Two Bouts of Exercise before Meals, but Not after Meals, Lower Fasting Blood Glucose

KATARINA T. BORER1, ELIZABETH C. WUORINEN1, JAMIE R. LUKOS1, JOHN W. DENVER3, STEPHEN W. PORGES3, and CHARLES F. BURANT1,2

1School of Kinesiology, University of Michigan, Ann Arbor, MI; 2Department of Internal Medicine, University of Michigan, Ann Arbor, MI; and 3Brain-Body Center, Department of Psychiatry, University of Illinois at Chicago, Chicago, IL

ABSTRACT


Introduction: Reduced counterregulatory responses to a next-day hypoglycemia challenge and hypoglycemia result from two spaced episodes of moderate-intensity exercise and have been characterized as exercise-associated autonomic failure. We hypothesized that this phenomenon is caused by postabsorptive state at the time of exercise rather than by autonomic failure. Methods: Participants were nine healthy postmenopausal women in a crossover study. Two hours of treadmill exercise at 43% of maximal effort were performed twice a day, separated by 5 h, either 1 h before (Before-Meals trial) or 1 h after a meal (After-Meals trial). Plasma insulin, counterregulatory hormones (glucagon, growth hormone, cortisol), and metabolites (glucose, free fatty acids, ketones) were measured to evaluate the effects of nutritional timing. Analyses of HR and vagal tone were measured to assess autonomic function. Results: Before-Meals exercise, but not After-Meals exercise, reduced postabsorptive plasma glucose by 20.2% during a 16-h period, without a change in counterregulatory response, and elicited postexercise ketosis. A 49% increase in insulin–glucagon ratio during meals, a 1 mM decline in glucagon glycemic threshold, and a reduced vagal tone during exercise were associated with Before-Meals but not with After-Meals trials. Conclusions: These results demonstrate that exercise performed in postabsorptive, but not in postprandial state, lowers glucoregulatory set point and glucagon glycemic threshold and is accompanied by reduced vagal tone, counterregulatory responses, and glucagon glycemic threshold and by increased insulin–glucagon ratio. Reduced counterregulatory response, altered neuroendocrine function, and sustained lowering of blood glucose are most likely the consequences of reduced carbohydrate availability during exercise. Key Words: RESPIRATORY SINUS ARRHYTHMIA, COUNTERREGULATION, HYPOGLYCEMIA, GLUCAGON GLYCEMIC THRESHOLD, POSTEXERCISE KETOSIS, VAGAL TONE

Diet and exercise are integral to the prevention of type 2 diabetes (16) by normalizing blood glucose level and preventing damage to peripheral organs that results from chronic hyperglycemia. When diet and exercise fail to reduce persistent hyperglycemia, pharmacological agents such as metformin (35) are added to increase peripheral insulin sensitivity, to enhance insulin secretion, or to reduce elevated hepatic glucose production. Improvements in the efficacy of diet and exercise could aid the prediabetic without the side effects of medication and with additional health benefits of exercise.

Dysregulation of insulin action entails reduction in the insulin-stimulated glucose uptake and increase in hepatic glucose output (5,29). Hepatic glucose output is increased by glucagon, epinephrine, growth hormone (GH), and cortisol, and this provides adequate circulatory glucose supply during fasting and during up to 3 h of continuous moderate-intensity exercise. Exercise causes faster glucose clearance from plasma owing to increased insulin-independent muscle glucose uptake (27) even when additional glucose is concurrently introduced through food or drink (22). After 4 h of continuous low-intensity exercise (3) or 3 h of moderate-intensity exercise (2), plasma glucose gradually declines toward the hypoglycemic level, despite robust increases in counterregulatory response. This likely reflects a limit in hepatic glucose production capacity rather than an altered neuroendocrine response, as supplying glucose orally (6) or intravenously (10,12) normalizes plasma glucose and appropriately suppresses counterregulatory response.

Glucoregulation is altered when moderate-intensity exercise of 90-min duration is repeated after 3 h of rest. This treatment reduces or eliminates counterregulatory response to a hyperinsulinemic hypoglycemic challenge reducing plasma glucose to 2.8 mM administered 6 to 24 h later (8,9,30–33). Hepatic glucose output declines, and plasma glucose concentration declines toward hypoglycemic levels in both healthy (8,9,32,33) and type 1 diabetic subjects (30,31). This

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phenomenon has been characterized as exercise-associated autonomic failure (EAAF) because of reduced of absent counterregulatory response (33). A postabsorptive state in combination with an extended interval between two exercise bouts seems to be necessary for the appearance of sustained hypoglycemia and the reduction in counterregulation because neither continuous exercise after a 72-h fast (11) nor repeated exercise bouts of 30-min duration at 30-min intervals (18) suppress vigorous counterregulatory response in the face of plasma glucose declines.

We hypothesized that sustained hypoglycemia seen in the spaced exercise studies is an adaptation to reduced circulating carbohydrate availability during exercise. The preferential glucose uptake into the exercising and postexercise muscle does not allow adequate glucose flux to restore liver glycogen when exercise is carried out in fasted or in postabsorptive state (7). Increased non–insulin-dependent muscle glucose uptake during exercise (27) and substantial dependence on carbohydrate fuel during moderate-intensity exercise (28), combined with sustained increases in muscle insulin sensitivity after exercise (13,39), channel ample amounts of carbohydrate to muscle for glycogen synthesis. Postexercise muscle glycogen synthesis is further facilitated by the activation of glycogen synthase in proportion to the level of muscle glycogen depletion (17) and of the enzyme hexokinase that is capable of sequestering glucose at hypoglycemic concentrations so long as it is used for glycogen synthesis (25). Because of the difference in the $K_m$ of muscle hexokinase and liver glucokinase, muscle takes precedence over liver in glucose uptake and glycogen resynthesis for between several hours (7) and for up to 3 d after a glycogen-depleting exercise. This produces not only compensation but also supercompensation of muscle glycogen stores (4) before the full recovery of liver glycogen.

To test the hypothesis that sustained lowering of blood glucose after two spaced bouts of exercise performed in postabsorptive state is due to inadequate circulating carbohydrate availability rather than to autonomic failure, we scheduled exercise either 1 h before (Before-Meals) or 1 h after (After-Meals) a meal containing 63% carbohydrate. We also measured plasma ketone body formation after exercise to functionally evaluate hepatic carbohydrate depletion (19,21). Finally, to assess whether the reduced carbohydrate availability was responsible for autonomic failure, we examined changes in global autonomic function during exercise under differing prandial states by measuring HR and its variability. Our aim was to gain a better understanding of the mechanism of exercise-associated blood lowering in healthy subjects before such lifestyle strategy could be tested in prediabetic and type 2 diabetic subjects.

**MATERIALS AND METHODS**

The study was approved by the University of Michigan Medical School Institutional Review Board, and all subjects provided informed consent. Subjects were nine postmenopausal women, seven white and two African American. Eight were nondiabetic, and one African American subject was prediabetic. They were $58.5 \pm 1.7$ yr of age, with body mass of $74.9 \pm 4.3$ kg, body mass index of $27.0 \pm 1.4$ kg·m$^{-2}$, and $37.3 \pm 2.7$% body fat, and four were on hormone replacement therapy. All engaged in less than 60 min of exercise per week. Their maximal oxygen consumption was $1.9$ L O$_2$·min$^{-1}$ or $25.1 \pm 1.5$ mL O$_2$·kg$^{-1}$·min$^{-1}$. A health questionnaire, that is, and a detailed physical examination that included measurements of weight, height, body fat by a bioimpedance apparatus (RJL Quantum II, Clinton, MI), laboratory chemistries, and thyroid function, constituted a health screen. An exercise screen consisted of indirect calorimetric measurements (Physio-Dyne, Quoque, NY) during a treadmill test consisting of 0.64-km·min$^{-1}$ speed increments until maximal effort was achieved on the basis of the respiratory quotient, RQ of 1 as the criterion.

**Study protocol.** Subjects participated in two trials that were assigned in a random order and separated by at least 1 wk (Fig. 1). After admission to the General Clinical Research Center at 1800 h on the day before a trial, subjects were provided at 1900 h with a standardized meal consisting of 63% carbohydrates, 23% fat, and 14% protein that provided 25 kcal·kg$^{-1}$ body weight. A chest electrode below the right clavicle and another one at the left lower rib cage were connected to a Polar HR receiver and a Mini-Logger2000 recorder (Respiration, Inc, Bend, OR), worn around the waist, for measurement of HR and its variability. At 0545 h the following morning, an indwelling Teflon catheter was inserted into a forearm vein.

**Meals.** Two meals containing $22.7 \pm 0.9$ kcal·kg$^{-1}$ body weight and consisting of 63% carbohydrates, 23% fat, and 14% protein were provided at 1000 and at 1700 h of the study day. The food provided and any food left uneaten were weighed to allow calculation of macronutrient energy.

**Exercise.** Two 2-h bouts of treadmill walking at $43 \pm 2.5$% of $V_{02max}$ were completed by each subject. The times of Before-Meals exercise were 0700 to 0900 h and 1400 to 1600 h, and those of After-Meals exercise were 1100 to 1300 h and 1800 to 2000 h.

**Metabolic measurements.** RMR was measured between 0600 and 0630 h at the start and the end of the 24-h trials with a Delta Trac II metabolic cart (SensorMedics, Yorba Linda, CA). Metabolism was also measured immediately after exercise and meals at midnight and 0400 h. Exercise metabolism was measured (Physio-Dyne, Quoque, NY) during minutes 0 to 30 and minutes 60 to 90 of each exercise bout. Energy cost of exercise and relative use of carbohydrates and lipids were estimated using the Weir equation (38).

**Blood collection.** Serial blood samples (3 to 5 mL, $n = 42$) were collected into ice-chilled EDTA-coated tubes containing $50$ µL·mL$^{-1}$ blood of aprotinin (Sigma Chemical, St. Louis, MO) at hourly intervals except for 15- and
30-min intervals at the start of meals and exercise. Plasma was kept frozen at −80°C.

**Hormone and metabolite measurements.** Concentrations of insulin, glucagon (Linco Research, St. Louis, MO), and cortisol (Diagnostic Systems Laboratories, Webster, TX) were measured with radioimmunoassays. Measurements for both trials were performed in the same assay. The intra- and interassay coefficients of variation (CV) were 2.2% and 20% for insulin, 10.1% and 11.8% for glucagon, and 9.1% and 14.2% for cortisol, respectively. GH was measured with a chemiluminescent immunoassay (Nichols Institute Diagnostics, St. Juan Capistrano, CA) with intra- and interassay CV of 10.7% and 15.9%, respectively. Plasma glucose, nonesterified free fatty acid (FFA), and the ketone body 3-β-hydroxybutyrate concentrations were measured with enzymatic spectrophotometric procedures provided by Thermo DMA (Arlington, TX), WACO Diagnostics (Richmond, VA), and RANDOX (Ardmore, Antrim, United Kingdom; Randox Laboratories, Oceanside, CA), respectively.

**Assessment of autonomic function.** This used measurement of HR and respiratory sinus arrhythmia (RSA). HR acceleration during exercise is caused by vagal withdrawal and sympathetic stimulation of β1-adrenergic receptors and thus reflects global sympathetic function. RSA reflects spontaneous HR oscillations at the frequency of breathing, and its amplitude is a component of the beat-to-beat HR variability. It is controlled by the parasympathetic vagal tone (23). Duration of interbeat (R-wave) intervals was timed by the Mini-Logger2000 in milliseconds and stored as sequential heart periods. A 250-s series of interbeat intervals during the last 5 min of each hour were analyzed by MxEdit software (Brain–Body Center, Chicago). MxEdit applied time series analyses to the interbeat interval data to extract the HR variability in the frequencies of spontaneous breathing during resting and exercise conditions (0.12–1.00 Hz) after removing lower-frequency trends and periodicities. The natural log of the variance of the HR data was a measure of cardiac vagal tone (14,26).

**Statistical analysis.** A two-way mixed-model repeated-measures ANOVA (factors: trial and time) was performed with SAS software version 9.1 (SAS Institute, Cary, NC) for hormone and metabolite areas under the curve (AUC) calculated with trapezoidal rule. AUC were calculated for insulin and insulin–glucagon ratio during the 4.5-h postprandial periods, for GH and cortisol during exercise-associated hormone surges, and for FFA and ketone bodies during 2 h before and 2 h after the meals. Glucose concentrations, energy expenditure, use of carbohydrates and fats, HR, and RSA were averaged during the 2 h of exercise. Mixed-model repeated-measures ANOVA was performed on all time points for glucagon and postabsorptive glucose concentrations during the entire period and the final 16 h of study. Glucagon glycemic threshold was identified at glucose concentrations that elicited increases in hormone concentration. RMR and total nonexercise metabolism also were analyzed. Data are presented as means and SEM. α ≤ 0.05 was the criterion of significant difference.

**RESULTS**

All subjects had normal hemoglobin (13.1 ± 0.3 g·dL⁻¹), hematocrit (38.0 ± 1%), fasting insulin (10.3 ± U·mL⁻¹), and TSH (2.4 ± 0.3 mU·L⁻¹). Eight subjects had normal fasting glucose (4.1 ± 0.2 mM) and total–HDL cholesterol ratio (2.9 ± 0.3), whereas one subject was prediabetic with above-normal fasting glucose (7.5 mM) and total–HDL cholesterol ratio (4.7).

**Energy metabolism.** No between-trial differences were found for any measures of energy expenditure or intake (Table 1). In both trials, RMR was 53.3 ± 2.5 kcal·h⁻¹, total 24-h nonexercise energy expenditure ranged between 1570 ± 82 and 1475 ± 76 kcal, and exercise energy expenditure above the RMR was between 761.5 ± 37 and 762.1 ± 25 kcal in each trial. Carbohydrate use during exercise was the same in the two trials but was significantly lower in the morning (43% to 48% of total) compared with that in the afternoon (approximately 53% of total; $F_{1,8} = 13.31$,
There also were no between-trial differences in energy intake (752 to 785 kcal per meal) or in carbohydrate intake. Carbohydrate intake (462 to 488 kcal per meal) matched carbohydrate use during exercise (463.9 ± 28.5 kcal in Before-Meals and 516.6 ± 37.7 kcal in After-Meals trials).

Total daily energy intakes were 1542.4 ± 79 and 1508.4 ± 71 kcal in the Before-Meals and After-Meals trials, respectively. Daily energy intake matched the total sedentary energy expenditures of between 1475 and 1570 kcal but did not replace the 762 kcal energy cost of exercise.

### Plasma glucose, insulin, FFA, and ketone bodies.

The pattern of changes in one prediabetic subject was comparable to that in eight nondiabetic subjects and was included in data analysis. There was no between-trial difference in fasting glucose concentration (4.5 mM) or the initial postprandial rise in plasma glucose (Fig. 2A), but differences appeared during the postabsorptive state. Postabsorptive glucose levels remained between 4.0 and 5.5 mM in the After-Meals trial. In contrast, plasma glucose decreased to approximately 3.7 mM after both meals in the Before-Meals trial and remained at between 3.8 and 4 mM throughout the night. Between-trial difference in plasma glucose concentration was significant during 2 h of exercise ($F_{1,8} = 6.66, P = 0.03$). Postabsorptive plasma glucose in the Before-Meals trial was 16.4 ± 0.2% lower than that in the After-Meals trial when the entire 24-h period was considered (4.0 ± 0.1 vs 4.8 ± 0.1 mM, $F_{1,520} = 22.47, P < 0.0001$) and 20.2 ± 0.2% lower during the final 16 h of the study ($F_{1,376} = 25.98, P < 0.0001$). A greater between-trial difference in glucose concentration during the second compared with the first exercise bout ($F_{1,8} = 12.9, P = 0.007$) caused a significant interaction between meals and trials ($F_{1,8} = 8.32, P = 0.02$). Thus, postabsorptive plasma glucose was approximately 20% lower in the Before-Meals compared with that in the After-Meals trials and was maintained at this reduced level throughout the night.

Postprandial insulin AUC in the Before-Meals trial was 48% and 52% higher compared with the After-Meals trial despite similar initial rises (Fig. 2B). In addition to the overall between-trial difference in insulin AUC ($F_{1,8} = 6.67, P = 0.03$), the interaction between trials and meals was significant after both meals (morning, $F = 6.28, P = 0.037$ and afternoon, $F = 5.39, P = 0.049$).

There was no between-trial difference in FFA AUC (Fig. 2C), but exercise caused significant premeal FFA rises ($F_{1,8} = 5.21, P = 0.048$). A smaller morning rise in FFA concentration during the After-Meals trial contributed to a significant interaction between the trials and time ($F_{1,8} = 6.36, P = 0.03$).

An increase in the AUC of the 3-β-hydroxybutyrate (Fig. 2D) was 137% greater after the morning exercise bout and 314% greater after the afternoon exercise bout in the Before-Meals trial than in the After-Meals trials ($F_{1,8} = 13.39, P = 0.005$). A disproportionate increase in ketone body AUC after the afternoon exercise trial ($F_{1,8} = 9.01, P = 0.015$) accounted for a significant interaction between the trials and exercise. Thus, a significant postexercise ketosis, especially after the second exercise bout, occurred in the Before-Meals but not in the After-Meals trials.

### Counterregulatory hormone response.

No overall between-trial difference was found in the counterregulatory response to exercise despite a between-trial difference in plasma glucose (Fig. 3). There only was a significant effect of time for glucagon ($F_{21,344} = 2.26, P = 0.001$) and cortisol AUC ($F_{2,16} = 7.45, P = 0.005$). GH secretory pulses were amplified by exercise, and an interaction between the trials and exercise was of borderline significance ($F_{1,8} = 5.3, P = 0.05$).

### Insulin–glucagon ratio and glucagon glycemic threshold.

The AUC for the postprandial insulin–glucagon ratio (Fig. 4A) after the morning and afternoon meals were 50% and 48% greater, respectively, in the Before-Meals trials compared with the After-Meals trials ($F_{1,8} = 8.01, P = 0.02$). This effect was significant at both times (morning, $F = 6.74, P = 0.03$ and afternoon, $F = 8.33, P = 0.02$). Glucagon glycemic threshold was approximately 1 mM lower in the Before-Meals compared with the After-Meals trial. In the After-Meals trial, declines in glucose below 5.5 to 5.3 mM during both morning and afternoon exercises (Fig. 4B, right) triggered increases in glucagon concentration. Plasma glucagon concentration was unaffected during the first Before-Meals exercise bout with plasma glucose at 4.3 to 4 mM. It increased during the second exercise bout (Fig. 4B) when plasma glucose declined below 4.3 mM. Increased insulin–glucagon ratio and reduced glucagon glycemic threshold indicated reduced contribution of glucagon to glucose counterregulation during Before-Meals compared with After-Meals trials.

### Assessment of autonomic function.

There was no between-trial difference in HR during exercise, reflecting equal sympathetic activation of the heart (Fig. 5A). However, vagal tone during exercise was significantly lower in the Before-Meals trial but not in the After-Meals trial.

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### Table 1. Energy intake and expenditure.

<table>
<thead>
<tr>
<th></th>
<th>Energy Intake (Meal)</th>
<th>Resting Metabolism (2 h)</th>
<th>Exercise Metabolism (2 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before-Meals Trial</td>
<td>After-Meals Trial</td>
<td>Before-Meals Trial</td>
</tr>
<tr>
<td><strong>Morning</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>757.8 ± 43</td>
<td>751.8 ± 33</td>
<td>106.3 ± 5</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>466.2 ± 22</td>
<td>462.5 ± 20</td>
<td>51.0 ± 8</td>
</tr>
<tr>
<td>Fat</td>
<td>76.1 ± 6</td>
<td>75.9 ± 1</td>
<td>55.4 ± 8</td>
</tr>
<tr>
<td><strong>Afternoon</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>764.8 ± 36</td>
<td>756.8 ± 38</td>
<td>475.7 ± 20</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>487.9 ± 18</td>
<td>470.1 ± 22</td>
<td>202.1 ± 14*</td>
</tr>
<tr>
<td>Fat</td>
<td>77.0 ± 1</td>
<td>77.2 ± 1</td>
<td>273.7 ± 24</td>
</tr>
</tbody>
</table>

* Different from afternoon values ($F_{1,8} = 13.31, P = 0.007$).
Before-Meals trial compared with the After-Meals trial ($F_{1,8} = 75.63$, $P < 0.0001$), and the effect was seen during both exercise bouts (bout 1, $F = 40.93$, $P = 0.001$ and bout 2, $F = 17.67$, $P = 0.0023$; Fig. 5B). Thus, the two trials did not differ in the sympathetic activation of the heart, whereas the parasympathetic influence only declined during postabsorptive exercise.

**DISCUSSION**

In this study, we tested the hypothesis that a previously described reduction in counterregulatory response and a sustained decline in plasma glucose after two bouts of spaced exercise in postabsorptive state in EAAF studies (8,9,30–33) was due to reduced circulating carbohydrate availability rather than to autonomic failure. By manipulating the timing of
meals and exercise and thus changing the abundance of circulating nutrients, we demonstrated that a postabsorptive state was a necessary condition for sustained 20% reduction in plasma glucose. In the spaced EAAF studies (8,9,30–33), exercise was performed after a 10-h overnight fast, and in some of them (8,9), glucose was provided in the amount of 1.5 g·kg⁻¹ body weight 30 to 45 min after exercise, the condition that, as demonstrated by our Before-Meals trial, does not prevent the decline in plasma glucose. Four- to sixfold increases in glucose infusion rates were required 24 h after exercise in spaced EAAF studies to maintain euglycemia during the hyperinsulinemic euglycemic clamps. The sustained reductions in plasma glucose and increases in glucose infusion rate required to maintain euglycemia are consistent with a downward shift in glucoregulatory set point in both the spaced exercise studies and our Before-Meals trials.

Dramatic increases in postexercise ketosis in the Before-Meals trial support the inference that deficiencies in circulating carbohydrates were critical in precipitating sustained lowering of plasma glucose. Postexercise ketosis is an indicator of hepatic glycogen depletion because it develops when liver glycogen declines in response to dietary carbohydrate scarcity (1,19–21). Postexercise ketosis is prevented by postexercise glucose ingestion or preexercise carbohydrate intake (20,21). Liver biopsies (15) or nuclear magnetic resonance measurements (36) reveal liver glycogen to contain approximately 90 g·1.4 kg⁻¹ liver weight approximately 5 h after a meal. Hepatic glycogen declines at a rate of 4.5 to 5 g·h⁻¹ during fasting. Therefore, at the start of both Before-Meals and spaced exercise studies (8,9,30–32), liver glycogen was probably reduced by approximately 33 g or was approximately 37% depleted. At exercise intensities where approximately 40% of energy cost of exercise is supplied by carbohydrates as was the case in this study, 20% of glucose is supplied by the liver and 80% by muscle glycogen (28). Therefore, oxidation of approximately 51 to

![FIGURE 4](image-url) - Effect of Before-Meals and After-Meals exercises (horizontal solid and open bars, respectively) on the insulin–glucagon ratio (A) and glucagon glycemic threshold (B) during the Before-Meals trial (center) and during the After-Meals trial (C, bottom). A. Solid circles, Before-Meals trials; open circles, After-Meals trials. C. Open symbols, morning values; solid symbols, afternoon values.

![FIGURE 5](image-url) - Effect of Before-Meals and After-Meals exercises (horizontal solid and open bars, respectively) on the HR (A) and vagal tone (B), as assessed by analysis of HR and its variability.
65 g of carbohydrates during exercise in fasted state (Table 1) could have produced further liver glycogen depletion possibly by between 48% to 51%. An acute postexercise increase in muscle insulin sensitivity (13,39), reduction in muscle glycogen concentration, and activation of the low-$K_m$ hexokinase should have secured abundant glucose uptake and glycogen resynthesis in the muscle after exercise in the postabsorptive state. This is likely to have left liver deprived of glucose and glycogen depleted for the remainder of the experimental period (7). In contrast, during the After-Meals trial, absorption of 116 to 122 g of carbohydrates into circulation was sufficient to support the oxidation of 57 to 67 g of glucose during postprandial exercise.

Finally, we show a significantly lower cardiac vagal tone and changed neuroendocrine function during exercise carried out in the postabsorptive state. Thus, selective suppression of parasympathetic, but not sympathetic function during postabsorptive exercise, is associated with two bouts of spaced exercise in the postabsorptive but not in the postprandial state. An increase in the insulin–glucagon ratio and a 1-mM decline in glucagon glyceemic threshold during spaced exercise in postabsorptive state are consistent with a downward shift in neuroendocrine glucoregulatory set point in response to inadequate circulating carbohydrate availability. Plasma-glycemic response to an acute postexercise meal may be an adaptive process to reduce the autonomic and neuroendocrine drive for hepatic glucose production when liver remains glycogen-depleted during postexercise replenishment of muscle glycogen stores.

Two concerns specific to this study need to be addressed to determine whether the results can be generalized to a wider range of subjects and conditions. An additional concern addresses the possibility that the phenomenon described could be attributed entirely or in part to circadian effects on the endocrine and autonomic functions. The first concern is whether the age or the postmenopausal status of our subjects would make the results less applicable to younger individuals of both gender. This concern is allayed by EAAF studies that show similar blood glucose lowering to two bouts of spaced exercise in 27- to 30-yr-old individuals of both gender (8,9,30,31) indicating that the phenomenon is not limited to a particular age range or gender. The second concern is the approximately 500 kcal daily negative energy balance under which our study was conducted. This concern would be minimized if the spaced EAAF studies that reported glucose lowering were also conducted under negative energy balance. The assessment of energy balance in EAAF studies is uncertain because the subject’s weight, exercise energy expenditure, and postexercise meal energy were not reported. Some estimates can be made from the reported exercise intensity, infusion rates of glucose, and occasional oral glucose supplementation in those studies. Their subjects entered their studies after a 10-h overnight fast and an additional 3-h equilibration period, which should have depleted approximately 69% of liver glycogen (8,9,30,31). In three of these studies, energy balance cannot be estimated further because an evening meal and an additional snack of unspecified caloric content were given (8,30,31). However, an estimate of energy balance can be made for one EAAF study where exercise was applied in the morning and a hyperinsulinemic hypoglycemic clamp was applied in the afternoon (9). In this study, total provision of energy is estimated to include 538 kcal in the form of carbohydrate: 105 g of oral carbohydrate after morning exercise, 7.9 g of glucose infused during morning exercise, and 21.5 g of glucose infused during the afternoon hyperinsulinemic hypoglycemic clamp. We estimate that the subjects expended approximately 750 kcal during 90 min of morning exercise at 50% of maximal effort and approximately 600 kcal in RMR and were therefore in approximately 800 kcal negative energy balance. Whether spaced exercise in postabsorptive state produces glucose-lowering effect only if a negative energy balance is maintained requires further examination.

The third concern addresses the possibility that the phenomenon we described in this study could be attributed entirely or in part to circadian effects on the endocrine and autonomic functions. Constant routine experiments, where masking and confounding influences of meal eating or lighting are removed, are appropriate tests of the contribution of endogenous rhythms to physiological processes. One such study addresses the role of circadian rhythms in glucoregulation (37). When the nutrient energy was provided through a continuous glucose infusion during a 24-h period at two rates, 5 and 8 g kg$^{-1}$h$^{-1}$ (corresponding to daily energy intakes of between 1000 and 2500 kcal), a distinct increase in plasma glucose was seen between 2300 and 0700 h, with a peak at 0300 h. Because no distinct circadian change in insulin concentration was seen, this circadian effect on glucoregulation in healthy subjects is more likely a result of reduced muscle glucose uptake during sleep rather than a result of reduced insulin sensitivity. This circadian effect could not have affected the changes in plasma glucose in our study because both bouts of exercise occurred before its onset. Further, sustained overnight reduction in plasma glucose in the Before-Meals trial and no elevation in plasma glucose at night in the After-Meals trial demonstrate that timing of meals and exercise overrode the nocturnal circadian effect on glucoregulation. The second study (34) exposed subjects to 8 h of darkness and 16 h of light under otherwise controlled conditions during which sympathetic and parasympathetic activities were assessed with power spectral analysis of HR variability at 4-h intervals. Within 4 h of light onset, the vagal tone declined and remained at reduced level during the next 8 h. Within 4 h of the light offset, the vagal tone increased and remained elevated during the remaining 4 h of darkness. The change in HR, a measure of sympathetic activation, followed a reciprocal pattern. In our study, the timing of morning exercise coincided with the last 2 h of autonomic shift toward diurnal function and that of our afternoon exercise coincided with the first 2 h of autonomic shift toward nocturnal function. Therefore, any contribution of circadian rhythms to the observed changes in vagal function would have been similar during both morning and afternoon.
exercises and could not have accounted for the differences in vagal tone observed between Before-Meals and After-Meals trials. Finally, a circadian influence has also been reported for counterregulatory hormones (24). The counterregulatory hormones epinephrine, cortisol, and glucagon were all more responsive to hypoglycemia at night than during the day. Our exercise and meal manipulations were carried out during the day, but the sustained reduction in plasma glucose was observed throughout the night. The circadian shift toward stronger nocturnal counterregulation at night cannot account for the selective reduction in nocturnal counterregulation in the Before-Meals trial.

This study shows that a behavioral strategy consisting of exercising twice a day before the meals in nondiabetic subjects can achieve a sustained reduction in fasting plasma glucose of comparable magnitude to that achieved by pharmacological approaches in type 2 diabetic subjects (33). Inclusion of one prediabetic subject in the study suggests that this behavioral strategy is likely to be effective not only in nondiabetic but also in prediabetic subjects. Because the present exercise paradigm requires exercise in excess of the amount likely to be acceptable to average individuals, it will be important in future studies to characterize the threshold amount of exercise sufficient for the glucose-lowering effect, the extent to which reduced dietary carbohydrates can substitute for exercise, and the mechanism through which reduced circulating carbohydrate availability during exercise affects neuroendocrine control of glucose regulation.

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**REFERENCES**


