusted intakes, as omnivores tended to be recruited in different seasons (more likely in winter and spring) compared to the vegetarians and vegans (more likely in early autumn). The beta-carotene intakes in vegans were similar in all three models and amounted to 5366 (mcg) of beta – carotene equivalents in model 2. Vegans differed significantly from omnivores. This suggests that fruit and vegetable intake was less seasonally affected in vegans than in vegetarians and omnivores. However, other explanations could also be relevant. Additionally, these changes were minor and could have been due to chance.

The median intakes of vitamin B12 were similar in all three models for omnivores and vegetarians and the adjusted levels in model 2 amounted to 7.1 mcg and 6.5 mcg respectively. This difference did not reach statistical significance. In vegans the levels meaningfully changed from model 1 (4.0 mcg) to model 2 (4.8 mcg) and 3 (5.2 mcg). The first change could be explained by adjusting for religion and parental education, both of which can be assumed to influence supplementation practices. The second change in model 3 can most likely be attributed to mediation by caloric intake. Vitamin B12 intakes in vegans came mainly from supplements and were independent of caloric intake. After adjustment (model 2, and 3), the difference in median vitamin B12 intakes between dietary groups lost statistical significance.

Vitamin D3 median intake levels were similar in omnivores in all three models. The adjusted intake level was 4.33 mcg. They increased in both vegetarians

and vegans from model 1 (2.35 mcg and 1.45 mcg respectively), to 3.23mcg and 2.67 mcg in model 2, which may most likely be explained by confounding by parental education and season on supplement intake.

Overall, the differences in nutrient intakes in comparison to omnivores were most pronounced in vegans, with vegetarians having intermediate values of all nutrients except for vitamin B2, where the intakes did not differ between groups; vitamin B3, where the intake was lowest in vegetarians and similar in the other dietary groups, and calcium, where intake was lowest in vegans and similar in the other two dietary groups.

There are no data on usual dietary intakes of vegans or vegetarians. However, these results are largely consistent with nutrient intakes of vegetarians reported in previous studies, discussed in chapter II, section 9.2.

There were no meaningful differences between the CC and MI analyses in nutrient intakes (see supplementary material).

Table 12e. Adjusted medians of usual intakes of nutrients in dietary groups

Outcome	Model 1						Model 2						Model 3					
	Meat-eaters		Vegetarians		Vegans		Meat-eaters		Vegetarians		Vegans		Meat-eaters		Vegetarians		Vegans	
	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI
Energy (kcal)	1632 ^a	1558, 1706	1571 ^a	1495, 1646	1616 ^a	1492, 1741	1608 ^a	1534, 1682	1587 ^a	1509, 1664	1609 ^a	1486, 1733	1592 ^a	1516, 1668	1623 ^a	1548, 1697	1652 ^a	1535, 1770
Protein (g)	56.6 ^a	53.5, 59.7	46.4^b	44.4, 48.4	42.6^c	39.4, 45.8	56.8 ^a	53.8, 59.9	46.5^b	44.3, 48.7	43.7^b	40.2, 47.3	56.4 ^a	52.3, 60.6	47.5^b	45.1, 49.9	43.6^c	40.9, 46.4
Carbohydrates (g)	221 ^a	212, 231	244^b	233, 256	267^b	245, 289	213 ^a	201, 225	245^b	230, 260	273^c	256, 290	223 ^a	210, 236	252^b	246, 259	270^c	261, 280
Starch (g)	98 ^a	93, 102	106^b	100, 112	117^c	108, 125	94 ^a	87, 100	107^b	100, 113	120^b	107, 133	94 ^a	85, 102	110^b	103, 116	119^b	108, 130
Sucrose (g)	53.0 ^a	47.7, 58.3	46.9 ^{ab}	42.8, 51.0	40.4^b	31.8, 49.1	53.5 ^a	47.1, 59.9	46.2 ^a	41.5, 50.9	37.7^b	30.7, 44.8	53.6 ^a	48.0, 59.3	45.0^b	40.4, 49.5	39.2^b	33.7, 44.6
Fibre (g)	16.0 ^a	14.5, 17.4	24.7^b	22.3, 27.2	32.4^c	28.3, 36.6	16.5 ^a	14.4, 18.6	24.2^b	21.8, 26.7	33.4^c	30.1, 36.7	16.8 ^a	15.0, 18.6	25.4^b	23.6, 27.2	32.0^c	29.5, 34.5
Fat (g)	65.2 ^a	61.5, 69.0	55.8^b	51.6, 59.9	54.3 ^{ab}	43.6, 65.1	65.1 ^a	60.5, 69.7	55.8^b	51.5, 60.1	54.4^b	46.5, 62.4	61.9 ^a	57.3, 66.6	56.7 ^{ab}	53.5, 60.0	54.3^b	50.1, 58.6
Saturated fat (g)	25.2 ^a	23.6, 26.7	18.2^b	16.7, 19.7	10.9^c	9.0, 12.7	24.7 ^a	22.8, 26.5	18.3^b	16.5, 20.2	12.2^c	10.3, 14.2	25.2 ^a	23.1, 27.2	17.7^b	16.0, 19.4	11.3^c	9.6, 13.0
MUFA (g)	25.3 ^a	23.6, 27.0	19.9^b	18.2, 21.6	20.5^b	16.6, 24.4	25.1 ^a	22.8, 27.4	19.8^b	18.1, 21.5	20.0^b	16.1, 23.9	24.5 ^a	22.9, 26.1	20.1^b	18.8, 21.5	20.6^b	18.8, 22.4
PUFA (g)	9.4 ^a	8.8, 10.1	11.7^b	10.5, 12.8	16.2^c	14.0, 18.3	9.0 ^a	7.8, 10.3	11.7^b	10.4, 12.9	16.4^c	13.5, 19.2	9.2 ^a	7.9, 10.6	13.1^b	11.6, 14.6	16.7^c	13.4, 20.0
Omega 3 PUFA (mg)	1.71 ^a	1.52, 1.89	1.82 ^a	1.47, 2.17	2.28 ^a	1.71, 2.84	1.67 ^a	1.34, 1.99	1.96 ^a	1.56, 2.37	2.26 ^a	1.53, 3.00	1.72 ^a	1.36, 2.07	2.07 ^a	1.70, 2.44	2.32 ^a	1.72, 2.91
Omega 6 PUFA (mg)	7.5 ^a	7.0, 8.1	9.7^b	8.7, 10.8	13.7^c	11.9, 15.5	7.0 ^a	6.2, 7.8	9.8^b	8.6, 11.1	13.7^c	12.1, 15.4	7.4 ^a	6.4, 8.4	10.8^b	9.6, 12.1	13.9^c	11.6, 16.3
Cholesterol (mg)	254 ^a	238, 269	154^b	140, 167	34^c	17, 50	253 ^a	228, 278	160^b	146, 175	34^c	20, 49	252 ^a	233, 270	163^b	143, 182	36^c	20, 52
Vitamin A (mcg RAE)	623 ^a	549, 696	588 ^a	513, 663	536 ^a	439, 633	618 ^a	523, 712	583 ^a	480, 685	535 ^a	416, 654	633 ^a	537, 729	629 ^a	517, 741	594 ^a	491, 697

Outcome	Model 1						Model 2						Model 3					
	Meat-eaters		Vegetarians		Vegans		Meat-eaters		Vegetarians		Vegans		Meat-eaters		Vegetarians		Vegans	
	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI
Beta - carotene equivalents (mcg)	3199 ^a	2721, 3678	3984 ^a	3323, 4644	5296^b	4325, 6267	3276 ^a	2689, 3862	3792 ^a	3114, 4470	5366^b	4139, 6594	3394 ^a	2685, 4104	3983 ^{ab}	2991, 4976	5354^b	4234, 6475
Vitamin B1 (mg)	1.00 ^a	0.93, 1.07	1.19^b	1.09, 1.29	1.52^c	1.37, 1.67	0.95 ^a	0.85, 1.05	1.24^b	1.09, 1.38	1.53^c	1.39, 1.67	0.97 ^a	0.90, 1.05	1.31^b	1.22, 1.40	1.60^c	1.49, 1.72
Vitamin B2 (mg)	1.39 ^a	1.29, 1.50	1.31 ^{ab}	1.19, 1.43	1.21^b	1.06, 1.36	1.35 ^a	1.22, 1.48	1.30 ^a	1.19, 1.41	1.24 ^a	1.06, 1.41	1.37 ^a	1.22, 1.52	1.39 ^a	1.25, 1.54	1.31 ^a	1.16, 1.46
Vitamin B3 (mg)	12.7 ^a	11.7, 13.7	9.4^b	8.3, 10.5	12.9 ^a	11.4, 14.4	12.8 ^a	11.5, 14.1	9.8^b	8.7, 10.9	12.6 ^a	11.0, 14.1	12.4 ^a	11.0, 13.8	10.0^b	8.8, 11.2	12.5 ^a	11.4, 13.6
Vitamin B6 (mg)	1.56 ^a	1.45, 1.68	1.66 ^a	1.52, 1.80	2.08^b	1.88, 2.27	1.53 ^a	1.37, 1.68	1.61 ^a	1.44, 1.77	2.16^b	1.95, 2.37	1.56 ^a	1.43, 1.69	1.62 ^a	1.46, 1.79	2.11^b	1.88, 2.33
Folate (mcg)	233 ^a	218, 249	306^b	284, 329	395^c	358, 431	236 ^a	215, 258	306^b	280, 332	396^c	358, 434	250 ^a	227, 274	312^b	282, 342	386^c	342, 429
Vitamin B12 (mcg)	7.3 ^a	7.0, 7.7	6.5^b	5.8, 7.2	4.0^c	2.5, 5.6	7.1 ^a	6.1, 8.0	6.5 ^a	5.4, 7.6	4.8 ^a	1.4, 8.2	7.2 ^a	6.4, 8.0	6.7 ^a	5.9, 7.5	5.2 ^a	2.6, 7.7
Vitamin C (mg)	114 ^a	100, 128	140^b	120, 160	192^b	137, 247	108 ^a	86, 130	139^b	121, 157	199^c	157, 241	109 ^a	89, 130	146^b	127, 166	194^b	133, 254
Vitamin D3 (mcg)	3.22 ^a	2.39, 4.05	2.35 ^{ab}	1.61, 3.08	1.45^b	0.65, 2.26	3.44 ^a	1.95, 4.93	3.23 ^a	2.05, 4.42	2.67 ^a	0.74, 4.61	3.50 ^a	1.89, 5.10	3.07 ^a	1.57, 4.56	2.32 ^a	0.08, 4.56
Vitamin E (mg)	9.0 ^a	8.4, 9.6	11.1^b	10.0, 12.2	14.2^c	11.5, 16.8	8.7 ^a	7.6, 9.7	11.0^b	9.9, 12.1	15.0^c	12.8, 17.1	8.9 ^a	7.8, 9.9	11.6^b	10.5, 12.7	14.4^c	12.6, 16.2
Calcium (mg)	559 ^a	508, 609	593 ^a	544, 641	407^b	353, 462	541 ^a	477, 605	591 ^a	528, 654	419^b	360, 479	540 ^a	470, 610	587^a	527, 647	421^b	368, 474
Copper (mg)	0.94 ^a	0.86, 1.01	1.52^b	1.40, 1.64	2.02^c	1.83, 2.22	0.94 ^a	0.82, 1.05	1.50^b	1.37, 1.63	2.08^c	1.88, 2.28	0.97 ^a	0.85, 1.08	1.54^b	1.40, 1.68	2.08^c	1.91, 2.26
Iron (mg)	8.5 ^a	7.9, 9.1	11.7^b	11.0, 12.3	13.1^c	12.2, 14.1	8.7 ^a	8.0, 9.3	11.5^b	10.6, 12.4	13.7^c	12.4, 15.0	9.0 ^a	8.3, 9.6	11.8^b	11.0, 12.5	14.1^c	13.2, 14.9
Magnesium (mg)	225 ^a	208, 241	306^b	284, 327	397^c	364, 429	218 ^a	197, 238	301^b	276, 326	411^c	373, 449	231 ^a	208, 253	315^b	288, 342	425^c	394, 456

Outcome	Model 1						Model 2						Model 3					
	Meat-eaters		Vegetarians		Vegans		Meat-eaters		Vegetarians		Vegans		Meat-eaters		Vegetarians		Vegans	
	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI
Manganese (mg)	2.80 ^a	2.46, 3.15	4.67^b	4.15, 5.19	6.54^c	5.78, 7.29	2.85 ^a	2.28, 3.43	4.60^b	4.09, 5.12	6.66^c	5.78, 7.53	3.07 ^a	2.53, 3.62	4.71^b	4.18, 5.24	6.58^c	5.50, 7.65
Potassium (mg)	2260 ^a	2129, 2392	2627^b	2475, 2780	3276^c	3042, 3510	2232 ^a	1990, 2474	2542 ^a	2369, 2716	3310^b	3046, 3574	2316 ^a	2183, 2449	2653^b	2507, 2799	3161^c	2887, 3435
Zinc (mg)	7.27 ^a	6.63, 7.91	7.98 ^{ab}	7.40, 8.55	8.90^b	7.85, 9.96	7.45 ^a	6.68, 8.23	8.19 ^{ab}	7.17, 9.21	9.36^b	8.33, 10.39	7.34 ^a	6.65, 8.03	8.32 ^a	7.48, 9.16	9.45^b	8.71, 10.19
Phosphorus (mg)	971 ^a	913, 1029	972 ^a	914, 1030	1019 ^a	936, 1102	936 ^a	870, 1002	989 ^a	926, 1051	1042 ^a	956, 1127	962 ^a	911, 1013	1033 ^{ab}	963, 1103	1051^b	994, 1109
Model 1:	Diet group, age, sex																	
Model 2:	Dietary group, age, sex, religion, urbanicity, seasonality, family history of obesity, parental education																	
Model 2:	Dietary group, age, sex, religion, urbanicity, seasonality, family history of obesity, parental education, breastfeeding at 6, 6–12 and over 12 months, fat mass z-score (DXA), lean mass z-score (DXA), birthweight (fifths), average movement count per minute internal z-score, usual caloric intake																	
^{abc} Pairs of estimated prevalences in the same row that do not have a common superscript are significantly different at $p < .05$.; * not adjusted for energy intake																		

Abbreviations: g, gram; mg, milligram; RAE, retinal activity equivalents; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids.

5. Health Outcomes

This section presents and discusses the results of health outcome differences between vegetarians or vegans in comparison to the omnivores.

Minimally adjusted models (models 1) are presented to illustrate the role of confounding in the associations. The multivariable-adjusted, multiply-imputed results for mean differences in health outcomes between vegetarians or vegans compared to the reference group of omnivores are presented in models 2 and 3. They aim to represent the causal effects (the limitations of cross-sectional study notwithstanding) of interest. Model 2 represents the total effect of dietary exposure on the health outcome and includes the presumed confounder set, model 3 represents the direct effect, eliminating the causal effect of a mediator, as explained in chapter V, section 9.3.

5.1. Anthropometry

Mean differences (mean, 95% CI) for anthropometric outcomes of vegetarians and vegans relative to omnivores are presented in Table 13a.

In multivariable model 2, vegetarianism and veganism were associated with -0.38 (95%CI -0.69, -0.08) and -0.65 (95%CI -1.01, -0.29) height z-score compared to omnivorous diet, respectively, which corresponds to -3.4 cm and -5.5 cm difference. Covariates included, age, sex, parental height, birthweight, gestational age, maternal pre-pregnancy BMI, physical activity level internal z-score, breastfeeding duration, parental education and urbanicity. The estimates in model 2 changed from the estimates in age and sex-adjusted model 1 in opposite directions. The difference decreased from -0.45 (95%CI -0.77, -0.12)

in vegetarians and increased in vegans from -0.55 (95CI -0.97, -0.12) height z-score in model 1. This suggests presence of positive confounding in vegetarians and negative confounding in vegans in the crude model 1.

Vegans, but not vegetarians, had lower BMI z-score than omnivores, and the difference in model 1 (-0.50 BMI z-score, 95%CI -0.82, -0.18) was not meaningfully changed after adding confounders in model 2 (-0.48 BMI z-score, 95%CI -0.81, -0.15). Additionally, vegans and not vegetarians, had lower suprailiac and triceps skinfold z-scores. The magnitude of these differences was similar in both models and amounted to -0.49 (95%CI -0.79, -0.20) and -0.52 (95%CI -0.84, -0.20) z-score for subscapular and -0.56 (95%CI -0.87, -0.24) and -0.54 (95%CI -0.87, -0.22) z-score for triceps skinfolds, in models 1 and 2 respectively. Hip girth z-score difference, again evident only in vegans, strengthened upon adding confounders from -0.59 (95%CI -0.86, -0.31) in model 1 to -0.68 (-0.98, -0.37) z-score in model 2. The lack of meaningful changes in the magnitude of the difference, as in the case of suprailiac and triceps skinfolds, or their increase upon adjustment, as in the case of hip girth, suggest that the differences are either operated by the main exposure (diet), or that other background exposures weaken ('cancel-out') some of the dietary effects.

There were differences relative to omnivores for both vegetarians and vegans in thigh girth z-score, with similar magnitudes in both models. The figures were -0.37 and -0.36 z-score for vegetarians in model 1 and 2 respectively, and -0.61 and -0.58 z-score in vegans. In relation to omnivores, biceps and subscapular skinfold z-score or waist girth z-score did not differ in either of the other dietary groups.

Table 13a. Crude and adjusted mean differences of vegetarian and vegan children relative to omnivore children in anthropometry

Outcome**	Model 1				Model 2			
	Vegetarian		Vegan		Vegetarian		Vegan	
	Δ^*	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI
Height z-score	-0.45	-0.77, -0.12	-0.55	-0.97, -0.12	-0.38	-0.69, -0.08	-0.65	-1.01, -0.29
BMI z-score	-0.24	-0.54, 0.06	-0.50	-0.82, -0.18	-0.25	-0.56, 0.05	-0.48	-0.81, -0.15
Biceps skinfold z-score***	0.03	-0.21, 0.27	-0.23	-0.53, 0.06	0.05	-0.20, 0.30	-0.17	-0.49, 0.14
Suprailiac skinfold z-score***	-0.06	-0.34, 0.23	-0.49	-0.79, -0.20	-0.08	-0.37, 0.22	-0.52	-0.84, -0.20
Subscapular skinfold z-score**	0.08	-0.20, 0.36	-0.31	-0.64, 0.03	0.08	-0.22, 0.37	-0.29	-0.65, 0.08
Triceps skinfold z-score***	-0.13	-0.43, 0.17	-0.56	-0.87, -0.24	-0.15	-0.45, 0.15	-0.54	-0.87, -0.22
Waist girth z-score	-0.24	-0.51, 0.04	-0.23	-0.50, 0.05	-0.24	-0.51, 0.04	-0.26	-0.56, 0.04
Hip girth z-score	-0.20	-0.53, 0.13	-0.59	-0.86, -0.31	-0.20	-0.52, 0.11	-0.68	-0.98, -0.37
Thigh girth z-score***	-0.37	-0.65, -0.09	-0.61	-0.90, -0.31	-0.36	-0.64, -0.08	-0.58	-0.88, -0.29
Model 1:	Diet group only							
Model 2:	Diet group, maternal height, paternal height, birthweight (fifths), gestational age (fifths), maternal pre-pregnancy BMI (fifths), average movement count per minute internal z-score, breastfeeding duration (<6, 6-12, >12 months), maternal education, paternal education, urbanicity; multiple imputation was used to account for missing data							
*difference, ** (Wells et al. 2012); ***outcomes affected by missing values of ≤ 4								

Overall, I found evidence of shorter height in both vegetarians and vegans, with the difference more pronounced in vegans. These results are broadly consistent with available evidence on vegetarian children, discussed in detail in chapter II, section 9.3. Furthermore, they provide novel evidence on the stature of contemporary vegan children. The exact reasons for shorter height in vegetarians and vegans are unknown. Potential explanations are discussed in chapter VII, Discussion.

Additionally, in comparison to omnivores, vegans had lower indices of peripheral and gluteo-femoral body fat, while vegetarians had lower values for the latter type of fat only. In vegans this might be another manifestation of overall lower body fatness, suggested by the majority of the remaining peripheral body fatness parameters. In vegetarians, who otherwise did not differ from the omnivores in any of the skinfold measures, it is a negative finding, as low subcutaneous thigh fat is a risk factor for adverse cardiometabolic risk profile (Snijder et al. 2005). The BMI z-score was lower than that of omnivores only in vegans. As this index, along with the skinfold and girth values, does not differentiate between fat and lean tissue, the results of body composition assessment methods distinguishing fat and fat free mass are discussed next.

5.2. Body composition

Mean differences (mean, 95% CI) for body composition outcomes measured by DXA and deuterium dilution method of vegetarians and vegans relative to omnivores are presented in Table 13b.

The results showed that vegans, but not vegetarians, had lower z-scores for lean mass measured by DXA in model 2 only (-0.36 z-score). When lean mass

was adjusted for height, giving lean mass index, this difference disappeared, suggesting it was attributable to smaller body stature. There were no differences in lean mass z-score measured by D₂O, which is regarded as a more accurate method relative to a 4C model (Wells and Fewtrell 2006).

Both vegans and vegetarians had lower fat mass z-score measured by DXA in minimally adjusted model 1 (-0.35 z-score, 95%CI -0.68, -0.02; for vegetarians, -0.84, 95%CI -1.18, -0.51; z-score for vegans). This difference in vegetarians changed minimally in model 2 to -0.32 z-score, however it lost statistical significance (95%CI -0.67, 0.02). It attenuated to -0.78 z-score (95%CI -1.14, -0.43) in vegans. Lower fat mass z-score in both vegetarians and vegans was also captured by the deuterium dilution method. The figures in model 1 were -0.36 z-score (95%CI -0.68, -0.05) and -0.78 z-score (95%CI -1.11, -0.46) for vegetarians and vegans respectively. These differences remained unchanged for vegetarians in model 2 (-0.36 z-score; 95%CI -0.71, -0.01), and changed minimally for vegans to -0.76 z-score (95%CI -1.20, -0.31). Therefore, the magnitude of fat mass z-score differences in vegetarians and vegans relative to omnivores was similar across the two methods.

When fat mass measured by deuterium dilution was adjusted for height, in fat mass index (FMI), the difference remained only in vegans and was practically unchanged upon adding the adjustment set to the model (-0.75 z-score in model 1 (95%CI -1.08, -0.42), and -0.72 z-score (95%CI -1.13, -0.30) in model 2), which suggests it might have been operated by the main exposure, diet. The difference in vegetarians was similar in both models (-0.32 and -0.33 z-score respectively), however it bordered on statistical significance.

The results of body composition analyses suggest that vegan diets exert their effect on body composition mainly by modifying fat mass rather than lean mass. Vegans have lower body fat independent of their height, and this association is virtually unconfounded by other background traits of that dietary group. Vegetarians' lower fat mass may be largely mediated by their lower height. Vegans, but not vegetarians, had lower lean mass measured by DXA, however the difference disappeared when lean mass was adjusted for height, suggesting a trend to lower lean mass in this group only in proportion to their shorter height. There was no evidence of difference in lean mass in vegetarians.

These results are consistent with nutrient intake profiles of children in this study. In comparison to both omnivores and vegetarians, the nutrient intake profile of vegans is suggestive of higher unprocessed carbohydrate, fruit and vegetable consumption (higher total carbohydrates accompanied by higher fibre, starch, and lower sucrose intake; higher intakes of vitamin C, beta carotene, folate, magnesium (USDA 2019). This dietary pattern, based on whole plant foods, is associated with lower risk of overweight and obesity in children (Sabaté and Wien 2010).

Table 13b. Crude and adjusted mean differences of vegetarian and vegan children relative to omnivore children in body composition

Outcome**	Model 1				Model 2			
	Vegetarian		Vegan		Vegetarian		Vegan	
	Δ^*	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI
Lean mass by DXA z-score	-0.24	-0.53, 0.04	-0.28	-0.68, 0.13	-0.19	-0.47, 0.10	-0.36	-0.71, -0.02
Fat mass by DXA z-score	-0.35	-0.68, -0.02	-0.84	-1.18, -0.51	-0.32	-0.67, 0.02	-0.78	-1.14, -0.43
Lean mass by D ₂ O z-score	-0.25	-0.53, 0.02	-0.19	-0.51, 0.13	-0.19	-0.49, 0.10	-0.27	-0.61, 0.07
Fat mass by D ₂ O z-score	-0.36	-0.68, -0.05	-0.78	-1.11, -0.46	-0.36	-0.71, -0.01	-0.76	-1.20, -0.31
Lean mass index D ₂ O z-score	0.00	-0.29, 0.28	0.18	-0.12, 0.48	0.01	-0.31, 0.33	0.15	-0.20, 0.50
Fat mass index D ₂ O z-score	-0.32	-0.66, 0.01	-0.75	-1.08, -0.42	-0.33	-0.70, 0.03	-0.72	-1.13, -0.30
Model 1:	Diet group only							
Model 2:	Diet group, maternal height, paternal height, birthweight (fifths), gestational age (fifths), maternal pre-pregnancy BMI (fifths), average movement count per minute internal z-score, breastfeeding duration (<6, 6–12, >12 months), maternal education, paternal education, urbanicity; multiple imputation was used to account for missing data							
*difference, ** (Wells et al. 2012);								

Abbreviations: D20, deuterium dilution

5.3. Bone status

Mean differences (mean, 95% CI) in bone outcomes of vegetarians and vegans relative to omnivores are presented in Table 14.

In comparison to omnivores, both vegetarians and vegans had lower TBLH BMC by 8.1% (95%CI -13.8, -2.4%) and 16.7% (95%CI -24.8, -8.6%) respectively in minimally adjusted models (age, sex). These differences remained practically unchanged (-8.2%,95%CI -14.2%, -2.3%; -16.6%,95%CI -24.6, -8.7 respectively) upon adding socioeconomic variables in model 2, which suggests that the diet was the primary driver of the BMC deficit. Since bone mineral content is dependent on bone size, which in turn, is determined by body size, and plant-based diets affect both height and weight, model 3 included presumed mediators of the effects of plant-based diets on bone BMC, height and weight z-score along with bone area. This eliminated the difference in vegetarians and attenuated it in vegans to -3.7% (95%CI -6.4%, -1.0). Therefore, the deficit in bone mass in vegetarians and vegans was largely explained by the effect of diet on body and bone size, but not entirely so in vegans. These results suggest that a vegan diet per se, independent of its effect on body dimensions, might lower bone mineral content.

The trend was similar for L2-L4 BMC. In the minimally adjusted model 1, the difference for vegetarians and vegans was -5.8% (95%CI -11%.0, -0.6%) and -10.6% (95%CI-17.3%, -4.0%). In model 2, the adjusted difference in vegetarians and vegetarians was of similar magnitude (-5.3%, 95%CI -10.5, -0.1; and -10.4%,95%CI -16.8%, -4%), which again implies the effect of diet, rather than socioeconomic traits, on the difference. Upon including mediators of

the dietary effect in model 3, the difference in vegetarians vanished entirely, and attenuated in vegans to -5.5% (95%CI -10.0%, -1.0%).

Similar trends in vegans for both bone sites suggest a direct influence of vegan diet on BMC. This assertion is strengthened, upon analysis of the results of another approach measuring BMC that corrects for bone size, bone mineral apparent density. As outlined in chapter 2, section 10.3, this method adjusts BMC content for calculated bone volume rather than bone area. The figures for lumbar spine (L2-L4) BMAD in vegans are consistent with the first approach, where the results of BMC were adjusted for body size and bone area. The differences in minimally adjusted models for L2-L4 BMAD z-scores were only significant for vegans, and amounted to -0.66 z-scores. (95%CI -1.07, -0.26). They attenuated only slightly to -0.61 z-scores (95%CI -1.06, -0.17) after adding confounders to the model. The corresponding figures for BMAD percentile were -12.7 (95%CI -22, -3.3) (model 1) and -11.1 (95%CI -21.1, -1.0) (model 2). As outlined in chapter V, section 5.4, in the absence of Polish reference data, UK reference data (Crabtree and et al. 2004) were used to obtain BMAD z-scores.

In summary, I found evidence of a deficit in bone mass in vegans, after adjusting for their smaller size. In vegetarians, adjustment for body and bone size eliminated the bone mass deficit found in unadjusted models. The results in vegetarians are consistent with the existing evidence, whereby BMD in vegetarian children unadjusted for bone and body size was lower than that of the omnivore reference group (Ambroszkiewicz et al. 2018b; Ambroszkiewicz et al. 2019). Additionally, they provide novel evidence on adjusted bone mass in both vegetarian and vegan children. Lower bone mass in vegans is consistent

with their lower or lack of intake of several bone-supporting nutrients or foods, like calcium, dairy, vitamin D or protein (Weaver et al. 2016). However, the exact mechanism underlying this finding is unknown, and potential explanations are discussed in chapter VII, Discussion.

Table 14. Crude and adjusted mean differences of vegetarian and vegan children relative to omnivore children in bone outcomes

Outcome**	Model 1				Model 2				Model 3			
	Vegetarian		Vegan		Vegetarian		Vegan		Vegetarian		Vegan	
	Δ^*	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI
TBLH BMC (%)***	-0.081	-0.138, -0.024	-0.167	-0.248, -0.086	-0.082	-0.142, -0.023	-0.166	-0.246, -0.087	0.011	-0.011, 0.033	-0.037	-0.064, -0.010
L2-L4 BMC (%)***	-0.058	-0.110, -0.006	-0.106	-0.173, -0.040	-0.053	-0.105, -0.001	-0.104	-0.168, -0.040	-0.006	-0.042, 0.031	-0.055	-0.100, -0.011
BMAD L2-L4 z-score ****	-0.11	-0.43, 0.22	-0.66	-1.07, -0.26	-0.06	-0.43, 0.30	-0.61	-1.06, -0.17				
BMAD L2-L4%ile	-4.0	-12.1, 4.2	-12.7	-22.0, -3.3	-2.2	-11.2, 6.8	-11.1	-21.1, -1.0				
Model 1:	Diet group, age, sex											
Model 2:	Diet group, age, sex, maternal education, religion, urbanicity											
Model 3:	Diet group, age, sex, maternal education, religion, urbanicity, height z-score (UK), weight z-score (UK), bone area											
* difference; ** outcomes affected by missing values of < 5; *** variable log-transformed, results represent percent difference; **** (Crabtree and et al. 2004)												

5.4. Cardiovascular risk

Tables 15a–c show that diet was associated with differences in several CVD risk factors. All these outcomes were measured around 2 weeks after the dietary exposure was recorded, and hence these associations might provide preliminary evidence for causal relationship. This is especially the case with blood lipids and hs-CRP, which have been shown to respond to dietary change in several days (Jenkins et al. 2003).

5.4.1. Lipid levels

The results of lipid levels in dietary groups are presented in Table 15a. Both vegans and vegetarians had lower total cholesterol levels. In minimally adjusted model 1, the values were -33.6 mg/dL for vegans (95%CI -42.5, -24.7), but the difference for vegetarians (-9.3 mg/dL, 95%CI -19.1, 0.4) did not reach statistical significance. After adding presumed confounders (birthweight, gestational age, maternal pre-pregnancy BMI, breastfeeding duration, parental education, religion, and urbanicity), the difference became significant for vegetarians (-12.6 mg/dL, 95%CI -22.7, -2.5) and strengthened in vegans (-36.1 mg/dL, 95%CI -46.4, -25.8). This suggests that other perinatal or socioeconomic factors acted as negative confounders, especially in vegetarians in model 1.

After including potential mediators of the dietary effect on cholesterol levels (fat, lean mass adjusted for height), the differences were slightly attenuated, which suggests that a small part of the effect was due to the influence of vegetarian and vegan diets on body size and composition. Vegans, but not vegetarians, had lower LDL-C levels. The trend was similar to that of total cholesterol. In minimally adjusted model 1 the difference for vegans was -23.4 mg/dL (95%CI-

30.9, -15.8), it increased upon adjustment to -25.3 mg/dL (95%CI-34.2, -16.4), and was attenuated to -22.0 mg/dL (95%CI-31.0, -13.0) after including presumed mediators. This suggests a predominant effect of the vegan diet, with minor negative confounding by socioeconomic or perinatal traits, and only slight mediation by fat and lean mass.

HDL cholesterol was lower in both vegans and vegetarians. The trend, again, was similar to the other cholesterol fractions in minimally- and confounder-adjusted models. Thus, in model 1, the difference was -5.0 mg/dL (95%CI -9.4, -0.6) and -10.6 mg/dL (95%CI -14.7, -6.5), and in model 2 -6.8 mg/dL (95%CI -11.3, -2.2) and -10.9 mg/dL (95%CI -16.0, -6.7), for vegetarians and vegans respectively. However, adding mediators to the analysis strengthened the difference to -7.0 mg/dL (95%CI -11.7, -2.3) in vegetarians and -11.7 mg/dL (95%CI -16.6, -6.8) in vegans. Although the magnitude of this change was relatively small, it suggests that the diet itself was driving the HDL difference and that the lower fat mass in children on meatless diets was acting as a negative mediator.

Vegetarians, but not vegans, had higher VLDL-C and triglyceride levels. In model 1, the differences for vegetarians were 14% (95%CI 3%, 25%) and 18% (95%CI 6%, 29%); in model 2 the figures were 15% (95%CI 3%, 28%) and 21% (95%CI 9%, 34%), and in model 3 they amounted to 17% (95%CI 4%,30%) and 23% (95%CI 10%, 0.36%) for VLDL-C and triglycerides respectively. This suggests that socioeconomic and perinatal factors negatively confounded dietary effect, and that predominantly diet was responsible for that difference, with lower fat mass in vegetarians 'cancelling out' some of the negative effects of their diets.

Table 15a. Crude and adjusted mean differences of vegetarian and vegan children relative to omnivore children in blood lipid levels

Outcome**	Model 1				Model 2				Model 3			
	Vegetarian		Vegan		Vegetarian		Vegan		Vegetarian		Vegan	
	Δ*	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI
Total cholesterol (mg/dL)	-9.3	-19.1, 0.4	-33.6	-42.5, -24.7	-12.6	-22.7, -2.5	-36.1	-46.4, -25.8	-11.6	-21.8, -1.4	-32.7	-43.1, -22.3
HDL cholesterol (mg/dL)	-5.0	-9.4, -0.6	-10.6	-14.7, -6.5	-6.8	-11.3, -2.2	-10.9	-16.0, -6.7	-7.0	-11.7, -2.3	-11.7	-16.6, -6.8
LDL cholesterol (mg/dL)	-6.2	-14.3, 1.9	-23.4	-30.9, -15.8	-7.9	-15.7, 0.0	-25.3	-34.2, -16.4	-6.8	-14.8, 1.2	-22.0	-31.0, -13.0
VLDL cholesterol (%mg/dL) ***	0.14	0.03, 0.25	0.00	-0.13, 0.14	0.15	0.03, 0.28	0.02	-0.13, 0.16	0.17	0.04, 0.30	0.05	-0.10, 0.21
Triglycerides (%mg/dL) ***	0.18	0.06, 0.29	0.03	-0.11, 0.17	0.21	0.09, 0.34	0.06	-0.09, 0.22	0.23	0.10, 0.36	0.11	-0.05, 0.27
Model 1:	Diet group, age, sex											
Model 2:	Diet group, age, sex, birthweight quintile, gestational age quintile, maternal pre-pregnancy BMI quintile, breastfeeding at 6, 6-12 and over 12 months, maternal education, paternal education, religion, urbanicity											
Model 3:	Diet group, age, sex, birthweight quintile, gestational age quintile, maternal pre-pregnancy BMI quintile, breastfeeding at 6, 6-12 and over 12 months, maternal education, paternal education, religion, urbanicity, height z-score (UK), fat mass z-score (DXA), lean mass z-score (DXA).											
* difference; ** outcomes affected by missing values of n=2; *** variable log-transformed, results represent percent difference.												

5.4.2. Glucose, insulin levels and insulin resistance

The results of fasting glucose, insulin levels along with insulin resistance index HOMA-IR, are presented in Table 15b. Fasting insulin levels did not differ in vegetarians or vegans in comparison to omnivores. However, vegetarians (but not vegans) had higher fasting glucose in all three models, with the magnitude of difference increasing from 3.2 mg/dL (95%CI 1.0, 5.4) in minimally adjusted model 1, to 3.4 mg/dL (95%CI 1.3, 5.5) in model 2, and finally to 3.8 mg/dL (95%CI 1.8, 5.9) in model 3. This suggests that socioeconomic and perinatal traits acted as negative confounders of the dietary exposure on fasting glucose and that lower fat levels in vegetarians might 'cancel-out' some of that adverse effect. Vegans had higher fasting glucose by 3 mg/dL (95%CI 0.1, 5.8) in model 3, which suggests that their lower body fat content counter-balanced this adverse trend. Vegetarians and vegans did not differ in their insulin sensitivity from the omnivores in multivariable adjusted models. HOMA-IR was higher for both vegetarians and vegans by 17% in model 3 (95% CI for vegetarians: 0.04, 0.30; for vegans: 0.03, 0.31), which suggest that if vegetarians and vegans had similar body composition as omnivores, they might have higher risk of insulin resistance. This is, however, only a hypothetical contemplation, as the final effects of plant-based diets need to be considered in their totality, i.e. including their effect on body composition. These effects, the limitations of cross-sectional study notwithstanding, are given in model 2, in which there is no evidence of adverse HOMA profile for vegetarians and vegans.

Table 15b. Crude and adjusted mean differences of vegetarian and vegan children relative to omnivore children in fasting glucose, insulin and HOMA-IR

Outcome**	Model 1				Model 2				Model 3			
	Vegetarian		Vegan		Vegetarian		Vegan		Vegetarian		Vegan	
	Δ^*	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI
Fasting insulin (μUI/mL)	0.23	-0.55, 1.02	-0.04	-0.85, 0.77	0.24	-0.63, 1.11	0.05	-0.83, 0.94	0.59	-0.23, 1.40	0.78	-0.07, 1.62
Fasting glucose (mg/dL)	3.2	1.0, 5.4	2.2	-0.1, 4.6	3.4	1.3, 5.5	2.2	-0.5, 4.8	3.8	1.8, 5.9	3.0	0.1, 5.8
HOMA-IR (%) ***	0.12	-0.01, 0.24	0.05	-0.08, 0.17	0.12	-0.02, 0.25	0.06	-0.08, 0.20	0.17	0.04, 0.30	0.17	0.03, 0.31
Model 1:	Diet group, age, sex											
Model 2:	Diet group, age, sex, birthweight quintile, gestational age quintile, maternal pre-pregnancy BMI quintile, breastfeeding at 6, 6–12 and over 12 months, maternal education, paternal education, religion, urbanicity											
Model 3:	Diet group, age, sex, birthweight quintile, gestational age quintile, maternal pre-pregnancy BMI quintile, breastfeeding at 6, 6–12 and over 12 months, maternal education, paternal education, religion, urbanicity, height z-score (UK), fat mass z-score (DXA), lean mass z-score (DXA).											
* difference; ** outcomes affected by missing values of < 3; *** variable log-transformed, results represent percent difference.												

Abbreviations: HOMA-IR, homeostatic model of insulin resistance

5.4.3. hs-CRP levels

Crude and adjusted mean differences of vegetarian and vegan children relative to omnivore children in hs-CRP, are presented in Table 15c. In comparison to omnivores, the hs-CRP difference was only evident in vegans. It ranged from -47% (95% CI -79%, -15%) in minimally adjusted models, increased to -56% (95% CI -95%, -17%) after including confounders in the analysis and decreased to -47% (95% CI -0.88, -0.05), when body composition was considered. This suggests that socioeconomic and perinatal characteristics negatively confounded the effects of the vegan diet on chronic inflammation and that some of that effect was mediated by lower body fat in vegans. The difference remained after excluding 3 outlier values (>1 mg/dL), in both model 1 (-32%, 95% CI -59%, -5%) and model 2 (-39%, 95% CI -72%, -6%), although it disappeared in model 3. The outlier values (<3.0 mg/L), however, were considered physiologically plausible and still within the normal range by Prof. Peter Whincup, director of the Population Health Research Institute, at St George's, University of London, an expert in this area whom I consulted on this matter in February 2019. Therefore, the results of the analysis with the outlier values are likely to be the ones representing the true associations of vegan diet with this inflammation marker, and the results of the model excluding outliers are presented as a sensitivity analysis.

5.4.4. cIMT

Crude and adjusted mean differences of vegetarian and vegan children relative to omnivore children in cIMT are presented in Table 15c. There was no evidence of differences in a surrogate marker of atherosclerosis (cIMT) across

the three diet groups, as displayed in Table 15c. The mean values (not shown in the table) and SDs were 0.364 mm (0.296) in omnivores, 0.364 mm (0.025) in vegetarians and 0.356 mm (0.033) in vegans. These figures were lower than those of 119 US children mean age 10 years, with no risk factors for cardiovascular disease (mean cIMT of 0.523 mm, with SD of 0.021) (White et al. 2017), cIMT of healthy 31 Brazilian children (Gazolla et al. 2015) aged 5–10 years old (median of 0.400 mm, inter quartile range 0.400, 0.500). This suggests that all children in this study were exposed to fewer risk factors than those from the above-mentioned studies, as the severity and extent of the cIMT is positively associated with (apart from age) the number of risk factors (McGill et al. 2000). The study aimed to recruit all children from health-conscious families, and the background characteristic comparison suggest that this was achieved. A potential explanation for the lack of difference might be that among young healthy children, the level of exposure to CVD risk factors was within physiological norms and was insufficient to affect the atherosclerotic disease process in any of the groups. Alternative explanation could be inadequate numbers of children to show significant group differences, which might, at this age group, be relatively small.

5.4.5. IGF-1, IGFBP-3, molar IGF1:IGFBP3 ratio

As shown in Table 15c, there was no evidence of differences in IGF-1, IGFBP-3 or molar IGF1:IGFBP3 ratio across the three diet groups. The mean and SDs values for IGF-1 were 210.0 ng/mL (86.3) for omnivores, 192.1 ng/mL (87.3) for vegetarians and 194.2 ng/mL (101.8) for vegans. The figures for IGFBP-3 were 3.5 (0.7) µg/mL for omnivores, 3.5 (0.6) for vegetarians and 3.4 (0.8) for vegans; and for the molar IGF1:IGFBP3 ratio 0.22 (0.07), 0.19 (0.07), and 0.20

(0.7) for omnivores, vegetarians and vegans respectively (values not shown in the tables). These values were comparable to findings in other populations (Xu et al. 2009; Löfqvist et al. 2005). The lack of differences between vegetarians, and especially vegans (who do not consume dairy, the main dietary determinant of the IGF-1 level (Hoppe et al. 2004)) and omnivores is difficult to explain.

Overall, there was evidence for a healthier CVD risk factor profile in vegans. This was demonstrated by their lower total and LDL-C levels along with lower levels of chronic inflammation marker, hs-CRP. These results are consistent with the dietary profiles of vegans. This dietary group had more optimal intakes of nutrients linked to healthy cholesterol levels (lower total fat, saturated fat, MUFA, cholesterol, sucrose; higher PUFA and fibre intakes (Varady and Jones 2005)). They also had higher intake of nutrients associated with fruits and vegetables (beta carotene, folate, vitamin C, magnesium (USDA 2019)), which are linked to lower inflammation status (Holt et al. 2009).

Table 15c. Crude and adjusted mean differences of vegetarian and vegan children relative to omnivore children in hs-CRP, cIMT, IGFBP3, IGF-1

Outcome**	Model 1				Model 2				Model 3			
	Vegetarian		Vegan		Vegetarian		Vegan		Vegetarian		Vegan	
	Δ^*	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI
hs-CRP (%) ***	-0.22	-0.56, 0.13	-0.47	-0.79, -0.15	-0.21	-0.59, 0.18	-0.56	-0.95, -0.17	-0.17	-0.58, 0.23	-0.47	-0.88, -0.05
hs-CRP values <1 (%mg/dL)***	-0.06	-0.36, 0.24	-0.32	-0.59, -0.05	-0.02	-0.36, 0.32	-0.39	-0.72, -0.06	0.02	-0.33, 0.37	-0.28	-0.62, 0.06
cIMT (mm)	0.000	-0.009, 0.010	-0.008	-0.021, 0.005	0.001	-0.010, 0.012	-0.006	-0.020, 0.007	0.003	-0.009, 0.014	-0.004	-0.017, 0.009
IGFBP3 ($\mu\text{g/mL}$)	0.06	-0.15, 0.28	-0.10	-0.35, 0.14	0.00	-0.22, 0.23	-0.19	-0.45, 0.07	0.08	-0.13, 0.30	-0.07	-0.32, 0.17
IGF-1 (ng/mL)	-14	-44, 16	-14	-45, 17	-12	-42, 18	-11	-48, 26	4	-23, 31	17	-15, 50
Molar IGF1:IGFBP3 ratio	-0.020	-0.044, 0.004	-0.014	-0.039, 0.011	-0.016	-0.041, 0.009	-0.006	-0.036, 0.023	-0.004	-0.027, 0.019	0.014	-0.013, 0.041
Model 1:	Diet group, age, sex											
Model 2:	Diet group, age, sex, birthweight quintile, gestational age quintile, maternal pre-pregnancy BMI quintile, breastfeeding at 6, 6–12 and over 12 months, maternal education, paternal education, religion, urbanicity											
Model 3:	Diet group, age, sex, birthweight quintile, gestational age quintile, maternal pre-pregnancy BMI quintile, breastfeeding at 6, 6–12 and over 12 months, maternal education, paternal education, religion, urbanicity, height z-score (UK), fat mass z-score (DXA), lean mass z-score (DXA).											
* difference; ** outcomes affected by missing values of < 7; *** variable log-transformed, results represent percent difference.												

Of particular interest is the vitamin E intake level of vegans, which compares to adult levels linked to regular nut consumption (O'Neil, Nicklas, and Fulgoni 2015). It is likely that high intakes of this vitamin were the result of regular nut consumption by the vegan children, as this was reported in other studies that measured food intakes (Sanders 1988). Nut consumption is inversely linked to adverse cardiovascular risk profile, including dyslipidaemia and metabolic syndrome (Guasch-Ferré et al. 2017). Additionally, vegans had the lowest sucrose intakes along with the highest starch and fibre intakes, which suggests that, in the context of high carbohydrate diets, they consumed less processed types of this macronutrient. Diets high in unprocessed carbohydrates are not linked to higher risk of insulin resistance, diabetes or adverse CVD risk, rather they are protective (De Munter et al. 2007; Wu et al. 2015). Although vegan HDL-C levels were also lower, this is a common finding in both adult vegetarians and vegans, whereby lower total and LDL-C cholesterol levels in conjunction with lower HDL-C levels are associated with lower CVD disease risk, as discussed in the first part of chapter II. Moreover, adult intervention trials applying low-fat high carbohydrate unprocessed plant-based diets demonstrate that HDL-C levels tend to decrease while all other measures of cardiovascular risk improve (Kent et al. 2013).

Contrasting with the findings for vegans, there was evidence of a worse CVD risk factor profile in vegetarians in comparison to omnivores. The only positive finding in this dietary group was their lower total cholesterol levels in comparison to omnivores. However, the magnitude of the difference was smaller than in vegans. Additionally, vegetarians had higher triglyceride, VLDL-C and fasting glucose levels, which in conjunction with lower HDL-C levels, are characteristics of a metabolic syndrome risk factor cluster (Alberti et al. 2009).

These results are consistent with the nutrient intake profiles of vegetarians, who – in comparison to the omnivores – had lower fat and higher carbohydrate intakes. However, in comparison to vegans, they also had lower intakes of fibre, starch and higher intakes of sucrose, which suggests higher consumption of processed carbohydrates. Their lowest consumption of vitamin B3 is another marker of low unprocessed plant food consumption, as the main sources of that nutrient, apart from meat, poultry and fish, are cereals, legumes, and seeds (USDA 2019).

Diets low in fat and high in processed carbohydrates are a risk factor for high triglyceride and VLDL-C levels (Ullmann et al. 1991). Diets higher in sugar increase risk of the metabolic syndrome and diabetes (Malik et al. 2010), raise LDL-C and triglycerides levels, and lower HDL-C levels (Welsh et al. 2010; Basiotis et al. 1987). This does not seem to be the case when the carbohydrates come from whole foods with higher fibre content, i.e. grains, vegetables, legumes, and fruit (Turley et al. 1998). Additionally, protein and fat reduce glycaemic responses in a dose-dependent fashion (Moghaddam, Vogt, and Wolever 2006), and lower intakes of those macronutrients, as observed in vegetarians in comparison to omnivores, might exacerbate the effects of habitual processed carbohydrates intakes.

5.5. Blood micronutrient status

Blood micronutrient outcomes were also measured around 2 weeks after the dietary exposure, hence any associations between diet exposure and markers of especially blood iron and vitamin B12 levels, provide preliminary evidence for a causal relationship. Dietary changes can impact blood iron status markers

within days (Wojciak 2014). Serum B12 levels start to normalize within several days after supplementation or the inclusion of food sources of this nutrient in the diet (Wang et al. 2018). Vitamin 25 (OH) D levels change in response to supplementation, diet and sun exposure over longer time periods, hence conclusions as to the nature of their association with diet require greater caution.

5.5.1. Differences in mean serum indicators of iron status

Mean differences between diet groups in selected serum indicators of iron status are presented in Table 16. Vegans, but not vegetarians, had lower mean RBC and haemoglobin levels in both minimally adjusted model 1 (-0.23 M/ μ l, 95%CI 0.33, -0.12; for RBC and -0.38 g/dL, 95%CI-0.70, -0.06 for HGB) and model 2 (-0.21 M/ μ l,95%CI -0.32, -0.11; for RBC and -0.35 g/dL, 95%CI -0.67, -0.03; for HGB), which additionally included maternal education and smoking along with urbanicity as independent variables. The magnitude of confounding was small, and the major effect, the limitations of cross-sectional study notwithstanding, is likely to be attributable to the diet itself.

Both vegetarians and vegans had lower haematocrit in minimally adjusted model 1 (-83%, 95%CI -160%, -7%; and -105%, 95%CI -203%, -8% respectively). This difference disappeared entirely for both of the dietary groups in adjusted model 2, which suggests that socioeconomic factors created a spurious association of the dietary exposure with this outcome. Ferritin was lower in vegetarians and vegans in model 1 (-19%, 95%CI -37%, -1%; and -28% 95%CI -48%, -7%), however in adjusted model 2, the difference remained only in vegans and was nearly of the same magnitude (-26%, 95%CI -45%, -

7%). This suggests that vegan dietary practices were associated with this iron-storage protein status, whereas in vegetarians, socioeconomic factors created a spurious association of their diet status and ferritin level.

Overall, there was evidence of lower body iron status in vegans only. This was evident across numerous iron metabolism parameters, including haemoglobin, blood marker most commonly used to assess iron deficiency anaemia, and confirmed with a more sensitive marker of iron deficiency, ferritin. This is the first study assessing these haematological parameters in vegan children.

My results are also broadly consistent with the available literature on iron deficiency risk of vegetarian children, discussed in chapter II, section 9.6. They support the US Institute of Medicine (US Institute of Medicine 2001) recommendations of increased iron intake for vegetarians due to its lower bioavailability from plant sources. At the same time, they contradict, at least for prepubertal vegan children, the assertion expressed in the latest position statement Academy of Nutrition and Dietetics on vegetarian and vegan diets (Melina, Craig, and Levin 2016), that individuals can adapt to a better absorption of non-haeme iron sources in the long term. Even though vegans had higher intakes of iron in comparison to omnivores, these higher levels were apparently not adequate to sustain normal haematopoiesis.

5.5.2. Differences in mean serum indicators of vitamin B12 status

Differences between the diet groups in selected serum indicators of B12 status (serum B12, homocysteine, MCV), addressing variation in supplementation and fortification practices, are presented in Table 17.

Table 16. Crude and adjusted mean differences of vegetarian and vegan children relative to omnivore children in selected serum indicators of the iron status

Outcome**	Model 1				Model 2			
	Vegetarian		Vegan		Vegetarian		Vegan	
	Δ^*	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI
RBC (M/μl)	-0.09	-0.18, 0.01	-0.23	-0.33, -0.12	-0.07	-0.17, 0.02	-0.21	-0.32, -0.11
HGB (g/dL)	-0.24	-0.50, 0.02	-0.38	-0.70, -0.06	-0.20	-0.47, 0.07	-0.35	-0.67, -0.03
HT (%)	-0.83	-1.60, -0.07	-1.05	-2.03, -0.08	-0.73	-1.52, 0.07	-0.98	-1.97, 0.01
Ferritin*** (%)	-0.19	-0.37, -0.01	-0.28	-0.48, -0.07	-0.14	-0.32, 0.03	-0.26	-0.45, -0.07
Model 1:	Diet group, age, sex							
Model 2:	Diet group, age, sex, maternal education, urbanicity, maternal smoking							
* difference; ** outcomes affected by missing values of 1; *** variable log-transformed, results represent percent difference.								

Table 17. Crude and adjusted mean differences of vegetarian and vegan children relative to omnivore children in serum vitamin B12, homocysteine and MCV levels

Model 1												
Outcome**	Vegetarian – no supplementation or fortification		Vegetarian – fortification only		Vegetarian – supplementation and fortification		Vegan – no supplementation or fortification		Vegan – fortification only		Vegan – supplementation and fortification	
	Δ^*	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI
Vit. B12 (pg/mL)	-82.8	-155.4, -10.3	2.8	-94.3, 99.9	116.4	-8.3, 241.1	-249.1	-341.4, -156.9	-141.0	-260.2, -21.7	90.7	-48.8, 230.3
Homocysteine*** (%)	0.14	0.00, 0.27	-0.05	-0.14, 0.04	-0.12	-0.24, 0.00	0.48	0.25, 0.71	0.14	-0.07, 0.36	-0.10	-0.23, 0.03
MCV (fl)	-0.27	-2.16, 1.60	-0.06	-2.10, 1.98	-0.63	-2.6, 1.33	4.25	1.35, 7.15	0.84	-1.64, 3.32	0.91	-0.65, 2.46
Model 2												
Vit. B12 (pg/mL)	-124.7	-214.7, -34.7	-41.1	-145.4, 63.1	94.7	-50.6, 240.0	-288.4	-411.6, -165.2	-172.1	-308.8, -35.4	60.1	-80.6, 200.8
Homocysteine*** (%)	0.15	0.00, 0.30	-0.02	-0.13, 0.09	-0.12	-0.26, 0.02	0.49	0.25, 0.72	0.15	-0.10, 0.40	-0.09	-0.24, 0.06
MCV (fl)	-0.34	-2.40, 1.70	-0.12	-2.51, 2.27	-0.52	-2.58, 1.54	4.43	1.69, 7.18	1.63	-1.03, 4.29	0.86	-0.96, 2.69
Model 1:	Dietary group categorised according to supplementation and fortification status											
Model 2:	Dietary group categorised according to supplementation and fortification status, maternal education, religion											
* difference; ** outcomes affected by missing values of < 3; *** variable log-transformed, results represent percent difference.												

Vegetarians and vegans had lower mean serum vitamin B12 levels than omnivores if they did not supplement vitamin B12 or use B12 fortified foods. The difference in minimally adjusted model 1 of -82.8 pg/mL (95%CI -155.4, -10.3) in vegetarians and -249.15 pg/mL (95%CI -341.4, -156.9) in vegans increased to -124.70 pg/mL (95%CI -214.7, -34.7) and -288.40 pg/mL (95%CI -411.6, -165.2) respectively, after adding maternal education and smoking along with urbanicity as confounders to model 2. This suggests that in both vegetarians and vegans these socioeconomic traits acted as negative confounders of dietary exposure on this outcome. Additionally, lower B12 vitamin levels were detected in vegans who used B12 fortified foods without B12 supplementation in model 1 (-141.0 pg/mL, 95% CI -260.2, -21.7) and this difference increased to -172.11 pg/mL (95%CI -308.8, -35.4), in model 2, which confirms the same effects of SES traits on serum vitamin B12 as discussed above.

Vegans who did not supplement or use fortified foods with B12 had higher mean homocysteine (48%, 95%CI 25%, 71%; in model 1 and 49%, 95%CI 25%, 72%; in model 2) and MCV (4.25 fl, 95%CI 1.35, 7.15; in model 1 and 4.43 fl, 95%CI 1.69, 7.18; in model 2) levels. Negative confounding by socioeconomic factors was also evident in these additional vitamin B12 status markers, but it was of a lesser magnitude. Vegetarians and vegans who supplemented or/and fortified their diet in vitamin B12 did not differ in serum MCV or homocysteine levels in comparison to omnivores.

Overall, there was evidence of vitamin B12 deficiency in vegans who did not supply this nutrient from outside sources, which was confirmed by three biomarkers, and hence this finding appears robust. The existence of abnormal levels of these three markers along with lower RBCs and haemoglobin is

suggestive of higher risk of megaloblastic anaemia (characterised by decreased Hgb and RBCs, increased MCV accompanied by low serum vitamin B12 levels) among this group of vegan children (Devalia, Hamilton, and Molloy 2014).

The evidence for deficiency in vegetarians with the same supplementation and fortification status was weaker, as it was only seen for serum vitamin B12 levels. However lower serum B12 levels in vegetarians did not affect their homocysteine metabolism or haematological indices, markers of physiological vitamin B12 deficiency. These results are consistent with the diet profiles of vegetarians, who consumed less, and vegans who consumed almost no dietary sources of this vitamin. They add to the available body of evidence of increased risk of vitamin B12 deficiency in vegetarians, and especially vegans who do not supplement or fortify their diets with that nutrient (Pawlak, Lester, and Babatunde 2014).

5.5.3. Differences in mean serum indicators of vitamin D3 status

Mean differences between groups in serum 25 (OH) D are presented in Table 18. Vegetarians and vegans who did not use supplements had lower 25 (OH) D concentrations (-2.8 ng/mL, 95%CI -5.5, -0.1; and -5.3 ng/mL, 95%CI -8.1, -2.5; respectively); supplementing vegetarians had higher concentrations (3.7 ng/mL, 95%CI 0.3, 7.1) than omnivores. These differences were the same in minimally adjusted model 1, which along with age and sex, included season of the blood draw, and in model 2, which additionally adjusted for maternal education. This suggests that the differences were predominantly the result of the association of dietary/supplemental exposure with this outcome. These results are consistent with the existing evidence on serum 25-hydroxy vitamin D levels in vegetarians discussed in chapter II section, 9.6, and provide novel evidence on serum levels of this nutrient in vegans.

Table 18. Crude and adjusted mean differences of vegetarian and vegan children relative to omnivore children in serum 25 (OH) D levels

Outcome**	Model 1								Model 2							
	Vegetarian – non-suppl.		Vegetarian – suppl.		Vegan – non-suppl.		Vegan – suppl.		Vegetarian – non-suppl.		Vegetarian – suppl.		Vegan – non-suppl.		Vegan – suppl.	
	Δ*	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI
Serum 25 (OH) D ng/mL	-2.8	-5.5, -0.1	3.7	0.3, 7.1	-5.3	-8.1, -2.5	-1.0	-4.6, 2.6	-2.8	-5.4, -0.1	3.7	0.3, 7.1	-5.3	-8.1, -2.5	-1.0	-4.7, 2.7
Model 1:	Dietary group categorised according to supplementation status, age, sex, seasonality (sine and cosine function of the day of blood draw)															
Model 2:	Dietary group categorised according to supplementation status, age, sex, seasonality (sine and cosine function of the day of blood draw), maternal education															
*difference; ** outcomes affected by missing values of 3.																

5.5.4. Prevalence of abnormal vitamin B12, HGB, LDL- and HDL cholesterol status

Estimated prevalences and pairwise comparisons of abnormal vitamin B12, HGB, LDL- and HDL cholesterol status in the dietary groups regardless of their supplementation or fortification status are presented in Table 19. The prevalence of probable vitamin B12 deficiency was 3% in omnivores, 4% among vegetarians and 12% in vegans. The prevalence of possible B12 deficiency was 17%, 20% and 38% in omnivores, vegetarians and vegans respectively. The prevalence of moderate iron deficiency anaemia was 0% among omnivores, and 2% in vegetarians and vegans. The prevalence of mild anaemia was 0% in omnivores, 7% in vegetarians, and 6% in vegans. There were no children with severe iron deficiency anaemia.

The prevalence of abnormal paediatric LDL cholesterol status with high (≥ 130 mg/dl) and borderline high (110–129 mg/dl) LDL-C levels was 12% and 16% for omnivores; 5% and 9% for vegetarians, and 0% and 1% for vegans. The prevalence of low (> 45 mg/dL) and borderline (40–45 mg/dL) HDL-C was 6% and 10% for omnivores, 17% and 21% for vegetarians and 27% and 25% for vegans. For most of these comparisons, the estimated prevalences differed significantly between the vegetarians or vegans in comparison to omnivores (Table 19).

To date, the prevalence of iron deficiency using haemoglobin levels in vegetarian children was reviewed in a 2017 paper, that reported a prevalence ranging from 2.5% to 47.5% (Roman Pawlak and Bell 2017). However, the studies cited used different criteria for what constituted normal haemoglobin levels. There are no published data in vegan children.

Table 19. Estimated prevalence of inadequate vitamin B12, iron and cholesterol status

Outcome	Omnivore	Vegetarian	Vegan
<u>Vitamin B12*</u>			
Probable deficiency (<200 pg/mL)	3.2% (0.5,5.9)	4.1% (0.1,7.4)	12.3% (1.9-22.6)
Possible deficiency (≥200–350 pg/mL)	16.5% (7.8, 25.0)	20.0% (10.7, 29.3)	38.4%^ (26.9, 49.8)
<u>Haemoglobin**</u>			
Moderate (8.00-10.9 g/dl)	0%	1.9% (-0.3,4.0)	1.6% (-1.2, 4.5)
Mild (11.–11.4 g/dl)	0%	6.5% (-0.02, 13.3)	5.6%^ (1.0,10.2)
<u>LDL cholesterol***</u>			
High (≥130 mg/dL)	12.1% (3.1,21.1)	5.3% (1.1, 9.5)	0.4%^ (-0.4, 1.4)
Borderline (110–129 mg/dL)	16.2% (8.8, 23.5)	9.2%^ (3.9, 14.5)	1.0%^ (-1.0, 3.1)
Acceptable (<110 mg/dL)	71.7% (59.9,83.)	85.5%^ (77.7, 93.2)	98.5%^ (95.6, 100.1)
<u>HDL cholesterol***</u>			
Acceptable (>45 mg/dL)	84.0% (75.4, 92.6)	62.2%^ (49.0, 75.3)	48.0%^ (34.1, 62.0)
Borderline (40–45 mg/dL)	10.2% (4.8, 15.7)	20.8%^ (13.3, 28.3)	24.8%^ (17.0, 32.6)
Low (<40 mg/dL)	5.7% (1.7, 9.8)	17.0%^ (7.9-26.2)	27.1%^ (14.7, 39.5)
^pairs of estimated prevalences between vegetarians or vegans and the reference group of omnivores are significantly different at $p < .05$ * (Devalia, Hamilton, and Molloy 2014); (WHO 2018);*** (Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents and National Heart, Lung, and Blood Institute 2011)			

Studies on the prevalence of vitamin B12 deficiency among prepubertal children on vegan or vegetarian diets from industrialised countries with more than 5 participants are lacking. Similarly, no data exists on the prevalence of abnormal cholesterol status in vegan and vegetarian children according to the criteria established by the Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents and National Heart, Lung, and Blood Institute.

VII. DISCUSSION

This chapter summarizes the principal findings, separately for vegan and vegetarian children, and compares them with existing studies. Next, it discusses the implications of this study in light of its results showing both benefits and risks. The benefits are discussed in the context of CVD prevention; the risks in the context of the findings of lower height, lower bone mineral content and micronutrient deficiencies. Two hypotheses are put forward as to why vegetarian and vegan diets constrain height in children. Next, unanswered questions are identified and discussed in the context of potential future research projects. Following, clinical significance of the findings is presented for both health professionals and medical and nutritional organisations. And lastly, this chapter concludes that this study generated new important data on the range of both benefits and risks of vegetarian and vegan diets in children that add to current political, public health and medical debates on planetary and human health.

1. Principal findings

This study aimed to quantify prevalence differences in several indicators of health, including body composition, CVD risk and micronutrient status, along with estimating the prevalence of inadequate micronutrient and abnormal cholesterol status in either vegetarian or vegan children relative to a reference

group of omnivore children. Additionally, it set out to assess dietary intake, and in particular, the prevalence of inadequate nutrient intakes and to calculate median intakes of nutrients in the same group of children. I put forward four main hypotheses, each of them relating the hypothesized outcome differences of vegetarian or vegan children to a comparable group of children consuming traditional diets:

- 1) Plant-based diets in childhood are associated with more favourable CVD risk profile including lipid levels, glucose homeostasis, lower markers of chronic inflammation, lower surrogate markers of atherosclerosis, however also lower levels of IGF-1.
- 2) Plant-based diets in childhood are associated with less favourable growth patterns and selected aspects of body composition, including height, lean mass, bone mineral content.
- 3) Plant-based diets in childhood are associated with increased risk of nutritional deficiencies especially that of iron, vitamin B12 and vitamin D.
- 4) Vegetarian and vegan children have higher prevalence of nutrient inadequacy and lower intakes of several nutrients, in particular vitamin B12, iron and vitamin D3.

I found evidence in support of most of hypotheses apart from those relating to lean mass, surrogate markers of atherosclerosis and IGF-1 levels, as there was no evidence of significant differences in those health parameters between dietary groups. There were cardiometabolic benefits, but also some nutritional harm detected in children following vegan diets. In vegetarians, the harm was intermediate, however the benefits were negligible and accompanied by less

healthy cardiometabolic risk profile. These findings are summarized in detail below.

1.1. Vegan children

The body composition differences in vegans in relation to the omnivore group included lower indices of peripheral body fat and lower fat mass independent of height, however also lower gluteo-femoral fat. Lower body fat content was accompanied by similar lean mass when adjusted for size. These body composition differences were apparent across several assessment methods, and hence appear robust.

Cardiovascular risk benefits for vegans included lower concentrations of total cholesterol, LDL cholesterol (however also lower HDL cholesterol), along with lower serum markers of chronic inflammation (hs-CRP). Almost no vegan children had abnormally high LDL cholesterol levels. The CVD risk differences persisted after adjusting for body composition, increasing confidence in the hypothesis that the diets themselves played a causal role. There was no evidence of significant difference in average cIMT in vegans, however their mean values were lower than those of found in omnivores. Additionally, the omnivores in this study had lower cIMT than those found in comparable healthy children from other countries.

Potential nutritional harm was evident in three categories of findings. Firstly, it was manifested in adverse body composition outcomes relating to stature and bone. Vegans were shorter and had lower bone mineral density. These bone deficits persisted after adjusting for body and bone size.

Secondly, vegans who did not supplement or fortify their diet with vitamin B12 had lower serum vitamin B12 concentrations, accompanied by higher levels of homocysteine and MCV, which is suggestive of vitamin B12 deficiency. The prevalence of vitamin B12 deficiency among the vegans, based on recently established serum vitamin B12 level cut-offs, was higher than in omnivores. Furthermore, vegans had lower levels of three markers of body iron status, RBC, haemoglobin and ferritin, and a higher prevalence of iron deficiency anaemia based on WHO haemoglobin cut-offs. Lastly, among the non-supplementing vegans, lower levels of serum 25 (OH) D were detected. Supplementation of both vitamin B12 and vitamin D3 in vegans seems to rectify these problems, as no abnormal values were found in the supplementing group.

Thirdly, in comparison to omnivores, vegans had a higher prevalence of inadequate consumption of numerous nutrients, including vitamins A, B2, B3, B12, along with calcium, phosphorus, iron and zinc. The number of nutrients with prevalence of inadequate consumption of more than 5% was highest in this dietary group.

The dietary analysis revealed that the nutrient profile of vegans was more characteristic for unprocessed plant-based dietary pattern than that of the remaining dietary groups. They consumed more carbohydrates, but also more starch and fibre, PUFA, beta-carotene, vitamin C, folate, vitamin E, magnesium, and potassium and less fat, saturated fat, protein and sucrose than both vegetarians and omnivores.

1.2. Vegetarian children

The evidence of lower body fat mass in vegetarians was suggestive, but did not reach statistical significance, other than the values for lower gluteo-femoral fat, which is a negative finding, as this type of fat is associated with lower cardiometabolic risk (Manolopoulos, Karpe, and Frayn 2010). At the same time, the vegetarians were shorter than the omnivores, although the height difference in comparison to omnivores was smaller than that of vegans. Additionally, the vegetarians had lower total body and L2-L4 bone mineral content, however this disappeared after adjusting for bone and body size.

The only cardiovascular benefit in vegetarians was a lower concentration of total cholesterol and lower prevalence of abnormally high LDL cholesterol levels. However, this was accompanied by higher concentrations of fasting glucose, triglycerides, VLDL cholesterol, and lower concentrations of HDL cholesterol than in omnivores. These differences were strengthened after adjusting for body composition, suggesting some of the adverse effects of their diet on CVD risk profile were mediated, and in this case, counter-balanced by their tendency to lower body fat. There was no evidence of difference in average cIMT in vegetarians in comparison to omnivores.

Similar to vegans, they had lower blood concentrations of vitamins B12 and 25 (OH) D, if they were not given supplements of these nutrients. Supplemented vegetarians had no evidence of deficits in these vitamin levels. They also had lower concentrations of markers of iron status (Ht, ferritin), however these differences disappeared after adjusting for socioeconomic traits. All the blood micronutrient differences in comparison to omnivores were less pronounced

than those of vegans. In comparison to omnivores, vegetarians had higher prevalences of inadequate intake of vitamins B2, B3 along with iron and zinc.

The vegetarians' intakes of nutrients linked to unprocessed plant-based dietary pattern (starch, fibre, PUFA, beta-carotene, vitamin B3, vitamin C, folate, vitamin E, magnesium, and potassium) were lower than those of vegans, and in the case of beta-carotene and potassium (markers of fruit and vegetable consumption) did not differ significantly from the omnivores. They did not consume significantly less sucrose than omnivores. This nutrient profile suggests a lower intake of unprocessed plant foods and a higher intake of processed carbohydrates, which might be the reason for the lack of substantial CVD benefits along with the less healthy cardiometabolic profile in this group.

2. Strengths and limitations of the study

The main strength of this study comprises the detailed and systematic approach to health assessment, spanning a broad range of outcomes, allowing me to identify both potential risks and potential benefits of different diets. A second strength is that the sample included had significant numbers of vegetarians and vegans allowing me to detect differences of magnitude >0.5 SD in all health outcomes between the plant-based diet groups and omnivores. Moreover, the sample was well-matched across the diet groups for age, sex, and several SES indices.

This is the first such study that included more than 50 participants in each dietary group assessing both diet and health outcomes in such a comprehensive way. Strict dietary inclusion criteria were applied, and children that were semi-vegetarian were excluded to allow for a clear distinction of the effect of excluding animal products on health outcomes. The study also provides the first evidence of usual nutrient intakes of vegetarian and vegan children, which were obtained by converting the observed nutrient data with the MSM method, discussed in chapter III, into estimates of the long-run average intakes. This is crucial for accurate estimation of nutrient inadequacy risk. All previous dietary analyses conducted in this group used observed data only.

A wide range of known potential confounders and presumed mediators was measured and included in statistical models as a separate set for each outcome group. The identification of confounders and mediators for each outcome was carried out with the Directed Acyclic Graphs method, put forward by the most recent theoretical and methodological developments in casual inference (Van

der Weele 2019). None of the previous studies have adjusted health-outcomes in vegetarian or vegan children for any confounders other than sex and age. This means that the results might not have represented the true associations of diet with the studied health parameters. This study also went beyond previous research in measuring physical activity objectively, and body composition via three independent techniques.

This project provides the first thorough evaluation of vegan children in comparison to well-matched omnivore counterparts, and the first study correcting for bone size in the measurements of bone status in children on plant-based diets.

Limitations of the study are as follows.

Firstly, there were some missing data, although in the case of most variables, the numbers of missing values were small ($\leq 2,1\%$). Additionally, due to a faulty operation of the blood pressure monitor, I was obliged to discard the blood pressure data collected.

I was unable to recruit the number of vegan children planned based on the power calculations. I attribute this mainly to the scarcity of vegan children in the Polish but also other industrialised populations and the reluctance of at least some vegan families to participate in medical examinations and research. The samples of vegetarian or vegan children in studies so far have generally been relatively small. As noted in a systematic review of studies on vegetarian and vegan children by Schuermann et al. 2017, the median sample size of the group consuming either vegetarian or vegan diets of 24 publications from 16 studies published from 1988 to 2013 was 35. Importantly, the only evidence on vegan

children older than 3 years of age comes from two studies. The first one by Sanders et al. 1988 included 23 vegan participants, while the second one included an unidentified number of strictly vegan children among 404 vegetarian children living in a closed- commune (O'Connell et al. 1989). Despite this, my study detected highly significant and sizeable differences in lipids - the primary outcomes for which power calculations had been made prior to recruitment. Post hoc power calculations using the observed means, standard deviations and group counts in vegan and meat-eating children suggested that the study had a 100% power to detect differences between these groups at $\alpha = 0.05$. Decrease in sample size necessarily leads to loss of power. Thus, false negatives in secondary outcomes cannot be excluded as a result of the failure to recruit the planned number of vegan participants.

Moreover, the study was not powered to detect differences smaller than 0.5 SD, which might still be of public health significance. I did not adjust for multiple testing, for the reasons given in chapter V, section 9.1.

Although one of the main consequences of vitamin B12 and iron deficiencies in childhood is neurocognitive impairment, the assessment of neurological or cognitive functions were not conducted in this study. There are several reasons for this. Firstly, the cognitive of vegan and vegetarian children was tested in our pilot project and no differences were detected in comparison to the control group (Książyk et al. 2012). Secondly, this study focused on growth, body composition, nutritional and cardiovascular risk, and adding cognitive assessment would prove unfeasible, given the considerable number of parameters assessed and financial constraints of this project.

Another limitation of this study is that its results cannot be generalised to children in middle- and low-income countries, other ethnic or age groups. The health effects of plant-based diets on children's health in non-industrialised countries are confounded by other factors, including limited choice of plant-based foods and other effects of poverty such as poor sanitation and frequent infections, as discussed in section 4.2.1 in the next part of this chapter. It is not known if the physiological responses to vegetarian and vegan diets in childhood are mediated by ethnic background, as informative studies in this area are lacking. And finally, nutritional needs vary throughout the periods of infancy, childhood and adolescence and separate studies are needed to establish the health outcomes of these diets in different age groups.

The method of sampling used, namely convenience sampling, is non-random in its nature, and hence we cannot generalize the results to the whole population of vegetarians and vegans with confidence. This is because the sample could have under- or overrepresented certain members of this population. Additionally, the results could be biased due to reasons why certain parents chose to take part in this study and others did not (Jager, Putnick, and Bornstein 2017). And finally, for the reasons outlined in chapter V, section 1, this study was not a randomised trial, and hence cannot prove causation. Utmost care has been undertaken to adjust for most the known confounders linked to adopting vegetarian diets, as discussed in chapter II, section 2.6. Nevertheless, some residual confounding cannot be excluded. This could include the personality traits of mothers adopting vegetarianism or veganism for themselves and hence their children, which could have affected the choice of healthier or low-calorie foods in the household.

3. Comparison with other studies

Most previous studies in this area have used cross-sectional design, had limited population samples and heterogeneous dietary group classification criteria, examined only a few isolated health parameters, and lacked well-matched (or indeed any) control groups. Only three previous studies examined vegan children, and two of them addressed anthropometry only, and lacked a reference group. Within those three studies, the Farm study did not specify the number of vegans at the time of data collection.

Where comparable, the results of this study are broadly consistent with previous research. Where the findings differ, the most likely explanation is differences in study design and definition or duration of the exposure (vegetarian diet). As described in detail in Chapter II, section 9.3, the majority of the other studies showed that anthropometric measures of children following meat-free diets were similar or reduced relative to the reference group. It has been hypothesized that differences in physical activity might have contributed to these results (Schürmann, Kersting, and Alexy 2017), but this study found no such difference, suggesting that diet itself is the likely causal factor (the limitations of cross-sectional study in inferring causality notwithstanding).

To date, iron status has been compared between vegetarian and omnivore children only (Chapter II, section 9.6) and none of the studies adjusted for any confounders other than age and sex. Most of them found lower indices or iron status in vegetarians, with the exception of one study in infants (Sievers et al. 1991) and one Polish study which concluded that markers of iron metabolism were within the normal physiological range, however the authors did not reveal details of how the data compared to the omnivore group (Laskowska-Klita et al.

2011). These findings are consistent with the results of my study, which found lower concentrations of haematocrit and ferritin in unadjusted model 1 among vegetarians. However, upon adding confounders to the analysis, the differences in mean serum concentrations of several iron metabolism parameters between vegetarians and omnivores disappeared, which suggests the influence of other, in this case SES, factors that are linked to both vegetarian diets and iron levels. Iron metabolism parameters were lower in vegans in both unadjusted and fully adjusted models, which suggests a causative effect of their diet. Therefore, this study provides new data on inadequate iron status in vegan children.

According to previous research, the prevalence of vitamin B12 deficiency in vegetarian and vegan children and adolescents ranges from 0 to 33% (Pawlak, Lester, and Babatunde 2014). However, these figures were based on 5 studies, some of which included fewer than seven participants of varying age, and the studies used lower deficiency thresholds (<96 pmol/l) for serum vitamin B12 levels. Therefore they might not describe the true prevalence of deficiency according to the higher thresholds (Oh 2019), and the wider range of biomarkers (Devalia, Hamilton, and Molloy 2014; Klee 2000), applied in this study. Our study included three biomarkers and both crude and adjusted analyses indicated inadequate B12 status in vegetarians, and outright deficiency in vegans who were not given supplements of this nutrient. The prevalence of probable and possible deficiency in vegetarians was 4.1% and 20% respectively, while the figures for vegans were 12.3% and 38.4%. Only the prevalence of possible deficiency in vegans was significantly different from the omnivores, however this might reflect the sample size of our study rather than the actual lack of prevalence difference between vegetarians and omnivores.

As summarized in Chapter II, section 9.6, previous studies found lower 25 (OH) D concentrations in vegetarian children, relative to omnivore controls or published reference values. Controlling for vitamin D supplementation practice and season, we demonstrated lower levels in vegetarians and vegans who did not supplement.

Regarding cardiometabolic risk, two studies showed more favourable blood lipid levels in paediatric vegetarians in comparison to omnivores (Krajcovicova-Kudlackova et al. 1997; Ambroszkiewicz et al. 2011) and one study, that included fish-eaters as vegetarians (Nathan, Hackett, and Kirby 1996b) did not. In contrast, our study found a consistently better lipid profile only in vegans. One study (Ambroszkiewicz et al. 2017) reported lower level of CRP in vegetarians than omnivores, whereas this study found lower hs-CRP only in vegans.

Two previous studies assessed BMD of vegetarian children (all prepubertal) and lower BMD z-scores for total and lumbar spine were reported. Neither of these studies considered vegans. However, these published data are difficult to interpret, as bone mineral content was not adjusted for smaller body size and bone area. To our knowledge, no group has previously assessed bone mass in vegan children, or cIMT in children on vegetarian and vegan diets.

4. Implications of this research

Plant-based diets are increasingly promoted around the world to improve human and planetary health. The World Cancer Research Fund (WCRF 2019b) has been recommending plant-based diets in order to lower cancer risk for the

last three decades. The British Dietetic Association has recently launched an Environmentally Sustainable Diet Project (BDA 2017b), an educational toolkit for registered dietitians, aimed at helping them teach their patients dietary sustainability. Its main principle involves the reduction of meat and other animal-based products, and replacing them with plant-based alternatives. In 2019, the EAT-Lancet Commission advocated a largely vegetarian diet for most people over 2 years old, in order to optimise both human and planetary health (Willett et al. 2019).

The call to adopt mostly plant-based diets comes not only from scientific sources. Numerous celebrities and politicians actively advocate this way of eating, in order to minimize climate change. In the UK, for example, the Duchess of Sussex declares being mostly vegan, and according to the popular press has announced that she will raise her child this way (Oehler 2019). A well-known child climate-change activist, Greta Thunberg, is a vegan and actively promotes this way of eating to lower our carbon footprint (Pritchett 2019). The Polish Nobel prize winner in literature for 2018, Olga Tokarczuk, is a vegetarian and promotes animal welfare and planetary sustainability in her work (Tokarczuk 2009) and interviews (Weryński 2019). The Polish European Parliament Member Sylwia Spurek (Spurek 2019) promotes veganism for planetary sustainability and animal welfare, actively lobbying for these issues in the headquarters of the European Union. Other examples in the UK include former Labour party leader Jeremy Corbyn (VIVA 2019a), and former leader of the Green party, Nathalie Bennett (VIVA 2019b). Even international fast-food chains are adopting this universal trend and starting to offer vegan options (BBC 2019).

These trends are likely to affect both parents and their children and despite the lack of formal estimates, numerous sources indicate that more people are adopting meat-free diets in industrialised countries, as outlined in chapter I. However, these co-exist with a sparsity of data on the health effects of vegetarian diet in children, and a virtual absence of any up-to-date data in the case of vegan children. The majority of information still comes from studies of adults, whose nutritional requirements differ from those of children. Therefore, this study will help to fill the knowledge gap, helping both parents and medical professionals make more informed decisions about adopting meatless diets in children.

4.1. Implications for cardiometabolic health

Adult vegetarians and vegans tend to have a better cardiometabolic risk profile than omnivores, which is a key reason why they have an approximately 25% lower risk of developing ischaemic heart disease (Desmond et al. 2018). However, as described in detail in Chapter II, section 3, atherosclerosis starts in childhood, and develops in relation to classical CVD risk factors, which track into adulthood. There is growing recognition that primordial prevention (i.e. avoiding the development of risk factors before disease onset through promotion of healthy lifestyle throughout childhood) should be embraced as a major component of global CVD prevention policies (Tanrikulu, Agirbasli, and Berenson 2016). So far, paediatric CVD prevention strategies have been focused on tackling childhood obesity, although success rates are unimpressive (Colquitt et al. 2016). Importantly, aside from BMI, other classical CVD risk factors affected by diet in children are also associated with adult atherosclerosis (Juonala et al. 2010). Additionally, components of the childhood diet themselves

track into adulthood (Mikkilä et al. 2005), and correlate with adulthood CVD risk factors, vascular markers of subclinical atherosclerosis, and CVD risk (Kaikkonen, Mikkilä, and Raitakari 2014). Therefore, improving the health aspects of childhood diets may play a key role in reducing adult CVD.

This study shows that vegan diets in children are associated with a better cardiovascular risk profile, and therefore might contribute to lowering adulthood CVD. This might be achieved not only by their effects on blood biochemistry and other CVD risk factors, but also via establishment of healthy eating patterns, which could offer an effective strategy of primordial CVD prevention, and a novel opportunity to promote a healthy childhood trajectory toward adult cardiovascular health and longevity. Nutrient intake profile of the studied vegan children is indicative of higher consumption of unprocessed plant foods, like whole grains, nuts and fruits and vegetables, than both vegetarians and omnivores and this could be the potential reason for their superior CVD risk profile.

At the same time, the results show that not all plant-based diets are associated with the same CVD benefits, and that a healthy diet means more than simply avoiding meat. Poorly planned plant-based diets might not bring any of the above-mentioned benefits, and might even worsen an individual's CVD risk factor profile or CVD risk itself (Satija et al. 2017). This was demonstrated among the vegetarian children in this study, who had higher levels of several parameters included in the metabolic syndrome risk factor cluster. The most likely explanation for this is their higher consumption of processed carbohydrates, in conjunction with lower fat and protein intakes, as both of the latter macronutrients reduce glycaemic responses in a dose-dependent fashion

(Moghaddam, Vogt, and Wolever 2006). This dietary pattern was associated with a less favourable CVD risk factor profile than that of omnivores. It seems that in conjunction with lower fat and protein intake, the 'penalties' of higher processed carbohydrate intakes were greater, and were not nullified by the somewhat higher fruit and vegetable intakes of vegetarians, suggested by their nutrient profiles (higher fibre, vitamin C, potassium, magnesium intakes than those of the omnivores).

Another potential reason contributing to the poorer cardiometabolic profile of vegetarians in this study (especially among girls), might be their 'reduced metabolic capacity', i.e. suboptimal organ development that occurs in foetal and early infant life. Birth weight, a marker of such capacity, was lower in vegetarian and vegan girls. Greater birth weight within the normal range, promotes greater metabolic capacity. Increased capacity lowers the risk of adverse cardiometabolic responses to greater 'metabolic load', which includes among other lifestyle factors, an unhealthy diet (Wells 2019). In the vegans, who followed a more 'unprocessed' plant-based dietary pattern, the reduced metabolic capacity was not exposed to higher metabolic load. In contrast, the vegetarians' exposed their lower metabolic capacity to a higher dietary metabolic load than that of the vegans (but lower than that of the omnivores), and this may account for their poorer cardiometabolic status.

4.2. Implications for nutritional risk

Beyond CVD risk, this study addresses knowledge gaps regarding the safety of plant-based diet in children. It suggests that the restriction of foods of animal

origin could prevent children from achieving optimal height or bone mineral status, and could lead to selected nutritional deficiencies.

4.2.1. Stature

The shorter stature of children consuming plant-based diets may have mixed implications for long term health. Taller height is associated with higher social status, and some studies suggest this is causal rather than just an artefact of social correlates (Stulp et al. 2015; Tyrrell et al. 2016). Taller adult height is also associated with lower risk of NCDs such as diabetes, heart disease, but is also a risk factor for diverse cancers (Sawada et al. 2017; Abar et al. 2018). Below I discuss two potential explanations for the lower height in children on plant-based diets.

4.2.1.1. Hypothesis 1 – vegetarian and vegan diets in childhood constrain growth during infancy and early childhood

4.2.1.1.1. Foods, nutrients and growth

Numerous nutrients affect growth, the most notable being energy, protein, iron, iodine, zinc and vitamin A (Rivera et al. 2003). All of these are known as ‘limiting’ (Poore and Nemecek 2018)(Poore and Nemecek 2018) nutrients (Poore and Nemecek 2018), and they might be potentially deficient or less bioavailable in plant-based diets (Melina, Craig, and Levin 2016; Millward 2017). Additionally, plant-based foods tend to have poorer quality protein, because their proteins are less digestible and contain lower amounts of some essential amino acids, in particular lower levels of lysine in

cereals and of methionine in legumes (US Institute of Medicine 2005). Therefore, in comparison to animal proteins, plant proteins have lower anabolic properties, and rather than used for protein synthesis, are more directed toward oxidation (Berrazaga et al. 2019).

In low-income countries, where predominantly plant-based diets are consumed, growth retardation is often observed (Rivera et al. 2003; Millward 2017). These diets are often limited to staples of grains or starchy root crops, and differ from the more versatile plant-based diets followed by children in high-income countries. Nevertheless, they are deficient in the same nutrients (and often energy), that are limiting in vegetarian and especially vegan diets in general (Keats et al. 2019; Millward 2017). Although poor sanitation and resulting frequent infections in low-income societies are considered as equally important factors negatively affecting growth (Millward 2017), multi-micronutrient interventions containing at least vitamin A, iron, zinc, B vitamins, can have a positive effect on children's growth in these settings (Ramakrishnan et al. 2004), however similar interventions in pregnancy did not affect growth prenatally (Devakumar et al. 2016). Moreover, observational studies in countries like Egypt, Kenya and Mexico, showed associations between animal-source foods (ASF) consumption and better growth, which persisted even after controlling for socioeconomic status, morbidity, parental education and nutritional status (Neumann, Harris, and Rogers 2002). In these studies, concentrating mainly on young and school-age children, the greatest deficits in linear growth were found in those with little animal source foods in their diet.

Interventions with ASF to improve growth have been implemented also in Europe, starting in the early 1920s. Most of those interventions were based on

milk and led to the recognition of its role as a key determinant of height growth in stunted and healthy children alike (Atkins 2005). In the Boyd-Orr study conducted in Scotland in 1920s (Leighton and Clark 1929), the intervention with milk, as opposed to biscuits with the same energy content, resulted in 20% greater growth in school children in the milk group but not in the biscuit group during 7-month intervention period, compared to controls. It is likely that the children in that period suffered from some degree of malnutrition (Mølgaard et al. 2011).

More recently, avoiding dairy has been linked to shorter stature in healthy pre-pubertal school children in New Zealand (Black et al. 2002). Similarly, the total intake of animal protein and milk, but not of vegetable or meat protein, was associated with height, along with increased serum concentration of IGF-1 in healthy Danish preschool children (Hoppe, Udam, et al. 2004). Higher IGF-1 concentrations were presumably induced by higher milk consumption. Although some studies have not found links of milk intake and growth (Cook et al. 1979; Grillenberger et al. 2003), the evidence that milk intake has growth-promoting effects is substantial (Mølgaard et al. 2011). The most supported explanation of the mechanisms through which milk promotes growth is the stimulation of IGF-I synthesis and insulin secretion (Mølgaard et al. 2011).

This study found no evidence of differences in insulin, IFG-1 or molar ratio of IFG-1/IGFBP-3 (which reflects free biologically active IGF-1) between the dietary groups. However, other hypotheses exist and link milk's bioactive peptides, calcium, phosphorus or magnesium content (or potentially, undiscovered components) to growth effects (Hoppe, Mølgaard, and Michaelsen 2006). Calcium in particular, which was consumed in significantly

lower amounts by vegans in this study, is linked to increases in bone strength and size and has been associated with height (Prentice et al. 2005). Additionally, vegetarians and especially vegans in this study, consumed less protein and their protein was of lower quality in the context of growth. Vegans, who had the lowest height in this study, also had a higher prevalence of inadequate iron and zinc intake and the highest prevalence of inadequate vitamin A intake. Beyond dietary intake analysis, there was also evidence of lower body iron status in this dietary group based on blood assays. All these nutrients are linked to growth (Rivera et al. 2003). Despite the fact that vegetarians consumed dairy products, and based on their calcium intake, they did so probably in similar amounts to the omnivores, they also had the highest prevalence of inadequate zinc intake, and other than that, their intake of growth-supporting nutrients was intermediate and lower than that of omnivores. This might therefore explain their intermediate height score.

4.2.1.1.2. Critical periods of growth affected by malnutrition

The first potential explanation of the lower height among the vegetarians and vegan children is that these diets constrain the whole process of growth through their lower quantity and quality of nutrients. This influences growth process at two early critical phases that may determine shorter height for the rest of life (Karlberg 2002). According to the infancy-childhood-puberty (ICP) growth model, which describes human growth from the latter half of intrauterine life through to maturity, the human growth process is divided into three additive and partly superimposed phases: infancy, childhood and puberty (Tse, Hindmarsh, and Brook 1989). The second phase, the childhood component, begins after the

6th month of life and takes place until 18 months. A normal age at onset of the childhood component is crucial for early linear growth, and a delayed onset will result in shortness, regardless of subsequent life circumstances (Karlberg 2002). In this growth phase, the mechanisms that regulate linear growth change from one system to another, i.e. from being nutritionally dependent but mostly growth hormone independent in the infancy phase, to being growth hormone dependent in the childhood phase. One of the hypothesized reasons for delayed onset of that phase is an inadequate supply of specific nutrients, including trace elements and vitamins (Karlberg 2002). It is likely that the shorter stature, at least for some vegetarian and vegan children following their respective diets from infancy, might have been 'programmed' during that phase of growth (Lucas 1991).

Additionally, although this explanation is less likely, as it does not apply to all study participants, the plant-based diets of the mothers might have influenced the infancy phase during the latter half of the intrauterine life. There was evidence of lower birth weight in vegetarians and especially vegans, though only in girls. The predictors of lower birth weight at term in industrialised countries include older maternal age, shorter maternal height, and low pre-pregnancy body mass index (Clausson, Cnattingius, and Axelsson 1998). Although we did not measure maternal age at birth, there was no difference in pre-pregnancy BMI or maternal height across the dietary groups. Neither was there evidence of differences in paternal height, which could have additionally influenced birthweight (Nahum and Stanislaw 2003). In fact, the average heights of mothers and fathers in all dietary groups were above the Polish average. Furthermore, all parents were of Caucasian ethnicity, which precludes

maternal or paternal contribution of different ethnicity to foetal growth (Wells et al. 2013).

At the same time, preliminary evidence suggests a negative influence of plant-based diets on foetal growth and an increased risk of SGA, as discussed in chapter VI, section 2.2. Therefore, it is likely that the plant-based diets followed by 42% of mothers of vegetarians and 29% of mothers of vegan children during pregnancy might have contributed to these birth weight differences, especially given the fact that more girls came from plant-based pregnancies than boys, and they were more affected by lower birth weight. Birth weight is well established to be positively associated with adult height (Eide et al. 2005), and is a strong predictor of childhood height. Infants with lower birth weights are likely to remain shorter and lighter throughout childhood, especially those who are intrauterine growth retarded rather than premature (Binkin et al. 1988; Hediger et al. 1998).

4.2.1.2. Hypothesis 2 – vegetarian and vegan diets in childhood delays puberty and modulates growth trajectory

The second potential reason for the height difference might be a different sexual maturation pattern and thus delayed puberty growth phase, which might eventually result in normal adult height, as suggested by (Sabaté, Llorca, and Sánchez 1992). These authors found that 11- to 12-year-old US Seven Day Adventist girls following a lacto-ovo-vegetarian diet were 3 to 3.5 cm shorter than omnivore girls of the same age. However, the same group of researchers also reported a delayed onset of menarche (up to 6 months) among vegetarian SDA girls in comparison to omnivore control group from the same area (Kissinger and Sanchez 1987). This finding was recently substantiated by larger

studies, whereby plant proteins were positively, and meat and total protein intake negatively, associated with the age of puberty onset or menarche (Günther et al. 2010; Rogers et al. 2010). On this basis, plant-based diets might slow the process of growth and maturation during childhood, without reducing final height in adulthood.

Limited evidence from adult studies exists to support the hypothesis of (Sabaté, Llorca, and Sánchez 1992). Data from the EPIC-Oxford study showed that life-long vegetarians (n = 125 men and 265 women) do not differ significantly in height from those who become vegetarian in adulthood (Rosell, Appleby, and Key 2005). Although this is the largest study on growth in vegetarians to date, the authors cautioned that statistical power to detect a difference of 1.0 cm between these groups was 0.76 in women and 0.38 in men, and hence the possibility of small differences in height between these groups, particularly in men, could not be excluded. There are no such data on the height of life-long vegans.

The children from my study were 5–10 years old and were recruited to represent the pre-pubertal population. Although pubertal stage was not ascertained, the median age at menarche for contemporary Polish girls is 12.77 years, and the mean age of puberty onset in boys (assessed by the Tanner second stage of pubic hair growth) is 11.80 years (Kryst et al. 2012). Therefore, any height differences are not likely to be the result of the earlier onset of puberty in omnivore children. However, an alternative scenario of the acceleration of height in early childhood and thus a faster transition to puberty among omnivores cannot be excluded and therefore definite conclusions cannot be made based on the data from this study.

In conclusion, based on the results of this study I hypothesise, that vegetarian and vegan diets, via their influences in the intra-uterine life, and especially during the critical phase of childhood growth, might constrain the whole process of growth. Further studies are needed to examine how this affects their attained adult height. Additionally, separate studies are warranted to determine if contemporary vegetarian and vegan children enter puberty later, and thus experience a longer period of prepubertal growth, and how this affects their adult stature.

4.2.2. Bone

The findings for BMC are a matter of some concern. It is recommended to maximise paediatric bone mass accrual in order to promote peak bone mass which is associated with long term bone health, and lower osteoporosis risk and fracture risk in adulthood (Sopher, Fennoy, and Oberfield 2015; Weaver et al. 2016). The results of this study show that vegans have lower bone mass even after taking into account their smaller body and bone size, which might increase the risk of adverse bone outcomes later in life. Although this finding comes from a cross-sectional observational study, it may provide preliminary evidence for a causal relationship. This is because there are several reasons to substantiate temporal directionality from the exposure to the outcome in this study. Firstly, the diet assignment precedes the bone outcome ascertainment, and secondly the duration of adherence to the respective diets was at least one year prior to the study commencement. Although the latter was self-reported rather than measured, the results of health outcomes that can be affected by plant-based diets from this study are consistent with the body of evidence in vegetarian and

vegan children and adults alike, and hence substantiate the accuracy of self-report.

The cross-sectional character of the study, however, prevents me from identifying the mechanisms or dietary determinants for lower mineral content in vegans. It is estimated that around 20% of the variation in peak bone mass is explained by lifestyle factors, including nutrition (Ferrari 2005). Among the nutrients identified as critical for bone health are calcium, vitamin D3, total and dairy protein (Weaver et al. 2016). A potential explanation for my findings could therefore be lower intake of several nutrients crucial for bone health like calcium, vitamin D3, and lack of higher-quality protein, especially that from dairy (Golden et al. 2014; Weaver et al. 2016) by the vegans in this study.

Inorganic bone matrix is composed of hydroxyapatite crystals involving compounds like calcium phosphate and calcium hydroxide, and vitamin D increases blood calcium levels which supports bone calcium deposition (Hoppe, Mølgaard, and Michaelsen 2006). Low calcium intakes in childhood have been implicated in low bone mineral mass (Institute of Medicine Food and Nutrition Board 2011). Additionally, evidence from calcium supplementation intervention trials supports the bone-building role of this mineral (Vatanparast and Whiting 2006). However, such non-milk calcium supplementation effects are usually short-term (Winzenberg et al. 2006).

More recently, attention has been diverted to milk proteins (casein and whey) as having unique effects on bone metabolism. Habitual milk protein, but not meat protein intake in prepubertal boys, was negatively associated with serum osteocalcin in prepubertal boys, which might be linked to greater BMD in children (Budek et al. 2007). Total and dairy, but not meat protein was positively

associated with size-adjusted bone mineral content in adolescents, and the positive association persisted after correction for energy, calcium, and physical activity and did not seem to be mediated via current serum IGF-I (Budek et al. 2007). The results of the latter study seem to confirm the associations seen in this study, whereby size-adjusted BMC was lower in children who did not consume dairy products in the absence of IGF-1 or energy intake differences in comparison to the omnivore reference group.

This study is also consistent with findings in adults. According to a Bayesian meta-analysis of nine studies of 2749 adult subjects (1880 women and 869 men) (Ho-Pham, Nguyen, and Nguyen 2009), in comparison to omnivores, bone mineral density was ca. 4% lower in vegetarians (95% CI: 2%, 7%) in both femoral neck and the lumbar spine respectively, and 6% lower (95% CI: 2%, 9%) lower at both sites in vegans. The authors noted that much of the effect of vegetarian diets on bone density was due to a vegan, not lacto-ovo-vegetarian diet, and that a complex interplay between dietary calcium and protein intakes (rather than their separate effects), could explain the results. Lower bone density in adult vegans does translate into higher fracture risk, however this effect seems to be limited to those who have inadequate calcium intakes (Appleby et al. 2007). This finding is relevant in the discussion of the potential future consequences of the BMC finding among vegan children in this study.

4.2.3. Micronutrient deficiencies

Vitamin B12 deficiency in children can lead to megaloblastic anaemia and neurocognitive deficits or delays (Sezgin, EM, Erdöl Ş, Özdemir Ö, Baytan B 2011; Jain et al. 2015). The blood assay results of the vegans were

characteristic of the diagnostic components of megaloblastic anaemia cluster (lower Hgb, RBC, serum vitamin B12, higher MCV). Neurocognitive damage can also be caused by iron deficiency (Georgieff 2011). This study contradicts the claim expressed in the latest position statement on the American Academy of Nutrition and Dietetics on vegetarian and vegan diets issued for all life stages, that individuals can adapt to a better absorption of non-haeme iron sources in the long term (Melina, Craig, and Levin 2016). This does not seem to be the case in vegan prepubertal children. Long-term developmental consequences of chronic inadequate status of these nutrients in children on plant-based diets in high-income countries are unknown. Although case reports of neurocognitive impairment due to vitamin B12 deficiency in unsupplemented vegan infants continue to be described in different countries (Roed C, Skovby F 2009; Guez et al. 2012), data on older children are sparse and are limited to two studies from industrialised countries. The first, a nested case–control study, compared Dutch macrobiotic vegan adolescents ($n = 93$; 10–16 years) who consumed vegan diets from birth until 6 years of age with controls who had consumed an omnivorous diet ($n = 102$; 10–18 y) during the same time period. The vegan group had significantly lower fluid intelligence scores than did adolescents in the omnivorous diet group (Dawson-Hughes et al. 2000). On the other hand, data from the pilot project to this study on 46 Polish vegetarians and vegans aged 1,4–7,6 years, who followed their respective diets for at least one year, showed normal cognitive development, with IQ higher than the population reference (Książyk et al. 2012).

In low-income settings, in a Kenyan trial that supplied children consuming inadequate intakes and/or with biochemical indices of micronutrient deficiencies

of iron, zinc, vitamins A and B12 along with B2 and calcium, with local snacks and meat, milk (or equivalent energy), the meat group improved cognitive function more than milk (Neumann, Harris, and Rogers 2002). Similarly, the Nutrition Collaborative Research Support Program project, a series of US government supported studies on food and nutrition issues in developing countries, found associations between the intake of animal source foods and cognitive function among school children in Egypt (Neumann, Bwibo, and Sigman 1992) and Mexico (Allen et al. 1992) controlling for an array of covariates. Finally, higher homocysteine levels have been postulated as a risk factor for cardiovascular disease, although current evidence remains inconclusive (Chrysant and Chrysant 2018).

Figure 12. Directionality and clinical significance of the main findings

Outcome	Vegetarian	Clinical significance	Vegan	Clinical significance
Prevalence of inadequate nutrient intakes	↑	Potential negative impact on growth and neuro-cognitive development.	↑↑	Potential negative impact on growth and neuro-cognitive development
Lean mass	≈	n/a	≈	n/a
Fat mass	≈	n/a	↓	Potential positive impact on future cardiometabolic risk
Bone mineral content	≈	n/a	↓	Potential negative influence on peak-bone mass and future risk of fractures
LDL-C	≈	Potential negative impact on future cardiometabolic risk.	↓	Potential positive impact on future cardiometabolic risk
HDL-C	↓		↓↓	
Triglycerides	↑		≈	
Glucose	↑		≈	
HOMA-IR	≈		≈	
hs-CRP	≈		↓	
Hgb	≈	n/a	↓	Increased risk of iron deficiency with potential negative consequences for growth and neuro-cognitive development.
Ferritin	≈	n/a	↓	
Vitamin B12	↓*	Increased risk of vitamin B12 deficiency with potential negative	↓↓*	Increased risk of vitamin B12 deficiency and megaloblastic anaemia with potential

		consequences for neuro-cognitive development.		negative consequences for neuro-cognitive development and other consequences of this deficiency.
25(OH)D	↓*	Increased risk of 25(OH)D vitamin deficiency and potential negative consequences for bone development.	↓↓*	Increased risk of 25(OH)D vitamin deficiency and potential negative consequences for bone development.
Designations: ↑ higher than omnivores, ↑↑ higher than omnivores, magnitude of the difference stronger than in the other plant-based group; ≈ no significant difference in relation to omnivores; ↓ lower than omnivores, ↓↓ lower than omnivores, magnitude of the difference stronger than in the other plant-based group; * if unsupplemented.				

Abbreviations: LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; hs-CRP, high sensitivity CRP; Hgb, haemoglobin, 25 (OH) D, 25-Hydroxy Vitamin D.

5. Unanswered questions and future research and clinical significance

There remain several unanswered questions that could stimulate future research.

- It is not clear what aspects of plant-based diets in children might be responsible for lower height and bone mineral outcomes (if these are causal) and at what stages of development they exert their influence.
- It is not known whether supplementation or altering diet composition can rectify these problems.
- Vitamin B12 supplementation guidelines for this paediatric population do not exist.
- We do not know the extent and consequences of long-term cardiometabolic benefits and nutritional risk.

Additional studies are, therefore, warranted, including intervention trials (with supplements or/and vegan diets with modified nutrient content) and longitudinal

observational research. Finally, these data relate to children aged 5–10 years, and the risks and benefits among children of different ages, especially infants, might be different and should be investigated as the critical phases for growth and development concentrate around infancy.

Based on the results from this study, several important messages can be communicated to health professionals, parents and researchers. Physicians and dietitians should educate their patients on both the potential benefits and risks of vegetarian and vegan diets in children, emphasizing their associations with stature and bone, as, unlike most other nutritional risks, these might not be preventable or reversible. Beyond supplementation, clear guidance on how to balance vegetarian and vegan diets so that they are healthy should be provided and regular surveillance of children's health status performed. Parents should be informed that plant-based diets might bring health benefits, however if they are not well planned, those benefits are not guaranteed and that they are associated with health risks. The priority for future research projects is to establish how to maximise the benefits and minimise the risks of these diets. Finally, these issues should be considered in current public health debates on plant-based diets, and in the position statements of expert organisations, which should tailor their recommendations to different age groups.

6. Conclusion

When I started this research project, the topic of vegetarianism and veganism was regarded mainly as a niche dietary choice of health-conscious people, animal welfare activists or a part of a religious practice. Since then it has become a matter of global significance in the context of planetary sustainability.

Plant-based diets are currently advocated internationally as a crucial strategy to counter balance climate change and feed the growing world population. This study brings new important data to this discussion, that so far, lacked substantial evidence of the health outcomes in vegetarian and especially vegan children. My results show that the associations of plant-based diets with health outcomes in children might not be the same than those in adults. Similarly to the adult population, they can provide substantial cardiometabolic benefit, provided they are based on minimally processed plant foods. At the same time, they might increase cardiometabolic risk if based on processed foods. I have also provided preliminary evidence that they might exert lasting and potentially irreversible effects during critical windows of growth and development, although more research is needed to understand this aspect fully and caution is needed when interpreting the results from observational study. Finally, vegetarian and vegan diets in children are also linked to higher risk of nutritional deficiencies, especially if not planned well. Hence, these diets provide a range of health benefits and risks, and future studies should concentrate on understanding how to capture these benefits while minimising the risks.

VIII. Supplementary material

1. Complete case analyses

Table 20. Adjusted medians of usual intakes of nutrients in dietary groups, complete case analyses

Outcome	Model 1						Model 2						Model 3					
	Meat-eaters		Vegetarians		Vegans		Meat-eaters		Vegetarians		Vegans		Meat-eaters		Vegetarians		Vegans	
	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI
Energy (kcal)	1615 ^a	1540, 1690	1648 ^a	1554, 1741	1593 ^a	1471, 1715	1602 ^a	1519, 1685	1610 ^a	1522, 1698	1595 ^a	1475, 1716	1571 ^a	1473, 1668	1652 ^a	1555, 1749	1623 ^a	1462, 1785
Protein (g)	57.1 ^a	53.7, 60.5	47.4^b	44.3, 50.4	42.9^c	39.5, 46.4	57.4 ^a	53.6, 61.1	48.8^b	45.7, 51.9	43.4^c	38.7, 48.1	57.6 ^a	54.9, 60.4	48.3^b	45.5, 51.0	44.5^c	40.7, 48.3
Carbohydrates (g)	221 ^a	211, 231	254^b	239, 269	262^c	241, 283	219 ^a	200, 238	251^b	226, 277	275^c	253, 296	219 ^a	209, 229	255^b	244, 266	270^c	260, 279
Starch (g)	99 ^a	94, 104	109 ^a	100, 117	117^b	108, 127	94 ^a	87, 101	107^b	97, 118	125^c	115, 135	92 ^a	86, 99	109^b	100, 118	123^c	115, 132
Sucrose (g)	50.9 ^a	44.9, 56.9	45.8 ^a	40.4, 51.2	37.5^b	28.0, 47.1	53.6 ^a	45.9, 61.2	45.4 ^a	39.7, 51.1	34.9^b	29.0, 40.8	52.6 ^a	46.5, 58.6	44.0^b	39.6, 48.5	38.4^c	33.7, 44.6
Fibre (g)	15.2 ^a	13.6, 16.8	24.9^b	21.9, 27.9	32.7^c	28.7, 36.7	16.6 ^a	14.2, 19.0	24.7^b	22.0, 27.5	33.7^c	30.0, 37.3	17.2 ^a	15.1, 19.2	25.1^b	22.9, 27.3	32.1^c	29.5, 34.7
Fat (g)	66.1 ^a	62.3, 69.9	57.5^b	52.4, 62.7	54.8 ^{ab}	42.0, 67.6	63.7 ^a	56.6, 70.8	54.2^b	49.2, 59.2	54.6 ^{ab}	42.2, 67.0	63.1 ^a	59.0, 67.2	56.8^b	53.4, 60.2	54.9^c	50.4, 59.3
Saturatedfat (g)	25.1 ^a	23.6, 26.6	18.7^b	17.1, 20.2	11.4^c	9.6, 13.2	25.0 ^a	22.8, 27.1	17.9^b	16.5, 19.4	12.6^c	10.1, 15.1	24.3 ^a	21.8, 26.7	17.7^b	16.1, 19.4	12.0^c	10.5, 13.5
MUFA (g)	25.1 ^a	23.4, 26.9	20.1^b	18.2, 22.1	20.3^c	16.9, 23.7	25.3 ^a	22.2, 28.4	20.0^b	17.4, 22.5	22.4 ^{ab}	18.2, 26.7	25.1 ^a	23.4, 26.8	20.3^b	18.8, 21.7	20.9^c	19.5, 22.4
PUFA (g)	9.6 ^a	8.9, 10.3	11.5^b	10.2, 12.8	16.2^c	13.3, 19.1	9.2 ^a	7.5, 10.9	11.7^b	10.0, 13.4	17.8^c	14.1, 21.4	9.4 ^a	8.1, 10.7	12.9^b	11.4, 14.4	17.8^c	15.2, 20.3
Omega 3 PUFA (mg)	1.74 ^a	1.52, 1.96	1.82 ^a	1.45, 2.19	2.35 ^a	1.77, 2.94	1.78 ^a	1.35, 2.22	1.94 ^a	1.58, 2.31	2.43 ^a	1.42, 3.43	1.89 ^a	1.56, 2.21	1.96 ^a	1.68, 2.24	2.36 ^a	1.72, 2.91
Omega 6 PUFA (mg)	7.5 ^a	6.9, 8.1	9.8^b	8.4, 11.2	13.4^c	11.1, 15.8	7.0 ^a	6.0, 8.0	9.7^b	8.3, 11.0	13.9^c	10.9, 16.9	7.6 ^a	6.0, 9.3	10.8^b	9.3, 12.4	14.5^c	11.6, 16.3
Cholesterol (mg)	252 ^a	236, 268	153^b	136, 169	34^c	18, 50	252 ^a	219, 285	157^b	140, 173	32^c	17, 46	252 ^a	216, 288	151^b	127, 175	33^c	19, 47

Outcome	Model 1						Model 2						Model 3					
	Meat-eaters		Vegetarians		Vegans		Meat-eaters		Vegetarians		Vegans		Meat-eaters		Vegetarians		Vegans	
	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI
Vitamin A (mcg RAE)	616 ^a	541, 691	585 ^a	491, 679	533 ^a	421, 645	605 ^a	506, 705	601 ^a	481, 722	550 ^a	437, 663	626 ^a	533, 719	621 ^a	490, 751	583 ^a	494, 671
Beta - carotene equivalents (mcg)	2982 ^a	2512, 3453	4033^b	3356, 4710	5382^c	4274, 6490	2943 ^a	2327, 3559	3813 ^a	3002, 4625	5507^b	4143, 6870	3270 ^a	2568, 3972	4396^b	3704, 5087	5687^c	4416, 6957
Vitamin B1 (mg)	1.01 ^a	0.93, 1.09	1.16 ^a	1.02, 1.29	1.56^b	1.39, 1.74	0.97 ^a	0.79, 1.15	1.25^b	0.99, 1.51	1.55^c	1.31, 1.79	1.01 ^a	0.92, 1.10	1.28^b	1.17, 1.39	1.60^c	1.50, 1.71
Vitamin B2 (mg)	1.35 ^a	1.22, 1.47	1.38 ^a	1.25, 1.50	1.14^b	0.99, 1.28	1.36 ^a	1.22, 1.50	1.35 ^a	1.20, 1.50	1.20 ^a	1.01, 1.40	1.38 ^a	1.24, 1.52	1.39 ^a	1.25, 1.52	1.29 ^a	1.11, 1.48
Vitamin B3 (mg)	12.6 ^a	11.5, 13.7	9.6^b	8.2, 11.0	13.0 ^{ab}	11.3, 14.7	12.2 ^a	11.0, 13.4	10.0^b	8.6, 11.3	12.3 ^{ab}	10.6, 14.0	12.6 ^a	11.0, 14.1	9.7^b	8.6, 10.9	12.7 ^{ab}	11.7, 13.8
Vitamin B6 (mg)	1.54 ^a	1.42, 1.65	1.69 ^a	1.49, 1.89	2.07^b	1.85, 2.30	1.53 ^a	1.35, 1.72	1.63 ^a	1.36, 1.90	2.18^b	1.98, 2.37	1.61 ^a	1.46, 1.76	1.60 ^a	1.38, 1.81	2.11^b	1.84, 2.38
Folate (mcg)	227 ^a	211, 243	305^b	277, 334	420^c	378, 463	242 ^a	218, 267	324^b	291, 357	404^c	359, 450	251 ^a	229, 272	318^b	288, 348	400^c	357, 443
Vitamin B12 (mcg)	7.2 ^a	6.8, 7.5	6.7 ^a	5.8, 7.5	3.9^b	2.0, 5.8	6.8 ^a	5.9, 7.8	6.5 ^a	5.0, 8.0	5.2 ^a	0.9, 9.5	7.3 ^a	6.2, 8.3	6.7 ^a	5.4, 8.0	6.7 ^a	3.4, 10.1
Vitamin C (mg)	110 ^a	95, 125	146^b	119, 173	182^c	129, 235	103 ^a	74, 131	149^b	131, 168	188^c	118, 258	109 ^a	84, 134	147 ^a	124, 171	189^b	135, 244
Vitamin D3 (mcg)	3.49 ^a	2.62, 4.35	2.75 ^a	1.51, 3.99	1.56^b	0.64, 2.48	3.62 ^a	2.43, 4.81	3.04 ^a	1.81, 4.28	2.62 ^a	0.34, 4.91	3.90 ^a	1.82, 5.98	3.39 ^a	1.39, 5.39	2.47 ^a	-0.15, 5.09
Vitamin E (mg)	9.0 ^a	8.3, 9.7	11.7^b	10.1, 13.2	14.1^c	11.4, 16.9	9.0 ^a	7.7, 10.3	11.5^b	10.2, 12.8	15.8^c	14.0, 17.6	9.2 ^a	8.3, 10.2	11.6^b	10.5, 12.8	14.2^c	12.5, 16.0
Calcium (mg)	532 ^a	484, 581	611^b	563, 659	398^c	346, 450	526 ^a	451, 601	598 ^a	531, 665	402^b	333, 471	528 ^a	454, 601	609 ^a	569, 648	425^b	381, 469
Copper (mg)	0.93 ^a	0.85, 1.01	1.54^b	1.39, 1.68	2.02^c	1.80, 2.24	0.95 ^a	0.84, 1.06	1.51^b	1.36, 1.67	2.09^c	1.91, 2.26	1.01 ^a	0.88, 1.13	1.57^b	1.37, 1.78	2.07^c	1.94, 2.21

Outcome	Model 1						Model 2						Model 3					
	Meat-eaters		Vegetarians		Vegans		Meat-eaters		Vegetarians		Vegans		Meat-eaters		Vegetarians		Vegans	
	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI
Iron (mg)	8.5 ^a	7.8, 9.2	11.8^b	11.0, 12.7	13.4^c	12.2, 14.6	8.5 ^a	7.8, 9.3	12.0^b	10.9, 13.2	13.9^c	12.5, 15.3	9.4 ^a	8.7, 10.0	11.7^b	10.9, 12.4	14.0^c	13.4, 14.7
Magnesium (mg)	225 ^a	206, 243	310^b	284, 335	405^c	371, 440	220 ^a	192, 248	309^b	273, 346	415^c	366, 463	239 ^a	213, 264	318^b	292, 344	426^c	388, 463
Manganese (mg)	2.78 ^a	2.41, 3.14	4.64^b	4.10, 5.18	6.69^c	6.02, 7.36	2.89 ^a	2.34, 3.43	4.62^b	3.99, 5.25	6.91^c	6.17, 7.65	3.10 ^a	2.54, 3.67	4.80^b	4.18, 5.41	6.59^c	5.74, 7.44
Potassium (mg)	2252 ^a	2128, 2376	2675^b	2478, 2871	3297^c	3032, 3563	2209 ^a	1991, 2428	2546^b	2368, 2724	3272^c	2955, 3589	2321 ^a	2127, 2514	2689^b	2450, 2929	3115^c	2786, 3444
Zinc (mg)	7.36 ^a	6.65, 8.08	8.19 ^a	7.40, 8.98	8.97^b	7.64, 10.29	7.51 ^a	6.69, 8.33	8.43 ^a	7.36, 9.50	8.95 ^a	7.23, 10.67	7.68 ^a	7.07, 8.30	8.30 ^a	7.10, 9.50	9.55^b	8.94, 10.16
Phosphorus (mg)	980 ^a	926, 1034	980 ^a	905, 1055	1022 ^a	941, 1102	919 ^a	863, 974	1033^b	948, 1118	1047^c	961, 1132	979 ^a	925, 1032	1043 ^a	958, 1129	1067^b	994, 1109
Model 1:	Diet group, age, sex																	
Model 2:	Dietary group, age, sex, religion, urbanicity, seasonality, family history of obesity, parental education																	
Model 2:	Dietary group, age, sex, religion, urbanicity, seasonality, family history of obesity, parental education, breastfeeding at 6, 6-12 and over 12 months, fat mass z-score (DXA), lean mass z-score (DXA), birthweight (fifths), average movement count per minute internal z-score, usual caloric intake																	

Abbreviations: g, gram; mg, milligram; RAE, retinal activity equivalents; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids.

Table 21. Crude and adjusted mean differences of vegetarian and vegan children relative to omnivore children in anthropometry, complete case analyses

Outcome**	Model 1				Model 2			
	Vegetarian		Vegan		Vegetarian		Vegan	
	Δ^*	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI
Height z-score	-0.45	-0.77, -0.12	-0.55	-0.97, -0.12	-0.38	-0.69, -0.08	-0.65	-1.01, -0.29
BMI z-score	-0.24	-0.54, 0.06	-0.50	-0.82, -0.18	-0.25	-0.56, 0.05	-0.48	-0.81, -0.15
Biceps skinfold z-score***	0.03	-0.21, 0.27	-0.23	-0.53, 0.06	0.05	-0.20, 0.30	-0.17	-0.49, 0.14
Suprailiac skinfold z-score***	-0.06	-0.34, 0.23	-0.49	-0.79, -0.20	-0.08	-0.37, 0.22	-0.52	-0.84, -0.20
Subscapular skinfold z-score**	0.08	-0.20, 0.36	-0.31	-0.64, 0.03	0.08	-0.22, 0.37	-0.29	-0.65, 0.08
Triceps skinfold z-score***	-0.13	-0.43, 0.17	-0.56	-0.87, -0.24	-0.15	-0.45, 0.15	-0.54	-0.87, -0.22
Waist girth z-score	-0.24	-0.51, 0.04	-0.23	-0.50, 0.05	-0.24	-0.51, 0.04	-0.26	-0.56, 0.04
Hip girth z-score	-0.20	-0.53, 0.13	-0.59	-0.86, -0.31	-0.20	-0.52, 0.11	-0.68	-0.98, -0.37
Thigh girth z-score***	-0.37	-0.65, -0.09	-0.61	-0.90, -0.31	-0.36	-0.64, -0.08	-0.58	-0.88, -0.29
Model 1:	Diet group only							
Model 2:	Diet group, maternal height, paternal height, birthweight (fifths), gestational age (fifths), maternal pre-pregnancy BMI (fifths), average movement count per minute internal z-score, breastfeeding duration (<6, 6–12, >12 months), maternal education, paternal education, urbanicity; multiple imputation was used to account for missing data							
*difference, ** (Wells et al. 2012); ***outcomes affected by missing values of ≤ 4								

Table 22. Crude and adjusted mean differences of vegetarian and vegan children relative to omnivore children in body composition, complete case analyses

Outcome**	Model 1				Model 2			
	Vegetarian		Vegan		Vegetarian		Vegan	
	Δ^*	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI
Lean mass by DXA z-score	-0.25	-0.60, 0.09	-0.36	-0.82, 0.10	-0.18	-0.53, 0.16	-0.41	-0.78, -0.04
Fat mass by DXA z-score	-0.35	-0.74, 0.05	-0.81	-1.18, -0.44	-0.34	-0.75, 0.07	-0.77	-1.14, -0.39
Lean mass by D₂O z-score	-0.26	-0.60, 0.08	-0.18	-0.59, 0.23	-0.22	-0.60, 0.16	-0.31	-0.69, 0.06
Fat mass by D₂O z-score	-0.31	-0.67, 0.05	-0.85	-1.27, -0.42	-0.36	-0.74, 0.01	-0.88	-1.33, -0.44
Lean mass index D₂O z-score	0.041	-0.306, 0.389	0.260	-0.123, 0.644	0.050	-0.361, 0.461	0.205	-0.181, 0.592
Fat mass index D₂O z-score	-0.25	-0.63, 0.13	-0.79	-1.21, -0.37	-0.32	-0.72, 0.08	-0.81	-1.27, -0.34
Model 1:	Diet group only							
Model 2:	Diet group, maternal height, paternal height, birthweight (fifths), gestational age (fifths), maternal pre-pregnancy BMI (fifths), average movement count per minute internal z-score, breastfeeding duration (< 6, 6–12, > 12 months), maternal education, paternal education, urbanicity; multiple imputation was used to account for missing data							
* difference, * (Wells et al. 2012)								

Table 23. Crude and adjusted mean differences of vegetarian and vegan children relative to omnivore children in bone outcomes, complete case analyses

Outcome**	Model 1				Model 2				Model 3			
	Vegetarian		Vegan		Vegetarian		Vegan		Vegetarian		Vegan	
	Δ^*	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI
TBLH BMC (%) ***	-0.081	-0.138, -0.024	-0.167	-0.248, -0.086	-0.082	-0.142, -0.023	-0.166	-0.246, -0.087	0.011	-0.011, 0.033	-0.037	-0.064, -0.010
L2-L4 BMC (%) ***	-0.058	-0.110, -0.005	-0.105	-0.172, -0.039	-0.052	-0.105, 0.001	-0.102	-0.166, -0.038	-0.004	-0.040, 0.032	-0.051	-0.096, -0.006
BMAD L2-L4 z-score ****	-0.058	-0.110, -0.006	-0.106	-0.173, -0.040	-0.053	-0.105, -0.001	-0.104	-0.168, -0.040	-0.006	-0.042, 0.031	-0.055	-0.100, -0.011
BMAD L2-L4%ile	-0.11	-0.43, 0.22	-0.66	-1.07, -0.26	-0.06	-0.43, 0.30	-0.61	-1.06, -0.17	.	., .	.	., .
Model 1:	Diet group, age, sex											
Model 2:	Diet group, age, sex, maternal education, religion, urbanicity											
Model 3:	Diet group, age, sex, maternal education, religion, urbanicity, height z-score (UK), weight z-score (UK), bone area											
*difference; ** outcomes affected by missing values of < 5;*** variable log-transformed, results represent percent difference; **** (Crabtree and et al. 2004)												

Table 24. Crude and adjusted mean differences of vegetarian and vegan children relative to omnivore children in blood lipid levels, complete case analyses

Outcome**	Model 1				Model 2				Model 3			
	Vegetarian		Vegan		Vegetarian		Vegan		Vegetarian		Vegan	
	Δ^*	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI
Total cholesterol (mg/dL)	-11	-22, 0	-36	-46, -27	-15	-26, -4	-40	-51, -28	-14	-25, -2	-36	-47, -25
HDL cholesterol (mg/dL)	-6.8	-11.5, -2.1	-11.2	-15.8, -6.6	-8.3	-13.2, -3.4	-11.8	-16.8, -6.8	-8.5	-13.4, -3.6	-12.1	-17.4, -6.9
LDL cholesterol (mg/dL)	-6.4	-15.4, 2.5	-25.4	-33.6, -17.3	-8.6	-17.0, -0.2	-28.3	-38.1, -18.4	-7.6	-16.3, 1.2	-25.3	-35.0, -15.6
VLDL cholesterol (%mg/dL)***	0.16	0.03, 0.28	0.00	-0.15, 0.15	0.17	0.05, 0.30	0.02	-0.14, 0.17	0.20	0.07, 0.32	0.07	-0.09, 0.22
Triglycerides (%mg/dL)***	0.20	0.07, 0.33	0.02	-0.14, 0.18	0.24	0.11, 0.37	0.06	-0.11, 0.22	0.27	0.14, 0.40	0.12	-0.04, 0.28
Model 1:	Diet group, age, sex											
Model 2:	Diet group, age, sex, birthweight quintile, gestational age quintile, maternal pre-pregnancy BMI quintile, breastfeeding at 6, 6-12 and over 12 months, maternal education, paternal education, religion, urbanicity											
Model 3:	Diet group, age, sex, birthweight quintile, gestational age quintile, maternal pre-pregnancy BMI quintile, breastfeeding at 6, 6-12 and over 12 months, maternal education, paternal education, religion, urbanicity, height z-score (UK), fat mass z-score (DXA), lean mass z-score (DXA).											
* difference; ** outcomes affected by missing values of n=2; *** variable log-transformed, results represent percent difference.												

Table 25. Crude and adjusted mean differences of vegetarian and vegan children relative to omnivore children in fasting glucose, insulin and HOMA-IR, complete case analyses

Outcome**	Model 1				Model 2				Model 3			
	Vegetarian		Vegan		Vegetarian		Vegan		Vegetarian		Vegan	
	Δ^*	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI
Fasting insulin (μUI/mL)	0.35	-0.53, 1.23	-0.04	-0.93, 0.84	0.34	-0.61, 1.29	0.03	-0.90, 0.95	0.65	-0.26, 1.56	0.74	-0.15, 1.62
Fasting glucose (mg/dL)	3.9	1.5, 6.3	2.0	-0.6, 4.6	3.8	1.5, 6.2	1.8	-1.1, 4.7	4.3	2.0, 6.7	2.7	-0.3, 5.8
HOMA-IR (%) ***	0.15	0.01, 0.28	0.04	-0.09, 0.18	0.14	0.00, 0.28	0.05	-0.09, 0.20	0.19	0.05, 0.33	0.16	0.02, 0.30
Model 1:	Diet group, age, sex											
Model 2:	Diet group, age, sex, birthweight quintile, gestational age quintile, maternal pre-pregnancy BMI quintile, breastfeeding at 6, 6–12 and over 12 months, maternal education, paternal education, religion, urbanicity											
Model 3:	Diet group, age, sex, birthweight quintile, gestational age quintile, maternal pre-pregnancy BMI quintile, breastfeeding at 6, 6–12 and over 12 months, maternal education, paternal education, religion, urbanicity, height z-score (UK), fat mass z-score (DXA), lean mass z-score (DXA).											
* difference; ** outcomes affected by missing values of < 3; *** variable log-transformed, results represent percent difference.												

Abbreviations: HOMA-IR, homeostatic model of insulin resistance

Table 26. Crude and adjusted mean differences of vegetarian and vegan children relative to omnivore children in hs-CRP, cIMT, IGFBP3, IGF-1, complete case analyses

Outcome**	Model 1				Model 2				Model 3			
	Vegetarian		Vegan		Vegetarian		Vegan		Vegetarian		Vegan	
	Δ^*	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI
hs-CRP (%) ***	-0.32	-0.66, 0.02	-0.52	-0.86, -0.18	-0.35	-0.72, 0.02	-0.58	-0.98, -0.19	-0.33	-0.71, 0.06	-0.53	-0.95, -0.12
hs-CRP values < 1 (%mg/dL)***	-0.15	-0.44, 0.14	-0.36	-0.64, -0.07	-0.15	-0.48, 0.17	-0.42	-0.75, -0.08	-0.13	-0.46, 0.20	-0.35	-0.70, -0.01
cIMT (mm)	0.002	-0.009, 0.012	-0.010	-0.024, 0.004	0.002	-0.008, 0.013	-0.007	-0.021, 0.007	0.003	-0.007, 0.014	-0.005	-0.018, 0.009
IGFBP3 ($\mu\text{g/mL}$)	89	-149, 327	-100	-364, 165	8	-245, 261	-182	-459, 95	80	-158, 319	-43	-307, 221
IGF-1 (ng/mL)	-11	-43, 22	-9	-41, 22	-10	-41, 20	-6	-42, 30	4	-24, 32	23	-8, 53
Molar IGF1:IGFBP3 ratio	-0.019	-0.045, 0.007	-0.009	-0.035, 0.017	-0.016	-0.042, 0.010	-0.002	-0.030, 0.026	-0.005	-0.030, 0.019	0.019	-0.007, 0.044
Model 1:	Diet group, age, sex											
Model 2:	Diet group, age, sex, birthweight quintile, gestational age quintile, maternal pre-pregnancy BMI quintile, breastfeeding at 6, 6–12 and over 12 months, maternal education, paternal education, religion, urbanicity											
Model 3:	Diet group, age, sex, birthweight quintile, gestational age quintile, maternal pre-pregnancy BMI quintile, breastfeeding at 6, 6–12 and over 12 months, maternal education, paternal education, religion, urbanicity, height z-score (UK), fat mass z-score (DXA), lean mass z-score (DXA).											
* difference; ** outcomes affected by missing values of < 7; *** variable log-transformed, results represent percent difference.												

Table 27. Crude and adjusted mean differences of vegetarian and vegan children relative to omnivore children in selected serum indicators of the iron status, complete case analyses

Outcome**	Model 1				Model 2			
	Vegetarian		Vegan		Vegetarian		Vegan	
	Δ^*	95% CI	Δ	95% CI	Δ	95% CI	Δ	95% CI
RBC (M/μl)	-0.09	-0.18, 0.01	-0.23	-0.33, -0.12	-0.07	-0.172, 0.023	-0.21	-0.316, -0.114
HGB (g/dL)	-0.24	-0.50, 0.02	-0.38	-0.70, -0.06	-0.20	-0.47, 0.07	-0.35	-0.67, -0.03
HT (%)	-0.83	-1.60, -0.07	-1.05	-2.03, -0.08	-0.73	-1.52, 0.07	-0.98	-1.97, 0.01
Ferritin*** (%)	-0.19	-0.37, -0.01	-0.28	-0.48, -0.07	-0.14	-0.32, 0.03	-0.26	-0.45, -0.07
Model 1:	Diet group, age, sex							
Model 2:	Diet group, age, sex, maternal education, urbanicity, maternal smoking							
* difference; ** outcomes affected by missing values of 1; *** variable log-transformed, results represent percent difference.								

2. Documents for study participants

Information Sheet for Subjects in Research Studies

You and your parents/guardians will be given a copy of this information sheet.

Title of Project: **How the diet that the children are eating affects the workings of their bodies**

(Body composition and cardiovascular risk in Polish children consuming vegetarian, vegan or omnivore diets)

We are inviting vegan, vegetarian and meat eating children aged 5-10 years and their parents to participate in a project that will help us understand how their diet affects the workings of their bodies.

Details of the project:

Please read this letter, or ask someone else to read it to you. Your parents will also have information about the study and you can ask them to help you understand it.

Why are you doing the study?

We want to find out if the sort of food that children eat affects how their body works and how healthy they are. We hope that we can use the results of our study to give children and their parents better advice about what to eat.

Do I have to take part?

No, it is up to you and your parents to decide if you want to take part. Even if you decide to take part, you can still decide to leave the study and you don't have to give a reason.

What do I have to do?

If you and your parents agree that you will take part in the study, we will ask your parents to write down everything you eat for 4 days. This is so we know what sorts of foods you eat. Then we will invite you to the clinic and do some measurements to see how your body works. We will ask you and your parents to come in the morning before you have your breakfast. At the beginning we will ask you and your parents if you have any questions about the study and check that you still want to take part. Then we will do some measurements and ask some questions. These are listed below and we have also put some pictures so you can see what the machines look like:

1. We will give you some special tracer water to drink, and ask you to give us some of your saliva (spit). We will ask you to give us some more saliva after 4 hours. When this special water mixes with your saliva, we can see how much water is in your body

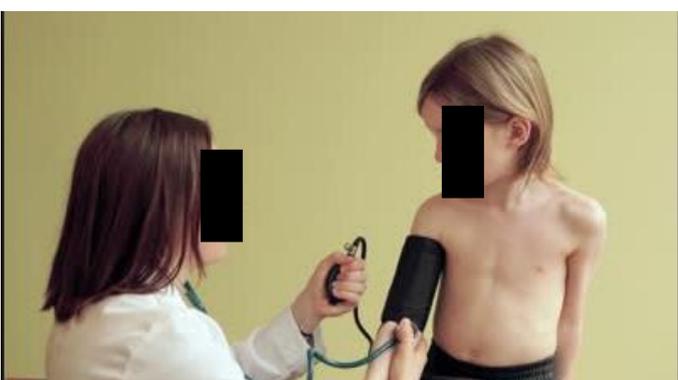


2. After this, we will take some of your blood to test the levels of iron, fat and vitamins. We will put some magic cream on your arm before the test so that it doesn't hurt. After the blood test, you will have time to rest and eat your breakfast.

3. Next, we will measure how big your head is, how tall and heavy you are and how thick your skin and muscles are by using calipers. These measurements are shown in the pictures. They do not hurt!



4. We will measure your blood pressure by putting a special sleeve on your arm and pumping it up so that it squeezes your arm for a few seconds.

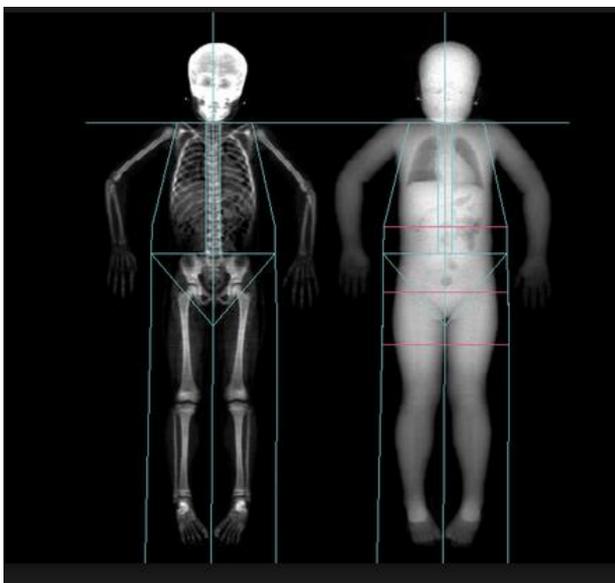


5. We will use an ultrasound probe on your neck, which can give us information about how your blood is flowing through your blood vessels.



6. At the end we will check how strong your muscles and your bones are. You will be asked to lie down on a bed and a camera will show your skeleton. We will give you a picture of your skeleton to keep like the one below!

7. We will ask you to do some exercises to show us how high you can jump and how hard you can squeeze a special 'grip' machine.



8. At the end we will give you a small red box to wear on a belt round your waist. This will record how much you move and exercise. We will ask you to wear this for 4 days after you leave the clinic. You can take it off when you go to bed or if you go swimming or have a bath or shower though!



When you visit the clinic, we will ask you to wear loose clothes without any metal – but you will not have to undress. Your Mum or Dad will be with you all the time.

At the end of the study, we will give you and your parents some information about how healthy your diet is and what can you do to make it even healthier. This will be the end of the project and you will be able to eat your lunch, as it will be a lunch time!

Will any of the measurements hurt?

Most of the measurements do not hurt at all. The only test that might hurt a little bit is the blood test – but this doesn't hurt as much as the other injections you will have had when you were younger. We will give

you some magic cream to numb the skin before the test. You do not have to do any of the tests if you do not like them.

Who can I ask if I have questions about the study?

You can speak to your parents, or you can contact one of the researchers – the details are given at the end of the letter.

Thank you for reading this information sheet!

Information Sheet for Subjects in Research Studies

You will be given a copy of this information sheet.

Title of Project: **Body composition and cardiovascular risk in Polish children consuming vegetarian, vegan or omnivore diets**

This study has been approved by the UCL Research Ethics Committee (Project ID Number): 0326/010

Name **Małgorzata Desmond**

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We would like to invite vegan and vegetarian children age 4-10 years old to participate in this research project.

Details of Study:

The aim of this study is to investigate how different diets: traditional, vegetarian and vegan, followed by children, affect the risk factors of heart disease in childhood. Additionally, the study will assess the adequacy and safety of the diet among study participants by conducting nutritional analysis and other non-nutritional tests.

We are recruiting 216 children (72 vegans, 72 vegetarians and 72 omnivores) aged 4-10.

We will invite vegan and vegetarian children by media and internet advertisements, and then we will ask them to bring their non-vegetarian friend to participate in the study.

We will first explain the study via an email or a postal contact to the parents of the participants. We will send an information sheet, consent form and a questionnaire that will qualify the participants according to age, sex, dietary status and health parameters. Those who will be eligible and whose parents/guardians agree to take part will be emailed or posted a 4-day food diary along with a dietary questionnaire that will have to be kept by parents (or guardians) not earlier than 2 weeks before the agreed visit at the hospital. The participants will be informed that while keeping the food diary, they will receive three phone calls from the researchers that will help them in filling in the diary. After a correctly kept diary has been received by an email or post, the participants will attend the Children's Memorial Health Institute's Paediatrics and Nutrition Department, arriving not later than 2 weeks from the date of the last food diary input and fasted for at least the previous 12 hours between 8 and 10 am.

Upon arrival, we will ask the parents of the participants to sign the consent form. Then the children will undergo blood tests carried out by experienced paediatric nurses. This will take approximately 15 minutes. After that, there will be a break and time for breakfast.

In the next part of the study we will measure children's' body weight, height, circumferences of the head, mid-upper arm, mid-thigh, waist and hip along with skinfolds of the biceps, triceps, subscapular and supriliac. This part can take up to 30 minutes. Subsequently, the children will be asked to consume a drink containing deuterium oxide (a non-toxic isotope of hydrogen) and then to give a sample of their saliva. This test will last no longer than 30 min.

Then their blood pressure will be measured three times (30 min). Next a radiologist will measure the thickness of their neck arteries, by a non –invasive ultrasound machine (20 min). This examination will be followed by tests that assess the musculoskeletal status: total body, lumbar spine and hand-wrist bone mineral density scans called DXA; peripheral quantitative computed tomography (pQCT) of leg and arm bones, quantitative ultrasound (QUS) measurement of the heel bone, grip strength, jumping mechanography and balance test. The DXA and QUS measurements will involve a total of approximately up to 20-30 minutes of lying down still (DXA) or sitting still (pQCT, QUS). The grip strength and jumping tests will involve active participation on child's part through moderate physical activity for approximately up to 10 minutes total, including short breaks between subsequent measurements.

Finally, the parents will be asked to give some general information on their education, income level, family size, the child's birth order, size at birth, gestational age, parental height, parental smoking, maternal pre-pregnancy BMI, breastfeeding practices, family history of heart disease, religion and if they have been on holidays with significant sun exposure recently. They along with the children will also be instructed on the correct way to wear an electronic monitor to measure physical activity and asked to wear the device for 4 days during waking hours. The monitor will have to be mailed back with a courier arranged by the researchers. The food diary and food questionnaire will be reviewed.

Parents will receive a short debriefing about the study, resource packs with the information on healthy eating practices pertaining to their dietary choice. Once the blood test results will be available, they will also receive information on all the parameters tested with a commentary in case of abnormalities. They will also receive a detailed dietary analysis.

We estimate that the data collection will finish at about 2.30 PM.

There are no known risks of participating in this study. A decision to withdraw at any time, or decision not to take part, will not affect the standard of care/education the participants will receive. If the parents agree to take part they will be asked whether they are happy to be contacted about participation in future studies. The participation in this study will not be affected should they choose not be re-contacted. If the parents decide to take part they will be given this information sheet to keep and will be asked to sign a consent form.

Please discuss the information above with others if you wish or ask us if there is anything that is not clear or if you would like more information.

It is up to you to decide whether to take part or not; choosing not to take part will not disadvantage you in any way. If you do decide to take part you are still free to withdraw at any time and without giving a reason.

All data will be collected and stored in accordance with the UK Data Protection Act 1998.

Informed Consent Form for Subjects in Research Studies

Please complete this form after you have read the Information Sheet and/or listened to an explanation about the research.

Title of Project: **Body composition and cardiovascular risk in Polish children consuming vegetarian, vegan or omnivore diets**

This study has been approved by the UCL Research Ethics Committee (Project ID Number): 0326/010

Thank you for your interest in taking part in this research. Before you agree to take part, the person organising the research must explain the project to you.

If you have any questions arising from the Information Sheet or explanation already given to you, please ask the researcher before you to decide whether to join in. You will be given a copy of this Consent Form to keep and refer to at any time.

Participant's Statement

I

- have read the notes written above and the Information Sheet, and understand what the study involves.
- understand that if I decide at any time that I no longer wish to take part in this project, I can notify the researchers involved and withdraw immediately.
- I agree to be contacted in the future by UCL researchers who would like to invite me to participate in follow-up studies.
- I understand that the information I have submitted will be published as a report and I will be sent a copy. Confidentiality and anonymity will be maintained and it will not be possible to identify me from any publications.
- consent to the processing of my personal information for the purposes of this research study.
- understand that such information will be treated as strictly confidential and handled in accordance with the provisions of the Data Protection Act 1998.
- agree that the research project named above has been explained to me to my satisfaction and I agree to take part in this study.

Signed:

Date:



**INSTYTUT „POMNIK – CENTRUM ZDROWIA DZIECKA”
AL. Dzieci Polskich 20, 04-730 Warszawa
KLINIKA PEDIATRII i ŻYWIENIA
Poradnia Żywienia**

**ATTENTION PARENTS OF CHILDREN ON VEGETARIAN AND VEGAN
DIETS.**

Studies show that adult vegetarians and vegans have lower risk of heart disease and several other chronic diseases. Our pilot study on vegan and vegetarian children from 2010 suggested that they might have lower cholesterol levels and lower body weight than their omnivore counterparts. This time we would like to study more children and test more parameters that could be affected by plant-based diets in order to evaluate their effects on children's health. Therefore, the Clinic of Paediatrics and Nutrition, Warsaw Memorial Health Institute in collaboration with the Institute of Child Health, University College London, are inviting children aged 5-10 years following vegetarian and vegan diets to participate in the study, that will include:

- Detailed nutritional analysis
- Anthropometric measurements
- Evaluation of body composition
- Evaluation of physical fitness
- Several blood tests (including tests assessing vitamin B12 and D status)
- Blood pressure measurement
- Evaluation of the health of arteries
- Evaluation of bone mineral density

Study results will be anonymous. Study participants from outside Warsaw County will be partially reimbursed for travel to and accommodation in Warsaw.

If you are interested in participation, please contact the staff of the clinic at:
e-mail: wege.poradnia@czd.pl ; tel. 663-918-818 (mgr Małgorzata Desmond, Jakub Sobiecki)



ATTENTION PARENTS OF CHILDREN AGED 5-10 YEARS OLD

Studies show that childhood diet can affect the risk of numerous diseases in adulthood. The Clinic of Paediatrics and Nutrition, Warsaw Memorial Children Health Institute is conducting a study that is trying to pinpoint the factors responsible for that risk. This is why we are trying to compare children on different diets: vegetarian, vegan and traditional.

You have most likely received that leaflet from your friends or family who have vegetarian or vegan children. We are specifically asking you to take part in this study, as you probably come from similar background as the vegetarian/vegan family. In order for the study to be meaningful, we need to compare children that do not differ much apart from their dietary habits. The fact that your child goes to the same school, lives in the same area or belongs to the same family, will let us analyse the health effects of the different diets more precisely.

Therefore, the Clinic of Paediatrics and Nutrition, Warsaw Memorial Health Institute in collaboration with the Institute of Child Health, University College London, are inviting friends of vegetarian and vegan children aged 4-10 years to participate in the study, that will include:

- **Detailed nutritional analysis**
- **Anthropometric measurements**
- **Evaluation of body composition**
- **Evaluation of physical fitness**
- **Several blood tests**
- **Blood pressure measurement**
- **Evaluation of the health of arteries**
- **Evaluation of the bone mineral density**

Study results will be anonymous. Study participants from outside Warsaw County will be reimbursed for travel to and accommodation in Warsaw.

If you are interested in participation, please contact the staff of the clinic at: e-mail: m.desmond@czd.pl ; tel. 663918818 (mgr Małgorzata Desmond, Jakub Sobiecki)

IX. REFERENCES

- Abar, L. et al. Height and body fatness and colorectal cancer risk: an update of the WCRF–AICR systematic review of published prospective studies. *European Journal of Nutrition* 57, 1701–1720 (2018).
- Aburto, N. J. et al. Effect of increased potassium intake on cardiovascular risk factors and disease: systematic review and meta-analyses. *BMJ* 346, f1378–f1378 (2013).
- Acosta-Navarro, J. et al. Reduced subclinical carotid vascular disease and arterial stiffness in vegetarian men: The CARVOS Study. *Int. J. Cardiol.* 230, 562–566 (2017).
- Albanese, C. V., Diessel, E. & Genant, H. K. Clinical Applications of Body Composition Measurements Using DXA. *J. Clin. Densitom.* 6, 75–85 (2003).
- Alberti, C. et al. Serum concentrations of insulin-like growth factor (IGF)-1 and IGF binding protein-3 (IGFBP-3), IGF-1/IGFBP-3 ratio, and markers of bone turnover: Reference values for French children and adolescents and z-score comparability with other references. *Clin. Chem.* 57, 1424–1435 (2011).
- Alberti, K. et al. Harmonizing the metabolic syndrome: A joint interim statement of the international diabetes federation task force on epidemiology and prevention; National heart, lung, and blood institute; American heart association; World heart federation; International. *Circulation* 120, 1640–1645 (2009).
- al-Dlaigan, Y. H., Shaw, L. & Smith, A. J. Vegetarian children and dental erosion. *Int. J. Paediatr. Dent.* 11, 184–92 (2001).
- Allen, L. H., Backstrand, J. R., Chivez, A. & Pelto, G. H. People Cannot Live by Tortillas Alone: The Results of the Mexico Nutrition CRSP. (1992).
- Allender, S., Scarborough, P., O’Flaherty, M. & Capewell, S. Patterns of coronary heart disease mortality over the 20th century in England and Wales: Possible plateaus in the rate of decline. *BMC Public Health* 8, (2008).
- Ambroszkiewicz, J. et al. Anti-Inflammatory and Pro-Inflammatory Adipokine Profiles in Children on Vegetarian and Omnivorous Diets. *Nutrients* 10, 1241 (2018).

- Ambroszkiewicz, J. et al. Bone status and adipokine levels in children on vegetarian and omnivorous diets. *Clin. Nutr.* 38, 730–737 (2019).
- Ambroszkiewicz, J. et al. Serum concentration of adipocytokines in prepubertal vegetarian and omnivorous children. *Med. Wieku Rozwoj.* 15, 326–34 (2011).
- Ambroszkiewicz, J. et al. Serum Hcpidin and Soluble Transferrin Receptor in the Assessment of Iron Metabolism in Children on a Vegetarian Diet. *Biol. Trace Elem. Res.* 180, 182–190 (2017).
- Ambroszkiewicz, J. et al. The Assessment of Bone Regulatory Pathways, Bone Turnover, and Bone Mineral Density in Vegetarian and Omnivorous Children. *Nutrients* 10, 183 (2018a).
- Ambroszkiewicz, J., Klemarczyk, W., Chełchowska, M., Gajewska, J. & Laskowska-Klita, T. Serum homocysteine, folate, vitamin B12 and total antioxidant status in vegetarian children. *Adv. Med. Sci.* 51, 265–8 (2006).
- Ambroszkiewicz, J., Klemarczyk, W., Gajewska, J., Chełchowska, M. & Laskowska-Klita, T. Serum concentration of biochemical bone turnover markers in vegetarian children. *Adv. Med. Sci.* 52, 279–82 (2007).
- Ambroszkiewicz, J., Laskowska-Klita, T. & Klemarczyk, W. Low levels of osteocalcin and leptin in serum of vegetarian prepubertal children. *Med. Wieku Rozwoj.* 7, 587–91 (2003).
- Andersen, L. B. et al. Physical activity and clustered cardiovascular risk in children: a cross-sectional study (The European Youth Heart Study). *Lancet* 368, 299–304 (2006).
- Andersen, P. *Modern Epidemiology*. 2nd edn. Kenneth J. Rothman and Sander Greenland, Lippincott-Raven, U.S.A., 1998. No. of pages: 737. Price: \$74.50. ISBN 0-316-75780-2. *Stat. Med.* 19, 881–882 (2000).
- Appleby, P., Crowe, F., Bradbury, K., Travis, R. & Key, T. Mortality in vegetarians and comparable nonvegetarians in the United Kingdom. *Am. J. Clin. Nutr.* 103, 218–230 (2016).
- Appleby, P., Roddam, A., Allen, N. & Key, T. Comparative fracture risk in vegetarians and nonvegetarians in EPIC-Oxford. *Eur. J. Clin. Nutr.* 61, 1400–1406 (2007).
- Arslanian, S. & Suprasongsin, C. Insulin Sensitivity, Lipids, and Body Composition in Childhood: Is 'Syndrome X' Present? *J. Clin. Endocrinol. Metab.* 81, 1058–1062 (1996).
- Atkins, P. The Milk in Schools Scheme, 1934-45: 'Nationalization' and resistance. *History of Education* 34, 1–21 (2005).
- Aune, D., Norat, T., Romundstad, P. & Vatten, L. J. Whole grain and refined grain consumption and the risk of type 2 diabetes: a systematic review and dose–response meta-analysis of cohort studies. *Eur. J. Epidemiol.* 28, 845–858 (2013).

- Bachrach, L. K. Acquisition of optimal bone mass in childhood and adolescence. *Trends Endocrinol. Metab.* 12, 22–8 (2001).
- Bailey, R. L. et al. Estimation of total usual calcium and vitamin D intakes in the United States. *J. Nutr.* 140, 817–22 (2010).
- Barnard, N. D. et al. A Low-Fat Vegan Diet Improves Glycemic Control and Cardiovascular Risk Factors in a Randomized Clinical Trial in Individuals With Type 2 Diabetes. *Diabetes Care* 29, 1777–1783 (2006).
- Baş, M., Karabudak, E. and Kiziltan, G. Vegetarianism and eating disorders: Association between eating attitudes and other psychological factors among Turkish adolescents'. *Appetite* 44(3), pp. 309–315 (2005).
- Basiotis, P. P., Welsh, S. O., Cronin, F. J., Kelsay, J. L. & Mertz, W. Number of Days of Food Intake Records Required to Estimate Individual and Group Nutrient Intakes with Defined Confidence. *J. Nutr.* 117, 1638–1641 (1987).
- BBC. McDonald's latest fast food chain to join vegan craze - BBC News. (2019). Available at: <https://www.bbc.com/news/business-50726619>. (Accessed: 17th December 2019)
- BDA. British Dietetic Association confirms well-planned vegan diets can support healthy living in people of all ages. (2017a). Available at: <https://www.bda.uk.com/news/view?id=179>. (Accessed: 2nd July 2019)
- BDA. Environmentally Sustainable Diet Toolkit - One Blue Dot. (2017b). Available at: https://www.bda.uk.com/professional/resources/environmentally_sustainable_diet_toolkit_-_one_blue_dot. (Accessed: 17th December 2019)
- Beaton, G. H. Criteria of an adequate diet. in *Modern Nutrition in Health and Disease* (ed. Shils ME, editor; Olson JA, editor; Shike M, editor. eds.) 1491–1505. (Lea & Febiger, 1994).
- Bekkers, M. B. M. et al. BMI and Waist Circumference; Cross-Sectional and Prospective Associations with Blood Pressure and Cholesterol in 12-Year-Olds. *PLoS One* 7, (2012).
- Belgique, A. R. de M. de. Le Veganisme proscrit pour les enfants, femmes enceintes et allaitantest. (2019). Available at: <https://updlf-asbl.be/articles/avis-de-larmb-sur-le-veganisme-des-enfants>. (Accessed: 2nd July 2019)
- Ben-Shlomo, Y. et al. Prenatal and postnatal milk supplementation and adult insulin-like growth factor I: Long-term follow-up of a randomized controlled trial. *Cancer Epidemiol. Biomarkers Prev.* 14, 1336–1339 (2005).
- Berenson, G. S. et al. Association between Multiple Cardiovascular Risk Factors and Atherosclerosis in Children and Young Adults. *N. Engl. J. Med.* 338, 1650–1656 (1998).
- Berger, A. Bone mineral density scans. *BMJ* 325, 484 (2002).

- Berrazaga, I., Micard, V., Gueugneau, M. & Walrand, S. The role of the anabolic properties of plant-versus animal-based protein sources in supporting muscle mass maintenance: a critical review. *Nutrients* 11, (2019).
- Bertuccio, P. et al. Coronary heart disease and cerebrovascular disease mortality in young adults: Recent trends in Europe. *Eur. J. Cardiovasc. Prev. Rehabil.* 18, 627–634 (2011).
- Bibbins-Domingo, K. et al. Statin Use for the Primary Prevention of Cardiovascular Disease in Adults. *JAMA* 316, 1997 (2016).
- Binkin, N. J., Yip, R., Fleshood, L. & Trowbridge, F. L. Birth Weight and Childhood Growth. *Pediatrics* 82, (1988).
- Black, R. E., Williams, S. M., Jones, L. E. & Goulding, A. Children who avoid drinking cow milk have low dietary calcium intakes and poor bone health. *Am. J. Clin. Nutr.* 76, 675–680 (2002).
- Bonanome, A. & Grundy, S. M. Effect of Dietary Stearic Acid on Plasma Cholesterol and Lipoprotein Levels. *N. Engl. J. Med.* 318, 1244–1248 (1988).
- Bonjour, J. P. et al. Calcium-enriched foods and bone mass growth in prepubertal girls: A randomized, double-blind, placebo-controlled trial. *J. Clin. Invest.* 99, 1287–1294 (1997).
- Borai, A., Livingstone, C., Kaddam, I. & Ferns, G. Selection of the appropriate method for the assessment of insulin resistance. *BMC Med. Res. Methodol.* 11, 158 (2011).
- Borga, M. et al. Advanced body composition assessment: from body mass index to body composition profiling. *J. Investig. Med.* 66, 1.10-9 (2018).
- Bosy-Westphal, A. et al. Measurement Site for Waist Circumference Affects Its Accuracy As an Index of Visceral and Abdominal Subcutaneous Fat in a Caucasian Population. *J. Nutr.* 140, 954–961 (2010).
- Bradbury, K. E. et al. Serum concentrations of cholesterol, apolipoprotein A-I and apolipoprotein B in a total of 1694 meat-eaters, fish-eaters, vegetarians and vegans. *Eur. J. Clin. Nutr.* 68, 178–183 (2014).
- Budek, A. Z. et al. Dietary protein intake and bone mineral content in adolescents - The Copenhagen Cohort Study. *Osteoporos. Int.* 18, 1661–1667 (2007).
- Budek, A., Hoppe, C., Michaelsen, K., Bügel, S. & Mølgaard, C. Associations of Total, Dairy, and Meat Protein with Markers for Bone Turnover in Healthy, Prepubertal Boys. *J. Nutr.* 137, 930–934 (2007).
- Buzanovskii, V. A. Methods for the determination of glucose in blood. Part 1. *Rev. J. Chem.* 5, 30–81 (2015).
- Cabral, M., Araújo, J., Lopes, C. & Ramos, E. Food intake and high-sensitivity C-reactive protein levels in adolescents. *Nutr. Metab. Cardiovasc. Dis.* 28, 1067–1074 (2018).

- Capewell, S. & Buchan, I. Why have sustained increases in obesity and type 2 diabetes not offset declines in cardiovascular mortality over recent decades in Western countries? *Nutr. Metab. Cardiovasc. Dis.* 22, 307–311 (2012).
- Capewell, S. & O’Flaherty, M. Rapid mortality falls after risk-factor changes in populations. *Lancet* 378, 752–753 (2011).
- Capewell, S. et al. Cardiovascular risk factor trends and potential for reducing coronary heart disease mortality in the United States of America. *Bull. World Health Organ.* 88, 120 (2010).
- Carrquiry, A. Assessing the prevalence of nutrient inadequacy. *Public Health Nutr.* 2, 23–33 (1999).
- Carrquiry, A. Estimation of Usual Intake Distributions of Nutrients and Foods. *J. Nutr.* 133, 601S-608S (2003).
- Carter, D. R., Bouxsein, M. L. & Marcus, R. New approaches for interpreting projected bone densitometry data. *J. Bone Miner. Res.* 7, 137–145 (1992).
- Cercato, C. & Fonseca, F. A. Cardiovascular risk and obesity. *Diabetology and Metabolic Syndrome* 11, (2019).
- Chang, V. W., Langa, K. M., Weir, D. & Iwashyna, T. J. The obesity paradox and incident cardiovascular disease: A population-based study. *PLoS One* 12, (2017).
- Chaput, J.-P. et al. Inequality in physical activity, sedentary behaviour, sleep duration and risk of obesity in children: a 12-country study. *Obes. Sci. Pract.* 4, 229–237 (2018).
- Chen, X. & Wang, Y. Tracking of Blood Pressure From Childhood to Adulthood. *Circulation* 117, 3171–3180 (2008).
- Chevalley, T., Bonjour, J. P., Ferrari, S., Hans, D. & Rizzoli, R. Skeletal site selectivity in the effects of calcium supplementation on areal bone mineral density gain: A randomized, double-blind, placebo-controlled trial in prepubertal boys. *J. Clin. Endocrinol. Metab.* 90, 3342–3349 (2005).
- Christie, D. et al. A community-based motivational personalised lifestyle intervention to reduce BMI in obese adolescents: Results from the Healthy Eating and Lifestyle Programme (HELP) randomised controlled trial. *Arch. Dis. Child.* 102, 695–701 (2017).
- Chrysant, S. G. & Chrysant, G. S. The current status of homocysteine as a risk factor for cardiovascular disease: a mini review. *Expert Review of Cardiovascular Therapy* 16, 559–565 (2018).
- Chuang, S.-Y. et al. Vegetarian diet reduces the risk of hypertension independent of abdominal obesity and inflammation. *J. Hypertens.* 34, 2164–2171 (2016).
- Cirmanová, V., Bayer, M., Stárka, L. & Zajícková, K. The effect of leptin on bone: an evolving concept of action. *Physiol. Res.* 57 Suppl 1, S143-51 (2008).

- Clausson, B., Cnattingius, S. & Axelsson, O. Preterm and term births of small for gestational age infants: A population-based study of risk factors among nulliparous women. *BJOG An Int. J. Obstet. Gynaecol.* 105, 1011–1017 (1998).
- Clemens, T. L. Vitamin B12 deficiency and bone health. *N. Engl. J. Med.* 371, 963–964 (2014).
- Cliff, D. P., Reilly, J. J. & Okely, A. D. Methodological considerations in using accelerometers to assess habitual physical activity in children aged 0–5 years. *J. Sci. Med. Sport* 12, 557–567 (2009).
- CNBC.COM. Nestle gears up to launch its own plant-based burger in the US. (2019). Available at: <https://www.cnbc.com/2019/06/03/nestle-gears-up-to-launch-its-own-plant-based-burger-in-the-us.html>. (Accessed: 2nd July 2019)
- Cole, T. J. & Altman, D. G. Statistics Notes: Percentage differences, symmetry, and natural logarithms. *BMJ* 358, j3683 (2017).
- Cole, T. J., Flegal, K. M., Nicholls, D. & Jackson, A. A. Body mass index cut offs to define thinness in children and adolescents: international survey. *BMJ* 335, 194 (2007).
- Cole, T., Freeman, J. & Preece, M. Body mass index reference curves for the UK, 1990. *Arch. Dis. Child.* 73, 25–29 (1995).
- Colquitt, J. L. et al. Diet, physical activity, and behavioural interventions for the treatment of overweight or obesity in preschool children up to the age of 6 years. *Cochrane Database of Systematic Reviews* 2016, (2016).
- Connor, W. E. & Connor, S. L. Importance of diet in the treatment of familial hypercholesterolaemia. *Am. J. Cardiol.* 72, 42D-53D (1993).
- Cook, J. et al. The influence of availability of free school milk on the height of children in England and Scotland. *J. Epidemiol. Community Health* 33, 171–176 (1979).
- Cooper, A. J. et al. Fruit and vegetable intake and type 2 diabetes: EPIC-InterAct prospective study and meta-analysis. *Eur. J. Clin. Nutr.* 66, 1082–1092 (2012).
- Corder, K., Ekelund, U., Steele, R. M., Wareham, N. J. & Brage, S. Assessment of physical activity in youth. *Journal of Applied Physiology* 105, 977–987 (2008).
- Corpeleijn, E., Saris, W. H. M. & Blaak, E. E. Metabolic flexibility in the development of insulin resistance and type 2 diabetes: Effects of lifestyle: Etiology and Pathophysiology. *Obesity Reviews* 10, 178–193 (2009).
- Crabtree, N. J. & et al. UK Paediatric} reference data (GE Lunar Prodigy). *Osteoporos. Int* 15, (S2): S6 (2004).
- Craig, W. J. Nutrition Concerns and Health Effects of Vegetarian Diets. *Nutr. Clin. Pract.* 25, 613–620 (2010).

Craigie, A. M., Lake, A. A., Kelly, S. A., Adamson, A. J. & Mathers, J. C. Tracking of obesity-related behaviours from childhood to adulthood: A systematic review. *Maturitas* 70, 266–284 (2011).

Cramer, H. et al. Characteristics of Americans Choosing Vegetarian and Vegan Diets for Health Reasons. *Journal of Nutrition Education and Behavior*. 49(7), 561-567 (2017).

Crowe, F. L. et al. The association between diet and serum concentrations of IGF-I, IGFBP-1, IGFBP-2, and IGFBP-3 in the European prospective investigation into cancer and nutrition. *Cancer Epidemiol. Biomarkers Prev.* 18, 1333–1340 (2009).

Crowe, F. L., Appleby, P. N., Travis, R. C. & Key, T. J. Risk of hospitalization or death from ischemic heart disease among British vegetarians and nonvegetarians: results from the EPIC-Oxford cohort study. *Am. J. Clin. Nutr.* 97, 597–603 (2013).

Cundy, T., Reid, I. R. & Grey, A. Metabolic bone disease. *Clin. Biochem. Metab. Clin. Asp.* 604–635 (2014).

Curtis, M. J. and Comer, L. K. Vegetarianism, dietary restraint and feminist identity. *Eating Behaviors*. 7(2), 91–104 (2006).

Dahm, C. C. et al. Adolescent Diet Quality and Cardiovascular Disease Risk Factors and Incident Cardiovascular Disease in Middle-Aged Women. *J. Am. Heart Assoc.* 5, (2016).

Dalla Pozza, R. et al. Intima media thickness measurement in children: A statement from the Association for European Paediatric Cardiology (AEPC) Working Group on Cardiovascular Prevention endorsed by the Association for European Paediatric Cardiology. *Atherosclerosis* 238, 380–387 (2015).

Daniels, S. R., Khoury, P. R. & Morrison, J. A. The Utility of Body Mass Index as a Measure of Body Fatness in Children and Adolescents: Differences by Race and Gender. *Pediatrics* 99, 804–807 (1997).

Davis, P. H., Dawson, J. D., Riley, W. A. & Lauer, R. M. Carotid intimal-medial thickness is related to cardiovascular risk factors measured from childhood through middle age the Muscatine Study. *Circulation* 104, 2815–2819 (2001).

Dawson-Hughes, B. et al. Signs of impaired cognitive function in adolescents with marginal cobalamin status. *American Journal of Clinical Nutrition*, 72(3), 762–769 (2000).

Day, N., McKeown, N., Wong, M., Welch, A. & Bingham, S. Epidemiological assessment of diet: a comparison of a 7-day diary with a food frequency questionnaire using urinary markers of nitrogen, potassium and sodium. *Int. J. Epidemiol.* 30, 309–317 (2001).

De Medécine de Belgique, A. R. Le Veganisme proscrit pour les enfants, femmes enceintes et allaitantest. (2019).

- De Munter, J. S. L., Hu, F. B., Spiegelman, D., Franz, M. & Van Dam, R. M. Whole grain, bran, and germ intake and risk of type 2 diabetes: A prospective cohort study and systematic review. *PLoS Medicine* 4, 1385–1395 (2007).
- De Vries, S. I. et al. Validity and Reproducibility of Motion Sensors in Youth. *Med. Sci. Sport. Exerc.* 41, 818–827 (2009).
- Degerud, E. et al. Cosinor modelling of seasonal variation in 25-hydroxyvitamin D concentrations in cardiovascular patients in Norway. *Eur. J. Clin. Nutr.* 70, 517–522 (2016).
- Desmond, M. A., Sobiecki, J., Fewtrell, M. & Wells, J. C. K. Plant-based diets for children as a means of improving adult cardiometabolic health. *Nutr. Rev.* 76, (2018).
- Deurenberg, P., Yap, M. & van Staveren, W. Body mass index and percent body fat: a meta-analysis among different ethnic groups. *Int. J. Obes.* 22, 1164–1171 (1998).
- Devakumar, D. et al. Maternal antenatal multiple micronutrient supplementation for long-term health benefits in children: A systematic review and meta-analysis. *BMC Med.* 14, (2016).
- Devalia, V., Hamilton, M. S. & Molloy, A. M. Guidelines for the diagnosis and treatment of cobalamin and folate disorders. *Br. J. Haematol.* 166, 496–513 (2014).
- Die Deutsche Gesellschaft für Ernährung. Vegane Ernährung: Nährstoffversorgung und Gesundheitsrisiken im Säuglings- und Kindesalter. Available at: <https://www.dge.de/wissenschaft/weitere-publikationen/dge-position/vegane-ernaehrung/>. (Accessed: 15th July 2019)
- Din-Dzietham, R., Liu, Y., Bielo, M.-V. & Shamsa, F. High Blood Pressure Trends in Children and Adolescents in National Surveys, 1963 to 2002. *Circulation* 116, 1488–1496 (2007).
- Dinu, M., Abbate, R., Gensini, G. F., Casini, A. & Sofi, F. Vegetarian, vegan diets and multiple health outcomes: A systematic review with meta-analysis of observational studies. *Crit. Rev. Food Sci. Nutr.* 57, 3640–3649 (2017).
- Doumatey, A. P., Zhou, J., Adeyemo, A. & Rotimi, C. High sensitivity C-reactive protein (Hs-CRP) remains highly stable in long-term archived human serum. *Clin. Biochem.* 47, 315–8 (2014).
- Doyon, A. et al. Carotid artery intima-media thickness and distensibility in children and adolescents: reference values and role of body dimensions. *Hypertens. (Dallas, Tex. 1979)* 62, 550–556 (2013).
- Driessler, F. & Baldock, P. A. Hypothalamic regulation of bone. *J. Mol. Endocrinol.* 45, 175–181 (2010).
- EFCOVAL. Multiple Source Method (MSM) for estimating usual dietary intake from short-term measurement data, user guide. (2011).

- EFSA. Scientific Opinion on principles for deriving and applying Dietary Reference Values. *EFSA J.* 8, (2010).
- Eide, M. G. et al. Size at birth and gestational age as predictors of adult height and weight. *Epidemiology* 16, 175–181 (2005).
- Emberson, J., Whincup, P., Morris, R., Walker, M. & Ebrahim, S. Evaluating the impact of population and high-risk strategies for the primary prevention of cardiovascular disease. *Eur. Heart J.* 25, 484–491 (2004).
- Ernst, E., Pietsch, L., Matrai, A. & Eisenberg, J. Blood rheology in vegetarians. *Br. J. Nutr.* 56, 555–60 (1986).
- Escobar-Morreale, H. F. et al. Body iron stores are increased in overweight and obese women with polycystic ovary syndrome. *Diabetes Care* 28, 2042–4 (2005).
- Ettehad, D. et al. Blood pressure lowering for prevention of cardiovascular disease and death: a systematic review and meta-analysis. *Lancet* 387, 957–967 (2016).
- Evans, C. E. L. et al. Effects of dietary fibre type on blood pressure. *J. Hypertens.* 33, 897–911 (2015).
- Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents, E. P. O. I. G. F. C. H. A. R. R. I. C. A. & National Heart, Lung, and Blood Institute. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. *Pediatrics* 128 Suppl 5, S213-56 (2011).
- Fanidi, A. et al. Circulating vitamin {D} in relation to cancer incidence and survival of the head and neck and oesophagus in the {EPIC} cohort. *Sci Rep* 6, 36017 (2016).
- Ference, B. A. et al. Effect of Long-Term Exposure to Lower Low-Density Lipoprotein Cholesterol Beginning Early in Life on the Risk of Coronary Heart Disease. *J. Am. Coll. Cardiol.* 60, 2631–2639 (2012).
- Ferrari, S. L. Osteoporosis: a complex disorder of aging with multiple genetic and environmental determinants. *World review of nutrition and dietetics* 95, 35–51 (2005).
- Ferron, M. & Lacombe, J. Regulation of energy metabolism by the skeleton: Osteocalcin and beyond. *Arch. Biochem. Biophys.* 561, 137–146 (2014).
- Fewtrell, M. S. Bone densitometry in children assessed by dual x ray absorptiometry: uses and pitfalls. *Arch. Dis. Child.* 88, 795–798 (2003).
- Fomon, S. J., Haschke, F., Ziegler, E. E. & Nelson, S. E. Body composition of reference children from birth to age 10 years. *Am. J. Clin. Nutr.* 35, 1169–1175 (1982).

- Foo, J.-P., Hamnvik, O.-P. R. & Mantzoros, C. S. Optimizing bone health in anorexia nervosa and hypothalamic amenorrhea: new trials and tribulations. *Metabolism*. 61, 899–905 (2012).
- Ford, E. S. & Capewell, S. Coronary Heart Disease Mortality Among Young Adults in the U.S. From 1980 Through 2002. Concealed Leveling of Mortality Rates. *J. Am. Coll. Cardiol.* 50, 2128–2132 (2007).
- Fraser, G. E. Vegetarian diets: what do we know of their effects on common chronic diseases? *Am. J. Clin. Nutr.* 89, 1607S-1612S (2009).
- Freedman, D. S. et al. Relation of BMI to fat and fat-free mass among children and adolescents. *Int. J. Obes.* 29, 1–8 (2005).
- Friedemann, C. et al. Cardiovascular disease risk in healthy children and its association with body mass index: Systematic review and meta-analysis. *BMJ* 345, (2012).
- Frontini, M. G., Srinivasan, S. R. & Berenson, G. S. Longitudinal changes in risk variables underlying metabolic Syndrome X from childhood to young adulthood in female subjects with a history of early menarche: The Bogalusa Heart Study. *Int. J. Obes.* 27, 1398–1404 (2003).
- Fung, T. T., Schulze, M., Manson, J. E., Willett, W. C. & Hu, F. B. Dietary Patterns, Meat Intake, and the Risk of Type 2 Diabetes in Women. *Arch. Intern. Med.* 164, 2235 (2004).
- Gafni, R. I. & Baron, J. Overdiagnosis of osteoporosis in children due to misinterpretation of Dual-energy x-ray absorptiometry (DEXA). *J. Pediatr.* 144, 253–257 (2004).
- Galan, P. et al. Low Total and Nonheme Iron Intakes Are Associated with a Greater Risk of Hypertension. *J. Nutr.* 140, 75–80 (2010).
- Gallagher, D. et al. How Useful Is Body Mass Index for Comparison of Body Fatness across Age, Sex, and Ethnic Groups? *Am. J. Epidemiol.* 143, 228–239 (1996).
- Gallelli, L. et al. 25-Hydroxy Vitamin D Detection Using Different Analytic Methods in Patients with Migraine. *J. Clin. Med.* 8, (2019).
- Ganguly, P. & Alam, S. F. Role of homocysteine in the development of cardiovascular disease. *Nutrition Journal* 14, (2015).
- Ganz, T. Hepcidin and the global burden of iron deficiency. *Clin. Chem.* 61, 577–8 (2015).
- Gazolla, F. M. et al. Association between cardiovascular risk factors and carotid intima-media thickness in prepubertal Brazilian children. *J. Pediatr. Endocrinol. Metab.* 28, 579–587 (2015).
- Georgieff, M. K. Long-term brain and behavioral consequences of early iron deficiency. *Nutr. Rev.* 69, (2011).

- Gilting, A. M. et al. The Netherlands Cohort Study - Meat Investigation Cohort; A population-based cohort over-represented with vegetarians, pescetarians and low meat consumers. *Nutrition Journal* 12(1),156 (2013).
- Golden, N. H. et al. Optimizing bone health in children and adolescents. *Pediatrics* 134, e1229–e1243 (2014).
- Gorczyca, D., Paściak, M., Szponar, B., Gamian, A. & Jankowski, A. An impact of the diet on serum fatty acid and lipid profiles in Polish vegetarian children and children with allergy. *Eur. J. Clin. Nutr.* 65, 191–195 (2011).
- Gorczyca, D., Prescha, A., Szeremeta, K. & Jankowski, A. Iron Status and Dietary Iron Intake of Vegetarian Children from Poland. *Ann. Nutr. Metab.* 62, 291–297 (2013).
- Gosmanova, E. O. & Kovesdy, C. P. Adherence to antihypertensive medications: is prescribing the right pill enough? *Nephrol. Dial. Transplant.* 30, 1649–1656 (2015).
- Grillenberger, M. et al. Food Supplements Have a Positive Impact on Weight Gain and the Addition of Animal Source Foods Increases Lean Body Mass of Kenyan Schoolchildren. *J. Nutr.* 133, 3957S-3964S (2003).
- Grundy, S. M. & Denke, M. A. Dietary influences on serum lipids and lipoproteins. *Journal of Lipid Research* 31, 1149–1172 (1990).
- Guasch-Ferré, M. et al. Nut Consumption and Risk of Cardiovascular Disease. *J. Am. Coll. Cardiol.* 70, 2519–2532 (2017).
- Günther, A. L. B., Karaolis-Danckert, N., Kroke, A., Remer, T. & Buyken, A. E. Dietary Protein Intake throughout Childhood Is Associated with the Timing of Puberty. *J. Nutr.* 140, 565–571 (2010).
- Guran, O., Akalin, F., Ayabakan, C., Dereli, F. Y. & Haklar, G. High-sensitivity C-reactive protein in children at risk for coronary artery disease. *Acta Paediatr.* 96, 1214–1219 (2007).
- Guez, S. et al. Severe vitamin B12 deficiency in an exclusively breastfed 5-month-old Italian infant born to a mother receiving multivitamin supplementation during pregnancy. *BMC Pediatr.* 12 (2012).
- GUS. Życie religijne w Polsce. (2015). Available at: <https://stat.gov.pl/obszary-tematyczne/inne-opracowania/wyznania-religijne/zycie-religijne-w-polsce,3,1.html>. (Accessed: 17th December 2019)
- Guthold, R., Stevens, G. A., Riley, L. M. & Bull, F. C. Global trends in insufficient physical activity among adolescents: a pooled analysis of 298 population-based surveys with 1.6 million participants. *Lancet Child Adolesc. Heal.* 1–13 (2019).
- Guzman-Castillo, M. et al. The contribution of primary prevention medication and dietary change in coronary mortality reduction in England between 2000 and 2007: A modelling study. *BMJ Open* 5, (2015).

- Haddad, E. H. & Tanzman, J. S. What do vegetarians in the United States eat? *Am. J. Clin. Nutr.* 78, 626S-632S (2003).
- Haider, B. A. et al. Anaemia, prenatal iron use, and risk of adverse pregnancy outcomes: Systematic review and meta-analysis. *BMJ (Online)* 347, (2013).
- Hamrick, M. W. Leptin and Bone: A Consensus Emerging? *International Bone and Mineral Society Knowledge Environment* 4(3):99-107 (2007).
- Harris, R. D., Phillips, R. L., Williams, P. M., Kuzma, J. W. & Fraser, G. E. The child-adolescent blood pressure study: I. Distribution of blood pressure levels in Seventh-Day-Adventist (SDA) and non-SDA children. *Am. J. Public Health* 71, 1342–9 (1981).
- Haubrock, J. et al. Estimating Usual Food Intake Distributions by Using the Multiple Source Method in the EPIC-Potsdam Calibration Study. *J. Nutr.* 141, 914–920 (2011).
- He, J. et al. Effect of Soybean Protein on Blood Pressure: A Randomized, Controlled Trial. *Ann. Intern. Med.* 143, 1 (2005).
- Hebbelinck, M., Clarys, P. & De Malsche, A. Growth, development, and physical fitness of Flemish vegetarian children, adolescents, and young adults. *Am. J. Clin. Nutr.* 70, 579s-585s (1999).
- Hediger, M. L. et al. Growth of infants and young children born small or large for gestational age: Findings from the Third National Health and Nutrition Examination Survey. *Arch. Pediatr. Adolesc. Med.* 152, 1225–1231 (1998).
- Hermansen, K., Sondergaard, M., Hoie, L., Carstensen, M. & Brock, B. Beneficial Effects of a Soy-Based Dietary Supplement on Lipid Levels and Cardiovascular Risk Markers in Type 2 Diabetic Subjects. *Diabetes Care* 24, 228–233 (2001).
- Higashi, Y., Gautam, S., Delafontaine, P. & Sukhanov, S. IGF-1 and cardiovascular disease. *Growth Hormone and IGF Research* 45, 6–16 (2019).
- Hills, A. P., Andersen, L. B. & Byrne, N. M. Physical activity and obesity in children. *British Journal of Sports Medicine* 45, 866–870 (2011).
- Hodis, H. N. et al. The Role of Carotid Arterial Intima-Media Thickness in Predicting Clinical Coronary Events. *Ann. Intern. Med.* 128, 262 (1998).
- Hoek, A. C., Luning, P. A., Stafleu, A. & de Graaf, C. Food-related lifestyle and health attitudes of Dutch vegetarians, non-vegetarian consumers of meat substitutes, and meat consumers. *Appetite* 42, 265–272 (2004).
- Holt, E. M. et al. Fruit and Vegetable Consumption and Its Relation to Markers of Inflammation and Oxidative Stress in Adolescents. *J. Am. Diet. Assoc.* 109, 414–421 (2009).
- Hong, I., Coker-Bolt, P., Anderson, K. R., Lee, D. & Velozo, C. A. Relationship between physical activity and overweight and obesity in children: Findings from

the 2012 National Health and Nutrition Examination Survey National Youth Fitness Survey. *American Journal of Occupational Therapy* 70, (2016).

Ho-Pham, L. T., Nguyen, N. D. & Nguyen, T. V. Effect of vegetarian diets on bone mineral density: a Bayesian meta-analysis. *Am. J. Clin. Nutr.* 90, 943–950 (2009).

Hoppe, C. et al. Animal protein intake, serum insulin-like growth factor I, and growth in healthy 2.5-y-old Danish children. *Am. J. Clin. Nutr.* 80, 447–452 (2004).

Hoppe, C., Mølgaard, C. & Michaelsen, K. Cow's Milk and Linear Growth in Industrialized and Developing Countries. *Annu. Rev. Nutr.* 26, 131–173 (2006).

Hoppe, C., Mølgaard, C., Juul, A. & Michaelsen, K. F. High intakes of skimmed milk, but not meat, increase serum IGF-I and IGFBP-3 in eight-year-old boys. *Eur. J. Clin. Nutr.* 58, 1211–1216 (2004).

Huffman, M. D. et al. Quantifying Options for Reducing Coronary Heart Disease Mortality By 2020. *Circulation* 127, 2477–2484 (2013).

Hughes, J. et al. Modelling Future Coronary Heart Disease Mortality to 2030 in the British Isles. *PLoS One* 10 (2015).

Institute of Medicine (U.S.). Committee on Military Nutrition Research., Newberry, S. J. & Costello, R. B. Emerging technologies for nutrition research: potential for assessing military performance capability. (National Academy Press, 1997).

Institute of Medicine (US) Subcommittee on Interpretation and Uses of Dietary Reference Intakes; Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Adjustment of Observed Intake Data to Estimate the Distribution of Usual Intakes in a Group. in *Dietary Reference Intakes: Applications in Dietary Planning*. (The National Academies Press, 2003).

Institute of Medicine Food and Nutrition Board. Dietary Reference Intakes for calcium and vitamin D. (The National Academies Press, 2011).

IOM, Fuster, V. & Kelly, B. B. Promoting cardiovascular health in the developing world: a critical challenge to achieve global health. (National Academies Press, 2010).

Jackson, A. et al. The effect of sex, age and race on estimating percentage body fat from body mass index: The Heritage Family Study. *Int. J. Obes.* 26, 789–796 (2002).

Jager, J., Putnick, D. L. and Bornstein, M. H. More than Just Convenient: The Scientific Merits of Homogeneous Convenience Samples. *Monographs of the Society for Research in Child Development* 82(2), pp. 13–30 (2017).

Jain, R., Singh, A., Mittal, M. & Talukdar, B. Vitamin B12 deficiency in children: A treatable cause of neurodevelopmental delay. *J. Child Neurol.* 30, 641–643 (2015).

Jain, V. & Singhal, A. Catch up growth in low birth weight infants: Striking a healthy balance. *Rev. Endocr. Metab. Disord.* 13, 141–147 (2012).

Janssen, I. & LeBlanc, A. G. Systematic review of the health benefits of physical activity and fitness in school-aged children and youth. *International Journal of Behavioral Nutrition and Physical Activity* 7, (2010).

Janz, K. F. et al. Sustained Effect of Early Physical Activity on Body Fat Mass in Older Children. *Am. J. Prev. Med.* 37, 35–40 (2009).

Janz, K. F., Burns, T. L. & Levy, S. M. Tracking of activity and sedentary behaviors in childhood: The Iowa bone development study. *Am. J. Prev. Med.* 29, 171–178 (2005).

Järvisalo, M. J. et al. Elevated serum C-reactive protein levels and early arterial changes in healthy children. *Arterioscler. Thromb. Vasc. Biol.* 22, 1323–8 (2002).

Järvisalo, M. J. et al. Increased Aortic Intima-Media Thickness. *Circulation* 104, 2943–2947 (2001).

Jenkins, D. J. A. et al. Effects of a Dietary Portfolio of Cholesterol-Lowering Foods vs Lovastatin on Serum Lipids and C-Reactive Protein. *JAMA* 290, 502 (2003).

Jourdan, C. et al. Normative values for intima-media thickness and distensibility of large arteries in healthy adolescents. *J. Hypertens.* 23, 1707–1715 (2005).

Juonala, M. et al. Childhood C-Reactive Protein in Predicting CRP and Carotid Intima-Media Thickness in Adulthood. *Arterioscler. Thromb. Vasc. Biol.* 26, 1883–1888 (2006).

Juonala, M. et al. Influence of Age on Associations Between Childhood Risk Factors and Carotid Intima-Media Thickness in Adulthood. *Circulation* 122, 2514–2520 (2010).

Juonala, M. et al. Risk Factors Identified in Childhood and Decreased Carotid Artery Elasticity in Adulthood. *Circulation* 112, 1486–1493 (2005).

Júrez-López, C. et al. Insulin resistance and its association with the components of the metabolic syndrome among obese children and adolescents. *BMC Public Health* 10, (2010).

Juul, A., Scheike, T., Davidsen, M., Gyllenborg, J. & Jørgensen, T. Low serum insulin-like growth factor I is associated with increased risk of ischemic heart disease: A population-based case-control study. *Circulation* 106, 939–944 (2002).

Kahleova, H. & Pelikanova, T. Vegetarian Diets in the Prevention and Treatment of Type 2 Diabetes. *J. Am. Coll. Nutr.* 34, 448–458 (2015).

Kahleova, H. et al. Vegetarian diet improves insulin resistance and oxidative stress markers more than conventional diet in subjects with Type 2 diabetes. *Diabet. Med.* 28, 549–559 (2011).

- Kaikkonen, J. E. et al. Childhood Serum Fatty Acid Quality Is Associated with Adult Carotid Artery Intima Media Thickness in Women but Not in Men. *J. Nutr.* 143, 682–689 (2013).
- Kaikkonen, J. E., Mikkilä, V. & Raitakari, O. T. Role of Childhood Food Patterns on Adult Cardiovascular Disease Risk. *Curr. Atheroscler. Rep.* 16, 443 (2014).
- Kanbur, N. Ö., Derman, O. & Kinik, E. The relationships between pubertal development, IGF-1 axis, and bone formation in healthy adolescents. *J. Bone Miner. Metab.* 23, 76–83 (2005).
- Karlberg, J. Secular Trends in Pubertal Development. *Horm. Res. Paediatr.* 57, 19–30 (2002).
- Karlsson, A.-K. et al. Measurements of total and regional body composition in preschool children: A comparison of MRI, DXA, and anthropometric data. *Obesity* 21, 1018–1024 (2013).
- Keats, E. C., Neufeld, L. M., Garrett, G. S., Mbuya, M. N. N. & Bhutta, Z. A. Improved micronutrient status and health outcomes in low-and middle-income countries following large-scale fortification: Evidence from a systematic review and meta-Analysis. *Am. J. Clin. Nutr.* 109, 1696–1708 (2019).
- Kelishadi, R., Zadegan, N. S., Naderi, G. A., Asgary, S. & Bashardoust, N. Atherosclerosis risk factors in children and adolescents with or without family history of premature coronary artery disease. *Med. Sci. Monit.* 8, CR425-9 (2002).
- Kent, L. et al. The effect of a low-fat, plant-based lifestyle intervention (CHIP) on serum HDL levels and the implications for metabolic syndrome status - A cohort study. *Nutr. Metab.* 10, (2013).
- Kesmodel, U. S. Cross-sectional studies - what are they good for? *Acta Obstet. Gynecol. Scand.* 97, 388–393 (2018).
- Ketha, H. & Singh, R. J. Clinical assays for quantitation of insulin-like-growth-factor-1 (IGF1). *Methods* 81, 93–98 (2015).
- Key, T. J. et al. Mortality in vegetarians and nonvegetarians: Detailed findings from a collaborative analysis of 5 prospective studies. in *American Journal of Clinical Nutrition* 70, (1999).
- Khan, S. S. et al. Association of body mass index with lifetime risk of cardiovascular disease and compression of morbidity. *JAMA Cardiol.* 3, 280–287 (2018).
- Kiens, B. Skeletal muscle lipid metabolism in exercise and insulin resistance. *Physiological Reviews* 86, 205–243 (2006).
- Kim, J. et al. Development of a food frequency questionnaire in Koreans. *Asia Pac. J. Clin. Nutr.* 12, 243–50 (2003).
- Kissinger, D. G. & Sanchez, A. The association of dietary factors with the age of menarche. *Nutr. Res.* 7, 471–479 (1987).

- Kitsios, K. et al. High-sensitivity C-reactive protein levels and metabolic disorders in obese and overweight children and adolescents. *J. Clin. Res. Pediatr. Endocrinol.* 5, 44–9 (2013).
- Kjøllestad, M. & Holmboe-Ottesen, G. Dietary Patterns and Birth Weight—a Review. *AIMS Public Heal.* 1, 211–225 (2014).
- Klee, G. G. Cobalamin and folate evaluation: Measurement of methylmalonic acid and homocysteine vs vitamin B12 and folate. in *Clinical Chemistry* 46, 1277–1283 (2000).
- Klein, S. et al. Clinical Implications of Obesity With Specific Focus on Cardiovascular Disease. *Circulation* 110, 2952–2967 (2004).
- Kleinman, R. E. & American Academy of Pediatrics Committee on Nutrition. *Pediatric nutrition handbook.* (American Academy of Pediatrics, 2009).
- Krajcovicova-Kudlackova, M. et al. Plasma Fatty Acid Profile and Alternative Nutrition. *Ann. Nutr. Metab.* 41, 365–370 (1997).
- Krajcovicová-Kudláčková, M., Simoncic, R., Béderová, A., Grancicová, E. & Magálová, T. Influence of vegetarian and mixed nutrition on selected haematological and biochemical parameters in children. *Nahrung* 41, 311–4 (1997).
- Kristensen, N. B. et al. Intake of macro- and micronutrients in Danish vegans. *Nutr. J.* 14, (2015).
- Królak-Olejnik, B., Błasiak, I. & Szczygieł, A. Promotion of breastfeeding in Poland: the current situation. *J. Int. Med. Res.* 45, 1976–1984 (2017).
- Kryst, Ł., Kowal, M., Woronkiewicz, A., Sobiecki, J. & Cichocka, B. A. Secular changes in height, body weight, body mass index and pubertal development in male children and adolescents in Krakow, Poland. *J. Biosoc. Sci.* 44, 495–507 (2012).
- Książyk, J. et al. Intellectual development and anthropological assessment of nutritional status in children on vegetarian diets. *Standardy medyczne/Pediatrics.* 9:100–4 (2012).
- Kuhl, J. E. et al. Exercise training decreases the concentration of malonyl-CoA and increases the expression and activity of malonyl-CoA decarboxylase in human muscle. *Am. J. Physiol. Endocrinol. Metab.* 290, (2006).
- Kułaga Z, Rózdzyńska-Świątkowska, Grajda A, E. al. Percentile charts for growth and nutritional status assessment in Polish children and adolescents from birth to 18 year of age. *Stand. Med.* 1, (2015).
- Kułaga, Z. et al. Polish 2010 growth references for school-aged children and adolescents. *Eur. J. Pediatr.* 170, 599–609 (2011).
- Kunachowicz, H., Przygoda, B. & Iwanow, K. Tabele wartości odżywczej produktów spożywczych i potraw: komputerowa baza danych. (Instytut Żywności i Żywienia, 2005).

- Kuromori, Y., Okada, T., Miyashita, M. & Harada, K. Determination of Lipid Composition of Plasma Lipoproteins in Children with a Rapid Agarose Gel Electrophoresis Method. *J. Atheroscler. Thromb.* 13, 227–230 (2006).
- Laitinen, J., Power, C. & Järvelin, M. R. Family social class, maternal body mass index, childhood body mass index, and age at menarche as predictors of adult obesity. *Am. J. Clin. Nutr.* 74, 287–294 (2001).
- Laitinen, T. T. et al. Childhood lifestyle and clinical determinants of adult ideal cardiovascular health. *Int. J. Cardiol.* 169, 126–132 (2013).
- Lakshman, R. et al. Association between age at menarche and risk of diabetes in adults: Results from the EPIC-Norfolk cohort study. *Diabetologia* 51, 781–786 (2008).
- Larnkjaer, A. et al. Early programming of the IGF-I axis: Negative association between IGF-I in infancy and late adolescence in a 17-year longitudinal follow-up study of healthy subjects. *Growth Horm. IGF Res.* 19, 82–86 (2009).
- Larnkjær, A., Mølgaard, C. & Michaelsen, K. F. Early nutrition impact on the insulin-like growth factor axis and later health consequences. *Current Opinion in Clinical Nutrition and Metabolic Care* 15, 285–292 (2012).
- Laskowska-Klita, T., Chełchowska, M., Ambroszkiewicz, J., Gajewska, J. & Klemarczyk, W. The effect of vegetarian diet on selected essential nutrients in children. *Med. Wieku Rozwoj.* 15, 318–25 (2011).
- Lauer, R. & Clarke, W. Use of cholesterol measurements in childhood for the prediction of adult hypercholesterolaemia. The Muscatine Study. *JAMA* 264, 3034–8 (1990).
- Lauer, R. M., Burns, T. L. & Daniels, S. R. Pediatric prevention of atherosclerotic cardiovascular disease. (Oxford University Press, 2006).
- Lauer, R., Lee, J. & Clarke, W. Factors affecting the relationship between childhood and adult cholesterol levels: the Muscatine Study. *Pediatrics* 82, 309–18 (1988).
- Laughlin, G. A., Barrett-Connor, E., Criqui, M. H. & Kritz-Silverstein, D. The Prospective Association of Serum Insulin-Like Growth Factor I (IGF-I) and IGF-Binding Protein-1 Levels with All Cause and Cardiovascular Disease Mortality in Older Adults: The Rancho Bernardo Study. *J. Clin. Endocrinol. Metab.* 89, 114–120 (2004).
- Lavrakas, P. *Encyclopedia of Survey Research Methods*. (Sage Publications, Inc. 2012).
- Lazarou, C., Panagiotakos, D. B., Chrysohoou, C., Andronikou, C. & Matalas, A.-L. C-Reactive protein levels are associated with adiposity and a high inflammatory foods index in mountainous Cypriot children. *Clin. Nutr.* 29, 779–783 (2010).
- Le Roith, D. Insulin-like growth factors. in *New England Journal of Medicine* 336, 633–640 (1997).

- Lee, I. & Shiroma, E. Using accelerometers to measure physical activity in large-scale epidemiological studies: issues and challenges. *Br. J. Sports Med.* 48, 197–201 (2014).
- Lee, K. & Carlin, J. Multiple Imputation for Missing Data: Fully Conditional Specification Versus Multivariate Normal Imputation. *Am. J. Epidemiol.* 171, 624–632 (2010).
- Lee, S. & Gallagher, D. Assessment methods in human body composition. *Curr. Opin. Clin. Nutr. Metab. Care* 11, 566–72 (2008).
- Leighton, G. & Clark, M. L. Milk Consumption and the Growth of School Children. *Br. Med. J.* 1, 23–25 (1929).
- Leonard, M. B. & Zemel, B. S. Current concepts in pediatric bone disease. *Pediatric Clinics of North America* 49, 143–173 (2002).
- Levy-Marchal, C. et al. Insulin resistance in children: Consensus, perspective, and future directions. *J. Clin. Endocrinol. Metab.* 95, 5189–5198 (2010).
- Lewiecki, E. M. et al. International Society for Clinical Densitometry 2007 Adult and Pediatric Official Positions. *Bone* 43, 1115–1121 (2008).
- Li, D. Chemistry behind Vegetarianism. *J. Agric. Food Chem.* 59, 777–784 (2011).
- Little, R. J. A. & Rubin, D. B. *Statistical Analysis with Missing Data.* (John Wiley & Sons, Inc., 2002).
- Liu, A. et al. Validation of bioelectrical impedance analysis for total body water assessment against the deuterium dilution technique in Asian children. *Eur. J. Clin. Nutr.* 65, 1321–1327 (2011).
- Locatelli, V. & Bianchi, V. E. Effect of GH/IGF-1 on Bone Metabolism and Osteoporosis. *Int. J. Endocrinol.* 2014, (2014).
- Löfqvist, C. et al. Reference Values for Insulin-Like Growth Factor-Binding Protein-3 (IGFBP-3) and the Ratio of Insulin-Like Growth Factor-I to IGFBP-3 throughout Childhood and Adolescence. *J. Clin. Endocrinol. Metab.* 90, 1420–1427 (2005).
- Lombard, K. A., Olson, A. L., Nelson, S. E. & Rebouche, C. J. Carnitine status of lactoovo vegetarians and strict vegetarian adults and children. *Am. J. Clin. Nutr.* 50, 301–306 (1989).
- Lucas, A. Programming by early nutrition in man. *Ciba Foundation symposium* 156, (1991).
- Lucas, A. Programming by Early Nutrition: An Experimental Approach. *J. Nutr.* 128, 401S-406S (1998).
- Ludwig, M., v. Petzinger-Kruthoff, A., v. Buquoy, M. & Stumpe, K. Intima-Media-Dicke der Karotisarterien: Früher Indikator für Arteriosklerose und therapeutischer Endpunkt. *Ultraschall der Medizin* 24, 162–174 (2003).

- Lustig, R. H. Which Comes First? The Obesity or the Insulin? The Behavior or the Biochemistry? *Journal of Pediatrics* 152, 601–602 (2008).
- Macknin, M. et al. Plant-Based, No-Added-Fat or American Heart Association Diets: Impact on Cardiovascular Risk in Obese Children with Hypercholesterolaemia and Their Parents. *J. Pediatr.* 166, 953-959.e3 (2015).
- Maffeis, C., Pietrobelli, A., Grezzani, A., Provera, S. & Tatò, L. Waist Circumference and Cardiovascular Risk Factors in Prepubertal Children. *Obes. Res.* 9, 179–187 (2001).
- Magnussen, C. G. et al. Utility of Currently Recommended Pediatric Dyslipidemia Classifications in Predicting Dyslipidemia in Adulthood. *Circulation* 117, 32–42 (2008).
- Mahoney, L. T. et al. Coronary risk factors measured in childhood and young adult life are associated with coronary artery calcification in young adults: The muscatine study. *J. Am. Coll. Cardiol.* 27, 277–284 (1996).
- Maldonado-Araque, C. et al. Iron deficiency is associated with Hypothyroxinemia and Hypotriiodothyroninemia in the Spanish general adult population: Di@bet.es study. *Sci. Rep.* 8, (2018).
- Malik, V. S. et al. Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: A meta-analysis. *Diabetes Care* 33, 2477–2483 (2010).
- Manolopoulos, K. N., Karpe, F. & Frayn, K. N. Gluteofemoral body fat as a determinant of metabolic health. *Int. J. Obes.* 34, 949–959 (2010).
- Marketingchina.com. Vegan Food Industry is Booming in China (Let's go Now!) - Marketing China. Available at: <https://www.marketingtochina.com/vegan-food-industry-is-booming-in-china-lets-go-now/>. (Accessed: 2nd July 2019).
- Martínez-Mesa, J. et al. Sampling: How to select participants in my research study?. *Anais Brasileiros de Dermatologia.* 91(3), pp. 326–330 (2016).
- Martins, V. J. B. et al. Long-lasting effects of undernutrition. *Int. J. Environ. Res. Public Health* 8, 1817–46 (2011).
- Matthews, D. et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28, 412–9 (1985).
- Matthews, V., Wien, M. & Sabaté, J. The risk of child and adolescent overweight is related to types of food consumed. *Nutr. J.* 10, 71 (2011).
- Maynard, L. M. et al. Childhood Body Composition in Relation to Body Mass Index. *Pediatrics* 107, 344–350 (2001).
- McCarron, P., Okasha, M., McEwen, J. & Smith, G. D. Height in young adulthood and risk of death from cardiorespiratory disease: A prospective study of male former students of Glasgow University, Scotland. *Am. J. Epidemiol.* 155, 683–687 (2002).

- McCarthy, H., Jarrett, K. & Crawley, H. The development of waist circumference percentiles in British children aged 5.0–16.9 y. *Eur. J. Clin. Nutr.* 55, 902–907 (2001).
- McGill, H. C. Fatty streaks in the coronary arteries and aorta. *Lab. Invest.* 18, 560–4 (1968).
- McGill, H. et al. Origin of atherosclerosis in childhood and adolescence. *Am. J. Clin. Nutr.* 72, 1307s-1315s (2000).
- McGreevy, K. M., Lipsitz, S. R., Linder, J. A., Rimm, E. & Hoel, D. G. Using median regression to obtain adjusted estimates of central tendency for skewed laboratory and epidemiologic data. *Clin. Chem.* 55, 165–169 (2009).
- Melina, V., Craig, W. & Levin, S. Position of the Academy of Nutrition and Dietetics: Vegetarian Diets. *J. Acad. Nutr. Diet.* 116, 1970–1980 (2016).
- Meng, X.-H., Tan, L.-J., Xiao, H.-M., Tang, B.-S. & Deng, H.-W. Examining the causal role of leptin in bone mineral density: A Mendelian randomization study. *Bone* 125, 25–29 (2019).
- Merkiel-Pawłowska, S. & Chalcarz, W. Gender differences and typical nutrition concerns of the diets of preschool children - the results of the first stage of an intervention study. *BMC Pediatr.* 17 (2017).
- Michels, K. B. & Willett, W. C. Self-administered semiquantitative food frequency questionnaires: patterns, predictors, and interpretation of omitted items. *Epidemiology* 20, 295–301 (2009).
- Mikkilä, V., Räsänen, L., Raitakari, O. T., Pietinen, P. & Viikari, J. Consistent dietary patterns identified from childhood to adulthood: The Cardiovascular Risk in Young Finns Study. *Br. J. Nutr.* 93, 923–931 (2005).
- Millward, D. J. Nutrition, infection and stunting: The roles of deficiencies of individual nutrients and foods, and of inflammation, as determinants of reduced linear growth of children. *Nutrition Research Reviews* 30, 50–72 (2017).
- Moghaddam, E., Vogt, J. A. & Wolever, T. M. S. The Effects of Fat and Protein on Glycemic Responses in Nondiabetic Humans Vary with Waist Circumference, Fasting Plasma Insulin, and Dietary Fiber Intake. *J. Nutr.* 136, 2506–2511 (2006).
- Mølgaard, C., Larnkjær, A., Arnberg, K. & Michaelsen, K. F. Milk and growth in children: Effects of Whey and Casein. in *Nestle Nutrition Workshop Series: Pediatric Program* 67, 67–78 (2011).
- Molloy, A. M., Kirke, P. N., Brody, L. C., Scott, J. M. & Mills, J. L. Effects of folate and vitamin B12 deficiencies during pregnancy on fetal, infant, and child development. in *Food and Nutrition Bulletin* 29 (2008).
- Montenegro, M. R. & Eggen, D. A. Topography of atherosclerosis in the coronary arteries. *Lab. Invest.* 18, 586–93 (1968).

Morrison, J. A., Glueck, C. J. & Wang, P. Childhood risk factors predict cardiovascular disease, impaired fasting glucose plus type 2 diabetes mellitus, and high blood pressure 26 years later at a mean age of 38 years: the Princeton-lipid research clinics follow-up study. *Metabolism*. 61, 531–41 (2012).

Mozaffarian, D. Dietary and Policy Priorities for Cardiovascular Disease, Diabetes, and Obesity. *Circulation* 133, 187–225 (2016).

MRC. DAPA Measurement Toolkit. Available at: <https://dapa-toolkit.mrc.ac.uk/diet/subjective-methods/24-hour-dietary-recall>. (Accessed: 11th October 2019)

MSM. Available at: <https://msm.dife.de/>. (Accessed: 17th January 2020)

Mulligan, A. A. et al. A new tool for converting food frequency questionnaire data into nutrient and food group values: FETA research methods and availability. *BMJ Open* 4 (2014).

Multiple risk factor intervention trial. Risk factor changes and mortality results. Multiple Risk Factor Intervention Trial Research Group. *JAMA* 248, 1465–77 (1982).

Nahum, G. G. & Stanislaw, H. Relationship of paternal factors to birth weight. *J. Reprod. Med.* 48, 963–8 (2003).

Naska, A., Lagiou, A. & Lagiou, P. Dietary assessment methods in epidemiological research: current state of the art and future prospects. *F1000Research* 6, 926 (2017).

Naspi, A. et al. Insulin-Like-Growth-Factor-Binding-Protein-3 (IGFBP-3) Contrasts Melanoma Progression In Vitro and In Vivo. *PLoS One* 9 (2014).

Nathan, I., Hackett, A. & Kirby, S. The dietary intake of a group of vegetarian children aged 7-11 years compared with matched omnivores. *Br. J. Nutr.* 75, 533–544 (1996).

Nathan, I., Hackett, A. F. & Kirby, S. A longitudinal study of the growth of matched pairs of vegetarian and omnivorous children, aged 7-11 years, in the north-west of England. *Eur. J. Clin. Nutr.* 51, 20–5 (1997).

Nathan, I., Hackett, A. F. & Kirby, S. The dietary intake of a group of vegetarian children aged 7-11 years compared with matched omnivores. *Br. J. Nutr.* 75, 533–44 (1996).

National Cancer Institute. 24-hour Dietary Recall (24HR) At a Glance | Dietary Assessment Primer. 24-hour Dietary Recall (24HR) At a Glance (2018a). Available at: <https://dietassessmentprimer.cancer.gov/profiles/recall/>. (Accessed: 17th October 2019)

National Cancer Institute. ASA24® Dietary Assessment Tool | EGRP/DCCPS/NCI/NIH. (2019). Available at: <https://epi.grants.cancer.gov/asa24/>. (Accessed: 11th October 2019)

National Cancer Institute. Food Frequency Questionnaire at a Glance | Dietary Assessment Primer. (2018b). Available at: <https://dietassessmentprimer.cancer.gov/profiles/questionnaire/>. (Accessed: 13th October 2019)

National Cancer Institute. Food Record at a Glance | Dietary Assessment Primer. (2018c). Available at: <https://dietassessmentprimer.cancer.gov/profiles/record/index.html>. (Accessed: 13th October 2019)

National Research Council. Nutrient Adequacy. Assessment Using Food Consumption Surveys. in (National Academy Press, 1986).

Navar-Boggan, A. M. et al. Hyperlipidemia in Early Adulthood Increases Long-Term Risk of Coronary Heart Disease. *Circulation* 131, 451–458 (2015).

Neumann, C. G., Bwibo, N. O. & Sigman, M. The Human Cost of Moderate Malnutrition in an Egyptian Village, Final Report Phase II. (Nutrition CRSP., 1992).

Neumann, C., Harris, D. M. & Rogers, L. M. Contribution of animal source foods in improving diet quality and function in children in the developing world. *Nutrition Research* 22, 193–220 (2002).

Newman, T. B., Garber, A. M., Holtzman, N. A. & Hulley, S. B. Problems with the report of the Expert Panel on blood cholesterol levels in children and adolescents. *Arch. Pediatr. Adolesc. Med.* 149, 241–7 (1995).

Newman, W. P. et al. Relation of Serum Lipoprotein Levels and Systolic Blood Pressure to Early Atherosclerosis. *N. Engl. J. Med.* 314, 138–144 (1986).

Nguyen, Q. M. & Berenson, G. S. Utility of Childhood Glucose Homeostasis Variables in Predicting Adult Diabetes and Related Cardiometabolic Risk Factors: The Bogalusa Heart Study: Response to d'Annunzio, Russo, and Lorini. *Diabetes Care* 33, e124–e124 (2010).

NICE. Technology overview | Active B12 assay for diagnosing vitamin B12 deficiency | Advice | NICE. (2015). Available at: <https://www.nice.org.uk/advice/mib40/chapter/Technology-overview>. (Accessed: 23rd November 2019)

Nichols, M., Townsend, N., Scarborough, P. & Rayner, M. Trends in age-specific coronary heart disease mortality in the European Union over three decades: 1980-2009. *Eur. Heart J.* 34, 3017–3027 (2013).

Nicklaus, S. & Remy, E. Early Origins of Overeating: Tracking Between Early Food Habits and Later Eating Patterns. *Curr. Obes. Rep.* 2, 179–184 (2013).

NIHR. DAPA Measurement Toolkit. Available at: <https://dapa-toolkit.mrc.ac.uk/anthropometry/objective-methods/hydrometry>. (Accessed: 20th November 2019)

- NIHRa. DAPA Measurement Toolkit. Available at: <https://www.measurement-toolkit.org/anthropometry/objective-methods/multi-component-models>. (Accessed: 25th November 2019)
- NRC. Nutrient Adequacy. (National Academies Press, 1986).
- Nusser, S. M., Carriquiry, A. L., Dodd, K. W. & Fuller, W. A. A Semiparametric Transformation Approach to Estimating Usual Daily Intake Distributions. *J. Am. Stat. Assoc.* 91, 1440–1449 (1996).
- O’Connell, J. M. et al. Growth of vegetarian children: The Farm Study. *Pediatrics* 84, 475–81 (1989).
- O’Flaherty, M. et al. Exploring potential mortality reductions in 9 European countries by improving diet and lifestyle: A modelling approach. *Int. J. Cardiol.* 207, 286–291 (2016).
- O’Flaherty, M. et al. The decline in coronary heart disease mortality is slowing in young adults (Australia 1976-2006): A time trend analysis. *Int. J. Cardiol.* 158, 193–198 (2012).
- O’Neil, C. E., Nicklas, T. A. & Fulgoni, V. L. Tree nut consumption is associated with better nutrient adequacy and diet quality in adults: National health and nutrition examination survey 2005–2010. *Nutrients* 7, 595–607 (2015).
- OECD. Education at a Glance 2019. Education at a Glance: OECD Indicators (OECD, 2019).
- Oehler, C. Meghan Markle May Want to Raise Her Baby on a Vegan Diet—Is That Safe? *Health*. (2019). Available at: <https://www.health.com/nutrition/archie-sussex-vegan-diet>. (Accessed: 17th December 2019)
- Oh, R. C. Vitamin B12 deficiency - Symptoms, diagnosis and treatment | *BMJ Best Practice*. (2019). Available at: <https://bestpractice.bmj.com/topics/en-gb/822>. (Accessed: 16th December 2019)
- Oliver, J. & Webb, D. Noninvasive Assessment of Arterial Stiffness and Risk of Atherosclerotic Events. *Arterioscler. Thromb. Vasc. Biol.* 23, 554–566 (2003).
- Oliver, M., Schofield, G. M. & Kolt, G. S. Physical Activity in Preschoolers. *Sport. Med.* 37, 1045–1070 (2007).
- Ondrak, K. S. & Morgan, D. W. Physical activity, calcium intake and bone health in children and adolescents. *Sports Medicine* 37, 587–600 (2007).
- Orlich, M. J. & Fraser, G. E. Vegetarian diets in the Adventist Health Study 2: a review of initial published findings. *Am. J. Clin. Nutr.* 100, 353S-358S (2014).
- Ormazabal, V. et al. Association between insulin resistance and the development of cardiovascular disease. *Cardiovascular Diabetology* 17, (2018).
- Owens, S. et al. Visceral adipose tissue and cardiovascular risk factors in obese children. *J. Pediatr.* 133, 41–45 (1998).

- Øygdagen, H. Carotid Intima-Media Thickness and Prediction of Cardiovascular Disease. *J. Am. Heart Assoc.* 6, (2017).
- Pan, A. et al. Red meat consumption and risk of type 2 diabetes: 3 cohorts of US adults and an updated meta-analysis. *Am. J. Clin. Nutr.* 94, 1088–1096 (2011).
- Pandis, N. Cross-sectional studies. *Am. J. Orthod. Dentofac. Orthop.* 146, 127–129 (2014).
- Park, M. H. et al. Adiposity and carotid-intima media thickness in children and adolescents: a systematic review. *BMC Pediatr.* 15, 161 (2015).
- Pate, R. R., O'Neill, J. R. & Mitchell, J. Measurement of Physical Activity in Preschool Children. *Med. Sci. Sport. Exerc.* 42, 508–512 (2010).
- Paul, T. K. et al. Cardiovascular risk profile of asymptomatic healthy young adults with increased femoral artery intima-media thickness: The Bogalusa Heart Study. *Am. J. Med. Sci.* 330, 105–110 (2005).
- Pawlak, R. & Bell, K. Iron Status of Vegetarian Children: A Review of Literature. *Ann. Nutr. Metab.* 70, 88–99 (2017).
- Pawlak, R., Lester, S. E. & Babatunde, T. The prevalence of cobalamin deficiency among vegetarians assessed by serum vitamin B12: A review of literature. *European Journal of Clinical Nutrition* 68, 541–548 (2014).
- Pawlus, B. et al. Birth body length, birth body weight and birth head circumference in neonates born in a single centre between 2011 and 2016. *Ginekol. Pol.* 88, 599–605 (2017).
- PCRM. Physician's Committee for Responsible Medicine Health Topics. Available at: <https://www.pcrm.org/health-topics>. (Accessed: 2nd July 2019)
- Persky, V. W. et al. Hormone levels in vegetarian and nonvegetarian teenage girls: potential implications for breast cancer risk. *Cancer Res.* 52, 578–83 (1992).
- Piccoli, G. B. et al. Vegan-vegetarian diets in pregnancy: Danger or panacea? A systematic narrative review. *BJOG: An International Journal of Obstetrics and Gynaecology* 122, 623–633 (2015).
- Pierce, M. B. & Leon, D. A. Age at menarche and adult BMI in the Aberdeen Children of the 1950s Cohort Study. *Am. J. Clin. Nutr.* 82, 733–739 (2005).
- Poore, J. & Nemecek, T. Reducing food's environmental impacts through producers and consumers. *Science* 360, 987–992 (2018).
- Prentice, A. et al. Calcium supplementation increases stature and bone mineral mass of 16- to 18-year-old boys. *J. Clin. Endocrinol. Metab.* 90, 3153–3161 (2005).
- Prentice, A., Parsons, T. J. & Cole, T. J. Uncritical use of bone mineral density in absorptiometry may lead to size- related artifacts in the identification of bone

mineral determinants. *American Journal of Clinical Nutrition* 60, 837–842 (1994).

Priest, J. R., Nead, K. T., Wehner, M. R., Cooke, J. P. & Leeper, N. J. Self-Reported History of Childhood Smoking Is Associated with an Increased Risk for Peripheral Arterial Disease Independent of Lifetime Smoking Burden. *PLoS One* 9, e88972 (2014).

Pritchett, L. Greta Thunberg Promotes Veganism to 4 Million Ellen Viewers | LIVEKINDLY. (2019). Available at: <https://www.livekindly.co/greta-thunberg-promotes-veganism-ellen-viewers/>. (Accessed: 17th December 2019)

Proudfoot, N. A. et al. Physical activity and trajectories of cardiovascular health indicators during early childhood. *Pediatrics* 144, (2019).

R&D Systems. Human IGF1P-4 DuoSet ELISA DY804: R&D Systems. Available at: https://www.rndsystems.com/products/human-igfbp-4-duo-set-elisa_dy804. (Accessed: 23rd November 2019)

Raitakan, O. T. et al. Effects of persistent physical activity and inactivity on coronary risk factors in children and young adults the cardiovascular risk in young Finns study. *Am. J. Epidemiol.* 140, 195–205 (1994).

Ramakrishnan, U., Aburto, N., McCabe, G. & Martorell, R. Multimicronutrient Interventions but Not Vitamin A or Iron Interventions Alone Improve Child Growth: Results of 3 Meta-Analyses. *J. Nutr.* 134, 2592–2602 (2004).

Rasmussen, O. W. et al. Effects on Blood Pressure, Glucose, and Lipid Levels of High-Monounsaturated Fat Diet Compared With a High-Carbohydrate Diet in NIDDM Subjects. *Diabetes Care* 16, 1565–1571 (1993).

Reguła, J. Description and the evaluation of chosen alternative diets. *Forum Zaburzeń Metab.* 4, (2013).

Reichenheim, M. E. & Coutinho, E. S. F. Measures and models for causal inference in cross-sectional studies: arguments for the appropriateness of the prevalence odds ratio and related logistic regression. *BMC Med. Res. Methodol.* 10, 66 (2010).

Reilly, J. J. et al. Objective measurement of physical activity and sedentary behaviour: review with new data. *Arch. Dis. Child.* 93, 614–619 (2008).

Ried-Larsen, M., Grøntved, A., Kristensen, P. L., Froberg, K. & Andersen, L. B. Moderate-and-vigorous physical activity from adolescence to adulthood and subclinical atherosclerosis in adulthood: prospective observations from the European Youth Heart Study. *Br. J. Sports Med.* 49, 107–112 (2015).

Riso, E. M., Toplaan, L., Viira, P., Vaiksaar, S. & Jürimäe, J. Physical fitness and physical activity of 6-7-year-old children according to weight status and sports participation. *PLoS One* 14, (2019).

Rivera, J. A., Hotz, C., González-Cossío, T., Neufeld, L. & García-Guerra, A. The Effect of Micronutrient Deficiencies on Child Growth: A Review of Results

from Community-Based Supplementation Trials. *J. Nutr.* 133, 4010S-4020S (2003).

Roberts, B. Insulin. Association of Clinical Biochemistry (2012). Available at: [www.acb.org.uk/Nat Lab Med Hbk/Insulin.pdf](http://www.acb.org.uk/Nat%20Lab%20Med%20Hbk/Insulin.pdf) (Accessed: 23rd November 2019).

Roed C, Skovby F, L. A. Vaer vitamin B12-mangel hos spaedbørn brystnaeret af veganere [Severe vitamin B12 deficiency in infants breastfed by vegans]. *Ugeskr Laeger.*, 171(43),3099–3101 (2009).

Rogers, I. et al. Diet throughout childhood and age at menarche in a contemporary cohort of British girls. *Public Health Nutr.* 13, 2052–2063 (2010).

Rogers, W. Regression standard errors in clustered samples. *Stata Tech. Bull.* 3, (1994).

Romaszko, J. et al. An assessment of the duration of breastfeeding in north-eastern Poland. *Cent. Eur. J. Med.* 8, 75–79 (2013).

Rosell, M., Appleby, P. & Key, T. Height, age at menarche, body weight and body mass index in life-long vegetarians. *Public Health Nutr.* 8, 870–875 (2005).

Rosenbush, S. W. & Parker, J. M. Height and heart disease. *Rev. Cardiovasc. Med.* 15, 102–8 (2014).

Rothausen, B. W., Matthiessen, J., Andersen, L. F., Brockhoff, P. B. & Tetens, I. Dietary patterns on weekdays and weekend days in 4–14-year-old Danish children. *Br. J. Nutr.* 109, 1704–1713 (2013).

Rothkrantz-Kos, S., Schmitz, M. P. J., Bekers, O., Menheere, P. P. C. A. & van Dieijen-Visser, M. P. High-Sensitivity C-Reactive Protein Methods Examined. *Clin. Chem.* 48, (2002).

Roy Morgan Research. The slow but steady rise of vegetarianism in Australia - Roy Morgan Research. Available at: <http://www.roymorgan.com/findings/vegetarianisms-slow-but-steady-rise-in-australia-201608151105>. (Accessed: 2nd July 2019)

Ruys, J. & Hickie, J. B. Serum cholesterol and triglyceride levels in Australian adolescent vegetarians. *Br. Med. J.* 2, 87 (1976).

Sabaté, J. & Wien, M. Vegetarian diets and childhood obesity prevention. *Am. J. Clin. Nutr.* 91, 1525S-1529S (2010).

Sabaté, J., Lindsted, K. D., Harris, R. D. & Sanchez, A. Attained height of lacto-ovo vegetarian children and adolescents. *Eur. J. Clin. Nutr.* 45, 51–8 (1991).

Sabaté, J., Lindsted, K., Harris, R. & Johnston, P. Anthropometric Parameters of Schoolchildren With Different Life-styles. *Arch. Pediatr. Adolesc. Med.* 144, 1159 (1990).

- Sabaté, J., Llorca, M. C. & Sánchez, A. Lower height of lacto-ovovegetarian girls at preadolescence: an indicator of physical maturation delay? *J. Am. Diet. Assoc.* 92, 1263–4 (1992).
- Sacri, A.-S. et al. Stability of serum ferritin measured by immunoturbidimetric assay after storage at -80°C for several years. *PLoS One* 12 (2017).
- Sanders, T. A. Growth and development of British vegan children. *Am. J. Clin. Nutr.* 48, 822–825 (1988).
- Satija, A. et al. Healthful and Unhealthful Plant-Based Diets and the Risk of Coronary Heart Disease in U.S. Adults. *J. Am. Coll. Cardiol.* 70, 411–422 (2017).
- Sawada, N. et al. The association between adult attained height and sitting height with mortality in the European prospective investigation into cancer and nutrition (EPIC). *PLoS One* 12, (2017).
- Sawaya, A. L., Sesso, R., Florencio, T. M. de M. T., Fernandes, M. T. B. & Martins, P. A. Association between chronic undernutrition and hypertension. *Matern. Child Nutr.* 1, 155–163 (2005).
- Schaefer, F., Georgi, M., Wühl, E. & Schärer, K. Body mass index and percentage fat mass in healthy German schoolchildren and adolescents. *Int. J. Obes.* 22, 461–469 (1998).
- Schisterman, E. F., Cole, S. R. & Platt, R. W. Overadjustment bias and unnecessary adjustment in epidemiologic studies. *Epidemiology* 20, 488–95 (2009).
- Scholes, S. et al. Quantifying Policy Options for Reducing Future Coronary Heart Disease Mortality in England: A Modelling Study. *PLoS One* 8, e69935 (2013).
- Schürmann, S., Kersting, M. & Alexy, U. Vegetarian diets in children: a systematic review. *Eur. J. Nutr.* 56, 1797–1817 (2017).
- Schwingshackl, L. & Hoffmann, G. Comparison of Effects of Long-Term Low-Fat vs High-Fat Diets on Blood Lipid Levels in Overweight or Obese Patients: A Systematic Review and Meta-Analysis. *J. Acad. Nutr. Diet.* 113, 1640–1661 (2013).
- Sciarrone, S. E. et al. Biochemical and neurohormonal responses to the introduction of a lacto-ovovegetarian diet. *J. Hypertens.* 11, 849–60 (1993).
- Scoffin, K. Hematology Analyzers—From Complete Blood Counts to Cell Morphology | Labcompare.com. (2014). Available at: <https://www.labcompare.com/10-Featured-Articles/162042-Hematology-Analyzers-From-Complete-Blood-Counts-to-Cell-Morphology/>. (Accessed: 23rd November 2019)
- Seery, T., Moodie, D., Cephus, C. & Sexson, K. Lipid profiles performed at the time of routine examinations in 9-11 year olds within Texas children’s pediatric associates primary care pediatric clinics. *J. Am. Coll. Cardiol.* 63, A1461 (2014).

- Sezgin, EM, Erdöl Ş, Özdemir Ö, Baytan B, G. A. Long-term outcome in children with nutritional vitamin B12 deficiency. *Turk J Haematol.* 28, 286–93 (2011).
- Shepherd, J. A., Ng, B. K., Sommer, M. J. & Heymsfield, S. B. Body composition by DXA. *Bone* 104, 101–105 (2017).
- Shim, J.-S., Oh, K. & Kim, H. C. Dietary assessment methods in epidemiologic studies. *Epidemiol. Health* 36, e2014009 (2014).
- Sievenpiper, J. L. et al. Effect of non-oil-seed pulses on glycaemic control: a systematic review and meta-analysis of randomised controlled experimental trials in people with and without diabetes. *Diabetologia* 52, 1479–1495 (2009).
- Sievers, E., Dörner, K., Hamm, E., Hanisch, C. & Schaub, J. Vergleichende Untersuchungen zur Eisenversorgung lakto-ovo-vegetabil ernährter Säuglinge. *Ärztezeitschr Naturheilverf* 32, 106-112. (1991).
- Singh, A. S., Mulder, C., Twisk, J. W. R., Van Mechelen, W. & Chinapaw, M. J. M. Tracking of childhood overweight into adulthood: a systematic review of the literature. *Obes. Rev.* 9, 474–488 (2008).
- Singhal, A. & Lucas, A. Early origins of cardiovascular disease: Is there a unifying hypothesis? *Lancet* 363, 1642–1645 (2004).
- Skelly, A. C., Dettori, J. R. & Brodt, E. D. Assessing bias: the importance of considering confounding. *Evid. Based. Spine. Care. J.* 3, 9–12 (2012).
- Slimani, N. et al. The standardized computerized 24-h dietary recall method EPIC-Soft adapted for pan-European dietary monitoring. *Eur. J. Clin. Nutr.* 65, S5–S15 (2011).
- Smith, C. Y. et al. Contributions of increasing obesity and diabetes to slowing decline in subclinical coronary artery disease. *J. Am. Heart Assoc.* 4, (2015).
- Snijder, M. et al. Associations of hip and thigh circumferences independent of waist circumference with the incidence of type 2 diabetes: The Hoorn Study. *Am. J. Clin. Nutr.* 77, 1192–1197 (2003).
- Snijder, M. et al. Low subcutaneous thigh fat is a risk factor for unfavourable glucose and lipid levels, independently of high abdominal fat. The Health ABC Study. *Diabetologia* 48, 301–308 (2005).
- Snowdon, D. A. & Phillips, R. L. Does a vegetarian diet reduce the occurrence of diabetes? *Am. J. Public Health* 75, 507–12 (1985).
- Soare, A. et al. A 6-month follow-up study of the randomized controlled Ma-Pi macrobiotic dietary intervention (MADIAB trial) in type 2 diabetes. *Nutr. Diabetes* 6, e222 (2016).
- Sobiecki, J. G., Appleby, P. N., Bradbury, K. E. & Key, T. J. High compliance with dietary recommendations in a cohort of meat eaters, fish eaters, vegetarians, and vegans: results from the European Prospective Investigation into Cancer and Nutrition–Oxford study. *Nutr. Res.* 36, 464–477 (2016).

Sopher, A. B., Fennoy, I. & Oberfield, S. E. An update on childhood bone health: Mineral accrual, assessment and treatment. *Current Opinion in Endocrinology, Diabetes and Obesity* 22, 35–40 (2015).

Spolidoro, J. V. et al. Waist circumference in children and adolescents correlate with metabolic syndrome and fat deposits in young adults. *Clin. Nutr.* 32, 93–97 (2013).

Spurek, S. dr Sylwia Spurek posłanka do Parlamentu Europejskiego. (2019).

Srinivasan, S. R., Bao, W., Wattigney, W. A. & Berenson, G. S. Adolescent overweight is associated with adult overweight and related multiple cardiovascular risk factors: the Bogalusa Heart Study. *Metabolism.* 45, 235–40 (1996).

Stamler, J., Dyer, A. R., Shekelle, R. B., Neaton, J. & Stamler, R. Relationship of Baseline Major Risk Factors to Coronary and All-Cause Mortality, and to Longevity: Findings from Long-Term Follow-Up of Chicago Cohorts. *Cardiology* 82, 191–222 (1993).

Sтары, H. Evolution and progression of atherosclerotic lesions in coronary arteries of children and young adults. *Arteriosclerosis* 9, 119-32 (1989).

Sтары, H. Lipid and macrophage accumulations in arteries of children and the development of atherosclerosis. *Am. J. Clin. Nutr.* 72, 1297s-1306s (2000).

Stehbens, W. E. The epidemiological relationship of hypercholesterolaemia, hypertension, diabetes mellitus and obesity to coronary heart disease and atherogenesis. *J. Clin. Epidemiol.* 43, 733–41 (1990).

Stiglic, N. & Viner, R. M. Effects of screentime on the health and well-being of children and adolescents: A systematic review of reviews. *BMJ Open* 9, (2019).

Stokes, A. & Preston, S. H. Smoking and reverse causation create an obesity paradox in cardiovascular disease. *Obesity* 23, 2485–2490 (2015).

Stulp, G., Buunk, A. P., Verhulst, S. & Pollet, T. V. Human height is positively related to interpersonal dominance in dyadic interactions. *PLoS One* 10, (2015).

Su, T.-C. et al. Homocysteine, circulating vascular cell adhesion molecule and carotid atherosclerosis in postmenopausal vegetarian women and omnivores. *Atherosclerosis* 184, 356–362 (2006).

Suttorp, M. M., Siegerink, B., Jager, K. J., Zoccali, C. & Dekker, F. W. Graphical presentation of confounding in directed acyclic graphs. *Nephrol. Dial. Transplant.* 30, 1418–1423 (2015).

Szeto, Y. T., Kwok, T. C. Y. & Benzie, I. F. F. Effects of a long-term vegetarian diet on biomarkers of antioxidant status and cardiovascular disease risk. *Nutrition* 20, 863–866 (2004).

Szulc, P., Seeman, E. & Delmas, P. D. Biochemical Measurements of Bone Turnover in Children and Adolescents. *Osteoporos. Int.* 11, 281–294 (2000).

- Taittonen, L. et al. Insulin and blood pressure among healthy children: Cardiovascular risk in young Finns. *Am. J. Hypertens.* 9, 193–199 (1996).
- Talarico, R. & Janssen, I. Compositional associations of time spent in sleep, sedentary behavior and physical activity with obesity measures in children. *Int. J. Obes.* 42, 1508–1514 (2018).
- Tanner, J. M. & Whitehouse, R. H. Revised standards for triceps and subscapular skinfolds in British children. *Arch. Dis. Child.* 50, 142–145 (1975).
- Tanrikulu, M. A., Agirbasli, M. & Berenson, G. Primordial Prevention of Cardiometabolic Risk in Childhood. in *Advances in experimental medicine and biology* 956, 489–496 (2016).
- Tantamango-Bartley, Y., Jaceldo-Siegl, K., Fan, J. & Fraser, G. Vegetarian Diets and the Incidence of Cancer in a Low-risk Population. *Cancer Epidemiol. Biomarkers Prev.* 22, 286–294 (2013).
- Taylor, A., Redworth, E. W. & Morgan, J. B. Influence of Diet on Iron, Copper, and Zinc Status in Children Under 24 Months of Age. *Biol. Trace Elem. Res.* 97, 197–214 (2004).
- Telama, R. Tracking of physical activity from childhood to adulthood: A review. *Obesity Facts* 2, 187–195 (2009).
- Textor, J. Drawing and Analyzing Causal DAGs with DAGitty. (2015).
- Textor, J., van der Zander, B., Gilthorpe, M. S., Liškiewicz, M. & Ellison, G. T. H. Robust causal inference using directed acyclic graphs: the R package ‘dagitty’. *Int. J. Epidemiol.* dyw341 (2017).
- The Emerging Risk Factors Collaboration et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet* 375, 2215–2222 (2010).
- The Sentience Institute. Sentience Institute | Animal Farming Attitudes Survey 2017. (2017). Available at: <https://www.sentienceinstitute.org/animal-farming-attitudes-survey-2017>. (Accessed: 2nd July 2019)
- The Vegan Society. Find out how many vegans are in Great Britain | The Vegan Society. (2016). Available at: <https://www.vegansociety.com/whats-new/news/find-out-how-many-vegans-are-great-britain>. (Accessed: 2nd July 2019)
- The Vegetarian Resource Group. The Market for Vegetarian Foods. Available at: <https://www.vrg.org/nutshell/market.htm#market>. (Accessed: 2nd July 2019)
- ThermoFisher. IGFBP3 Human ELISA Kit - Invitrogen. Available at: <https://www.thermofisher.com/elisa/product/IGFBP3-Human-ELISA-Kit/EHIGFBP3>. (Accessed: 23rd November 2019)
- Thorolfsdottir, R. B. et al. Population assessment of future trajectories in coronary heart disease mortality. *PLoS One* 9, (2014).

- Tirosh, A. et al. Adolescent BMI trajectory and risk of diabetes versus coronary disease. *N. Engl. J. Med.* 364, 1315–1325 (2011).
- Tokarczuk, O. *Drive your Plow over the Bones of the Dead.* (Fitzcarraldo Editions, 2009).
- Tonstad, S., Butler, T., Yan, R. & Fraser, G. E. Type of Vegetarian Diet, Body Weight, and Prevalence of Type 2 Diabetes. *Diabetes Care* 32, 791–796 (2009).
- Touboul, P.-J. Intima-Media Thickness of Carotid Arteries. in *Frontiers of neurology and neuroscience* 36, 31–39 (2014).
- Tremblay, M. S. et al. Systematic review of sedentary behaviour and health indicators in school-aged children and youth. *International Journal of Behavioral Nutrition and Physical Activity* 8, (2011).
- Tse, W. Y., Hindmarsh, P. C. & Brook, C. G. D. The infancy-childhood-puberty model of growth: Clinical aspects. *Acta Paediatr. Scand. Suppl.* 78, 38–43 (1989).
- Tsuji, K., Maeda, T., Kawane, T., Matsunuma, A. & Horiuchi, N. Leptin stimulates fibroblast growth factor 23 expression in bone and suppresses renal $1\alpha,25$ -dihydroxyvitamin D₃ synthesis in leptin-deficient ob/ob Mice. *J. Bone Miner. Res.* 25, 1711–1723 (2010).
- Turley, M. L., Skeaff, C. M., Mann, J. I. & Cox, B. The effect of a low-fat, high-carbohydrate diet on serum high density lipoprotein cholesterol and triglyceride. *Eur. J. Clin. Nutr.* 52, 728–732 (1998).
- Twig, G. et al. Body-Mass Index in 2.3 Million Adolescents and Cardiovascular Death in Adulthood. *N. Engl. J. Med.* 374, 2430–2440 (2016).
- Tyrrell, J. et al. Height, body mass index, and socioeconomic status: mendelian randomisation study in UK Biobank. *BMJ* 352, i582 (2016).
- U.S. Department of Health and Human Services and U.S. Department of Agriculture. *Dietary Guidelines for Americans 2015-2020.* (2015).
- Ullmann, D., Connor, W. E., Hatcher, L. F., Connor, S. L. & Flavell, D. P. Will a high-carbohydrate, low-fat diet lower plasma lipids and lipoproteins without producing hypertriglyceridemia? in *Arteriosclerosis and Thrombosis* 11, 1059–1067 (1991).
- Umer, A. et al. Childhood obesity and adult cardiovascular disease risk factors: A systematic review with meta-analysis. *BMC Public Health* 17, (2017).
- Upadhyay, J., Farr, O. M. & Mantzoros, C. S. The role of leptin in regulating bone metabolism. *Metabolism.* 64, 105–13 (2015).
- Urbina, E. M. et al. Noninvasive Assessment of Subclinical Atherosclerosis in Children and Adolescents. *Hypertension* 54, 919–950 (2009).

US Institute of Medicine. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. (National Academic Press, 2005).

US Institute of Medicine. Dietary reference intakes. Applications in dietary assessment: a report of the Subcommittees on Interpretation and Uses of Dietary Reference Intakes and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition. (National Academy Press, 2000a).

US Institute of Medicine. Iron - Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc - NCBI Bookshelf. in (The National Academies Press., 2001).

US Institute of Medicine. Using the Estimated Average Requirement for Nutrient Assessment of Groups. in Dietary reference intakes. Applications in dietary assessment: a report of the Subcommittees on Interpretation and Uses of Dietary Reference Intakes; Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. (National Academic Press, 2000b).

USDA. USDA Food Composition Databases. Available at: <https://ndb.nal.usda.gov/ndb/> (accessed 5 May 2019). (Accessed: 25th November 2019)

van der Ploeg, G. E., Withers, R. T. & Laforgia, J. Percent body fat via DEXA: comparison with a four-compartment model. *J. Appl. Physiol.* 94, 499–506 (2003).

Van Gaal, L. F., Mertens, I. L. & De Block, C. E. Mechanisms linking obesity with cardiovascular disease. *Nature* 444, 875–880 (2006).

Van der Weele, T. J. Principles of confounder selection. *Eur. J. Epidemiol.* 34, 211–219 (2019).

Varady, K. A. & Jones, P. J. H. Combination Diet and Exercise Interventions for the Treatment of Dyslipidemia: an Effective Preliminary Strategy to Lower Cholesterol Levels? *J. Nutr.* 135, 1829–1835 (2005).

Vatanparast, H. & Whiting, S. Calcium Supplementation Trials and Bone Mass Development in Children, Adolescents, and Young Adults. *Nutr. Rev.* 64, 204–209 (2006).

Verduin, W. M., Van Den Helder, R., Doodeman, H. J., Struijf, E. & Houdijk, A. P. J. Dexa Body Composition Assessment in 10-11 Year Healthy Children. *PLoS One* 11 (2016).

VIVA. Jeremy Corbyn, leader of the Labour Party | Viva! - The Vegan Charity. (2019a). Available at: <https://www.viva.org.uk/what-we-do/celebrity-supporters/jeremy-corbyn-leader-labour-party>. (Accessed: 17th December 2019)

- VIVA. Natalie Bennett - Former leader of the Green Party | Viva! - The Vegan Charity. (2019b). Available at: <https://www.viva.org.uk/what-we-do/celebrity-supporters/natalie-bennett-green-party-leader>. (Accessed: 17th December 2019)
- Wallace, T. M. & Matthews, D. R. The assessment of insulin resistance in man. *Diabet. Med.* 19, 527–534 (2002).
- Wang, A. et al. Cumulative Exposure to High-Sensitivity C-Reactive Protein Predicts the Risk of Cardiovascular Disease. *J. Am. Heart Assoc.* 6, (2017).
- Wang, F. et al. Effects of Vegetarian Diets on Blood Lipids: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *J. Am. Heart Assoc.* 4 (2015).
- Wang, H. et al. Oral vitamin B12 versus intramuscular vitamin B12 for vitamin B12 deficiency. *Cochrane Database of Systematic Reviews* 2018, (2018).
- Wang, S.-F. et al. Birth weight and risk of coronary heart disease in adults: a meta-analysis of prospective cohort studies. *J. Dev. Orig. Health Dis.* 5, 408–419 (2014).
- Wang, Z., Shen, W., Withers, R. & S., H. Multicomponent molecular level model of body composition analysis. in *Human Body Composition. Human Kinetics* (ed. Heymsfield SB, Lohman TG, G. S.) 163–76 (2005).
- Wądołowska, L. et al. Stanowisko Komitetu Nauki o Żywieniu Człowieka PAN w sprawie wartości odżywczej i bezpieczeństwa stosowania diet wegetariańskich e. (2019).
- WCRF. Cancer Prevention Recommendations | World Cancer Research Fund International. (2019a). Available at: <https://www.wcrf.org/dietandcancer/cancer-prevention-recommendations>. (Accessed: 2nd July 2019)
- WCRF. Eat a diet rich in wholegrains, vegetables, fruit and beans | WCRF UK. (2019b). Available at: <https://www.wcrf-uk.org/uk/preventing-cancer/cancer-prevention-recommendations/enjoy-more-grains-veg-fruit-and-beans>. (Accessed: 17th December 2019)
- Weaver, C. M. et al. The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations. *Osteoporos. Int.* 27, 1281–1386 (2016).
- Webber, C. Reproducibility of DXA Measurements of Bone Mineral and Body Composition: Application to Routine Clinical Measurements. in *Handbook of Anthropometry* 151–165 (Springer New York, 2012).
- Webber, L. S., Srinivasan, S. R., Wattigney, W. A. & Berenson, G. S. Tracking of Serum Lipids and Lipoproteins from Childhood to Adulthood. *Am. J. Epidemiol.* 133, 884–899 (1991).
- Weber, D. R., Leonard, M. B. & Zemel, B. S. Body composition analysis in the pediatric population. *Pediatr. Endocrinol. Rev.* 10, 130–9 (2012).

- Weber, D. R., Leonard, M. B., Shults, J. & Zemel, B. S. A Comparison of Fat and Lean Body Mass Index to BMI for the Identification of Metabolic Syndrome in Children and Adolescents. *J. Clin. Endocrinol. Metab.* 99, 3208 (2014).
- Weber, D. R., Moore, R. H., Leonard, M. B. & Zemel, B. S. Fat and lean BMI reference curves in children and adolescents and their utility in identifying excess adiposity compared with BMI and percentage body fat. *Am. J. Clin. Nutr.* 98, 49–56 (2013).
- Weder, S., Hoffmann, M., Becker, K., Alexy, U. & Keller, M. Energy, Macronutrient Intake, and Anthropometrics of Vegetarian, Vegan, and Omnivorous Children (1–3 Years) in Germany (VeChi Diet Study). *Nutrients* 11, 832 (2019).
- Weigensberg, M. J. et al. Dietary fat intake and insulin resistance in black and white children. *Obes. Res.* 13, 1630–1637 (2005).
- Weiss, N. S. *Clinical epidemiology: the study of the outcome of illness.* (Oxford University Press, 2006).
- Wells, J. & Fewtrell, M. Measuring body composition. *Arch. Dis. Child.* 91, 612–7 (2006).
- Wells, J. & Siervo, M. Obesity and energy balance: Is the tail wagging the dog. *European Journal of Clinical Nutrition* 65, 1173–1189 (2011).
- Wells, J. A Hattori chart analysis of body mass index in infants and children. *Int. J. Obes.* 24, 325–329 (2000).
- Wells, J. et al. Body-composition reference data for simple and reference techniques and a 4-component model: a new UK reference child. *Am. J. Clin. Nutr.* 96, 1316–1326 (2012).
- Wells, J. et al. Four-component model of body composition in children: density and hydration of fat-free mass and comparison with simpler models. *Am. J. Clin. Nutr.* 69, 904–912 (1999).
- Wells, J. et al. Pediatric reference data for lean tissue properties: Density and hydration from age 5 to 20 y. *Am. J. Clin. Nutr.* 91, 610–618 (2010).
- Wells, J. et al. Prediction of total body water in infants and children. *Arch. Dis. Child.* 90, 965–971 (2005).
- Wells, J. The diabesity epidemic in the light of evolution: insights from the capacity–load model. *Diabetologia* 62, 1740–1750 (2019).
- Wells, J. The limitations of DXA for body composition assessment | SFEBES2009. *Endocr. Abstr.* 19, S78 (2009).
- Wells, J., Sharp, G., Steer, P. & Leon, D. Paternal and Maternal Influences on Differences in Birth Weight between Europeans and Indians Born in the UK. *PLoS One* 8, (2013).

- Welsh, J. A. et al. Caloric sweetener consumption and dyslipidemia among US adults. *JAMA - J. Am. Med. Assoc.* 303, 1490–1497 (2010).
- Weryński, M. Olga Tokarczuk - Vege | weganizm, ochrona zwierząt, zdrowy styl życia. (2019). Available at: <https://vege.com.pl/2018/05/24/olga-tokarczuk/>. (Accessed: 17th December 2019)
- White, D. et al. The Relationship between Coronary Artery Disease Risk Factors and Carotid Intima-Media Thickness in Children. *J. Pediatr.* 190, 38–42 (2017).
- Whitley, E., Martin, R. M., Davey Smith, G., Holly, J. M. P. & Gunnell, D. Childhood stature and adult cancer risk: The Boyd Orr cohort. *Cancer Causes Control* 20, 243–251 (2009).
- WHO WHF WSO. Global Atlas on cardiovascular disease prevention and control. (2011).
- WHO. Iron Deficiency Anaemia, Assessment, Prevention, and Control. A guide for programme managers. (2001).
- WHO. Noncommunicable diseases. (2018b). Available at: <https://www.who.int/en/news-room/fact-sheets/detail/noncommunicable-diseases>. (Accessed: 29th December 2019)
- WHO. Nutrition, Physical Activity and Obesity, Poland. (2013). Available at: http://www.euro.who.int/__data/assets/pdf_file/0020/243317/Poland-WHO-Country-Profile.pdf. (Accessed: 7th January 2020)
- WHO. Disability-adjusted life years (DALYs). (2019b). Available at: https://www.who.int/gho/mortality_burden_disease/daly_rates/text/en/. (Accessed: 29th December 2019)
- WHO. Global status report on noncommunicable diseases 2010. (World Health Organization, 2011).
- WHO. A healthy lifestyle. (2019). Available at: www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle. (Accessed 7th July 2019)
- WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. WHO (World Health Organization, 2018).
- WHO. Tobacco control in Poland. WHO (2015). Available at: <https://www.who.int/tobacco/about/partners/bloomberg/pol/en/%0D>. (Accessed: 8th January 2020)
- Willett, W. et al. Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. *Lancet* 393, 447–492 (2019).
- Williams, J. et al. Evaluation of Lunar Prodigy dual-energy X-ray absorptiometry for assessing body composition in healthy persons and patients by comparison with the criterion 4-component model. *Am. J. Clin. Nutr.* 83, 1047–1054 (2006).

Williams, T. C., Bach, C. C., Matthiesen, N. B., Henriksen, T. B. & Gagliardi, L. Directed acyclic graphs: a tool for causal studies in paediatrics. *Pediatr. Res.* 84, 487–493 (2018).

Winzenberg, T., Shaw, K., Fryer, J. & Jones, G. Effects of calcium supplementation on bone density in healthy children: Meta-analysis of randomised controlled trials. *Br. Med. J.* 333, 775–778 (2006).

Wojciak, R. W. Effect of short-term food restriction on iron metabolism, relative well-being and depression symptoms in healthy women. *Eat. Weight Disord.* 19, 321–327 (2014).

Wolf, A. M. et al. Activity, inactivity, and obesity: racial, ethnic, and age differences among schoolgirls. *Am. J. Public Health* 83, 1625–7 (1993).

Wong, W. W. et al. Evaluating body fat in girls and female adolescents: Advantages and disadvantages of dual-energy X-ray absorptiometry. *Am. J. Clin. Nutr.* 76, 384–389 (2002).

Wrenn, C., Wrenn & Lee, C. Trump Veganism: A Political Survey of American Vegans in the Era of Identity Politics. *Societies* 7, 32 (2017).

Wu, H. et al. Association between dietary whole grain intake and risk of mortality: Two large prospective studies in US Men and Women. *JAMA Intern. Med.* 175, 373–384 (2015).

Xu, S. et al. Reference Ranges for Serum IGF-1 and IGFBP-3 Levels in Chinese Children During Childhood and Adolescence. *Endocr. J.* (2009).

Yang, S.-Y. et al. Relationship of carotid intima-media thickness and duration of vegetarian diet in Chinese male vegetarians. *Nutr. Metab. (Lond)*. 8, 63 (2011).

Yen, C.-E., Yen, C.-H., Huang, M.-C., Cheng, C.-H. & Huang, Y.-C. Dietary intake and nutritional status of vegetarian and omnivorous preschool children and their parents in Taiwan. *Nutr. Res.* 28, 430–436 (2008).

Yokoyama, Y. et al. Vegetarian Diets and Blood Pressure. *JAMA Intern. Med.* 174, 577 (2014).

Zulyniak, M. A. et al. Does the impact of a plant-based diet during pregnancy on birth weight differ by ethnicity? A dietary pattern analysis from a prospective Canadian birth cohort alliance. *BMJ Open* 7 (2017).