Short Communication

Serum fatty acid composition and indices of stearoyl-CoA desaturase activity are associated with systemic inflammation: longitudinal analyses in middle-aged men

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Altered fatty acid (FA) composition is related to insulin resistance and CVD. One possible mediator may be inflammation, but longitudinal data relating FA composition to inflammation taking insulin resistance into account are limited. We investigated the long-term association between FA composition and C-reactive protein (CRP) concentrations in a large population-based cohort study in 767 men followed for 20 years. The association between FA composition in serum cholesteryl esters at age 50 and CRP concentrations at age 70 was investigated using linear regression. In addition, desaturase activities (stearoyl-CoA desaturase-1 (SCD-1), $\Delta 5$- and $\Delta 6$-desaturase) were estimated using FA product-to-precursor ratios. Insulin resistance was measured directly at follow-up by euglycaemic clamp. After adjusting for confounders (smoking, physical activity, alcohol intake, obesity and erythrocyte sedimentation rate) CRP concentrations were inversely associated with the proportion of $18:2n$-6 ($P=0.002$) and positively associated with $16:1n$-7 ($P=0.008$), $18:1n$-9 ($P=0.0003$), $20:5n$-3 ($P=0.04$) and estimated SCD-1 ($P=0.005$) and $\Delta 6$-desaturase ($P=0.02$) activities. After adding insulin resistance to the model, $18:1n$-9, $18:2n$-6 and SCD-1 remained significant predictors of CRP. A FA composition indicating low intake of $18:2n$-6, high intake of SFA and high SCD-1 activity is, in a Swedish population of middle-aged men, associated with CRP concentrations 20 years later, even independently of obesity and insulin resistance.

C-reactive protein: Fatty acids: SCD-1: Inflammation

Serum fatty acid (FA) composition is a consequence of dietary fat[1,2] but also of endogenous FA desaturation[3]. Desaturation is catalysed by enzymes introducing a double bond in the FA chains. Stearoyl-CoA desaturase-1 (SCD-1) synthesize MUFA from SFA, whereas $\Delta 5$- and $\Delta 6$-desaturases catalyse the synthesis of highly unsaturated FA[3].

FA composition in cholesteryl esters (CE) has been associated with insulin resistance[4] and CVD[5,6]. This FA pattern is often characterized by increased proportion of SFA and $16:1n$-7, decreased proportion of $18:2n$-6 and indications of increased activity of SCD-1 and $\Delta 6$-desaturase and reduced activity of $\Delta 5$-desaturase[4,6,7]. A potential mediating role of low-grade inflammation in the association between FA composition and insulin resistance is still unknown.

Low-grade inflammation as assessed by C-reactive protein (CRP) is related to insulin resistance[8] and CVD[9]. A potential anti-inflammatory effect of *n*-3 FA has previously been indicated[10–13]. No larger studies have, however, investigated the long-term relationship between overall FA composition and CRP. Furthermore, no studies have examined the role of

FA desaturases on inflammation, despite high SCD-1 activity index being linked to obesity and insulin resistance[3,7]. We investigated the longitudinal association between dietary fat quality as assessed by CE FA composition at age 50, and CRP concentrations 20 years later in 767 healthy men, also taking several important confounders into account, including insulin resistance measured by a gold standard technique.

Methods

Subjects

Subjects participated in the Swedish cohort, Uppsala Longitudinal Study of Adult Men (http://www.pubcare.uu.se/ULSAM/). Subjects (*n* 1020) had measures of serum CE FA composition at age 50 and CRP at age 70. Exclusion criteria were CRP concentration >10 mg/l, diabetes (fasting blood glucose ≥ 6.1 mmol/l), CVD (ICD-8 codes 401–443) or malignancy at baseline, and usage of lipid-lowering medicine or glucocorticoids at age 50 or 70. After exclusions, the

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population consisted of 767 participants. The study was approved by the Ethics Committee of Uppsala University. All subjects gave written informed consent.

Investigations at 50 years

All measurements were performed under standardized conditions as previously described[14]. All blood samples were drawn after an overnight fast. Blood glucose was measured using the glucose oxidase method. Serum insulin was determined with the Phadebas Insulin Test (Pharmacia AB, Uppsala, Sweden), using a radioimmunosorbent technique. Insulin resistance was estimated by the homeostasis model assessment of insulin resistance index (HOMA-IR)[15]. Analysis of the FA composition in serum CE was performed as previously described[16]. The percentage composition of methylated FA was determined by GC. The erythrocyte sedimentation rate was determined by Westergren's method. Drug use, smoking habits (smoker or non-smoker) and physical activity (sedentary, moderate, regular and athletic) were obtained through a questionnaire.

Investigations at 70 years

The investigation was performed in the same manner as at baseline. Alcohol consumption was assessed by questionnaire. High-sensitivity CRP was measured by latex-enhanced reagent (Dade Behring, Deerfield, IL, USA) using a Behring BN ProSpec analyser. Intra-assay CV of the CRP method was 1·4 % at both 1·23 and 5·49 mg/l. Insulin sensitivity was determined by the euglycaemic clamp according to DeFronzo *et al.* [17], slightly modified. Insulin infusion rate was 56 mU/min per body surface area (m^2). Glucose disposal M (mg/kg body weight per min) was the tissue glucose disposal during the last 60 min of the clamp.

Desaturase estimations

Desaturase activities were estimated by FA product-to-precursor ratios according to the following: Δ5-desaturase $= (20:4n-6/20:3n-6)$, Δ6-desaturase $= (18:3n-6/18:2n-6)$ and SCD-1 $= (16:1n-7/16:0)$.

Statistical analyses

A JMP software package was used for statistics (SAS Institute, Cary, NC, USA). CRP, $16:1n-7$, $18:0$, $18:3n-6$, $20:5n-3$, Δ6-desaturase, SCD-1, HOMA-IR, alcohol consumption and erythrocyte sedimentation rate were logarithmically transformed to achieve normal distribution. Univariate associations between FA composition and CRP concentrations were investigated by linear regression. Three multivariate models were used: one adjusting for BMI, physical activity, smoking and erythrocyte sedimentation rate at age 50 and alcohol consumption at age 70 (alcohol intake was not available at baseline) and two models additionally adjusting for insulin resistance (either as HOMA-IR at baseline or as M at follow-up). $P < 0·05$ was considered as statistically significant.

Results

Baseline characteristics are presented as means and standard deviations; variables with skewed distribution are presented as median (Q1–Q3): BMI, 24·7 (SD 2·9) kg/m^2; $14:0$, 1·1 (SD 0·2) %; $16:0$, 11·6 (SD 1·0) %; $16:1n-7$, 3·4 (2·9–4·1) %; $18:0$, 1·1 (1·0–1·3) %; $18:1n-9$, 19·0 (SD 2·4) %; $18:2n-6$, 54·9 (SD 4·7) %; $18:3n-6$, 0·6 (0·5–0·8) %; $18:3n-3$, 0·7 (SD 0·2) %; $20:3n-6$, 0·6 (SD 0·1) %; $20:4n-6$, 4·8 (SD 0·9) %; $20:5n-3$, 1·2 (0·9–1·6) %, $22:6n-3$, 0·7 (SD 0·2) %; Δ5-desaturase, 8·8 (SD 2·2); Δ6-desaturase, 0·011 (0·008–0·015); SCD-1, 0·29 (0·26–0·35); CRP concentration at age 70, 1·9 (0·9–3·8) mg/l.

In univariate analyses, the proportions of $16:1n-7$ (r 0·13, $P = 0·0002$), $18:1n-9$ (r 0·20, $P < 0·0001$) and $18:3n-6$ (r 0·09, $P = 0·012$) were positively correlated, whereas $18:2n-6$ ($r − 0·18, P < 0·0001$) was inversely related to CRP concentrations 20 years later. Δ6-Desaturase and SCD-1, but not Δ5-desaturase, were significantly related to CRP (r 0·11, $P = 0·0014$ and r 0·13, $P = 0·0004$, respectively).

In the multivariate model (n 594) including BMI, smoking, physical activity and erythrocyte sedimentation rate at age 50 and alcohol consumption at age 70, the positive correlation with CRP remained for $16:1n-7$ ($P = 0·008$), $18:1n-9$ ($P = 0·0003$), Δ6-desaturase ($P = 0·022$) and SCD-1 ($P = 0·005$), as well as the inverse correlation with $18:2n-6$ ($P = 0·002$). When additionally adjusting for insulin resistance, $16:1n-7$ ($P = 0·008$), $18:1n-9$ ($P = 0·010$), $18:2n-6$ ($P = 0·012$) and SCD-1 ($P = 0·003$) were associated with CRP in the model with HOMA-IR at baseline (n 482), whereas only $18:1n-9$ ($P = 0·002$), $18:2n-6$ ($P = 0·023$) and SCD-1 ($P = 0·047$) were related in the model with M at follow-up (n 570). $20:5n-3$ was positively related to CRP in the multivariate models without insulin resistance and with HOMA-IR ($P = 0·036$ and $P = 0·015$, respectively). The univariate association between $20:5n-3$ and CRP was, however, non-significant.

The univariate associations remained after excluding subjects with CRP concentrations > 5 mg/l (n 635), except for $16:1n-7$ ($P = 0·062$). Only $18:1n-9$ was related in multivariate analysis. Excluding subjects with CVD or non-steroidal anti-inflammatory drug use at follow-up did not change the results appreciably.

When analysing the relationship between CE FA composition at age 70 and CRP, only 320 subjects were included and 14:0 was not assessed. $18:2n-6$ ($r − 0·11, P = 0·041$), $20:3n-6$ (r 0·25, $P < 0·0001$), Δ5-desaturase ($r − 0·13, P = 0·020$) and Δ6-desaturase (r 0·13, $P = 0·026$) were correlated to CRP. Only $20:3n-6$ was correlated when adjusting for BMI, smoking, physical activity and alcohol intake at age 70 (n 221) as well as when additionally adjusting for insulin sensitivity (M) at age 70 (n 220).

Discussion

In this longitudinal study, serum FA composition in CE at age 50 was related to CRP concentrations 20 years later among 767 Swedish men. It is the first study to demonstrate a link between estimated SCD-1 and inflammation, which is of interest since high SCD-1 activity has been linked to diabetes and obesity[18,19]. CRP were positively associated with SCD-1 and the proportion of $18:1n-9$, and inversely associated with $18:2n-6$, independently of BMI, smoking, physical activity, alcohol intake, erythrocyte sedimentation rate and insulin

resistance. The present results accord with previous findings from this population where a similar FA pattern was related to the metabolic syndrome[7], insulin resistance[4] and CVD[5,6].

Since this is an observational study, only speculations about possible mechanisms can be made. The positive correlations between MUFA and CRP may first seem surprising since MUFA generally is associated with beneficial health effects. However, MUFA in CE in Swedish populations at the time of the study reflects intake of foods containing high SFA rather than olive oil which was rarely consumed[5]. Similarly, in US populations, MUFA in CE reflected SFA intake rather than MUFA intake[1], suggesting that the present correlation between CRP and MUFA probably reflects high meat and dairy fat intake. Why then was SFA not significantly related to CRP? Increased intake of SFA may increase the SCD-1 activity to keep the membrane concentration of $16:0$ low to retain fluidity and prevent impaired cell signalling[3,20]. Inflammation could also be promoted by the metabolic disordered state associated with elevated SCD-1 activity rather than by the SFA intake *per se*. The SCD-1 activity index ($16:1/16:0$), however, seems to be an excellent marker of SFA intake, at least as good as individual serum SFA[21] (E Warensjö, unpublished results). A role of SFA in inflammation is in line with *in vitro* data showing that stimulation of cells with $16:0$ increases IL-6 mRNA expression and protein production[22,23]. IL-6 in turn induces CRP production. Interestingly, $18:2n$-6 inhibited $16:0$-induced IL-6 up-regulation[22]. Since $18:2n$-6 in plasma reflects the dietary intake[2], a diet high in this FA may contribute to lower CRP concentrations.

When associating FA composition at age 70 to CRP, the cross-sectional results partly differed from those assessed at age 50, possibly due to the lower power at that time-point. The data should therefore be interpreted cautiously. The divergence may, however, also depend on a different response to diet caused by ageing. High proportion of $20:3n$-6 and low $\Delta5$-desaturase activity have previously been associated with the metabolic syndrome[7] and CVD[6].

Little data exist concerning CE FA composition and CRP concentrations. In a Spanish study, $18:2n$-6 and n-3 FA were inversely associated with CRP[11]. Similarly, in a French population CRP was inversely associated with $18:3n$-3 and $20:5n$-3[12]. A large Italian study also reported an inverse relationship between $18:3n$-3 and CRP[13]. We found no inverse association between n-3 FA and CRP, a result that accords with controlled trials[24]. Divergences in results may depend on differences in background diet and inflammation status among populations.

There are limitations to the present study. In this observational study, no conclusions regarding causality can be drawn. Desaturase activities were only estimated but may give an indication of the FA desaturation pattern. There are studies suggesting that these ratios reflect desaturase activity. In man, both SCD-1 mRNA expression in adipose tissue and serum SCD-1 ratio increased after rosiglitazone treatment[25]. This ratio is also markedly reduced in mice lacking SCD-1[26], and inhibition of SCD-1 is reflected by $16:1/16:0$ in human hepatoma cells[18]. Since we had no measure of CRP at age 50, we adjusted for erythrocyte sedimentation rate to address the possibility that subclinical inflammation at baseline explained the longitudinal correlations. Interestingly the relationships remained, supporting the possibility that FA composition may precede inflammation. Another limitation is that no food records were assessed at baseline to support FA composition data. Only men at the same age participated, with no data for women or other ethnic groups. Strengths include the longitudinal design, the large sample with complete FA composition data and adjustments of several relevant covariates including directly measured insulin resistance at follow-up. Serum FA composition is, compared to dietary registration, a more objective method that limits reporting bias. FA composition was assessed in CE which may not be directly translated to FA composition in phospholipids, but overall differences between fractions should probably not play a significant role when interpreting or comparing data.

In conclusion, among healthy middle-aged men, a serum FA pattern indicating high intake of SFA and low PUFA ($18:2n$-6) in a Swedish population predicted CRP concentrations 20 years later, even independently of obesity and insulin resistance. This dietary pattern was accompanied by increased estimated SCD-1 activity, which may be a consequence of such a FA pattern and/or reflect a novel link between lipogenic activity and inflammation independent of diet. Studies measuring SCD-1 activity directly will be required to answer the latter question. Our independent associations over a long time period motivate investigation of whether a specific FA intake could prevent or decrease inflammation, i.e. investigation in controlled studies of whether a diet relatively high in $18:2n$-6 and low in SFA may decrease low-grade inflammation.

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