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Endothelial function and insulin sensitivity during acute non-esterified fatty acid elevation: Effects of fat composition and gender

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Abstract *Background and aims:* We have reported that adverse effects on flow-mediated dilation of an acute elevation of non-esterified fatty acids rich in saturated fat (SFA) are reversed following addition of long-chain (LC) *n-3* polyunsaturated fatty acids (PUFA), and hypothesised that these effects may be mediated through alterations in insulin signalling pathways. In a subgroup, we explored the effects of raised NEFA enriched with SFA, with or without LC *n-3* PUFA, on whole body insulin sensitivity (SI) and responsiveness of the endothelium to insulin infusion.
Methods and results: Thirty adults (mean age 27.8 y, BMI 23.2 kg/m^2) consumed oral fat loads on separate occasions with continuous heparin infusion to elevate NEFA between 60 and 390 min. For the final 150 min, a hyperinsulinaemic-euglycaemic clamp was performed, whilst FMD and circulating markers of endothelial function were measured at baseline, pre-clamp (240 min) and post-clamp (390 min). NEFA elevation during the SFA-rich drinks was associated with impaired FMD ($P = 0.027$) whilst SFA + LC *n-3* PUFA improved FMD at 240 min ($P = 0.003$). In males, insulin infusion attenuated the increase in FMD with SFA + LC *n-3* PUFA ($P = 0.049$), with SI 10% greater with SFA + LC *n-3* PUFA than SFA ($P = 0.041$).
Conclusion: This study provides evidence that NEFA composition during acute elevation influences both FMD and SI, with some indication of a difference by gender. However our findings are not consistent with the hypothesis that the effects of fatty acids on endothelial function and SI operate through a common pathway.
This trial was registered at clinical trials.gov as NCT01351324 on 6th May 2011.

Introduction

Non-esterified fatty acids (NEFA) have been proposed to be a mediator of insulin signalling defects in both skeletal muscle and endothelial tissue [1,2]. Elevation of NEFA in healthy subjects by co-infusing Intralipid (a commercial lipid preparation) and heparin has been reported to impair glucose uptake and the phosphoinositide 3 kinase (P13K) signalling pathway in skeletal muscle [3–5], as well as reduce endothelial function. This pathway in endothelial cells regulates vascular tone via activation of endothelial

Abbreviations used: bw, body weight; eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; FAME, fatty acid methyl ester; FFM, fat-free mass; FMD, flow-mediated dilatation; iAUC, incremental AUC; LC, long-chain; NEFA, non-esterified fatty acid; NO, nitric oxide; NOx, total nitrites; PI3K, phosphoinositide 3 kinase; SFA, saturated fatty acid; SI, insulin sensitivity; TG, triglyceride.

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nitric oxide synthase (eNOS) with production of the vasodilator, nitric oxide (NO). Lind et al. reversed the NEFA-induced impairment of forearm blood flow in response to methacholine [5] by infusion of insulin, supporting the notion that elevated NEFA impair endothelial function via induction of insulin resistance in this tissue. Dietary fat quality may be a contributory factor in both impaired insulin sensitivity [6] and endothelial function [7]. *In vitro* studies report more adverse effects of saturated (SFA) than unsaturated fatty acids on the endothelial PI3K insulin signalling pathway and NO production [8–10]. In human studies, the impact of SFA is less clear, however, chronic supplementation with the long chain *n-3* polyunsaturated fatty acids (LC *n-3* PUFA) found in fish oil has been consistently shown to improve endothelial function in a variety of populations [11–13]. We have previously reported that adverse effects of acute elevation of NEFA rich in SFA on flow-mediated dilatation (FMD) are reversed following addition of LC *n-3* PUFA [14]. Here using an experimental protocol, we test the hypothesis that SFA and LC n-3 PUFA differentially affect both whole body insulin sensitivity (SI) and the responsiveness of the endothelium to insulin infusion. For this study, we chose to focus on the eNOS Glu298 subgroup only, thereby excluding subjects carrying the less common allele, and providing a more homogeneous and representative population for carrying out this intensive experimental investigation.

Methods

Study population

From a larger cohort genotyped for a common polymorphism in the eNOS gene (rs1799983, Glu298Asp) [15], fifteen males and fifteen females homozygous for Glu298 were matched for age (mean ± SD, 27.8 ± 11.9 y) and BMI (23.2 ± 3.0 kg/m^2). All subjects were healthy non-smokers who were not taking greater than 1 g eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) per day, or any medication known to influence blood clotting, lipids or blood pressure. The subjects were screened for fasting cholesterol (mean ± SD 4.62 ± 0.76 mmol/L), triglyceride (TG) (1.06 ± 0.28 mmol/L) and glucose (5.15 ± 0.64 mmol/L). Subjects were recruited between March 2009 and January 2010.

Study design

This was a single-blind crossover study; subjects attended the Hugh Sinclair Unit of Human Nutrition on two occasions separated by four weeks for females (to control for possible effects of the menstrual cycle on FMD) or at least one week for males. Subjects were randomly assigned to one of the fat loads on each day using an online number generator. Investigators responsible for performing and analysing the FMD and insulin clamp measures were blinded to the allocation and were not involved in the preparation or serving of the fat loads.

Protocol

The study protocol has been described elsewhere [14]. Briefly, on each study day participants arrived fasted and following a baseline FMD measurement, a cannula was inserted at the wrist for venous blood sampling. A bolus fat load (66 g) was consumed at 0 min, followed by smaller volumes (22 g) every 30 min for a further 390 min. At 60 min, a second cannula was inserted into the antecubital vein in the sampling arm for the infusion of heparin. A bolus of heparin (500 IU) was followed by a continuous infusion (0.4 IU/kg body weight/min) for the remainder of the study day. At 240 min, a 150 min hyperinsulinaemic-euglycaemic clamp was performed; both insulin and glucose were co-infused into the same cannula as the heparin. Measurements of FMD were also performed immediately prior to (240 min) and at the end (390 min) of the insulin clamp.

The procedures followed in the current study were in accordance with the ethical standards of the University of Reading Research and Ethics Committee. Written informed consent was obtained from all subjects.

Test drinks

Oral fat loads were prepared according to bodyweight (Table 1) using palm stearin (AarhusKarlshman Ltd, UK) with or without the addition of DHA-rich fish oil (Croda Healthcare, UK), 30 g skimmed milk powder (Premier International Foods Ltd, UK), 15 g chocolate powder (The Spanish Chocolate Co Ltd, UK) and 0.5 g monoglyceride emulsifier (Danisco, Denmark). Water was added to achieve a final weight of 352 g. The SFA and SFA + LC n-3 PUFA test drinks were identical in protein (11.2 g) and carbohydrate (27.1 g) content.

FMD

FMD of the brachial artery was measured by trained researchers using an ATL Ultrasound HDI5000 broadband ultrasound system (ATL Ultrasound, Bothell, Washington)

Table 1 Formulation of the test drinks.

	SFA	SFA + LC *n-3* PUFA
Palm stearin (g/kg bw)[a]	0.75	0.65
Fish oil concentrate (g/kg bw)[a]	–	0.1
Composition of oils (%)		
Palmitic acid; 16:0	59	51
Stearic acid; 18:0	5	4
Oleic acid; 18:1 n-9	28	24
Linoleic acid; 18:2 n-6	6	5
Arachidonic acid; 20:4 n-6	–	0.3
Eicosapentaenoic acid; 20:5 n-3	–	1.2
Docosapentaenoic acid; 22:5 n-3	–	0.4
Docosapentaenoic acid; 22:5 n-6	–	0.7
Docosahexaenoic acid; 22:6 n-3	–	10.4

[a] A 70 kg individual would receive 53 g palm stearin or 46 g palm stearin +7 g fish oil concentrate.