Exercise training increases lipid metabolism gene expression in human skeletal muscle

Rebecca J. Tunstall, Kate A. Mehan, Glenn D. Wadley, Gregory R. Collier, Arend Bonen, Mark Hargreaves, and David Cameron-Smith. Exercise training increases lipid metabolism gene expression in human skeletal muscle. Am J Physiol Endocrinol Metab 283: E66–E72, 2002.—The effects of a single bout of exercise and exercise training on the expression of genes necessary for the transport and β-oxidation of fatty acids (FA), together with the gene expression of transcription factors implicated in the regulation of FA homeostasis were investigated. Seven human subjects (3 male, 4 female, 28.9 ± 3.1 yr of age, range 20–42 yr, body mass index 22.6 kg/m², range 17–26 kg/m²) underwent a 9-day exercise training program of 60 min cycling per day at 65% peak oxygen uptake (V\(\text{O}_2\) peak, 104 ± 14 W). On days 1 and 9 of the program, muscle biopsies were sampled from the vastus lateralis muscle at rest, at the completion of exercise, and again 3 h postexercise. Gene expression of key components of FA transport [FA translocase (FAT/CD36), plasma membrane-associated FA-binding protein (FABPpm), β-oxidation [carnitine palmitoyltransferase (CPT) I, β-hydroxyacyl-CoA dehydrogenase] and transcriptional control [peroxisome proliferator-activated receptor (PPAR\(\alpha\), PPAR\(\gamma\), PPAR\(\gamma\) coactivator 1, sterol regulatory element-binding protein-1c] were unaltered by exercise when measured at the completion and at 3 h postexercise. Training increased total lipid oxidation by 24% (\(P < 0.05\)) for the 1-h cycling bout. This increased capacity for lipid oxidation was accompanied by an increased expression of FAT/CD36 and CPT I mRNA. Similarly, FAT/CD36 protein abundance was also upregulated by exercise training. We conclude that enhanced fat oxidation after exercise training is most closely associated with the genes involved in regulating FA uptake across the plasma membrane (FAT/CD36) and across the mitochondrial membrane (CPT I).

β-oxidation (17, 38). With exercise training, these increments have been associated with the upregulation of membrane-associated FA transport proteins, FA-binding protein (FABPpm) (22), FA translocase (FAT/CD36) (5), the mitochondrial transporter carnitine palmitoyltransferase I (CPT I) (4), and a key enzyme of β-oxidation, β-hydroxyacyl-CoA dehydrogenase (β-HAD) (38).

The molecular mechanisms initiated by increased physical activity that enable the increased protein abundance of components of the FA uptake and β-oxidative pathway are undoubtedly complex. There are multiple steps important in the regulation of cellular protein, including gene transcription, mRNA stability, protein translation rate, translation efficiency, post-translational modifications, and protein degradation. As a significant and sustained physiological stressor, it is not surprising that endurance exercise may regulate protein level by modifications at multiple sites. Many studies have now demonstrated that a bout of sustained muscular activity exerts the capacity to transiently activate the expression of many genes (2). For example, GLUT4, hexokinase, and uncoupling protein (UCP)-3 gene expression are observed to peak from within 30 min to 3 h after exercise (23, 24).

Whether the expression of genes involved in FA uptake and β-oxidation is upregulated rapidly after exercise is not known. Moreover, it has not yet been determined whether prior short-term training has any impact on the regulation of these genes. Therefore, in the present study, we have examined the transient and chronic changes in key genes integral to FA uptake (FAT/CD36, FABPpm) and oxidation (CPT I, β-HAD) in skeletal muscle. The transient changes in gene expression were measured before and after 9 days of exercise training, a period known to increase FA oxidation in human skeletal muscle (38).

Transcriptional control of the key genes necessary for FA uptake and oxidation has been shown to be under the regulation of several transcription factors, including the peroxisome proliferator-activated recep-
tor (PPAR) isoforms PPARα and PPARγ (8, 30), the recently identified PPARγ coactivator-1 (PGC-1) (35, 40), and the sterol regulatory element-binding protein-1c (SREBP-1c) (12, 29). It has yet to be established whether changes in expression of these nuclear transcription factors precede the changes in the expression of genes involved in FA metabolism. A further aim of the present study was to examine the expression of FA-related transcription factors (PPARα, PPARγ, PGC-1, and SREBP-1c) before and after 9 days of exercise training.

**METHODS**

**Subjects.** Seven healthy, untrained subjects (3 male, 4 female, 28.9 ± 3.1 yr of age, range 20–42 yr, body mass index 22.6 kg/m² (range 17–26 kg/m²), peak oxygen uptake (VO\textsubscript{2 peak}) 37.1 ± 2.7 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}) volunteered to participate in the study (Table 1). Female subjects were premenopausal, and subjects were not on medication at the time of the intervention. Informed, written consent was obtained from each subject after a verbal and written explanation of the experimental protocol and its potential risks. The study was approved by the Deakin University Ethics Committee and was in accordance with National Health and Medical Research Council guidelines.

**Experimental protocol.** Seven days before commencement of the training protocol, subjects attended the laboratory for VO\textsubscript{2 peak} determination. This was measured with a metabolic cart (Gould Metabolic Systems, Dayton, OH) during incremental exercise to exhaustion on a cycle ergometer (Quinton Excilibur, Groningen, The Netherlands).

The exercise protocol consisted of cycle ergometer training at 63 ± 2% VO\textsubscript{2 peak} (104 ± 14 W) for 60 min/day for 9 consecutive days under direct supervision. On days 1 and 9 of the training period, subjects attended the laboratory in the morning having fasted overnight. For the 24 h preceding the test sessions, subjects consumed a standard diet (71% carbohydrate, 17% fat, 12% protein), abstained from the consumption of alcohol and caffeine, and refrained from strenuous exercise. The subjects had the lateral portion of one thigh prepared for needle biopsy sampling of the vastus lateralis muscle. Three small incisions were made through the skin to the deep fascia under local anesthesia. A resting venous blood sample from an antecubital vein and a muscle sample were immediately frozen and stored in liquid N\textsubscript{2} for subsequent analysis.

**Body mass, kg 67.7
BMI, kg/m\textsuperscript{2} 22.6
VO\textsubscript{2 during exercise trial, ml·kg\textsuperscript{-1}·min\textsuperscript{-1}} 23.1 ± 0.4
HR during exercise trial, beats/min 158 ± 2
Glucose, mmol/l 4.7 ± 0.1
Lactate, mmol/l 3.1 ± 0.7

Values are reported as means ± SE. BMI, body mass index; VO\textsubscript{2}, oxygen uptake; HR, heart rate. Comparison of 2 means was performed using t-test analysis (BM, BMI). Two-way ANOVA was performed to discriminate between differences due to the effect of training and/or time (VO\textsubscript{2} during exercise trial, HR during exercise trial, glucose, and lactate). A significant treatment effect for training was observed for HR. *P < 0.01.

**Table 1. Subject characteristics**

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**Minimum requirements for natural text:**

- The text should be readable and free of any errors.
- It should be structured in a way that is easy to follow and understand.
- It should use clear and concise language.
- It should be free of jargon and technical terms.

**Table 1. Subject characteristics**
membranes. Membranes were incubated for 2 h with either the monoclonal CD36 antibody (1:500) or the polyclonal FABPPM antibody (1:200). Secondary complexes were generated using anti-mouse IgG horseradish peroxidase secondary antibody (1:10,000; Santa Cruz Biotechnology, Santa Cruz, CA) for FAT/CD36 and donkey anti-rabbit IgG horseradish peroxidase-conjugated secondary antibody (1:3,000; Amersham) for FABPPM. Enhanced chemiluminescence detection (Hyperfilm-ECL; Amersham, Oakville, ON, Canada) was performed, with band densities obtained by densitometry.

Blood analysis. Blood samples were drawn from the intravenous catheter, placed into heparinized vials, and spun at 4°C. Plasma was stored at −80°C. Plasma glucose and lactate were measured in duplicate using an automated glucose/lactate analyzer (EML 105, Rodiometer, Copenhagen, Denmark). The mean intra-assay CV for the glucose and lactate assays were 0.1% and 3%, respectively.

Statistical analysis. All data are presented as means ± SE. Two-way ANOVA with repeated measures was used to determine the main effects of time and/or training on the response (VO2 during exercise, heart rate during exercise, and glucose, lactate, and gene expression). Post hoc analysis was performed to determine differences between groups with the Newman-Keuls test where appropriate. Paired-sample t-tests were performed to reveal differences pre- and posttraining. A value of P ≤ 0.05 was considered statistically significant.

RESULTS

Physiological variables. Mean intensity of the 60-min exercise bout was 63 ± 2% VO2 peak. Heart rate significantly decreased posttraining (P = 0.009), whereas plasma lactate and glucose levels remained unchanged (Table 1). The average RER during the 60-min exercise was significantly lower posttraining (P = 0.016) (Fig. 1). This corresponded to a small, but significant, increase in fat oxidation (4.5 g) during the 60-min exercise (P = 0.008).

FA transporter mRNA and protein. In untrained subjects, a single bout of cycle ergometer exercise for 1 h did not alter the mRNA abundance of FAT/CD36 immediately after the completion of the exercise bout or again when measured 3 h postexercise (Fig. 2A). However, after 9 days of exercise there was a significant (36%, P = 0.04, treatment effect) increase in FAT/CD36 gene expression compared with the untrained state (Fig. 2A). With this exercise training, FAT/CD36 was again unaltered by an acute exercise bout, with no alteration in gene expression compared with the resting values, of the samples analyzed immediately and 3 h after the exercise bout. The increased level of gene expression posttraining was matched with a significant increase in FAT/CD36 protein (Fig. 2A).
protein abundance (Fig. 3A, \( P = 0.03 \)). In contrast, \( \text{FABP}_{\text{PM}} \) gene expression was unaltered by both acute exercise bout and exercise training (Fig. 2B). Similarly, \( \text{FABP}_{\text{PM}} \) protein content did not differ in the trained, compared with untrained, muscle samples (Fig. 3B).

**Oxidative enzymes.** \( \text{CPT} \) I gene expression did not change after the 60-min bout of exercise or at 3 h postexercise. However, after 9 days of exercise, there was a significant (57%, \( P = 0.03 \), treatment effect) increase in \( \text{CPT} \) I gene expression (Fig. 2C) compared with the untrained state. \( \beta \)-HAD gene expression was unchanged after the acute exercise bout and remained unchanged 3 h postexercise. No change in \( \beta \)-HAD gene expression was observed after exercise training.

**Transcription factors in skeletal muscle.** Analysis of four FA-associated transcription factors demonstrated no acute regulation of their gene expression immediately and 3 h after the single exercise bout (Fig. 4). The expression of \( \text{PPAR}_{\alpha} \), \( \text{SREBP}-1c \), and \( \text{PGC}-1 \) genes all remained unaltered by prior training and after the last exercise bout. However, the gene expression of \( \text{PPAR}_{\gamma} \) was significantly (\( P = 0.04 \), treatment effect) reduced after training (Fig. 4B).

**DISCUSSION**

In the present study, we have examined, in human skeletal muscle, the effects of a single bout of exercise and exercise training on the expression of genes involved in FA uptake and oxidation, as well as their regulatory transcription factors. Several novel observations were made in this study. First, an acute bout of exercise (60 min, 63% \( \dot{V} \text{O}_2 \) peak), whether performed in the untrained or the trained state, did not significantly alter the expression of genes involved in FA uptake and metabolism (\( \text{FAT/CD36}, \text{FABP}_{\text{PM}}, \text{CPT} \) I, \( \beta \)-HAD) or their transcription factors (\( \text{PPAR}_{\alpha}, \text{PPAR}_{\gamma}, \text{PGC}-1 \), \( \text{SREBP}-1c \)) measured immediately at the cessation of exercise or 3 h postexercise. Second, the exercise training (9 days, 60 min/day, 63% \( \dot{V} \text{O}_2 \) peak) increased FA oxidation during exercise; yet this was accompanied by an increase in the expression of just two of the selected genes (\( \text{FAT/CD36} \) and \( \text{CPT} \) I), with the expression of all remaining genes remaining constant. The exception was the expression of \( \text{PPAR}_{\gamma} \), which was lowered by training. Therefore, the capacity for increased FA oxidation induced by exercise training is most closely associated with genes involved in regulating FA uptake across the plasma membrane (\( \text{FAT/CD36} \)) and across the mitochondrial membrane (\( \text{CPT} \) I).
In this study, 9 days of repeated exercise increased total fat oxidation over the course of 1 h by 24%, which equated to 4.5 g of additional fat oxidized. Adaptations of a similar magnitude have been reported previously in comparable short-term training programs (38), as well as in training programs in excess of 8 wk (14, 19). Despite the current debate regarding the relative contributions of intracellular lipids and blood-borne FA to lipid oxidation in muscle (11), there is evidence to suggest that increases in muscle fat oxidative capacity require a concurrent increase in FA uptake into the skeletal muscle cell (21). The results of the present study demonstrated a significant increase (36%) in FAT/CD36 gene expression in response to the moderate-intensity exercise training. This was accompanied by a comparable increase in the protein abundance of FAT/CD36 in trained, compared with untrained, skeletal muscle. These data are consistent with the observation that mRNA abundance of FAT/CD36 is upregulated by muscle contractile activity (5), which in this study was demonstrated to enhance FAT/CD36 protein abundance. Thus an increase in FAT/CD36 gene expression, translated into increased protein content, provides a plausible mechanism whereby FA uptake and oxidation are increased in trained skeletal muscle of humans.

Skeletal muscle FA transport may involve a family of membrane-bound putative transporters. An additional member of this family, FABP_Pm was also investigated in this study. FABP_Pm protein abundance has been shown to increase in human skeletal muscle after single-legged exercise training (22). In the present study, there was no evidence of increased FABP_Pm mRNA or protein after short-term training in humans. It is possible, then, that the absence of the response in this study is due to the lower intensity of exercise stimulation or the total time period of activation. Alternatively, it might also be suggested that the current study provides evidence of selectivity in the regulation of the analyzed FA transport proteins FABP_Pm and FAT/CD36. It has been speculated that these FA transporters may operate cooperatively (28), although recent data demonstrate differential regulation in insulin-resistant tissues (27). Further analysis of the actions of FAT/CD36 and FABP_Pm to determine their independent and possible cooperative roles in the transport of FA across the muscle plasma membrane is required.

Mitochondrial biogenesis is an important component of the adaptive response to endurance exercise training (18); yet the impact of sustained muscular activity on the expression of nuclear gene-encoded mitochondrial proteins is highly variable. In chronically electrically stimulated rat tibialis anterior, the expression of a nuclear-encoded subunit of cytochrome c increased only marginally after 5 days (13). This increase was due to increased mRNA stability rather than to the activation of mRNA transcription. In contrast, it has recently been demonstrated that there is a rapid induction of nuclear genes involved in mitochondrial function, including CPT I and UCP-3, after an exhaustive exercise bout at the completion of 5 days of training (34). The results of the present study demonstrate that a single exercise bout, of 1 h duration, failed to elicit increased mRNA abundance of CPT I postexercise, although with training, CPT I mRNA levels were increased. It is not possible to conclude whether these increases in CPT I gene expression are the net result of increased mRNA synthesis or mRNA stability. Furthermore, it is difficult to ascertain why there was no adaptive increase in β-HAD mRNA, given the downstream role of this enzyme in FA oxidation. These data suggest that the increased availability of CPT I mRNA, and thus protein synthesis, represents the key site for the adaptive regulation of β-oxidative capacity with exercise training. The downstream enzymes, such as β-HAD, are potentially in sufficient excess to catalyze the increased FA flux after exercise training.

From the present study, the genes upregulated by training (FAT/CD36 and CPT I) may have been acti...
vated progressively subsequent to each exercise bout. It could be hypothesized that the expression of these genes, which were responsive only to repeated exercise (training), may have occurred as a function of the cumulative actions of a sensitive transcription factor pathway (32). Thus this study sought to examine whether the gene expression of several transcriptional regulators of FA uptake and oxidation could account for the increased abundance of FAT/CD36 and CPT I mRNA. Important in the regulation of FA homeostasis is the PPAR family of transcription factors, of which there are three distinct subtypes (α, γ, and δ) (9). Recently, Horowitz et al. (19) demonstrated a twofold induction in skeletal muscle PPARα protein content after 12 wk of endurance training in women. This may reflect the role of PPARα in regulating lipid-sensitive gene expression (27) but also the possible link between PPARα activation and improved skeletal muscle insulin action (42). However, the present study failed to demonstrate a change in the expression of mRNA levels of PPARα, either after a single exercise bout or after 9 days of training. Although the training protocol adopted by Horowitz et al. was longer (12 wk) and was undertaken at a lower intensity (50% \( V_{\text{O2 peak}} \)), it is unlikely that these differences would significantly modulate the synthesis of PPARα, suggesting that significant posttranslational control might be important in the regulation of PPARα protein abundance.

The PPARγ isoform has been identified as a potential regulator of skeletal muscle FA metabolism, with the PPARγ-specific agonist troglitazone upregulating UCP3 mRNA in rat (31) and FAT/CD36 protein in human skeletal muscle cell lines (8). Additionally a significant positive relationship has been demonstrated between the abundance of the PPARγ and CPT I genes in human skeletal muscle (25). However, the present study has demonstrated a significant (20%) decrease in PPARγ mRNA expression after 9 days of training, demonstrating a dissociation of the relationship between PPARγ and CPT I with exercise training. These data suggest that factors other than PPARγ mRNA abundance are important in the adaptive control of CPT I, but does not exclude the possibility of a role for the PPARγ protein.

The transcriptional control of FA-specific genes has been shown to extend beyond PPAR regulation. Although there have apparently been no specific examination of the actions of PGC-1 and SREBP-1c in human skeletal muscle, expression of these proteins has been identified in this tissue (6, 26). Goto et al. (16) examined the responsiveness of PGC-1 gene expression to exercise in rat skeletal muscle. Two hours of swimming exercise training per day for 7 days resulted in a 163% increase in epitrochlearis muscle PGC-1 mRNA. In contrast, we were unable to demonstrate any change in PGC-1 mRNA levels in human skeletal muscle either acutely or after 9 days of endurance training. Additionally, no impact of cycle ergometer exercise was demonstrated on the gene expression of SREBP-1c.

Recently, there has been much interest in sex differences in lipid metabolism after endurance exercise, with many (15, 20, 39) studies demonstrating that females have a lower RER during submaximal exercise. However, the impact of endurance training on enzymatic adaptations appears to be similar between the sexes (7). For this reason, we sought not to examine sex differences in detail. On analysis, no significant sex differences were observed in gene expression responses to exercise training.

In the present study, the mRNA abundance of FAT/CD36, a plasma membrane FA transporter, and CPT I, the rate-limiting mitochondrial FA transporter, were both increased after 9 days of moderate-intensity exercise training. These data were further corroborated with the analysis of FAT/CD36 protein abundance matching the activation of the gene. However, genes coding for complementary components of the FA transport and oxidation pathways, FABPPM and β-HAD, were unaltered by exercise training. Thus the adaptive increase in FA uptake and oxidation after exercise training in humans is linked to activation of key genes that encode responsive components of the FA-uptake and oxidative pathway. Further analysis was made of the gene expression of regulatory transcription factors implicated in the regulation of skeletal muscle FA metabolism. Of the measured transcription factor genes (PPARα, PPARγ, PGC-1, and SREBP-1c), all failed to increase either after an acute exercise bout or after training. Therefore, the results of the present study were unable to provide evidence that increased gene expression of transcription factors mediates the increased mRNA abundance of FAT/CD36 and CPT I after exercise training.

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