A short-term, high-fat diet up-regulates lipid metabolism and gene expression in human skeletal muscle[1–3]

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ABSTRACT

Background: Dietary fatty acids may be important in regulating gene expression. However, little is known about the effect of changes in dietary fatty acids on gene regulation in human skeletal muscle.

Objective: The objective was to determine the effect of altered dietary fat intake on the expression of genes encoding proteins necessary for fatty acid transport and β-oxidation in skeletal muscle.

Design: Fourteen well-trained male cyclists and triathletes with a mean (± SE) age of 26.9 ± 1.7 y, weight of 73.7 ± 1.7 kg, and peak oxygen uptake of 67.0 ± 1.3 $mL \cdot kg^{-1} \cdot min^{-1}$ consumed either a high-fat diet (HFat: > 65% of energy as lipids) or an isoenergetic high-carbohydrate diet (HCho: 70–75% of energy as carbohydrate) for 5 d in a crossover design. On day 1 (baseline) and again after 5 d of dietary intervention, resting muscle and blood samples were taken. Muscle samples were analyzed for gene expression [fatty acid translocase (*FAT/CD36*), plasma membrane fatty acid binding protein (*FABPpm*), carnitine palmitoyltransferase I (*CPT I*), β-hydroxyacyl-CoA dehydrogenase (*β-HAD*), and uncoupling protein 3 (*UCP3*)] and concentrations of the proteins FAT/CD36 and FABPpm.

Results: The gene expression of *FAT/CD36* and *β -HAD* and the gene abundance of FAT/CD36 were greater after the HFat than after the HCho diet ($P < 0.05$). Messenger RNA expression of *FABPpm*, *CPT I*, and *UCP-3* did not change significantly with either diet.

Conclusions: A rapid and marked capacity for changes in dietary fatty acid availability to modulate the expression of mRNA-encoding proteins is necessary for fatty acid transport and oxidative metabolism. This finding is evidence of nutrient-gene interactions in human skeletal muscle. *Am J Clin Nutr* 2003;77:313–8.

KEY WORDS Diet-gene interaction, messenger RNA, high-fat diet, skeletal muscle, metabolism, dietary intervention, fat oxidation, carbohydrate oxidation, gene expression

INTRODUCTION

Skeletal muscle, by virtue of its mass and metabolic activity, is the major site of whole-body fatty acid and carbohydrate oxidation (1). The relative contribution of these fuels to the energetic demands of skeletal muscle is subject to complex regulation at multiple levels, including substrate availability, hormonal concentrations, and the allosteric regulation of enzyme activities by intracellular metabolic intermediates (for review, *see* reference 2). Under most physiologic conditions, glucose availability and flux exert the dominant influence on the oxidized fuel mix (3). However, with isoenergetic dietary manipulations, increases in dietary lipids elevate the contribution of fatty acids to oxidative metabolism, such that within 1 wk the imbalance between fat intake and oxidation is minimal in lean and obese persons (4–6) and in endurance-trained athletes (7, 8). Despite these marked changes in the rate of fatty acid oxidation after short-term high-fat diets, the mechanisms underlying this adaptation are yet to be fully elucidated.

In addition to their role as important oxidative substrates, fatty acids regulate the expression of many genes (9, 10). Several rodent studies indicate that dietary lipids modulate the expression of genes in skeletal muscle, with an increase in the messenger RNA (mRNA)expression of genes involved in fatty acid metabolism after isoenergetic high-fat diets compared with low fat, high-carbohydrate diets (11, 12). More recently, the actions of dietary fatty acids have been extended to human skeletal muscle; it was shown that dietary fatty acids have a significant effect on the expression of the gene encoding for uncoupling protein 3 (*UCP3*) (13). However, that study did not examine the response of those genes central to the regulation of fatty acid transport and mitochondrial β-oxidation, whose actions are likely to be pivotal in the increased capacity to oxidize fatty acids.

Therefore, the aim of the current study was to determine the effect of marked changes in the proportion of dietary fat and carbohydrate intakes of persons undergoing extensive daily endurance training on the abundance of fatty acid–sensitive genes encoding

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[2] Supported by grants from Kellogg (Australia) Pty Ltd, the Australian Institute of Sport, Deakin University, and the Canadian Institutes of Health Research.

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Received January 24, 2002.

Accepted for publication May 15, 2002.

proteins necessary for fatty acid transport and β-oxidation in skeletal muscle. The genes chosen for investigation included the putative fatty acid transporters fatty acid translocase (*FAT/CD36*) and the plasma membrane fatty acid binding protein (*FABPpm*) (14), the mitochondrial fatty acid transporter carnitine palmitoyltransferase I (*CPT I*; EC2.3.1.21) (15), the β-oxidation pathway enzyme β-hydroxyacyl-CoA dehydrogenase (*β-HAD*; EC 1.1.1.211) (16), and *UCP3* (13). We also determined the protein abundance of the fatty acid–transport proteins FAT/CD36 and FABPpm.

SUBJECTS AND METHODS

Subjects and preliminary testing

Fourteen well-trained male cyclists and triathletes were recruited for this study. The subjects were informed of all potential risks and procedures before giving their written informed consent to participate in the study. All procedures were approved by the Ethics Committee of the Australian Institute of Sport. An incremental cycling ergometer (Lode, Groningen, Netherlands) test to exhaustion, with expired air analysis, was used to determine peak pulmonary oxygen uptake ($\dot{V}O_{2peak}$) for each subject according to the methods previously described in detail (7, 8). The subjects had a mean (± SE) age of 26.9 ± 1.7 y, weight of 73.7 ± 1.7 kg, and $\dot{V}O_{2peak}$ of 67.0 ± 1.3 mL · kg^{-1} · min^{-1}.

Dietary intervention

Each subject underwent 2 trials in a randomized crossover design with a 2-wk washout period separating each trial as described in detail previously (7, 8). Briefly, subjects received either a high-carbohydrate diet (HCho) or an isoenergetic high-fat diet (HFat) for 5 d. The HCho diet provided 70–75% of energy as carbohydrate and < 15% of energy as lipids, whereas the HFat diet provided > 65% of energy as lipids and < 20% of energy as carbohydrate. To ensure compliance, all meals and snacks were supplied to the subjects, and the diets were individualized on the basis of food preferences and body mass. At least one meal each day was eaten under supervision in the laboratory; the remaining food for each 24-h period was provided in preprepared packages. The subjects were required to keep a food diary, in which they reported the daily intake of all food and drink to maximize compliance with the designed diets. Food intake data (7) and sample menus (8) were reported previously.

Experimental intervention and muscle collection

An identical protocol was followed for each diet. For both trials, the subjects reported to the laboratory on day 1, after they had fasted overnight. After voiding, the subjects were weighed, and a resting blood sample (10–12 mL) was drawn from an antecubital vein. A muscle sample (baseline) was then obtained from the vastus lateralis by using the percutaneous needle biopsy technique with suction applied (17). The excised muscle was immediately frozen in liquid nitrogen. After 10–15 min of rest, the subjects then completed 20 min of cycling at 70% of $\dot{V}O_{2peak}$. For the last 10 min of cycling, the subjects breathed through the previously described gas analysis system so that whole-body rates of carbohydrate and fat oxidation (g/min) could be estimated (7). Over the 5 d of the dietary intervention, the subjects maintained the same intensive training program, which was described previously (7). On the morning of day 6, the subjects reported to the laboratory after fasting overnight for 12–14 h, and a resting muscle biopsy was taken. After a 10–15-min rest, the subjects underwent 20 min of cycling at 70% $\dot{V}O_{2peak}$, as described for day 1. Because the muscle samples (30–40 mg) were of insufficient size to enable analysis of both gene expression and protein abundance, they were randomized for either gene analysis ($n = 6$) or protein analysis ($n = 8$).

Gene quantitation with real-time reverse transcriptase–polymerase chain reaction

Total RNA was extracted from 30 mg frozen muscle by using RNAzol (Bresatec, Adelaide, Australia). First-strand complementary DNA (cDNA) was generated from 1 μg RNA by using avian myeloblastosis virus reverse transcriptase (RT) (Promega, Madison, WI). cDNA was stored at −20 °C for subsequent analysis.

Primer and probes for each gene were designed by using PRIMER EXPRESS software package version 1.0 (Applied Biosystems, CA) from gene sequences obtained from GenBank (National Library of Medicine, Bethesda, MD; Internet: www.ncbi.nlm.nih.gov/blast/): *FAT/CD36*—L06850, *FABPpm*/mASPAT—M22632, *CPT I*—Y08683, *β-HAD*—NM_000182, and *UCP3* – XM_055241. A BLAST search for each primer and probe confirmed homologous binding to the desired mRNA of skeletal muscle (18).

Real-time RT–polymerase chain reaction (RT-PCR) was performed with the use of the ABI PRISM 5700 sequence detection system (Applied Biosystems, Foster City, CA). All samples for each gene were run simultaneously, in duplicate, to control for amplification efficiency. Fluorescent emission data were captured, and mRNA concentrations were quantified by using the critical threshold value (19). For mRNA quantification, a real-time PCR mix of 0.5 × TaqMan Universal PCR master mix (Applied Biosystems), TaqMan oligonucleotide probe (1 μmol/L; Applied Biosystems), forward and reverse primers (3 μmol/L), and cDNA (12 ng) were run for 40 cycles of RT-PCR in a total volume of 25 μL. To compensate for variations in the amount of input RNA and the efficiency of reverse transcription, *β-actin* (GenBank-X00351) mRNA was quantified, and the results were normalized to these values.

Immunoblot analysis

For the detection of FAT/CD36 and FABPpm, we used a monoclonal antibody against human CD36 (provided by NN Tandon) and a rabbit polyclonal anti-FABPpm antiserum (provided by J Calles-Escandon), respectively, as previously described (20). Briefly, muscle samples were solubilized and electrophoresed on 10% sodium dodecyl sulfate–polyacrylamide gels and then transferred onto immobilon polyvinylidene difluoride membranes. The membranes were incubated for 2 h with either the monoclonal CD-36 antibody (1:500) or the polyclonal FABPpm antibody) (1:200). Secondary complexes were generated by using an anti-mouse immunoglobulin G horseradish peroxidase secondary antibody (1:10 000; Santa Cruz Biotechnology, Santa Cruz, CA) for FAT/CD36 and donkey anti-rabbit immunoglobulin G horseradish peroxidase–conjugated secondary antibody (1:3000; Amersham Biosciences Corp, Piscataway, NJ) for FABPpm. Enhanced chemiluminescence detection (Hyperfilm-ECL; Amersham, Oakville, Canada) was performed according to the instructions of the manufacturer; band densities were obtained by scanning the blots on a densitometer connected to a Macintosh LC computer (Apple Computer, Inc, Cupertino, CA) with appropriate software.

TABLE 1
Glucose, lactate, fatty acid, and insulin concentrations before (baseline) and after 5 d of the high-carbohydrate (HCho) and high-fat (HFat) diets[1]

	Baseline	HCho	HFat
Glucose (mmol/L)	5.0 ± 0.1	4.8 ± 0.2	4.6 ± 0.1
Lactate (mmol/L)	1.0 ± 0.1	1.0 ± 0.1	0.8 ± 0.1
Fatty acids (mmol/L)	0.39 ± 0.04	0.41 ± 0.05	0.85 ± 0.06[2,3]
Insulin (mIU/L)	27.4 ± 3.3	32.4 ± 6.6	27.0 ± 3.2

[1] $\bar{x}$ ± SE; n = 14.
[2] Significantly different from baseline, $P < 0.05$.
[3] Significantly different from HCho, $P < 0.05$.

Blood analyses

Blood was portioned into tubes containing fluoride-heparin, lithium-heparin, or a preservative solution containing EGTA and reducing glutathione in isotonic sodium chloride solution. All tubes were spun, and the plasma aliquots were stored at −80 °C. The plasma prepared with fluoride-heparin was used for the analysis of plasma glucose and lactate (EML-105; Radiometer, Copenhagen). The plasma prepared with lithium-heparin was used for the analysis of plasma insulin by radioimmunoassay (Incstar, Stillwater, MN). The plasma preserved with EGTA and glutathione was analyzed for plasma fatty acids (Wako, Toyko).

Statistical analysis

All data are presented as means ± SEs. The baseline data (muscle, blood, and rates of substrate oxidation), collected before each dietary intervention (HCho and HFat), did not differ significantly; therefore for the purpose of data analysis, these values were pooled. One-way analysis of variance was used to compare data across dietary groups (baseline, HCho, and HFat). Post hoc analysis was performed to determine differences between groups with the use of Tukey's test, where appropriate. Significance was accepted at $P < 0.05$. SPSS version 11.0.0 (SPSS Inc, Chicago) was used for the analyses.

RESULTS

All subjects complied with the dietary and exercise regimens throughout each study period.

Plasma glucose, lactate, fatty acid, and insulin concentrations

The fasting plasma glucose, lactate, and insulin concentrations did not change significantly after the dietary interventions (**Table 1**). Fatty acid concentrations increased after both diets and were significantly greater after 5 d of the HFat diet than after the baseline or HCho diet.

Substrate oxidation

During the 20-min exercise bout, fat oxidation was 1.7-fold greater after 5 d of the HFat diet than after the HCho diet; conversely, carbohydrate oxidation was significantly lower after 5 d of the HFat diet than after the baseline diet (**Figure 1**).

mRNA analysis

The abundance of *FAT/CD36* mRNA increased significantly after 5 d of the HFat diet and was significantly greater than the value after the HCho diet (**Figure 2**). Furthermore, the expression of *β-HAD*

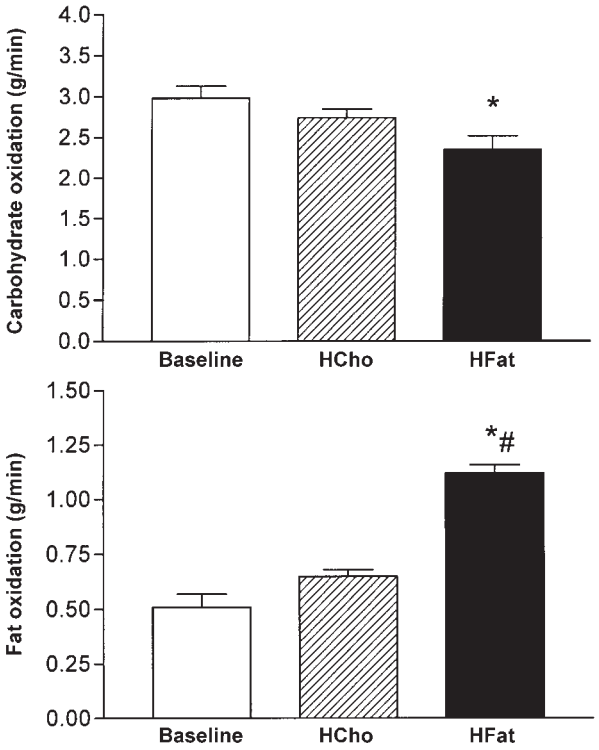

FIGURE 1. Mean (±SE) carbohydrate and fat oxidation rates measured in 14 male athletes during cycling at 70% of peak oxygen uptake before (baseline) and after 5 d of either a high-carbohydrate (HCho) or a high-fat (HFat) diet. *Significantly different from baseline, $P < 0.05$. #Significantly different from HCho, $P < 0.05$.

mRNA was significantly greater after the HFat diet than after both the baseline and HCho diets. The expression of *FABPpm* tended to be highest after the HCho diet, although there was considerable interindividual variation in the measurement of this gene, possibly because of the low abundance relative to the housekeeping gene, *β-actin*. *CPT I* and *UCP-3* did not change significantly after the dietary treatments.

FAT/CD36 and FABPpm protein abundance

Protein concentrations of FAT/CD36 were significantly greater after the HFat diet than after the baseline diet (**Figure 3**). The abundance of FABPpm did not change significantly by either of the dietary treatments.

DISCUSSION

Fatty acids, in addition to their important role as energy-yielding nutrients, may exert a significant influence on the regulation of gene expression (9, 10). We provide novel data that show the marked capacity for changes in dietary fat intake to increase mRNA concentrations of encoded genes necessary for fatty acid transport and oxidative metabolism in skeletal muscle. Thus, fatty acid–mediated interactions on gene abundance may be a significant component of the adaptive capacity of skeletal muscle that contributes to the rapid matching of the oxidative profile of the muscle to the predominant dietary fuel source. In well-trained subjects undertaking a matched exercise regimen, consumption of the

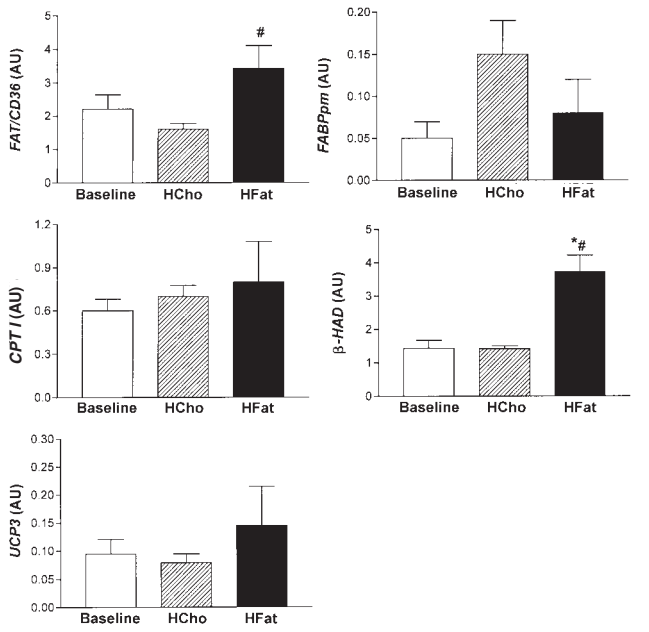

FIGURE 2. Mean (±SE) expression of genes encoding for fatty acid translocase (*FAT/CD36*), plasma membrane fatty acid binding protein (*FABPpm*), carnitine palmitoyltransferase I (*CPT I*), β-hydroxyacyl-CoA dehydrogenase (*β-HAD*), and uncoupling protein 3 (*UCP3*) measured in 6 male athletes before (baseline) and after 5 d of either a high-carbohydrate (HCho) or a high-fat (HFat) diet. The data were normalized for amounts of the input RNA and the efficiency of reverse transcription with *β-actin* expression. *Significantly different from baseline, $P < 0.05$. #Significantly different from HCho, $P < 0.05$. AU, arbitrary units.

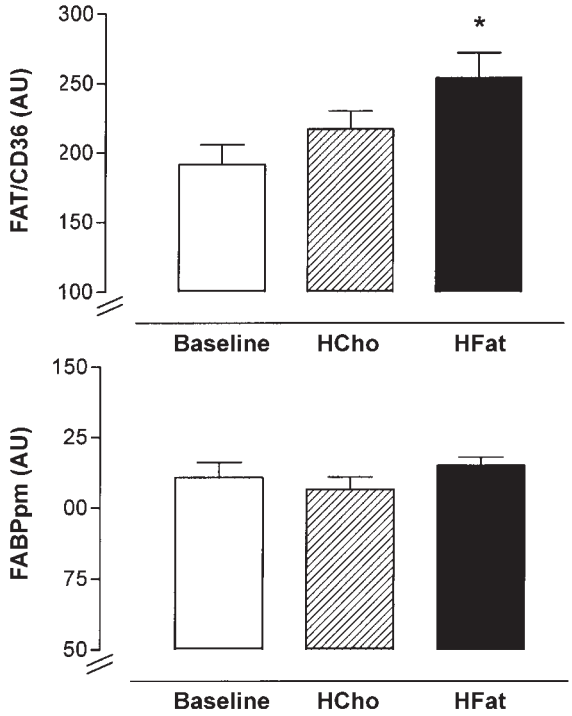

FIGURE 3. Mean (±SE) abundance of the fatty acid translocase protein (FAT/CD36) and plasma membrane fatty acid binding protein (FABPpm) in 8 male athletes before (baseline) and after 5 d of either a high-carbohydrate (HCho) or a high-fat (HFat) diet. *Significantly different from baseline, $P < 0.05$.

HFat diet for 5 d markedly increased the expression of *FAT/CD36* and *β-HAD* when compared with the isoenergetic HCho diet. The increased abundance of *FAT/CD36* was accompanied by a concomitant increase in the cellular content of the transcribed protein. The abundance of skeletal muscle *FABPpm*, *CPT I*, and *UCP3* expression in mRNA did not change significantly after either dietary intervention.

The importance of nutrient-gene interactions is well recognized as a key feature of single cellular organisms, in which adaptation to differing nutrient supplies is necessary for survival (21). The capacity of fatty acids to regulate gene expression in complex cellular systems in which fatty acid availability is a major determinant of cellular function is now well described (9, 10). The current data, together with data from earlier studies in rodents and humans (13, 22), provide strong evidence for a functional role of dietary fatty acid in the regulation of gene expression as one step in skeletal muscle cellular adaptation. Although mRNA abundance is a significant determinant of protein synthesis, this relation is neither simple nor linear (23). The half-lives of many mRNAs are short compared with their transcribed proteins, with transient gene activation occurring before sustained increases in protein (24). Furthermore, many intermediary steps between gene and functional protein might also be subject to fatty acid regulation (25). Thus, further studies aimed at elucidating the relation between the increased mRNA abundance after high-fat feeding reported in the current study and downstream protein synthesis rates and enzyme activities are required.

The current study was performed in highly trained athletes who maintained a matched exercise program while consuming either the HFat or HCho diet. Their high aerobic fitness and intense training schedule might have influenced the extent and degree of gene expression adaptation observed. Indeed, our group and others reported on the capacity of exercise to activate muscle gene expression (26–29). However, the sustained nature of the exercise training undertaken by these subjects, ie, competing for an average of 8 y (range: 2–20 y), together with the precise matching of the exercise workload during both trials would act to limit this potential confounding factor.

FAT/CD36 is present in tissues with high fatty acid demands, including heart, adipose tissue, intestine, and skeletal muscle (14). Currently, the contribution of FAT/CD36 to skeletal muscle fatty acid uptake is not well described. Of the available data, compelling evidence has emerged of a significant role of FAT/CD36 in fatty acid transport when the actions of FAT/CD36 are analyzed by using either selective chemical inhibition or muscle-specific gene overexpression (30, 31). A further notable feature of FAT/CD36 is the recruitment of this protein to the plasma membrane from intracellular sites by muscle contraction, highlighting a role as an adaptable regulator of intracellular fatty acid uptake during periods of increased demand (32). In support of the adaptable nature of FAT/CD36, the current study showed 215% and 17% increases in gene and protein abundance, respectively, when the HFat diet was compared with the HCho diet. These data

are consistent with rapid increases in the expression of *FAT/CD36* after lipid infusion in the adipocytes of human subjects (33) and in the skeletal muscle of rats (34). Similarly, increases in FAT/CD36 protein have been shown after high-fat feeding and in disorders in which lipid metabolism is dysregulated, including obesity and diabetes (20, 35). These data indicate that an increase in dietary fatty acids results in the up-regulation of *FAT/CD36* and FAT/CD36 protein, suggesting a role in facilitating greater skeletal muscle fatty acid uptake.

Along with FAT/CD36 protein, FABPpm protein is also an important mediator of fatty acid plasma membrane transport, present in tissues with high fatty acid flux (14). In addition to facilitating fatty acid transport, FABPpm is identical to mitochondrial aspartate aminotransferase (EC 2.6.1.1) and thus can perform multiple cellular functions (36). An increased abundance of skeletal muscle FABPpm protein is observed in conditions of increased fatty acid utilization, including fasting (37) and endurance training (38), which supports its role as an adaptable regulator of fatty acid transport. In contrast, the current study did not show increased expression of mRNA *FABPpm* or abundance of FABPpm protein after 5 d adaptation to a high-fat diet. Therefore, FABPpm is differentially regulated from FAT/CD36, with no evidence of an effect of diet on FABPpm.

Currently, there is little known about the regulation of *β-HAD* in skeletal muscle. The 2.6-fold greater abundance of *β-HAD* after the HFat diet than after the HCho diet indicates the marked transcriptional activation of this gene. However, the significance of the induced expression of *β-HAD* relative to the abundance and enzyme activity of the β-HAD protein remains unclear. In a previous study, the increase in β-HAD enzyme activity was 120% greater after 7-wk of a high-fat diet (62% of energy as fat) than after an isoenergetic high-carbohydrate diet (37% of energy as fat) (16). However, the consumption of a high-fat diet for 6 d by untrained subjects had little effect on β-HAD enzyme activity relative to the effect of a low-fat control diet (33% of energy as fat) (39). Furthermore, a diet moderately high in fat (53% of total energy), relative to a lower-fat diet (43% of energy as fat), fed for 4 wk also did not alter β-HAD enzyme activity (40). Taken collectively, these data suggest that with the short duration of the current study, it is unlikely that an increase in *β-HAD* would have been translated into significantly increased functional protein concentrations. However, with prolonged high-fat feeding, the increased gene transcript may support the maintenance of increased steady state enzyme abundance.

A surprising feature of the current study was the absence of a diet-mediated action on the expression of *CPT I* and *UCP3*, particularly because these genes are responsive to fatty acid exposure in vitro with the use of a variety of cell types, including myocytes (15, 41, 42). The absence of dietary-induced changes in gene expression in human skeletal muscle suggests that significant regulation of *CPT I* function is exerted via posttranslational control mechanisms (2).

An unresolved issue is the possible molecular mechanism selectively activating gene expression after a high-fat diet. Signaling to the nucleus by many fatty acids has been shown to occur via the multiple isoforms of the peroxisome proliferators–activated receptor (PPAR) family of transcription factors (9, 10, 43). PPAR-α and PPAR-γ isoform selective agonists activate the expression of the gene *FAT/CD36* (44, 45), suggesting a role for increased activity of these transcription factors after a high-fat diet. However, PPAR activation alone cannot fully account for the observed regulation of the genes *β-HAD*, *CPT I*, and *UCP3*. Interestingly, there is no evidence for a PPAR response element, upstream from *β-HAD* in humans (46), yet this gene was markedly activated by the HFat diet in the current study. In contrast, despite functional PPAR response elements, which can regulate the expression of *CPT I* (47) and *UCP3* (42), the expression of these genes did not change significantly with the HFat diet. Thus, the mechanisms linking the increased availability of dietary fatty acid to the regulation of *FAT/CD36* and *β-HAD* in human skeletal muscle are far from being understood; thus, more detailed molecular biological investigations are needed.

In summary, the results of the current study indicate that short-term consumption of a high-fat diet selectively increases the gene expression of *FAT/CD36* and *β-HAD* in human skeletal muscle. These actions failed to extend to the expression of *FABPpm*, *CPT I*, and *UCP3*. Consistent with the gene data, the abundance of FAT/CD36 protein increased significantly, but that of FABPpm did not change significantly. In addition to the significant regulation of enzyme activity by high-fat diets, these data show that one additional component in the adaptation to high-fat diets may be regulated by changes in the expression of genes in skeletal muscle.

We thank NN Tandon (Otsuka Research Maryland, Bethesda) and J Calles-Escandon (Smith Kline Beecham, Miami) for providing the antibodies for FAT/CD6 and FABPpm, respectively; Peter Fricker, Kieran Fallon, Andrew Garnham, and Bruce Hamilton for their excellent medical assistance; and Fabian Gargano, Robyn Hunter, and Bronywn Jones for their technical expertise. Special thanks are extended to the subjects for their participation in this study and to Nicola Cummings, Michelle Minehan, and Ben Desbrow for their care in preparing the diets. LMB, JAH, and MH designed the study; LMB, DJA, JAH, GRC collected the data; DC-S, RJT, and AB performed the data analysis; and DC-S wrote the manuscript.

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