Obesity, Metabolic Health, and History of Cytomegalovirus Infection in the General Population

Mark Hamer,[1,2,3] G. David Batty,[3] Mika Kivimäki[3]

[1] School of Sport, Exercise & Health Sciences, Loughborough University; [2]NIHR Leicester-Loughborough Diet, Lifestyle and Physical Activity Biomedical Research Unit, Leicester, UK; [3]Department of Epidemiology & Public Health, University College London, London, UK.

Corresponding author: Mark Hamer, PhD; School of Sport, Exercise & Health Sciences, Loughborough University, LE11 3TU, United Kingdom

E-mail: m.hamer@lboro.ac.uk ; phone: +44 (0) 1509 228473

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Abstract

Context: Common community-acquired infections, such as cytomegalovirus (CMV), may contribute to the development of obesity and metabolic dysfunction, but empirical evidence is scarce.

Objective: We examined the associations between CMV, obesity and metabolic characteristics in a large, general population-based sample of adults.

Design and setting: An observational study in community dwelling adults from the general population, 'Understanding Society -- the UK Household Longitudinal Study'.

Participants: 9,517 men and women (aged 52.4 ± 16.4 yrs; 55.3% female).

Measures: CMV infection was measured using Immunoglobulin G (IgG) from serum. Obesity was defined as body mass index ≥30 kg/m^2. Based on blood pressure, HDL-cholesterol, triglycerides, glycated haemoglobin A1c, and C-reactive protein, participants were classified as 'healthy' (0 or 1 metabolic abnormality) or 'unhealthy' (≥2 metabolic abnormalities).

Results: A positive CMV test was recorded in 47.5% of the sample. There was no association between CMV and obesity. Of the individual metabolic risk factors, CMV was positively associated with glycated haemoglobin and HDL-cholesterol. In combination, only 'unhealthy non-obese' participants had modestly increased odds of CMV (odds ratio compared to healthy normal-weight = 1.12, 95% confidence interval 1.00 – 1.26) after adjusting for a range of variables. CMV was associated with an increased prevalence of cardiovascular diseases (odds ratio=1.67; 1.07 – 2.60) independently of obesity, metabolic risk factors, and other covariates.

Conclusion: Our findings suggest a weak but statistically significant association between CMV and metabolic dysfunction in non-obese adults. This relationship appears to be masked in the obese, possibly by the effects of excess adiposity on metabolism.

Key words: cytomegalovirus; epidemiology; infection; obesity

Cytomegalovirus (CMV) is one of the most well-characterised infections in humans. This infection is typically acquired in childhood and is lifelong. Although CMV rarely causes symptoms, it has been linked to adverse metabolic characteristics, including obesity[1-4] and factors that accompany this condition, such as impaired glucose control and dyslipidaemia.[5-12] These associations are biologically plausible because infection provokes immune responses, such as the release of inflammatory cytokines that have been linked to the etiology of metabolic disorders including diabetes.[13] Infection with CMV might also contribute to features of immune-senescence, such as the accumulation of differentiated cytotoxic T cells. Some evidence suggests that the accumulation of these cells could drive an unfavourable metabolic profile.[14] In addition, it has been postulated that excess adipose tissue may lead to susceptibility to infections, such as CMV,[2,4] through influencing a variety of immune mediators. However, the adverse effects of excess adiposity on metabolism might also mask any association between CMV and metabolic parameters.

Existing data on obesity, metabolic dysfunction and acquired infections is generally sparse. Most studies have suffered from methodological weaknesses such as small sample sizes ($n<150$),[5,7-8] case-control rather than prospective designs,[5,7-8,11] and inadequate adjustment for sociodemographic factors. To the best of our knowledge, no large-scale studies to date have simultaneously examined the associations of CMV with both obesity and metabolic health, controlling for potential confounding factors, such as poor lifestyle and social disadvantage,[12,15] to evaluate the strength of these associations in the general population and to separate the possible underlying mechanisms. These associations may have important clinical implications as CMV infections, although common, are not routinely

subject to screening and treatment is considered only in the rare event the infection is activated and symptomatic.

Methods

Understanding Society -- the UK Household Longitudinal Study (UKHLS) -- is a large, longitudinal survey of households in the United Kingdom (England, Scotland, Wales and Northern Ireland). In 2010-2012, participants completed a face-to-face interview and nurse health assessments were conducted approximately five months following completion of the survey interview.[16] In brief, in the general population sample there was a 58.6% response for the nurse assessment component and full blood samples were successfully collected in 10,175 participants. Participants gave full informed written consent to participate in the study and ethical approval was obtained from the Ethics Committee of the University of Essex (main survey) and National Research Ethics Service Oxfordshire REC A (nurse health assessment).

Nurse health assessment

Nurses collected anthropometric data (weight, height, waist circumference), blood pressure (BP), and non-fasting blood samples using standard protocols. Body weight was measured using Tanita BF 522 scales without shoes and in light clothing, and height was measured using a Stadiometer with the Frankfort plane in the horizontal position. Body mass index (BMI) was calculated as weight (kilograms)/height (meters) squared. Waist circumference was recorded twice using measuring tape mid-way between the iliac crest and lower rib. An average of the first two measurements was used provided these differed by no more than

3cm; otherwise a third reading was taken and the two closest results utilised. Systolic and diastolic BP was measured with an Omron HEM-907 BP monitor three times in the sitting position after 5-minute rest between each reading. The initial reading was discarded and an average of the second and third BP recordings was used for the present analyses. All respondents were eligible to give blood except pregnant women, individuals who volunteered that they are HIV positive or had hepatitis B or C, persons with clotting or bleeding disorder such as haemophilia, or those with a self-declared low platelet count. Additionally, people who had ever had a fit, or those taking anti-clotting medication (e.g., warfarin) were also excluded. Blood samples were analyzed for C-reactive protein (CRP), high density lipoprotein (HDL) cholesterol, triglycerides, and glycated haemoglobin (HbA1c). Detailed information on the technicalities of the blood analysis have been described elsewhere.[17]

Measurement of Cytomegalovirus (CMV) antibodies

Immunoglobulin G (IgG) and IgM were measured from serum samples with an electrochemiluminsecent immunoassay (Roche E170 analyser). Inter- and intra-assay coefficients of variation were acceptable, less than 4%. A positive CMV IgG result indicates a CMV infection at some point in time, while a negative CMV IgG indicates that the participant has never been exposed to, or been infected with, CMV. A positive Immunoglobulin M (IgM) indicates a recent or current infection. Indeterminate CMV occurs during current or acute infection or may be due to non-specific binding. For those people who had a positive IgM test or whose result was indeterminate, an additional test was performed to confirm recent CMV infection. This confirmatory assay was an avidity test on the Mini VIDAS immunoassay analyser.

Covariables

Health-related questions included cigarette smoking (current; previous ; non-smoker), the frequency of participation in sports and exercise (more than three times per week; 1 – 3 times per week; once per month or less; never), and the frequency of alcohol intake (at least 5-6/week; 1-4/week; monthy; rarely /never). Participants were also asked to state their highest educational attainment (Degree; A-level/GCSE; other; none) and to rate their health (excellent; very good; good; fair; poor).

Statistical analyses

Body mass index was categorised into four groups (normal: from 18.5 to <25 kg/m^2 ; overweight: from 25 to <30 kg/m^2 ; obese I: from 30 to <35kg/m^2 ; obese II and more severe forms: ≥ 35 kg/m^2). Based on existing criteria[18] unhealthy metabolic status was defined as having two or more of the following metabolic risk factors: high BP (systolic/diastolic BP ≥130/85 mmHg, or hypertension diagnosis, or use of anti-hypertensive medication), impaired glycaemic control (HbA1c > 6.0% [42.1 mmol/mol] or doctor's diagnosed diabetes), systemic inflammation (CRP ≥ 3mg/l), low HDL cholesterol (<1.03 mmol/l in men and <1.30 mmol/l in women), and high triglycerides (≥ 1.7 mmol/l). Participants were then categorized into four groups: 'healthy non-obese'; 'unhealthy non-obese'; 'healthy obese '; and 'unhealthy obese'.

We calculated odds ratios (OR) and 95% confidence intervals (CI) for the odds of CMV in relation to obesity, metabolic status and their combination. We tested for sex interactions, but as none were present, men and women were pooled in the same analysis. Initially, we adjusted our effect estimates for sex and age (model 1). We further adjusted the models for

education, sports and exercise participation, self-rated health, smoking, and alcohol (model 2). Analyses were conducted using SPSS version 22.

Results

The analytic sample comprised 9,517 participants (aged 52.4 ± 16.4 yrs; 55.3% female). A positive CMV test was apparent in 47.5% of the sample. Participants testing positive for CMV tended to be older, female, smokers, have no educational qualifications, and poorer self-rated health (Table 1). In logistic regression models mutually adjusted for all variables, per year increase in age (OR; 95% confidence interval: 1.02, 1.01 – 1.03), being female (1.26; 1.16 – 1.38), a smoker (1.21; 1.07 – 1.37), and no qualifications (1.72; 1.46 – 2.02) remained associated with CMV positive status.

There was no association between BMI and CMV (Table 2), nor did we observe any association when using waist circumference as a measure of central obesity (OR per unit increase = 1.00; 0.99 – 1.01, P=0.94). Metabolic health was associated with the status of CMV in models adjusted for age and sex, although after further adjustments the association was attenuated to the null (Table 2). In analyses that combined obesity and metabolic health, participants defined as “unhealthy non-obese” had increased odds of being CMV positive (Table 2). In further analyses to examine associations between individual metabolic risk factors and CMV we observed significant associations for HbA1C and HDL-cholesterol (Table 3).

We further examined these associations in relation to a clinically meaningful outcome; 105 self-reported physician-diagnosed cases of cardiovascular diseases (CVD) (including congestive heart failure, angina/ myocardial infarction/coronary heart disease, and stroke)

were reported. In analyses (Table 4) in which we adjust our effect estimates for covariates, CMV was associated with higher odds of CVD (OR = 1.67, 95% CI, 1.07 – 2.60) independently of obesity and metabolic risk factors.

Discussion

Our main finding was an association between CMV and the individual metabolic risk factors of high glycated haemoglobin and low HDL-cholesterol. However, only metabolically 'unhealthy non-obese' participants had an increased prevalence of the acquired infection. In contrast, CMV was not associated with metabolic health in obese participants and there was no association between obesity and CMV. In further analyses using a clinical endpoint, CMV was associated with CVD independently of obesity and metabolic risk factors.

Existing data on metabolic health and acquired infections is generally sparse. Most studies have suffered from methodological weaknesses such as small sample sizes (n<150),[5,7-8] case-control rather than prospective designs,[5,7-8,11] and inadequate adjustment for sociodemographic factors. With over 9000 participants, our study is, to the best of our knowledge, the largest population-based study on CMV in relation to a range of metabolic factors and obesity.

Obesity is thought to influence the immune response that has been hypothesised to increase susceptibility to infections.[2,4] However, the most plausible interpretation of our

findings is that the accumulation of viral load and associated immune activation is driving an unfavourable metabolic profile among non-obese. Obesity often precedes metabolic dysfunction,[19] thus in obese participants is likely to be the strongest driver of metabolic risk and might explain why the 'unhealthy obese' were seemingly not at elevated risk of CMV infection in contrast to their non-obese counterparts.

Associations between CMV and metabolic health were attenuated after adjustment for social and lifestyle factors, suggesting these relationships could be part of a causal pathway starting from social determinants of health. This is consistent with findings from a previous population sample of US adults demonstrating that the association between CMV and diabetes was attenuated to the null in models accounting for social and lifestyle factors.[12] CMV was, however, associated with CVD independently of covariates; this is consistent with prior evidence.[20] CMV is known to increase experimental atherosclerosis and to modulate vascular-wall activity,[21,22] thus the association is likely to be independent of adiposity and metabolic dysfunction.

Infection causes immune responses, such as the release of inflammatory cytokines that have been linked to the etiology of metabolic disorders including diabetes.[13] Interestingly, we found no association between C-reactive protein and CMV, but the link with metabolic health was driven by HDL-cholesterol and HbA1C. This suggests mechanisms other than inflammatory response related to innate immunity may primarily drive the association between CMV and metabolic dysfunction. Recent evidence has shown the accumulation of

differentiated cytotoxic T cells in CMV positive participants was associated with HbA1C and cholesterol,[14] suggesting a direct role of the immune cells related to the adaptive immune system.

There are several limitations. Firstly this is a cross-sectional study thus we can only speculate on the causality and direction of our findings. Second, our measurement of pathogen infection was based on seropositivity to IgG antibodies, which reflects prior infection, but are not sensitive indicators of current infection or the chronicity of prior infections. Nevertheless, active pathogen infection is unlikely to have influenced our results as recent infection (measured through positive IgM and confirmatory avidity test) was apparent in less than 0.5% of the sample and removal of these participants did not influence the present results (data not shown). Detailed assessments of immune activity were not possible in the present study. An assessment of T cell pattern in participants with positive or negative CMV test would provide further hints as to how CMV infection impacts on immune cell function driving an unfavourable metabolic profile.[23]

In summary, we demonstrated no association between obesity and CMV. We identified a weak but statistically significant association between CMV and metabolic dysfunction in non-obese adults, but not in their obese counterparts. We speculate that in the non-obese CMV infection may drive metabolic dysfunction whereas in the obese population excess adiposity is the main cause of metabolic disturbance. As any associations observed with metabolic risk factors were weak, our findings do not justify universal screening of CMV to

prevent diabetes, although there appears to be a stronger association between CMV and CVD.

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Table 1. Characteristics of the sample according to CMV status (N=9,517)

Variable	**CMV positive** (n=4,524)	**CMV negative** (n=4,993)	**p-value**
Age (yrs)	56.4 ± 16.0	48.6± 15.8	<0.001
Sex (%)			<0.001
Men	41.9	47.3	
Women	58.1	52.7	
Education (%)			<0.001
Degree	30.3	38.8	
A-level/GCSE only	37.7	43.3	
Other	13.3	9.4	
No qualification	18.7	8.5	
Smoking (%)			0.002
Never	37.9	41.5	
Ex-smoker	42.3	40.0	
Current	19.7	18.4	
Sports and exercise participation (%)			<0.001
Never	39.7	27.8	
Once a month or less	27.4	33.7	
At least once a week	19.3	22.3	
More than three times a week	13.6	16.4	
Frequency of Alcohol intake (%)			<0.001
At least 5-6 times a week	16.4	14.9	
Weekly	41.4	47.8	
Monthly	14.4	15.6	
Rarely/never	27.8	21.7	
Self–rated health (%)			<0.001
Excellent/very good	47.2	55.1	
Good	29.9	28.3	
Poor/fair	23.0	16.6	
Body mass index (kg/m^2)	28.4 ± 5.3	28.0 ± 5.6	<0.001
Number of metabolic risk factors	1.4 ± 1.2	1.2 ± 1.1	<0.001

Table 2: Odds ratios (95% confidence interval) for the relation between obesity, metabolic health and history of CMV infection (N=9,517)

	CASES/N	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Obesity			
Normal (18.5 to <25 kg/m^2)	1198/2679	1.0 (Ref)	1.0 (Ref)
Overweight (25 to < 30 kg/m^2)	1871/3893	1.02 (0.92 – 1.13)	1.03 (0.93 – 1.15)
Obese I (30 to <35 kg/m^2)	976/1957	1.08 (0.95 – 1.22)	1.06 (0.93 – 1.20)
Obese II (≥35 kg/m^2)	499/993	1.12 (0.96 – 1.30)	1.04 (0.89 – 1.22)
p-linear trend		0.10	0.52
*Metabolic health*ǂ			
Healthy (0 or 1 risk factor)	2658/5956	1.0 (Ref)	1.0 (Ref)
Unhealthy (> 1 risk factor)	1891/3584	1.15 (1.05 – 1.25)	1.05 (0.95 – 1.15)
p-linear trend (continuous score)		0.002	0.41
Metabolic health/ obesity			
Healthy non-obese	2105/4785	1.0 (Ref)	1.0 (Ref)
Unhealthy non-obese	963/1784	1.22 (1.08 – 1.36)	1.12 (1.00 – 1.26)
Healthy Obese	546/1151	1.13 (0.99 – 1.29)	1.11 (0.97 – 1.27)
Unhealthy Obese	927/1797	1.14 (1.02 – 1.28)	1.04 (0.92 – 1.17)

Model 1: adjusted for age and sex

Model 2: adjusted for age, sex, education, sports and exercise participation, self rated health, smoking, alcohol.

ǂdefined from: High blood pressure (clinic BP ≥130/85 mmHg, or hypertension diagnosis, or use of anti-hypertensive medication), impaired glycaemic control (HbA1c > 6.0% or doctor's diagnosed diabetes), systemic inflammation (C-reactive protein≥ 3mg/l), low HDL cholesterol (<1.03 mmol/l in men and <1.30 mmol/l in women), and high triacylglycerol (≥ 1.7 mmol/l).

Table 3: Odds ratios (95% confidence interval) for the relation between individual metabolic risk factors and CMV infection

Risk factor (per standard deviation increase)†	Model 1 (OR, 95% CI)	Model 2 (OR, 95% CI)
HbA1c (8.0 mmol/mol)	1.08 (1.03 – 1.13)	1.01 (1.00 – 1.02)
HDL-Cholesterol (0.46 mmol/l)	0.91 (0.87 – 0.95)	0.80 (0.71 – 0.90)
Triglycerides (1.10 mmol/l)	1.03 (0.98 – 1.08)	0.99 (0.94 – 1.03)
C-Reactive Protein (6.75 mg/l)	1.02 (0.99 – 1.07)	1.00 (0.99 – 1.02)
Systolic Blood Pressure (16.3 mmHg)	0.95 (0.81 – 1.12)	0.94 (0.80 – 1.11)

†a standard deviation increase denoted after variable

Model 1: adjusted for age and sex

Model 2: adjusted for age, sex, education, sports and exercise participation, self rated health, smoking, alcohol, BMI, and mutually for other metabolic risk factors.

Table 4: Odds ratios (95% confidence interval) for the associations of CMV infection, obesity, metabolic health with cardiovascular disease.

	CVD cases/N	Model 1 OR (95% CI)	Model 2 OR (95% CI)
CMV infection			
No	30/4976	1.0 (Ref)	1.0 (Ref)
Yes	75/4541	1.81 (1.17 – 1.80)	1.67 (1.07 – 2.60)
Obesity			
Normal (18.5 to <25 kg/m^2)	11/2679	1.0 (Ref)	1.0 (Ref)
Overweight (25 to < 30 kg/m^2)	38/3893	1.60 (0.81 – 3.17)	1.64 (0.82 – 3.28)
Obese I (30 to <35 kg/m^2)	34/1957	2.63 (1.30 – 5.33)	2.35 (1.14 – 4.84)
Obese II (≥35 kg/m^2)	22/993	3.91 (1.81 – 8.42)	2.83 (1.29 – 6.24)
Metabolic health			
Healthy (0 or 1 risk factor)	32/5956	1.0 (Ref)	1.0 (Ref)
Unhealthy (> 1 risk factor)	73/3584	1.97 (1.26 – 3.08)	1.59 (1.00 – 2.51)

Model 1: adjusted for age, sex, and mutually for CMV, obesity category, or metabolic health.

Model 2: adjusted for age, sex, education, sports and exercise participation, self rated health, smoking, alcohol, and mutually for CMV, obesity, or metabolic health.