17β-Estradiol Supplementation Decreases Glucose Rate of Appearance and Disappearance with No Effect on Glycogen Utilization during Moderate Intensity Exercise in Men

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Context and Objective: Women use less carbohydrate during endurance exercise, as compared with men. In rodents, 17β-estradiol (E2) supplementation robustly increases lipid use and lowers muscle and liver glycogen use during exercise. E2 supplementation has been found to influence substrate selection by decreasing glucose rate of appearance (Ra), disappearance (Rd), and metabolic clearance rate during exercise in humans; however, neither a change in total carbohydrate use nor a sparing of muscle glycogen was demonstrated.

Subjects and Methods: We investigated the effect of E2 supplementation on glucose turnover and net muscle glycogen use in 11 men using a randomized, double-blind, placebo-controlled, crossover design. Subjects underwent primed constant infusion of [6,6-2H]glucose, and muscle biopsies were taken before and after 90 min of cycling at 65% maximal oxygen uptake.

Results: E2 supplementation decreased the respiratory exchange ratio (P = 0.03) and glucose Ra and Rd (both P = 0.04) during exercise, as compared with placebo. E2 supplementation lowered proglycogen (P < 0.05) and total glycogen (P = 0.04) concentration, as compared with placebo; however, there was no effect of E2 on net muscle glycogen use during exercise.

Conclusions: These findings show that E2 supplementation alters fuel selection in exercising men by increasing lipid use and reducing carbohydrate use, glucose Ra (primarily liver glucose production), and Rd (primarily muscle glycogen uptake). Furthermore, E2 reduces the basal level of total muscle glycogen, particularly the proglycogen form. (J Clin Endocrinol Metab 90: 6218–6225, 2005)
or low dose could be a factor in the aforementioned mixed findings in the human studies (22, 23).

In previous studies involving E2 administration, either a transdermal E2 patch (21, 23) or an oral E2 tablet (22) was used to increase plasma E2 levels. In the studies that used the transdermal patch, plasma E2 levels doubled (21, 23), mimicking E2 levels seen in the follicular phase (FP) of the menstrual cycle. However, these levels were well below those observed in the animal studies. Consequently, Carter et al. (22) used an oral tablet to achieve higher plasma E2 levels, similar to those seen in the luteal phase (LP) of the menstrual cycle. However, because this was the first study using the E2 tablet, it was not known what dosage to administer to obtain such levels, and the plasma E2 levels that were reached were well above what would normally be observed in the LP of the menstrual cycle (22).

Previous studies attempting to determine the effect of E2 supplementation on glycogen metabolism have investigated E2 effect only on either glucose turnover or muscle glycogen use during exercise, not both. To date, no study has been conducted using both stable isotope tracers and muscle samples to investigate the effect of E2 supplementation on whole-body and muscle glucose/glycogen metabolism. The purpose of the present study was to determine the effect of E2 administration on whole-body glucose turnover and skeletal muscle glycogen use during moderate intensity endurance exercise in young men. Furthermore, we used a dosage that more closely mimics E2 levels observed in the LP, which is higher than that used previously (23), and increased the sample size as compared with our previous research (22) to determine whether previously observed trends were artifacts of type II error. In addition, we investigated the effect of E2 administration on the proglycogen (PG) and macroglycogen (MG) fractions of glycogen as the effects of E2 on muscle glycogen use and storage may be more apparent in the PG form because it is more dynamic (24, 25), and failure to measure both forms may miss a shift in glycogen use and/or storage.

**Subjects and Methods**

**Participants**

Eleven healthy, recreationally active male volunteers participated in this study [age 23 ± 2 yr (mean ± sd); weight 80 ± 3 kg; height 178 ± 1 cm; body mass index 25 ± 1 kg/m²; fat-free mass 64 ± 2 kg (80 ± 1% of body weight); maximal oxygen uptake (VO₂max) 44 ± 2 ml O₂ per kilogram body weight per minute]. Informed consent was obtained before commencing the study following a description of the study and advisement of the risks and benefits of participation. This study was conducted under approval of the Research Ethics Committee of McMaster University.

**Protocol**

At least 1 wk before the first trial date, subjects performed a progressive exercise test on an electronically braked bike (Ecalibur Sport, Lode, Groningen, The Netherlands) to determine their VO₂max, as previously described (26). The VO₂max was used to determine the work intensity needed to elicit 65% of the subject’s VO₂max for subsequent testing and was confirmed by measuring oxygen uptake at the calculated workload intensity for each subject approximately 30 min after the test. Subjects were randomly assigned to receive either placebo (400 mg/d glucose polymer, Polycose; Abbott Laboratories, Ross Division, St. Laurent, Quebec, Canada) or E2 [1 mg/d for 2 d and 2 mg/d for 6 d (Estrace; Shire BioChem, Inc., St. Laurent, Quebec, Canada)] for 8 d in a randomized, double-blind, crossover manner. On the morning of the ninth day, subjects reported to the laboratory and performed 90 min of exercise on a cycle ergometer at 65% VO₂max. At least a 10-d washout period was allowed between trials for the hormone levels to return to baseline. After the washout period, the subjects repeated the 9-d protocol on the alternate treatment. The tablets were placed in gelatin-filled capsules and filled with a glucose polymer and taken once per day. Subjects were instructed to take the pills at the same time each day and return any unused pills. All subjects reported 100% compliance.

During the 8-d dosage period, subjects were asked to maintain and record their normal activity level. Food intake was recorded in all subjects for at least 3 d of the dosage period (minimum of 2 weekdays and 1 weekend day) and was returned to the subjects during the second arm of the study to allow them to keep their diet similar between trials. Three subjects completed diet records on both arms of the study in order to ensure that habitual diet did not change between trials. Diet records were analyzed using commercially available analysis software (Nutritionist Pro, version 2.2; First DataBank, Inc., San Bruno, CA). Subjects were not tested on or around major holidays or during times when their diets deviated from normal.

Subjects consumed the same meal on the evening before both test days. On the morning of the test day, subjects reported to the laboratory 10–12 h postabsorptive. Body weight was recorded and body composition was determined via bioelectric impedance analysis (RJL Systems BIA-101A, Mt. Clemens, MI). A 20-gauge plastic catheter (Becton Dickinson, Lincoln Park, NJ) was placed into the antecubital vein of the right arm and a 10-cm piece of blood was allowed to flow for 3 min before the withdrawal of each blood sample when the subjects were not exercising. Another plastic catheter was placed into the antecubital vein of the left arm to allow for infusion of stable isotopes with a constant infusion pump (model 74900; Cole-Parmer Instrument Co., Vernon Hills, IL). Subjects then underwent primed constant infusion of [6,6-²H]glucose (99% enriched; Cambridge Isotope Laboratories, Inc., Cambridge, MA) for 2.5 h. Glucose was mixed with 0.9% saline and filtered through a 0.2-μm filter (sterile, nonpyrogenic, Acrodisc; Pall Gelman Corp., Ann Arbor, MI) into single-use vials at the McMaster University Medical Center pharmacy. The solutions were cultured for 5 d at room temperature and 35 C to ensure there was no bacterial contamination. Before initiating the infusion protocol, a baseline blood sample was taken to allow for determination of the natural background enrichment of [6,6-²H]glucose. A priming dose of [6,6-²H]glucose (17 μmol/kg) was given followed immediately by a constant infusion at a rate of 0.22 μmol/kg/min for 60 min to reach steady state. At the onset of exercise, the infusion rate was increased in a stepwise fashion at t = 0, 5, and 10 min of exercise to 0.33 μmol/kg/min, 0.44 μmol/kg/min, and 0.55 μmol/kg/min. The infusion rate remained at 0.55 μmol/kg/min for the remainder of the 90-min exercise session.

Before and after exercise, a muscle biopsy was taken under local anesthetic from the vastus lateralis muscle approximately 20 cm proximal to the knee joint using a modified Bergström needle (5 mm diameter) with suction modification, as previously described (23, 27). The vastus lateralis muscle was chosen because it is one of the four quadriceps muscles, which is one of the primary muscles used during cycling activity and has been used in similar studies (23, 27). Biopsies were taken from the same leg before and after exercise and from the contralateral leg on the subsequent test day. The site of the second biopsy was approximately 3 cm above the first biopsy site to ensure homogeneity for muscle sampling source. Approximately 30 mg was immediately snap frozen and stored in liquid nitrogen until transferred to –86 C for subsequent PG/MG analyses.

Blood samples were taken at baseline and 15 min before commencing exercise and at t = 60, 75, and 90 min of exercise. For glucose and lactate analysis, blood samples were collected in heparinized tubes, placed on ice, centrifuged at 1750 × g at 4 C for 10 min and stored at −50 C for subsequent analysis. For determination of hormone levels (E2, testosterone, progesterone), blood samples were collected and allowed to stand for 30 min in untreated test tubes, centrifuged at 1200 × g at 4 C for 30 min and the serum stored at −50 C for subsequent analysis.

Respiratory measures (oxygen uptake, volume of carbon dioxide expired, RER) were taken at t = 0, 5, 30, 60, 75, and 90 min during exercise using a computerized open-circuit gas collection system (Moxus Mod-
ulotor VO2 system with O2 analyzer S-3A/I and CO2 analyzer CD-3A; AEI Technologies, Inc., Pittsburgh, PA).

Biochemical glycogen determination

Muscle PG and MG content was analyzed as described by Adamo and Graham (24). Briefly, snap-frozen muscle samples were freeze dried for 24 h, powdered, and dissected free of any blood and connective tissue and weighed. Ice-cooled perchloric acid (200 μl, 1.5 m) was added to 1.5–2.5 mg tissue and pressed with a plastic inoculating loop for 20 min on ice. Samples were then centrifuged for 15 min at 3000 rpm, and an aliquot of 100 μl of the supernatant was removed for MG determination. The remaining supernatant was aspirated off. HCl (1 ml, 1 m) was added to each sample, and PG samples were briefly pressed and MG samples were vortexed for several seconds. Samples were hydrolyzed for 2 h at 100 C and neutralized with 2 ml Tris base, vortexed, centrifuged at 3000 rpm for 5 min, and stored at −86 C until subsequent determination of glucosyl units. Muscle PG and MG content was determined as described previously (25). Values for PG and MG were then added to give total muscle glycogen content. Muscle glycogen use during exercise was calculated as follows:

\[
glycogen = [\text{glycogen}]_{\text{pre}} - [\text{glycogen}]_{\text{post}}\]

Isotopic enrichment, glucose Ra and Rd, and MCR

Isotopic enrichment of glucose was determined using gas chromatography (GC)-mass spectrometry (MS; GC: model 6890; Hewlett-Packard, Palo Alto, CA; and MS model 5973; Agilent Technologies, Inc., Palo Alto, CA) of the pentaacetate derivative, as described previously (22). To isolate the pentaacetate derivative, the samples were first deproteinized using barium hydroxide and zinc sulfate. The supernatant was passed through an anion-exchange (AG 1X-400, and AG 50X-400; Sigma Chemicals, St. Louis, MO) exchange column, and the eluted extract was evaporated using a rotary evaporator (SpeedVac Plus SC210A; ThermoSavant, Holbrook, NY). The derivative was prepared for GCMS analysis by adding 100 μl of 2:1 acetic anhydride and pyridine solution to each sample. A 15-m fused silica capillary column with 0.2 mm diameter and 0.2 μm film thickness (Supelco, Bellefonte, PA) was used in the GC oven. The modified Steele equation is as follows:

\[
MCR = \frac{Rd}{F - Vd + \frac{Cm}{1 + E}\frac{dE}{dt}} \frac{dCm}{dt} + \frac{Vd}{(1 + E) - Cm}\frac{dE}{dt}
\]

where \(C_1\) and \(C_2\) are plasma glucose concentrations at sampling times 1 and 2.

Blood samples

Plasma was analyzed for lactate and glucose and serum for estrogen, testosterone, and progesterone levels. Plasma lactate and glucose concentrations were analyzed with an automated lactate and glucose analyzer (2300 STAT plus; YSI, Farnborough, UK). Serum E2, testosterone, and progesterone were analyzed using a single-incubation RIA (Coat-a-Count, kit TKE21, kit TKE1 and kit TKN5; Diagnostics Products Corp., Los Angeles, CA).

Statistical analysis

For RER and indirect calorimetry, rest samples were analyzed using paired t tests. For all other analyses, including RER and indirect calorimetry exercise time points, two-way, repeated-measures ANOVA with treatment and time being the experimental variables was used. One-tailed tests were used for RER, glucose oxidation, and substrate oxidation because we hypothesized a priori that E2 supplementation would decrease RER and carbohydrate and glucose oxidation and increase lipid oxidation. When significance was attained, Tukey’s honestly significant difference post hoc test was used to determine the location of the difference. Analyses were performed using a computerized statistics program (STATISTICA for Windows, version 5.1; StatSoft, Tulsa, OK). Statistical significance was set at \(P \leq 0.05\). Data are presented as means ± SEM unless otherwise indicated. All data were checked for normality using the Kolmogorov-Smirnov test.

Results

Diet and body weight

Subjects consumed 2,416 ± 331 kcal (mean ± sd, 10,080 ± 1,381 kJ), 298 ± 69 g of CHO, 94 ± 28 g of protein, and 87 ± 18 g of fat per day. CHO, protein, and fat made up 49 ± 16 %, 16 ± 1, and 33 ± 2% of total dietary intake, respectively. The caloric intake and macronutrient composition of the three subjects who completed diet records on both arms of the study did not change between trials. Body weight did not change between trials.

Hormone concentrations

E2 supplementation for 8 d significantly increased serum E2 concentrations (\(P < 0.001\)) and significantly decreased serum testosterone (\(P = 0.001\)) and serum progesterone (\(P = 0.03\)) (Table 1).

RER and lipid and carbohydrate metabolism

Data from one subject were not included in the statistical analysis due to an error that occurred when taking breath samples; hence, data presented are for 10 subjects. RER, CHO, and lipid oxidation increased during exercise, as compared with rest (\(P < 0.001\)). E2 supplementation decreased

<table>
<thead>
<tr>
<th>TABLE 1. Serum hormone concentrations for 11 men after 8 d of placebo or E2 supplementation</th>
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<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>Total testosterone (nmol/liter)</td>
</tr>
<tr>
<td>E2 (pmol/liter)</td>
</tr>
<tr>
<td>Progesterone (nmol/liter)</td>
</tr>
</tbody>
</table>

Data are means ± SEM; \(n = 11\). Repeated-measures ANOVA: E2 is significantly different from placebo.

\(a P < 0.002.\)

\(b P = 0.03.\)
RER ($P = 0.03$) during exercise, as compared with placebo (Fig. 1). E2 supplementation also decreased CHO oxidation ($P = 0.03$) and increased lipid oxidation ($P = 0.03$) (Fig. 1). There was no difference in energy expenditure between trials.

**Plasma glucose and lactate concentrations**

Plasma glucose concentration was lower at $t = 75$ and 90 min exercise as compared with the resting levels ($P = 0.01$) (Table 2). Plasma lactate concentration was higher during exercise as compared with rest ($P < 0.001$). There was no effect of E2 supplementation on plasma glucose or lactate concentrations at rest or during exercise.

**Glucose Ra and Rd and MCR**

Data from a different subject were not included in the statistical analysis due to technical problems with the infusion on one of the test days; hence, data are presented for 10 subjects. Glucose Ra and Rd were equivalent, indicating that subjects were in steady-state glucose flux for the duration of the exercise bout. Glucose Ra and Rd and MCR all increased during exercise, as compared with rest ($P < 0.001$) (Fig. 2). E2 supplementation decreased glucose Ra ($P = 0.04$) and Rd ($P = 0.04$) during exercise with no effect on MCR, as compared with placebo (Table 3).

**Muscle glycogen concentration and use**

Muscle PG, MG, and total glycogen ($G_{tot}$) concentration decreased significantly during 90 min of endurance exercise at 65% VO$_{2\text{max}}$ ($P < 0.001$) (Fig. 3). E2 supplementation

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**TABLE 2.** Plasma glucose and lactate concentrations in 11 men at rest and during 90 min of cycling at 65% VO$_{2\text{max}}$

<table>
<thead>
<tr>
<th></th>
<th>0 min</th>
<th>60 min</th>
<th>75 min</th>
<th>90 min</th>
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</thead>
<tbody>
<tr>
<td>Glucose (mmol/liter)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrogen</td>
<td>4.9 ± 0.1</td>
<td>4.7 ± 0.1</td>
<td>4.5 ± 0.1</td>
<td>4.5 ± 0.2</td>
</tr>
<tr>
<td>Placebo</td>
<td>5.1 ± 0.1</td>
<td>4.8 ± 0.2</td>
<td>4.7 ± 0.1</td>
<td>4.7 ± 0.2</td>
</tr>
<tr>
<td>Lactate (mmol/liter)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrogen</td>
<td>0.9 ± 0.1</td>
<td>2.3 ± 0.3</td>
<td>2.4 ± 0.4</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>Placebo</td>
<td>1.0 ± 0.1</td>
<td>2.5 ± 0.3</td>
<td>2.4 ± 0.2</td>
<td>2.6 ± 0.3</td>
</tr>
</tbody>
</table>

Data are means ± SEM, $n = 11$. Repeated-measures ANOVA.
FIG. 3. Muscle PG (A), MG (B), and Gtot in 11 men at rest (pre) and after 90 min of cycling at an intensity of 65% VO2max on placebo (PL, ■) or after 8 d of E2 supplementation (△). Data are means ± SEM. *, Rest higher than after exercise, P < 0.001. †, E2 lower than PL, P < 0.05. DW, Dry weight.

significantly lowered PG by 18% (P < 0.05) and Gtot by 20% (P = 0.04) with a strong trend toward a lowered MG (24%, P = 0.059) at rest and after exercise, as compared with placebo (Table 3). E2 supplementation had no effect on PG (172 ± 17 vs. 191 ± 8, E2 vs. placebo), MG (118 ± 27 vs. 113 ± 8), or Gtot (290 ± 42 vs. 304 ± 8) use.

Discussion

The main findings of this study were that 8 d of E2 supplementation lowered RER, glucose Ra and Rd, and muscle PG and total glycogen concentration with no effect on glucose MCR or net muscle glycogen use. These findings suggest that E2 influences CHO use during endurance exercise through an effect on glucose turnover (hepatic glucose production and peripheral uptake). E2 supplementation also reduced basal muscle glycogen stores but not net muscle use during exercise.

The E2 supplementation regimen used in the current study was somewhat different from that used in studies conducted previously. Two previous studies used a patch delivery method to administer E2 to subjects (21, 23), and serum E2 concentrations varied dramatically between studies due in part to differences in the duration of supplementation. In one study, a 72- and 144-h E2 supplementation regimen to amenorrheic women significantly increased serum E2 concentrations (21), but the levels were still well below what is typically observed in the FP of the menstrual cycle (31–33). The second study used a patch delivery method during an 11-d protocol that resulted in serum E2 concentrations between what is typically observed in the late FP and early LP of the menstrual cycle (23). Another investigation used a novel delivery method that resulted in serum E2 concentrations five times the values typically observed in the LP of the menstrual cycle (22). With the oral E2 delivery method used in the current study (dose and duration), the serum E2 concentrations were at the upper limit of the range for the LP of the menstrual cycle and less than the supraphysiological levels seen in our previous study (22) and was enough to induce metabolic changes in line with previous research (21–23).

There were also obvious gender differences in the amenorrheic women recruited in the aforementioned studies for Ruby et al. (21), whereas we used males in our previous studies (22) and in the current one. Because it is likely that any metabolic effects of E2 are to be receptor mediated, it is important to note that E2 receptor-β mRNA and protein are present in the skeletal muscle of men (34, 35). The only study that we are aware of that has looked at E2 receptor mRNA and protein content in men and women used a small number of subjects (three men, three women), and thus no gender comparisons were conducted (35), although, subjective examination of the presented data suggested that the men had consistently lower E2 receptor mRNA content and percent E2 receptor-positive nuclei (35). Consequently, it is likely that higher levels of serum E2 are required in studies using men before a change in substrate oxidation is seen. This could partially explain why we did not find changes in exercise metabolism when we supplemented men with an E2 dose that resulted in much lower serum E2 concentrations than those found in the current study (23).

In previous animal studies, E2 was given to male (13, 15, 16) or oophorectomized female rats (12, 14). E2 supplementation decreased the reliance on CHO and increased reliance on lipid during endurance exercise, regardless of whether male or female mice were used (12–16). Essentially, oophorectomized rats are the animal equivalent to amenorrheic women because both models lack ovarian hormones. A concern associated with amenorrheic women as subjects is that their energy intake is lower, as compared with eumenorrheic women (37). Lower energy intake results in lower body stores and may alter substrate selection during exercise. For example, lower caloric intake results in lower glycogen stores, which in turn promotes lipid oxidation during exercise (38). As such, it may be more appropriate to use males as subjects to determine the effects of E2 administration per se on substrate use during exercise. The only potentially confounding issue with using males in E2 supplementation studies is the effect testosterone may have on substrate selection during exercise. In this and other studies (22, 23), E2...
supplementation in men resulted in a significant lowering of testosterone; however, the levels were still well above female levels and within the normal range for adult males. However, in the animal studies, both male and oophorectomized female rats were used, conditions characterized by high and low levels of testosterone, and no differences in liver or muscle glycogen use, performance, or any metabolic blood parameters were observed (12, 14–16). Most importantly, a recent study involving human subjects found that pharmacologic manipulation of testosterone in men had no influence on substrate metabolism (39). As such, it is most likely that the increase in E2 is the predominant hormonal factor that caused the observed shift in fuel selection during exercise in the current study.

There may be a dose-response effect of E2 on substrate use and storage. In previous studies, E2 had no effect on RER (21–23). However, in two of these studies (21, 23), the E2 dose that was administered was lower than what was used in the current study and may explain the lack of change in RER. However, no change in RER was observed in the study conducted by Carter et al. (22), even though serum E2 concentrations were well above those observed in the current study. One possible explanation could be the relatively small sample size (type II error) used in that study (22). Another possible explanation could be that E2 is known to increase IMCL content and lipoprotein lipase activity in rats (17, 19, 20). Given that there is simultaneous esterification of FFAs to IMCL and IMCL hydrolysis during exercise (40) and that an increase in IMCL synthesis would tend to elevate the RER, it is possible that the RER could be falsely elevated with E2. Animal studies have shown that E2 supplementation initially increased fat oxidation followed by a subsequent decrease in glucose oxidation during exercise (14). Furthermore, Rooney et al. (16) found that E2 supplementation in rats increased resting IMCL concentration causing a subsequent down-regulation of CHO use during exercise. These two studies further support that an initial change in fat availability and oxidation needs to occur before a shift in CHO use is observed after E2 supplementation. Future studies should investigate the effect of E2 supplementation on IMCL content and whether an increase in lipid content is a requisite to promote lipid use and whether a longer E2 dosing regimen promotes muscle glycogen sparing during exercise.

Ours is the first study to investigate the effect of E2 supplementation on muscle PG, MG, and Gtot storage and use. Previous studies have failed to find a reduction in muscle glycogen use after E2 treatment (23); therefore, we decided to investigate whether there was a fraction-specific decrease in glycogen use that was not translated to whole-muscle glycogen use. We anticipated a decrease in muscle PG use if there was an effect of E2 on muscle glycogen fraction use because the PG fraction is more dynamic than the MG fraction (24, 25). Interestingly, the only effect of E2 on muscle glycogen was that 8 d of E2 supplementation decreased PG and Ghot with a strong trend toward a decrease in MG stores. However, it is likely that we lacked sufficient power due to the high variability in MG concentrations to detect a significant decrease in MG stores because such a strong trend (24%, \( P = 0.059 \)) was observed; however, it still remains that PG is the more dynamic form of glycogen because the shift in storage is observed in this pool more readily.

### TABLE 3. Glucose Ra, Rd, and MCR and muscle proglycogen, MG, and total glycogen values before and after 90 min of cycling at 65% VO\(_{2,\text{max}}\)

<table>
<thead>
<tr>
<th>E2</th>
<th>Placebo</th>
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<tbody>
<tr>
<td>Pre</td>
<td>During/post</td>
</tr>
<tr>
<td>Glucose Ra (mg/kg-min)</td>
<td>22.6 ± 0.6(^{a})</td>
</tr>
<tr>
<td>Glucose Rd (mg/kg-min)</td>
<td>22.6 ± 0.7(^{a})</td>
</tr>
<tr>
<td>Glucose MCR (ml/min)</td>
<td>4.7 ± 0.2(^{a})</td>
</tr>
<tr>
<td>PG (mmol/kg DW)</td>
<td>279 ± 22(^{a})</td>
</tr>
<tr>
<td>MG (mmol/kg DW)</td>
<td>152 ± 29</td>
</tr>
<tr>
<td>Total glycogen (mmol/kg DW)</td>
<td>431 ± 48(^{a})</td>
</tr>
</tbody>
</table>

Data are means ± SEM. DW, Dry weight.

\(^{a}\) Repeated-measures ANOVA, pre lower than exercise, \( P < 0.001 \).

\(^{b}\) Repeated-measures ANOVA, E2 lower than placebo, \( P = 0.04 \).

\(^{c}\) Repeated-measures ANOVA, E2 lower than placebo, \( P < 0.05 \).

\(^{d}\) Repeated-measures ANOVA, post exercise lower than pre, \( P < 0.001 \).
Our study confirms the previous observations that E2 supplementation caused a decrease in glucose Ra and Rd (21, 22). The consistency of this finding reaffirms that E2 acts to promote whole-body CHO sparing during exercise by preferentially attenuating hepatic glucose production (Ra) and glucose uptake (Rd). Animal studies support a net sparing of hepatic glycogen use during exercise in response to E2 supplementation (12, 15, 16). From the current data, we cannot differentiate between an effect of E2 on reducing hepatic gluconeogenesis and/or glycolgenolysis; however, a previous study showed that women had a lower glucose recycling rate than men (2), suggesting a lesser flux of CHO precursor through gluconeogenesis. The main precursors for gluconeogenesis are lactate, glyceral, and alanine. No effect of E2 supplementation has been found on glycerol concentration (21–23), and the current study and others (21–23) found no effect of E2 supplementation on plasma lactate concentration, suggesting that E2 does not decrease gluconeogenesis. Furthermore, amino acid oxidation in skeletal muscle during exercise, the main source for alanine, is lower in women (4, 42). The possible reasons that E2 appears to have a predominant effect on net hepatic, but not skeletal muscle, glycolgenolysis are unclear. To date, no study has investigated differences in E2 receptor number or affinity between the two tissues, which could account for the observed differences. Alternatively, it could be due to differences in the rate of glycogen turnover between liver and muscle; because liver has a quicker protein turnover rate as compared with muscle (44), it could be more sensitive to E2 supplementation and/or show effects more readily in a short-term study.

Unlike previous studies (21, 22), we failed to find an effect of E2 on MCR. It is possible that we did not have the statistical power necessary to find an effect of E2 on MCR, therefore making a type II error. However, the two studies that found an effect of E2 on MCR were slightly different from the current study. Our dose was lower than that used in one study (22), which achieved supraphysiological E2 concentrations and the effect on MCR thus may be a dose-response relation, and our dose may not have been high enough to elicit this response. Although the dose in the other study that found an effect of E2 on MCR was lower than that used in the current study, the subjects were women, who, as previously discussed, may respond more readily to E2 treatment because of gender differences in estrogen receptor number and affinity. From the current data, we are unable to determine whether we have made a type II error or whether there was no effect of our E2 dose on MCR.

In the current study, we used 11 men to investigate the effect of E2 on glucose kinetics and muscle glycogen use. In this randomized, double-blind, crossover, placebo-controlled design, the subjects acted as their own control, hence reducing errors due to small sample size and the high between-subject variability. The statistical analyses that we used complemented the strong study design to reduce both type I and type II errors. First, we used a higher number of subjects than in the previously published articles addressing similar issues. Second, we observed significant differences due to estrogen supplementation in RER, glucose Ra and Rd, and total glycogen and PG concentrations, indicating that, at least with respect to these outcome variables, we achieved sufficient power. Furthermore, alpha (also known as $P$ value, which indicates the probability of a type I error) would be the same, even with 10 times the number of subjects because alpha would remain at a level of 0.05. This confirms and extends the findings by other researchers. Indeed, this manuscript complements our observations of the effect of estrogen on whole-body substrate oxidation and leucine kinetics (26). Alternatively, a low sample size will alter power (1–$\beta$), and this may have resulted in the lack of significant findings in glycogen use. However, we calculated the number of individuals necessary to elicit an effect of E2 on glycogen use and found it to be 300. The differences in glycogen use between the estrogen and placebo groups were 4.2% for macroglycogen and 4.6% for total glycogen, which is unlikely to be statistically significant or, with a sample size of 300 subjects, physiologically significant. Furthermore, it is unlikely that the lack of difference in glycogen use was due to a type I error because our results fall in line with results observed in previous estrogen supplementation studies.

In summary, we have demonstrated that E2 administration decreases RER, glucose Ra and Rd, and muscle glycogen content without altering muscle glycogen use during exercise. Future studies should concentrate on longer duration supplementation regimens to determine whether a longer dosage period would decrease muscle glycogenolysis. Also, future research should focus on determining whether the decreased glucose Ra after E2 supplementation is due to a decrease in glycogenolysis or gluconeogenesis. Lastly, research needs to be conducted to investigate the effect of E2 supplementation on IMCL stores and whether E2 controls lipid availability and oxidation primarily, resulting in a subsequent shift in CHO use.

Acknowledgments

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