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The association of red meat intake with inflammation and circulating intermediate biomarkers of type 2 diabetes is mediated by central adiposity

Mohsen Mazidi[1*], Andre Pascal Kengne[1], Elena S. George[2] and Mario Siervo[3]

[1]*Non-Communicable Disease Research Unit, South African Medical Research Council and University of Cape Town, Cape Town, South Africa*

[2]*Institute for Physical Activity and Nutrition, School of Exercise and Nutrition Sciences, Deakin University, Geelong, Australia*

[3]*School of Life Sciences, The University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH, UK*

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Abstract

We explored the role of lipid accumulation products and visceral adiposity on the association between red meat consumption (RMC) and markers of insulin resistance (IR) and inflammation in USA adults. Data on RMC and health outcome measurements were extracted from the 2005–2010 US National Health and Nutrition Examination Surveys. Overall 16 621 participants were included in the analysis (mean age = 47·1 years, 48·3 % men). ANCOVA and 'conceptus causal mediation' models were applied while accounting for survey design. In adjusted models, a lower RMC was significantly associated with a cardio-protective profile of IR and inflammation. BMI had significant mediation effects on the association between RMC and C-reactive protein (CRP), apo B, fasting blood glucose (FBG), insulin, homoeostatic model assessment of IR and β-cell function, glycated Hb (HbA1c), TAG:HDL ratio and TAG glucose (TyG) index (all Ps < 0·05). Both waist circumference and anthropometrically predicted visceral adipose tissue mediated the association between RMC and CRP, FBG, HbA1c, TAG:HDL ratio and TyG index (all Ps < 0·05). Our findings suggest that adiposity, particularly the accumulation of abdominal fat, accounts for a significant proportion of the associations between red meat consumption, IR and inflammation.

Key words: **Meat intake: Inflammation: Glucose haemostasis: Insulin resistance: Adiposity**

Red meat consumption (RMC) has been associated with a pro-inflammatory status, which in turn has been related to a higher risk of type 2 diabetes[1], the metabolic syndrome[2] and CHD[2]. The Multi-Ethnic Cohort Study and Nurses' Health Study reported that a high RMC was linked to approximately 40 % greater risk of type 2 diabetes over a follow-up time of 14 and 4 years[3,4]. However, findings are not consistent across studies as a non-significant association was also found between type 2 diabetes risk and red meat intake, especially between unprocessed red meat and diabetes risk[5,6].

Despite the conflicting literature surrounding RMC and risk of type 2 diabetes, there is evidence to suggest that increased RMC is associated with increased whole-body and central adiposity[7]. These findings may contribute to explaining the significant association between RMC and type 2 diabetes as central adiposity is a more sensitive predictor of type 2 diabetes and other obesity-related chronic diseases compared with BMI[8].

The role of visceral fat as a causal factor connecting obesity and weight gain to the pathogenesis of insulin resistance (IR) and atherosclerosis is established[9]. Waist circumference (WC) has been proposed as a rapid and simple measurement for the assessment of abdominal adiposity but, like BMI, is not able to discriminate between subcutaneous and visceral abdominal fat depots[10]. Hence, additional simple and integrated indexes have been recently proposed by combining physical (i.e. WC, BMI, thigh circumference, age) and biochemical measures (i.e. TAG, blood glucose or HDL). The lipid accumulation product (LAP) index is a marker of central fat accumulation derived from the measurements of WC and circulating TAG[11], which has been proposed as a predictor of IR, the metabolic syndrome

Abbreviations: AMPM, Agriculture Automated Multiple-Pass Method; apVAT, anthropometrically predicted visceral adipose tissue; CRP, C-reactive protein; FBG, fasting blood glucose; HbA1c, glycated Hb; HOMA, homoeostatic model assessment; IR, insulin resistance; LAP, lipid accumulation product; MEC, mobile examination centres; MET, metabolic equivalent of task; MetS, metabolic syndrome; NCHS, National Center for Health Statistics; NHANES, National Health and Nutrition Examination Survey; RMC, red meat consumption; VAI, visceral adiposity index; TyG, TAG glucose.

* **Corresponding author:** M. Mazidi, email mazidi_ns@yahoo.com

(MetS), type 2 diabetes mellitus and CVD[12,13]. The visceral adiposity index (VAI) is another indicator of adipose tissue distribution which has been used in the stratification of adult obesity phenotypes[14] and to improve the prediction of cardio-metabolic risk[15].

Regression analysis is frequently used to evaluate the association between dietary factors and disease risk, but it may be characterised by a limited capacity to identify putative biological mechanisms that could possibly explain the association between red meat intake and the risk of high inflammation and impaired glucose control[16]. Mediation analysis is a more sensitive statistical approach that can be used to explore and quantify the extent to which the relationship between an exposure and an outcome of interest is established through the effect of a third variable[16,17]. The traditional approach to mediation analysis tends to produce a bias when the interaction between exposure and mediator is undefined[18,19]. In addition, unbiased valid estimates of direct and indirect effects can be obtained using the counterfactual framework in causal mediation analysis[18,19]. It is unclear to what extent the adjustment for adiposity modifies or attenuates the association between meat consumption and cardio-protective parameters. Mediation analysis could clarify the role of adiposity underlying the relation between meat consumption and cardio-protective factors.

Previous studies have investigated the association between red meat intake with biomarkers of inflammation and glucose/insulin metabolism with mixed results[1,2,20,21]; however, none of these studies has attempted to identify the intermediate factors that connect the exposure to red meat with the selected health outcomes. The present analysis aims to specifically investigate the link between red meat intake with C-reactive protein (CRP) and glucose/insulin haemostasis and identify adiposity factors that may mediate these associations. These factors include markers of adiposity (WC, BMI, visceral adipose tissue (VAT)), LAP and VAI in a representative adult population of USA using National Health and Nutrition Examination Survey (NHANES) database. We hypothesised that a higher red meat intake would be associated with unfavourable concentrations of inflammatory and glucose/insulin haemostasis biomarkers among adults and that these associations would be partly or fully mediated by adiposity markers.

Methods

Population characteristics

The NHANES programme is implemented by the US National Center for Health Statistics (NCHS)[22]. NHANES uses a complex, multistage and stratified sampling design to select a representative sample of the civilian and non-institutionalised resident population of the USA. The NCHS Research Ethics Review Board approved the NHANES protocol, and consent was obtained from all participants[22]. The present study was based on analysis of data collected from 2005 to 2010. Data collection on demographics occurs through in-home-administered questionnaires, while anthropometric and biochemistry data are collected by trained personnel using mobile examination centres (MEC). More detailed information is available elsewhere[22,23].

For the assessment of height and weight during the physical examination, participants were dressed in underwear, disposable paper gowns and foam slippers. A digital scale ('Mettler Toledo, Panther') was used to measure weight to the nearest 100 g, a fixed stadiometer was used to measure height to the nearest millimetre. BMI was calculated as weight in kilograms divided by the square of height in metres. WC was measured at the iliac crest to the nearest millimetre[23].

A blood specimen was drawn from the participant's antecubital vein by a trained phlebotomist. Glycated Hb (HbA1c) was measured using a Tosoh A1C 2.2 Plus Glycohemoglobin Analyzer. Fasting blood glucose (FBG) was measured by a hexokinase method using a Roche/Hitachi 911 Analyzer and Roche Modular P Chemistry Analyzer. Insulin was measured using an ELISA immunoassay (Merocodia)[24]. Other laboratory test details are available in the NHANES Laboratory/Medical Technologists Procedures Manual[25]. Apo B was measured by radial immunodiffusion[23]. Details on the measurement of CRP concentrations are available elsewhere[23]. Homoeostatic model assessment of IR (HOMA-IR), β-cell function (HOMA-B) and insulin sensitivity (HOMA-IS) were calculated as follows: HOMA-IR = (FBG (nmol/l) × insulin (mU/ml)/22·5), and HOMA-β = (20 × insulin (μU/ml))/(FBG (mmol/l) − 3·5)[26]. The TAG glucose (TyG) index was calculated as the ln(TAG (TAG, mg/dl) × FBG (mg/dl)/2)[27]. Anthropometrically predicted VAT (apVAT) was predicted with sex-specific validated equations that included age, BMI, WC and thigh circumference [28]. The equation for men was 6 × WC − 4·41 × proximal thigh circumference + 1·19 × age − 213·65; and the equation for women was 2·15 × WC − 3·63 × proximal thigh + 1·46 × age + 6·22 × BMI − 92·713[28]. VAI was calculated using sex-specific formulas: males (WC/39·68 + (1·88 × BMI)) × (TAG/1·03) × (1·31/HDL); females: (WC/36·58 + (1·89 × BMI)) × (TAG/0·81) × (1·52/HDL), where both TAG and HDL levels are expressed in mmol/l[15]. LAP was calculated as (WC − 65) × TAG in men, and (WC − 58) × TAG in women[11]. Smoking status was self-reported and participants classified as current smoker or not. Metabolic equivalent of task (MET) is used to measure the intensity level of physical activity and indicated the rate of energy consumption for a specific activity. MET is defined as 1 kcal/kg per h (4·184 kJ/kg per h) that is roughly equal to the energy cost of being at rest. Physical activity was categorised into three intensity levels based on MET score: light, moderate and vigorous[29]. Subjects with diabetes were excluded from the study.

Red meat consumption

Dietary intake was assessed via a 24-h recall obtained by a trained interviewer during the MEC visit, using a computer-assisted dietary interview system with standardised probes, that is, the US Department of Agriculture Automated Multiple-Pass Method (AMPM)[30,31]. Briefly, information on the type and quantity of all food and beverages consumed in a 24-h period before the dietary interview (from midnight to midnight) was collected using the AMPM. The AMPM is designed to enhance complete and accurate data collection while reducing respondent burden[31,32]. Detailed descriptions of the dietary interview

Table 1. Age-, sex- and race-adjusted mean of markers of insulin resistance and inflammation across quartiles of red meat consumption (Mean values with their standard errors)

	Quartiles of red meat consumption								
	1 (*n* 4153)		2 (*n* 4158)		3 (*n* 4166)		4 (*n* 4144)		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	*P**
Median and 25th–75th percentiles of meat consumption (g/d)	5·5	2·2–7·8	11·1	9·8–19·7	32·4	27·6–41·9	58·4	46·3–66·9	
Serum hs-CRP (mg/dl)	0·29	0·01	0·36	0·01	0·39	0·03	0·48	0·01	<0·001
Serum apo B (mg/dl)	91·3	0·86	93·9	0·82	96·4	0·98	97·1	1·04	<0·001
Fasting blood glucose (mg/dl)	97·4	0·65	97·6	0·82	100·2	0·49	102·3	0·76	<0·001
Plasma insulin (μU/ml)	1·86	0·01	1·93	0·02	2·06	0·01	2·19	0·01	<0·001
HOMA-IR	0·69	0·03	0·89	0·01	1·06	0·01	1·18	0·01	<0·001
HOMA-β	4·29	0·01	4·41	0·01	4·62	0·02	4·79	0·01	<0·001
HbA1c (%)	5·44	0·02	5·49	0·01	5·34	0·01	5·28	0·02	0·28
TyG index	8·46	0·01	8·59	0·01	8·71	0·03	8·89	0·01	<0·001

hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, homoeostatic model assessment of insulin resistance; HOMA-β, homoeostatic model assessment of β-cell function; HbA1c, Hb A1c; TyG index, TAG glucose index.
**P* values for linear trend across quartiles of hs-CRP. Variables were compared across quartiles of red meat consumption using ANCOVA test.

methods are provided in the NHANES Dietary Interviewer's Training Manual[33]. The MyPyramid Equivalents Database for USDA Survey Food Codes was used to calculate RMC[33]. In the present study, red meat intake was calculated as the sum of beef, pork, lamb, veal and game consumption and expressed as g/d.

Statistical analysis

Analyses were conducted using the SPSS software (version 22) according to the guidelines set forth by the Centers for Disease Control and Prevention for analysis of complex NHANES data sets, accounting for the masked variance and using the proposed weighting methodology[34]. We used means and standard errors of the mean for continuous measures (ANOVA) and percentages for categorical variables (χ^2). ANCOVA was used to compute age, race, energy intake and sex-adjusted means of IR markers or inflammation across quartiles of meat consumption.

The counterfactual framework assessed the total, direct and indirect effects of RMC on markers of IR or inflammation with BMI, WC, apVAT, VAI and LAP as mediators[35,36]. In this approach, the 'total effect' can be decomposed into a 'direct effect' (not mediated by BMI, WC, apVAT, VAI and LAP; online Supplementary Fig. S1) and an 'indirect effect' (mediated by BMI, WC, apVAT, VAI and LAP; online Supplementary Fig. S1). The analysis was conducted using the SPSS Macro developed by Preacher and Hayes[37]. A product-of-coefficients test was used as it has the potential to detect significant mediation effects in the absence of a significant intervention effect[35,36]. In brief, the macro generates outputs that include the following steps. First, the total effect (γ-coefficient) of the exposure on the outcome variable (i.e. markers of IR or inflammation) is estimated by regressing the markers of IR or inflammation (outcomes) on RMC (independent variable) while adjusting for the covariates used in the first step but without adjusting for mediators. The 'action theory' test is then used to examine the effect of the exposure (meat consumption) on the hypothesised mediators (α-coefficient, BMI, WC, apVAT, VAI and LAP; online Supplementary Fig. S1). The 'conceptual theory' test examines the association between changes in the hypothesised mediators and changes in outcome variables (i.e. markers of IR or inflammation; β-coefficient, online Supplementary Fig. S1). The programme also estimates the direct (γ'-coefficient) and indirect ($\alpha \times \beta$ product of coefficients) effects. The proportion of the mediation effect was calculated using the following equation ($\alpha \times \beta / (\alpha \times \beta + \gamma)$). Full or complete mediation is present when the total effect (the γ' path) is significant, the direct effect (the γ' path) is NS and $\alpha \times \beta$ is significant, whereas partly or incomplete mediation is present when the direct effect (the γ' path) is also significant. Inconsistent mediation is when neither total nor direct effect is significant and $\alpha \times \beta$ is significant[38]. All estimates were adjusted for age, sex, race/ethnicity, educational, smoking and level of physical activity.

Results

General characteristics

A total of 16 621 subjects met the criteria for inclusion in the present analyses. Overall 8607 (48·3 %) participants were men and the mean age was 47·1 years. Non-Hispanic White (69·4 %) was the largest racial group and other Hispanic (4·5 %) the smallest racial group. Furthermore, 56·1 % of the participants were married, while 56·4 % had achieved more than high school education. Means for BMI, WC and apVAT were 28·7 (SE 0·05) kg/m^2, 98·2 (SE 0·12) cm and 179·2 (SE 1·18), respectively. Overall, 20·1 % were current smokers including 24·7 % of men and 15·7 % of women. Participants engaging in vigorous physical activity represented 5·2 % of the participants, and those engaging in little/no physical activity represented 24·3 %. Age, sex and race-adjusted mean of markers of IR and inflammation (high-sensitivity CRP, apo B, FBG, insulin, HOMA-IR, HOMA-B and TyG index) significantly increased across quartiles of RMC (all *P*s <0·001; Table 1); HbA1c was not associated with RMC (Table 1).

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Table 2. Estimates of regression coefficients for the association between red meat consumption (g/d), BMI, waist circumference (WC), anthropometrically predicted visceral adipose tissue (apVAT), visceral adiposity index (VAI) and lipid accumulation product (LAP) (action theory) and markers of insulin resistance and inflammation (total effect) among adults in USA using National Health and Nutrition Examination Survey database*
(Regression coefficients and 95 % confidence intervals)

Mediator	Estimate	95 % CI	*P*
BMI	0·34	0·21, 0·48	<0·001
WC	0·91	0·59, 1·24	<0·001
apVAT	3·27	0·95, 5·42	<0·001
LAP	0·06	0·04, 0·08	<0·001
VAI	0·05	0·03, 0·07	<0·001
Outcome			
Serum hs-CRP (mg/dl)	0·03	0·02, 0·07	0·01
Serum apo B (mg/dl)	0·96	0·45, 1·12	0·01
Fasting blood glucose (mg/dl)	0·69	0·33, 1·01	0·04
Plasma insulin (μU/ml)	0·03	0·02, 0·06	<0·001
HOMA-IR	0·04	0·02, 0·07	<0·001
HOMAβ	0·01	–0·005, 0·04	0·14
HbA1c (%)	0·01	–0·003, 0·04	0·12
TyG index	0·04	0·03, 0·06	<0·001

hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, homoeostatic model assessment of insulin resistance ; HOMA-β, homoeostatic model assessment of β-cell function; HbA1c, glycated Hb; TyG index, TAG glucose index.
*All estimates were adjusted for age, sex, race/ethnicity, education, smoking and level of physical activity. Estimates for mediator and outcomes correspond to the regression coefficients α and γ, respectively, in online Supplementary Fig. S1.

Red meat intake, anthropometry, insulin resistance and inflammation

Action theory. After adjusting for covariates, a significant association was found between red meat intake and BMI (β: 0·345, $P < 0·001$), WC (β: 0·912, $P < 0·001$), apVAT (β: 3·27, $P < 0·001$), VAI (β: 0·054, $P < 0·001$) and LAP (β: 0·066, $P < 0·001$) (Table 2).

Total effect. This was calculated by examining the association between red meat intake and markers of IR or inflammation in multivariate models without adjusting for potential mediators. Results showed that, with the exception of HbA1c and HOMA-β, all the markers of IR or inflammation were positively and significantly associated with red meat intake (all *P*s < 0·04; Table 2).

Conceptual theory. This analysis tested the association between mediators (BMI, WC, apVAT, VAI and LAP) and markers of IR or inflammation; all potential mediators had significant and positive association with markers of IR or inflammation (all *P*s < 0·001; Table 3).

Direct and indirect effects of red meat consumption on insulin resistance and inflammation

Table 4 shows the *direct effect*, *indirect effect*, proportion of mediation effect and Sobel statistics for testing indirect effects. Both BMI and WC significantly mediated the association between markers of IR and inflammation and red meat intake (all *P*s < 0·001); BMI and WC showed the greatest effect on FBG ($\beta = 0·312$ and $\beta = 0·371$, respectively). apVAT was a significant mediator for the association between red meat intake and CRP, FBG, HbA1c and TyG index (all *P*s < 0·001); similarly FBG was the variable with the strongest association with apVAT

Table 3. Estimates of regression coefficients for the association between BMI, waist circumference (WC), anthropometrically predicted visceral adipose tissue (apVAT), visceral adiposity index (VAI) and lipid accumulation product (LAP) with markers of insulin resistance and inflammation (conceptual theory) among USA adults*
(Regression coefficients and 95 % confidence intervals)

	BMI			WC			apVAT			VAI			LAP		
Outcomes	Estimate	95 % CI	*P*	Estimate	95 % CI	*P*	Estimate	95 % CI	*P*	Estimate	95 % CI	*P*	Estimate	95 % CI	*P*
Serum hs-CRP (mg/dl)	0·08	0·080, 0·085	<0·001	0·03	0·036, 0·038	<0·001	0·009	0·0089, 0·0096	<0·001	0·40	0·38, 0·42	<0·001	0·56	0·52, 0·56	<0·001
Serum apo B (mg/dl)	0·54	0·460, 0·630	<0·001	0·29	0·260, 0·330	<0·001	0·09	0·073, 0·102	<0·001	14·23	13·95, 15·26	<0·001	13·62	12·52, 14·63	<0·001
Fasting blood glucose (mg/dl)	0·77	0·690, 0·880	<0·001	0·36	0·320, 0·400	<0·001	0·07	0·064, 0·095	<0·001	8·25	7·62, 9·12	<0·001	7·42	6·39, 8·54	<0·001
Plasma insulin (μU/ml)	0·05	0·054, 0·059	<0·001	0·02	0·024, 0·026	<0·001	0·006	0·005, 0·007	<0·001	0·40	0·38, 0·42	<0·001	0·44	0·43, 0·46	<0·001
HOMA-IR	0·06	0·061, 0·065	<0·001	0·02	0·028, 0·030	<0·001	0·007	0·006, 0·008	<0·001	0·47	0·44, 0·49	<0·001	0·51	0·49, 0·52	<0·001
HOMA-β	0·03	0·034, 0·039	<0·001	0·01	0·016, 0·018	<0·001	0·004	0·003, 0·005	<0·001	0·23	0·21, 0·25	<0·001	0·28	0·26, 0·30	<0·001
HbA1c (%)	0·02	0·023, 0·027	<0·001	0·01	0·010, 0·012	<0·001	0·002	0·001, 0·003	<0·001	0·21	0·19, 0·23	<0·001	0·20	0·19, 0·22	<0·001
TyG index	0·02	0·027, 0·031	<0·001	0·01	0·013, 0·015	<0·001	0·004	0·003, 0·005	<0·001	0·77	0·72, 0·80	<0·001	0·64	0·63, 0·66	<0·001

hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, homoeostatic model assessment of insulin resistance; HOMA-β, homoeostatic model assessment of β-cell function; HbA1c, glycated Hb; TyG index, TAG glucose index.
*All estimates were adjusted for age, sex, race/ethnicity, educational, smoking and level of physical activity. Regression coefficient β is shown in online Supplementary Fig. S1.

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Table 4. Direct and indirect effects of red meat consumption on markers of insulin resistance and inflammation with BMI, waist circumference (WC), anthropometrically predicted visceral adipose tissue (apVAT), visceral adiposity index (VAI) and lipid accumulation product (LAP) as mediators among USA adults*
(Regression coefficients and percentages)

Mediator and outcomes	Direct effect (γ')		Indirect effect ($\alpha \times \beta$)3		Proportion of mediation (%)
BMI	Estimate	*P*	Estimate	Sobel test statistic	
Serum hs-CRP (mg/dl)	0·004	0·692	0·029	<0·001	82·1
Serum apo B (mg/dl)	0·888	0·023	0·201	<0·001	21·1
Fasting blood glucose (mg/dl)	0·382	0·312	0·312	<0·001	27·2
Plasma insulin (μU/ml)	0·016	0·079	0·022	<0·001	66·9
HOMA-IR	0·020	0·032	0·025	<0·001	58·1
HOMA-β	0·002	0·795	0·013	<0·001	12·3
HbA1c (%)	0·055	0·623	0·009	<0·001	26·1
TyG index	0·039	<0·001	0·010	<0·001	5·3
WC					
Serum hs-CRP (mg/dl)	0·008	0·865	0·034	<0·001	69·1
Serum apo B (mg/dl)	0·695	0·046	0·266	<0·001	22·3
Fasting blood glucose (mg/dl)	0·203	0·562	0·371	<0·001	23·1
Plasma insulin (μU/ml)	0·014	0·112	0·024	<0·001	52·1
HOMA-IR	0·019	0·043	0·028	<0·001	46·5
HOMA-β	0·001	0·956	0·015	<0·001	4·23
HbA1c (%)	0·001	0·846	0·011	<0·001	20·4
TyG index	0·033	<0·001	0·013	<0·001	34·5
apVAT					
Serum hs-CRP (mg/dl)	−0·023	0·336	0·030	<0·001	95·1
Serum apo B (mg/dl)	−0·022	0·762	0·212	0·166	72·1
Fasting blood glucose (mg/dl)	0·112	0·623	0·293	<0·001	16·3
Plasma insulin (μU/ml)	0·031	0·042	0·016	0·166	40·6
HOMA-IR	0·030	0·109	0·019	0·143	36·1
HOMA-β	0·022	0·245	0·011	0·145	33·1
HbA1c (%)	0·003	0·831	0·009	<0·001	13·1
TyG index	0·020	0·032	0·013	<0·001	20·1
VAI					
Serum hs-CRP (mg/dl)	0·016	0·205	0·021	<0·001	6·2
Serum apo B (mg/dl)	0·215	0·563	0·682	<0·001	43·1
Fasting blood glucose (mg/dl)	0·105	0·777	0·452	<0·001	4·1
Plasma insulin (μU/ml)	0·021	0·039	0·018	<0·001	3·7
HOMA-IR	0·021	0·019	0·022	<0·001	3·4
HOMA-β	0·007	0·653	0·011	<0·001	6·2
HbA1c (%)	0·001	0·635	0·11	<0·001	2·9
TyG index	0·005	0·038	0·042	<0·001	19·7
LAP					
Serum hs-CRP (mg/dl)	0·003	0·865	0·036	<0·001	16·2
Serum apo B (mg/dl)	0·101	0·723	0·808	<0·001	16·2
Fasting blood glucose (mg/dl)	0·033	0·986	0·542	<0·001	8·2
Plasma insulin (μU/ml)	0·011	0·214	0·027	<0·001	18·6
HOMA-IR	0·016	0·123	0·031	<0·001	14·8
HOMA-β	0·001	0·865	0·017	<0·001	31·2
HbA1c (%)	−0·011	0·911	0·014	<0·001	6·3
TyG index	0·004	0·320	0·043	<0·001	6·32

hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, homoeostatic model assessment of insulin resistance; HOMA-β, homoeostatic model assessment of β-cell function; HbA1c, glycated Hb; TyG index, TAG glucose index.
*All estimates were adjusted for age, sex, race/ethnicity, educational, smoking and level of physical activity. Regression coefficients α, β and γ' are shown in online Supplementary Fig. S1.

($\beta = 0{\cdot}293$). Both VAI and LAP mediated the association between red meat intake and markers of IR and inflammation (all *P*s < 0·001); serum apo B was the variable with the strongest association with VAI and LAP ($\beta = 0{\cdot}682$ and $\beta = 0{\cdot}808$, respectively).

Discussion

In the present study, we demonstrated that red meat intake was significantly associated with all anthropometric outcomes (BMI, WC, apVAT, VAI and LAP) in fully adjusted models. Red meat intake was also significantly associated with markers of IR (except for HbA1c and HOMA-B) and with inflammation. In addition, mediation analyses suggested that these significant associations were partly or fully mediated by central adiposity.

A systematic review reported a significant link between red meat intake, especially processed varieties, with risk of breast cancer[39]. Existing observational and intervention studies testing the association between RMC and CRP levels have reported mixed results. Findings were significant in some studies[2,6,21], which is in line with that found in the present study. However, other studies have findings contradictory to our study

findings, which demonstrated that while processed meat was positively associated with CRP, RMC alone was not[1] and in another study where lean red meat was not associated with CRP levels[40]. The content of cholesterol[41], Fe[42] and SFA[41,43] in red meat to some extent explains the association between red meat and adverse health outcomes[44,45]. Previous studies on the effect of meat consumption on glucose/insulin homoeostasis have been inconsistent, with some finding an association, in line with our study[5,46–48], and others failing to show such an association[20,21]. It has been reported that RMC may have an impact on glucose/insulin metabolism through Fe-related metabolic pathways[49]. Fe is a strong pro-oxidant that catalyses several cellular reactions involved in the production of reactive oxygen species and hence increases the oxidative stress level[50]. This can cause damage to cellular structures, including pancreatic beta cells, and high body Fe stores have been shown to be associated with an elevated risk of diabetes[50]. Once Fe is accumulated in the liver, it could interrupt with role of insulin and also constrain glucose production[51]. Increased Fe accumulation might lead to IR by constraining glucose uptake in different tissues[49]. Clinical studies have shown no significant effect of Fe supplementation on CRP levels[42].

Additionally, the effect of red meat on uric acid levels could constitute another pathway linking this dietary component with glucose/insulin homoeostasis dysregulation[52]. It has been reported that uric acid could play a role in oxidative stress[53] and inflammatory factors[53], which are both linked to the progress of unfavourable glucose/insulin homoeostasis[52,53]. Further, an experiment in animal models reported that fructose-induced hyperuricaemia plays a pathogenic role in the development of cardio-metabolic risk factors[54].

A prolonged intake of SFA is correlated with the MetS and is known to contribute to weight gain and inflammation if consumed in excess[55,56]. In particular, SFA are known to contribute to increases in influence of white adipose tissue increasing inflammatory response[57–59]. Hence, we hypothesised that a high energy diet which contains excess red meat and is high in SFA may be associated with weight gain and thus increased adiposity and subsequently contribute to developing unfavourable glucose/insulin homoeostasis enhancing low-grade inflammation, which is strongly linked to the pathogenesis of CVD and other non-communicable diseases. The role of dietary factors, such as excess refined sugar or saturated fat intake, in triggering low-grade chronic inflammatory response has received further scientific support recently, reiterating the link with age-related chronic conditions. These inflammatory responses are thought to interact with the ageing process and, if persisting, could play a key role in the pathogenic mechanisms, leading to the onset of chronic metabolic and CVD[60].

The main strength of the present study is the investigation of the mediation effects using various markers of adiposity including not only BMI and WC, which are the markers of general and abdominal obesity, but also apVAT, VAI and LAP. Moreover, we have used a randomly selected, large and representative sample, and our results can be extrapolated to the general population. Lastly, the analyses included extensive adjustment for potential confounders which reduces the chance of the residual confounders.

The study has limitations. The study is cross-sectional and focused on adults only. Cohort studies may better address the causal relation between red meat intake and relevant health outcomes but may not be feasible because of the nature of the exposure and ethical issues. Consumption reported associations do not necessarily mean causation. Consumption of red meat in different life stages (childhood, adolescents adulthood and during ageing) has been previously shown to affect risk estimates[61]. Furthermore, different patterns of exposure over time could also affect the results. Cooking methods and variations in animal farming and meat preparation can alter the quality as well as the health effects of red meats[62,63]. This introduces additional residual confounding into the analyses[64–66]. This information was not available and therefore could not be controlled. The mediating effect of WC may be affected by BMI, or vice versa, because of the high colinearity between these two variables. This issue could be resolved by adding BMI and WC simultaneously to the mediation model[42]. However, this approach was not possible in our analyses because of the complex survey design of the present study. Hence, in an attempt to overcome this limitation, we have added other validated adiposity factors to the models such as apVAT, LAP and VAI, which provide an independent evaluation of the mediating role of central adiposity in red meat intake and markers of diabetes risk. Lastly, although BMI and WC are regularly applied to determine adiposity, these indicators are still imprecise and can lead to bias in determining obesity. For instance, BMI, is usually limited compared with direct measures of obesity due to the fact that it does not consider age, sex, bone structure, fat distribution or muscle mass info[67]. However, we also used other markers of adiposity (apVAT and LAP) which are sensitive to the age and sex.

Conclusion

Our study finding suggests that high RMC could negatively affect glucose/insulin homoeostasis and inflammatory profile, via mechanisms involving central fat accumulation. Future research is warranted to explore the effect of reducing red meat intake on glucose/insulin homoeostasis, which in turn could inform dietary strategies to reduce the risk of type 2 diabetes mellitus.

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The authors have no conflicts of interest to declare.

For the data collection and physical examination of the NHANES, informed consent was obtained from all adult participants, and the National Centre for Health Statistics Research Ethics Review Board approved the protocol.

For the data collection and physical examination of the NHANES, informed consent (publication) was obtained from

all adult participants, and the National Centre for Health Statistics Research Ethics Review Board approved the protocol.

All the data are from a public access database.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114519002149

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