Fish oil omega-3 fatty acids partially prevent lipid induced insulin resistance in human skeletal muscle without limiting acylcarnitine accumulation

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Abstract
Acylcarnitine accumulation in skeletal muscle and plasma has been observed in numerous models of mitochondrial lipid overload and insulin resistance. Fish oil omega-3 polyunsaturated fatty acids (n3PUFA) are thought to protect against lipid-induced insulin resistance. The present study tested the hypothesis that addition of n3PUFA to an intravenous lipid emulsion would limit muscle acylcarnitine accumulation and reduce the inhibitory effect of lipid overload on insulin action. On three occasions, six healthy young men underwent a 6-hour euglycaemic hyperinsulinaemic clamp accompanied by intravenous infusion of saline (Control), 10% Intralipid (n6PUFA), or 10% Intralipid + 10% Omegaven (2:1; n3PUFA). The decline in insulin-stimulated whole-body glucose infusion rate, muscle pyruvate dehydrogenase complex activation (PDCa), and glycogen storage associated with n6PUFA compared to Control, was prevented with n3PUFA. Muscle acetyl-CoA accumulation was greater following n6PUFA compared to Control and n3PUFA, suggesting that mitochondrial lipid overload was responsible for the lower insulin action observed. Despite these favourable metabolic effects of n3PUFA, accumulation of total muscle acylcarnitine was not attenuated when compared with n6PUFA. These findings demonstrate that n3PUFA exert beneficial effects on insulin-stimulated skeletal muscle glucose storage and oxidation independently of total acylcarnitine accumulation, which does not always reflect mitochondrial lipid overload.

Abbreviations
CAT, carnitine acetyltransferase; CPT2, carnitine palmitoyltransferase 2; n3PUFA, fish oil omega-3 polyunsaturated fatty acids; PDC, pyruvate dehydrogenase complex.

INTRODUCTION

It is generally accepted that the accumulation of lipid-derived intermediates within skeletal muscle are a major cause of insulin resistance in sedentary individuals [1, 2]. However, although lipid-intermediates such as diacylglecerol, ceramides, and long-chain acyl-CoAs have the potential to impair insulin-stimulated glucose uptake, the mechanisms by which this may occur *in vivo*, if at all, and the characteristics (e.g. degree of saturation, chain length) of any aberrant species are still not known [1, 2]. Recent studies suggest that lipid-induced insulin resistance may arise secondary to mitochondrial overload, whereby excess entry of fatty acids into mitochondria results in an imbalance between β-oxidation and the demands of the tricarboxylic acid cycle [2, 3, 4, 5]. Under such circumstances free carnitine, acting predominantly via carnitine acetyltransferase (CAT) and the reverse carnitine palmitoyltransferase (CPT) 2 reactions, sequesters excess acyl-groups from β-oxidation in the form of acylcarnitine, which can then be efficiently exported across the otherwise impermeable mitochondrial membrane to the cytosol [6]. Although muscle and plasma acylcarnitine accumulation has been observed in numerous models of lipid overload and insulin resistance, it is uncertain to what extent this incomplete β-oxidation can impair glucose uptake and metabolism in human skeletal muscle [5].

The intravenous infusion of lipid emulsion combined with heparin in healthy individuals during euglycaemic hyperinsulinaemia results in impaired insulin-stimulated oxidative and non-oxidative glucose disposal, with inhibition of the pyruvate dehydrogenase complex (PDC) by increased fatty acid β-oxidation and/or lipid intermediate accumulation as a primary event [7]. Interestingly, slightly varying the lipid composition of the infused emulsion in individuals with type 2 diabetes mellitus by the addition of fish oil omega-3 long-chain polyunsaturated fatty acids (n3PUFA) has been demonstrated to partially reduce the lipid infusion-induced increase in fatty acid oxidation [8]. Furthermore, n3PUFA are thought to protect against lipid-induced skeletal muscle insulin resistance in rodents [9], and preincubation of primary myotubes from obese individuals with or without type 2 diabetes mellitus with n3PUFA has been demonstrated to increase glucose oxidation compared to oleic acid [4]. Studies in human myotubes have also suggested that n3PUFA improves insulin sensitivity by diverting fatty acids away from mitochondrial β-oxidation towards cellular lipid incorporation [10]. Indeed, daily n3PUFA supplementation for 1-2 weeks has been demonstrated to reduce fat oxidation in individuals with type 2 diabetes mellitus [11] and improve insulin-stimulated glucose uptake [12]. However, findings from n3PUFA supplementation studies lasting 3-9 weeks are ambiguous with most [e.g. 11, 13, 14] demonstrating impaired glucose uptake in individuals with type 2 diabetes mellitus, perhaps suggesting a time dependent effect [11]. Thus, the aim of the present study was to provide further insight to the relationship between incomplete β-oxidation (skeletal muscle acylcarnitine accumulation) and insulin resistance *in vivo*, by testing the hypothesis that addition of n3PUFA to an intravenous lipid emulsion during 6-hours of hyperinsulinaemia would limit the inhibitory effect of excessive β-oxidation on PDC activation and, therefore, improve insulin-stimulated glucose uptake compared to lipid emulsion alone.

MATERIALS AND METHODS

Subjects

Six healthy, male volunteers (age 25.7 ± 2.3 years, body mass 84.0 ± 7.8 kg, BMI 26.8 ± 2.4 kg/m^2) gave their written informed consent to participate in the present study, which was approved by the University of Nottingham Medical School Ethics Committee in accordance with the Declaration of Helsinki.

Protocol

Volunteers reported to the laboratory following an overnight fast at 0800 on three randomized occasions at least one week apart (range 1 to 3 weeks). On arrival, subjects were asked to rest in a supine position on a bed while cannulae were inserted into a vein in the hand for arterialized-venous blood sampling, the forearm for the infusion of insulin (Actrapid; Novo Nordisk, Denmark) and 20% dextrose, and the contralateral forearm for the infusion of a lipid emulsion (Intralipid or Omegaven; Fresenuis Kabi, Germany) or 0.9% saline as described previously [7]. On each visit a 6-hour euglycaemic hyperinsulinaemic (50 $mU \cdot m^{-2} \cdot min^{-1}$) clamp was performed in combination with the infusion of saline (Control), 10% Intralipid (n6PUFA), or 10% Intralipid + 10% Omegaven (2:1 ratio; n3PUFA) at a rate of 100 ml/h. This insulin infusion rate was chosen as it has been previously demonstrated to completely supress endogenous (hepatic) glucose production under insulin resistant conditions known to affect acylcarnitine metabolism [15]. Thus, the variable glucose infusion rate required to maintain euglycaemia (4.52 ± 0.02 mmol/l) was equivalent to peripheral glucose disposal and, therefore, peripheral insulin sensitivity. The total Intralipid infusion provided 60 g of omega-6 soybean oil (2515 kJ), whereas the Omegaven + Intralipid infusion provided 20 g of highly refined omega-3 fish oil and 40 g soybean oil (2610 kJ). This equated into approximately 1 g of palmitic acid, 1 g of oleic acid, 10 g of linoleic acid, and 1 g linolenic acid in n6PUFA being replaced with 1 g of palmitoleic acid, 1 g of arachidonic acid, 5 g of eicosapentaenoic acid (EPA), 1 g of docosapentaenoic acid (DPA), and 5 g of docosahexanoic acid (DHA). During each lipid infusion heparin sodium was infused at rate of 600 U/h to elevate plasma non-esterified fatty acid (NEFA) availability.

Sample collection and analysis

Arterialized-venous blood was obtained every hour for the analysis of plasma NEFA (NEFA C kit, WAKO Chemicals, Germany) after the addition of tetrahydrolipostatin (30 μg/ml plasma) on an automated analyzer (ABX Pentra 400, Horiba Medical Ltd., France). Plasma insulin was measured by ELISA (DRG diagnostics, Germany). Muscle samples were also obtained from the *vastus lateralis* before and after each clamp using the Bergström needle biopsy technique, and immediately frozen in liquid nitrogen cooled isopentane. One portion of the frozen muscle sample (~30 mg) was freeze-dried, separated free of visible blood, fat, and connective tissue, and powdered. Acylcarnitines were then extracted using a modified version of the method described by Sun *et al* [16]. Briefly, powdered samples were vigorously vortexed for 2 min in 500 μl of 1M KH_2PO_4 buffer (pH 4.9)/isopropanol (1:1), and then for a further 5 min following the addition of 500 μl of acetonitrile. After centrifugation at 14,000 *g* for 20 min at 4°C the supernatant was removed, dried under gentle N_2 flow, and resuspended in 100 μl of isopropanol/1 mM acetic acid (4:1) for subsequent liquid chromatography-mass spectrometry (LC-MS) analysis [17]. Acylcarnitines were screened (electrospray positive mode) for the common carnitine moiety m/z 85 (4000 QTRAP, ABSciex, USA) and sensitively quantified in multiple reaction monitoring mode against a dilution series of known acylcarnitine standards of varying chain lengths (C2-C20). Muscle glycogen, glucose-6-phoshate, and acetyl-CoA content were also determined in a portion of freeze-dried muscle powder, whereas 5-10 mg portion of frozen muscle was used to determine PDC activation status (PDCa) all as previously described [7]. Intramyocellular lipid

(IMCL) content was determined in 10 μm sections cut from frozen muscle and fixed in 4% paraformaldehyde phosphate buffered saline (pH 7.4). Samples were incubated at room temperature for 1 hour in 3 μM LD540/dimethyl sulfoxide (DMSO) and, following 3 washes in PBS (pH 7.4), embedded in an antifade reagent (ProLong Gold, Life Technologies, Paisley, UK) for subsequent visualisation of LD540 stained lipid droplets using a TCS SP2 confocal microscope (Leica Microsystems, Heidelberg, Germany). Briefly, 1 μm z-stacks using a 561nm laser were captured at x40 magnification in order to control for sample depth and background noise, and the area of the fibre covered by fluorescence was calculated using Volocity software (Volocity 6.3, PerkinElmer, Cambridge, UK). LD540 is a lipophilic dye similar to Bodipy, and was manufactured by the University of Nottingham School of Chemistry according to the method of Spandl et al [18].

In addition, total RNA was extracted from approximately 20 mg of wet muscle tissue by the method of Chomczynski and Sacchi [19] using Trizol reagent (Life Technologies, Paisley, UK). Following spectrophotometric quantification, first-strand cDNA was generated from 2 μg of RNA using the SuperScript III cDNA kit (Life Technologies, Paisley, UK) and stored at -80°C. Thereafter, the relative mRNA abundance of 24 genes from pathways involved in fatty acid metabolism and insulin signalling/carbohydrate metabolism was determined using custom designed low density RT-PCR array microfludic cards (Applied Biosystems Inc., Foster City, CA, USA) in combination with the ABI PRISM 7900T sequence detection system and SDS 2.1 software (Applied Biosystems Inc., Foster City, CA, USA). The candidate genes were selected from PubMed literature searches and data obtained from our laboratory. A complete list of details for each gene assay is available in Supplemental Table 1. The threshold cycle C_T was automatically given by the SDS software RQ manager, and relative mRNA abundance was calculated using the $\Delta\Delta C_T$ method with one of the subjects' baseline sample from their first visit as the calibrator and cyclophillin (*PPIA*) as the endogenous control. C_T values for *PPIA* did not change across time points (data not shown).

Statistics

All blood and muscle data, along with acylcarnitine species grouped according to their chain length into acetyl- (C2), short- (C3-C5), medium- (C6-C10), and long-chain (C12-C20), were analysed using a two-way ANOVA (GraphPad Prism 6, GraphPad Software Inc, USA). When a significant main effect was detected data were further analysed with a Student's *t* test using the Sidak-Bonferroni correction. Pearson's correlation coefficients with a Holm-Bonferroni stepwise correction were used to analyse associations between post-clamp acylcarnitine content and glucose disposal, glycogen accumulation, and PDCa. Statistical significance was declared at $P<0.05$, and all the values presented are means ± standard error.

RESULTS

Insulin resistance

Similar steady-state (120-360 min) serum insulin concentrations of 242 ± 12, 256 ± 41 and 274 ± 36 pmol/l, were obtained during the hyperinsulinaemic clamps in Control, n6PUFA, and n3PUFA, respectively. This resulted in a complete suppression of steady-state plasma NEFA concentration during Control (0.01 ± 0.002 mmol/l), but not during n6PUFA and n3PUFA, where lipid infusion maintained NEFA at similar steady-state (120-360 min) concentration of 0.42 ± 0.06 mmol/l. Despite the greater circulating NEFA concentration, IMCL content did not change following Control, n6PUFA, or n3PUFA (Table 1). However, whereas steady-state (240-360) whole-body glucose infusion rate was 28% lower in n6PUFA compared to Control (41.8 ± 2.5 vs. 57.3 ± 3.0 $\mu mol \cdot m^{-2} \cdot min^{-1}$; P<0.01), it was no different in n3PUFA such that it was 24% greater than n6PUFA (51.4 ± 2.4 $\mu mol \cdot m^{-2} \cdot min^{-1}$; P<0.05; Figure 1A). Furthermore, the greater insulin-stimulated glucose infusion rate in Control was associated with a 108 ± 25% and 18 ± 6% increase in PDCa (P<0.01) and muscle glycogen content (P<0.05), respectively (Figure 1B and C). These effects were impaired following n6PUFA, where there was no increase in muscle glycogen or PDCa, but not following n3PUFA, where muscle glycogen increased by 20 ± 8% (P<0.05) and PDCa increased by 49 ± 10% (P<0.05) such that it was 70 ± 24% greater than n6PUFA (P<0.05). However, there were no differences in muscle G6P content between n6PUFA and n3PUFA, which increased around 2-fold (P<0.05) following both (Figure 1D).

Acylcarnitine metabolism

Insulin infusion suppressed total muscle acylcarnitine (sum of C3 to C20) content in Control (53.2 ± 11.2 to 21.3 ± 4.7 µmol/kg dm; P<0.001), but not in n6 or n3PUFA (58.2 ± 11.5 to 52.0 ± 14.3 and 44.5 ± 6.7 to 49.6 ± 6.8 µmol/kg dm, respectively; Figure 2 inset). The suppression of acylcarnitine in Control was predominantly attributable to around a 75% decrease in medium- (C6-C10; P<0.05) and long-chain (C12-C20; P<0.05) acylcarnitines (Table 1). Interestingly, this suppressive effect of insulin on long-chain acylcarnitine was also observed in n6PUFA (P<0.05; Table 1), but not in n3PUFA such that it was 3.1-fold greater than Control (P<0.05; Table 1). Similarly, medium- and short-chain acylcarnitine was 4.9- and 1.9-fold greater, respectively, than Control in n3PUFA (P<0.01). However, only post-clamp short-chain acylcarnitine was negatively correlated with steady-state glucose disposal across all three trials (r^2=0.38, P<0.01). Of the short-chain acylcarnitines, isovaleryl (r^2=0.37, P<0.05), hydroxy/hydroxyisobutyryl (r^2=0.31, P<0.05), and propionylcarnitine (r^2=0.31, P<0.05) were negatively correlated with glucose disposal, but only isovalerylcarnitine was negatively correlated with muscle glycogen accumulation (r^2=0.42, p<0.01). Short-chain acylcarnitine did not correlate with PDCa (r^2=0.16). Despite muscle acetyl-CoA accumulation being 2.5-fold greater (P<0.05) following n6PUFA compared to Control and n3PUFA trials (Table I), there was no significant changes in muscle acetylcarnitine (C2) content following Control, n6PUFA, or n3PUFA (Table 1).

Gene expression

Insulin infusion per se reduced the expression of genes encoding insulin receptor substrate 1 (*IRS1*) and 2 (*IRS2*), and increased the expression of phosphatidylinositol 3-kinase regulatory subunit alpha gene (*PIK3R1*), irrespective of lipid infusion (all time effects, P<0.01; Supplemental Table 1). On the other hand, lipid infusion per se reduced the expression of genes encoding uncoupling protein 3 (*UCP3*) and GLUT4 (*SLC2A4*), and prevented the insulin stimulated increase in sterol regulatory element binding protein gene expression (*SREBF1*) observed in Control (all treatment or interaction effects, P<0.05; Supplemental Table I). Interestingly, the expression of several genes responded differently to the infusion of n6PUFA and n3PUFA. The gene encoding nicotinamide phosphoribosyl

transferase (*NAMPT*) did not change following Control or n6PUFA, but increased in n3PUFA above baseline by 35% such that it was around 50% greater than Control and n6PUFA (Figure 3A). Furthermore, genes encoding peroxisome proliferator activated receptor alpha (*PPARA*; Figure 3B; $P<0.05$) and its targets lipoprotein lipase (*LPL*; Figure 3D; $P<0.01$) and medium chain acyl-CoA dehydrogenase (*ACADM*; Figure 3E; $P<0.05$) were reduced following n3PUFA but not n6PUFA, whereas peroxisome proliferator activated receptor delta (*PPARD*; Figure 3C; $P<0.05$) was reduced in n6PUFA but not n3PUFA, and fatty acid synthase (*FASN* Figure 3E; $P<0.05$) was increased in n6PUFA only.

DISCUSSION

In agreement with our hypothesis, replacement of one third of an n6PUFA lipid infusion with an equimolar fish oil based n3PUFA emulsion prevented much of the decline in insulin-stimulated whole-body glucose disposal and muscle PDC activation, and the inhibition of muscle glycogen accumulation associated with n6PUFA. These findings provide the first evidence in humans that acute administration of n3PUFA can have beneficial effects on skeletal muscle insulin sensitivity in the face of lipid excess, albeit at a 3-fold greater dose (11 vs. 3 g of EPA + DPA + DHA) than previously described in patients with type 2 diabetes mellitus [8]. Given the finding that some [12], but not all [11, 13, 14], previous studies have demonstrated improved insulin stimulated glucose uptake with fish-oil supplementation in insulin resistant individuals, and that beneficial effects on insulin sensitivity are generally seen in glucose tolerant individuals over a short supplementation period (<2 weeks [12]), or low dose (<3g/day [20]), further research is warranted to ascertain the optimal dose and duration of fish-oil supplementation if it is to be used as a nutritional tool to improve skeletal muscle insulin sensitivity and glycaemic control in individuals with type 2 diabetes mellitus. For example, it is unlikely that the beneficial metabolic effects of acute fish-oil administration are lost during more prolonged supplementation due to progressive n3PUFA incorporation into the skeletal muscle plasma membrane, as there is a relationship between insulin sensitivity and the n3PUFA composition of skeletal muscle phospholipids [21, 22]. However, as acute n3PUFA administration has also been shown to divert fatty acids away from mitochondrial β-oxidation towards IMCL storage [10], one may predict that prolonged fish-oil supplementation may begin to have a detrimental effect on insulin sensitivity by increasing IMCL content [1, 2].

This study also provides a novel scenario of a similar amount of circulating NEFA producing a markedly different whole-body glucose disposal, allowing greater insight to the role of excessive lipid and β-oxidation in insulin resistance in humans *in vivo*. Thus, in line with several observational studies in insulin resistant conditions [2, 3, 4, 5, 23], the greater muscle acetyl-CoA (allosteric inhibitor of the PDC) accumulation following n6PUFA suggests that excessive intramuscular β-oxidation was responsible for the lower insulin action on oxidative glucose disposal (inhibited muscle PDCa) observed compared to n3PUFA. However, the findings that muscle total and medium-chain acylcarnitine content was similar, and that long-chain acylcarnitine was actually suppressed, following n6PUFA infusion compared to n3PUFA would suggest that longer-chain length acylcarnitines (C6-C20) do not accurately reflect excessive β-oxidation and insulin resistance. This is in agreement with the finding of Soeters et al. [15] that muscle long-chain acylcarnitines did not reflect fasting induced insulin resistance in humans. On the other hand, muscle short-chain acylcarnitines tended to be greater after n6PUFA infusion and negatively correlated with whole-body glucose disposal, with C5 (isovaleryl), C4OH (hydroxybutyryl/ hydroxyisobutyryl), and C3 (propionyl) carnitine having the strongest relationship. However, it is important to note that these acylcarnitines are derived from branched-chain amino acid (BCAA) catabolism and not fatty acid β-oxidation, proving further evidence against incomplete β-oxidation (i.e. acylcarnitine accumulation per se as opposed to excessive acetyl-CoA accumulation) causing or reflecting insulin resistance. This is consistent with other studies demonstrating a strong association of hydroxybutyrylcarnitine [24, 25] and other short chain acylcarnitines of BCAA catabolism [26, 27] with insulin resistant states and the development of type 2 diabetes [28]. Indeed, the ability of insulin to suppress BCAA catabolism is impaired in insulin resistance [29], and fatty acids have been shown to activate branch-chain keto-acid dehydrogenase (the rate limiting step in BCAA oxidation; 30]). It is also important to note that, unlike short-chain acyl-CoA products of mitochondrial BCAA catabolism, short-chain acylcarnitines do not inhibit PDC activity *in vitro* [31]. In support of the latter, short-chain acylcarnitine products of mitochondrial BCAA catabolism did not negatively correlate with PDCa in the present study, suggesting that acetyl-CoA per se is more important for inducing insulin resistance at the level of glucose oxidation. Of course, we also observed a beneficial

effect of n3PUFA infusion on non-oxidative glucose disposal in that there was a complete prevention of the inhibition of net glycogen synthesis observed in n6PUFA. Thus, whether short-chain acylcarnitines that are exported from mitochondria directly inhibited glucose uptake or storage and caused insulin resistance in the present study clearly requires further investigation, particularly as isovalerylcarnitine had a negative correlation with glycogen accumulation and acylcarnitines have previously been demonstrated to stimulate key cytosolic pathways implicated in lipid-induced skeletal muscle insulin resistance [32].

The differences in skeletal muscle fatty acid metabolism between n6PUFA and n3PUFA in the present study are in agreement with previous cell and animal studies [4, 9, 10], and are supported by the finding that n3PUFA supressed the expression of genes encoding peroxisome proliferator activated receptor alpha (PPARa), which is a key transcription factor controlling the expression of genes involved in fatty acid oxidation, and medium chain acyl-CoA dehydrogenase (MCAD), which is a rate limiting step in β-oxidation downstream of medium- and long-chain acylcarnitine accumulation. Indeed, the PPAR transcription factors are known to have different affinities for saturated, mono-unsaturated and poly-unsaturated fatty acids [33], and this differential expression of genes between n6PUFA and n3PUFA infusion was also observed for other genes involved in fatty acid metabolism namely *PPARD*, *NAMPT*, *LPL*, and *FASN*. Also, although not significantly different, IMCL content increased by around 50% (range -13.4 to 264.4%) in n3PUFA in the present study, which would fit with the suggestion of fatty acids being diverted from oxidation toward cellular lipid incorporation [10], particularly as nicotinamide phosphoribosyl transferase (NAMPT) has been shown to be responsive to changes in cellular triglyceride and phospholipid metabolism [34], the gene expression of which increased in every volunteer during the n3PUFA visit. However, genes involved in insulin signalling and glucose metabolism that changed in the present study such as *IRS1*, *IRS2*, *PIK3R1, SREBF1, and SLC2A4* were not differentially expressed between n6PUFA and n3PUFA, which is in line with cell studies [10], and possibly because the proteins encoded by these genes are regulated by phosphorylation or translocation.

In conclusion, replacement of predominantly n6PUFA linoleic acid with n3PUFA fish-oil fatty acids in an intravenous lipid emulsion infusion during 6-hours of hyperinsulinaemia improves insulin-stimulated oxidative and non-oxidative glucose disposal compared to lipid emulsion alone. This was likely due to a reduction in the inhibitory effect of excessive β-oxidation on PDC activation, and possibly the inhibitory effect of lipid on glucose transport and storage by an unidentified mechanism. The model used in the present study provides further human *in vivo* evidence of excess entry of fatty acids into the mitochondria per se causing insulin resistance, but is in contrast to that speculated in the literature that skeletal muscle total acylcarnitine accumulation from incomplete β-oxidation may be a causative factor. Nevertheless, the effect of short-chain acylcarnitines from aberrant amino acid metabolism on insulin action in skeletal muscle requires further investigation.

CLINICAL PERSPECTIVES

Recent clinical studies suggest that skeletal muscle insulin resistance may arise secondary to mitochondrial lipid overload, as reflected by plasma acylcarnitine accumulation. The present study provided further insight by demonstrating that addition of fish oil to an intravenous lipid infusion in healthy male participants partially prevented lipid-induced mitochondrial overload and insulin resistance. However, these effects were not reflected by skeletal muscle total acylcarnitine content. The findings confirm that acute fish-oil fatty acid administration can have positive effects in insulin resistant conditions. They also suggested that whilst total acylcarnitine accumulation might not reflect mitochondrial lipid overload, a short-chain acylcarnitine might cause skeletal muscle insulin resistance. This latter target requires further investigation.

AUTHOR CONTRIBUTION

F.B.S, P.M., and K.T. designed research. F.B.S. drafted the manuscript and had primary responsibility for final content. All of the authors contributed to acquisition, analysis and interpretation of data, revised the manuscript for important intellectual content, and approved the final version. The authors have nothing to disclose.

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Table 1. Skeletal muscle metabolite content before and after 6 h of euglycaemic hyperinsulinaemia (~260 pmol/l) accompanied by saline (Control), Intralipid (n6PUFA) or Intralipid:Omegaven (2:1 ratio; n3PUFA) infusions.

	Control		n6PUFA		n3PUFA	
	Pre	Post	Pre	Post	Pre	Post
Intramyocellular lipid (IMCL)	0.066 ± 0.011	0.079 ± 0.012	0.081 ± 0.008	0.082 ± 0.009	0.063 ± 0.012	0.093 ± 0.023
Acetyl-CoA	3.9 ± 0.9	3.9 ± 0.5	3.8 ± 0.9	10.1 ± 3.3*	4.2 ± 0.9	3.3 ± 0.6
Acetylcarnitine	2.6 ± 0.6	2.0 ± 0.7	2.5 ± 0.8	3.7 ± 1.2	2.2 ± 1.0	3.2 ± 1.4
Short-chain acylcarnitine (C3-5)	26.5 ± 6.1	15.7 ± 4.2	25.4 ± 4.7	40.3 ± 15.5	27.5 ± 4.4	29.3 ± 3.0**
Medium-chain (C6-10)	5.8 ± 1.4	1.7 ± 0.5†	8.1 ± 2.3	4.8 ± 0.8	5.6 ± 1.8	8.3 ± 2.0**
Long-chain (C12-20)	20.9 ± 8.8	3.9 ± 0.8†	24.6 ± 9.6	6.9 ± 2.2†	11.4 ± 2.8	12.0 ± 2.4*

Values represent means ± SEM expressed as % of fibre area covered for IMCL, mmol·(kg dry muscle)$^{-1}$ for acetylcarnitine, and μmol·(kg dry muscle)$^{-1}$ for acetyl-CoA and acylcarnitine (n = 6). †† P<0.01, † P<0.05, different from Pre infusion value. * P<0.05, different to corresponding Control value. ‡ P<0.05, different to corresponding n6PUFA value.

FIGURE LEGENDS

Figure 1. Whole-body glucose disposal (A) during 6 h of euglycaemic hyperinsulinaemia (~260 pmol/l) accompanied by saline (Control; white squares), Intralipid (n6PUFA; white circles) or Intralipid:Omegaven (2:1 ratio; n3PUFA; black circles) infusions, and skeletal muscle pyruvate dehydrogenase complax activation status (PDCa; B), glycogen content (C), and glucose-6-phophate content (D) pre (white bars) and post (black bars) each infusion. Values represent means ± SEM. ** $P<0.01$, n6PUFA steady-state (240-360 min) glucose disposal (A) and PDC activation status (B) less than corresponding Control. ‡ $P<0.05$, n3PUFA steady-state (240-360 min) glucose disposal (A) and PDCa (B) greater than corresponding n3PUFA. †† $P<0.01$, † $P<0.05$, post different from pre infusion value.

Figure 2. Skeletal muscle acylcarnitine species, and total acylcarnitine content (insert), before (Pre; horizontal hatched bars) and after 6 h of euglycaemic hyperinsulinaemia (~260 pmol/l) accompanied by saline (Control; white bars), Intralipid (n6PUFA; black bars) or Intralipid:Omegaven (2:1 ratio; n3PUFA; cross hatched bars) infusions. The Pre infusion value has been presented as the mean of all three experimental visits for clarity. Values represent means ± SEM. † $P<0.05$, post different from pre infusion value.

Figure 3. Skeletal muscle expression of genes pre (white bars) and post (black bars) 6 h of euglycaemic hyperinsulinaemia (~260 pmol/l) accompanied by saline (Control; white bars), Intralipid (n6PUFA; black bars) or Intralipid:Omegaven (2:1 ratio; n3PUFA; cross hatched bars) infusions. Values represent means ± SEM. Only gene that were differentially expressed between n6PUFA and n3PUFA have been illustrated. * $P<0.05$, different to corresponding Control value. ‡ $P<0.05$, different to corresponding n6PUFA. †† $P<0.01$, † $P<0.05$, post different from pre infusion value.

Figure 1

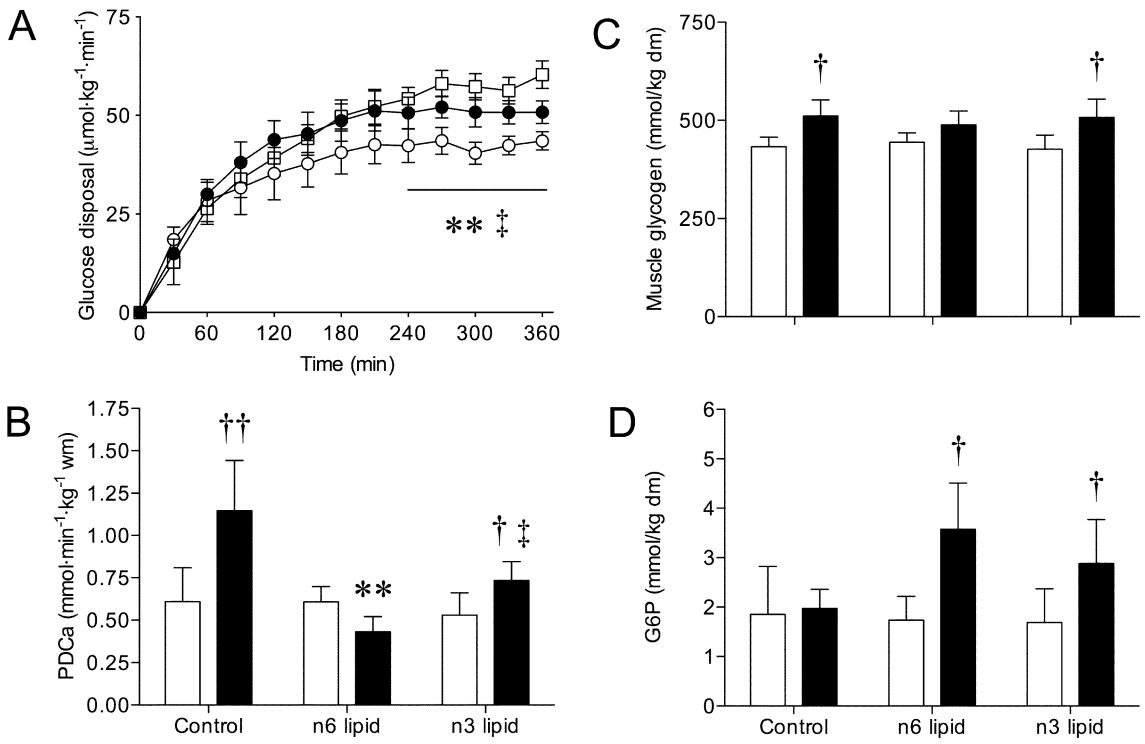

A
Glucose disposal (µmol·kg-1·min-1)
75
50
25
0
0
60
120
180
240
300
360
Time (min)
** ‡
C
Muscle glycogen (mmol/kg dm)
750
500
250
0
†
†
B
PDCa (mmol·min-1·kg-1 wm)
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
†† **
† ‡
Control
n6 lipid
n3 lipid
D
G6P (mmol/kg dm)
6
5
4
3
2
1
0
†
†
Control
n6 lipid
n3 lipid

Figure 2

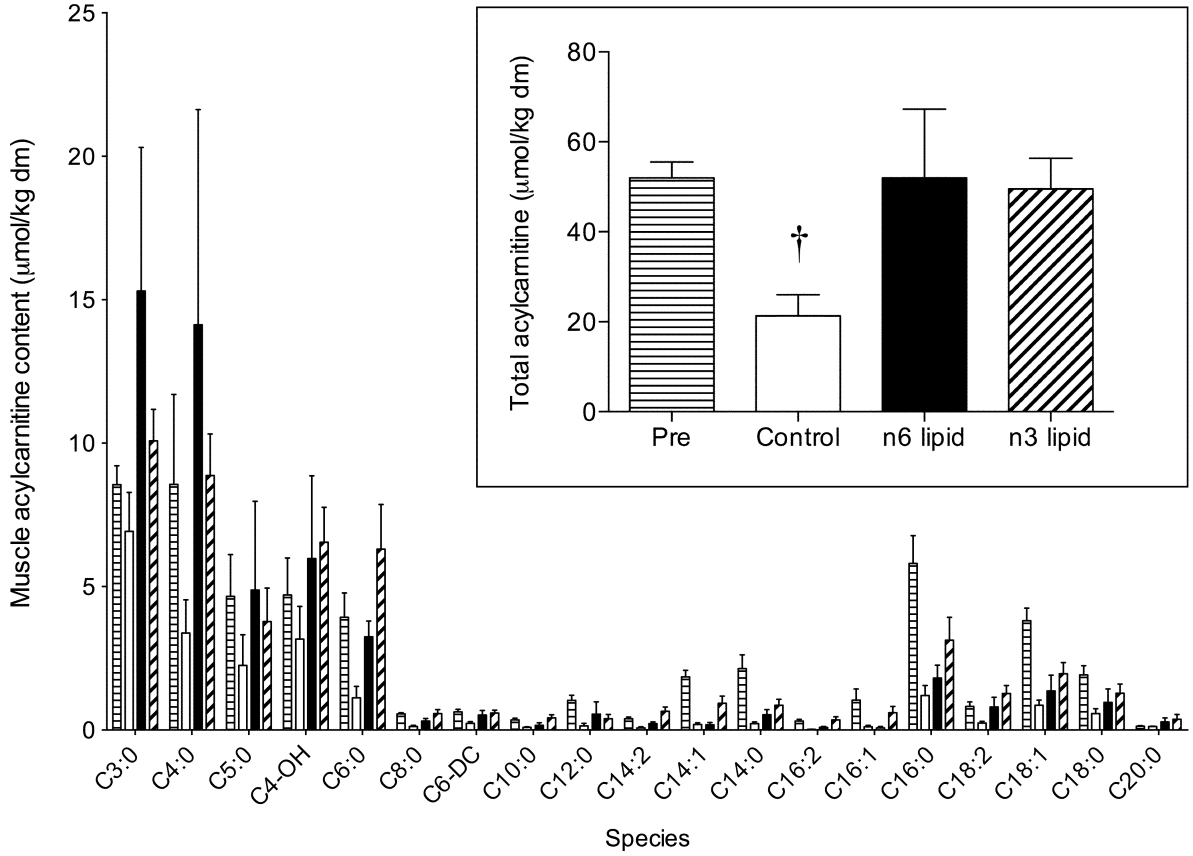
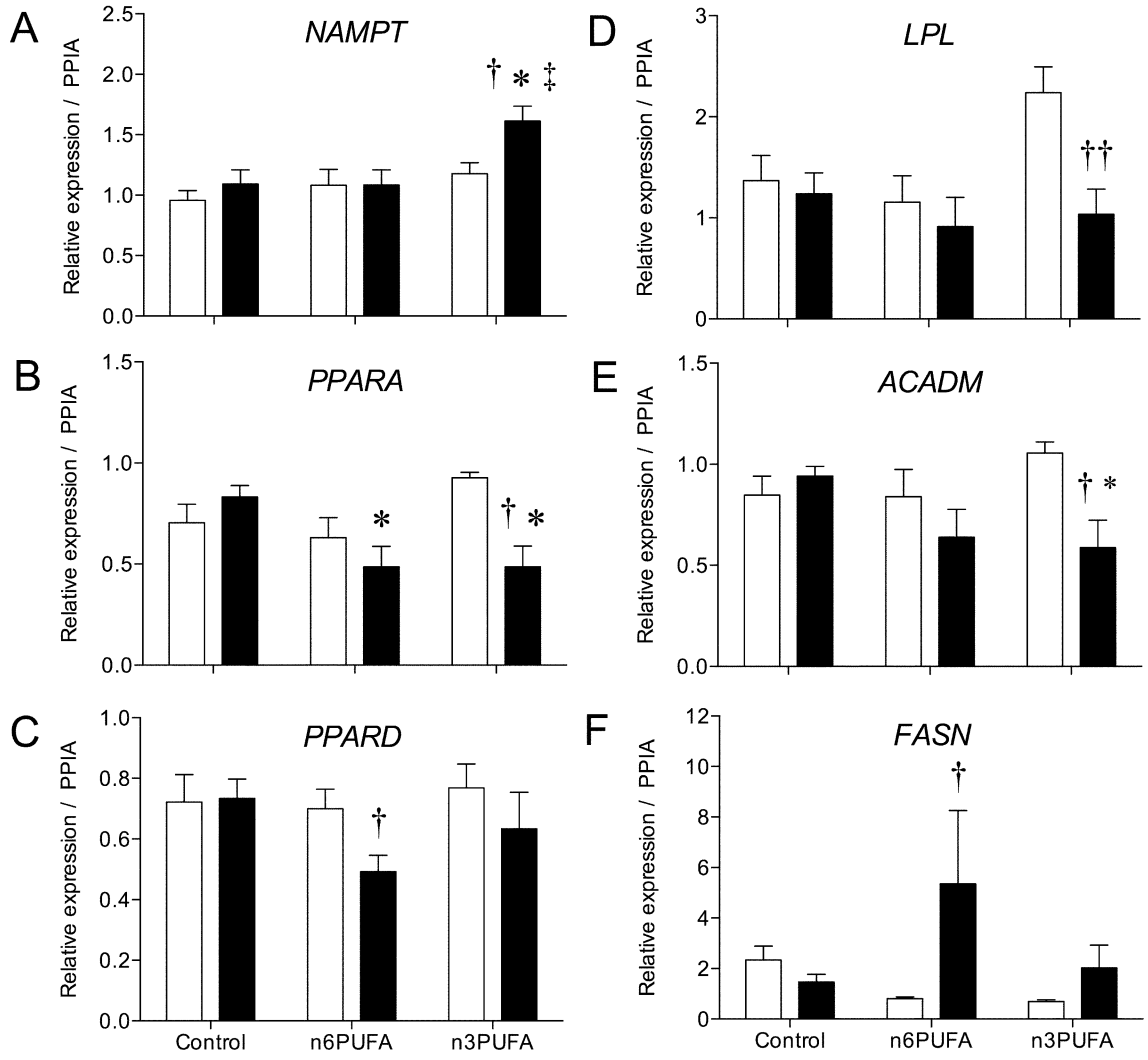

Muscle acylcarnitine content (µmol/kg dm)
25
20
15
10
5
0
C3:0
C4:0
C5:0
C4-OH
C6:0
C8:0
C6-DC
C10:0
C12:0
C14:2
C14:1
C14:0
C16:2
C16:1
C16:0
C18:2
C18:1
C18:0
C20:0
Species
Total acylcarnitine (µmol/kg dm)
80
60
40
20
0
Pre
Control
n6 lipid
n3 lipid

Figure 3


A
NAMPT
Relative expression / PPIA
2.5
2.0
1.5
1.0
0.5
0.0
† * ‡
D
LPL
3
2
1
0
††
B
PPARA
1.5
1.0
0.5
0.0
*
† *
E
ACADM
1.5
1.0
0.5
0.0
† *
C
PPARD
1.0
0.8
0.6
0.4
0.2
0.0
†
F
FASN
12
10
8
6
4
2
0
†
Control
n6PUFA
n3PUFA

Supplemental Table 1. Skeletal muscle gene expression before and after 6 h of euglycaemic hyperinsulinaemia (~260 pmol/l) accompanied by saline (Control), Intralipid (n6PUFA) or Intralipid:Omegaven (2:1 ratio; n3PUFA) infusions.

Pathway	Gene	Control		n6lipid		n3lipid	
		Pre	Post	Pre	Post	Pre	Post
Fat metabolism	ACADM-Hs00936580_m1	0.85 ±0.09	0.94 ±0.05	0.84 ±0.13	0.64 ±0.14	1.06 ±0.06	0.59 ±0.14†*
	ANGPTL4-Hs01101127_m1	9.58 ±6.08	3.60 ±1.65	10.36 ±6.33	23.43 ±9.70	12.30 ±5.90	24.74 ±8.19
	FASN-Hs00188012_m1	2.34 ±0.54	1.47 ±0.30	0.81 ±0.07	5.36 ±2.90†	0.69 ±0.07	2.03 ±0.90
	IL6-Hs00985639_m1	1.32 ±0.50	2.06 ±1.04	0.34 ±0.06	15.32 ±6.28	0.93 ±0.26	13.32 ±8.78
	LIPE-Hs00193510_m1	1.34 ±0.36	1.39 ±0.55	0.75 ±0.24	0.67 ±0.23	0.97 ±0.28	0.58 ±0.23
	LPL-Hs01012571_m1	1.37 ±0.25	1.24 ±0.21	1.15 ±0.26	0.92 ±0.29	2.24 ±0.26	1.04 ±0.25
	NAMPT-Hs00237184_m1	0.96 ±0.08	1.09 ±0.12	1.08 ±0.13	1.08 ±0.13	1.18 ±0.09	1.61 ±0.12†*‡
	PNPLA2-Hs00386101_m1	0.98 ±0.07	1.33 ±0.14	1.00 ±0.20	0.67 ±0.11	1.22 ±0.18	0.91 ±0.30
	PPARA-Hs00231882_m1	0.70 ±0.09	0.83 ±0.06	0.63 ±0.10	0.49 ±0.10*	0.93 ±0.03	0.48 ±0.10†*
	PPARD-Hs00602622_m1	0.72 ±0.09	0.73 ±0.07	0.70 ±0.06	0.49 ±0.05†	0.77 ±0.08	0.63 ±0.12
	PPARG-Hs01115513_m1	1.41 ±0.31	1.01 ±0.08	0.82 ±0.14	1.15 ±0.31	1.12 ±0.09	0.91 ±0.21
	PPARGC1A-Hs00173304_m1	0.78 ±0.11	1.15 ±0.14	0.71 ±0.13	0.69 ±0.11	0.89 ±0.06	0.69 ±0.23
	UCP3-Hs01106052_m1	1.35 ±0.13	1.27 ±0.26	1.23 ±0.28	0.67 ±0.07†*	1.86 ±0.25	0.66 ±0.20††*
Insulin signalling and carbohydrate metabolism	AKT1-Hs00178289_m1	0.77 ±0.11	0.80 ±0.08	0.70 ±0.11	0.57 ±0.07	0.90 ±0.04	0.59 ±0.15
	AKT2-Hs00609846_m1	0.73 ±0.08	0.96 ±0.12	0.69 ±0.11	0.57 ±0.08	0.77 ±0.09	0.49 ±0.15
	G6PD-Hs00166169_m1	1.21 ±0.11	1.19 ±0.10	1.15 ±0.13	1.52 ±0.30	1.16 ±0.12	1.21 ±0.16
	IRS1-Hs00178563_m1	0.49 ±0.18	0.40 ±0.04	0.62 ±0.11	0.31 ±0.08†	0.76 ±0.12	0.22 ±0.06††
	IRS2-Hs00275843_s1	0.98 ±0.14	0.47 ±0.09	0.97 ±0.11	0.56 ±0.15	0.87 ±0.10	0.39 ±0.10
	PIK3R1-Hs00381459_m1	0.63 ±0.11	2.25 ±0.59†	0.68 ±0.12	1.99 ±0.36	0.63 ±0.05	1.76 ±0.53
	PDK4-Hs01037712_m1	3.59 ±1.27	0.93 ±0.65	2.86 ±0.70	0.88 ±0.57	4.40 ±1.22	2.07 ±1.08
	SLC2A4-Hs00168966_m1	0.77 ±0.08	0.97 ±0.08	0.74 ±0.13	0.46 ±0.07†*	1.01 ±0.03	0.47 ±0.13††*
	SREBF1-Hs01088691_m1	0.77 ±0.12	1.29 ±0.25†	0.68 ±0.10	0.58 ±0.11**	0.73 ±0.08	0.59 ±0.17**

Values represent means ± SEM (n = 5). ††† $P<0.001$, †† $P<0.01$, † $P<0.05$, different from Pre infusion value. ** $P<0.01$, * $P<0.05$, different to corresponding Control value. ‡ $P<0.05$, different to corresponding n6PUFA value

Summary Statement

Intravenous infusion of lipid into healthy males caused insulin resistance. Addition of fish oil omega-3 fatty acids to the lipid infusion partially prevented the insulin resistance. This effect was not due to differences in muscle acylcarnitine content.