Exercise, sex, menstrual cycle phase, and 17β-estradiol influence metabolism-related genes in human skeletal muscle

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Fu MH, Maher AC, Hamadeh MJ, Ye C, Tarnopolsky MA. Exercise, sex, menstrual cycle phase, and 17β-estradiol influence metabolism-related genes in human skeletal muscle. Physiol Genomics 40: 34–47, 2009. First published October 6, 2009; doi:10.1152/physiolgenomics.00115.2009.—Higher fat and lower carbohydrate and amino acid oxidation are observed in women compared with men during endurance exercise. We hypothesized that the observed sex difference is due to estrogen and that menstrual cycle phase or supplementation of men with 17β-estradiol (E2) would coordinately influence the mRNA content of genes involved in lipid and/or carbohydrate metabolism in skeletal muscle. Twelve men and twelve women had muscle biopsies taken before and immediately after 90 min of cycling at 65% peak oxygen consumption (V\textsubscript{O2peak}). Women were studied in the midfollicular (Fol) and midluteal (Lut) phases, and men were studied after 8 days of E2 or placebo supplementation. Targeted RT-PCR was used to compare mRNA content for genes involved in transcriptional regulation and lipid, carbohydrate, and amino acid metabolism. Sex was the greatest predictor of substrate metabolism gene content. Sex affected the mRNA content of FATm, FABPc, SREBP-1c, mtGPAT, PPAR\textsubscript{0}, PPAR\textsubscript{α}, CPTI, TFP-α, GLUT4, HKII, PFK, and BCOADK (P < 0.05). E2 administration significantly (P < 0.05) affected the mRNA content of PGC-1α, PPAR\textsubscript{α}, TFP-α, CPTI, SREBP-1c, GLUT4, GS-1, and AST. Acute exercise increased the mRNA abundance for PGC-1α, HSL, FABPc, CPTI, GLUT4, HKII, and AST (P < 0.05). Menstrual cycle had a small effect on PPAR\textsubscript{α}, GP, and glycogenin mRNA content. Overall, women have greater mRNA content for several genes involved in lipid metabolism, which is partially due to an effect of E2 on lipid metabolism; protein metabolism; carbohydrate metabolism; messenger RNA

A number of studies have found that women oxidize more fat and less carbohydrates (CHO) during endurance exercise compared with men (9, 20, 26, 32, 43, 49, 54–56, 66, 68). Specifically, women have higher whole body lipolysis and greater skeletal muscle uptake of plasma free fatty acids (FFAs) (44), potentially the result of sex, acute exercise, menstrual cycle phase, and E2 supplementation on the mRNA content of genes involved in lipid, CHO, and protein metabolism in human skeletal muscle. Specifically, we evaluated the mRNA content of genes involved in the transcriptional regulation of mitochondrial biogenesis (PGC-1α) and lipid metabolism (PPAR\textsubscript{α}, PPAR\textsubscript{γ}, and PPAR\textsubscript{δ}), sarcolemmal [FATm, cytosolic fatty acid binding protein (FABPc)], and mitochondrial (CPTI, CPTII) FFA transport, IMCL [sterol regulatory element binding protein (SREBP)-1c], membrane lipid (SREBP-2), and mitochondrial glycerol phosphate acyltransferase (mGPAT) synthesis, IMCL hydrolysis [hormone-sensitive lipase (HSL)], β-oxidation [very long-chain acyl-CoA dehydrogenase (VLCAD)], trifunctional protein α-subunit (TFP-α), sarcolemmal glucose transport [glucose transporter 4 (GLUT4)], glucose phosphorylation [hexokinase II (HKII)], glycogen hydrolysis [glycogen phosphorylase (GP)], glycolysis [phosphofructokinase (PFK)], glycogen synthesis [glycogenin, glycogen synthase 1 (GS-1), glycogen synthetase kinase-3α (GSK3α)], transamination mechanisms for the observed differences in substrate oxidation between men and women.

Given that sex differences in lipid metabolism are predominantly apparent only during exercise (9, 20, 26, 32, 43, 49, 54–56, 66, 68), and that acute exercise is a potent stimulus for mRNA induction (6), it is important to consider sex differences in the response to exercise. Acute exercise increases the mRNA content for some of the known regulators of mitochondrial biogenesis: peroxisome proliferator-activated receptor (PPAR\textsubscript{γ}) coactivator-1α (PGC-1α) (41, 47, 51, 75), PPAR\textsubscript{α} (51, 78), PPAR\textsubscript{δ} (78), and lipid transporters FAT/CDF3 (31), FABPpm (31), and carnitine palmitoyltransferase (CPTI), plus LPL (50, 51).

It has been hypothesized that sex hormones may contribute to observed metabolic differences. This can be achieved by studying menstrual cycle differences in women and/or 17β-estradiol (E2) administration in men. The influence of menstrual cycle phase on fat oxidation during endurance exercise has revealed inconsistent results (14, 22, 25, 30, 79, 80), although some studies found that FFA oxidation was greater during the luteal (Lut) compared with the follicular (Fol) phase (22, 79, 80). Administration of E2 to amenorrheic women (61) and men (8, 13, 23) resulted in a lower respiratory exchange ratio (RER) during exercise, and more specifically lower whole body CHO and leucine oxidation and higher lipid oxidation (23), in men. E2 supplementation increased plasma FFA concentration (61) and reduced glucose rate of appearance (R\textsubscript{a}) (8, 13, 61), rate of disappearance (R\textsubscript{d}), and metabolic clearance rate (8, 13). The consistency of the aforementioned results and the directional similarity to the sex differences seen in metabolism during exercise implies that E2 is likely an important mediator of substrate selection favoring higher fat oxidation.

The purpose of this study was to comprehensively evaluate the result of sex, acute exercise, menstrual cycle phase, and E2 supplementation on the mRNA content of genes involved in lipid, CHO, and protein metabolism in human skeletal muscle. Specifically, we evaluated the mRNA content of genes involved in the transcriptional regulation of mitochondrial biogenesis (PGC-1α) and lipid metabolism (PPAR\textsubscript{α}, PPAR\textsubscript{γ}, and PPAR\textsubscript{δ}), sarcolemmal [FATm, cytosolic fatty acid binding protein (FABPc)], and mitochondrial (CPTI, CPTII) FFA transport, IMCL [sterol regulatory element binding protein (SREBP)-1c], membrane lipid (SREBP-2), and mitochondrial glycerol phosphate acyltransferase (mGPAT) synthesis, IMCL hydrolysis [hormone-sensitive lipase (HSL)], β-oxidation [very long-chain acyl-CoA dehydrogenase (VLCAD)], trifunctional protein α-subunit (TFP-α), sarcolemmal glucose transport [glucose transporter 4 (GLUT4)], glucose phosphorylation [hexokinase II (HKII)], glycogen hydrolysis [glycogen phosphorylase (GP)], glycolysis [phosphofructokinase (PFK)], glycogen synthesis [glycogenin, glycogen synthase 1 (GS-1), glycogen synthetase kinase-3α (GSK3α)], transamination

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[aspartate transaminase (AST)], dehydrogenation [branched-chain 2-oxo-acid dehydrogenase (BCOAD)], and phosphorylation/inactivation of BCOAD kinase (BCOADK). We hypothesized that 1) the mRNA content for genes involved in lipid metabolism would be higher in women compared with men before and after acute exercise; 2) $E_2$ supplementation in men would alter genes in a directionally consistent manner to those found to be differentially expressed between the sexes; and 3) menstrual cycle would have a less robust influence on mRNA content.

**MATERIALS AND METHODS**

**Subjects**

Twenty-four young (22 ± 2 yr) healthy, nonsmoking, nonobese, recreationally active men (n = 12) and women (n = 12) participated in the present study. The female subject characteristics were age 23 ± 2 yr, weight 62 ± 3 kg, fat-free mass (FFM) 44 ± 1 kg, and peak oxygen consumption ($\dot{V}$O$_{2\text{peak}}$) 55 ± 3 ml O$_2$·kg$^{-1}$·min$^{-1}$. The male subject characteristics were age 23 ± 2 yr, weight 78 ± 3 kg, FFM 63 ± 2 kg, and $\dot{V}$O$_{2\text{peak}}$ 55 ± 2 ml O$_2$·kg$^{-1}$·min$^{-1}$. All subjects gave informed written consent before participation. The study was approved by the McMaster University Hamilton Health Sciences Human Research Ethics Board and conformed to Declaration of Helsinki guidelines.

**Study Design**

The study design and testing procedures have been described previously (13, 14, 23). In brief, women were tested in the mid-Fol (days 7–9) and mid-Lut (days 19–21) phases of the menstrual cycle, and to a lesser extent oral contraceptive use, had only minor influences on substrate selection (14).

**Preparation of RNA**

Total RNA was extracted from frozen human muscle according to Turnpolsky et al. (67) and Mahoney et al. (41). Briefly, 25 mg of human skeletal muscle biopsy was homogenized in 1 ml of TRIzol reagent (Invitrogen, Carlsbad, CA) at 4°C and left at room temperature for 5–10 min. To this, 0.2 ml of chloroform was added, and the mixture was vortexed for 15 s and centrifuged at 12,000 g and 4°C for 15 min. The supernatant aqueous layer was transferred to a fresh tube and mixed with 0.5 ml of isopropanol ethanol, left to stand at ~22°C for 10 min, and centrifuged at 12,000 g and 4°C for 10 min. The RNA pellet was washed twice with 0.5 ml of 75% ethanol, air dried, and dissolved in 14 μl of diethyl pyrocarbonate-treated double-distilled H$_2$O. Aliquots of 2 μl each were made and stored at ~80°C. The concentration and purity of the RNA were determined with a UV spectrophotometer (Shimadzu UV-1201; Mandel Scientific, Guelph, ON, Canada) by measuring the absorbance at 260 [optical density (OD$_{260}$)] and 280 (OD$_{280}$) nm. Measurements were done in duplicate and had an average coefficient of variation (CV) of <10%. The purity (OD$_{260}$/OD$_{280}$) for each of the samples was >1.5. RNA integrity was assessed in a randomly chosen subset of samples by agarose gel electrophoresis, and the OD ratio of 28S to 18S rRNA was consistently >1 for each sample checked, indicating high-quality RNA. Because of technical difficulties we could only use the mRNA from 11 men and 11 women.

**TaqMan Real-Time RT-PCR**

Before analysis by TaqMan real-time RT-PCR, RNA samples were treated with DNase 1 for 25 min at 37°C to remove any contaminating DNA with DNA-free (Ambion, Austin, TX) according to the manufacturer’s instructions. Specific primers and probe to each target gene (Table 2) were designed based on the cDNA sequence in GenBank (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) with primer 3 designer (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Their specificity was checked with Blast (http://www.ncbi.nlm.nih.gov/BLAST/). Their thermal dynamics was manipulated by calculating delta G with Analyzer of Oligo (http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/Default.aspx). All target gene probes were labeled with FAM at their 5’ ends and BHQ1 at their 3’ ends. Human β2-microglobulin (β2-M) was used as an internal standard. Human β2-M primers and probe were same as those previously described by Mahoney et al. (41). To validate human β2-M as an internal standard for quantification of mRNA content of the target genes with TaqMan real-time RT-PCR (41), we tested the effect of acute exercise, sex, and menstrual cycle phase on the mRNA content of β2-M in the same samples used for this study under the same RT-PCR reaction conditions as used by Mahoney et al. (41) and found no effect of any of these parameters on mRNA content. Duplex RT-PCR was performed on the iCycler real-time PCR system (Bio-Rad Laboratories, Hercules, CA) with One-Step TaqMan RT-PCR Master Mix Reagents (Roche, Branchburg, NJ, no. 4309169) according to the manufacturer’s instruction with target gene primers and probe and internal standard gene primers and probe in the same reaction. We optimized each PCR reaction condition so that the amplification efficiency for both target gene and internal reference was close to 1. The difference between the slopes of the regression curves of each target gene and the corresponding internal standard gene [threshold cycle (C$_T$) vs. amount of RNA] was <10%. Agarose gel electrophoresis was used to confirm the specificity of the priming. RT-PCR profiling was 1 cycle at 48°C for 30 min; 1 cycle at 95°C for 10 min; 50 cycles at 95°C for 15 s and at 60°C for 1 min; and 1 cycle at 4°C for ∞. All samples were run in duplicate simultaneously with RNA- and RT-negative controls. Fluorescence emission was detected through a filter corresponding to the reporter dye at the 5’ end of each probe, and C$_T$ was automatically calculated and displayed.

**Statistical Analysis**

Statistical analyses on mRNA expression of the genes tested were performed on linear data 2$^{-C_T}$ for evaluation of internal standards and 2$^{-ΔC_T}$ for target gene normalized with internal reference (39). Data on sex differences about target gene mRNA expression were analyzed

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**Table 1. Serum hormone concentrations for women during follicular and luteal phase of menstrual cycle and men after 8 days of placebo or $E_2$ supplementation**

<table>
<thead>
<tr>
<th></th>
<th>Fol Women</th>
<th>Lut Women</th>
<th>Pl Men</th>
<th>$E_2$ Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>17β-Estradiol, nmol/l</td>
<td>125±45</td>
<td>203±75</td>
<td>129±13</td>
<td>946±167‡</td>
</tr>
<tr>
<td>Testosterone, nmol/l</td>
<td>0.9±0.1</td>
<td>0.8±0.0</td>
<td>21±1*</td>
<td>16±1‡</td>
</tr>
<tr>
<td>Progesterone, nmol/l</td>
<td>2.0±0.1</td>
<td>6.0±2.0</td>
<td>3.0±0.3</td>
<td>2.3±0.2‡</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 13 women and 11 men. Fol, follicular phase; Lut, luteal phase; Pl, placebo; $E_2$, 17β-estradiol supplemented. *Significantly different from women in both Fol and Lut phases, P < 0.05; ‡significantly different from women in Fol phase, P < 0.05; †significantly different from Pl men, P < 0.05. Data are summarized and combined from Refs. 13 and 14.
with a two-way ANOVA of sex by exercise, with sex as a between-group factor and exercise as a within-group factor. Data on menstrual cycle phase effect and E2 supplementation on the target gene mRNA expression were analyzed with a two-way repeated-measurement ANOVA, of menstrual cycle phase/E2 by exercise, with both cycle phase and E2 supplementation as within-group factors. Tukey’s honestly significant difference (HSD) post hoc test was performed only when the interaction between sex and exercise or between menstrual phase and exercise was significant (P < 0.05). All data are expressed as mean ± SE fold change between sexes, menstrual cycle phases, E2 supplementation (±SE) or pre- and postexercise, using 2^−ΔΔCt. All analyses were done with computerized statistics software (Statistica; Statsoft, Tulsa, OK).

RESULTS

Mitochondrial Biogenesis: PGC-1α

Acute exercise increased PGC-1α mRNA content in men (7.9 ± 3.7-fold, P = 0.047) and Lut women (3.6 ± 1.7-fold, P = 0.021) but not Fol women (Fig. 1A). There was no significant difference between men and women, Fol and Lut, or before and after exercise. E2 significantly attenuated the increase in PGC-1α mRNA content during exercise (P = 0.041) but not the resting/basal PGC-1α mRNA content (Fig. 1B).
**Lipid Metabolism**

**PPARα.** Fol women had higher PPARα mRNA content compared with men (1.6 ± 0.3-fold, *P = 0.04*) (Fig. 1C). After exercise, Lut women had higher PPARα mRNA content compared with men (1.4 ± 0.2-fold, *P = 0.04*). In men, E2 supplementation increased the mRNA content of PPARα at rest and after exercise (1.4 ± 0.3-fold, *P = 0.038*) (Fig. 1D). Exercise alone did not significantly alter PPARα mRNA content in men (Pl and E2) or women (Fol and Lut) (Fig. 1, C and D). There was no significant difference between Fol and Lut, before or after exercise (Fig. 1C).

**PPARδ.** Fol women had higher PPARδ mRNA content compared with men at rest (2.3 ± 0.4-fold, *P = 0.014*) but not after exercise (Fig. 1E). Fol women had higher PPARδ mRNA content compared with Lut women, before and after exercise (1.7 ± 0.3-fold, *P = 0.0007*) (Fig. 1E). E2 supplementation increased the mRNA content of PPARδ at rest (2.1 ± 0.5-fold, *P = 0.034*) (Fig. 1F). Exercise did not significantly alter PPARδ content in women (Fol and Lut) (Fig. 1E). There was no significant difference between Lut women and men, before or after exercise (Fig. 1E).

**PPARγ.** Sex, exercise, menstrual cycle phase, and E2 supplementation did not significantly alter PPARγ mRNA content (Fig. 1, G and H).

**β-Oxidation**

**VLCAD.** VLCAD mRNA content was not significantly altered by exercise, sex, menstrual phase, or E2 supplementation (Fig. 2, A and B).

**TFP-α.** Lut women had higher TFP-α mRNA content compared with men (2.4 ± 0.5-fold, *P = 0.021*) (Fig. 2C). E2 significantly increased mRNA content of TFP-α (1.5 ± 0.3-fold, *P = 0.033*) (Fig. 2D). Exercise did not significantly alter the TFP-α mRNA content in men and women (Fol and Lut) (Fig. 2, C and D). There was no significant difference between Fol and Lut, before or after exercise (Fig. 2C).

**Sarcocellular Fatty Acid Transport**

**FATm.** Women had higher FATm mRNA content compared with men (Fol 3.3 ± 1.1-fold, *P = 0.039*; Lut 2.2 ± 0.6-fold, *P = 0.05*) (Fig. 3A). Exercise, menstrual cycle phase, and E2 did not significantly alter FATm mRNA content in men or women (Fig. 3, A and B).
**FABPc.** Exercise increased FABPc mRNA content in men (1.2 ± 0.1-fold, \(P = 0.004\)) and women (Fol 1.1 ± 0.2-fold, \(P = 0.005\); Lut 1.6 ± 0.2-fold, \(P = 0.005\)), with Lut women increasing to a greater extent than men (\(P = 0.021\)). Exercise increased FABPc mRNA content in men on E2 and Fol, before and after exercise. \(\beta_2\)-Microglobulin was used as an internal standard. Values are means ± SE.

**Mitochondrial Fatty Acid Transport: CPTI and CPTII**

Exercise increased CPTI mRNA content in men (1.2 ± 0.1-fold, \(P = 0.014\)) and Lut women (1.5 ± 0.3-fold, \(P = 0.014\)) (Fig. 4A). Fol women had higher CPTI mRNA content compared with men (1.5 ± 0.2-fold, \(P = 0.023\)). There was no significant difference between Fol and Lut women, before or after exercise (Fig. 4A). E2 significantly increased CPTI mRNA content before and after exercise (1.3 ± 0.2-fold, \(P = 0.041\)) (Fig. 4B).

CPTII mRNA content was not significantly altered by exercise, sex, menstrual phase, or E2 supplementation (Fig. 4, C and D).

**IMCL Synthesis**

**SREBP-1c.** Women had higher SREBP-1c mRNA content compared with men (Fol 3.1 ± 1.0-fold, \(P = 0.014\); Lut 4.0 ± 1.5 fold, \(P = 0.011\)) (Fig. 5A). E2 significantly increased SREBP-1c before and after exercise (2.9 ± 1.3-fold, \(P = 0.05\)) (Fig. 5B). Exercise and menstrual cycle phase did not significantly alter SREBP-1c mRNA content (Fig. 5, A and B).

**mtGPAT.** Women had higher mtGPAT mRNA content compared with men (Fol 2.0 ± 0.3-fold, \(P = 0.002\); Lut 1.6 ± 0.2-fold, \(P = 0.004\)) (Fig. 5C). E2 significantly increased mtGPAT (1.3 ± 0.2-fold, \(P = 0.043\)) (Fig. 5D). Exercise did not significantly alter mtGPAT mRNA content in men or women (Fol and Lut) (Fig. 5, C and D). There was no significant difference between Fol and Lut women, before or after exercise (Fig. 5C).

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**Fig. 2.** Skeletal muscle mRNA and protein content of very long-chain acyl-CoA dehydrogenase (VLCAD) and trifunctional protein α-subunit (TFP-α). A: VLCAD mRNA content in men and women (Fol and Lut). B: VLCAD mRNA content in men on Pl and E2, before and after exercise. C: TFP-α mRNA content in men and women (Fol and Lut). ËLut women higher than men (\(P = 0.021\)). D: TFP-α mRNA content in men on Pl and E2, before and after exercise. †Men on E2 higher than men on Pl (\(P = 0.033\)). \(n = 11\) men and 11 women. ËMicroglobulin was used as an internal standard. Values are means ± SE.

**Fig. 3.** Skeletal muscle mRNA content of membrane fatty acid transport protein 1 (FATm) and cytosolic fatty acid binding protein (FABPc). A: FATm mRNA content in men and women (Fol and Lut). †Fol (\(P = 0.039\)) and Lut (\(P = 0.05\)) women higher than men. B: FATm mRNA content in men on Pl and E2, before and after exercise. C: FABPc mRNA content in men and women (Fol and Lut). *Postexercise higher than preexercise (men, \(P = 0.004\); Fol women, \(P = 0.005\); Lut women, \(P = 0.005\)). †Postexercise Lut women increased to a greater extent than men (\(P = 0.001\)); ‡Fol (\(P = 0.05\)) and Lut (\(P = 0.008\)) women higher than men. D: FABPc mRNA content in men on Pl and E2, before and after exercise. *Postexercise higher than preexercise (\(P = 0.004\)). \(n = 11\) men and 11 women. ËMicroglobulin was used as an internal standard. Values are means ± SE.
Membrane Lipid Synthesis: SREBP-2

SREBP-2 mRNA content was not significantly altered by exercise, sex, menstrual phase, or E2 supplementation (Fig. 6, A and B).

IMCL Hydrolysis: HSL

Exercise significantly increased HSL mRNA content in men only (1.4±0.4-fold, *P = 0.037) (Fig. 6, C and D). There was no significant difference between men and women (Fol and Lut) or between Fol and Lut women, before or after exercise (Fig. 6C). E2 supplementation had no effect on HSL mRNA content (Fig. 6D).

Glucose Transport and Glucose Phosphorylation

GLUT4. Acute exercise significantly increased the mRNA content of GLUT4 in men (2.1±0.5-fold, *P = 0.002) and Lut women (2.0±0.3-fold, *P = 0.002) (Fig. 7A). No significant exercise effect was detected for the mRNA content of GLUT4 in Fol women (Fig. 7A). In contrast, Fol women had a significantly higher GLUT4 mRNA content than men at rest (2.4±0.7-fold, *P = 0.032) (Fig. 7A). Fol women tended to have a higher mRNA content of GLUT4 than Lut women at rest (2.2±0.6-fold, *P = 0.056) (Fig. 7A). E2 supplementation significantly increased the mRNA content of GLUT4 (2.0±0.5-fold, *P = 0.025) (Fig. 7B). There was no significant difference in GLUT4 mRNA content between Lut women and men before and after exercise (Fig. 7, A and B).

Hexokinase II. Exercise significantly increased the mRNA content of HKII in men (2.0±0.5-fold, *P = 0.03), Fol women (1.5±0.3-fold, *P = 0.04), and Lut women (2.4±0.4-fold, *P = 0.04) (Fig. 7, C and D). Fol women had higher HKII mRNA content than men at rest (2.4±0.6-fold, *P = 0.016) before and after exercise (Fig. 7C). There was no significant difference in mRNA content of HKII between Lut women and men.

Fig. 4. Skeletal muscle mRNA content of carnitine palmitoyltransferase (CPT)I and CPTII. A: CPTI mRNA content in men and women (Fol and Lut). *Postexercise higher than preexercise (*P = 0.014); ‡Fol women higher than men (*P = 0.023). B: CPTI mRNA content in men on Pl and E2, before and after exercise. †Men on E2 higher than men on Pl (*P = 0.041). C: CPTII mRNA content in men and women (Fol and Lut). D: CPTII mRNA content in men on Pl and E2, before and after exercise. n = 11 men and 11 women. β2-Microglobulin was used as an internal standard. Values are means ± SE.

Fig. 5. Skeletal muscle mRNA content of sterol regulatory element binding protein (SREBP)-1c and mitochondrial glycerol phosphate acyltransferase (mtGPAT). A: SREBP-1c mRNA content in men and women (Fol and Lut). †Fol (*P = 0.014) and Lut (*P = 0.011) women higher than men. B: SREBP-1c mRNA content in men on Pl and E2, before and after exercise. †Men on E2 higher than men on Pl (*P = 0.05). C: mtGPAT mRNA content in men and women (Fol and Lut). †Fol (P = 0.002) and Lut (P = 0.004) women higher than men. D: mtGPAT mRNA content in men on Pl and E2, before and after exercise. †Men on E2 higher than men on Pl (P = 0.043). n = 11 men and 11 women. β2-Microglobulin was used as an internal standard. Values are means ± SE.
Menstrual cycle phase and E2 supplementation had no influence on HK II mRNA content (Fig. 7, C and D). Glycogenolysis and Glycolysis

**GP.** GP mRNA content was significantly higher in Fol versus Lut women (1.6 ± 0.4-fold, *P* = 0.041) (Fig. 8A). There was no significant difference due to sex, exercise, or E2 supplementation (Fig. 8, A and B).

**PFK.** Men had a significantly higher PFK mRNA content than Lut women (3.4 ± 1.5-fold, *P* = 0.031) (Fig. 8C). Exercise, menstrual cycle phase, and E2 supplementation did not affect PFK mRNA content (Fig. 8, C and D).

**Glycogen Synthesis**

**Glycogenin.** The mRNA content of glycogenin in Lut women was significantly higher than that in Fol women (1.2 ± 0.2-fold, *P* = 0.02) (Fig. 9A); however, there was no significant effect due to exercise, sex, or E2 supplementation (Fig. 9, A and D).

**GS-1 and GSK3α.** E2 supplementation significantly increased the mRNA content of GS-1 (1.4 ± 0.2-fold, *P* = 0.02) (Fig. 9D), but there were no exercise, sex, or menstrual phase effects on the mRNA content of GS-1 (Fig. 9, C and D). No sex, exercise, menstrual phase, or E2 supplementation effects were seen on the mRNA content of GSK3α (Fig. 9, E and F).

**Amino Acid Metabolism**

**AST.** Exercise increased AST mRNA content in men (1.2 ± 0.2-fold, *P* = 0.038), Fol women (1.3 ± 0.2-fold, *P* = 0.032), and Lut women (1.3 ± 0.3-fold, *P* = 0.063) (Fig. 10, A and B). E2 may attenuate the exercise-induced increase in the mRNA content of AST, since exercise slightly, but significantly, increased the mRNA content of AST in men on Pl only (1.1 ± 0.2-fold, *P* = 0.038) and Lut women (1.1 ± 0.3-fold, *P* = 0.063) (Fig. 10, C and D).
There were no effects of sex or menstrual cycle (Fig. 10A).

BCOAD. There was no significant effect of sex, exercise, menstrual phase, or E2 supplementation on BCOAD mRNA content (Fig. 10, C and D).

BCOADK. The mRNA content of BCOADK was significantly higher in Fol and Lut women than men before and after exercise (Fol 1.4 ± 0.2-fold, \( P = 0.026 \); Lut 1.3 ± 0.2-fold, \( P = 0.05 \)) (Fig. 10E). Exercise, menstrual phase, and E2 supplementation did not significantly influence the mRNA content of BCOADK (Fig. 10, E and F).

**DISCUSSION**

This study evaluated the independent and interactive influences of sex, acute endurance exercise, menstrual cycle phase,
and E₂ supplementation on the mRNA content of genes involved in fat, CHO, and protein metabolism, specifically, mitochondrial biogenesis, transcriptional regulation, fatty acid transport, IMCL hydrolysis, IMCL synthesis, β-oxidation, glucose transport and glucose phosphorylation, glycogenolysis and glycolysis, glycogen synthesis, and amino acid metabolism. We found sex differences in the mRNA content of genes involved in fat oxidation that support previous findings that women oxidize more fat during endurance exercise (48, 55, 59, 70). In contrast, we did not find a coordinate or directional difference between sexes in the mRNA abundance of most of the measured genes involved in CHO and protein metabolism, which would be supportive of a lower CHO and protein oxidation in women during exercise (Table 3). Moderate-intensity endurance exercise increased the mRNA content of genes involved in mitochondrial biogenesis, cytosolic and mitochondrial fatty acid transport, β-oxidation, glucose transport and phosphorylation, and amino acid deamination in men and women. The menstrual cycle phase differences in mRNA content of PPARγ, GP, and glycogenin did not coordinately support that women use more fat and less CHO during the mid-Lut phase than the mid-Fol phase (22, 79, 80). We also found that some genes involved in fat and CHO oxidation showed different patterns of mRNA expression in response to exercise during the different phases of the menstrual cycle (PGC-1α, CPTI, GLUT4, and AST). E₂ altered both basal and exercise-induced mRNA content for several genes, which was directionally supportive of the observation of higher lipid and lower CHO oxidation in women compared with men (5, 71).

Robust Sex Differences in mRNA Content Account for Higher Lipid Oxidation in Women Compared with Men

Women (Fol and Lut) at rest and after exercise maintained higher mRNA content of FATm, FABPc, SREBP-1c, mtGPAT, and BCOADK. Women in the Fol phase only maintained higher mRNA content of PPARα, CPTI, and HKII before and after exercise and had higher PPARγ and GLUT4 mRNA both at rest and after exercise. Exercise significantly increased the abundance of PPARα and FABPc mRNA in Lut women compared with men. Interestingly, the Fol phase blunted the exercise-induced increase in PGC-1α, CPTI, and GLUT4 compared with men and Lut women, perhaps because of the slightly higher (although not significant) resting levels. Together with the findings of a sex difference in FATm mRNA (3), FABPpm protein and mRNA, FAT/CD36 protein and mLPL (31), and β-HAD (56), these results indicate that compared with men, Fol and Lut women at rest and during exercise have a higher pretranslational abundance of the genes responsible for fat oxidation downstream of PPARα or PPARγ. PPARα transcriptionally activates medium-chain acyl-CoA dehydrogenase (MCAD), CPTI, CPTII, FABP, and FATP (42, 76), whereas PPARγ transcriptionally activates FABPc, FAT/CD36, LPL, CPTI, and genes involved in β-oxidation (16, 19, 40). At the physiological level these results suggest that women have a higher capacity for sarcolemmal, cytosolic, and mitochondrial fatty acid transport, synthesis of IMCL, and oxidation of fatty acid within mitochondria at rest and during exercise. These results are in agreement with our hypothesis.

Fig. 10. Skeletal muscle mRNA content of aspartate transaminase (AST), branched-chain 2-oxo-acid dehydrogenase (BCOAD), and BCOAD kinase (BCOADK). A: AST mRNA content in men and women (Fol and Lut). *Significant increase during exercise (P = 0.038). B: AST mRNA content in men on PI and E₂, before and after exercise. *Postexercise higher than preexercise (P = 0.03). C: BCOAD mRNA content in men and women (Fol and Lut). D: BCOAD mRNA content in men on PI and E₂, before and after exercise. E: BCOADK mRNA content in men and women (Fol and Lut). †Both Fol (P = 0.026) and Lut (P = 0.05) women higher than men. F: BCOADK mRNA content in men on PI and E₂, before and after exercise. n = 11 men and 11 women. β₂-Microglobulin was used as an internal standard. Values are means ± SE.
and directly support previous observations that women have higher IMCL content than men (15, 48, 55, 59, 70) and oxidize more fat during endurance exercise (9, 14, 20, 26, 32, 43, 49, 54, 58, 66, 68, 70) (Fig. 11).

In apparent contradiction to the observation of lower muscle glucose uptake (12), metabolic clearance rate (9, 20), and attenuation of muscle glycogen use during endurance exercise (9, 14, 20, 26, 32, 43, 49, 54, 58, 66, 68, 70) (Fig. 11), we found that women in the follicular phase had significantly higher muscle glycogen use during endurance exercise in women versus men (36, 43, 49, 54, 66). Therefore, the sex difference in CHO utilization (9, 14, 20, 26, 32, 43, 49, 54, 66). Therefore, the sex difference in CHO metabolism was not coordinately or directionally supportive of the sex difference in CHO oxidation in human skeletal muscle during aerobic activity. Little is known about the pretranslational regulation of GLUT4, HKII, and PFK in regard to their sex differential expression in human skeletal muscle, and it is not possible to determine whether or not the protein for the respective mRNA species was directionally consistent, although the PFK mRNA expression in human skeletal muscle, and it is not possible to determine whether or not the protein for the respective mRNA species was directionally consistent, although the PFK mRNA expression in human skeletal muscle, and it is not possible to determine whether or not the protein for the respective mRNA species was directionally consistent, although the PFK mRNA expression in human skeletal muscle, and it is not possible to determine whether or not the protein for the respective mRNA species was directionally consistent, although the PFK mRNA expression in human skeletal muscle, and it is not possible to determine whether or not the protein for the respective mRNA species was directionally consistent, although the PFK mRNA expression in human skeletal muscle, and it is not possible to determine whether or not the protein for the respective mRNA species was directionally consistent, although the PFK mRNA expression in human skeletal muscle.

In apparent contradiction to the observation of less amino acid oxidation during exercise in women versus men (36, 43, 49), we

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**Table 3. Summary of fold difference in mRNA abundance for metabolism-related genes**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sex Women vs. Men</th>
<th>Menstrual Phase Fol vs. Lut</th>
<th>Estradiol E2 vs. Pl</th>
<th>Exercise Post vs. Pre</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transcriptional regulators</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGC-1α</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>7.9±1.8 Pl, 3.6±0.8 L</td>
</tr>
<tr>
<td>PPARα</td>
<td>1.4±0.1 F</td>
<td>NS</td>
<td>1.4±0.1</td>
<td>NS</td>
</tr>
<tr>
<td>PPARδ</td>
<td>2.3±0.1 F</td>
<td>2.2±0.1</td>
<td>2.1±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>PPARγ</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS &gt; Fat metabolism</td>
</tr>
<tr>
<td>VLCAD</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>TFPI</td>
<td>2.4±0.3 L</td>
<td>NS</td>
<td>1.5±0.1</td>
<td>NS</td>
</tr>
<tr>
<td>FATm</td>
<td>4.6±0.5 F, 2.9±0.2 L</td>
<td>NS</td>
<td>1.3±0.1 all</td>
<td>1.2±0.05 Pl, 1.5±0.1 L</td>
</tr>
<tr>
<td>FABPc</td>
<td>1.7±0.1 F, 1.7±0.1 L</td>
<td>NS</td>
<td>1.5±0.1</td>
<td>1.2±0.05 Pl, 1.5±0.1 L</td>
</tr>
<tr>
<td>CPTI</td>
<td>1.7±0.1 F</td>
<td>NS</td>
<td>1.5±0.1</td>
<td>NS</td>
</tr>
<tr>
<td>CPT2</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>SREBP1c</td>
<td>3.1±0.4 F, 4.1±0.7 L</td>
<td>NS</td>
<td>3.0±0.3</td>
<td>NS</td>
</tr>
<tr>
<td>SREBP2</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>mtGPAT</td>
<td>2.0±0.1 F, 1.7±0.1 L</td>
<td>NS</td>
<td>1.4±0.1</td>
<td>NS</td>
</tr>
<tr>
<td>HSL</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>1.4±0.2 Pl, 1.2±0.2 E2</td>
</tr>
<tr>
<td><strong>Carbohydrate metabolism</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLUT4</td>
<td>2.4±0.3 F</td>
<td>NS</td>
<td>3.9±0.5</td>
<td>2.1±0.2 Pl, 2.0±0.2 L</td>
</tr>
<tr>
<td>HKII</td>
<td>2.9±0.3 F</td>
<td>NS</td>
<td>NS</td>
<td>1.9±0.2 all</td>
</tr>
<tr>
<td>GP</td>
<td>2.2±0.2</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>PFK</td>
<td>-3.7±0.0 L</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Glycogenin</td>
<td>NS</td>
<td>-1.2±0.1</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>GS1</td>
<td>NS</td>
<td>1.3±0.1</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>GSK3α</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Amino acid metabolism</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>1.2±0.1 Pl, 1.3±0.1 F</td>
</tr>
<tr>
<td>BCOAD</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>BCOADK</td>
<td>1.3±0.1 F, 1.2±0.1 L</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are presented as fold difference ± SE. *P < 0.05. Post, postexercise; Pre, preexercise; NS, not significant; F and Fol, follicular phase; L and Lut, luteal phase; Pl, placebo; FABPpm, membrane-associated fatty acid binding protein.
did not find sex differences in mRNA content of AST or BCOAD between men and women during either the Fol or Lut phase. Our finding of higher mRNA abundance for BCOADK in Fol and Lut women implicates a higher phosphorylation potential for BCOAD (hence lower activity) and is consistent with the observation of a lower BCOAD activity in women compared with men only in the pre- but not postexercise samples (43). A lower oxidation of amino acids in women versus men could also be a response to higher fat oxidation leading to lower CHO oxidation and resultant “sparing” of amino acid oxidation in women compared with men (9, 14, 20, 26, 32, 43, 49, 54, 66). The pretranslational regulatory mechanism(s) underlying the sex differential BCOADK mRNA expression is not clear but does not appear to be due to energy charge or AMP-activated protein kinase (AMPK) signaling, given that these are not different between sexes in the resting state (58) and acute exercise did not alter the mRNA content of BCOADK in men (present study) even though AMPK activation increased during exercise in men only (58).

**Acute Exercise Increases mRNA Capacity for Fatty Acid, Carbohydrate, and Protein Metabolism and Mitochondrial Biogenesis**

Acute and chronic endurance exercise training increase the proportionate utilization of fat (1). We found that a single bout of moderate-intensity endurance exercise increased the mRNA content of FABPc and HKII in men and women (Fol and Lut); PGC-1α, CPTI, and GLUT4 in men and Lut women; AST in men and Fol women; and HSL in men only. Our observations are consistent with previous findings that acute exercise increases the mRNA content of PGC-1α (41, 47), PPARδ (78), and CPTI (50, 51) in human skeletal muscle. The coordinate induction of the mRNA content of these genes suggests that there may be some regulatory relationship in the corresponding group of subjects. PGC-1α is a transcriptional coactivator of PPARα, which in turn increases gene expression of enzymes involved in mitochondrial fatty acid β-oxidation including MCAD, CPTII (42, 76), CPTII (42), and FATm and FABP (62). Even in the absence of PPARα mRNA induction, increased PGC-1α expression may still increase the expression of PPARα target genes through coactivation (76). The coordinate induction of the mRNA content for genes involved in mitochondrial biogenesis, hydrolysis of IMCL, cytosolic and mitochondrial fatty acid transport, and β-oxidation is directionally supportive of the fact that acute endurance exercise increases the transcriptome capacity for fat oxidation in men and women.

In contrast to the immediate postexercise increase in mRNA abundance for many genes involved in fat oxidation, we did not find higher abundance of mRNA species for genes involved in IMCL synthesis (SREBP-1c, mtGPAT). Endurance exercise training increases IMCL content in skeletal muscle (70), and acute exercise appears to reduce IMCL content, primarily in women (55, 59, 77). These results are consistent with studies investigating the mRNA abundance of SREBP-1c (75) in response to acute exercise in human skeletal muscle. Our finding of no change in mtGPAT is also consistent with a lack of an acute exercise-induced increase in GPAT activity in exercising men (77). In addition to the mtGPAT mRNA data, we are also the first to measure and report the lack of an acute exercise effect on SREBP-2 abundance (involved in membrane biosynthesis). Together, the above data imply that acute endurance exercise does not change the mRNA capacity for synthesis of IMCL or membrane lipids in men or women.

Exercise also increased the mRNA content of GLUT4 in men and Lut (but not Fol) women and HKII and AST in men and women. We are the first to measure and report that exercise did not influence the mRNA content of GS-1, GSK3α, or GP in human skeletal muscle. Our observations are consistent with studies measuring the mRNA content of GLUT4 (49) and HKII (12, 33, 34, 46, 52) but inconsistent with studies showing an acute effect of exercise increasing GS-1 activity (12, 33) because of dephosphorylation by activation of phosphatase and inactivation of kinases (45). The menstrual cycle phase differential response of GLUT4 to exercise suggests that sex hormonal influences may be involved in the regulation of GLUT4 mRNA expression in women at different phases of the menstrual cycle phases during exercise (7, 24). We confirm that acute endurance exercise influences the mRNA content of the genes involved in glucose transport (GLUT4), phosphorylation, and trapping (HKII) in human skeletal muscle, which may increase the capacity for glucose uptake, disposal, and oxidation. Our observations are also in agreement with findings that training increased AST activity in human muscle (53) and show that this is a very early response induced immediately after a single bout of exercise.

Given that men and women have similar mitochondrial volume densities (10) and enzyme activities (2, 10), it was surprising that we consistently found acute induction of PGC-1α following endurance exercise. It was robustly higher...
in men (7.9-fold) and in Lut women (3.6-fold) and not seen in
women during the Fol phase of the menstrual cycle. These
results suggest that the response of PGC-1α mRNA expression
to exercise may depend on the ratio of progesterone to estro-
gen. In Lut women the level of progesterone is greater than
estrogen, while the level of progesterone is less than estrogen
in Fol women (27).

**E2-Mediated Effects on Lipid Metabolism**

This is the first study to examine the effect of short-term (8
days) E2 supplementation on basal and exercise-induced changes
in mRNA content of genes involved in substrate metabolism in
human skeletal muscle. E2 supplementation in men increased the
mRNA content of PPARδ and, together with the small but
significant effects of menstrual cycle phase on PPARδ, sug-
gests that E2 increases the capacity for the pretranslational
abundance of genes involved in lipid utilization (42, 76) and
β-oxidation (16, 19, 40) as stated above. Our results are
consistent in that we found an increase in mRNA content for
TPPα and CPTI. Consistent with the observation that IMCL
content is higher in women compared with men (64), we found
an increase in both SREBP-1c and mtGPAT mRNA content
with E2 supplementation. These latter results implicate E2 as
the key factor underlying the sex difference in IMCL content
(64), given that SREBP-1c and mtGPAT are involved in the
transcriptional regulation of and biochemical pathways for
IMCL synthesis, respectively (65, 73). Further support for
an E2-mediated effect is the observation that ovariectomy in
mice decreases the mRNA content of genes involved in
lipogenesis (29).

Changes in mRNA content are directionally consistent with
physiological measures of substrate utilization. Men given E2
had a lower RER, which reflects a reduced reliance on CHO
substrate utilization and an increase in lipid metabolism (13).
Administration of E2 in both men and women attenuated
hepatic glucose production during endurance exercise (8, 61).
Administration of E2 in men reduced proglycogen, total gly-
cogen, hepatic glucose production, and glucose uptake at rest
and after exercise, suggesting whole body glycogen sparing
(13). Although the direct mechanism involved has yet to be
determined, estrogen has been shown to elicit its effects in two
different ways (Fig. 11). Estrogen can function in a “non-
genomic” manner by binding to estrogen receptors on the cell
surface and rapidly inducing calcium flux (72), phospholipase
C activation (37), and inositol 1,4,5-trisphosphate (IP3) signal-
ing (63), which can alter gene transcription. Alternatively,
estrogen can mediate “genomic” actions by binding to estrogen
response element (ERE) upstream of target genes. It is pre-
picted that PPARα, PPARδ, CPTI, SREBP-1c, and GLUT4
have upstream ERE (4), suggesting the genomic regulation of
E2 in this study. Further studies are required to determine
whether estrogen directly binds ERE to upregulate these genes,
and to determine whether changes in mRNA correspond to
changes in protein and protein function.

E2 also unexpectedly abolished the exercise-induced induc-
tion of mRNA content for PGC-1α in men, which correlates to
the lack of significant upregulation of PGC-1α in Fol women.
The similarity of the PPARδ response to that of PGC-1α,
combined with the known interaction between PGC-1α and
PPARs (38, 76), suggests that E2 may influence the mRNA
content of these species via similar mechanisms. The basal
elevation of PPAR6 with E2 supplementation is directionally
consistent with higher fat oxidation in women (5, 71).

The increase in both GLUT4 and GS-1 mRNA content in
our study would predict that muscle glycogen would be higher
in men after E2 administration; however, this is in contrast to
our published findings that E2 lowers basal muscle glycogen
content (13) and reduces glucose Rm, Rg, and metabolic clear-
ance rate (8, 13) and to several studies that have not found sex
differences in basal glycogen content (57). In contrast, E2 did
not alter the mRNA content of GP, indicating no change in the
potential for glycogen breakdown, which is consistent with the
observation that E2 did not attenuate the use of glycogen during
exercise (8, 13). The directionality and consistency of changes
in the mRNA content of genes involved in fat metabolism
suggest that fat metabolism is the primary regulated process.

**Conclusions**

We conclude that both Fol and Lut women have a higher
capacity for pretranslational abundance of genes responsible
for fat oxidation and a higher transcriptional capacity for
sarcolemmal, cytosolic, and mitochondrial fatty acid transport,
synthesis of IMCL, and oxidation of fatty acids at rest and
during exercise compared with men. These observations di-
rectly support previous research demonstrating that women
have more IMCL than men and oxidize more fat during
endurance exercise. The sex differences in the mRNA content
of the genes involved in CHO and protein metabolism do not
coordinate or directionally support a lower oxidation of CHO
and protein in women compared with men. Exercise increases
the transcriptional capacity of fatty acid, CHO, and protein metab-
olism in men and women, specifically in the Lut phase. The
menstrual cycle influences the profile of mRNA involved in
lipid and CHO metabolism at rest and its response to endurance
exercise. The present data support E2 as a determinant of
steady-state mRNA levels for genes primarily involved in
skeletal muscle fat metabolism. The lack of consistency be-
 tween the directional change in the mRNA content of genes
involved in CHO metabolism and physiological observations
in men versus women and E2-supplemented men indirectly
suggests that fat metabolism appears to be the main regulated
process and CHO metabolism follows by physiological regu-
lation (5).

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**DISCLOSURES**

No conflicts of interest are declared by the author(s).

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