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Short Communication

Greater impact of dietary fat manipulation than apolipoprotein E genotype on *ex vivo* cytokine production – Insights from the SATgenε study

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ABSTRACT

Apolipoprotein E (*APOE*) genotype is believed to play an important role in cardiovascular risk. *APOE4* carriers have been associated with higher blood lipid levels and a more pro-inflammatory state compared with *APOE3*/*E3* individuals. Although dietary fat composition has been considered to modulate the inflammatory state in humans, very little is known about how *APOE* genotype can impact on this response. In a follow-up to the main SATgenε study, we aimed to explore the effects of *APOE* genotype, as well as, dietary fat manipulation on *ex vivo* cytokine production. Blood samples were collected from a subset of SATgenε participants (n = 52/88), prospectively recruited according to *APOE* genotype (n = 26 E3/E3 and n = 26 E3/E4) after low-fat (LF), high saturated fat (HSF) and HSF with 3.45 g docosahexaenoic acid (DHA) dietary periods (each diet eight weeks in duration assigned in the same order) for the measurement of *ex vivo* cytokine production using whole blood culture (WBC). Concentrations of IL-1beta, IL-6, IL-8, IL-10 and TNF-alpha were measured in WBC supernatant samples after stimulation for 24 h with either 0.05 or 1 μg/ml of bacterial lipopolysaccharide (LPS). Cytokine levels were not influenced by genotype, whereas, dietary fat manipulation had a significant impact on TNF-α and IL-10 production; TNF-α concentration was higher after consumption of the HSF diet compared with baseline and the LF diet (P < 0.05), whereas, IL-10 concentration was higher after the LF diet compared with baseline (P < 0.05). In conclusion, our study has revealed the amount and type of dietary fat can significantly modulate the production of TNF-α and IL-10 by *ex vivo* LPS-stimulated WBC samples obtained from normolipidaemic subjects.

1. Introduction

The aetiology and progression of cardiovascular disease (CVD) is affected by both environmental and genetic factors [1]. The most widely researched common gene variants, with respect to CVD risk are the apolipoprotein E (*APOE*) single nucleotide polymorphisms (SNP) [2]. Although the literature is not fully consistent [3,4] *APOE4* carriers (approximately 25% of the Caucasian population), have been reported to have a higher risk of CVD. This was originally attributed to elevated blood lipid levels in this subgroup [4]. However, the mechanisms that relate *APOE4* to increased CVD risk may be more complex than solely a lipid effect [1,2] with studies largely conducted in transgenic animals or cell lines indicating that the *APOE4* allele is related to a more pro-oxidative and pro-inflammatory state compared with the *APOE3* allele [1,2].

Dietary fat manipulation may play an important role in the inflammatory response [5,6]; several studies indicated increasing levels of pro-inflammatory cytokines after a high fat meal or

Abbreviations: APOE, apolipoprotein E; BMI, body mass index; CHO, carbohydrate; CRP, C-reactive protein; CVD, cardiovascular disease; DHA, docosahexaenoic acid; GM-CSF, granulocyte-macrophage colony-stimulating factor; HSF, high saturated fat; ICAM-1, inter-cellular adhesion molecule-1; IFN, interferon; IL, interleukin; LDL-C, low density lipoprotein-cholesterol; LF, low fat; LPS, lipopolysaccharide; MUFA, monounsaturated fatty acid; NF-κB, nuclear factor kappa B; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; SNP, single nucleotide polymorphism; TNF-α, tumor necrosis factor-alpha; VCAM-1, vascular cell adhesion molecule; WBC, whole blood culture.

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chronic consumption of a high fat diet [5,7,8]. However, few studies have focused on the impact of dietary fat composition on these markers in response to iso-energetic diets. Recently, we have reported that *APOE* genotype influences the C-reactive protein (CRP) response to dietary fat intake, with higher concentrations after diets rich in saturated fat (HSF) and HSF with 3.45 g/d of docosahexaenoic acid (DHA) in *APOE4* carriers, relative to a low fat (LF) diet [9]. Yet, there are no studies investigating the combined effect of *APOE* genotype and fat manipulation on cytokine production in normolipidaemic subjects.

The aim of the present study was to investigate the effect of these three iso-energetic diets differing in fat quantity and quality on *ex vivo* whole blood culture (WBC) cytokine production according to *APOE* genotype. The WBC technique measures cytokine production following a pro-inflammatory stimulant and is considered a more physiologically meaningful and informative measure of inflammatory status in humans relative to the more traditional assessment of plasma cytokines in fasting blood.

2. Material and methods

2.1. Subjects and study design

A subset of the normolipidaemic participants from the SATgenε study ($n = 52/88$), who were prospectively recruited according to *APOE* genotype ($n = 26$ E3/E3 and $n = 26$ E3/E4) provided blood samples at the beginning of the study (baseline) and eight weeks after the low fat (LF), high saturated fat (HSF) and HSF with 3.45 g/day DHA (HSF-DHA) diet for the determination of *ex vivo* cytokine production using whole blood culture. The target macronutrient composition of the diets are shown in Table 1 and a detailed description of study design and dietary manipulation are presented in Carvalho-Wells et al. [9].

2.2. Stimulation of whole blood cultures

Blood samples collected in EDTA tubes were diluted 1:9 with RPMI 1640 medium (Sigma, UK) supplemented with 1% antibiotics, 1% L-glutamine and 1% non-essential amino acids (BioScience, UK). Subsequently, the diluted blood sample was cultured in 12-well plates (Greiner bio-one, UK), with 10 or 0.5 μg/ml of bacterial lipopolysaccharide (LPS) (*E. coli* 026:B6, Sigma, UK), leading to a final concentration of 1 or 0.05 μg/ml, respectively. Cultures were incubated at 37 °C for 24 h before centrifugation at 700×*g* to isolate the supernatant, which was stored at −20 °C until analysis. The monocyte count of each sample was measured by the Pathology Department at the Royal Berkshire Hospital in Reading.

2.3. Measurement of the cytokine concentration using the Luminex method

A Human Cytokine 10-Plex Panel (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IFN-γ, TNF-α, and GM-CSF; Invitrogen, Life Technologies) was used to measure the concentration of cytokines in the whole blood culture supernatant using the Luminex 200. Only IL-1β, IL-6, IL-8, IL-10 and TNF-α were detectable in the whole blood culture samples. Cytokine production was expressed as $\mu g/10^3$ monocytes as previously reported by Nagata et al. [10] and Rohleder et al. [11].

Table 1
Target daily macronutrient composition of the 3 isoenergetic diets.

Target macronutrient composition	LF diet	HSF diet	HSF-DHA diet
Energy from fat (%)	24	38	38
SFA (%)	8	18	18
MUFA (%)	8	12	12
PUFA (%)	6	6	6
Energy from carbohydrates (%)	59	45	45

Abbreviations: LF, low fat diet; HSF, high saturated fat diet; HSF-DHA, high saturated fat diet with 3.45 g/d docosahexaenoic acid (DHA); SFA, saturated fat; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; %, % of the total energy intake.

2.4. Statistical analysis

A one-within, one-between repeated measures ANOVA was used to analyse the effects of the different diets on the whole blood culture cytokine concentrations in the two genotype groups. Logarithmic or square-root transformation was applied to the variables that were not normally distributed. When statistical differences were found, data were further tested by the least significant difference (LSD) post hoc test. The statistical analysis was performed using SPSS version 17.0 (Statistical Package for Social Sciences, SPSS Inc., Chicago, Illinois, USA). $P \leqslant 0.05$ was considered statistically significant.

3. Results

Two subjects (*APOE4* carriers) were excluded because of missing monocyte population data. Cytokine production by either 0.05 μg/ml or 1 μg/ml LPS-stimulated WBC was not significantly affected by genotype (Table 2, Fig. 1). In the data for the two genotype groups combined, dietary fat manipulation significantly affected TNF-α and IL-10 production, for both 0.05 μg/ml ($P = 0.012$ and $P = 0.036$, respectively, repeated measures ANOVA) and 1 μg/ml of LPS ($P = 0.006$ and $P = 0.049$, respectively, repeated measures ANOVA) (Fig. 1). Post hoc analysis revealed that TNF-α production was significantly higher in the WBC supernatant after the subjects consumed the HSF diet compared with baseline ($P = 0.004$ and $P = 0.002$, for 0.05 and 1 μg/ml LPS, respectively) and the LF diet ($P = 0.020$, for 1 μg/ml LPS only) (Fig. 1). The HSF-DHA diet resulted in higher TNF-α concentration in comparison to baseline ($P = 0.012$ and $P = 0.021$, for 0.05 and 1 μg/ml LPS, respectively) (Fig. 1). For both LPS concentrations, the consumption of the LF diet resulted in significantly higher concentrations of IL-10, compared with baseline ($P = 0.013$ and $P = 0.015$, for 0.05 and 1 μg/ml LPS, respectively) and the HSF-DHA diet ($P = 0.026$ and $P = 0.050$, for 0.05 and 1 μg/ml LPS, respectively) (Fig. 1). The levels of IL-1β, IL-6 and IL-8 were not significantly modulated by dietary fat composition (Table 2).

4. Discussion

The present study investigated the impact of *APOE* genotype on the *ex vivo* cytokine response of normolipidaemic subjects to chronic dietary fat manipulation. It has been indicated that *APOE* has immuno-modulatory properties that could affect the risk of CVD [2]. *APOE* genotype has been reported to affect macrophage cytokine secretion [1,2] and, thus, may be predicted to influence inflammatory responses. As we previously reviewed [2] animal and cell culture models and limited human study evidence showed that pro-inflammatory cytokine levels, such as TNF-α and IL-6, were higher whereas the anti-inflammatory cytokine IL-10 concentrations were lower in *APOE4*-expressing cells or genotype groups compared to *APOE3* homozygotes. In contrast, in the current study, cytokine production was not significantly affected by *APOE* genotype suggesting that in normolipidaemic adults *APOE* genotype may not be a significant determinant of the circulating pro-inflammatory cytokine status [2].