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Title: Dietary fat, abdominal obesity and smoking modulate the relationship between plasma complement component 3 concentrations and metabolic syndrome risk

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Abstract

Objective: Chronic inflammation plays a role in the pathogenesis of metabolic syndrome (MetS) and cardiovascular disease (CVD). Complement component 3 (C3) is a novel cardiometabolic risk factor. Whether dietary fat intake modulates MetS risk conferred by elevated C3 concentrations is unknown. Our objective is to investigate the relationship between C3 concentrations and risk of the MetS and its phenotypes, and to further examine whether dietary fat intake modulates these relationships.

Methods: Biochemical, dietary and lifestyle measurements were determined in the LIPGENE-SU.VI.MAX study of MetS cases and matched controls (n = 1754).

Results: Elevated C3 concentrations (> median) were associated with increased risk of impaired insulin sensitivity [OR 1.78, CI 1.34-2.36, $P < 0.0001$], insulin resistance [OR 1.73, CI 1.31-2.89, $P = 0.0001$], abdominal obesity [OR 2.15, CI 1.43-3.24, $P = 0.0002$] and low HDL cholesterol [OR 1.40, CI 1.05-1.86, $P = 0.02$] compared to low C3 concentrations. Increased MetS risk conferred by elevated C3 concentrations [OR 3.11, 95% CI 2.52-3.82, $P < 0.0001$] was further accentuated among high dietary fat consumers [OR 4.80, 95% CI 2.77-8.33, $P < 0.0001$] (particularly of saturated [OR 4.05, 95% CI 2.33-7.05, $P < 0.0001$] and monounsaturated fat [OR 4.48, 95% CI 2.62-7.56, $P < 0.0001$]), and smokers [OR 3.83, 95% CI 2.12-6.94, $P < 0.0001$], however this effect was abolished in abdominally lean individuals [OR 1.46, 95% CI 0.69-3.14, $P = 0.33$].

Conclusions: Dietary fat (intake and composition), abdominal obesity and smoking modulate the relationship between elevated plasma C3 concentrations and MetS risk.

Supplementary key words: Metabolic syndrome, inflammation, cardiovascular risk factors, diet, obesity, smoking, LIPGENE.

Dietary fat, abdominal obesity and smoking modulate the relationship between plasma complement component 3 concentrations and metabolic syndrome risk

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Abbreviations:

BMI	Body mass index
C3	Complement component 3
CRP	C reactive protein
CVD	Cardiovascular disease
HOMA	Homeostasis model assessment
MetS	Metabolic syndrome
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
QUICKI	Quantitative insulin-sensitivity check index
SFA	Saturated fatty acid
T2DM	Type 2 diabetes mellitus
TAG	Triacylglycerol

1. Introduction

The metabolic syndrome metabolic syndrome (MetS) is a common, multi-component, condition characterised by abdominal obesity, insulin resistance, dyslipidaemia and hypertension, that is associated with increased risk of type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) [1]. In addition the National Cholesterol Education Program's Adult Treatment Panel III report (NCEP ATP III) identified pro-inflammatory status as being another key MetS characteristic [2]. Obesity is a chronic low-grade inflammatory state that predisposes to the development of insulin resistance, the hallmark of obesity and the MetS [3]. It has been suggested that insulin resistance and ultimately T2DM may be a manifestation of a chronic acute-phase response [4]. Elevated circulating concentrations of complement component 3 (C3), an acute-phase response protein with a central role in the innate immune system, have been associated with insulin resistance, fasting and postprandial triacylglycerol (TAG) concentrations, diabetes, the MetS and CVD [5-8]. Adipose tissue has been recognised as an important organ contributing to the inflammatory phenotype and adipocytes are an important source of C3 [9]. There is a progressive increase in C3 concentrations with BMI in individuals with severe, morbid and extreme obesity [10]. C3 concentrations are also elevated in lean and obese diabetic individuals relative to both lean and obese non-diabetic subjects [11]. In that study, C3 concentrations correlated with insulin, glucose and insulin resistance, suggesting that metabolic perturbations such as hyperglycaemia and insulin resistance augment C3 mediated inflammation, which is the case for other components of the inflammatory response [12]. Although a relationship between plasma C3 concentrations and BMI has been examined, this has not been investigated in the context of the MetS.

Genetic and environmental factors contribute to susceptibility to diet-related polygenic disorders such as the MetS. Recently we reported novel genetic associations between *C3* polymorphisms with MetS risk [13]. Dietary fat composition represents an important

environmental factor which may alter MetS risk [14-16]. Fasting and postprandial lipid metabolism is disturbed in the MetS [17]. The complement system is activated in the postprandial phase and both insulin and chylomicrons stimulate adipocyte C3 production [18, 19]. However it is not known whether dietary fat intake (either quantity or composition) directly influences C3 activation or modulates C3 concentrations. In addition smoking and physical activity are important cardiometabolic risk factors which may influence MetS risk, but little is known regarding their influence on C3 concentrations, with inconsistent reports perhaps reflective of differences in cohort demographics [20-23]. Therefore the aim of this study was to investigate the relationship between plasma C3 concentrations and risk of the MetS and its phenotypes. An additional novel objective was to examine whether this relationship is affected by habitual dietary fat intake and fatty acid composition and other cardiometabolic risk factors including obesity, smoking and physical activity.

2. Methods

2.1. Subjects, MetS classification and study design

This study is part of a prospective case control study of LIPGENE, an EU Sixth Framework Programme Integrated Project entitled "Diet, genomics and the metabolic syndrome: an integrated nutrition, agro-food, social and economic analysis". Subjects were selected from an existing national French SU.VI.MAX cohort including 13,000 subjects who were followed over 7.5 years (from 1994 to 2002) [24]. The LIPGENE-SU.VI.MAX study is a nested case control study of MetS consisting of women (35-60 years of age) and men (45-60 years of age) recruited from SU.VI.MAX. Additional approval from the ethical committee, CCPPRB, of Paris-Cochin Hospital included an additional clause (number Am 2840-12-706) to perform the biochemical analysis required for the LIPGENE study. LIPGENE

subjects were informed of the study objectives and provided signed informed consent using protocol approved by this ethical committee. Participants were invited to provide a 24 h dietary record every two months, for a total of six records per year as previously described [25].

Baseline and 7.5 year follow up data including full clinical examination records were made available to LIPGENE. This data was used to identify cases, individuals who developed ≥3 elements of the MetS, over the 7.5 year follow up period and control subjects. MetS cases were selected based on the NCEP-ATP III criteria for the MetS, with some modifications [26]. MetS cases were required to fulfil at least three of the following five criteria: increased waist circumference [>94cm (men) or >80cm (women)], increased fasting blood glucose [≥5.5 mmol/L or treatment for diabetes], increased TAG [≥ 1.5 mmol/L or treatment for dyslipidaemia], decreased high density lipoprotein cholesterol (HDL-C) [<1.04 mmol/L (men) or < 1.29 mmol/L (women)] and increased systolic/diastolic blood pressure [≥ 130/85 mmHg or antihypertensive treatment]. Cases were defined as both men and women with ≥ 3 abnormalities, and controls were defined as men and women with no abnormalities or men with ≤1 abnormality. Cases and controls (n = 1754) were matched according to age (± 5 y), gender and number of dietary records available. For the purpose of the work detailed herein all data relate to the follow-up time point.

2.2. Biochemical analysis

Fasting glucose, TAG, HDL-C and total cholesterol were measured as previously described [24]. Insulin and C-peptide were determined by electrochemiluminescence immunoassays (Roche Diagnostics, France). Non-esterified fatty acids (NEFA) and LDL cholesterol (LDL-C) were measured by enzymatic colorimetric methods (Randox Laboratories, UK and Roche Diagnostics, France). Total plasma C3 and C reactive protein

(CRP) were measured on a Dade Behring BN II nephelometer (Dade Behring Diagnostics, Marburg, Germany). Homeostasis model assessment (HOMA), a measure of insulin resistance, was calculated as: [(fasting plasma glucose x fasting serum insulin)/ 22.5] [27]. Quantitative insulin-sensitivity check index (QUICKI), a measure of insulin sensitivity, was calculated as = [1/(log fasting insulin + log fasting glucose + log fasting NEFA)] [28].

2.3. Statistical analysis

Statistical analysis was performed using SAS for Windows™, version 9.0 (SAS Institute, Cary, North Carolina, USA). Data is expressed as means ± SEM. After checking for skewness and kurtosis, glucose, insulin, NEFA, TAG, QUICKI and HOMA were normalised by logarithmic transformation. Plasma C3 concentrations were dichotomised based on control subject median. Logistic regression was used to determine associations between C3 status (</> median) and risk of the MetS and its risk phenotypes (high TAG, low HDL-C, high HOMA, low QUICKI, fasting hyperglycaemia, abdominal obesity and high blood pressure). Cut off points for these MetS risk phenotypes were determined by the MetS criteria. To determine modulation by dietary fat consumption, logistic analyses were repeated using the median of control subjects to dichotomize intakes and to examine associations in low and high consumers (i.e. below and above dietary fat medians). To determine effect modification by abdominal obesity, individuals were stratified according to the waist circumference cut-offs employed in the MetS criteria (>94cm (men) or >80cm (women). In a separate analysis individuals were also categorised according to BMI, those with a BMI > 25 kg/m^2 were classified as overweight including obese and those with a BMI ≤ 25 kg/m^2 were defined as lean. To assess the influence of current smoking status on C3 concentrations and MetS risk, individuals were categorised as non-smokers (never plus former smokers) and current

smokers. Similarly for physical activity individuals were categorised as irregularly active and active based on their daily level of physical activity (<1 hour/day plus ≥1 hour/day). The generalised estimating equation (GEE) linear regression [29] was used to investigate interactions between continuous MetS phenotypes and SFA intake. Potential confounding factors used in the adjusted multivariate analysis included age, gender, energy intake, smoking status, physical activity and use of medications including lipid lowering, hypertension and diabetes treatments. The correlation between variables was verified using Spearman's correlation coefficient. For all analyses a *P*-value of < 0.05 was considered significant.

3. Results

3.1. Associations between plasma C3 concentrations and metabolic characteristics

Table 1 details the characteristics of the study population stratified by C3 median concentrations. In terms of their phenotype, individuals with higher plasma C3 concentrations had greater BMI and abdominal obesity ($P < 0.0001$), displayed numerous metabolic perturbations (elevated insulin concentrations, lower QUICKI and higher HOMA, $P < 0.0001$), were more dyslipidaemic (higher TAG, NEFA, LDL-C and total cholesterol and lower HDL-C concentrations, $P < 0.0001$), more hypertensive ($P < 0.0001$) and had higher CRP concentrations ($P < 0.0001$) compared to individuals with low C3 concentrations. Not surprisingly, more MetS cases comprised the top 50th percentile of C3 concentrations. No differences were observed between groups with respect to age, gender distribution, dietary fatty acid profiles, smoking status and physical activity concentrations. Correlation analysis verified the reported associations. C3 concentrations were significantly and positively correlated with CRP ($r = 0.51$, $P < 0.0001$), MetS score derived from the MetS criteria (r =

0.43, $P < 0.0001$), insulin (r = 0.41, $P < 0.0001$), insulin resistance (r = 0.41, $P < 0.0001$), waist circumference (r = 0.40, $P < 0.0001$), BMI (r = 0.40, $P < 0.0001$), TAG (r = 0.34, $P < 0.0001$), systolic and diastolic blood pressure (r = 0.34 and 0.24 respectively, $P < 0.0001$) and glucose (r = 0.21, $P < 0.0001$). Negative correlations were found for C3 and insulin sensitivity (r = -0.41, $P < 0.0001$) and for C3 and HDL-C (r = -0.33, $P < 0.0001$).

3.2. C3 concentrations and risk of metabolic syndrome and its phenotypes

Individuals with elevated C3 concentrations (above the median) had 3 fold higher risk of MetS [OR 3.11, 95% CI 2.52-3.82, $P < 0.0001$] compared to individuals with C3 concentrations in the bottom 50th percentile (**Table 2**). Among all individuals increased risk of abdominal obesity, hyperinsulinaemia, impaired insulin sensitivity, reduced insulin resistance and low HDL-C was identified in subjects with higher C3 concentrations relative to individuals with C3 concentrations below the median. Similar results for these parameters were observed when males and females were analysed separately (data not shown), with greater MetS risk identified in females [OR 5.67, CI 4.02-7.99, $P < 0.0001$] than in males [OR 2.16, CI 1.67-2.82, $P < 0.0001$]. In addition increased risk of low HDL-C concentrations was only evident among the male subjects [OR 1.63, CI 1.12-2.37, $P = 0.01$]. Interestingly when MetS case and control subjects were analysed separately (**Table 2**), higher C3 concentrations were associated with increased risk of hyperinsulinaemia, impaired insulin sensitivity and abdominal obesity in both groups. MetS cases also had greater risk of low HDL-C concentrations whereas individuals without the MetS with higher C3 concentrations displayed increased risk for a number of other MetS phenotypes including insulin resistance and hypertension.

3.3. Modulation of MetS risk conferred by high C3 concentrations by dietary fat composition

Dietary fat consumption modulated the relationship between plasma C3 concentrations and MetS risk (**Table 3**), whereby the increased MetS risk conferred by elevated C3 concentrations was further accentuated among high fat consumers [OR 4.80, 95% CI 2.77-8.33, $P < 0.0001$] which appeared to be due to high intake of both saturated fatty acids (SFA) [OR 4.05, 95% CI 2.33-7.05, $P < 0.0001$] and monounsaturated fatty acids (MUFA) [OR 4.48, 95% CI 2.62-7.56, P < 0.0001]. MetS risk was also increased among individuals with elevated C3 levels who habitually consumed a low-fat diet, or diets low in SFA or MUFA, however the observed odds ratios were all below that identified for MetS risk associated with elevated C3 levels alone (OR 3.11), suggesting an additive effect of elevated C3 levels and high fat intake. Dietary polyunsaturated fatty acid (PUFA) intake did not influence MetS risk, nor were any gender differences noted for this analysis when males and females were analysed separately. Interaction analysis confirmed these findings whereby higher dietary intake of total fat ($P_{\text{interaction}} = 0.003$), SFA ($P_{\text{interaction}} = 0.02$) and MUFA ($P_{\text{interaction}} = 0.02$) in individuals with elevated C3 concentrations was predictive of increased MetS score. Further examination of interactions between SFA intake and MetS phenotypes (**Figure 1A-1C**) according to C3 status identified significant effects on glucose ($P_{\text{interaction}} = 0.0003$) and CRP concentrations ($P_{\text{interaction}} = 0.003$) and on abdominal obesity ($P_{\text{interaction}} = 0.02$) only among subjects with high C3 concentrations. Examination of measures of insulin resistance and sensitivity (**Figure 2A-2B**) revealed interactions between SFA and HOMA ($P_{\text{interaction}} = 0.01$) and QUICKI ($P_{\text{interaction}} = 0.04$) again only among subjects with elevated C3 concentrations. Similar interactions and P values were identified between C3 concentrations and these parameters with MUFA (data not shown).

3.4. Effect modification of MetS risk conferred by elevated C3 concentrations by obesity, smoking and physical activity

When stratified by BMI, similar odds ratios for the MetS were observed in the overweight (including obese) and the lean individuals with elevated C3 levels relative to their counterparts with C3 concentrations below the median, suggesting that BMI did not modulate the increased MetS risk associated with elevated plasma C3 concentrations. However when abdominal obesity based on increased waist circumference (1 of the 5 MetS criteria) was used to stratify subjects MetS risk was abolished in those classified as abdominally lean [OR 1.46, 95% CI 0.69-3.14, P=0.33]. MetS risk was increased in the abdominally obese individuals with elevated C3 levels [OR 2.89, 95% CI 1.87-4.39, $P < 0.0001$]; however this OR was in keeping with MetS risk identified in the whole population [OR 3.11]. Interestingly when stratified by abdominal obesity (**Figure 3**), obese MetS subjects demonstrated significantly increased C3 concentrations (ANOVA $P < 0.0001$) compared to lean MetS subjects ($P = 0.002$) and to the obese and lean control subjects ($P = 0.0001$). Similar patterns were observed for TAG concentrations and insulin resistance, with inverse relationships identified for insulin sensitivity and HDL-C concentrations (ANOVA $P < 0.0001$). In relation to smoking status, higher C3 levels among smokers were associated with increased MetS risk [OR 3.83, 95% CI 2.12-6.94, $P < 0.0001$] compared to smokers with C3 levels below the median. Plasma C3 levels did not seem to affect MetS risk among non-smokers, with odds ratios for MetS [OR 3.05, 95% CI 2.45-3.82, $P < 0.0001$] in line with that of the whole population [OR 3.11]. Physical activity levels did not modulate MetS risk associated with elevated C3 levels with similar findings in irregularly active individuals [OR 3.89, 95% CI 2.47-6.14, $P < 0.0001$] and those active on a daily basis [OR 3.64, 95% CI 2.47-5.35, $P < 0.0001$].

4. Discussion

MetS is associated with increased risk of T2DM and CVD [1]. Increasing numbers of studies have reported associations between elevated C3 concentrations, MetS phenotypes and CVD risk [5-8]. This study adds to current knowledge in terms of determining how this association is further modified by dietary fat composition and obesity. We identified a three-fold increased risk of the MetS and its phenotypes including abdominal obesity, impaired insulin sensitivity, reduced insulin resistance and low HDL-C concentrations in individuals with elevated C3 concentrations (> median). To our knowledge, no information exists in relation to potential modulation of MetS risk conferred by C3 concentrations by habitual dietary fat intake. We have shown for the first time that MetS risk associated with higher C3 concentrations was subject to a significant effect modification by dietary fat intake, with greater risk identified among individuals with high total dietary fat, SFA and MUFA intake. While increased MetS risk was still evident in their low-fat consuming counterparts with elevated C3 concentrations, one interpretation could be that individuals predisposed to the MetS display a greater sensitivity to high intake of total dietary fat, SFA and MUFA which further accentuates their risk.

Chronic inflammation has been recognised by the NCEP ATP III as a key MetS characteristic [2]. Recently the metabolic inflammatory state associated with obesity and insulin resistance has been termed 'meta-inflammation' and has been defined as "low-grade, chronic inflammation orchestrated by metabolic cells in response to excess nutrients and energy" [30]. Dietary fat is an important nutrient, wherein excessive exposure has been suggested to play a key role in the development of MetS [14-16]. Cross-sectional, intervention and experimental data suggest that high-fat diets, in particular high SFA diets, promote obesity, insulin resistance and inflammation, promoting the development of MetS, T2DM and CVD [16, 31]. In insulin resistant subjects, replacing SFA with MUFA to attenuate insulin resistance was only

effective in subjects habitually consuming a high fat diet (>36% energy from fat) [32]. It has been suggested that dietary oleic acid (the major MUFA) may be more readily oxidised than SFA which may in turn have a negative effect on insulin sensitivity [33]. Interestingly C3a receptor (C3aR) knockout mice fed high fat diets displayed resistance to diet-induced obesity and insulin resistance. Examination of their adipocytes revealed reduced macrophage infiltration and pro-inflammatory status [34]. This data provides evidence that the C3aR is responsive to dietary fat, at least in mice, and that the C3aR plays an important role in insulin resistance and obesity. Unfortunately that study did not examine the influence of dietary fat composition. In a mouse model of atherosclerosis ($LDLR^{-/-}$) hepatic gene expression profiling revealed induction of the alternative complement pathway and down-regulated expression of C3, in conjunction with greater aortic lesion C3, in high-fat relative to low-fat fed mice [35].

In the current study we did not find any evidence that dietary PUFA modulates the association between C3 concentrations and MetS risk, suggesting specific effects of SFA and MUFA. As oleic acid is mostly derived from animal products and not olive oil, at least outside of the Mediterranean region, it is difficult to fully differentiate the effects of SFA from MUFA. Thus the SFA and MUFA specific effects may be inter-linked. In a recent small study of abdominally overweight individuals (n=20) consumption of a SFA-rich diet increased pro-inflammatory gene expression in adipose tissue, whereas a MUFA-rich diet induced a more anti-inflammatory profile [36]. However there was no difference in associated plasma inflammatory biomarker concentrations including C3 concentrations between diets [36]. To our knowledge there is only one paper in the literature which reports a diet related change in C3 concentrations in humans, in that study obese subjects (n=30) randomly assigned to a hypocaloric legume-rich diet achieved a greater reduction in C3 concentrations compared to a legume-free diet [37]. Chylomicrons are the strongest stimulators of adipocyte C3 production [19], thus dietary fat composition could potentially indirectly influence C3 activation. The

molecular mechanisms underlying modulation of MetS risk by dietary fat in individuals with elevated C3 concentrations are currently unknown and warrants further investigation. Toll-like receptor-4 (TLR4) is another innate immune molecular link between fatty acids, obesity, inflammation and insulin resistance [38]. SFA activate TLR4 mediated inflammation whereas MUFA or PUFA do not [39, 40]. TLR4 deficiency protects against high SFA diet-induced obesity, inflammation and insulin resistance [39, 41]. Complement and TLR pathways are both activated by lipopolysaccharide, suggesting potential cross-talk between the two systems [42]. Initial experiments indicate a regulatory effect of complement on TLR signalling mediated, at least in part, via the C3a receptor [43]. Thus potential synergism between C3 and TLR4 in response to high SFA intake to promote inflammation and insulin resistance should be examined.

While Mets risk was greater in females, we did not observe any differences in C3 concentrations between genders. Higher C3 concentrations have been reported in women [44] and the C3 pathway is thought to be more active in subcutaneous adipose tissue [45], which is more abundant in females than in males. Thus this finding perhaps reflects gender-specific differences in intra- and extra- peritoneal adipose tissue mass. Interestingly abdominal obesity, but not BMI, modulated MetS risk associated with elevated C3 concentrations. MetS risk was abolished in those classified as abdominally lean. When we further stratified the cohort obese MetS subjects demonstrated the highest C3 concentrations, which were significantly higher compared to lean MetS subjects and to both obese and lean control subjects, suggesting an additive effect. Adipose tissue is an important source of C3 production [9]. Thus it may not be surprising that a previous examination of C3 concentrations across degrees of obesity revealed a relationship between C3 concentrations and the progressive increase of BMI in individuals with severe, morbid and extreme obesity [10]. In our study the increased C3 concentrations observed in the lean MetS subjects relative to the lean

control subjects also indicate that the MetS, or at least some of its associated metabolic perturbations, rather than obesity per say may also be responsible.

In the current study higher C3 concentrations among smokers were associated with further increased MetS risk compared to smokers with low C3 concentrations, whereas C3 concentrations did not seem to affect MetS risk among non-smokers. We found no evidence of modulation by physical activity concentrations, with similar MetS risk in the top 50th C3 percentile regardless of whether they were irregularly active or active on a daily basis. Conflicting data exists in the literature in relation to smoking status, physical activity and C3 concentrations. In middle-aged and elderly men and women (n=1220) C3 concentrations were inversely associated with smoking status [21]. Similarly in a large study of middle-aged non-diabetic men the proportion of smokers was lower in those with high C3 concentrations, with no difference in physical activity concentrations across C3 quartiles [20]. More recently a cross-sectional association between C3 concentrations and coronary heart disease was reported, but only in heavy smokers [23]. It is unknown whether smoking has a direct effect on C3 concentrations or whether other factors / mechanisms may account for these findings.

Several features of this study (comprehensive phenotypic characterisation, large number of male and female cases and matched controls from all socio-economical categories and areas in the country) make this study particularly robust. Nevertheless, some limitations can be identified. As dietary consumption was self-reported by food-frequency questionnaire, some misclassification of exposure, due to deficiencies in nutrient databases, accuracy of memories or willingness to divulge these details, was inevitable. The number of dietary records used was minimal (3 in a small number of subjects) but was necessitated in order to maximise the number of matched cases and controls. The focus of the current analysis was on dietary fat composition but other food components such as carbohydrate or fibre can play a role in the development of the MetS.

In conclusion, this study provides new data on modulation of MetS risk associated with elevated C3 concentrations by dietary fat intake, abdominal obesity and smoking. We demonstrated that individuals with C3 concentrations in the top 50th percentile had increased risk of impaired insulin sensitivity, hyperinsulinaemia and abdominal obesity, regardless of whether they had the MetS or not. Indeed further metabolic perturbations existed in the control subjects with elevated C3 levels (insulin resistance and hypertension). Interestingly increased MetS risk was further augmented in high dietary fat consumers and smokers, suggesting that these individuals could derive most benefit from current public health guidelines to reduce dietary fat intake and stop smoking. While the underlying molecular mechanisms are unknown and require further investigation, such data add to the current knowledge and may be useful in terms of developing personalised dietary recommendations wherein an individuals' meta-inflammatory profile may determine choice of dietary therapy to improve responsiveness and cardiometabolic health.

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FIGURE LEGENDS

FIGURE 1

Interaction between dietary SFA intake and C3 status (< and > median) on glucose concentrations (Figure 1a), CRP concentrations (Figure 1b) and abdominal obesity (Figure 1c) in all subjects.

Increasing dietary SFA intake was predictive of increasing fasting glucose ($P_{interaction}$ = 0.0003) and CRP ($P_{interaction}$ = 0.003) concentrations and greater abdominal obesity ($P_{interaction}$ = 0.02) in individuals with elevated C3 levels (depicted as open circles) but not in those with low C3 levels (depicted as closed circles). The *P* values and predicted values were calculated from the generalised estimating equation (GEE) linear regression [29] model.

FIGURE 2

Interaction between dietary SFA intake and C3 status (< and > median) on insulin resistance (Figure 2a) and insulin sensitivity (Figure 2b).

Among individuals with high C3 levels (depicted as open circles) increasing dietary SFA intake was predictive of increasing insulin resistance ($P_{interaction}$ = 0.01) and deteriorating insulin sensitivity ($P_{interaction}$ = 0.04). The *P* values and predicted values were calculated from the generalised estimating equation (GEE) linear regression [29] model. The open circles represent individuals with C3 levels > median and the closed circles represent individuals with C3 levels < median.

FIGURE 3

Relationship between plasma C3 concentrations according to abdominal obesity in MetS cases and control subjects.

When stratified by abdominal obesity, as defined in the MetS criteria, obese MetS subjects demonstrated significantly increased C3 levels (ANOVA $P < 0.0001$) compared to lean MetS subjects ($P = 0.002$), obese ($P = 0.0001$) and lean individuals without the MetS ($P = 0.0001$). The black bars represent obese individuals and the white bars represent lean individuals.

Fig. 1A

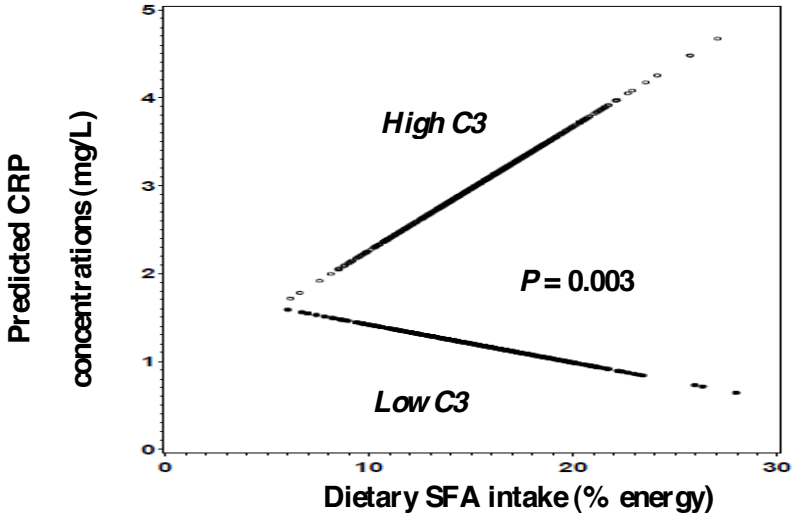

Fig. 1B

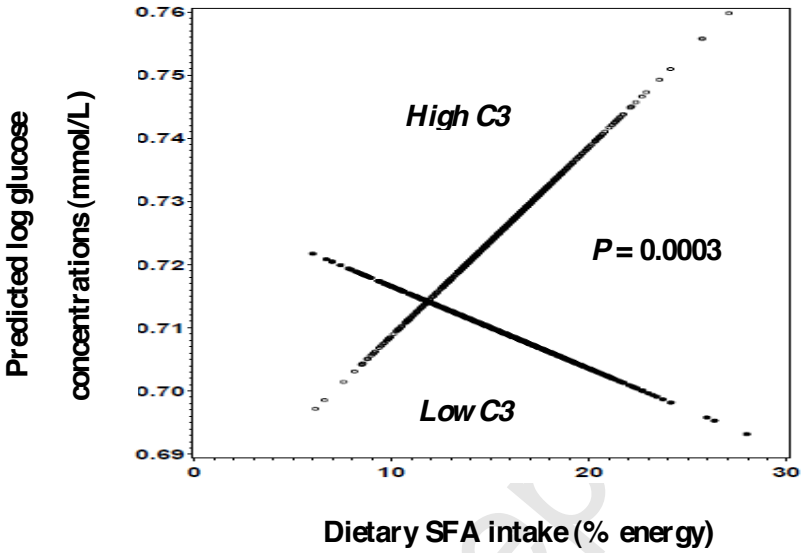

Fig. 1C

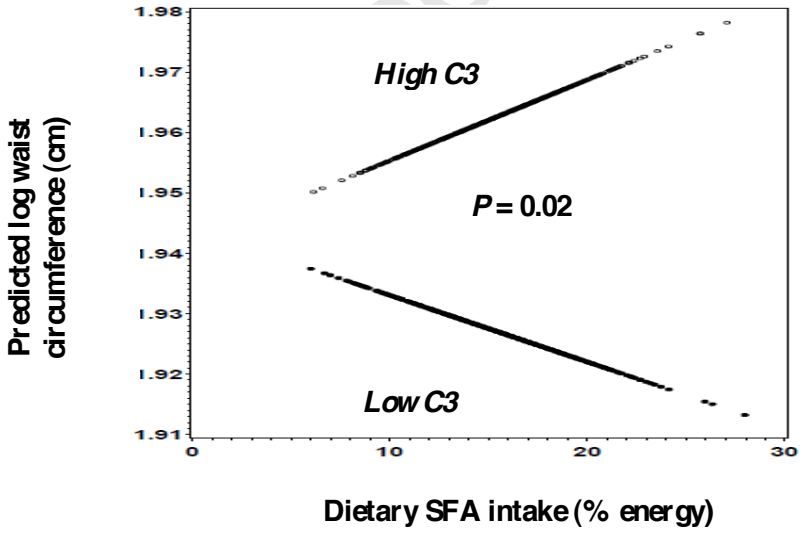

Fig. 2A

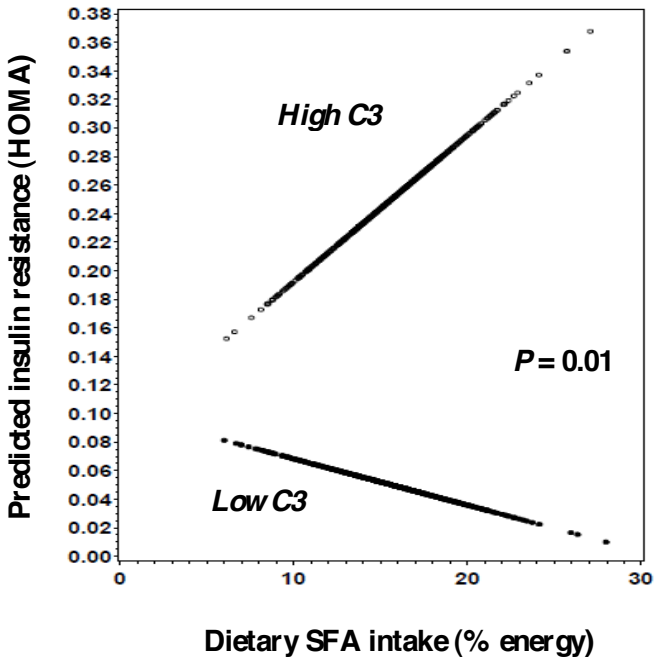

Fig. 2B

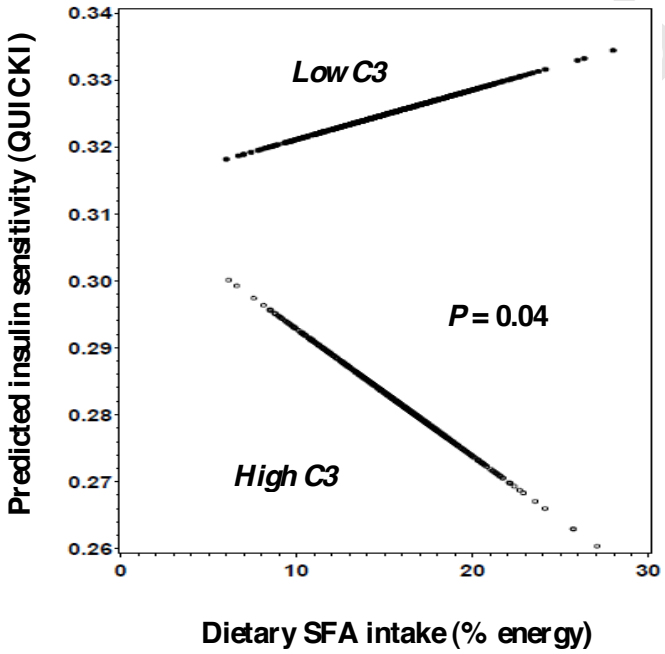

Figure 3

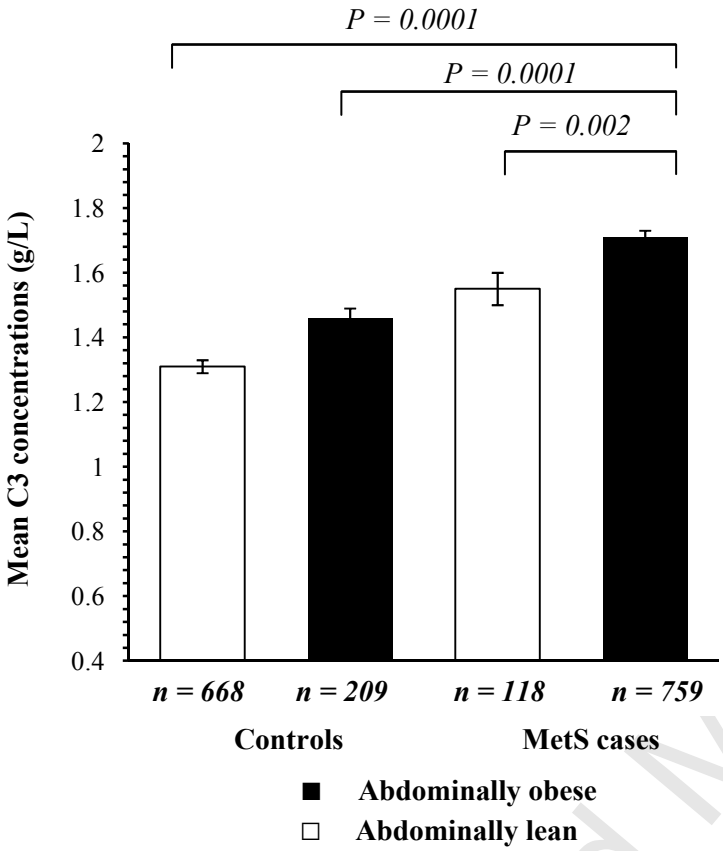

Table 1

Clinical characteristics and dietary profiles of the population at follow-up categorised according to high versus low plasma C3 median concentrations

	< C3 median	**> C3 median**
n	871	883
MetS case/control *%*	39/61	67/33 *
Male/female *%*	61/39	59/41
Age, *yrs*	58.44±0.20	58.01±0.16
BMI, kg/m^2	24.98±0.12	27.55±0.18 *
Waist, *cm*	85.44±0.38	92.36±0.47 *
Glucose, *mmol/L*	5.17±0.03	5.37±0.04
Insulin, *mU/L*	6.27±0.16	9.07±0.24 *
HOMA	1.53±0.05	2.26±0.07 *
QUICKI	0.35±0.01	0.30±0.01 *
Total cholesterol, *mmol/L*	5.62±0.03	5.85±0.03 *
HDL-C, *mmol/L*	1.55±0.01	1.35±0.01*
LDL-C, *mmol/L*	3.31±0.03	3.88±0.03 *
Triglycerides, *mmol/L*	1.12±0.03	1.46±0.03 *
NEFA, *mmol/L*	0.82±0.02	1.06±0.03 *
SBP, *mm Hg*	128.1±0.45	135.6±0.61 *
DBP, *mm Hg*	80.86±0.27	84.04±0.37 *
C3, *g/L*	1.24±0.01	1.90±0.05 *

Total fat intake (% energy)	33.34±0.32	33.43±0.45
Total SFA intake (% energy)	14.12±0.18	13.86±0.22
Total MUFA intake (% energy)	12.29±0.13	12.50±0.21
Total PUFA intake (% energy)	4.81±0.08	4.94±0.11
Protein intake (% energy)	17.05±0.25	16.54±0.17
Carbohydrate intake (% energy)	42.03±0.57	43.00±0.37
Total fibre intake (g/day)	20.08±0.53	20.16±0.40
Soluble fibre intake (g/day)	3.94±0.08	3.92±0.11
Non-soluble fibre intake (g/day)	16.14±0.35	16.24±0.37
Alcohol intake (% energy)	7.58±0.30	7.03±0.49
Physical activity: irregular/daily (%)	22/78	20/80
Smoking: never/former & current (%)	87/13	86.5/13.5

Values are means ± SEM. * $P < 0.0001$ compared to < C3 median (1.42 *g/L*).

Table 2

Odds ratios and 95% confidence intervals for risk of MetS and related phenotypes according to C3 concentrations in all subjects, MetS cases and controls

	All subjects	*P* value	MetS cases	*P* value	Controls	*P* value
MetS	3.11 (2.52-3.82)	< 0.0001	-	-	-	-
Impaired insulin sensitivity	1.78 (1.34-2.36)	< 0.0001	1.75 (1.05-2.91)	0.033	1.96 (1.41-2.75)	< 0.0001
Reduced insulin resistance	1.73 (1.31-2.89)	0.0001	1.57 (0.94-2.63)	0.089	1.90 (1.36-2.65)	0.0002
Abdominal obesity	2.15 (1.43-3.24)	0.0002	2.69 (1.66-4.37)	< 0.0001	2.17 (1.17-4.03)	0.014
Low HDL-C	1.40 (1.05-1.86)	0.02	1.37 (1.03-1.83)	0.034	2.33 (0.50-4.59)	0.282
Hyperinsulineamia	1.84 (1.41-2.41)	< 0.0001	1.88 (1.18-2.99)	0.008	1.81 (1.30-2.52)	0.0005
Hypertension	1.36 (0.96-1.94)	0.089	0.87 (0.50-1.52)	0.610	1.65 (1.06-2.59)	0.025

Odds ratios and 95% confidence intervals for the association between plasma C3 concentration and the MetS and related phenotypes were determined by logistic regression

analyses comparing individuals with high C3 concentrations (> median) to those with low C3 concentrations (< median). Potential confounding factors included in the analyses included age, gender, smoking status, physical activity and medication use.

Table 3

Odds ratios and 95% confidence intervals for MetS risk associated with elevated C3 concentrations, with effect modification by dietary fat intake

Dietary fat (median % energy)	**< dietary fat median**	***P***	**> dietary fat median**	***P***	$P_{interaction}$ *C3 levels x dietary fat on MetS score*
Total fat (33.40 %)	2.86 (2.29-3.56)	< 0.0001	4.80 (2.77-8.33)	< 0.0001	0.003
Total SFA (14.20 %)	2.96 (2.37-3.71)	< 0.0001	4.05 (2.33-7.05)	< 0.0001	0.02
Total MUFA (12.30%)	2.88 (2.30-3.62)	< 0.0001	4.48 (2.62-7.56)	< 0.0001	0.02
Total PUFA (4.67 %)	2.95 (2.35-3.69)	< 0.0001	2.81 (1.86-4.03)	< 0.0001	0.07

Odds ratios and 95% confidence intervals for the association between plasma C3 concentrations and the MetS, stratified according to dietary fatty acid composition and status (median of fatty acids expressed as % energy), were determined by logistic regression analyses comparing individuals with high C3 concentrations (> median) to those with low C3 concentrations (< median). $P_{interaction}$ values were calculated from the GEE linear regression model [36] using the MetS score based on criteria used to define the MetS. Potential confounding factors included in the analyses included age, gender, smoking status, physical activity and medication use.