Effect of morning exercise on counterregulatory responses to subsequent, afternoon exercise

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Received 20 October 2000; accepted in final form 12 February 2001

Galassetti, Pietro, Stephnie Mann, Donna Tate, Ray A. Neill, David H. Wasserman, and Stephen N. Davis.
Effect of morning exercise on counterregulatory responses to subsequent, afternoon exercise. J Appl Physiol 91: 91–99, 2001.—The aim of this study was to determine whether a bout of morning exercise (EXE1) can alter neuroendocrine and metabolic responses to subsequent afternoon exercise (EXE2) and whether these changes follow a gender-specific pattern. Sixteen healthy volunteers (8 men and 8 women, age 27 ± 1 yr, body mass index 23 ± 1 kg/m², maximal O₂ uptake 31 ± 2 ml·kg⁻¹·min⁻¹) were studied after an overnight fast. EXE1 and EXE2 each consisted of 90 min of cycling on a stationary bike at 48 ± 2% of maximal O₂ uptake separated by 3 h. To avoid the confounding effects of hypoglycemia and glycogen depletion, carbohydrate (1.5 g/kg body wt po) was given after EXE1, and plasma glucose was maintained at euglycemia during both episodes of exercise by a modification of the glucose-clamp technique. Basal insulin levels (7 ± 1 μU/ml) and exercise-induced insulin decreases (−3 μU/ml) were similar during EXE1 and EXE2. Plasma glucose was 5.2 ± 0.1 and 5.2 ± 0.1 mmol/l during EXE1 and EXE2, respectively. The glucose infusion rate needed to maintain euglycemia during the last 30 min of exercise was increased during EXE2 compared with EXE1 (32 ± 4 vs. 7 ± 2 μmol·kg⁻¹·min⁻¹). Although this increased need for exogenous glucose was similar in men and women, gender differences in counterregulatory responses were significant. Compared with EXE1, epinephrine, norepinephrine, growth hormone, pancreatic polypeptide, and cortisol responses were blunted during EXE2 in men, but neuroendocrine responses were preserved or increased in women. In summary, morning exercise significantly impaired the body’s ability to maintain euglycemia during later exercise of similar intensity and duration. We conclude that antecedent exercise can significantly modify, in a gender-specific fashion, metabolic and neuroendocrine responses to subsequent exercise.

glucose clamp; epinephrine; glucagon

DURING EXERCISE, a complex pattern of neuroendocrine and autonomic nervous system (ANS) counterregulatory responses protects against the occurrence of hypoglycemia (5, 12, 20). These mechanisms effectively maintain euglycemia in healthy individuals over a broad range of exercise intensities and begin to fail only when exercise is performed for a prolonged period of time (19, 35). Although the counterregulatory responses to a single episode of exercise have been investigated extensively (8, 22–24), only incomplete and conflicting information exists regarding the effects of a prior bout of exercise on the counterregulatory responses to subsequent exercise performed later during the same day. Consequently, increased or unchanged neuroendocrine responses have been reported during a second bout of same-day exercise (9, 29, 30, 33, 34). Furthermore, in the majority of the above-mentioned studies, only a limited number of neuroendocrine responses were measured. More importantly, in no study utilizing a multiple exercise protocol were attempts made to compensate for the decline in blood glucose that occurs during exercise or to replenish glycogen stores that were depleted during earlier exercise bouts. Blood glucose falls during submaximal exercise, the magnitude of the drop being directly related to the absolute intensity of the work performed and the duration of the exercise bout (19). The fall in blood glucose, however, is greater during successive bouts of exercise, even if duration and workload are identical (29). Exercise performed during hypoglycemic conditions elicits counterregulatory responses that are greater than those induced by exercise per se (19). Therefore, quantitative comparative assessments of neuroendocrine responses during similar episodes of exercise could be confounded by the presence of hypoglycemia.

Gender differences in counterregulatory responses have been reported after a single bout of exercise, although with somewhat conflicting conclusions (15, 38). Furthermore, after antecedent stress (hypoglycemia), healthy women display less blunting of counterregulatory responses to subsequent hypoglycemia than do men (17). Hypoglycemia and exercise have many qualitative similarities in the pattern of counterregulatory responses they generate. It is unknown, however, whether prior exercise would induce sexually dimorphic alterations in counterregulatory responses to later bouts of exercise.

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We therefore designed the present study with the aims of determining 1) whether an earlier bout of exercise may alter the neuroendocrine and ANS responses to a second, equivalent bout of same-day exercise and 2) whether any alterations in counterregulatory responses induced during the later bout of exercise would follow a gender-specific pattern. We hypothesized that if subjects were maintained strictly euglycemic and glycogen stores were replenished between exercise bouts, then counterregulatory responses would be blunted by prior exercise, with a proportionally greater reduction in men than in women. To test this hypothesis, we studied 16 healthy subjects of both genders who performed two 90-min submaximal (~50% of maximal O\(_2\) uptake (V\(_{O2\ max}\)) cycle ergometer tests, separated by a 3-h interval. An integrative assessment of metabolic and neuroendocrine responses was performed during both episodes of exercise, so that a comparison of key counterregulatory responses could be determined.

**RESEARCH DESIGN AND METHODS**

**Subjects.** We studied 16 healthy volunteers, 8 men and 8 women, age 27 ± 1 yr, body weight 74 ± 4 kg (83 ± 4 and 62 ± 2 kg for men and women, respectively), body mass index 23 ± 1 kg/m\(^2\) (24 ± 41 and 22 ± 1 kg/m\(^2\) for men and women, respectively), and glycosylated hemoglobin (HbA\(_1c\)) 5.3 ± 0.1% (normal range 4.0–6.5%). None of the subjects were taking medications, nor did any of the subjects have a family history of diabetes. Each subject had a normal blood count, plasma electrolytes, and liver and renal function. All gave written informed consent. Studies were approved by the Vanderbilt University Human Subjects Institutional Review Board. The subjects were asked to avoid any exercise and consume their usual weight-maintaining diet for 3 days before each study. Female subjects were studied during the midfollicular phase of their menstrual cycle. Each subject was admitted to the Vanderbilt Clinical Research Center at 5 PM on the evening before an experiment. All subjects were studied after an overnight 10-h fast.

**Experimental design.** At least 2 wk before the initial study, subjects performed an incremental work test on a stationary cycle ergometer to determine V\(_{O2\ max}\) and anaerobic threshold (AT) (36). Airflow and O\(_2\) and CO\(_2\) concentrations in inspired and expired air were measured by a computerized open-circuit indirect calorimetry cart (Medical Graphics CPX-D) with a mouthpiece-and-nose clip system. AT was determined by the V-slope method (4). Experimental work rate was established by calculating 80% AT. The AT was detected at 59 ± 5% V\(_{O2\ max}\) and 80% of O\(_2\) uptake at AT corresponded to 47 ± 2% of the subject’s V\(_{O2\ max}\). This workload was chosen because it is close enough to the AT to produce a physically challenging stress (i.e., large experimental signal) but is sustainable for a prolonged period of time. Subjects ranged from sedentary to regularly exercising, although not actively participating in competitive sports. Mean V\(_{O2\ max}\) for the group was 31 ± 2 ml·kg\(^{-1}\)·min\(^{-1}\) (range 21–43 ml·kg\(^{-1}\)·min\(^{-1}\)).

**Experimental procedures.** On the morning of the study day, after an overnight fast, two intravenous cannulas were inserted under 1% lidocaine local anesthesia. One cannula was placed in a retrograde fashion into a vein on the back of the hand. This hand was placed in a heated box (55–60°C) so that arterialized blood could be obtained (1). The other cannula was placed in the contralateral arm so that 20% glucose could be infused via a variable-rate volumetric infusion pump (I-med, San Diego, CA).

After insertion of venous cannulas, a period of 90 min was allowed to elapse, followed by a 30-min basal period and a 90-min morning exercise period (EXE\(_1\)), a 180-min resting period, and a second 90-min exercise period (EXE\(_2\); Fig. 1). Each exercise bout consisted of 90 min of continuous submaximal exercise (at 60–70 rpm) on an upright cycle ergometer (Medical Graphics, Yorba Linda, CA) at 80% of the individual’s AT. Plasma glucose was maintained equivalent to basal levels throughout the study by a glucose-clamp technique, according to which glucose levels were measured every 5 min during both exercise periods and every 20 min during the rest interval between EXE\(_1\) and EXE\(_2\). A variable infusion of 20% dextrose was adjusted so that plasma glucose levels were held constant at the desired concentration (3). During the first 45 min after EXE\(_1\), a drink containing carbohydrate (1.5 g/kg body wt) was administered orally to replenish glycogen stores depleted during EXE\(_1\).

**Analytic methods.** The collection and processing of blood samples have been described elsewhere (11). Plasma glucose concentrations were measured in triplicate using the glucose oxidase method with a glucose analyzer (Beckman, Fullerton, CA). Glucagon was measured according to a modification of the method of Aguilar-Parada et al. (2), with an interassay coefficient of variation (CV) of 12%. Insulin was measured as previously described (37), with an interassay CV of 9%. Catecholamines were determined by HPLC (10), with an interassay CV of 12% for epinephrine and 8% for norepinephrine. We made two modifications to the procedure for catecholamine determination: 1) we used a five-point, rather than a one-point, standard calibration curve, and 2) we spiked the initial and final samples of plasma with known amounts of epinephrine and norepinephrine so accurate identification of the relevant respective catecholamine peaks could be made. Cortisol was assayed using the Clinical Assays Gamma Coat RIA kit, with an interassay CV of 8%. Growth hormone (GH) was determined by RIA (28) using the Nichols Institute Diagnostics kit (San Juan Capistrano, CA), with a CV of 8.6%. Pancreatic polypeptide was measured by RIA using the method of Hagopian et al. (26), with an interassay CV of 8%. Lactate, glycerol, alanine, and β-hydroxybutyrate were measured in deproteinized whole blood using the method of Lloyd et al. (31). Nonesterified fatty acids (FFA) were measured by

![Fig. 1. Schematic diagram of experimental protocols. V\(_{O2\ max}\), maximal O\(_2\) uptake.](http://jap.physiology.org/DownloadedFrom/10.1152/jappl.02334.17)
using the WAKO kit adapted for use on a centrifugal analyser (27).

Blood for hormones and intermediary metabolites was drawn twice during the basal period and every 15 min during the experimental period. Cardiovascular parameters (pulse, systolic, diastolic, and mean arterial pressure) were measured noninvasively by a Dinamap (Critikon, Tampa, FL) every 10 min during EXE1 and EXE2. Gas-exchange measurements were performed during the 30 min preceding each exercise bout, as well as during the last 30 min of each exercise bout.

Statistical analysis. Values are means ± SE unless otherwise stated and were analyzed using standard, parametric, two-way analysis of variance with repeated-measures design. When appropriate, Newman-Keuls post hoc test was performed to delineate at which time points statistical significance was reached. \( P < 0.05 \) indicated significant difference.

RESULTS

Plasma glucose and insulin levels. Plasma glucose was maintained at euglycemia throughout both episodes of exercise (morning baseline, 5.2 ± 0.1 mmol/l; EXE1 steady state, 5.2 ± 0.1 mmol/l; afternoon baseline, 5.0 ± 0.1 mmol/l; EXE2 steady state, 5.1 ± 0.1 mmol/l). Plasma insulin levels were similar at baseline (morning, 6.3 ± 0.8 μU/ml; afternoon, 8.8 ± 1.2 μU/ml) and during exercise steady state [last 30 min of exercise, 3.8 ± 0.7 (EXE1) and 4.7 ± 0.6 (EXE2) μU/ml]. No difference between genders was measured for glucose or insulin levels at any time during the study.

Counterregulatory hormone levels. Baseline plasma epinephrine was 53 ± 9 pg/ml in the morning and 37 ± 4 pg/ml in the afternoon (Fig. 2, Table 1). During both exercise bouts, epinephrine increased similarly and
significantly ($P < 0.01$) over basal values ($\Delta = 141 \pm 32$ and $161 \pm 35$ pg/ml during EXE$_1$ and EXE$_2$, respectively). Baseline plasma norepinephrine was $315 \pm 34$ pg/ml in the morning and $459 \pm 45$ pg/ml in the afternoon. During both exercise bouts, norepinephrine increased significantly over basal values, but the increase was blunted during EXE$_2$ ($\Delta = 351 \pm 92$ pg/ml) compared with EXE$_1$ ($\Delta = 597 \pm 102$ pg/ml, $P < 0.05$). When data were separated by gender, in women the epinephrine response was increased in the afternoon, whereas the norepinephrine response was unchanged. In men, both catecholamine responses were reduced in the afternoon.

Basal levels of plasma glucagon were $57 \pm 12$ pg/ml in the morning and $41 \pm 4$ pg/ml in the afternoon (Table 1). Increments over baseline (mean values of the last 30 min of exercise) in plasma glucagon were similar during EXE$_1$ and EXE$_2$ ($\Delta = 17 \pm 4$ and $17 \pm 3$ pg/ml, respectively), with no difference between genders.

Basal levels of plasma cortisol (Table 1, Fig. 3) were $15 \pm 3$ µg/dl in the morning and increased to $23 \pm 4$ µg/dl during EXE$_1$. Although basal levels were lower in the afternoon than in the morning ($9 \pm 1$ µg/dl), the exercise-induced increments during both bouts of exercise were identical ($6 \pm 2$ µg/dl). The afternoon cortisol response to exercise was significantly greater than the morning response in women, while it was reduced in men.

GH (Table 1, Fig. 3) increased from a basal level of $2 \pm 1$ ng/ml to $10 \pm 3$ ng/ml during the last 30 min of EXE$_1$ and from $2 \pm 1$ to $7 \pm 1$ ng/ml during the last 30 min of EXE$_2$. The exercise-induced increment in the hormone, therefore, was smaller, although not significantly, during EXE$_2$ ($5 \pm 1$ ng/ml) than during EXE$_1$ ($8 \pm 3$ ng/ml). Pancreatic polypeptide (Fig. 3) increased from $93 \pm 15$ to $253 \pm 41$ pg/ml during EXE$_1$ and from $146 \pm 27$ to $267 \pm 38$ pg/ml during EXE$_2$. For these two latter hormones, the afternoon exercise response was similar to the morning response in women, whereas it was reduced in men.

Glucose infusion and substrate oxidation rates. No exogenous glucose was infused at the time of morning baseline measurements. During EXE$_1$, exogenous glucose was gradually needed to maintain euglycemia, with an average infusion rate of $7 \pm 2$ µmol·kg$^{-1}$·min$^{-1}$ (9 ± 3 in men and 4 ± 2 in women) during the last 30 min of exercise (Fig. 4). In the afternoon, the basal glucose infusion rate was $6 \pm 2$ µmol·kg$^{-1}$·min$^{-1}$ (8 ± 3 in men and 4 ± 2 in women) and progressively increased to $32 \pm 4$ µmol·kg$^{-1}$·min$^{-1}$ (34 ± 8 in men and 30 ± 7 in women) during the last 30 min of EXE$_2$ ($P < 0.001$ vs. EXE$_1$).

The morning baseline respiratory exchange ratio (RER) was $0.84 \pm 0.02$ ($0.84 \pm 0.02$ in men and $0.83 \pm 0.03$ in women); the rate of carbohydrate oxidation (Carb$_{ox}$) was $8 \pm 2$ µmol·kg$^{-1}$·min$^{-1}$ ($8 \pm 1$ in men and $8 \pm 3$ in women), and the rate of fat oxidation (FFA$_{ox}$) was $0.7 \pm 0.2$ mg·kg$^{-1}$·min$^{-1}$ ($0.7 \pm 0.2$ in men and $0.8 \pm 0.2$ in women). At the end of EXE$_1$, RER was $0.90 \pm 0.01$ ($0.91 \pm 0.02$ in men and $0.89 \pm 0.01$ in women), Carb$_{ox}$ was $98 \pm 8$ µmol·kg$^{-1}$·min$^{-1}$ ($111 \pm 15$ in men and $86 \pm 7$ in women), and FFA$_{ox}$ was $3.1 \pm 0.3$ mg·kg$^{-1}$·min$^{-1}$ ($3.2 \pm 0.4$ in men and $2.9 \pm 0.7$ in women). During afternoon baseline measurements, RER was $0.92 \pm 0.03$ ($0.89 \pm 0.06$ in men and $0.95 \pm 0.03$ in women), Carb$_{ox}$ was $18 \pm 3$ µmol·kg$^{-1}$·min$^{-1}$ ($15 \pm 5$ in men and $21 \pm 2$ in women), and FFA$_{ox}$ was $0.3 \pm 0.2$ mg·kg$^{-1}$·min$^{-1}$ ($0.4 \pm 0.3$ in men and $0.2 \pm 0.2$ in women). At the end of EXE$_2$, RER was $0.90 \pm 0.01$ ($0.90 \pm 0.02$ in men and $0.90 \pm 0.01$ in women),
Intermediary metabolism. In the morning during the first 30 min of EXE1, blood lactate levels increased from the basal level of 1.1 ± 0.1 mmol/l to a peak value of 2.9 ± 0.3 mmol/l and gradually decreased to 1.7 ± 0.2 mmol/l at the end of EXE1 (Fig. 4). Lactate excursions were reduced during EXE2 (basal, 1.5 ± 0.1 mmol/l; 30-min peak, 2.1 ± 0.2 mmol/l; end exercise, 1.4 ± 0.1 mmol/l; *P* < 0.05 vs. EXE1). Blood alanine levels (Table 2) increased moderately over baseline during EXE1 and remained unchanged during EXE2.

Basal blood glycerol levels tended to be higher in the afternoon (74 ± 12 μmol/l) than in the morning (53 ± 5 μmol/l, *P* = 0.06) and increased similarly during both exercise bouts (by 166 ± 15 and 162 ± 15 μmol/l during EXE1 and EXE2, respectively; Table 2). Basal plasma FFA levels, on the other hand, were lower in the afternoon (335 ± 68 μmol/l) than in the morning (486 ± 40 μmol/l, *P* < 0.05) and increased significantly more during EXE2 (by 504 ± 81 μmol/l) than during EXE1 (by 248 ± 38 μmol/l, *P* < 0.005; Table 2). Basal levels of β-hydroxybutyrate also tended to be lower in the afternoon (54 ± 26 μmol/l) than in the morning (99 ± 22 μmol/l) and increased significantly more during EXE2 (by 109 ± 27 μmol/l) than during EXE1 (by 29 ± 10 μmol/l, *P* < 0.01; Table 2).

Cardiovascular parameters. Heart rate and systolic, diastolic, and mean arterial pressures were similar at baseline in the morning and afternoon and increased equivalently during both exercise bouts (Table 3).
DISCUSSION

This study has investigated the effects of moderate antecedent morning exercise on counterregulatory responses to subsequent afternoon exercise of comparable intensity and duration. Euglycemia was maintained during exercise, and carbohydrate utilization was equalized during both exercise bouts via oral administration of glucose (1.5 g/kg body wt) after morning exercise. Compared with morning exercise, an almost five-times-greater exogenous glucose infusion was needed during afternoon exercise to maintain euglycemia. Although the afternoon increase in this metabolic response was similar in both genders, concomitant neuroendocrine responses were altered according to a clear gender-specific pattern. In men, catecholamines, GH, pancreatic polypeptide, and cortisol were altered.

Table 2. Blood lactate, alanine, glycerol, and plasma FFA during two 90-min bouts of cycling exercise at 50% \( \dot{V}O_{2\max} \) in healthy humans

<table>
<thead>
<tr>
<th></th>
<th>Morning Baseline</th>
<th>Morning Last 30 min</th>
<th>Afternoon Baseline</th>
<th>Afternoon Last 30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood alanine, mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>0.35 ± 0.05</td>
<td>0.44 ± 0.06</td>
<td>0.38 ± 0.04</td>
<td>0.37 ± 0.05</td>
</tr>
<tr>
<td>Women</td>
<td>0.28 ± 0.03</td>
<td>0.34 ± 0.04</td>
<td>0.36 ± 0.04*</td>
<td>0.33 ± 0.02</td>
</tr>
<tr>
<td>Blood glycerol, ( \mu )M</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>44 ± 6</td>
<td>196 ± 31</td>
<td>76 ± 21</td>
<td>227 ± 24</td>
</tr>
<tr>
<td>Women</td>
<td>63 ± 8*</td>
<td>242 ± 21</td>
<td>72 ± 16</td>
<td>245 ± 34</td>
</tr>
<tr>
<td>Plasma FFA, ( \mu )M</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>423 ± 40</td>
<td>629 ± 74</td>
<td>402 ± 140</td>
<td>944 ± 188</td>
</tr>
<tr>
<td>Women</td>
<td>548 ± 70*</td>
<td>838 ± 79*</td>
<td>267 ± 41†</td>
<td>735 ± 85</td>
</tr>
<tr>
<td>Blood ( \beta )-hydroxybutyrate, ( \mu )M</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>73 ± 20</td>
<td>89 ± 22</td>
<td>82 ± 56</td>
<td>185 ± 43</td>
</tr>
<tr>
<td>Women</td>
<td>125 ± 41</td>
<td>166 ± 53</td>
<td>26 ± 7</td>
<td>141 ± 47</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 16 \) (8 men and 8 women). FFA, free fatty acid. *\( P < 0.05 \) vs. men. †\( P < 0.05 \) vs. corresponding morning value.
reduced; in women, these responses were unchanged or increased.

During physical exercise of moderate intensity, such as that performed in this study, plasma glucose levels are maintained relatively stable by an equilibrium between endogenous glucose production and glucose utilization by the working muscle. In our study, Carbox was virtually identical during morning and afternoon exercise (98 ± 9 and 94 ± 10 μmol·kg⁻¹·min⁻¹, respectively). The exogenous glucose infusion needed to maintain a plasma glucose level of 52 mmol/l, on the other hand, was 31 ± 4 μmol·kg⁻¹·min⁻¹ during the last 30 min of the afternoon exercise, as opposed to only 6 ± 2 μmol·kg⁻¹·min⁻¹ during the earlier bout of exercise. Because of similar morning and afternoon Carbox, it would therefore appear that the increased rate of exogenous glucose infusion needed in the afternoon to maintain euglycemia was the result of decreased endogenous glucose production.

Gender-related differences in counterregulatory responses to stress are being increasingly recognized. Women typically display reduced neuroendocrine and metabolic responses to hypoglycemia (14) and exercise (16), possibly reflecting a greater sensitivity to ANS drive (14). Furthermore, exercise responses may be influenced in women by the phase of their menstrual cycle: FFA responses have been reported to be lower, and GH responses higher, during the luteal phase than during the follicular phase (6). Our laboratory has previously demonstrated that, after antecedent stress, counterregulatory responses to hypoglycemia are significantly blunted in men and women, but the magnitude of the blunting is consistently less in women than in men (16, 17, 21). Consistent with these previous observations, in the present study, key counterregulatory responses in men, such as epinephrine, norepinephrine, glucagon, cortisol, and pancreatic polypeptide, were attenuated in the afternoon, compared with the morning, exercise (Figs. 2 and 3). These responses were either unchanged or increased in women, resulting in a statistically significant gender difference in the pattern of change of these parameters between morning and afternoon exercise. It is interesting that these gender differences in counterregulatory responses were not paralleled by proportional differences in endogenous glucose infusion. Although explanations for this discrepancy remain speculative, we believe that the acute increase in insulin sensitivity induced by prior exercise may have overridden differences in counterregulatory responses and resulted in similar metabolic effects.

The lower peak lactate levels observed with the afternoon than with the morning exercise may be due to residual vasodilation from morning exercise, favoring O₂ delivery and glucose oxidation over glycolysis. Although different levels of glycogen stores may also have contributed to the reduced lactate levels during afternoon compared with morning exercise, we believe that the oral glucose load administered at the end of morning exercise minimized the impact of this variable.

Although absolute values of FFA concentrations differed considerably between genders (Table 2), lipolytic responses appeared to be similar during both exercise bouts in men and women. It would therefore appear that the antecedent bout of exercise in women resulted in an acceleration of peripheral FFA disposal and/or increased FFA reesterification. The mechanisms responsible for this observation, which may include gender-specific regional hypersensitivity to insulin at the adipocyte, remain speculative.

The secretion of some counterregulatory hormones is modulated by circadian patterns. Cortisol levels, in particular, are normally high in the morning and progressively decrease during the day. Raised levels of cortisol (2- to 3-fold) do not have an effect on increasing endogenous glucose production for 180 min during hypoglycemia-inducing stress, during which time glucagon and epinephrine play the greatest counterregulatory role (13). Our exercise bouts, on the other hand, lasted only 90 min. We therefore believe that in our particular experimental setting the relatively small circadian changes in basal cortisol secretion would have had a negligible effect on our findings.

### Table 3. Heart rate and systolic, diastolic, and mean arterial blood pressures during two 90-min bouts of cycling exercise at 50% V₀₂max in healthy humans

<table>
<thead>
<tr>
<th></th>
<th>Morning</th>
<th>Afternoon</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>Last 30 min</td>
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<tr>
<td>Heart rate, beats/min</td>
<td></td>
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</tr>
<tr>
<td>Men</td>
<td>69 ± 4</td>
<td>144 ± 6</td>
</tr>
<tr>
<td>Women</td>
<td>74 ± 6</td>
<td>139 ± 7</td>
</tr>
<tr>
<td>Systolic arterial blood pressure, mmHg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>120 ± 4</td>
<td>152 ± 5</td>
</tr>
<tr>
<td>Women</td>
<td>112 ± 4</td>
<td>136 ± 4*</td>
</tr>
<tr>
<td>Diastolic arterial blood pressure, mmHg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>76 ± 4</td>
<td>70 ± 4</td>
</tr>
<tr>
<td>Women</td>
<td>73 ± 2</td>
<td>68 ± 4</td>
</tr>
<tr>
<td>Mean arterial blood pressure, mmHg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>91 ± 4</td>
<td>97 ± 3</td>
</tr>
<tr>
<td>Women</td>
<td>86 ± 4</td>
<td>91 ± 2*</td>
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</tbody>
</table>

Values are means ± SE; n = 16 (8 men and 8 women). *P < 0.05 vs. men.
The degree of depletion of the body’s glycogen stores may alter substrate metabolism during exercise. To replenish glycogen stores, at the end of morning exercise, we administered an oral load of carbohydrate calculated to equal the amount oxidized during morning exercise. It could be argued, however, that a greater depletion of glycogen stores was present at the start of afternoon exercise because of continued glucose oxidation during the 3-h interval between exercise bouts. We believe, however, that if any additional glycogen depletion was present before afternoon exercise, it would have been of negligible magnitude for the following reasons: 1) glycogen depletion during morning exercise was less than estimated from Carb ox, inasmuch as hepatic gluconeogenesis would have contributed toward endogenous glucose production (7); 2) additional glucose was infused during the last 30 min of morning exercise (7 μmol·kg⁻¹·min⁻¹) and during the last 90 min of the rest period (6 μmol·kg⁻¹·min⁻¹), providing an additional ~10 g of carbohydrate; and 3) gluconeogenesis probably also contributed 5–10 g of glucose during the rest period, as previously observed in similar experimental conditions by Maehlum et al. (32). Differences in the degree of glycogen depletion may also have existed between genders. Before afternoon exercise was started, Carb ox was ~40% higher in women than in men. Had this difference been present for most of the interval between exercises, it may indicate that a smaller portion of exogenous glucose was channeled toward glycogen replenishment in women.

Direct comparison of our results with the work of other investigators is confounded by marked differences in experimental design and subject characteristics. Nevertheless, it is worth noting that, in several previous studies investigating counterregulatory responses to repeated bouts of exercise, neuroendocrine responses were unchanged or increased during later bouts of exercise compared with earlier exercise. Knaley et al. (30) reported a progressive increase in GH secretion during three 30-min bouts of exercise at 70% of peak VO₂ (VO₂peak); Marliss et al. (33) reported similar increases in glucagon and catecholamines during two short bouts of exercise at 100% VO₂peak; Brennan et al. (8) reported unchanged catecholamine, GH, and cortisol responses during two 30-min bouts of exercise at 50% VO₂peak, whereas Kaciuba-Uscilko et al. (29) reported progressively increased catecholamines, cortisol, GH, and glucagon during four consecutive 30-min exercise bouts at 50% VO₂peak, separated by 30-min intervals. It should be noted that in these studies all subjects were men. Furthermore, in two of the above-mentioned studies (30, 33), the experimental workload was 70–100% VO₂peak. At these elevated workloads, the ANS drive is very high, the increase in endogenous glucose production is disproportionately more than the increase in glucose utilization, and hyperglycemia occurs. This generates metabolic circumstances completely different from those in our study. Independent of the workload used, however, levels of circulating glucose in all the above-mentioned studies were lower during later bouts of exercise. Felig et al. (19) reported that, of 19 healthy men exercising at 60–65% VO₂peak to exhaustion, 7 experienced severe hypoglycemia (blood glucose <2.5 mmol/l). In this group of subjects, plasma epinephrine levels correlated with the degree of hypoglycemia, and in a control group the increase in catecholamines was prevented by glucose administration that maintained euglycemia. In the study by Kaciuba-Uscilko et al., which used an exercise workload similar to that of our study, the greatest increases in counterregulatory hormones were measured during the fourth exercise bout, when blood glucose had dropped to 3.3 mmol/l from an initial level of 4.6 mmol/l. We therefore believe that unless euglycemia is carefully maintained, as in the present study, the confounding effect of concomitant hypoglycemia prevents the identification of counterregulatory responses induced by exercise per se.

One implication of our findings is that hypoglycemia would have occurred during the afternoon bout of exercise had exogenous glucose not been administered to our subjects. This could be of particular relevance for patients with diabetes, who suffer a high prevalence of exercise-associated hypoglycemia (18, 25). Although the mechanisms causing exercise-associated hypoglycemia are unclear, studies in healthy subjects indicate that acute counterregulatory failure induced by prior stress is likely to play a role (16, 21). Further studies are needed to ascertain whether these concepts also apply to patients with diabetes.

In summary, this study has demonstrated that one episode of prior moderate, prolonged morning exercise markedly increased the body’s need for exogenous glucose to maintain euglycemia during subsequent, afternoon exercise. Compared with morning exercise responses, the epinephrine, norepinephrine, GH, pancreatic polypeptide, and cortisol responses were decreased in men, whereas in women these parameters were unchanged or increased.

We conclude that, in overnight-fasted healthy men, prior moderate prolonged exercise markedly impaired the ability to maintain euglycemia during subsequent, comparable same-day exercise. Neuroendocrine counterregulatory responses in women are resistant to the blunting effects of antecedent exercise relative to men.

We thank Eric Allen and Pam Venson for expert technical assistance. We also appreciate the skill and help of the nurses of Vanderbilt General Clinical Research Center.

This work is supported by a grant from the Juvenile Diabetes Foundation International, National Institutes of Health Grants R01 DK-45369, Diabetes Research and Training Center Grant 5P60-AM-20593, and Clinical Research Center Grant M01-RR-00095, and a Veterans Affairs/Juvenile Diabetes Foundation International Diabetes Research Center Grant.

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