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High-Fat/Low-Carbohydrate Diet Reduces Insulin-Stimulated Carbohydrate Oxidation but Stimulates Nonoxidative Glucose Disposal in Humans: An Important Role for Skeletal Muscle Pyruvate Dehydrogenase Kinase 4

K. Chokkalingam,* K. Jewell,* L. Norton, J. Littlewood, L. J. C. van Loon, P. Mansell, I. A. Macdonald, and K. Tsintzas

Center for Integrated Systems Biology and Medicine (K.C., K.J., L.N., J.L., I.A.M., K.T.), Institute of Clinical Research, School of Biomedical Sciences, University of Nottingham, Nottingham NG7 2UH, United Kingdom; Department of Diabetes and Endocrinology (K.C., P.M.), Queen's Medical Center, Nottingham NG7 2UH, United Kingdom; and Department of Movement Sciences (L.J.C.v.L.), Nutrition and Toxicology Research Institute Maastricht, Maastricht University, 6200 MD Maastricht, The Netherlands

Aim: The aim of this report was to study the effect of high-fat (HF)/low-carbohydrate (CHO) diet on regulation of substrate metabolism in humans.

Methods: Ten healthy men consumed either a HF (75% energy as fat) or control (35%) diet for 6 d in random order. On d 7, blood glucose disappearance rate (R_d) was determined before and during a hyperinsulinemic euglycemic clamp. Substrate oxidation was determined by indirect calorimetry. Muscle biopsies were obtained prediet, postdiet, and postclamps.

Results: R_d was similar under basal conditions but slightly elevated (~10%, $P < 0.05$) during the last 30 min of the clamp after the HF diet. HF diet reduced CHO oxidation under basal (by ~40%, $P < 0.05$) and clamp conditions (by ~20%, $P < 0.05$), increased insulin-mediated whole-body nonoxidative glucose disposal (by 30%, $P < 0.05$) and muscle glycogen storage (by ~25%, $P < 0.05$). Muscle pyruvate dehydrogenase complex activity was blunted under basal and clamp conditions after HF compared with control ($P < 0.05$) and was accompanied by an approximately 2-fold increase ($P < 0.05$) in pyruvate dehydrogenase kinase 4 (PDK4) mRNA and protein expression.

Conclusion: Short-term HF/low-CHO dietary intake did not induce whole-body insulin resistance, but caused a shift in im glucose metabolism from oxidation to glycogen storage. Insulin-stimulated CHO oxidation and muscle pyruvate dehydrogenase complex activity were blunted after the HF diet. Up-regulation of muscle PDK4 expression was an early molecular adaptation to these changes, and we showed for the first time in healthy humans, unlike insulin-resistant individuals, that insulin can suppress PDK4 but not PDK2 gene expression in skeletal muscle. **(*J Clin Endocrinol Metab* 92: 284–292, 2007)**

UNDERSTANDING THE ETIOLOGY of insulin resistance is of major clinical importance, not least because this is the main feature of type 2 diabetes (1). One potentially modifiable nutritional determinant of insulin sensitivity is diet composition. Several studies have shown that when nonesterified fatty acid (NEFA) availability was profoundly increased by Intralipid infusions, impaired whole-body insulin sensitivity was observed in humans (2–6). However, high-fat (HF) feeding in humans has produced contradictory results. HF diets for just 3 d have been shown to induce whole-body insulin resistance (7, 8). In contrast, HF feeding for 11–21 d does not induce whole-body insulin resistance, although the partitioning of glucose metabolism is altered with decreased oxidation and increased nonoxidative glucose disposal (9, 10). Therefore, further detailed *in vivo* studies are required to investigate the potentially more subtle changes in insulin-mediated muscle metabolism that are associated with a high dietary fat intake in humans.

The cellular mechanisms by which increased availability of NEFA may induce insulin resistance are unclear. The mitochondrial pyruvate dehydrogenase enzyme complex (PDCa) occupies a central role in muscle intermediary metabolism and has been proposed to play a primary role in the development of insulin resistance (11, 12). The activity of this complex is down-regulated when there is an increased availability of NEFA, which promotes fat oxidation and suppresses glucose metabolism, and this is mediated through changes in the activity of pyruvate dehydrogenase kinase (PDK) (13, 14). Administration of a HF diet is associated with significant increases in muscle PDK4 expression in healthy humans (15). Insulin down-regulates transcript levels of

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* K.C. and K.J. are joint first authors.

Abbreviations: CHO, Carbohydrate; ChREBP, CHO response element-binding protein; CON, control; C_{ox}, CHO oxidation; GIR, glucose infusion rate; HF, high fat; HKII, hexokinase II; NEFA, nonesterified fatty acid; PCA, perchloric acid; PDCa, pyruvate dehydrogenase complex; PDK, pyruvate dehydrogenase kinase; PK, pyruvate kinase; PPAR, peroxisome proliferator-activated receptor; R_a, glucose appearance rate; R_d, glucose disappearance rate; SREBP-1c, sterol regulatory element binding protein-1c.

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PDK2 and PDK4 in insulin-resistant nondiabetic Pima Indians (16), but no study has examined the effect of insulin on their expression in healthy humans. Furthermore, the signaling mechanisms by which NEFA and insulin regulate these kinases *in vivo* are not very well characterized in humans.

This study examined the effect of 6 d of isoenergetic HF/low-carbohydrate (CHO) diet on insulin-mediated whole-body and muscle intermediary metabolism. To develop our understanding of nutrient-gene interactions further, we also examined the impact of a HF/low-CHO diet on mRNA expression and protein abundance of key genes involved in the uptake and oxidation of both CHO and fat in skeletal muscle biopsies obtained from healthy humans.

Subjects and Methods

Ten healthy males [age 25.6 (2.5) yr and BMI 23.7 (0.9) kg/m^2] were recruited and informed of all procedures and risks associated with the experimental trials before obtaining informed consent. All procedures used in this study were performed according to the Declaration of Helsinki and approved by the University of Nottingham Medical School Ethics Committee.

Study design and protocol

All subjects underwent two 7-d trials, at least 2 wk apart, in a randomized crossover design. On each occasion, subjects consumed for 6 d either a HF (75% energy as fat) or normal diet [control (CON) 35% fat]. On d 7, subjects were infused with [6,6-$^2H_2$]glucose for 2 h before and during a 4-h hyperinsulinemic euglycemic clamp to quantify insulin sensitivity. Muscle biopsies were obtained from the vastus lateralis before and after each diet and after the clamps.

On d 1, subjects reported to the laboratory at 0800 h after an overnight fast, and after recording the body weight a baseline blood sample was drawn. Subsequently, under local anesthetic, the vastus lateralis muscle was biopsied as described previously (17). One piece of fresh muscle tissue was immediately used for mitochondrial extraction, and the remaining part was snap frozen in liquid nitrogen for subsequent enzyme, RNA, and protein extraction. Subjects then consumed either a CON or a HF diet for 6 consecutive days (see *Dietary intervention*).

On d 7 of both treatments, subjects reported to the laboratory at 0800 h after an overnight fast. Subjects were weighed, a baseline urine sample was acquired, and subjects then rested on a reclined couch. Resting $\dot{V}CO_2$ and $\dot{V}O_2$ were measured for 30 min using a ventilated hood system (GEM, Nutren Technologies, Manchester, UK), and values were used for the calculation of energy expenditure and substrate oxidation rates. Measurements were made while the subjects were lying supine, undisturbed, and awake. A muscle biopsy was then obtained and, at 0900 h, a Teflon catheter was inserted into the antecubital vein of one arm for glucose tracer, 20% glucose (spiked with glucose tracer, 1%), and insulin infusion. Another catheter was placed into a dorsal hand vein in a retrograde fashion to obtain arterialized blood samples. The hand was placed in a heated box (55 C) for blood sampling. Subjects were infused with [6, 6-$^2H_2$]glucose in a primed (22 μmol/kg body mass)/continuous (13.75 μmol/kg·h) fashion for 2 h before (basal period) and during a 4-h hyperinsulinemic (50 mU/m^2·min) euglycemic (4.5 mmol/liter) clamp for the determination of glucose appearance (R_a) and glucose disappearance (R_d) rates. Total glucose tracer infusion rate averaged 677 ± 34 nmol/kg·min. A second indirect calorimetry measurement was made during the last 30 min of the insulin clamp, and a muscle sample was obtained at the end of the clamp. Blood samples were collected at 20-min intervals during the study. Urine samples were collected during the study day for glucose and nitrogen measurements and were stored at −20 C in 10% thymol until analysis.

Dietary intervention

A minimum 2-wk interval between the two diets was allowed in each subject. The HF and CON diets were designed based on the subjects' usual dietary habits and energy intake to ensure palatability and adherence to the study protocol. The HF diet was designed to provide 75% of energy from fat (of which 35% from saturated fat) and 10% of energy as CHO. The CON diet was designed to provide 50% of energy from CHO and 35% from fat. Both diets and menus for the 6 d were designed using a dietary analysis program (Microdiet, version 1.1; Downlee Systems Limited, High Peak, UK). All food, beverage (noncaffeinated), and snack requirements for both diets were purchased and delivered to the study participants. Written instructions on cooking methods and ingredients were also provided. Ready-made meals with known nutritional content were provided to subjects who were unfamiliar with cooking methods. Subjects were requested to adhere to the items on the menu and to record intake. Subjects were also requested to abstain from alcohol consumption, smoking, and intense exercise for 3 d before and during each 6-d dietary intervention. Food records were analyzed at the end of the study using the dietary analysis program.

Blood and urine analysis

Blood and urine glucose and blood lactate were measured shortly after collection using a Yellow Springs analyser (YSI 2300 Stat Plus-D, Yellow Springs Instruments, Yellow Springs, OH). Whole blood β-hydroxybutyrate was measured in perchloric acid (PCA)-treated blood samples (18). Plasma and serum were separated by low-speed centrifugation (15 min at 3000 × *g*). Serum insulin was measured using a RIA kit from Diagnostic Products Corp. (Wales, UK). Plasma NEFA and urea were measured using kits from WAKO Chemicals (Neuss, Germany) and Randox Laboratories (Crumlin, UK), respectively. Two-milliliter blood samples were collected in EDTA tubes, immediately centrifuged (4 min at 1000 × *g* at 4 C), and plasma was separated and stored at −80 C until analysis for [6,6-$^2H_2$]glucose enrichment. After derivatization, plasma [6,6-$^2H_2$]glucose enrichment was determined by electron ionization gas chromatography-mass spectrometry (INCOS-XL; Finnigan, Bremen, Germany). Plasma cytokines (IL-1β, IL-8, IL-10, IL-12, and TNFα) were measured using a cytokine bead array (BD Biosciences, San Jose, CA) as previously described (19). Urine nitrogen was determined using the Kjeldahl method.

Muscle metabolites

Ten to 20 mg of frozen muscle was freeze-dried and washed with 40% petroleum ether to remove fat. Muscle metabolites were extracted using PCA and determined enzymatically (20). Acetylcarnitine was determined using a RIA (21). Acid hydrolyzes of the muscle extract and the muscle pellet left over after PCA extractions were carried out to measure macroglycogen and proglycogen, respectively (22). Five to 10 mg of frozen muscle was used to determine the active form of pyruvate dehydrogenase complex (PDCa) (23).

RNA extraction and real-time quantitative PCR

Total RNA was extracted from 10–20 mg of frozen muscle tissue as described previously (24) using TRIzol reagent (Invitrogen, Paisley, UK). Human cDNA sequences of the genes of interest were obtained from GenBank. TaqMan probes and primer sets were designed using Primer Express version 2.0 Software (Applied Biosystems, Warrington, UK). The sequences and PCR methodology are reported in a previous publication (19).

Protein extractions and Western blotting

Total protein extracts were prepared from 20–30 mg of frozen muscle as previously described (19) and used for the determination of hexokinase II (HKII), sterol regulatory element binding protein-1c (SREBP-1c), peroxisome proliferator-activated receptor (PPAR) α and PPARδ protein expression. Mitochondria were extracted from 20–40 mg of fresh muscle tissue as previously described (25) and used for the determination of PDK2 and PDK4 protein expression. Protein concentrations of mitochondrial suspensions and whole tissue extracts were measured using the Bradford method (Bio-Rad Laboratories, Hemel Hempstead, UK).

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TABLE 1. Blood metabolite concentrations before (Pre) and after each diet (After diet)

	CON		HF	
	Pre	After diet	Pre	After diet
Blood glucose (mmol/liter)	4.8 (0.1)	4.5 (0.1)	4.5 (0.1)	4.4 (0.2)
Serum insulin (mU/liter)	7.4 (1.3)	6.4 (0.8)	6.1 (0.6)	5.1 (0.7)
Plasma NEFA (mmol/liter)	0.47 (0.08)	0.48 (0.04)	0.42 (0.04)	0.53(0.04)
Blood β-hydroxybutyrate (mmol/liter)	0.08 (0.02)	0.13 (0.03)	0.07 (0.01)	0.56(0.19)[a]

Data are mean (SEM); n = 10.
[a] $P = 0.05$ from pre-HF.

Proteins were separated, blocked, Western blotted, and quantified as previously described (19).

Calculations

Calculations of glucose disposal and substrate oxidation were made at steady state during the clamp (last 30 min). CHO oxidation (C_{ox}) and fat oxidation rates were calculated from the $\dot{V}CO_2$ and $\dot{V}O_2$ measurements (26) after correcting for protein oxidation. Rates of protein oxidation were estimated from urinary nitrogen excretion after correction for changes in blood urea nitrogen pool size. It was assumed that, for each gram of nitrogen excreted in the urine, 6.04 liters of O_2 were consumed and 4.89 liters of CO_2 were produced. The glucose stable isotope dilution technique was used to determine glucose turnover during basal and insulin-stimulated states. Modified Steele equations (27) were used to calculate R_d and R_a. Glucose disposal was also calculated from the glucose infusion rate (GIR) during the clamp (28). Hepatic glucose output was calculated as the difference between R_a and GIR during the clamp. Nonoxidative glucose disposal was calculated as the difference between R_d and C_{ox}.

Statistics

Repeated measures were analyzed using two-way (treatment × time) ANOVA (SPSS, version 11.5; SPSS, Chicago, IL). Bonferroni multiple comparisons *post hoc* tests were used to compare paired data where appropriate. *P* values less than 0.05 were considered as significant. All data are expressed as mean (SEM). Statistical comparisons were made at steady state during the clamps.

Results

Diet analysis

There was a small but significant ($P < 0.01$) excess energy intake in the HF treatment compared with CON [11.9 (0.2) *vs.* 11.0 (0.2) MJ/d] although this did not result in changes in body mass and resting energy expenditure. All subjects experienced symptoms of lethargy and hunger during the HF dietary treatment. The mean daily proportion of energy as

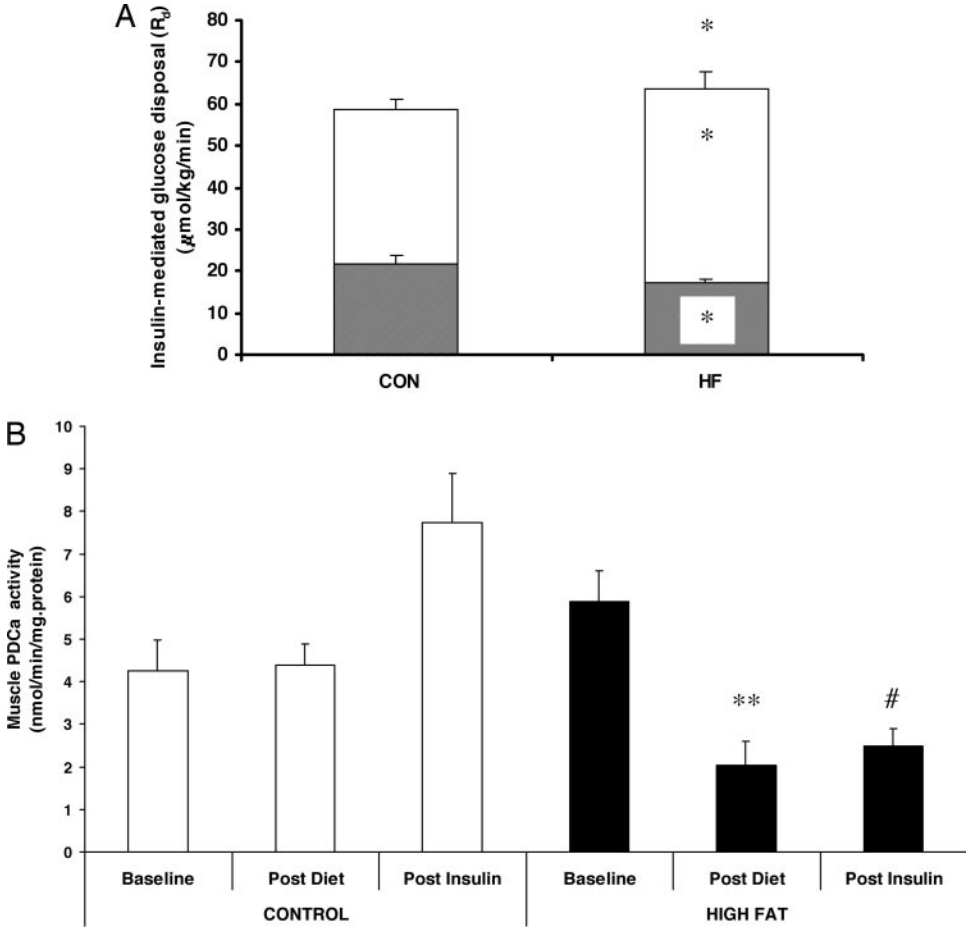

FIG. 1. A, Insulin-mediated whole-body glucose disposal (R_d) before and after the CON and HF diets. The *open part of column* denotes nonoxidative disposal and the *closed part* denotes CHO oxidation. *, $P < 0.05$ when compared with CON diet for differences in R_d, nonoxidative disposal, and CHO oxidation. B, Muscle PDCa activity at baseline, postdiet, and postinsulin infusion. **, $P < 0.05$ for HF diet-induced reduction in PDCa; and #, $P < 0.05$ for blunted PDCa activity postinsulin when compared with CON diet (treatment × time interaction, two-way ANOVA). Data are mean (SEM); n = 10 for R_d and n = 8 for PDCa.

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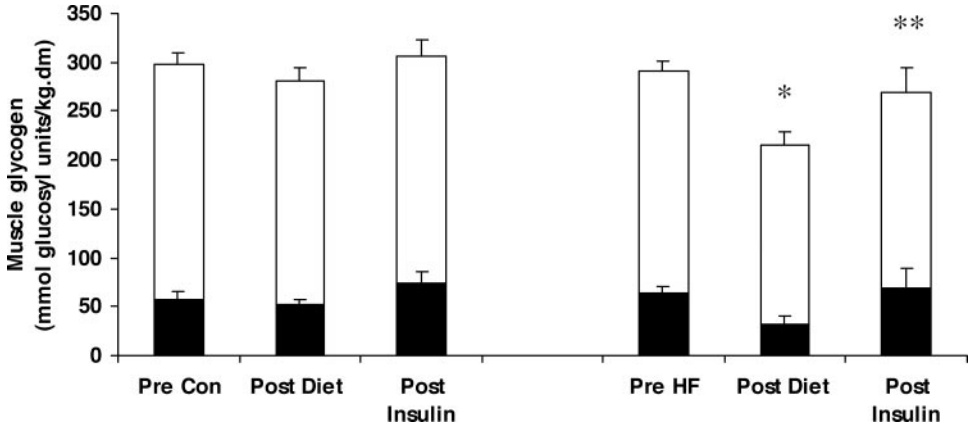

FIG. 2. Muscle glycogen concentrations prediet, postdiet, and after insulin clamp. *Open columns* represent proglycogen and *closed columns* represent macroglycogen. Data are mean (SEM); n = 10. *, $P < 0.05$ for reduction in glycogen concentrations after HF diet when compared with CON. **, $P < 0.05$ for increase in glycogen concentrations postinsulin in HF treatment.

CHO was 7.4% (0.2) in the HF diet and 49.8% (0.8) in the CON diet ($P < 0.01$), whereas the fat intake was 76.7% (0.4) *vs.* 32.3% (0.7) ($P < 0.01$), respectively. There was no difference in protein intake between the two diets. Furthermore, it should be noted that there was no difference in daily energy intake and macronutrient composition between the subjects' prescribed CON diet and their recorded habitual diet [10.8 (1.2) MJ/d, of which 47.0% (1.5) was derived from CHO, 34.3% (1.7) was derived from fat, and 18.7% (1.7) was derived from protein].

Circulating metabolites and hormones

Fasting blood glucose and serum insulin concentrations before and after each diet were not different (Table 1). There were no differences in insulin concentrations at steady state during the hyperinsulinemic clamp [CON 71.8 (3.5) *vs.* HF 70.0 (3.5) mU/liter]. There were no diet-induced differences in fasting plasma NEFA concentrations, but they were markedly suppressed during both clamps. Fasting β-hydroxybutyrate concentrations were increased after the HF diet compared with the CON diet ($P = 0.05$; Table 1). During the clamp, β-hydroxybutyrate concentrations were completely suppressed with no differences between diets. Baseline prediet concentrations of IL-12, TNF-α, IL-1β, IL-10, and IL-8 were 5.5 (1.1), 2.6 (0.3), 63.9 (12.7), 3.7 (0.4), and 2.9 (0.5) pg/ml, respectively, and these levels were not altered after either diet.

Whole-body substrate metabolism

There was a difference between diets in nonprotein respiratory exchange ratio at basal [CON 0.79 (0.01) *vs.* HF 0.75 (0.01), $P < 0.05$] and insulin-mediated conditions [CON 0.91 (0.01) *vs.* HF 0.87 (0.01), $P < 0.01$]. There was no diet-induced difference in resting metabolic rate [CON 5.65 (0.17) *vs.* HF 5.65 (0.17) kJ/min]. Insulin increased ($P < 0.01$) the metabolic rate after both diets, but there were no differences between diets [CON 5.99 (0.17) *vs.* HF 6.07 (0.17) kJ/min].

There was no difference in R_d between diets under basal conditions [CON 10.6 (2.6) *vs.* HF 8.8 (2.9) μmol/kg·min]. Hepatic glucose output was comparable under basal conditions after both diets [CON 10.9 (0.9) *vs.* HF 8.8 (0.8) μmol/kg·min] and was completely suppressed during clamp conditions in both trials. Under clamp conditions, R_d was higher during the last 30 min of the clamp after the HF diet [CON 57.5 (3.8) *vs.* HF 64.5 (4.9) μmol/kg·min, $P < 0.05$; Fig. 1A]. C_{ox} was reduced ($P < 0.05$) after HF when compared with CON under basal [CON 8.0 (1.2) *vs.* HF 4.6 (1.4)] and clamp conditions [CON 21.5 (2.4) *vs.* HF 17.2 (1.0) μmol/kg·min]. Nonoxidative glucose disposal under clamp conditions was greater after the HF diet [CON 36.0 (2.5) *vs.* HF 47.3 (4.6) μmol/kg·min, $P = 0.01$]. Fat oxidation was higher under clamp conditions after the HF diet compared with CON diet (CON 3.3 ± 0.5 *vs.* HF 5.3 ± 0.3 μmol/kg·min, $P < 0.05$), with a trend for a difference under basal conditions (CON 7.2 ± 0.2 *vs.* HF 8.7 ± 0.7 μmol/kg·min, $P = 0.06$).

TABLE 2. Muscle metabolite concentrations before (Pre) and after each diet (After diet) and after the hyperinsulinemic clamp (After insulin)

	CON			HF		
	Pre	After diet	After insulin	Pre	After diet	After insulin
ATP	23.4 (0.7)	26.2 (1.4)	24.2 (0.8)	25.4 (1.9)	23.5 (0.6)	25.7 (0.9)
PCr	71.9 (3.4)	70.8 (4.9)	61.3 (3.8)	64.7 (4.2)	65.4 (3.2)	70.1 (3.5)
Creatine	43.9 (4.6)	40.6 (4.9)	45.3 (4.5)	41.1 (4.6)	45.0 (3.8)	37.6 (4)
Lactate	7.0 (0.7)	7.2 (0.9)	9.0 (1.0)	7.2 (1.3)	5.5 (0.8)	11.2 (1.4)
Acetylcarnitine	4.3 (0.4)	4.5 (0.5)	4.7 (0.6)	4.7 (0.5)	7.9 (1.3)[a]	5.3 (1.0)[b]
Glucose-6-phosphate	3.4 (0.9)	4.7 (1.7)	1.2 (1.1)	3.9 (1.1)	3.9 (1.2)	2.9 (1.1)

Data are expressed as mean (SEM) as millimoles per kilogram of dry muscle. n = 10 for ATP, PCr, creatine, and glucose-6-phosphate, and n = 9 for lactate and acetylcarnitine.

[a] $P < 0.05$ for increase in acetylcarnitine concentrations post-HF diet.

[b] $P < 0.05$ for elevated acetylcarnitine concentrations postinsulin after HF compared with CON (treatment × time interaction, two-way ANOVA).

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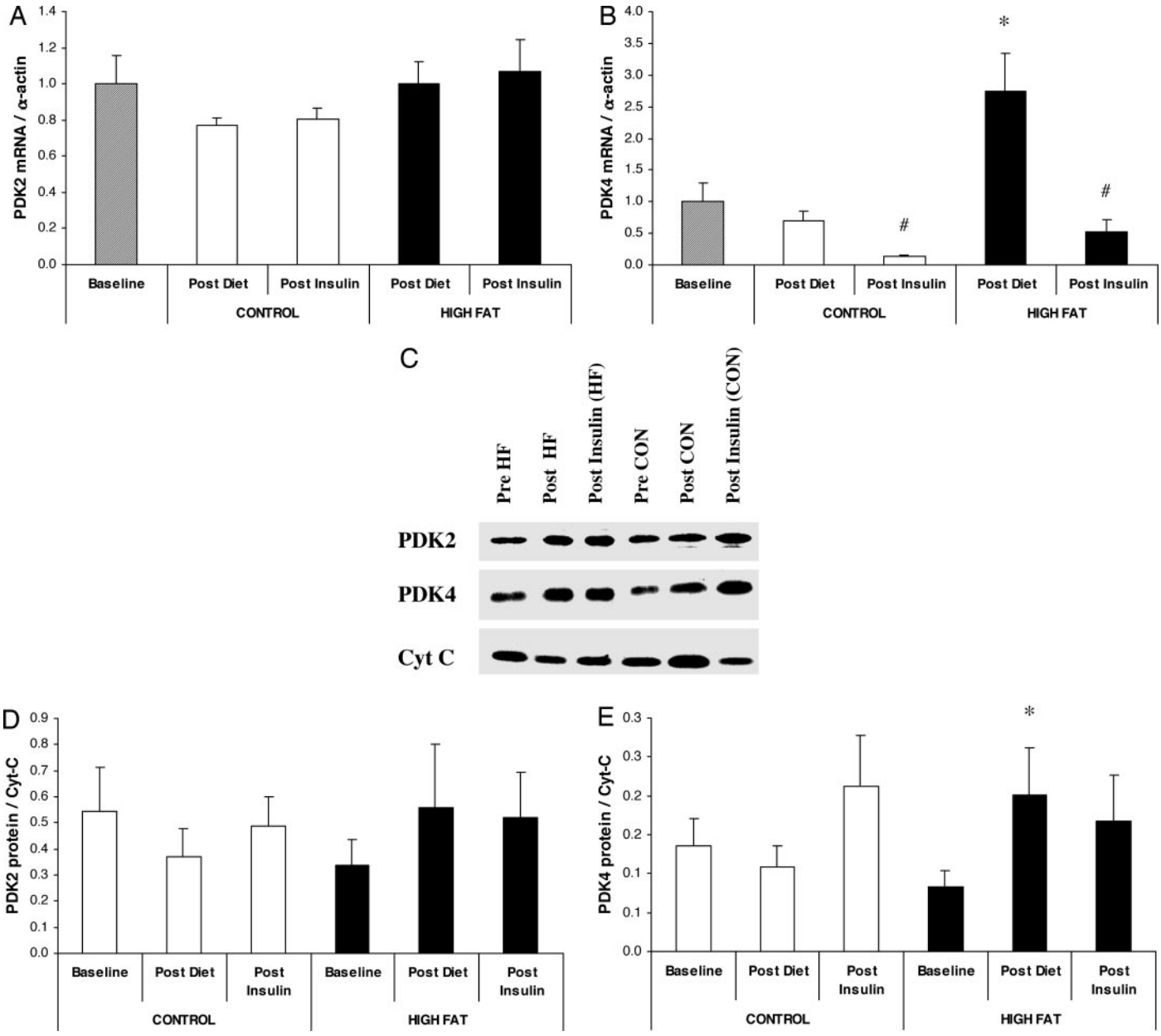

FIG. 3. A and B, Muscle PDK2 and PDK4 mRNA expression; C, representative Western blots of PDK2, PDK4, and cytochrome C (control); D and E, PDK2 and PDK4 mitochondrial protein expression. *Open columns* denote CON diet and *closed columns* denote HF diet. All mRNA changes are relative to baseline value = 1 (denoted by the *hatched column*). Data are mean (SEM); A, n = 10; B, n = 9; D, n = 8; E, n = 7. *, $P < 0.05$ (treatment effect, ANOVA). #, $P < 0.01$ when compared with the postdiet value.

Muscle metabolism

There was no difference in baseline PDCa activity between treatments (Fig. 1B). The HF diet induced a 65% reduction in PDCa activity ($P < 0.05$) when compared with CON. The insulin-mediated increase in PDCa activity was blunted after the HF diet when compared with CON diet ($P < 0.05$). Muscle glycogen concentrations were similar before both diets (Fig. 2). The HF diet caused a 26% decline in muscle glycogen content, which was different from the change after the CON diet ($P < 0.05$). Insulin-mediated muscle glycogen deposition was greater ($P < 0.05$) after the HF diet when compared with CON. There were no differences between diets in muscle ATP, PCr, lactate, glucose-6-phosphate, and creatine concentrations (Table 2). Muscle acetylcarnitine concentrations were unchanged after the CON diet but increased after the HF diet and remained high during the clamp when compared with CON (Table 2).

Muscle expression of metabolic genes and proteins

The HF diet induced an approximately 2-fold increase ($P < 0.05$) in both PDK4 mRNA and protein content when compared with CON (Fig. 3, B, C, and E). Insulin infusion down-

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regulated the expression of PDK4 mRNA, but not protein, after both diets ($P < 0.01$). There were no diet- or insulin-induced changes in PDK2 mRNA and protein expression (Fig. 3, A, C, and D). There were significant increases ($P < 0.05$) in insulin-mediated HKII and SREBP-1c mRNA (Fig. 4A) expression after the CON diet, whereas nonsignificant increases were observed after the HF diet. There were no diet- or insulin-induced changes in mRNA expression of pyruvate kinase (PK) and CHO response element-binding protein (ChREBP) (Fig. 4B), PPARα and δ, PPARδ coactivator-1α (PGC1α), carnitine palmitoyl transferase 1 (CPT1), fatty acid translocase/CD36, long chain acyl-CoA dehydrogenase (LCAD) and the protein expression of HKII, SREBP-1c, PPARα and δ (data not shown).

Discussion

In the present study, 6 d of HF feeding led to a reduction of basal and insulin-mediated CHO oxidation without inducing whole-body insulin resistance. In fact, an increase in R_d during the last 30 min of the clamp was observed after the HF diet compared with the CON diet. As a consequence, there was an increase in insulin-mediated nonoxidative glucose disposal both at the whole body and skeletal muscle level. These responses were associated with impaired muscle PDCa activity, most likely as a result of selective up-regulation of PDK4 expression. Furthermore, it is shown for the first time that insulin infusion can suppress PDK4 but not PDK2 gene expression *in vivo* in human skeletal muscle.

HF feeding in humans has produced contradictory results. In agreement with the findings from the present study, HF dietary treatment for 11–21 d did not induce whole-body insulin resistance, although the partitioning of glucose metabolism was altered with decreased oxidation and increased nonoxidative glucose disposal (9, 10). Furthermore, a study employing a HF diet for 16 d reported no effect on GIR during a 3-h hyperinsulinemic clamp although 12 of 25 subjects had greater GIRs during the last hour of the infusion after the HF diet than after the CON diet (29). This is in agreement with the results from the present study because a slight increase in R_d during the last 30 min of the clamp was observed after the HF than the CON diet. In contrast, HF feeding for just 3 d appears to induce whole-body insulin resistance (7, 8). It is possible that acute changes in dietary fat availability (several hours up to 3 d) might induce insulin resistance because of a greater imbalance between plasma NEFA availability and their muscle oxidation, whereas after several days, an increase in NEFA availability can be compensated by a greater im lipid storage and/or use. On the other hand, this difference may also be due to methodological differences in determining insulin resistance in the aforementioned studies (oral glucose tolerance test *vs.* insulin clamps; clamp duration and ambient insulin concentrations). Therefore, further studies are required to elucidate the precise sequence of adaptations to HF diets in humans.

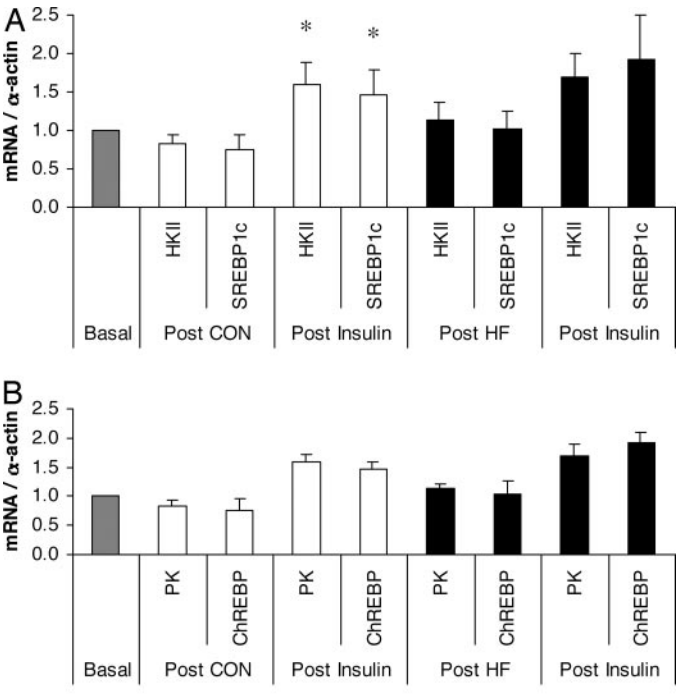

FIG. 4. Baseline, postdiet, and postinsulin mRNA expression changes of HKII and SREBP-1c (A), PK and ChREBP (B). All mRNA changes are relative to basal value = 1 (denoted by the *hatched column*). *Open columns* denote CON diet and *closed columns* denote HF diet. Data are mean (SEM); n = 10. *, $P < 0.05$ when compared with the postdiet value.

In the present study, the differential partitioning of intracellular glucose metabolism (with decreased oxidative and increased nonoxidative glucose disposal) observed in the HF dietary treatment extends previous findings at the whole-body level (9, 10) and may constitute one of the earliest im adaptations to HF diet in healthy humans. Moreover, the reduction in CHO oxidation was not readily reversible even after 4 h of insulin and glucose infusion. Glucose transport and oxidation, along with glycogen synthesis, are also impaired in patients with type 2 diabetes (30). Interestingly, when the defect in glucose transport was normalized by hyperglycemia and hyperinsulinemia, only glucose oxidation remained impaired (30). Therefore, it is likely that the impairment in insulin-mediated glucose oxidation is an early adaptation in metabolic states characterized by elevated lipid metabolism.

In the present study, we observed a reduction in muscle glycogen after the HF diet as observed previously (31, 32), and this is likely to have increased muscle glycogen synthase activity (10). This, in turn, may have resulted in greater insulin-mediated muscle glycogen storage after the HF diet. Alternatively, the increase in muscle glycogen content may also be attributed to the increase in insulin-mediated whole-body nonoxidative glucose disposal observed after the HF diet. Perhaps this is not surprising if one considers that under conditions of impaired CHO oxidation when glucose is made available intracellularly, either by feeding or during a hyperinsulinemic clamp, it will be directed toward either glycogen synthesis or nonoxidative glycolysis (5, 10). However, under hyperinsulinemic conditions, the contribution of nonoxidative glycolysis is rather small (3–5%) although it may double under conditions of elevated fat availability and impaired CHO oxidation (5, 10). In support of this notion, muscle lactate concentration (an indirect measure of glycolytic flux) tended to increase more during the clamp after the HF diet. Furthermore, it should be noted that, under hyper-

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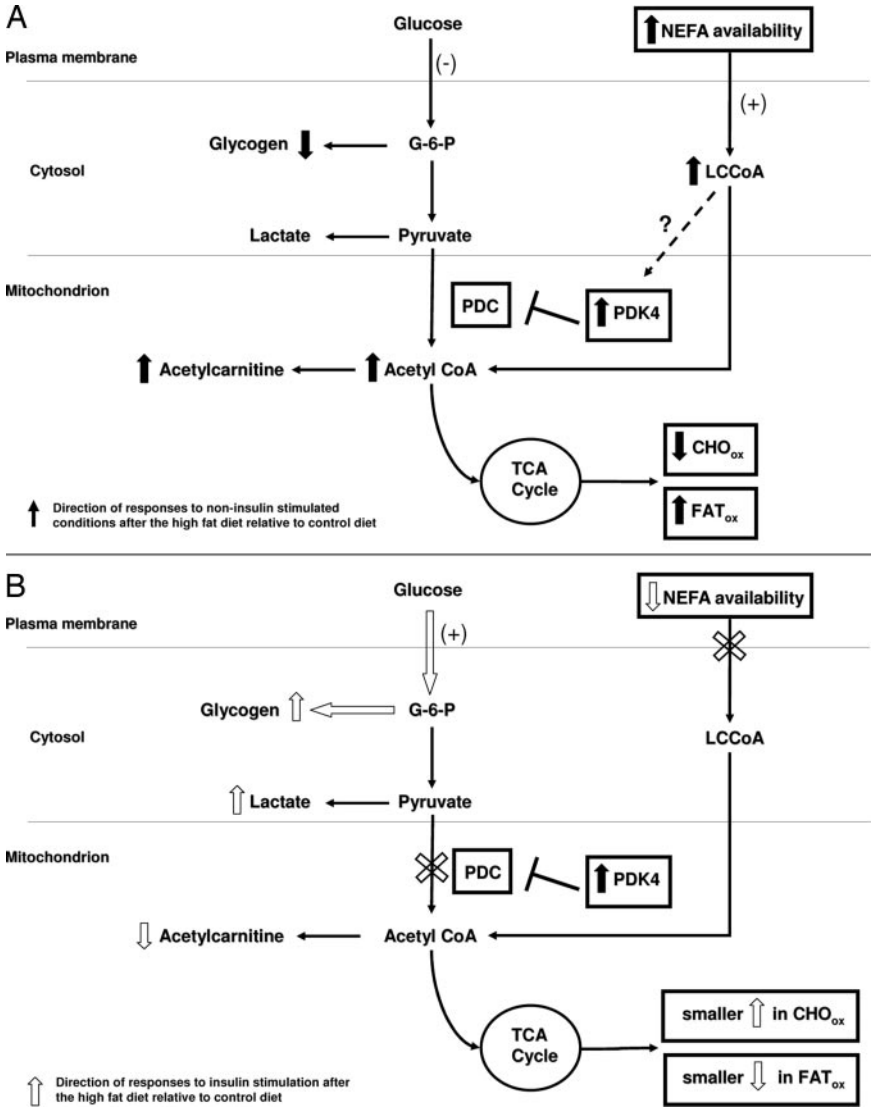

FIG. 5. Schematic diagram to explain HF diet-induced changes in key steps of CHO metabolism in human skeletal muscle. The *arrows* denote direction of responses to both noninsulin (*closed arrows*, A) and insulin-stimulated (*open arrows*, B) conditions after the HF diet relative to CON diet. Under noninsulin-stimulated conditions (A), the key initiating events are a HF-induced decrease in CHO availability (resulting in a decrease in muscle glycogen stores) and an increase in circulating fat availability, which cause a shift from CHO to fat metabolism. Up-regulation of muscle PDK4 protein expression (possibly as a result of elevated cytosolic concentration of long-chain fatty acyl COA-LCCoA) is most likely responsible for the inhibition of muscle PDC activity (the rate limiting step in CHO oxidation), and hence, the subsequent reduction in glucose oxidation. Under those conditions, the increase in muscle acetylcarnitine concentration indicates an increase in fat-derived acetyl CoA content, which reflects increased im fat oxidation. Under insulin-stimulated conditions (B), the key initiating events are an increase in CHO availability and a decrease in circulating NEFA. When compared with CON, glucose disposal during the clamp increases after the HF diet, whereas the increase in CHO oxidation and the decrease in fat oxidation (normally observed under those conditions) are smaller. Although insulin readily suppresses PDK4 gene expression, it does not affect PDK4 protein expression within the time limits of this study, which may explain the blunted PDC activity, and hence CHO oxidation, even after 4 h of insulin infusion after the HF diet when compared with the CON diet. The HF diet also causes an increase in insulin-mediated whole-body nonoxidative glucose disposal, which (along with the fat-induced reduction in preclamp glycogen content) may explain the increase in muscle glycogen storage and the lactate content (an indirect measure of glycolytic flux) during the clamp after the HF diet. G-6-P, Glucose-6-phosphate.

insulinemic conditions, glucose disposal is not restricted to skeletal muscle tissue. Thus, the increase in insulin-stimulated whole-body nonoxidative metabolism after the HF diet would also be expected to replenish nonmuscle, *e.g.* hepatic, glycogen stores, although the latter was not determined in the present study. Further studies are required to examine the detailed partitioning of nonoxidative glucose disposal in humans after consumption of a HF diet.

The changes in whole-body oxidative metabolism were also mirrored by corresponding changes in intracellular oxidative metabolism. After the HF diet, muscle PDCa activity was reduced, indicating reduced CHO oxidation, and muscle acetylcarnitine concentrations were increased reflecting increased fat oxidation. During the hyperinsulinemic clamp, muscle PDCa activity remained lower, whereas both muscle lactate and glycogen concentrations increased (reflecting an increase in intracellular nonoxidative glucose disposal). Thus, it would appear that, at the cellular level, the reduced flux through PDCa facilitates the increase in both nonoxidative glucose metabolism and oxidative fat metabolism (Fig. 5).

Skeletal muscle PDCa activity is inhibited by PDK, and four PDK isoenzymes have been identified in skeletal muscle, but only PDK4 expression is increased in starvation and diabetes in animal models (33–35) and starvation in healthy humans (19). Similarly, in the present study, administration of a HF diet for 6 d was associated with significant increases in muscle PDK4 mRNA and protein expression, which is in agreement with a previous 3-d study in healthy humans (15). However, we did not observe any diet or insulin-induced changes in gene and protein expression of PDK2, the other major isoform in human skeletal muscle. Insulin was shown to down-regulate transcript levels of PDK2 and PDK4 in insulin-resistant nondiabetic Pima Indians (16). In contrast, this is the first study to show that, in healthy humans, insulin can readily suppress PDK4 but not PDK2 gene expression in skeletal muscle although there was no effect on protein expression, most likely due to the short-term nature of the insulin infusion.

The selective increase in PDK4 expression most likely precedes the decrease in muscle PDCa activity after the HF diet. The fact that insulin infusion did not affect PDK4 protein

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expression indicates no suppression of PDK activity by insulin, thus explaining the blunted PDCa activity after 4 h of insulin infusion. Contrary to evidence from cell lines and animal studies that HF feeding-induced up-regulation of PDK4 is mediated by PPAR signaling (36–38), we did not observe any changes in gene expression of PPARα, PPARδ, their coactivator PGC1α, or some of their known transcriptional targets (CD36, CPT1,and LCAD). This is in line with the findings from a recent study from our laboratory indicating that the selective up-regulation of skeletal muscle PDK4 expression in fasted humans occurs in a novel manner distinct, at least in part, from the PPARs and Akt/FOXO1 pathways (19). It is possible that either posttranscriptional or allosteric factors such as acyl-CoA derivatives of NEFA might be responsible for the up-regulation of muscle PDK4 expression and, thus, blunting of insulin-mediated PDCa activity and further investigation is required. Our findings also suggest that, during short-term HF feeding, NEFA and/or their metabolites could allosterically activate the transporters and enzymes involved in the uptake and oxidation of fatty acids without invoking the transcriptional machinery. These findings indicate that there is sufficient capacity in resting skeletal muscle of healthy, nonobese subjects to use the substantial increase in fatty acid influx that occurs during short-term HF feeding.

In conclusion, short-term HF/low-CHO dietary intake does not induce whole-body insulin resistance in healthy humans but causes a shift in intracellular glucose metabolism by reducing insulin-mediated glucose oxidation and stimulating nonoxidative glucose disposal. Up-regulation of muscle PDK4 expression precedes these changes and is responsible for the inhibition of muscle PDCa and the subsequent reduction in glucose oxidation. The latter appears to precede changes in glucose uptake, further highlighting a key role for PDK4 in substrate metabolism and insulin action in human skeletal muscle.

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Address all correspondence and requests for reprints to: K. Tsintzas, Center for Integrated Systems Biology and Medicine, School of Biomedical Sciences, University of Nottingham, Nottingham NG7 2UH, United Kingdom. E-mail: kostas.tsintzas@nottingham.ac.uk.

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