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Abstract: Background and aims: Sex differences in measures of cardiovascular health in adults are well documented. However, the sex-specific aetiology of cardiovascular health across childhood and adolescence is poorly understood.
Material and methods: We examined sex differences in trajectories of 11 measures of cardiovascular health from birth to 18 years, in a contemporary birth cohort study in England (N participants per outcomes: 662-13,985, N repeated measures per outcome: 1,831-112,768). Outcomes were measured over varying time spans from birth or mid-childhood to age 18 and with different numbers of repeated measures per outcome. Analyses were performed using fractional polynomial and linear spline multilevel models.
Results: Females had higher mean BMI, height-adjusted fat mass, pulse rate, insulin, triglycerides, and non-high-density lipoprotein cholesterol (HDL-c) and lower mean height-adjusted lean mass from birth or from mid-childhood to age 18. For example, mean non-HDL-c was 0.07 mmol/l (95% Confidence Interval (CI), 0.04, 0.10) higher in females compared with males at birth. By age 18, this difference persisted and widened to 0.19 mmol/l (95% CI, 0.16, 0.23) higher non-HDL-c in females compared with males. Females had lower levels of glucose from mid-childhood and developed lower systolic blood pressure and higher HDL-c from mid-adolescence onward. For example, females had 0.08 mmol/l (95% CI, 0.05, 0.10) lower mean glucose compared with males at age 7 which widened to a difference of 0.22 mmol/l (95% CI, 0.25, 0.19) at age 18.
Conclusions: Sex differences in measures of cardiovascular health are apparent from birth or mid-childhood and change during early life. These differences may have implications for sex-specific disease risk in future adult populations.

***Highlights**

- Sex differences in measures of cardiovascular health are well established in adulthood.
- Few studies have examined sex-specific change in cardiovascular risk in childhood.
- Our findings show that sex differences in cardiovascular health begin at birth.
- These sex differences change further throughout childhood and adolescence.
- Early life factors may play a role in sex differences in cardiometabolic disease.

# Sex-specific trajectories of measures of cardiovascular health during childhood and adolescence: A prospective cohort study

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**Keywords:** sex-specific; cardiovascular; childhood; adolescence; longitudinal

## Abstract

*Background and aims:* Sex differences in measures of cardiovascular health in adults are well documented. However, the sex-specific aetiology of cardiovascular health across childhood and adolescence is poorly understood.

*Methods:* We examined sex differences in trajectories of 11 measures of cardiovascular health from birth to 18 years, in a contemporary birth cohort study in England (N participants per outcomes: 662-13,985, N repeated measures per outcome: 1,831-112,768). Outcomes were measured over varying time spans from birth or mid-childhood to age 18 and with different numbers of repeated measures per outcome. Analyses were performed using fractional polynomial and linear spline multilevel models.

*Results:* Females had higher mean BMI, height-adjusted fat mass, pulse rate, insulin, triglycerides, and non-high-density lipoprotein cholesterol (HDL-c) and lower mean height-adjusted lean mass from birth or from mid-childhood to age 18. For example, mean non-HDL-c was 0.07 mmol/l (95% confidence interval (CI), 0.04, 0.10) higher in females compared with males at birth. By age 18, this difference persisted and widened to 0.19 mmol/l (95% CI, 0.16, 0.23) higher non-HDL-c in females compared with males. Females had lower levels of glucose from mid-childhood and developed lower systolic blood pressure and higher HDL-c from mid-adolescence onward. For example, females had 0.08 mmol/l (95% CI, 0.05, 0.10) lower mean glucose compared with males at age 7 which widened to a difference of 0.22 mmol/l (95% CI, 0.25, 0.19) at age 18.

*Conclusions:* Sex differences in measures of cardiovascular health are apparent from birth or mid-childhood and change during early life. These differences may have implications for sex-specific disease risk in future adult populations.

## Introduction

Cardiovascular disease (CVD) is a leading cause of death worldwide and its prevalence continues to increase globally. (1, 2) Women and men do not experience cardiometabolic diseases (CVD and type 2 diabetes mellitus (T2DM)) equally. For instance, at age 40, the remaining lifetime risk of CVD is one in two for women and two in three for men. (3, 4) Women are also less insulin resistant than men and develop T2DM at higher levels of adiposity. (5) However, amongst people with T2DM, coronary heart disease (CHD) and stroke risk are up to 50% higher in women compared with men. (6, 7) Despite these well-established sex differences, the sex-specific aetiology of cardiovascular risk remains poorly understood. Recent guidelines and scientific statements emphasise the importance of studying sex differences in cardiovascular risk in adults. (8-11) Given that cardiovascular risk

originates in early life (12-14) and tracks through the life course (15-17), there is also a need to study potential sex differences during childhood and adolescence. Longitudinal studies of sex differences in measures of cardiovascular health during childhood and adolescence can help to establish when sex differences emerge and how sex differences change over time, contributing to understanding the mechanisms underlying sex differences in cardiovascular disease risk across the life course.

To date, sex differences in selected measures of cardiovascular health have been examined during childhood and adolescence in a small number of US studies, including change in blood pressure (18) and lipids (19) in 678 children aged eight to 18 years in the Project Heartbeat! and change in glucose and insulin from 5 to 17 years (20) and lipids from 5 to 26 years (21) in the Bogalusa Heart Study. A more recent study in 507 children in Minneapolis examined change over time in 10 conventional measures of cardiovascular health measured up to 3 times from 11 to 19 years. (22) Recent analyses, combining data from several cohorts across the life course have also examined sex differences in trajectories of blood pressure over time. (23) However, large contemporary studies with repeated measures of all key measures of cardiovascular health together from early life through adolescence are lacking. Contemporary studies of measures of cardiovascular health in early life are of particular importance given the high prevalence of overweight and obesity during childhood and adolescence compared with previous generations. (24) Studies of successive generations are also important given the significant changes in lifestyle that have occurred over time, (25) in both sexes but particularly among women, which will likely impact future sex-specific disease burden in the population.

We examine sex-specific trajectories of a range of measures of cardiovascular health measured at multiple time points across childhood and adolescence in a prospective birth cohort study of participants born in 1991-2 in the South West of England. The risk factors we consider are body mass index (BMI) measured repeatedly from age 1 to 18 years, fat mass and lean mass measured from 9 to 18 years, systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse rate and glucose

measured from 7 to 18 years, and insulin, triglycerides, high density lipoprotein cholesterol (HDL-c), and non-HDL-c measured from birth to 18 years.

## Patients and methods

### Study participants

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a prospective birth cohort study in Southwest England. (26, 27) Pregnant women resident in one of the three Bristol-based health districts with an expected delivery date between April 1, 1991 and December 31, 1992 were invited to participate. The study has been described elsewhere in detail. (26, 27) ALSPAC initially enrolled a cohort of 14,451 pregnancies, from which 13,867 live births occurred in 13,761 women. Follow-up has included parent and child completed questionnaires, links to routine data and clinic attendance. Research clinics were held when the participants were approximately 7, 9, 10, 11, 13, 15, and 18 years old. Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. The study website contains details of all the data that is available through a fully searchable data dictionary http://www.bristol.ac.uk/alspac/researchers/access/. (28)

### Study outcomes

*Anthropometry*

Height and weight were modelled previously and are not included here.(29-31) BMI (weight (kg) divided by height squared ($m^2$)) was calculated from 1 to 18 years using data from several sources including research clinics, routine child health clinics, health visitor records and questionnaires. Whole body less head, and central fat and lean mass were derived from whole body dual energy X-ray absorptiometry (DXA) scans assessed 5 times at ages 9, 11, 13, 15, and 18 using a Lunar prodigy narrow fan beam densitometer.

*SBP, DBP and pulse rate*

At each clinic (ages 7, 9, 10, 11, 13, 15 and 18), SBP, DBP and pulse rate were measured at least twice each with the child sitting and at rest with the arm supported, using a validated device and a

cuff size appropriate for the child's upper arm circumference. The mean of the two final measures is used here.

*Blood based biomarkers*

Insulin was measured from cord blood at birth. Non-fasting glucose was measured at age 7 as part of metabolic trait profiling, using Nuclear Magnetic Resonance (NMR) spectroscopy. In a random 10% of the cohort at age 9 years, fasting glucose and insulin were also available; were taken as part of a continuation of an earlier sub-study called "Child in Focus" which included approximately 10% of the overall cohort. Fasting glucose and insulin were available from research clinics held when participants were 15 and 18 years old. Triglycerides, HDL-c and total cholesterol were measured in cord blood at birth and from venous blood subsequently. Samples were non-fasted at 7 and 9; fasting measures were available from clinics at 15 and 18 years. Non-HDL-c was calculated by subtracting HDL-c from total cholesterol at each measurement occasion. Trajectories of glucose, insulin, triglycerides, HDL-c, non-HDL are thus a combination of measures from cord blood, fasting bloods and non-fasting bloods, with most measures obtained through standard clinical chemistry assays, but one measure (glucose at age 7) by NMR spectroscopy.

Further details of measurement of study outcomes are available in ref (32).

**Statistical analysis**

We used multilevel models to examine the sex-specific patterns of change in each risk factor. (33, 34) Multilevel models estimate mean trajectories of the outcome while accounting for the non-independence (i.e. clustering) of repeated measurements within individuals, change in scale and variance of measures over time, and differences in the number and timing of measurements between individuals (using all available data from all eligible participants under a Missing at Random (MAR) assumption). (29, 35) All trajectories except BMI (fat mass, lean mass, SBP, DBP, pulse rate, glucose, insulin, triglycerides, HDL-c, non-HDL-c) were estimated using linear spline multilevel

models (two levels: measurement occasion and individual). Trajectories of BMI were modelled using fractional polynomials (36) (two levels: measurement occasion and individual), since change in BMI during childhood follows a complex pattern that cannot be parsimoniously modelled using linear splines. Linear splines allow knot points to be fit at different ages to derive periods in which change is approximately linear. Fractional polynomials involve raising age to many combinations of powers, resulting in a wide range of possible curves and offering more flexibility than standard polynomial approaches.

Trajectories were modelled separately for females and males to allow for different random effect variances between the sexes, i.e. allowing the between subject variability in outcomes to be different for males and females. Sex differences in the mean intercept and slopes of risk factors were examined by calculating the mean difference between the sexes for each risk factor and using the pooled standard error to calculate 95% confidence intervals for the difference. Values of cardiovascular risk factors that had a skewed distribution (BMI, fat mass, insulin and triglyceride) were (natural) log transformed prior to analysis. Differences between the sexes and confidence intervals were calculated on the log-scale. These values were then back-transformed and are interpreted as the ratio of geometric means. Graphs displayed for these outcomes are in original units and values were derived by back transforming from the log scale. Fat mass and lean mass were adjusted for height using the time- and sex-varying power of height that best resulted in a height-invariant measure (see Table 2 in ref (32) for further details). All trajectories were modelled in MLwiN version 2.36 (37), called from Stata version 14 (38) using the runmlwin command. (39) We performed a number of sensitivity analyses to examine the robustness of our findings. Further details of model selection (Table 3-13 of ref (32)) and sensitivity analyses performed are provided in Supplemental Material.

## Results

Sample sizes for different outcomes ranged from 662 participants (1,831 repeated measures) for insulin up to 13,985 participants (112,768 repeated measures) for BMI (Table 1). Mothers of participants included in the analysis of insulin tended to be more advantaged than mothers of participants excluded due to missing data but there were no differences in the distribution of sex between included and excluded participants (Table 14 in ref (32)).

### Anthropometry

Mean BMI was similar in females and males at 1 year but by age 3 BMI was lower in females compared with males (Fig. 1) and (Table 15 in ref (32)). From age 7 years onward, mean BMI was higher in females. At age 18, mean BMI was 2.4% (95% Confidence Interval (CI), 1.6, 3.1%) higher in females compared with males.

Height-adjusted fat mass was higher in females compared with males at age 9. This difference widened over time, particularly from age 13 onward due to an increase in the average height-adjusted fat mass in females. At age 18, mean height-adjusted fat mass was 77.8% (95% CI, 73.0, 82.8%) higher in females. In comparison, height-adjusted lean mass was lower in females at age 9 years. The trajectories converged at age 13 but widened again thereafter due to a slower rate of increase in height-adjusted lean mass in females. At age 18, mean height-adjusted lean mass was 18.4 kg (95% CI, 18.1, 18.7 kg) lower in females.

### SBP, DBP and pulse rate

At age 7 years, males and females had similar SBP (Fig. 2) and (Table 16 in ref (32)). SBP increased at a faster rate in females compared with males from 7 to 12 years but at a slower rate from 12 to 18 years resulting in a lower mean SBP in females from approximately age 13 onwards. At age 18, mean SBP was 10mmHg (95% CI, 10, 11 mmHg) lower in females. At age 7 years, DBP was higher in females. DBP increased at a similar rate in females and males up to age 12 whereas females

increased at a slower rate from 12 to 16. From 16 to 18, DBP decreased in both sexes but at a faster rate in males leading to a 1.7 mmHg (95% CI, 1.3, 2.0. mmHg) higher DBP in females at age 18. At age 7 years, females had a higher mean pulse rate compared with males. In both sexes, pulse rate decreased with age and rates of change were similar between the sexes. At age 18, mean pulse rate was 4.7 beats per minute (bpm) (95% CI, 4.1, 5.2 bpm) higher in females.

Glucose and insulin

At age 7 years, females had lower glucose levels compared with males (Fig. 3) and (Table 17 in ref (32)). Glucose increased similarly in both sexes from 7 to 15 years and decreased from 15 to 18 years, with a faster rate of decrease in females. At age 18, mean glucose was 0.2 mmol/l (95% CI, 0.2, 0.3 mmol/l) lower in females.

At birth, females and males had similar insulin levels. Insulin increased in both sexes at a broadly similar rate until age 15 and decreased thereafter at a similar rate in both sexes. At age 18, mean insulin was 23.8% (95% CI, 10.8, 36.4%) higher in females.

Lipids

Females had similar triglycerides at birth but higher HDL-c and non-HDL-c compared with males (Fig. 4) and (Table 18 in Ref (32)). From birth to 9 years, triglycerides and non-HDL-c increased in both sexes but at a faster rate in females. From birth to 7 years, HDL-c also increased in both sexes but at a slower rate in females.

From 9 to 18 years, rates of change in triglycerides and non-HDL-c did not differ substantially between females and males. In contrast, HDL-c decreased at a faster rate in males from 7 to 18. At age 18 years, females had 3.8% (95% CI, 1.6, 6.1%) higher triglycerides, 0.17 mmol/l (95% CI, 0.15, 0.18 mmol/l) higher HDL-c and 0.19 mmol/l (95% CI, 0.16, 0.23 mmol/l) higher non-HDL-c compared with males.

Sensitivity analyses

Our results examining sex differences in the observed data at the first occasion of measurement and last occasion of measurement (age 18) for each risk factor were similar to those predicted from the multilevel model at those ages and age 18 (Table 19 in Ref (32)). Results were not substantially different when including only individuals with at least one measure before and one measure after 11 years (Fig. 1-4 in Ref (32)). Results for BMI were not altered when the analysis was restricted to participants with 6 or more repeated measures (Fig. 5 in Ref (32)). Results were not altered when the observations of participants who did not fast in the four hours before the 15-and 18-year clinics were excluded from the models at those time points only (Fig. 6 and 7 in Ref (32)). Sex difference in glucose at ages 15 and 18 years, were similar whether standard clinical chemistry or NMR spectroscopy had been used to measure glucose (Table 1 in Ref (32)). This suggests that that the different glucose measure used at age 7 (NMR spectroscopy) and included in the trajectories compared to the measures used at later time points (from standard clinical chemistry) is unlikely to have influenced our findings. Our results for any measures of cardiovascular health were not altered when the observations of participants taking antihypertensive medications at the 18-year clinic (n=6) were excluded from analysis (data not shown).

## Discussion

In this paper, we examined longitudinal changes in 11 measures of cardiovascular health from early childhood through to 18 years in a large contemporary prospective birth cohort study. We found that sex differences in measures of cardiovascular health were apparent in early life and followed different patterns from childhood to early adulthood. Consistent with adult sex differences in contemporary populations (40, 41), females had higher height-adjusted fat mass, pulse rate and lower height-adjusted lean mass and glucose from mid-childhood through adolescence and up to age 18. Also consistent with adult sex differences (42-44), males developed higher SBP and lower HDL-c during adolescence. In contrast to adult sex differences (42, 45), we found that females had higher levels of insulin, triglycerides and non-HDL-c from birth or mid-childhood through adolescence and developed higher DBP by the end of adolescence. These findings have implications for understanding the sex-specific aetiology of cardiovascular risk across the life course and for sex differences in measures of cardiovascular health in future adults as contemporary child and adolescent populations mature.

### Implications

Sex differences in absolute levels of many risk factors (45, 46), rather than sex differences in their relative association with CVD risk (with some exceptions (47)) are thought to be the greatest contributor to sex differences in cardiovascular disease risk in adults. Thus, understanding the mechanisms underlying sex differences in measures of cardiovascular health and differentiating naturally arising sex differences (due to genetic and hormones) compared with those which are modifiable may provide sex-specific prevention opportunities. Several mechanisms have been proposed to underlie sex differences in measures of cardiovascular health but these remain poorly understood. (48) Our findings demonstrate that sex differences in measures of cardiovascular health in early life are each potentially driven by unique mechanisms due to substantial variation, between risk factors, in how sex differences emerge and change from birth to 18 years. For example,

sex differences in height-adjusted fat mass and lean mass were evident at age 9 and widened during adolescence (with girls having higher height-adjusted fat mass and lower height-adjusted lean mass). This suggests that sex differences in these are associated with mechanisms that pre-date adolescence but are further widened potentially due to hormonal changes associated with puberty. These hormonal changes, alongside changes in growth velocity and health behaviours, may also be associated with the emergence of the sex differences in SBP and HDL-c during adolescence. Our findings showed a rise in SBP and decrease in HDL-c in males in adolescence leading to a 10 mmHg higher SBP and 0.17 mmol/l lower HDL-c compared with females by age 18. However, this pattern of widening sex differences during adolescence was not common to all measures of cardiovascular health examined here. Sex differences that were present at birth and persisted throughout childhood and adolescence (non-HDL-c) may implicate genetics as an underlying mechanism, as the sex difference pre-dates exposure to the post-natal environment and gendered lifestyle behaviours. Further studies of the specific mediators of the sex differences identified here, such as pubertal timing, secondary sex characteristics, growth and lifestyle behaviours (smoking and physical activity) will be an important next step in understanding the sex-specific aetiology of cardiovascular risk. However, we acknowledge that we only have measures at birth for a small number of blood-based risk factors (insulin, triglycerides, HDL-c and non-HDL-c), with the next measure for these being several years later (between 7 to 9 years), preventing the modelling of change over time in infancy and early childhood with greater resolution.

We found several sex differences in measures of cardiovascular health in childhood and adolescence which were not comparable to sex differences in previous childhood generations or contemporary adult populations that warrant further follow-up. For example, females had higher DBP, triglycerides, and non-HDL-c at the end of adolescence in contrast to lower levels of these among females in other childhood cohorts (18, 21, 22, 49, 50) and contemporary adults. (42-44) It is possible that sex differences in these will change during early adulthood, and eventually lead to lower levels in females. However, it is also possible that the different patterns of these in this

population compared with previous generations are due to a cohort effect because of increasing overweight and obesity in contemporary child populations. (24) Studies with repeated measures of cardiovascular risk factors across adolescence and into adult life are needed to examine how these sex differences track into adulthood and whether the pattern of sex differences in contemporary child and adolescent populations differs from sex differences in previous generations.

## Comparison with existing studies

Few studies have examined sex differences in trajectories of measures of cardiovascular health through childhood and adolescence in contemporary populations. However, our findings are comparable with some earlier prospective studies. The Minneapolis Cohort Study (N=507) showed that whilst fat mass was higher in females from 11 to 19 years, similar rates of change in glucose, insulin, HDL-c, triglycerides, and non-HDL-c were observed for both sexes during adolescence such that sex differences remained stable during this period. (22) Project Heartbeat! (N=678) reported that sex differences in SBP began to emerge after age 11 years, with male SBP increasing at a faster rate, resulting in a lower SBP in females compared with males by age 18, consistent with our findings. (18) Our data support findings from the Bogalusa Heart Study (N=3,313), which showed that females had higher insulin and lower glucose than males from 5 to 17 years. (20) Project Heartbeat! and the Minneapolis Cohort Study found the same crossover from higher HDL-c in males to higher HDL-c in females in adolescence, as we have demonstrated here. However, both studies showed higher triglycerides and non-HDL-c in males compared with females by the end of adolescence, in contrast to our findings of higher levels of these in females at age 18.

## Strengths and Limitations

There are several strengths to our study, including its prospective design, availability of repeated measures, the ability to examine a range of measures of cardiovascular health, and the use of multi-level models which take account of clustering of repeated measures within individuals and the correlation between measures over time. We have also adjusted fat and lean mass using age-and

sex-specific powers of height; this approach is likely to result in a more accurate estimation of sex differences across childhood and adolescence. Limitations include combining non-fasting and fasting bloods for risk factors, the availability of measures from birth for only 4 out of the 11 risk factors, and the inclusion of glucose from NMR spectroscopy at age 7. We acknowledge that assays in cord-blood may not be directly comparable to those measured in serum or plasma later in life. Furthermore, with a period of 9 or more years after the cord blood measures before the next measure of insulin, triglycerides HDL-c and non-HDL-c, there is a strong assumption that these measures of cardiovascular health change in a linear way between birth and age 9. However, while different sources of blood-based measures may affect the estimated mean shape of the trajectories over time, different measurements and assay methods are unlikely to impact the direction and magnitude of the estimated sex difference. Supporting this, the sex differences in glucose measured using conventional clinical chemistry assays and NMR spectroscopy at age 15 and 18 were highly comparable. We have not explored the potential role of medications such as antihyperlipidemic drugs in this study; however, their prevalence is likely to be low in this population and the impact of medication use on our overall findings and the sex differences reported is likely to be minimal, as demonstrated when observations of individuals taking antihypertensive medications were excluded. A further limitation includes the use of BMI as a measure of adiposity which has several limitations in children despite its widespread use including, being unable to distinguish fat and lean mass, masking sex differences in these and its varying correlation with adiposity with age (as assessed directly by DXA scans) throughout childhood. However, we have included direct measures of height-adjusted fat mass and lean mass from DXA scans which provide more accurate insight into sex-and age-related change in body composition over time than BMI. The number of people with measurements of each measures of cardiovascular health varied, meaning that our analysis samples differed between measurements and are not directly comparable. Loss to follow-up is also a limitation; however, we have shown that sex is not associated with exclusion from our analysis and we have also aimed to minimise potential bias by including all participants with at least a single measure of a

risk factor. In addition, we have shown that participants included in our analysis were more advantaged than those excluded due to missing data and loss-to-follow-up. Thus, the generalisability of our findings to the wider population may be limited. Furthermore, our findings are not generalisable to non-White populations as 98% of ALSPAC participants are Caucasians.

## Conclusion

Sex differences in measures of cardiovascular health are apparent from birth or mid-childhood and change across the early life course, suggesting that early life factors may play a role in sex differences in cardiovascular disease. Further studies of the specific mechanisms underlying these sex differences and how sex differences in contemporary child and adolescenct populations track into adulthood are required

**Conflict of interest**

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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**Author contributions**

LMOK, LDH, and AF designed the study. LMOK performed the analysis and wrote the first draft of the manuscript. AS and ELA contributed to revision of analyses. LDH and AF supervised the analysis of the study. All authors contributed to critical revisions of the analysis and the manuscript.

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**Table 1 Number of participants with cardiovascular measures at each time point included in the analysis from birth to 18 years**

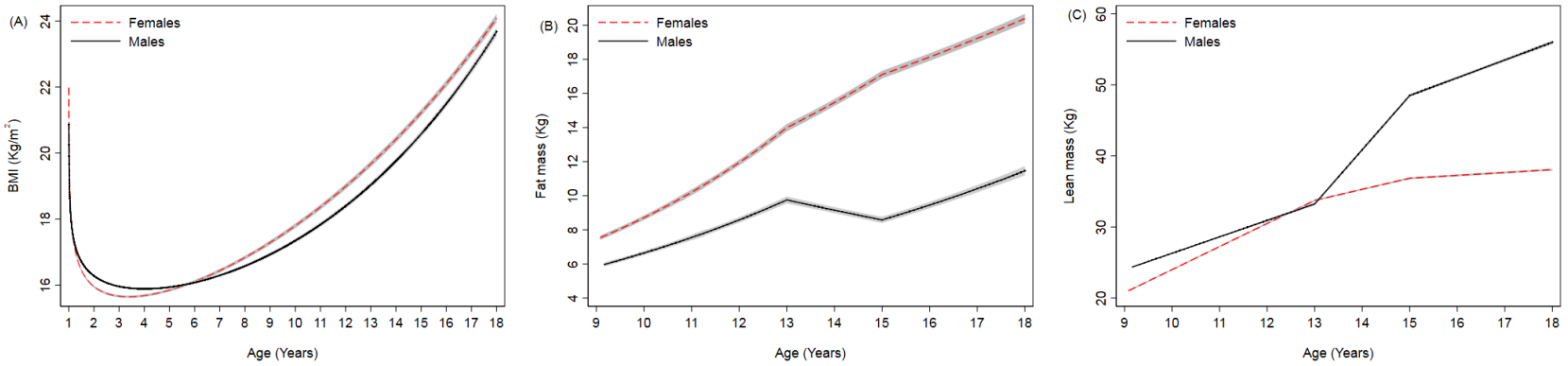
| | Birth | Age 7 | Age 9 | Age 10 | Age 11 | Age 12 | Age 13 | Age 15 | Age 18 | Total measures | Median measures (IQR) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **BMI** [a] | | x | x | x | x | x | x | x | x | 112,768 | 12 (9-15) |
| **Fat/lean mass** | | | 7,241 | | 6,963 | | 6,009 | 5,126 | 4,804 | 30,143 | 4 (3-5) |
| **SBP/DBP/pulse rate** | | 8,057 | 7,586 | 7,152 | 6,996 | 6,624 | | 5,277 | 4,629 | 45,961 | 5 (6-7) |
| **Glucose** | | 5,480 | 842 | | | | | 3,464 | 3,266 | 13,052 | 2 (1-3) |
| **Insulin** [b] | 262 | | 550 | | | | | 521 | 498 | 1,831 | 3 (3-3) |
| **Lipids** [c] | 4,770 | 5,394 | 5,048 | | | | | 3,460 | 3,254 | 21,926 | 3 (2-4) |

DBP, diastolic blood pressure; HDL-c, high density lipoprotein cholesterol; IQR, interquartile range; non-HDL-c, non-high-density lipoprotein cholesterol; SBP, systolic blood pressure.

[a] Measures available at each of these approximate ages and at several ages in between but exact timing and number of BMI measures not shown as a total of 112,768 BMI measures were available from questionnaires, routine child health records and research clinics at different mean ages from 1 to 18 years.

[b] Additional measures available at these ages but the model was restricted to participants with at least one measure before and after age 11 years to allow model convergence.

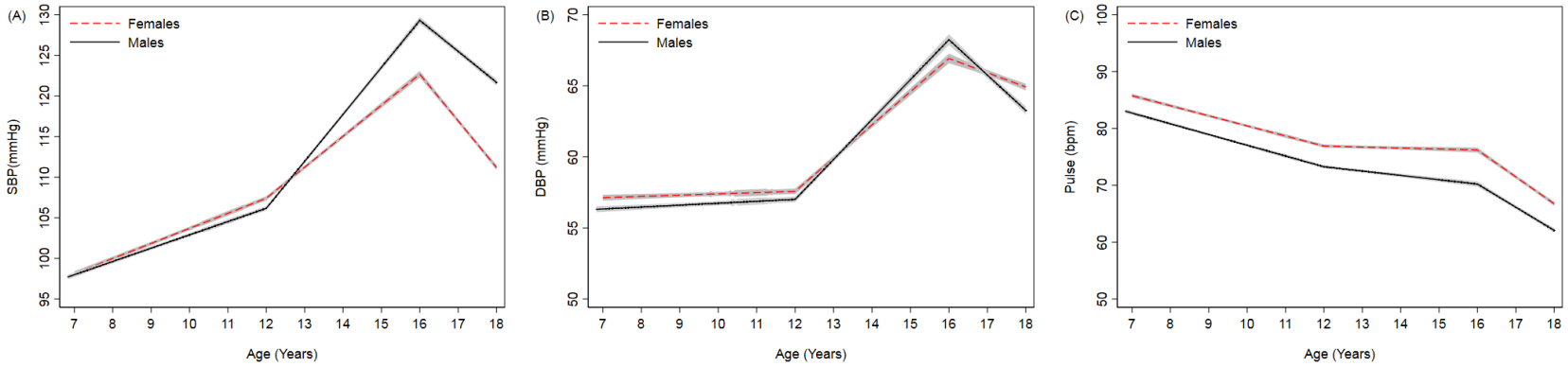
[c] Triglycerides, HDL-c and non-HDL-c.




**N=13,985** **N=8,802** **N=8,818**

**Fig. 1** Mean predicted sex-specific trajectories of anthropometry.

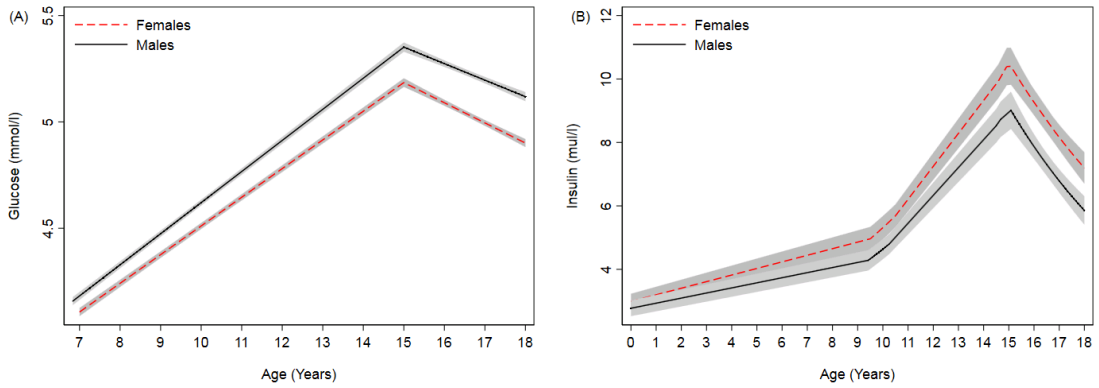
Mean trajectories of (A) BMI from 1 to 18 years, (B) height-adjusted fat mass, and (C) height-adjusted lean mass both from 9 to 18 years in the ALSPAC cohort, predicted from multilevel models. Shaded areas represent 95% confidence intervals. Note the different age range on the X axis for each outcome. Fat mass and lean mass were measured at 9, 11, 13, 15 and 18 years are adjusted for different age-and sex-specific powers of height. BMI was measured a median of 32 times from 1-18 years. Further details are included in (32).




**N=9,922** **N=9,922** **N=9,922**

**Fig. 2** Mean predicted sex-specific trajectories of blood pressure and pulse rate.

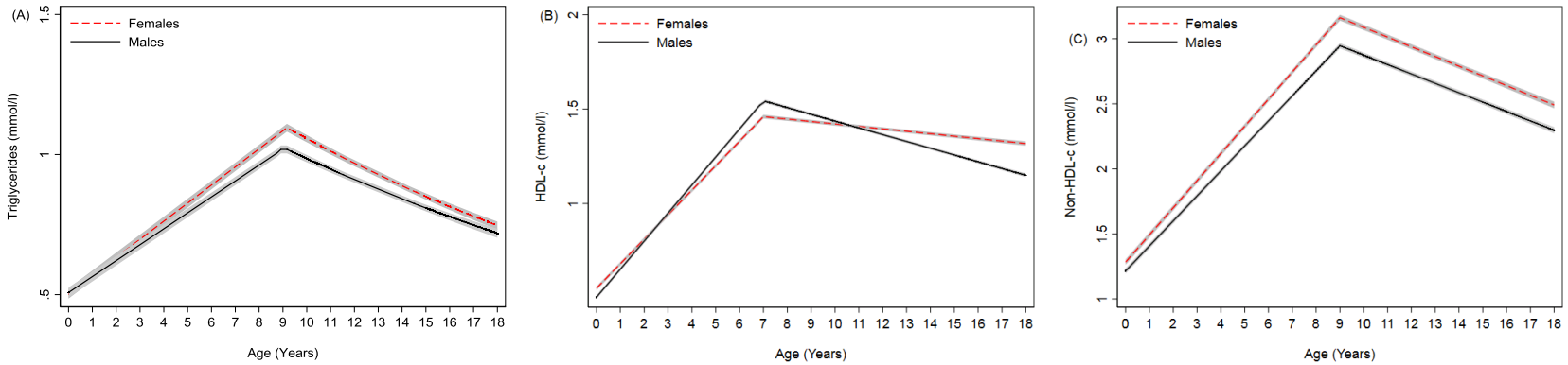
Mean trajectories of (A) SBP, (B) DBP and (C) pulse rate from 7-18 years in the ALSPAC cohort, predicted from multilevel models. Shaded areas represent 95% confidence intervals. Note the different age range on the X axis for each outcome. SBP, systolic blood pressure; DBP, diastolic blood pressure. SBP, DBP and pulse rate were measured at 7, 9, 10, 11, 13, 15 and 18 years. Further details are included in (32).




**N=7,255** **N=662**

**Fig. 3** Mean predicted sex-specific trajectories of glucose and insulin.

Mean trajectories of (A) glucose from 7 to 18 years and (B) insulin from birth to 18 years in the ALSPAC cohort, predicted from multilevel models. Shaded areas represent 95% confidence intervals. Note the different age range on the X axis for each outcome. Glucose was measured at 7, 9, 15 and 18 years. Insulin was measured at birth, 9, 15 and 18 years. Further details are included in (32).




**N=10,128** **N=10,115** **N=10,098**

**Fig. 4** Mean predicted sex-specific trajectories of lipids from birth to 18 years.

Mean trajectories of (A) triglyceride, (B) HDL-c and (C) Non-HDL-c from birth to 18 years in the ALSPAC cohort, predicted from multilevel models. Shaded areas represent 95% confidence intervals. HDL-c, high density lipoprotein cholesterol. All three lipids were measured at birth, 7, 9 15 and 18 years. Further details are included in (32).

***Point-by-Point Response**

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11th September 2018

Dear Prof. Von Eckardstein and Dr. Dallinga-Thie,

*Re: Requested revision of manuscript "Sex-specific trajectories of cardiometabolic risk factors during childhood and adolescence: a prospective cohort study"*

We thank the reviewers as well as the Manuscript Committee, for reviewing the revisions made to our manuscript and are very pleased that all reviewers find the changes made satisfactory. We have addressed the additional comment made by reviewer 2 below and we are happy to take further direction from the editor and/or the reviewer on this issue, if necessary.

We would be very pleased if you could consider our revised manuscript for publication in *Atherosclerosis* and we look forward to hearing from you.

With best wishes,
Dr Linda M O'Keeffe

## Reviewer #1

Many thanks for responding to the reviewers' comments. A reviewer has no further comment on the current manuscript.

**Response:** Thank you for taking the time to review our manuscript.

## Reviewer #2

The Authors answered adequately to the reviewers' questions and modified accordingly the manuscript.

**Concern:** Nonetheless, the concept of "measures of cardiovascular health" is still in my view highly questionable, if not confused. In the perception of most of us, cardiovascular health may be measured by other proxies, and not conceivably by the used biochemical measures. In addition, the use of heart rate - pulse - and of BP - seemingly "normal" - is hardly suitable to be considered a measure of cardiovascular health.

**Response:** We agree with the reviewer that the broad term “measures of cardiovascular health” could include other proxies. However, the term “measures of cardiovascular health” does indeed encompass all of the measures that we have included here such as blood pressure or biochemical measures. All of these measures, including blood pressure and heart rate are recognised as measures of cardiovascular health by clinical guidelines. (1,2) Our original submission of this manuscript to Atherosclerosis used the catch-all term “cardiometabolic risk factors” in the title and across the paper. We changed this to “measures of cardiovascular health” in response to reviewers’ comments, on the basis that this would be more intuitive and more accurately represent what measures were included in the paper. We are happy to revert to “cardiometabolic risk factors” again if the reviewer feels this would be useful or we are also happy to consider another more appropriate term which the editor or reviewer might have.

## Reviewer #5

The concerns raised were answered satisfactorily.

**Response:** Thank you for taking the time to review our manuscript.


1. Perret-Guillaume C, Joly L, Benetos A. Heart rate as a risk factor for cardiovascular disease. Progress in cardiovascular diseases. 2009; 52 (1): 6-10.
2. Kannel WB. Blood pressure as a risk factor for cardiovascular disease. JAMA. 1996; 275 (20): 1571-1576.

## Statement of Originality

We declare that the work herein is the original work of the listed authors and is not under consideration elsewhere for publication.

*Conflict of interest form

**Conflicts of Interest Statement**

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed.

We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript that has involved either experimental animals or human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). She is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from lo15992@bristol.ac.uk

**On behalf of all co-authors, I, Linda O'Keeffe sign that all of the information herein this document is true and that no authors have any conflicts of Interest. This statement can be taken as my signature.**

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| **Does your manuscript contain all the below essential elements, in this order?** (please stick to the headers as indicated below) | **Yes** | **No** |
|---|---|---|
| - Title | Y | |
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| - Abstract in the Atherosclerosis format (*Background and aims*, *Methods*, *Results*, *Conclusions*) | Y | |
| - Introduction | Y | |
| - Materials and methods (or Patients and methods) | Y | |
| - Results | Y | |
| - Discussion | Y | |
| - Conflict of interest (mandatory) | | |
| - Financial support (if applicable) | | |
| - Author contributions (mandatory) | | |
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|---|---|---|
| - *Background and aims* | Y | |
| - *Methods* | Y | |
| - *Results* | Y | |
| - *Conclusions* | Y | |

### Figure and table legends

| **Are figure and table legends formatted as described below?** | **Yes** | **No** |
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| Each figure and table legend should have a brief overarching title that describes the entire figure without citing specific panels, followed by a description of each panel, and all symbols used. | Y | |
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|---|---|---|
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## Mouse strains and cell lines

| **Are knock-out or transgenic mouse strains and cell lines italicized and the symbol superscripted?** | **Yes** | **No** |
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| (e.g. *ob/ob* , $p53^{+/+}$, $p53^{-/-}$) | NA | |

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