



# Energy deficit without reducing dietary carbohydrate alters resting carbohydrate oxidation and fatty acid availability

Jeffrey F. Horowitz, Amy E. Kaufman, Amanda K. Fox and Matthew P. Harber

*Journal of Applied Physiology* 98:1612-1618, 2005. First published Dec 17, 2004;

doi:10.1152/jappphysiol.00936.2004

## You might find this additional information useful...

---

This article cites 40 articles, 26 of which you can access free at:

<http://jap.physiology.org/cgi/content/full/98/5/1612#BIBL>

Updated information and services including high-resolution figures, can be found at:

<http://jap.physiology.org/cgi/content/full/98/5/1612>

Additional material and information about *Journal of Applied Physiology* can be found at:

<http://www.the-aps.org/publications/jappl>

---

This information is current as of July 7, 2005 .



## Energy deficit without reducing dietary carbohydrate alters resting carbohydrate oxidation and fatty acid availability

Jeffrey F. Horowitz, Amy E. Kaufman, Amanda K. Fox, and Matthew P. Harber

Division of Kinesiology, The University of Michigan, Ann Arbor, Michigan

Submitted 27 August 2004; accepted in final form 14 December 2004

**Horowitz, Jeffrey F., Amy E. Kaufman, Amanda K. Fox, and Matthew P. Harber.** Energy deficit without reducing dietary carbohydrate alters resting carbohydrate oxidation and fatty acid availability. *J Appl Physiol* 98: 1612–1618, 2005. First published December 17, 2004; doi:10.1152/jappphysiol.00936.2004.—Reduced carbohydrate (CHO) availability after exercise has a potent influence on the regulation of substrate metabolism, but little is known about the impact of fat availability and/or energy deficit on fuel metabolism when dietary CHO availability is not reduced. The purpose of this study was to determine the influence of a postexercise energy deficit, independent of CHO availability, on plasma substrate concentrations and substrate oxidation. Seven moderately trained men (peak oxygen uptake:  $56 \pm 2 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) performed exhaustive cycling exercise on two separate occasions. The two trials differed only by the meals ingested after exercise: 1) a high-fat diet designed to maintain energy balance or 2) a low-fat diet designed to elicit energy deficit. The CHO and protein contents of the diets were identical. The next morning, we measured plasma substrate and insulin concentrations and CHO oxidation, and we obtained muscle biopsies from the vastus lateralis for measurement of pyruvate dehydrogenase kinase (PDK)-2 and PDK-4 mRNA expression by using RT-PCR. Despite identical blood glucose ( $5.0 \pm 0.1$  and  $4.9 \pm 0.1 \text{ mM}$ ) and insulin ( $7.9 \pm 1.1$  and  $8.4 \pm 0.9 \text{ } \mu\text{U/ml}$ ) concentrations, plasma fatty acid and glycerol concentrations were elevated three- to fourfold during energy deficit compared with energy balance and CHO oxidation was 40% lower ( $P < 0.01$ ) the morning after energy deficit compared with energy balance ( $328 \pm 69$  and  $565 \pm 89 \text{ } \mu\text{mol/min}$ ). The lower CHO oxidation was accompanied by a  $7.3 \pm 2.5$ -fold increase in PDK-4 mRNA expression after energy deficit ( $P < 0.05$ ), whereas PDK-2 mRNA was similar between the trials. In conclusion, energy deficit increases fatty acid availability, increases PDK-4 mRNA expression, and suppresses CHO oxidation even when dietary CHO content is not reduced.

caloric restriction; pyruvate dehydrogenase kinase; fatty acid; exercise

INCREASING ENERGY EXPENDITURE through exercise can be an important contributor to the energy deficit necessary to lose weight. In addition to its weight-loss effects, energy deficit also triggers important changes in the mobilization and oxidation of endogenous fuels to protect against hypoglycemia (2, 26). Meals ingested after exercise are known to impact the metabolic responses to the exercise-induced energy deficit. Much of the emphasis has been placed on alterations in dietary carbohydrate content of these meals (8, 16, 43) because the human body is acutely sensitive to alterations in carbohydrate availability. However, little is understood about metabolic adaptations due to an energy deficit without carbohydrate restriction. Considering that energy deficit is required for weight loss, a

better understanding of the metabolic responses to exercise-induced energy deficit may help to develop improved nutritional and exercise models for losing weight.

An increase in the mobilization of endogenous fat (i.e., lipolysis) and a decrease in the oxidation of carbohydrate are critical to the preservation of blood glucose when exogenous energy availability is low. Lipolysis is primarily regulated by insulin and catecholamines, which are both very sensitive to carbohydrate availability (7, 17). However, the effect of changes in dietary fat and/or energy content on endogenous fatty acid mobilization and availability is not well understood. Carbohydrate oxidation is also affected by the availability of both exogenous and endogenous carbohydrate, but the details of the regulation of carbohydrate oxidation with energy restriction, per se, also remain unclear. Pyruvate dehydrogenase kinase (PDK) is a key factor in the regulation of carbohydrate oxidation by phosphorylating, and thereby inhibiting, pyruvate dehydrogenase (PDH), which catalyzes conversion of pyruvate to acetyl-CoA. Increasing carbohydrate availability and insulin have been found to reduce PDK expression and activity (24). Four isoforms of PDK have been identified; of these, PDK-2 and PDK-4 are the most abundant in human skeletal muscle (6, 36). However, the regulation of the different isoforms of PDK during energy deficit is complex, and an increase in lipid availability during energy deficit may be an important mediator of this regulation (21, 37).

Because endogenous and exogenous carbohydrate availability provide a potent influence on both lipolytic rate and substrate selection, it is critical to control for dietary carbohydrate content to delineate between the effects of energy deficit from carbohydrate deprivation. The primary purpose of this study was to determine the influence of an exercise-induced energy deficit without reducing dietary carbohydrate content on substrate availability and oxidation.

### MATERIALS AND METHODS

#### Subjects

Seven healthy, active men volunteered to participate in this study [age:  $31 \pm 3 \text{ yr}$ ; weight:  $76.4 \pm 1.3 \text{ kg}$ ; peak oxygen uptake ( $\dot{V}_{\text{O}_2 \text{ peak}}$ ):  $55.5 \pm 1.6 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ; percent body fat: 14.8%]. Body composition was assessed by using hydrostatic weighing. No subject had any evidence of metabolic or cardiovascular disorders, nor were any of them taking prescription medications. All subjects regularly performed aerobic exercise (i.e., 30–60 min of cycling or jogging at 60–80% maximal heart rate) at least three times per week. All subjects were fully informed of the possible risks associated with the study, and they signed an informed consent form approved by the

Address for reprint requests and other correspondence: J. F. Horowitz, Div. of Kinesiology, The Univ. of Michigan, 401 Washtenaw Ave., Ann Arbor, MI 48109-2214 (E-mail: jeffhoro@umich.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

University of Michigan Institutional Review Board. This study was performed in conjunction with another study from our laboratory examining the effects of high-fat and low-fat meals on glucose tolerance (13).

#### Preliminary Testing

Body fat for all subjects was measured by using the two-compartment method by hydrostatic weighing (3).  $\dot{V}O_{2\text{ peak}}$  was measured (PhysioDyne Technologies, Quogue, NY) during upright cycle ergometer exercise to assess cardiorespiratory fitness. The protocol consisted of a 4-min warm-up, after which the workload was progressively increased every minute until volitional fatigue.  $\dot{V}O_{2\text{ peak}}$  was affirmed by at least two of the following criteria: 1) a leveling off of the rate of oxygen consumption ( $\dot{V}O_2$ ), despite increases in workload; 2) respiratory exchange ratio  $>1.15$ ; and 3) attainment of age-predicted maximal heart rate.

#### Experimental Protocol

Subjects performed two separate trials, each lasting 2 days. These two trials differed only by the content of the meals ingested after exercise (see *Study Diets*). The day before each trial, subjects were provided with an evening meal (0.50 g fat/kg, 2.0 g carbohydrate/kg, 0.31 g protein/kg), which was eaten at home and completed at 1900. The next morning (*day 1*) subjects arrived at the laboratory at 0700 after an overnight fast (12 h). After the subjects rested supine for 30 min, resting  $\dot{V}O_2$  and carbon dioxide production ( $\dot{V}CO_2$ ) were measured by using a metabolic cart (PhysioDyne Technologies) to calculate resting energy expenditure and substrate oxidation. This indirect calorimetry data was collected for at least 20 min while the subjects were lying down, awake but undisturbed in a dark, quiet room. After this measurement, a blood sample was taken to measure basal plasma substrate and hormone concentrations. Subjects then exercised on a stationary bicycle (Lode ergometer, Gronigen, The Netherlands) for 90 min at  $\sim 65\%$   $\dot{V}O_{2\text{ peak}}$ , followed by a maximum of five high-intensity intervals (4 min at  $\sim 85\%$   $\dot{V}O_{2\text{ peak}}$ , interspersed by 2 min at  $\sim 50\%$   $\dot{V}O_{2\text{ peak}}$ ) lasting a maximum of 30 min (or to exhaustion). Exhaustion criterion was established as a subject's inability to maintain the power output necessary to elicit  $85\%$   $\dot{V}O_{2\text{ peak}}$ . Indirect calorimetry data were collected intermittently throughout exercise to ensure that subjects were exercising at the appropriate intensity and to calculate energy expenditure during exercise. Subjects received 200 ml of water every 20 min during exercise trials. After exercise, subjects were fed either a diet designed to maintain 24-h energy balance or a diet designed to create an energy deficit (see *Study Diets*). For both trials, equal portions of the diet were ingested at 1045, 1230, and 1900.

On *day 2* of each trial, subjects arrived at the laboratory at 0700 after an overnight fast. After 30 min of supine rest,  $\dot{V}O_2$  and  $\dot{V}CO_2$  were measured for 20 min to calculate resting energy expenditure and substrate oxidation while the subjects rested quietly. After this measurement, a blood sample was obtained for measurements of plasma substrates and hormones. Then a muscle biopsy was obtained from the vastus lateralis muscle of the thigh by using the percutaneous biopsy technique (4).

During each trial, subjects performed no additional activity other than the exercise performed in the laboratory on *day 1* and activities of daily living. Between trials, subjects were instructed to continue their regular exercise regimen. They performed identical exercise bouts 3 days before each experimental trial and did not exercise 2 days before each trial. Trial order was counterbalanced, and trials were separated by at least 1 wk.

#### Study Diets

Both the energy balance diet and the energy deficit diet contained exactly the same amount of carbohydrate (5 g/kg body wt) and protein

(1.1 g/kg body wt). The only difference between the trials was that a total of  $\sim 165$  g of fat were added to the diet during energy balance (i.e., total of 2.3 g/kg body wt for energy balance and 0.15 g/kg body wt for energy deficit) to maintain energy balance without altering carbohydrate content. Because lipolysis and the relative contribution of fat and carbohydrate to energy production are known to be greatly influenced by the amount of carbohydrate ingested (23, 34), it was critical to match the diets for carbohydrate content to determine the effect of energy balance on these processes without the confounding influence of differences in carbohydrate availability. The total caloric content of energy balance was designed to match the estimated total energy expenditure during the 1-day trial, whereas energy deficit was designed to create  $\sim 1,500$ -kcal energy deficit.

#### Blood and Muscle Sample Preparation

Blood samples were transferred into test tubes containing 0.03 mmol EDTA for analysis of plasma glucose, fatty acids, glycerol, triglycerides, insulin, and interleukin-6 (IL-6) concentrations. All samples were kept on ice and then centrifuged (1,600 g for 10 min at  $4^\circ\text{C}$ ) immediately after collection. After centrifugation, the plasma from each sample was transferred into  $12 \times 75$ -mm plastic culture tubes and immediately frozen and stored at  $-80^\circ\text{C}$  for later analysis. Muscle biopsy samples were frozen in liquid nitrogen within  $\sim 30$  s of removal and stored at  $-80^\circ\text{C}$  until biochemical analysis.

#### Analytical Procedures

**Plasma substrates.** Plasma glucose concentration was measured by using a glucose autoanalyzer (Analox Instruments, Lunenburg, MA). Plasma triacylglycerol (Sigma Chemical, St. Louis, MO) and fatty acid concentrations (Wako Chemicals, Neuss, Germany) were measured by using commercially available colorimetric assay kits, and plasma glycerol concentration was measured enzymatically (11).

**Plasma hormones.** Plasma insulin concentration was measured by radioimmunoassay kit (Linco Research, St. Charles, MO), and plasma IL-6 concentration was assessed by ELISA (R&D Systems, Minneapolis, MN).

**PDK-2 and PDK-4 mRNA expression in muscle.** Total RNA was isolated from  $\sim 25$  mg frozen muscle by using TRIzol Reagent (Invitrogen, Carlsbad, CA). RNA was DNase treated (DNA-free Ambion, Austin, TX) for purity and total RNA was determined spectrophotometrically at 260 nm. First-strand cDNA was generated from 2  $\mu\text{g}$  RNA by using Maloney murine leukemia virus reverse transcriptase (Invitrogen). Primer pairs for each gene were designed by using DNASTAR (Lazergene) computer software. Gene sequences were obtained from GenBank [PDK-2: NM\_002611, forward primer 5'-CCGGCCTCCTGGCTGGCTTTGGTTATG-3' and reverse primer 5'-CCGGCCTCCTGGATGGTCTGGT-3' (203 bp-product); and PDK-4: NM\_002612, forward primer 5'-CTGGTGCCCCGAGAGGTG-3' and reverse primer 5'-TACTAATTGGGTCCGGAGGATA-3' (186 bp-product)]. A BLAST search for each primer was conducted to confirm homologous binding to the target gene.

Real-time RT-PCR was performed with use of a DNA engine Opticon continuous fluorescence detection system (MJ Research, Reno, NV). Samples for each gene were run simultaneously and in duplicate to control for amplification efficiency. For mRNA quantitation, a real-time PCR mix of  $2 \times$  SYBR Green PCR Master Mix (Qiagen), forward and reverse primers (3  $\mu\text{mol/l}$ ), and cDNA (12 ng) was run for 36 cycles in a total volume of 25  $\mu\text{l}$ . After the final cycle, samples were subjected to melting curve analysis to ensure the detection of only one product (31). Additionally, all amplification products were separated by agarose gel electrophoresis to validate the presence and size of the appropriate product. To account for variations in RNA input amounts and transcription efficiency, 18S mRNA was determined and results were normalized to these values. mRNA levels were quantified for each gene via determination of the critical threshold value as described by Schmittgen et al. (33). Unfortunately,

because of limitations in muscle sample yield we were only able to quantify PDK-2 and PDK-4 mRNA expression in five of the seven subjects.

#### Calculations

**Energy expenditure.** Resting metabolic rate (RMR) was calculated from resting  $\dot{V}O_2$  and  $\dot{V}CO_2$  measurements using the Weir equation (41). To account for energy expenditure due to activities of daily living during the inactive period of the day, multiplying RMR by 1.2 has been found to provide an accurate estimation of daily energy expenditure when inactive (35). Exercise energy expenditure was calculated from the  $\dot{V}O_2$  and  $\dot{V}CO_2$  measured during exercise. Twenty-four-hour energy expenditure was calculated as the weighted sum of resting energy expenditure ( $\sim 22$  h) and exercise energy expenditure ( $\sim 2$  h).

**Carbohydrate oxidation.** Resting carbohydrate and fat oxidation rates were calculated from resting  $\dot{V}O_2$  and  $\dot{V}CO_2$  using the equations of Frayn (14).

#### Statistical Analysis

A two-way ANOVA for repeated measures (treatment  $\times$  day) with Tukey post hoc analysis was used to determine differences among trials and between days for plasma substrates and hormone concentrations and for substrate oxidation rates. A one-way ANOVA for repeated measures with Tukey post hoc analysis was used to assess differences among trials for mRNA expression of PDK-2 and PDK-4 in skeletal muscle. A  $P$  value of 0.05 was considered statistically significant.

## RESULTS

### Energy Balance

The exercise protocol was identical in both trials; subjects expended  $1,336 \pm 59$  kcal during exercise in each trial. Estimated 24-h resting energy expenditure was also the same in energy deficit and energy balance ( $2,157 \pm 68$  and  $2,127 \pm 55$  kcal, respectively). Therefore, total daily energy expenditure was identical between trials (Fig. 1). As designed, caloric intake was markedly higher during energy balance compared with energy deficit (Fig. 1) because of the added fat calories to the meals during energy balance (carbohydrate and protein contents were identical between trials). As a result, during energy balance subjects ingested approximately the same num-

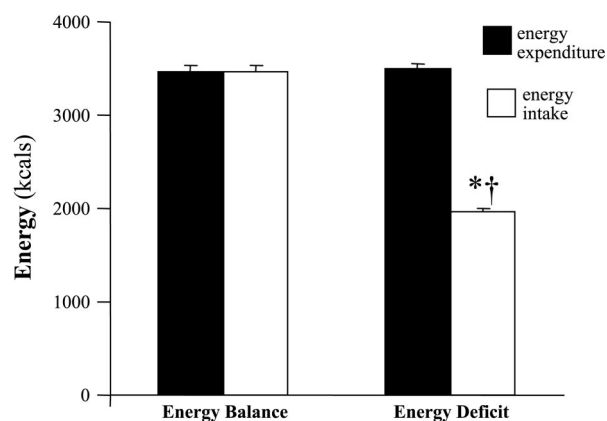


Fig. 1. Estimated 24-h energy expenditure and energy intake during the energy balance and energy deficit trials. Values are means  $\pm$  SE. \*Significantly different from energy balance trial,  $P < 0.05$ . †Significantly different from energy intake,  $P < 0.05$ .

ber of kilocalories that they expended (i.e., energy balance), whereas during energy deficit they were  $\sim 1,500$  kcal below energy balance (i.e.;  $\sim 1,500$ -kcal energy deficit) (Fig. 1).

### Plasma Glucose, Fatty Acid, Glycerol, and Triglyceride Concentrations

Despite the large difference in caloric content of the meals ingested after exercise on *day 1*, fasting plasma glucose concentrations were not affected the next morning (*day 1*:  $4.9 \pm 0.1$  and  $5.0 \pm 0.1$  mM, for energy balance and energy deficit, respectively; *day 2*:  $4.8 \pm 0.1$  and  $4.7 \pm 0.1$  mM, respectively). To the contrary, energy deficit increased plasma fatty acid and glycerol concentrations three- to fourfold above levels observed before exercise on *day 1* (Fig. 2, A and B; both  $P < 0.05$ ). The addition of fat calories to meals after exercise in energy balance prevented this increase in plasma glycerol and fatty acid concentrations (Fig. 2, A and B). Plasma triglyceride concentrations were reduced in the morning of *day 2* compared with *day 1* after both energy balance and energy deficit (both  $P < 0.05$ ) and the magnitude of reduction was the same in both trials (Fig. 2C).

### Plasma Hormone Concentrations

Plasma insulin concentration decreased slightly, but significantly ( $P < 0.05$ ) from before exercise on *day 1* to *day 2* during both trials (Table 1). However, plasma insulin concentrations were identical in energy balance and energy deficit on the morning of *day 2*. Neither treatment altered plasma IL-6 concentration (Table 1).

### Substrate Oxidation

Carbohydrate oxidation was significantly reduced on the morning of *day 2* compared with *day 1* during both energy balance and energy deficit (Fig. 3A). However, energy deficit induced a larger reduction, and carbohydrate oxidation on *day 2* was  $\sim 40\%$  lower during energy deficit than energy balance ( $P < 0.05$ ; Fig. 3A). Alternatively, fat oxidation was elevated on *day 2* compared with *day 1* during both trials ( $P < 0.05$ ) and the increase in fat oxidation was greater during energy deficit than energy balance ( $P < 0.05$ ; Fig. 3B).

### Muscle PDK mRNA expression

Energy deficit markedly increased the PDK-4 mRNA expression in skeletal muscle, as evidenced by more than a sevenfold greater expression of PDK-4 mRNA in muscle after energy deficit compared with energy balance ( $P < 0.05$ ; Fig. 4). Conversely, mRNA expression of muscle PDK-2 was not different between the trials.

## DISCUSSION

An acute energy deficit triggers essential metabolic alterations to mobilize endogenous lipids and preserve endogenous carbohydrate when exogenous energy availability is low (2, 26). However, because carbohydrate availability provides a potent influence on substrate selection, it is often very difficult to distinguish between the effects of energy deficit and carbohydrate restriction. The major finding of this study was that, without reducing dietary carbohydrate availability, an exercise-induced energy deficit increased plasma fatty acid concentra-

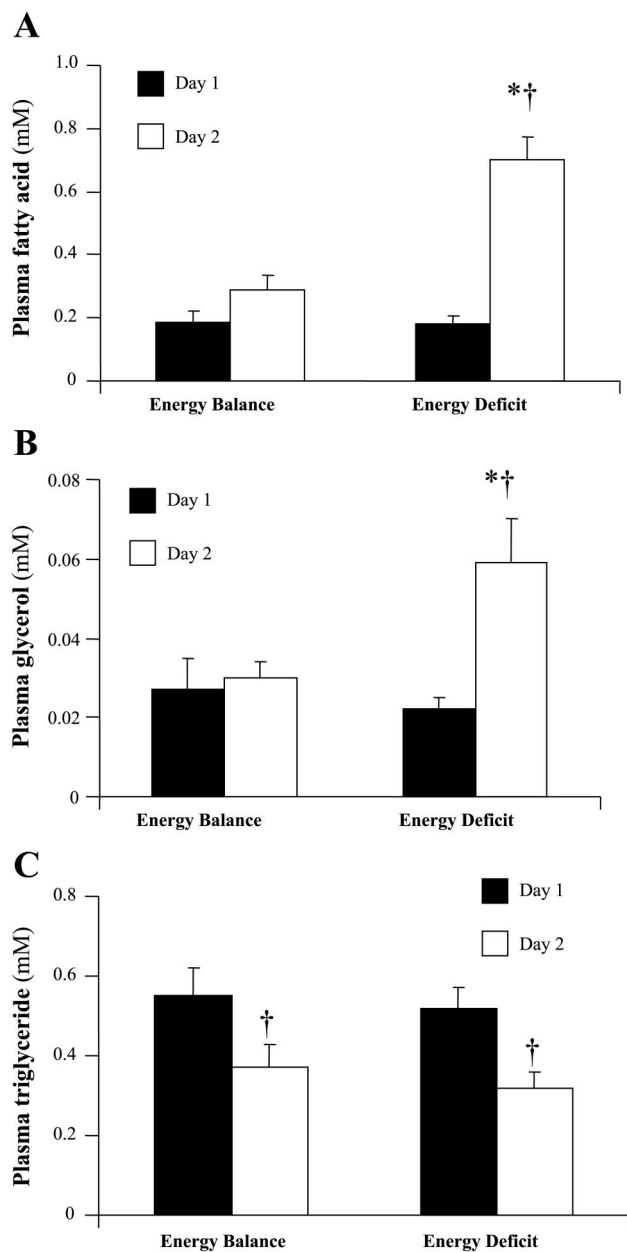


Fig. 2. Fasting plasma concentrations of fatty acids (A), glycerol (B), and triglycerides (C) before exercise on *day 1* and in the morning of *day 2* for energy balance and energy deficit trials. Values are means  $\pm$  SE. \*Significantly different from energy balance trial,  $P < 0.05$ . †Significantly different from *day 1*,  $P < 0.05$ .

tion and suppressed carbohydrate oxidation. Moreover, compared with an exercise-induced energy deficit, maintaining energy balance by ingesting extra dietary fat after exercise (without changing dietary carbohydrate content) prevented the rise in plasma fatty acid concentration and attenuated the decline in carbohydrate oxidation the next morning.

Exercise-induced energy deficit is known to suppress carbohydrate oxidation (1, 40). However, because the human body is keenly sensitive to alterations in dietary carbohydrate, much of this effect has been attributed to the reduction in dietary carbohydrate and the resultant low liver and muscle glycogen resynthesis, rather than the negative energy balance. Providing

Table 1. Plasma hormone concentrations

Hormone	Trial	Day 1	Day 2
Insulin, $\mu\text{U/ml}$	Energy balance	8.4 $\pm$ 0.9	6.7 $\pm$ 0.09*
	Energy deficit	7.9 $\pm$ 1.1	6.6 $\pm$ 0.8*
Interleukin-6, pg/ml	Energy balance	1.7 $\pm$ 0.3	2.3 $\pm$ 0.5
	Energy deficit	1.8 $\pm$ 0.3	2.0 $\pm$ 0.3

Values are means  $\pm$  SE. \*Significantly different from *day 1*,  $P < 0.05$ .

identical carbohydrate content during energy deficit and energy balance in our study enabled us to differentiate between the effects of energy deficit and the known potent influence of alterations in dietary carbohydrate (8, 16, 43). This study is the first to demonstrate that preventing energy deficit after exercise by ingesting extra dietary fat attenuated the reduction in carbohydrate oxidation the day after exercise was found despite identical plasma insulin and glucose concentrations during energy deficit and energy balance. Furthermore, in a companion study to this project using these same subjects (13), we found that adding extra dietary fat to meals after exercise did not affect muscle glycogen resynthesis. These findings indicate that the reduction in carbohydrate oxidation during energy deficit is not solely dependent on plasma insulin concentration or endoge-

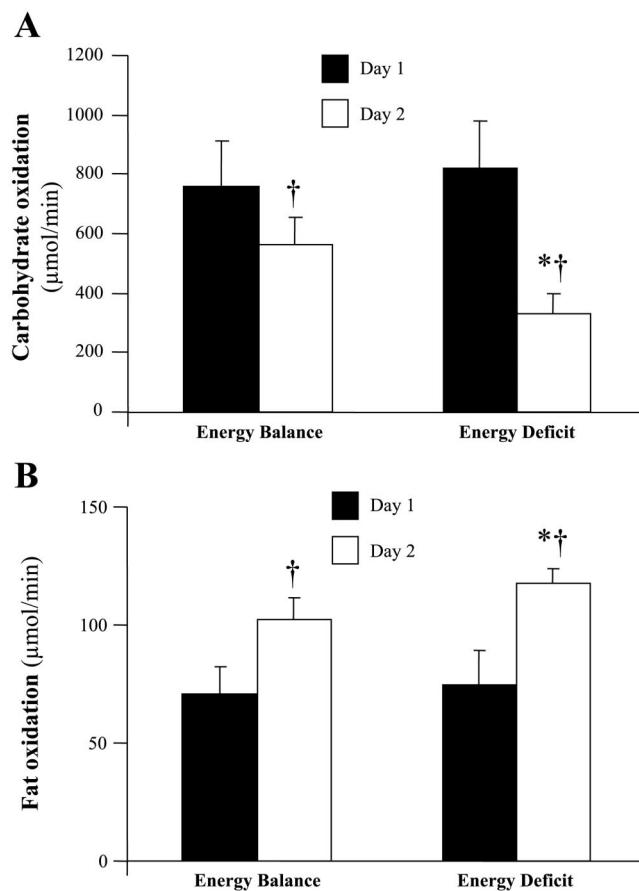


Fig. 3. Rates of fasting carbohydrate oxidation (A) and fat oxidation (B) before exercise on *day 1* and in the morning of *day 2* for the energy balance and energy deficit trials. Values are means  $\pm$  SE. \*Significantly different from energy balance trial,  $P < 0.05$ . †Significantly different from *day 1*,  $P < 0.05$ .

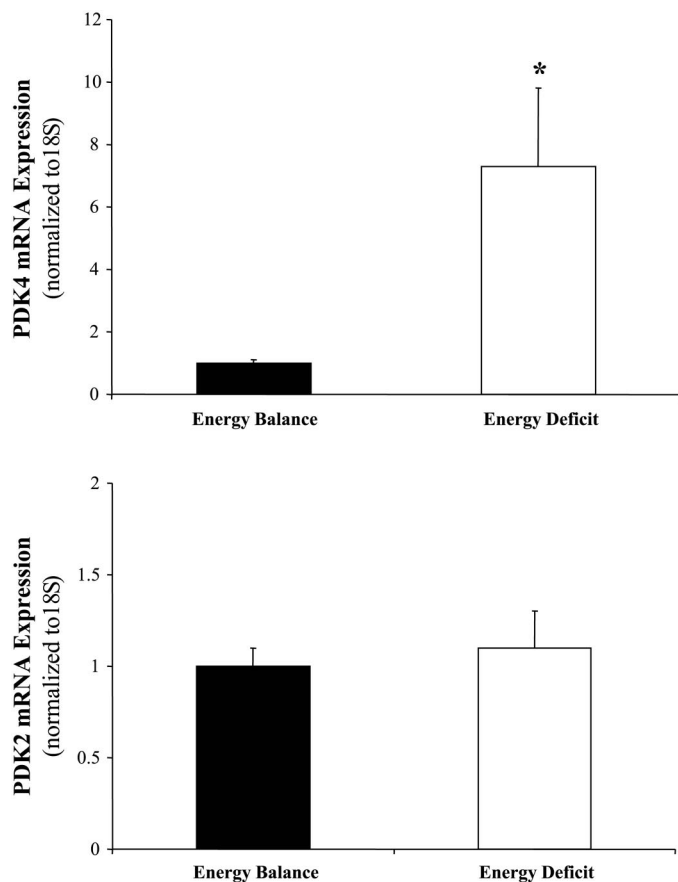


Fig. 4. mRNA expression of pyruvate dehydrogenase kinase 4 (PDK-4; *top*) and PDK-2 (*bottom*) in skeletal muscle samples from the vastus lateralis obtained in the morning of *day 2* during the energy balance and energy deficit trials. mRNA expression of PDK-4 and PDK-2 was normalized to 18S measured in each sample. Values are means  $\pm$  SE. \*Significantly different from energy balance trial,  $P < 0.05$ .

nous and exogenous carbohydrate availability, but that it can respond to dietary fat and/or energy availability, per se.

Carbohydrate oxidation is regulated in large part by the PDH complex. The conversion of pyruvate to acetyl-CoA is the first irreversible reaction in the regulation of carbohydrate oxidation, and this reaction is catalyzed by the enzyme PDH. The activity of PDH is tightly regulated by PDK, which phosphorylates and thereby inhibits PDH (6). The similar expression of PDK-2 in muscle samples in both of our trials was not surprising because the expression of the PDK-2 isoform is very sensitive to changes in insulin concentrations (24) and plasma insulin values were identical between the trials. Our finding that PDK-4 mRNA expression was sevenfold greater during energy deficit than energy balance suggests that changes in PDK-4 may have been involved in the observed reduction in carbohydrate oxidation. We recognize that mRNA expression is not a direct marker of protein activity. Unfortunately, because of limitations in our muscle biopsy yield, we did not have enough tissue available to measure enzyme activity of PDH or PDK. PDK-4 mRNA expression has been found to correlate well with PDK-4 activity after a high-fat diet (28) and has been reported to be inversely related to the activity of the active form of PDH with short-term fasting (36). However, this may not be the case under all conditions (30, 38).

PDK-4 mRNA expression has been found to be induced by fasting (30), exercise (29), or changes in dietary macronutrient content (i.e., high fat feeding) (28). However, it is not apparent from these previous studies whether the upregulation of PDK-4 mRNA is responding to reduced glycogen concentration, an increased free fatty acid supply to the peripheral tissues, or other factors associated with energy balance. In our study, dietary carbohydrate intake was matched and muscle glycogen levels were found to be identical (13), indicating that the marked increase in PDK-4 mRNA expression after energy deficit is not due to a reduction in carbohydrate availability, but instead it may be related to changes in fatty acid supply. PDK-4 has been postulated to be lipid responsive (21, 37). In absence of hyperinsulinemia, elevated fatty acid concentrations have been linked with a marked increase in PDK-4 expression (24). This increase in plasma fatty acid concentration may stimulate PDK-4 mRNA expression through activation of peroxisome proliferator-activated receptor- $\alpha$  (42). However, PDK-4 expression in skeletal muscle is not solely dependent on peroxisome proliferator-activated receptor- $\alpha$  (20). Although we measured PDK-4 expression in skeletal muscle, we hypothesize that the elevated plasma fatty acid concentrations may have increased PDK-4 expression in the liver as well because fatty acids markedly increase hepatic PDK-4 expression in hepatocytes (24).

Acute energy deficit, through diet and/or exercise, has been found to increase basal lipolytic rate (22, 32), with a resultant increase in plasma glycerol and fatty acid concentrations. Exogenous carbohydrate has been found to reverse the energy deficit-induced elevation in lipolytic rate (5, 26), and the present study demonstrates that preventing an energy deficit by ingesting extra dietary fat after exercise also prevents a rise in plasma fatty acid and glycerol concentrations the next day. Unlike the well-described metabolic responses to alterations in dietary carbohydrate, the lipolytic mechanism(s) responsible for the recognizing and responding to alterations in dietary fat and/or energy balance are not clear. Lipolytic rate in humans is primarily regulated by plasma insulin (7) and catecholamines (15). In the present study, the difference in plasma fatty acid concentrations observed between energy balance and energy deficit could not be attributed to the antilipolytic effects of insulin, because plasma insulin concentration was identical in the morning of *day 2* during both trials and the antilipolytic response to insulin has been found to be unaffected by differences in energy balance (25). Because we did not measure plasma concentrations of epinephrine and norepinephrine, we cannot rule out alterations in catecholamines as a cause for the differences in lipolytic rate. However, plasma catecholamine concentration is particularly sensitive to changes in plasma glucose concentrations (17), and by feeding the same carbohydrate content during both trials, plasma glucose concentrations were identical. We found no difference in IL-6 concentration between trials, suggesting that this putative lipolytic regulator (39) was also not responsible for the increase in plasma fatty acids during energy deficit. Contrary to our findings, Klein and Wolfe (26) reported that preventing energy deficit during fasting by infusing lipids intravenously did not attenuate the fasting-induced increase in lipolysis. The discrepancy between our findings and those of Klein and Wolfe may be explained by the role of factors associated with the gastrointestinal tract (e.g., autonomic control, gastric peptides) in

response to dietary fat. However, whether these factors directly affect lipolytic rate and their mechanisms of action have yet to be determined.

An additional finding from this study was that adding ~165 g of dietary fat to meals after exercise did not alter plasma triglyceride concentration. Our data agree with previous work (12, 18) indicating that a single session of exercise reduces plasma triglyceride concentration the next day. Our findings expand on this by demonstrating that adding a large amount of fat to meals after exercise does not impair the plasma triglyceride lowering effect of exercise. High-fat diets have been found to reduce plasma triglyceride concentration compared with low-fat diets (10, 19, 27). It is the high carbohydrate content of these "low-fat" diets that increases triglyceride production in very low-density lipoproteins, likely due to the increased availability of hepatic fatty acids resulting from a low rate of fat oxidation when carbohydrate intake is high (27). In the present study, identical carbohydrate content in the diets resulted in the same plasma triglyceride concentrations the day after exercise, independent of the fat or energy content of the meals.

It is important to note that although dietary carbohydrate was identical in our "energy deficit" and "energy balance" trials and muscle glycogen was found to be identical between the trials (13), the amount of carbohydrate provided after exercise was ~25% less than that recommended for complete glycogen replenishment after exhaustive exercise (9). Therefore, during both trials our subjects may have been in a "relative carbohydrate deficit" compared with their pretrial condition. However, because the magnitude of carbohydrate deficit was identical between the trials, this does not affect the interpretation of the major outcomes of the study.

In summary, an exercise-induced energy deficit elevated plasma fatty acid concentration, increased PDK mRNA expression, and suppressed carbohydrate oxidation the next day. Maintaining energy balance by ingesting enough dietary fat to compensate for the energy expenditure during exercise prevented the increase in plasma fatty acid concentration and attenuated the reduction in carbohydrate oxidation. These data indicate that alterations in substrate metabolism are responsive to changes in dietary fat and/or energy balance independently of changes in carbohydrate availability.

#### ACKNOWLEDGMENTS

We thank Nicolas Knuth and Simon Schenk for technical expertise, Sheryl Hansen Smith for assistance with designing the study diets, and the study subjects for participation.

#### REFERENCES

- Almeras N, Lavalley N, Despres JP, Bouchard C, and Tremblay A. Exercise and energy intake: effect of substrate oxidation. *Physiol Behav* 57: 995–1000, 1995.
- Barbe P, Stich V, Galitzky J, Kunesova M, Hainer V, Lafontan M, and Berlan M. In vivo increase in beta-adrenergic lipolytic response in subcutaneous adipose tissue of obese subjects submitted to a hypocaloric diet. *J Clin Endocrinol Metab* 82: 63–69, 1997.
- Behnke AR and Wilmore JH. *Evaluation and Regulation of Body Build and Composition*. Englewood Cliffs, NJ: Prentice-Hall, 1974.
- Bergstrom J. Muscle electrolytes in man. *Scand J Clin Lab Invest Suppl* 14: 1–110, 1962.
- Bisschop PH, de Sain-van der Velden MGM, Stellaard F, Kuipers F, Meijer AJ, Sauerwein HP, and Romijn JA. Dietary carbohydrate deprivation increases 24-hour nitrogen excretion without affecting post-absorptive hepatic or whole body protein metabolism in healthy men. *J Clin Endocrinol Metab* 88: 3801–3805, 2003.
- Bowker-Kinley MM, Davis WI, Wu P, Harris RA, and Popov KM. Evidence for existence of tissue-specific regulation of the mammalian pyruvate dehydrogenase complex. *Biochem J* 329: 191–196, 1998.
- Campbell PJ, Carlson MG, Hill JO, and Nurjhan N. Regulation of free fatty acid metabolism by insulin in humans: role of lipolysis and reesterification. *Am J Physiol Endocrinol Metab* 263: E1063–E1069, 1992.
- Cartee GD, Young DA, Sleeper MD, Zierath J, Wallberg-Henriksson H, and Holloszy JO. Prolonged increase in insulin-stimulated glucose transport in muscle after exercise. *Am J Physiol Endocrinol Metab* 256: E494–E499, 1989.
- Coyle EF. Substrate utilization during exercise in active people. *Am J Clin Nutr* 61: 968S–979S, 1995.
- Dreon DM, Fernstrom HA, Miller B, and Krauss RM. Low-density lipoprotein subclass patterns and lipoprotein response to a reduced-fat diet in men. *FASEB J* 8: 121–126, 1994.
- Eggstein M and Kuhlmann E. Triglycerides and glycerol. Determination after alkaline hydrolysis. In: *Methods of Enzymatic Analysis* (2nd ed.), edited by Bergmeyer HU. New York: Academic, 1974, p. 1825–1831.
- Ferguson MA, Alderson NL, Trost SG, Essig DA, Burke JR, and Durstine JL. Effects of four different single exercise sessions on lipids, lipoproteins, and lipoprotein lipase. *J Appl Physiol* 85: 1169–1174, 1998.
- Fox AK, Kaufman AE, and Horowitz JF. Adding fat calories to meals after exercise does not alter glucose tolerance. *J Appl Physiol* 97: 11–16, 2004.
- Frayn KN. Calculation of substrate oxidation rates in vivo from gas exchange. *J Appl Physiol* 55: 628–634, 1983.
- Galster AD, Clutter WE, Cryer PE, Collins JA, and Bier DM. Epinephrine plasma thresholds for lipolytic effects in man. Measurements of fatty acid transport with [ $^{1-13}C$ ]-palmitic acid. *J Clin Invest* 67: 1729–1738, 1981.
- Garcia-Roves PM, Han DH, Song Z, Jones TE, Hucker KA, and Holloszy JO. Prevention of glycogen supercompensation prolongs the increase in muscle GLUT4 after exercise. *Am J Physiol Endocrinol Metab* 285: E729–E736, 2003.
- Genter P, Berman N, Jacob M, and Ipp E. Counterregulatory hormones oscillate during steady-state hypoglycemia. *Am J Physiol Endocrinol Metab* 275: E821–E829, 1998.
- Gill JMR, Herd SL, Vora V, and Hardman AE. Effects of a brisk walk on lipoprotein lipase activity and plasma triglyceride concentrations in the fasted and post-prandial states. *Eur J Appl Physiol* 89: 184–190, 2003.
- Ginsberg H, Olefsky JM, Kimmerling G, Crapo P, and Reaven GM. Induction of hypertriglyceridemia by a low-fat diet. *J Clin Endocrinol Metab* 42: 729–735, 1976.
- Holness MJ, Bulmer K, Gibbons GF, and Sugden MC. Up-regulation of pyruvate dehydrogenase kinase isoform 4 (PDK4) protein expression in oxidative skeletal muscle does not require the obligatory participation of peroxisome-proliferator-activated receptor alpha (PPARalpha). *Biochem J* 366: 839–846, 2002.
- Holness MJ, Bulmer K, Smith ND, and Sugden MC. Investigation of potential mechanisms regulating protein expression of hepatic pyruvate dehydrogenase kinase isoforms 2 and 4 by fatty acids and thyroid hormone. *Biochem J* 369: 687–695, 2003.
- Horowitz JF, Coppack SW, Paramore D, Cryer PE, Zhao G, and Klein S. Effect of short-term fasting on lipid kinetics in lean and obese women. *Am J Physiol Endocrinol Metab* 276: E278–E284, 1999.
- Horowitz JF, Mora-Rodriguez R, Byerley LO, and Coyle EF. Lipolytic suppression following carbohydrate ingestion limits fat oxidation during exercise. *Am J Physiol Endocrinol Metab* 273: E768–E775, 1997.
- Huang B, Wu P, Bowker-Kinley MM, and Harris RA. Regulation of pyruvate dehydrogenase kinase expression by peroxisome proliferator-activated receptor-alpha ligands, glucocorticoids, and insulin. *Diabetes* 51: 276–283, 2002.
- Kashiwagi A, Mott D, Bogardus C, Lillioja S, Reaven GM, and Foley JE. The effects of short-term overfeeding on adipocyte metabolism in Pima Indians. *Metabolism* 34: 364–370, 1985.
- Klein S and Wolfe RR. Carbohydrate restriction regulates the adaptive response to fasting. *Am J Physiol Endocrinol Metab* 262: E631–E636, 1992.
- Mittendorfer B and Sidossis LS. Mechanism for the increase in plasma triacylglycerol concentrations after consumption of short-term, high-carbohydrate diets. *Am J Clin Nutr* 73: 892–899, 2001.

28. **Peters SJ, Harris RA, Wu P, Pehleman TL, Heigenhauser GJ, and Spriet LL.** Human skeletal muscle PDH kinase activity and isoform expression during a 3-day high-fat/low-carbohydrate diet. *Am J Physiol Endocrinol Metab* 281: E1151–E1158, 2001.
29. **Pilegaard H, Ordway GA, Saltin B, and Neufer PD.** Transcriptional regulation of gene expression in human skeletal muscle during recovery from exercise. *Am J Physiol Endocrinol Metab* 279: E806–E814, 2000.
30. **Pilegaard H, Saltin B, and Neufer PD.** Effect of short-term fasting and refeeding on transcriptional regulation of metabolic genes in human skeletal muscle. *Diabetes* 52: 657–662, 2003.
31. **Ririe KM, Rasmussen RP, and Wittwer CT.** Product differentiation by analysis of DNA melting curves during the polymerase chain reaction. *Anal Biochem* 245: 154–160, 1997.
32. **Romijn JA, Klein S, Coyle EF, Sidossis L, and Wolfe RR.** Strenuous endurance training increases lipolysis and triglyceride-fatty acid cycling at rest. *J Appl Physiol* 75: 108–113, 1993.
33. **Schmittgen TD, Zakrajsek BA, Mills AG, Gorn V, Singer MJ, and Reed MW.** Quantitative reverse transcription-polymerase chain reaction to study mRNA decay: comparison of endpoint and real-time methods. *Anal Biochem* 285: 194–204, 2000.
34. **Schwarz JM, Neese RA, Turner S, Dare D, and Hellerstein MK.** Short-term alterations in carbohydrate energy intake in humans. Striking effects on hepatic glucose production, de novo lipogenesis, lipolysis, and whole-body fuel selection. *J Clin Invest* 96: 2735–2743, 1995.
35. **Shils M, Olson JA, and Snike M.** Care of the patient with surgery, trauma, and sepsis. In: *Modern Nutrition in Health and Disease*. Philadelphia, PA: Lea & Febiger, 1994, p. 1225–1226.
36. **Spriet LL, Tunstall RJ, Watt MJ, Mehan KA, Hargreaves M, and Cameron-Smith D.** Pyruvate dehydrogenase activation and kinase expression in human skeletal muscle during fasting. *J Appl Physiol* 96: 2082–2087, 2004.
37. **Sugden MC, Bulmer K, Augustine D, and Holness MJ.** Selective modification of pyruvate dehydrogenase kinase isoform expression in rat pancreatic islets elicited by starvation and activation of peroxisome proliferator-activated receptor- $\alpha$ : implications for glucose-stimulated insulin Secretion. *Diabetes* 50: 2729–2736, 2001.
38. **Turvey EA, Heigenhauser GJF, Parolin M, and Peters SJ.** Elevated n-3 fatty acids in a high-fat diet attenuates the increase in PDH kinase but not PDH activity in human skeletal muscle. *J Appl Physiol* 98: 350–355, 2005.
39. **Van Hall G, Steensberg A, Sacchetti M, Fischer C, Keller C, Schjerling P, Hiscock N, Moller K, Saltin B, Febbraio MA, and Pedersen BK.** Interleukin-6 stimulates lipolysis and fat oxidation in humans. *J Clin Endocrinol Metab* 88: 3005–3010, 2003.
40. **Votruba SB, Atkinson RL, Hirvonen MD, and Schoeller DA.** Prior exercise increases subsequent utilization of dietary fat. *Med Sci Sports Exerc* 34: 1757–1765, 2002.
41. **Weir JB.** New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol* 109: 1–9, 1949.
42. **Wu P, Inskeep K, Bowker-Kinley M, Popov K, and Harris R.** Mechanism responsible for inactivation of skeletal muscle pyruvate dehydrogenase complex in starvation and diabetes. *Diabetes* 48: 1593–1599, 1999.
43. **Zderic TW, Schenk S, Davidson CJ, Byerley LO, and Coyle EF.** Manipulation of dietary carbohydrate and muscle glycogen affects glucose uptake during exercise when fat oxidation is impaired by  $\beta$ -adrenergic blockade. *Am J Physiol Endocrinol Metab* 287: E1195–E1201, 2004.

