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Effect of elevated lipid concentrations on human skeletal muscle gene expression

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Abstract

Dietary fatty acids regulate the abundance and activity of various proteins involved in the regulation of fat oxidation by functioning as regulators of gene transcription. To determine whether the transcription of key lipid metabolic proteins necessary for fat metabolism within human skeletal muscle are regulated by acute elevations in circulating free fatty acid (FFA) concentrations, 7 healthy men underwent 3 randomized resting infusions of Intralipid (20%) with heparin sodium, saline and heparin sodium, or saline only for 5 hours. These infusions significantly elevated plasma FFA concentrations by 15-fold (to 1.67 ± 0.13 mmol/L) in the Intralipid infusion trial, with modest elevations observed in the saline and heparin sodium and saline alone infusion groups (0.67 ± 0.09 and 0.49 ± 0.087 mmol/L, $P < .01$ both vs Intralipid infusion). Analysis of messenger RNA (mRNA) concentration demonstrated that pyruvate dehydrogenase kinase isoform 4 (PDK4) mRNA, a key negative regulator of glucose oxidation, was increased in all trials with a 24-fold response after Intralipid infusion, 15-fold after saline and heparin infusion, and 9-fold after saline alone. The PDK4 increases were not significantly different between the 3 trials. The mRNA concentration of the major uncoupling protein within skeletal muscle, uncoupling protein 3, was not elevated in parallel to the increased plasma FFA as similar (~2-fold) increases were evident in all trials. Additional genes involved in lipid transport (fatty acid translocase/CD36), oxidation (carnitine palmitoyltransferase I), and metabolism (1-acylglycerol-3-phosphate *O*-acyltransferase 1, hormone-sensitive lipase, and peroxisomal proliferator-activated receptor-γ coactivator-1α) were not altered by increased circulating FFA concentrations. The present data demonstrate that of the genes analyzed that encode proteins that are key regulators of lipid homeostasis within skeletal muscle, only the PDK4 gene is uniquely sensitive to increasing FFA concentrations after increased plasma FFA achieved by intravenous lipid infusion.

1. Introduction

Skeletal muscle is a complex tissue demonstrating considerable plasticity in its capacity to modify the contribution of energy derived from the macronutrients. While increased blood glucose availability induces a rapid compensatory promotion of the proportion of glucose oxidized by muscle, substantial control is also exercised to coordinately regulate lipid oxidation. As such, an increase in circulating free fatty acid (FFA) concentrations elicits a compensatory increase in fat oxidation rates [1,2]. Insulin-resistant states, including obesity and type 2 diabetes, are characterized by sustained elevations in plasma FFA concentrations and are accompanied by an impaired capacity for skeletal muscle fat oxidation [3-5]. There is also inflexibility in the capacity to alter fat oxidation rates after various metabolic perturbations in energy demand and FFA availability [6-8]. As such, investigations are required to determine which factors govern the acute adaptations necessary to regulate FFA oxidation within skeletal muscle.

Substrate oxidation after acute alterations in nutrient availability is dictated initially by downstream events mediated by the substrates themselves or resultant metabolites [9]. In addition to the substrate-dependent regulation of enzyme activities, an intricate picture of metabolic regulation has emerged, which involves many complex levels of activating or inactivating protein kinases. In a further level of metabolic control, FFAs, in addition to their primary role as oxidative substrates, appear to possess a marked capacity to modify the molecular mechanisms governing the synthesis of genes [10]. Indeed, emerging in vitro evidence in skeletal muscle cell cultures demonstrates the significant capacity of FFA to markedly regulate the expression of many genes [11,12]. Dietary and

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metabolic manipulations in human subjects that alter plasma FFA, including high-fat diets and short-term fasting, have also been shown to regulate lipid oxidative genes in skeletal muscle [13,14]. However, these studies result in many metabolic perturbations; consequently, the regulation of gene expression within skeletal muscle may be due to the combined actions of several factors. Therefore, this study aimed at acutely increasing circulating FFA concentrations using combined intravenous triglyceride and heparin infusion [2] to minimize the metabolic changes associated with oral fat feeding or fasting. Thus, the actions of acute elevations in circulating FFA concentrations on the messenger RNA (mRNA) concentration of proteins necessary for the transport and intracellular processing of FFA were assessed in the *vastus lateralis* muscle of healthy human subjects after a short-duration (5 hours) lipid infusion (Intralipid 20%). The combined infusion of Intralipid and heparin has previously been shown to acutely regulate fatty acid translocase (FAT/CD36) in rat skeletal muscle [15,16] and human adipose tissue [2].

Analysis of the gene expression was made for key components of intracellular lipid homeostasis and metabolism. These included the analysis of mRNA for proteins necessary for fatty acid (FA) (1) plasma membrane transport (FAT/CD36), (2) mitochondrial membrane transport (carnitine palmitoyltransferase I [CPT I]), (3) mitochondrial function (uncoupling protein 3 [UCP3]), (4) intramyocellular hydrolysis (hormone-sensitive lipase [HSL]), and (5) intramyocellular triacylglycerol (IMTG) reesterification (1-acylglycerol-3-phosphate *O*-acyltransferase 1 [AGPAT]). Additional analysis was performed on peroxisome proliferator–activated receptor (PPAR) γ coactivator 1 (PGC-1α) [17], which may act as an FA-induced transcriptional controller of a number of proteins involved in mitochondrial FA oxidation in adipocytes and cardiac cells [18,19]. A key determinant of carbohydrate flux, the pyruvate dehydrogenase (PDH) complex, is subject to inhibitory phosphorylation by pyruvate dehydrogenase kinase (PDK). Fasting and high-fat diets initiate marked induction of PDK isoform 4 (PDK4) mRNA and protein [20,21], suggesting that PDK4 is a rapid and FA-dependent target to down-regulate glucose oxidative flux. Therefore, the present study also measured the gene expression of PDK4.

2. Research design and methods

2.1. Subjects

Seven healthy men volunteered to participate in the study. The mean age, body mass, body mass index, and peak oxygen uptake (V_{O_2} peak) are shown in Table 1. The subjects were informed of all procedures and potential risks before giving their informed written consent to participate in the study. The Deakin University Human Research Ethics Committee approved all procedures.

Table 1
Subject characteristics

Characteristic	N = 7
Age (y)	26 ± 2
Mass (kg)	77 ± 3
BMI (kg/m^2)	24 ± 1
V_{O_2} peak (mL · kg^{-1} · min^{-1})	51 ± 1

BMI indicates body mass index.

2.2. Study design

Approximately 1 week before the first trial, subjects attended the laboratory for V_{O_2} peak determination. This was measured with a metabolic cart (AEI, Pittsburgh, Pa) during incremental exercise to exhaustion on a cycle ergometer (Lode, Quinton Excalibur, Groningen, The Netherlands). For 48 hours before each trial day, subjects consumed a standard high-carbohydrate diet (68% of energy from CHO, 18% energy fat, and 14% energy protein).

Each subject underwent 3 randomized trials with a washout period of 3 or more weeks separating each trial. On each occasion, subjects presented to the laboratory 3 hours after a standard breakfast meal (80% energy CHO, 9% energy fat, and 11% energy protein; total breakfast energy intake 2300 kJ). Trials were carried out in the postprandial state to limit the influence of prior changes in FFA availability on gene expression induced by the fasted state. Intravenous catheters were placed percutaneously in an antecubital vein in each arm. In 1 catheter, subjects received Intralipid 20% (Baxter Healthcare, Deerfield, Ill) at 1.5 mL/min with heparin sodium (200 U bolus and 0.4 U · min^{-1} · kg^{-1}) (Faulding, Parkville, Australia), saline at 1.5 mL/min (0.9% sodium chloride, Baxter Healthcare) and heparin sodium (200 U bolus and 0.4 U · min^{-1} · kg^{-1}), or saline only for 5 hours. Intralipid is a sterile nonpyrogenic fat emulsion for intravenous use composed of 20% wt/vol soybean oil, 1.2% wt/vol egg phospholipids, and 2.2% wt/vol glycerin in water. The FA composition has been described previously [22]. The contralateral catheter was used for collection of blood samples during the infusion. The subjects rested quietly without stress throughout the infusion periods.

2.3. Blood sampling and analysis

Venous blood (~7 mL) was obtained every hour during the infusion. Blood for glucose and insulin analysis was collected in a lithium heparin tube and spun at 14 000*g* for 3 minutes at 4°C and the plasma stored at −20°C until analysis. Plasma glucose was measured using an automated glucose analyzer (EML 105, Radiometer, Copenhagen, Denmark). Plasma insulin concentrations were determined by radioimmunoassay (Phadeseph, Pharmacia & Upjohn, Sweden). Blood for FFA analysis (1 mL) was mixed with 20 μL ethylene glycol-bis-N,N,N′ ,N′ -tetracetic acid and reduced glutathione and 10 μL tetrahydrolipstatin (120 mg/L) from Xenical (Orlistat) capsules (Roche, Nutley, NJ) to prevent in vitro lipolysis [23]. Samples were spun at 14°000*g* for 3 minutes at 4°C and the plasma stored at

−20°C before analysis. Free fatty acid was analyzed using a Wako NEFA C test kit (Wako Chemicals, Richmond, Va). Blood to be analyzed for glycerol was placed in a lithium heparin tube and spun at 14°000*g* for 3 minutes at 4°C and the plasma collected. Plasma was then deproteinized 1:1 with 3 mol/L PCA and spun at 14°000*g* for 3 minutes at 4°C. A 400-μL aliquot was added to 100 μL of 6 mol/L KOH; the sample spun as previously described and the supernatant stored at −80°C before analysis. Glycerol concentrations were determined enzymatically [24].

2.4. Muscle sampling

A muscle sample was obtained under local anesthesia (Xylocaine 1%, AstraZeneca, North Ryde, Australia) from the vastus lateralis using the percutaneous needle biopsy technique [25] modified to include suction [26] before (pre) and immediately after (post) the infusion. Tissue was immediately frozen in and stored in liquid nitrogen until analysis.

2.5. Total RNA isolation and reverse transcription

Total RNA from 8 to 10 mg of muscle was isolated using FastRNA Kit-Green (BIO 101, Vista, Calif) protocol and reagents. Total RNA concentration was determined spectrophotometrically at 260 nm. First-strand complementary DNA (cDNA) was generated from 0.5 μg of RNA using AMV RT (Promega, Madison, Wis) as previously described by Wadley et al [27]. The cDNA was stored at −20°C for subsequent analysis.

2.6. Real-time polymerase chain reaction analysis

Primers were designed using Primer Express software package version 1.0 (Applied Biosystems, Foster City, Calif) from gene sequences obtained from GenBank. A basic local alignment search tool [28] for each primer confirmed homologous binding to the desired mRNA of human skeletal muscle. Primer sequences are shown in Table 2.

Quantification of mRNA expression was performed (in triplicate) by real-time reverse transcription polymerase chain reaction (PCR) using the ABI PRISM 5700 sequence detection system (Applied Biosystems). A real-time PCR mix of Brilliant QPCR kit (Stratagene, La Jolla, Calif) with SYBR Green I dye, forward and reverse primers (3 μmol/L), and 12 ng of cDNA was run for 40 cycles of PCR in a volume of 20 μL. Fluorescent emission data were captured and mRNA levels were quantitated using the critical threshold value. To compensate for variations in input RNA amounts and efficiency of reverse transcription, β-actin mRNA was quantitated and results were normalized to these values as described previously [29]. To ensure the primers were detecting a single product, the samples were subjected to a heat dissociation protocol after the final cycle of PCR as described previously [30].

2.7. Statistical analysis

All data are presented as mean ± SEM. Two-way repeated measures analysis of variance (SigmaStat 2.03, Jandel Scientific, San Rafael, Calif) was used to determine the main effects of time and/or treatment on gene expression. Post hoc analysis was performed to determine differences between groups with the Tukey test where appropriate. Linear regression was used to model the effects of interaction and time and treatment on all metabolites measured. In all regression models, standard errors were adjusted to account for the repeated measures design. $P < .05$ was considered statistically significant.

3. Results

3.1. Plasma metabolites

Continuous Intralipid and heparin infusion (lipid) produced a 15-fold increase in plasma FFA concentrations at 5 hours compared with baseline (0 hours) (1.67 ± 0.13, 5 hours vs 0.11 ± 0.04 mmol/L, 0 hours) (Fig. 1). Saline and heparin infusion (saline heparin), stimulating lipoprotein lipase activity, elevated FFA 6-fold. Saline infusion alone resulted in a 2-fold rise in plasma FFA over the duration of the study (5 hours). The lipid infusion significantly elevated plasma FFA from 1 hour after the commencement of infusion and remained significantly higher at every time point thereafter. Plasma glycerol during the lipid infusion was significantly higher from 1 hour compared with the saline and saline heparin infusions, increasing to approximately 10-fold higher than baseline at 5 hours (0.27 ± 0.016, 5 hours vs 0.028 ± 0.006 mmol/L, 0 hours). Plasma glucose remained unchanged during the infusions in all treatment groups (4.77 ± 0.1, 4.9 ± 0.2, and 4.7 ± 0.8 mmol/L for lipid, saline heparin, and saline

Table 2
Gene primer sequences

Gene	Accession	Sense primer (5′ -3′)	Antisense primer (5′ -3′)
AGPAT	NM_006411	GAAGCACTCCCAGGCTCATC	AGGCATGAGACCTCTGAATTAGAAG
β-actin	NM_001101	AAGCCACCCCACTTCTCTCTAA	AATGCTATCACCTCCCCTGTGT
CPT I	Y08683	GAGGCCTCAATGACCAGAATG	GTGGACTCGCTGGTACAGGAA
FAT/CD36	L06850	AGTCACTGCGACATGATTAATGGT	CTGCAATACCTGGCTTTTCTCA
HSL	L11706	ACCTGCGCACAATGACACA	TGGCTCGAGAAGAAGGCTATG
PGC-1α	AF106698	CAAGCCAAACCAACAACTTTATCTCT	CACACTTAAGGTGCGTTCAATAGTC
PDK4	NM_002612	CCCGAGAGGTGGAGCATTT	GCATTTTCTGAACCAAAGTCCAGTA
UCP3	XM_055241	CGTGGTGATGTTCGTAACCTATG	CGGTGATTCCCGTAACATCTG

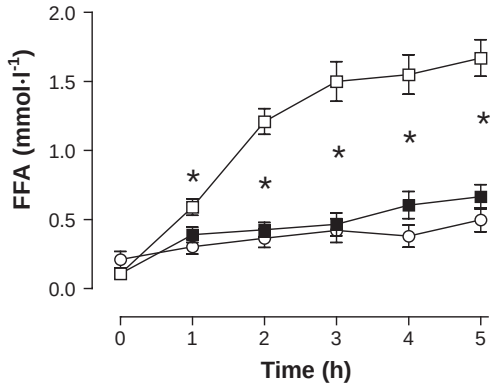

Fig. 1. Circulating plasma FFA concentrations during 5 hours of saline infusion (open circles), saline plus heparin (black squares), and Intralipid plus heparin (open squares). Results are presented as mean ± SEM of 7 subjects. Asterisk denotes a significant increase in Intralipid trial compared with saline and saline plus heparin trials.

infusions, respectively, at completion of 5-hour infusion). Plasma insulin was significantly elevated at the start of the lipid and saline heparin trials compared with saline (95.5 ± 18.3 and 86.0 ± 16.0 pmol/L for lipid and saline heparin trials, respectively, vs 43.17 ± 11.0 pmol/L for saline trial; $P < .05$). During the course of the 5-hour infusion, insulin significantly decreased in the saline heparin trial compared with the saline infusion, which resulted in no change in plasma insulin concentrations (64.4 ± 6.0, 35.7 ± 8.3, and 25.8 ± 7.8 pmol/L for lipid, saline heparin, and saline infusions, respectively, at completion of 5-hour infusion). At the end of the 5-hour infusion period, insulin was significantly elevated in the lipid trial compared with the saline and saline heparin trials ($P < .05$).

3.2. Messenger RNA abundance

The mRNA expression of PDK4 is shown in Fig. 2A. Pyruvate dehydrogenase kinase isoform 4 mRNA abundance was increased approximately 9-fold ($P < .05$, arbitrary units: pre, 0.002 ± 0.0005 vs post, 0.02 ± 0.0007) with only modest (2-fold) increases in circulating FFA concentrations induced by 5 hours of saline infusion. An approximately 15-fold induction ($P < .05$) in PDK4 mRNA expression was observed after the saline heparin trial, which produced a 6-fold increase in circulating FFA concentrations. Further increasing FFA concentrations to 15-fold from baseline by lipid infusion resulted in a 24-fold (range, 5- to 36-fold) up-regulation of PDK4 gene expression ($P < .001$, arbitrary units: pre, 0.002 ± 0.0009 vs post, 0.035 ± 0.008). This large difference in PDK4 abundance at the end of the infusion period between treatment groups failed to reach significance. This could be explained by the considerable interindividual variation of response after the lipid infusion, as there was a significant relationship ($P < .05$, data not shown) between change in plasma FFA and change in PDK4 above baseline.

The responsiveness of UCP3 was less marked than that of PDK4. There was a significant 2-fold rise in UCP3 mRNA after lipid infusion ($P < .05$) and saline infusion ($P < .05$), with a similar change also observed in the saline heparin infusion trial (Fig. 2B). Although there was a significant overall time effect, there was no effect of elevated lipid availability on UCP3 gene expression.

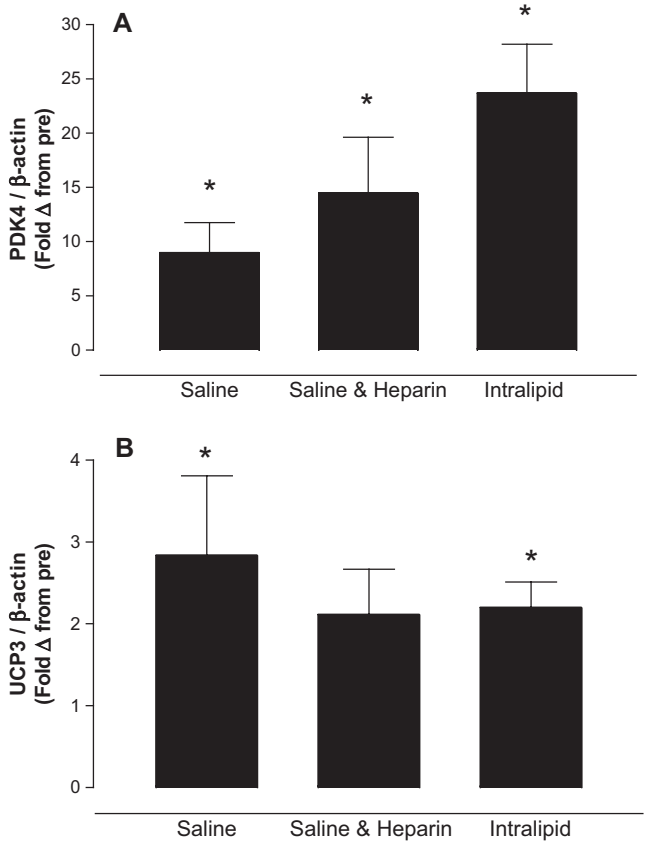

Fig. 2. Messenger RNA abundance of PDK4 (A) and UCP3 (B) in human skeletal muscle after 5 hours of saline infusion, saline plus heparin, and Intralipid plus heparin. Results are presented as mean ± SEM of fold changes from pre values for 7 subjects. Pre values represent a value of 1.0 (data not shown). Asterisk denotes significantly increased fold change in mRNA abundance after the 5-hour infusion period compared with preinfusion values. No significant differences in expression were observed between groups.

Markedly increasing FFA concentrations to 15 -fold from baseline concentrations resulted in no significant change in the mRNA expression of transcriptional coactivator PGC-1α

Table 3
Messenger RNA abundance (fold changes from pre) of lipid metabolic proteins in skeletal muscle after 5 hours of saline infusion, saline plus heparin, or Intralipid plus heparin

	Saline	Saline and heparin	Intralipid
FAT/CD36	1.6 ± 0.4	1.1 ± 0.2	1.1 ± 0.1
CPT I	1.5 ± 0.3	1.5 ± 0.2	1.7 ± 0.6
HSL	1.5 ± 0.1	1.0 ± 0.2	1.3 ± 0.5
AGPAT	1.5 ± 0.4	0.9 ± 0.2	1.4 ± 0.9
PGC-1α	1.8 ± 0.3	1.0 ± 0.2	2.3 ± 0.8

Pre values represented by a value of 1.0 (not shown). No statistical change in gene expression was demonstrated.

or proteins responsible for the uptake (FAT/CD36), mitochondrial transport (CPT I), reesterification (AGPAT), and mobilization (HSL) of FAs within skeletal muscle (Table 3).

4. Discussion

Fatty acids, in addition to their principal role as an oxidative substrate, are important mediators of cell signaling and gene expression. To examine the effect of acute elevations in circulating FFA on skeletal muscle gene expression, we undertook a short-duration infusion (5 hours) of a polyunsaturated-rich FA emulsion (Intralipid 20%). The resultant elevations in plasma FFA (15-fold increase from baseline) were in contrast to the normal postabsorptive rises in plasma FFA observed with saline treatment (2-fold increase) and the augmented response due to endogenous triglyceride cleavage with combined saline and heparin infusion (6-fold increase). Plasma insulin concentrations correspondingly fell with fasting, although there was a small unexplained elevation in the insulin concentration at the commencement of the saline and heparin infusion measured 3 hours after the breakfast consumption. Skeletal muscle gene abundance of PDK4 was increased approximately 24-fold (range, 5- to 36-fold) after the lipid infusion and to a lesser degree (9-fold increase) after 5 hours of saline infusion. These data demonstrate that the PDK4 gene was highly sensitive to increasing FFA concentrations. However, there were no differences in PDK4 mRNA abundance between treatment groups at the end of the infusion periods. Although every subject demonstrated a sensitivity of PDK4 to increased FFA concentrations, the extent of this sensitivity differed between individuals, resulting in a wide range of responses.

Activation of gene expression was not universally observed across all genes analyzed. Genes encoding proteins necessary for transcriptional coactivation (PGC-1α), FFA uptake (FAT/CD36), mitochondrial transport (CPT I), and intramuscular FA esterification and triglyceride storage (AGPAT and HSL) remained unchanged, whereas UCP3 mRNA expression was not elevated in parallel to the increased plasma FA. These data suggest that the actions of FFA on gene transcription are acutely targeted to a small subgroup needed for rapid adaptations of substrate metabolism.

PDK4 is 1 of 4 isoenzymes in skeletal muscle that catalyze the phosphorylation and inactivation of the E1α subunit of the PDH complex [31]. Pyruvate dehydrogenase kinase isoform 4 is expressed predominately in skeletal muscle and heart and is regulated by fasting and high-fat feeding [20,21]. The rapid and significant increase in PDK4 mRNA after either lipid infusion or saline (with or without heparin) infusion demonstrates a potential capacity for a marked translation of the PDK4 protein. The rapid activation of this gene without lipid infusion, where the subjects were maintained on a saline infusion, suggests that fasting for only 8 hours is sufficient to stimulate PDK4 gene expression. Previous studies using either a 24-hour fast or a single day on a high-fat diet have reported increases of a similar magnitude (2- to 5-fold) [20,21]. More recent data from our laboratory demonstrate a 3-fold increase in PDK4 mRNA in human skeletal muscle after 15 hours of fasting, a response that was unique when compared with other PDK isoforms [32]. Indeed, these studies also show a corresponding increase in PDK4 protein, implying that there is little reason to suggest a disassociation between the gene and the protein during acute interventions. The profound capacity for fat infusion to increase the gene abundance of PDK4 highlights the pivotal nature of this gene in providing a rapid inducible mechanism for the inhibition of PDH, thereby down-regulating glucose oxidative flux. These data complement studies in human skeletal muscle in which PDK4 mRNA is also activated by exercise [33], another metabolic perturbation in which the adaptations postexercise are to limit glucose oxidation in favor of lipid oxidation. Although PDK4 is regulated by PPAR-dependent signals, multiple pathways of regulation are evident. In addition, there are no studies that have examined which FAs species or metabolites are most potent in the regulation of skeletal muscle PDK4 gene expression. Therefore, further evaluation of the transcriptional factors and the most potent FA mediators that enable human skeletal muscle to markedly increase the expression of this gene to altered FFA concentrations in vivo is required.

The exact physiological function of UCP3, which is predominately expressed in skeletal muscle, is yet to be fully elucidated. Although UCP3 has sequence homology with the established uncoupling protein 1, metabolic states requiring greater oxidative coupling such as energy restriction and fasting increase the expression of UCP3 [14,34]. Moreover, the fasting-induced increase in UCP3 expression does not diminish phosphocreatine synthesis in vivo, highlighting the absence of an uncoupling action in human muscle [35]. However, there also appears to be a relationship between UCP3 and fat availability [11,17,36], which has led several groups to hypothesize a role for UCP3 in protecting the mitochondria against either superoxide damage [37] or excessive intramitochondrial FA anions [38] in metabolic situations of heightened FA availability. Consistent with data from a similar Intralipid infusion protocol also lasting 5 hours [17], we demonstrate an approximate doubling of UCP3 mRNA after Intralipid infusion. However, in our hands, there was an equivalent activation of UCP3 in the absence of a marked rise in FFA, with the saline only control infusion also doubling UCP3 mRNA. This might suggest that the UCP3 mRNA is sensitive to factors other than just FFA availability. Previously, we have demonstrated that 15 hours of fasting is capable of increasing UCP3 5-fold relative to postprandial expression (3 hours after the last meal) [14]. This suggests that factors related to the duration of fasting, in addition to FFA and insulin, exert a significant impact in UCP3 gene expression in human skeletal muscle. In the current study, the PCR oligonucleotide primers were used to measure total

UCP3 mRNA. Therefore, as the present study failed to account for the relative changes in the abundances of the constitutive isoforms of UCP3 (short and long), it is possible that selective differential activation of 1 or both of the isoforms exists in response to the differing infusions. Previously, the UCP3 long isoform was found to be activated only by Intralipid infusion, whereas the UCP3 short isoforms were increased by both the Intralipid and control infusions [17]. Although the current data confirm the previous evidence that UCP3 mRNA is increased by elevated plasma FFA, total UCP3 mRNA may not be tightly linked to circulating FFA concentrations.

PGC-1α is a transcriptional coactivator that participates as a key regulator of genes necessary for skeletal muscle lipid oxidative metabolism [16,18], mitochondrial biogenesis, and in the formation of type 1 (slow-twitch) muscle fibers [39]. Little is known of the in vivo response of PGC-1α to altered FFA availability. Recently, a central role for suppressed PGC-1, linked to decreased oxidative capacity, has been highlighted as a major metabolic disturbance in type 2 diabetes [40]. In the present study, acute elevations in FFA concentrations to 15-fold from baseline had limited effects on PGC-1α gene expression. These results suggest that the regulation of PGC-1α is not acutely sensitive to changing FFA concentrations. However, more chronic exposure to blood-borne FFA, as found in insulin-resistant states, may have differing actions. In very recent data, 48 hours of Intralipid infusion in healthy subjects has been reported to suppress the expression of PGC-1α and nuclear-encoded mitochondrial genes [41]. These data suggest that more chronic exposure to FAs has a detrimental action on mitochondrial function. The absence of suppression may indicate that reduced PGC-1α expression within skeletal muscle occurs overtime, potentially due to diminished transcript synthesis or accelerated degradation.

Carnitine palmitoyltransferase I is the rate-limiting enzyme of the CPT system integral to the mitochondrial influx of FA for β-oxidation. Carnitine palmitoyltransferase I is regulated by the transcription factor PPARα [42], a known transcriptional mediator of the actions of FAs. However, in the present study, no increase in CPT I gene abundance was demonstrated after any infusion condition. This suggests a requirement for greater than 5 hours between PPARα activation and increased CPT I expression in vivo, limited regulation of CPT I by FFA, or, alternatively, that in the short term, CPT I is regulated by alterations in enzyme activity rather than protein abundance. Indeed, acute enzymatic regulation has been demonstrated with CPT I activity increasing after 30 minutes of Intralipid infusion [43].

The high plasma FFA levels obtained in this study increased the availability of lipids for uptake into the muscle cell. In the present study, we were unable to demonstrate an increase in the transcription of key plasma membrane FA transporter, FAT/CD36. This is in contrast to previous in vivo studies using similar lipid infusion protocols, which have demonstrated markedly up-regulated FAT/CD36 mRNA in rat skeletal muscle after a 24-hour infusion [16] and in human adipose tissue after a 5-hour infusion [1]. However, we have previously demonstrated no change in skeletal muscle FAT/CD36 mRNA abundance after 40 hours of fasting in humans, a situation where FFA availability and oxidation are enhanced [14]. Therefore, if FAT/CD36 is central to the enhanced uptake of circulating FFA into human skeletal muscle under conditions of elevated lipid oxidation rates, it is likely to be a consequence of alterations in translational or posttranslational mechanisms such as increased activity or translocation of the transporter to the plasma membrane rather than transcriptional modifications [44].

The present study did not seek to examine the influence of differing FA composition on the regulation of key metabolic genes. However, several studies indicate that FA composition has the ability to differentially regulate gene expression, hence modifying partitioning of metabolic fuels. One element of obesity susceptibility is the preferential repartitioning of fat toward storage rather than oxidation, resulting in the intracellular accumulation of lipid derivatives [4,5,15]. Intramyocellular triacylglycerol levels are increased in obese and type 2 diabetic patients, and strong correlations between the levels of IMTG and the severity of insulin resistance are well documented [5,45]. A similar protocol using a 6-hour Intralipid infusion combined with a hyperinsulinemic-euglycemic clamp reported a 164% increase in the *tibialis anterior* IMTG level and a corresponding decrease in glucose infusion rate, demonstrating loss of insulin action [46]. Intralipid consists primarily of polyunsaturated FAs [22] that have been demonstrated to preferentially up-regulate the synthesis of key hepatic proteins to redirect FAs away from triglyceride storage and toward oxidation [47]. Fatty acyl coenzyme A partitioning between oxidation and storage within the cell is primarily regulated by HSL and the triacylglycerol synthesis pathway, of which AGPAT is a key enzyme [48]. The present study was unable to demonstrate transcriptional regulation of these genes in response to elevated circulating polyunsaturated FA concentrations; however, it remains to be seen whether a modified FA composition would have a different impact on the mRNA abundance of these genes.

In summary, the present study demonstrated that acute elevations in circulating FFA concentrations induced by a combined triglyceride and heparin infusion for 5 hours increased the mRNA abundance of PDK4 in human skeletal muscle. Our data also demonstrate the marked sensitivity of PDK4 to albeit modest increases in FFA with both control trials, pointing toward a unique role of this gene in the regulation of skeletal muscle substrate flux. Interestingly, several key genes integral to FA uptake, oxidation, and intramyofibrillar storage, including FAT/CD36, CPT I, AGPAT, HSL, and PGC-1α, were not altered by increased circulating FA concentrations induced by a combined Intralipid and heparin infusion. Thus, the actions of FA on

skeletal muscle gene expression is highly selective, with further work necessary to identify the transcriptional pathways regulating these selective responses and potentially which of the infused polyunsaturated FA species exerts predominant control on genes such as PDK4.

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