

The Role of FOXO and PPAR Transcription Factors in Diet-Mediated Inhibition of PDC Activation and Carbohydrate Oxidation During Exercise in Humans and the Role of Pharmacological Activation of PDC in Overriding These Changes

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High-fat feeding inhibits pyruvate dehydrogenase complex (PDC)-controlled carbohydrate (CHO) oxidation, which contributes to muscle insulin resistance. We aimed to reveal molecular changes underpinning this process in resting and exercising humans. We also tested whether pharmacological activation of PDC overrides these diet-induced changes. Healthy males consumed a control diet (CD) and on two further occasions an isocaloric high-fat diet (HFD). After each diet, subjects cycled for 60 min after intravenous infusion with saline (CD and HFD) or dichloroacetate (HFD+DCA). Quadriceps muscle biopsies obtained before and after 10 and 60 min of exercise were used to estimate CHO use, PDC activation, and mRNAs associated with insulin, fat, and CHO signaling. Compared with CD, HFD increased resting pyruvate dehydrogenase kinase 2 (*PDK2*), *PDK4*, forkhead box class O transcription factor 1 (*FOXO1*), and peroxisome proliferator-activated receptor transcription factor α (*PPAR α*) mRNA and reduced PDC activation. Exercise increased PDC activation and whole-body CHO use in HFD, but to a lower extent than in CD. Meanwhile *PDK4* and *FOXO1*, but not *PPAR α* or *PDK2*, mRNA remained elevated. HFD+DCA activated PDC throughout and restored whole-body CHO use during exercise. FOXO1 appears to play a role in HFD-mediated muscle PDK4 upregulation and inhibition of PDC and CHO oxidation in humans. Also, pharmacological activation of PDC restores HFD-mediated inhibition of CHO oxidation during exercise. *Diabetes* 61:1017–1024, 2012

Several days of high-fat diet (HFD) intake reduces the rate of muscle glycogen degradation and glucose oxidation during low to moderate-intensity exercise in humans (1,2), and has been attributed to the impairment of exercise-mediated activation of the pyruvate dehydrogenase complex (PDC) resulting from increased blood free fatty acid (FFA) availability (3). The PDC controls the rate of skeletal muscle carbohydrate (CHO) oxidation, and therefore this dietary-mediated impairment of PDC activation has been advocated as a

causative factor in skeletal muscle insulin resistance and the metabolic syndrome (4).

The activation status of the PDC is controlled by a covalent mechanism involving competing pyruvate dehydrogenase kinase (PDK) and phosphatase (PDP) reactions (5). The resulting interconversion cycle determines the amount of PDC existing in nonphosphorylated (active) form, i.e., PDCa (6). The PDK family is composed of four isoforms (PDK1–4) (7), whereas PDP has two isoforms (PDP1 and 2) (8). Although *PDK2* and *PDK4* mRNA are expressed in most tissues, including skeletal muscle and heart, the specific activity of PDK4 is eightfold greater than that of PDK2 (7), thereby assigning greater regulatory significance to PDK4. PDK1 and PDK3 appear to be limited to heart, pancreatic islets, and kidney (7). The principal physiological factors that regulate PDK4 expression in resting skeletal muscle are changes in FFA and insulin availability, which explains why *PDK4* mRNA is selectively upregulated in response to starvation and hormonal and substrate changes, and in pathologies such as insulin resistance and type 2 diabetes (9–11). Conversely, inhibition of PDK2 using synthetic inhibitors seems to improve blood glucose concentration in obese Zucker rats (12). However, the mechanism by which FFAs upregulate PDK4 expression, thereby inhibiting PDC-controlled CHO oxidation in humans, is still unclear.

Based upon knowledge acquired from mainly cell and animal-based studies, it has been suggested that activation of peroxisome proliferator-activated receptor transcription factors (*PPAR α* , δ , and γ) by ligands, such as FFAs (13), might be a mechanism responsible for the upregulation of muscle *PDK4* mRNA expression (3,14–17). However, the more rapid increase in *PDK4* mRNA expression compared with *PPAR α* mRNA expression after administration of a *PPAR α* receptor agonist partly speaks against this stance (18). Furthermore, more recently, the clear dissociation between increased plasma FFA levels and muscle *PDK4* mRNA expression, together with the lack of any change in muscle *PPAR α* mRNA or protein expression during a 40-h fast in humans (19), suggests other factor(s) could be responsible for the increase in *PDK4* mRNA expression under these conditions. Since FFAs can also indirectly induce the translocation of forkhead box class O (FOXO) transcription factors 1 and 3 to the nucleus (20,21), and FOXO1 can bind directly to the promoter region of the *PDK4* gene (20), it is plausible that FOXO factors could also play an important role in promoting the upregulation of *PDK4* mRNA in response to increased FFA availability.

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See accompanying commentary, p. 983.

The activation of PDC during muscle contraction is achieved by the accumulation of mitochondrial calcium and pyruvate (22). They function by activating PDP and inhibiting PDK2 and 4, respectively, and jointly appear to be able to fully activate PDC at exercise intensities of 75% maximal oxygen consumption and above (6,23). However, as outlined above, when exercise at this workload is preceded by several days of HFD intake, calcium and pyruvate seem unable to activate PDC to the same extent as in the control condition (1,2), although they may at lower exercise intensities (24), resulting in reduced CHO oxidation compared with control at exercise intensities where muscle glycogen is an important contributor to energy production. Dichloroacetate (DCA) is a more potent pharmacological inhibitor of PDK2 and 4 protein than pyruvate (7,25), and can fully activate muscle PDC at rest in humans (26). To date, however, no study has determined whether DCA administration at rest can offset the reported HFD-mediated PDK2 and/or PDK4 inhibition of PDC activation and CHO oxidation during subsequent exercise in humans.

The novelty of the current study is that we have concurrently determined changes in *PPAR* (α , δ , γ) and *FOXO* (1 and 3) transcription factor mRNA expression at rest and during exercise after 3 days of control or HFD intake. We have also attempted to interpret the significance of these diet-induced changes to muscle *PDK2* and *4* mRNA expression, PDC activation, and CHO oxidation during submaximal exercise in human volunteers. Furthermore, we have determined whether the pharmacological inhibition of PDK2 and 4 using DCA could offset any HFD-mediated inhibition of PDC activation and CHO oxidation during exercise. By revealing molecular changes associated with HFD-mediated inhibition of muscle CHO oxidation during exercise in humans, and testing a pharmacological approach to bypass and counteract this diet-induced effect, this work is of clear importance to our understanding and treatment of human muscle insulin resistance.

RESEARCH DESIGN AND METHODS

Subjects. Six untrained, healthy male volunteers (age, body mass, BMI [mean \pm SEM]: 22.0 \pm 0.6 years, 79.5 \pm 1.6 kg, 24.9 \pm 0.8 kg/m²) participated in the current study, which was approved by the University of Nottingham Medical School Ethics Committee in accordance with the Declaration of Helsinki. Before taking part in the study, all subjects underwent routine medical screening and completed a general health questionnaire.

Study protocol. After entry into the study, maximal oxygen consumption ($V_{O_{2max}}$) was determined and then verified on a separate occasion in all volunteers. Each subject then completed an exercise familiarization visit consisting of 60 min of cycling at 75% $V_{O_{2max}}$, which also allowed confirmation of the accuracy of this workload. Immediately after this, subjects received meal plans for a control diet (CD) for 3 days (55% CHO, 30% fat, and 15% protein) and recorded their dietary intake via diet diaries. Subjects had to weigh and record all food intake, which allowed the macronutrient and caloric intake to be calculated using the Microdiet program (Downlee System Ltd., Chapel-en-le-Grave, U.K.). On two further occasions, the volunteers consumed a prescribed isocaloric HFD (10% CHO, 75% fat, 15% protein) (see Fig. 1) each for 3 days. The energy content of this diet was based upon data collected during the 3-day CD. The fat diet trials were administered in a randomized design. All three trials were separated by 2-week washout intervals.

The morning after each 3-day period of dietary manipulation, subjects reported to the laboratory having fasted overnight. On each occasion, a cannula was inserted into a vein on the dorsum of a heated hand, after administration of local anesthetic (1% lignocaine), to allow infusion of saline or the PDC activator DCA and sampling of arterialized venous blood using a three-way tap. Subjects then rested in a semisupine position while they underwent 45 min of intravenous infusion with either saline (CD and HFD) or DCA (50 mg/kg body mass, HFD+DCA), and then rested for a further 45 min. Arterialized venous blood samples were collected at predetermined intervals throughout this 90-min period (Fig. 1). After this, a resting biopsy sample (27) was obtained from the vastus lateralis muscle, and subjects immediately began exercising at 75% $V_{O_{2max}}$ on an electrically braked cycle ergometer (Lode, Groningen, the Netherlands). Subjects momentarily stopped cycling after 10 min of exercise, and a second muscle biopsy sample was obtained while the subject remained seated (and supported) on the cycle ergometer. This biopsy was required because the exercise-mediated increase in PDC activation occurs predominantly within the first 10 min of exercise at this workload (6). Subjects then continued cycling at 75% $V_{O_{2max}}$ for a further 50 min, and a third muscle biopsy was obtained immediately after the termination of exercise while the subject was seated on the cycle ergometer. Expired gas (V_{max} Encore; SensorMedics, San Diego, CA) and blood samples were collected at predetermined intervals throughout exercise to determine oxygen consumption and carbon dioxide production rates and blood and plasma metabolites, respectively (Fig. 1).

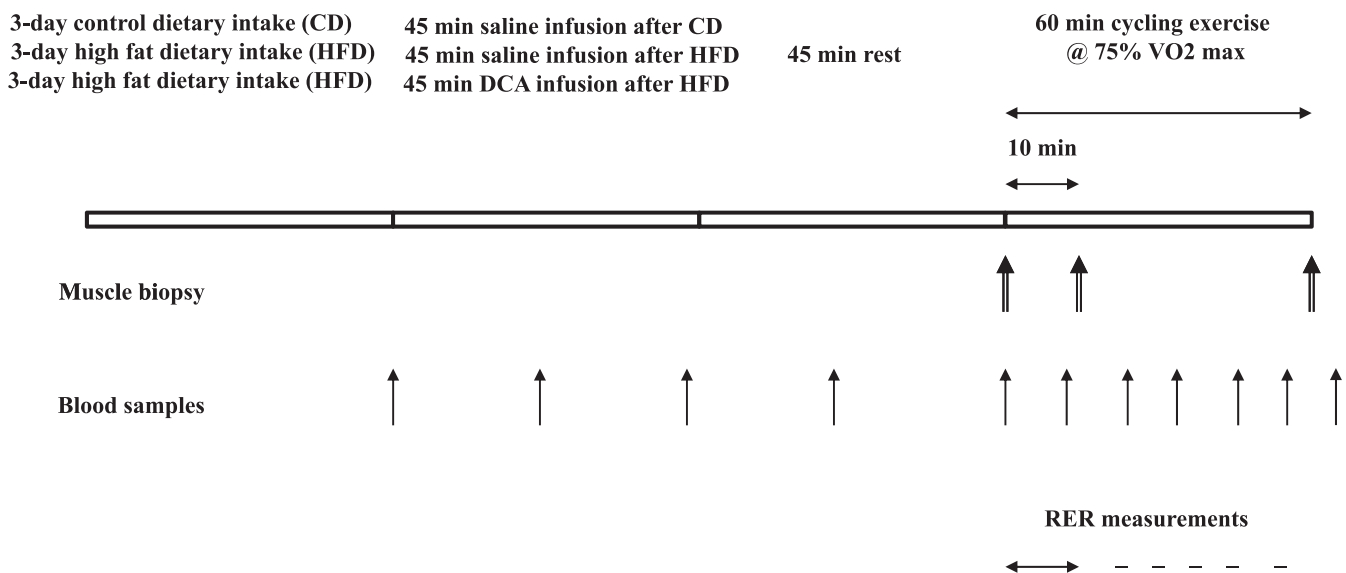


FIG. 1. Study protocol. Six healthy male subjects consumed a prescribed CD for 3 days (55% CHO, 30% fat, and 15% protein), and on two further occasions a prescribed isocaloric HFD (10% CHO, 75% fat, and 15% protein), each for 3 days. The fat diets were administered in a randomized manner separated by 2-week intervals. The morning after each period of dietary intervention, subjects cycled at 75% $V_{O_{2max}}$ for 60 min after intravenous infusion with either saline (CD and HFD) or the PDC activator DCA (50 mg/kg body mass; HFD+DCA). Quadriceps muscle biopsy samples were collected immediately before (0) and after 10 and 60 min of exercise. Throughout the infusion and subsequent exercise periods, blood samples were collected at the time points indicated in the figure.

Blood glucose and lactate. Immediately after blood sample collection, glucose and lactate concentrations were measured in whole blood using a glucose/lactate analyzer (YSI 2300 STATplus; Yellow Springs Instruments, Yellow Springs, OH).

Plasma FFAs. After collection into EDTANa₂ tubes, whole blood was immediately centrifuged and the plasma snap frozen in liquid nitrogen. This was used to determine plasma FFA concentrations at a later date using an enzymatic colorimetric assay kit (NEFA C kit; Wako Chemicals, Neuss, Germany).

Muscle metabolites and PDC activity. Snap-frozen vastus lateralis muscle was subsequently divided into two parts while under liquid nitrogen. One part was freeze dried, dissected free from visible connective tissue and blood, and used to extract muscle metabolites (28). Free carnitine and acetylcarnitine were measured in the muscle extract using enzymatic assays as previously described (29). Muscle glycogen and lactate concentrations were measured using a modification of a spectrophotometric method (28). The remainder of the frozen muscle was used to determine PDC activity as previously described (30).

Muscle mRNA expression. RNA was extracted from the snap-frozen muscle tissue using RNA Plus (Qiogene). First-strand cDNA was synthesized from a 1- μ g RNA sample using Powerscript reverse transcriptase (BD Biosciences). All reactions were performed using the ABI Prism 7000 Sequence Detection System (Applied Biosystems Foster City, CA). The genes analyzed were *PDK2* and *4*, *PDP1* and *2* (*PPM2C*), insulin receptor substrate 1 (*IRS1*), v-akt murine thymoma viral oncogene homolog 1 (*Akt1*), *FOXO1* and *3*, *PPAR α* , *β* , and *γ* , and *PPAR γ* coactivator-1 α isoform (*PGC-1 α*). The duplicate cycle threshold (Ct) values were averaged and the Δ Ct was calculated by subtracting the corresponding mean value of the housekeeping gene (porphobilinogen deaminase). $2^{-\Delta\Delta Ct}$ was measured by 2 to the power of the difference in Ct between samples. The control trial (CD) at rest was used as calibrator with a value of 1.

Calculations and statistics. CHO oxidation rates (g/min) were calculated using the equation of Frayn (31): $4.55 \times VCO_2$ (L/min) $- 3.21 \times Vo_2$ (L/min) $- 0.459 \times Pox$, where Pox is the protein oxidation rate, which was calculated using the following equation: Pox (g/min) = $0.12 \times$ energy expenditure (kJ/min)/16.74 kJ/g, assuming that protein oxidation contributed ~12% of energy expenditure (31). Energy expenditure was calculated from the rates of Vo_2 assuming 5 kcal or 22 kJ for each 1 L of oxygen consumed.

One- and two-way repeated ANOVA was used to determine treatment and time main effects. When a significant F ratio was obtained, a least significance difference post hoc test was applied to locate specific differences. Significance was set at the $P < 0.05$ level of confidence. Wherever indicated, the correlation coefficients between two variables or degree of linear relationship were obtained using Pearson product moment correlation. Unless otherwise stated, all data are expressed as mean \pm SEM.

RESULTS

Rates of whole-body CHO oxidation during exercise. The rates of CHO oxidation during exercise (g/min) are presented in Fig. 2. After an initial increase during the first 10 min of exercise in all treatment groups, the rate of CHO oxidation after the HFD was significantly lower throughout exercise compared with the CD and HFD+DCA treatments ($P < 0.01$ and $P < 0.05$, respectively; treatment effect). There was no difference between CD and HFD+DCA.

Blood and plasma metabolite concentrations. Resting blood glucose concentration during intravenous infusion of saline and DCA was no different between treatment groups (Fig. 3A). During exercise, blood glucose concentration declined progressively with time in HFD and HFD+DCA when compared with CD ($P < 0.05$; treatment effect). Resting blood lactate concentration declined steadily in HFD+DCA over the course of infusion compared with the other two treatment groups ($P < 0.05$; treatment effect) (Fig. 3A). During exercise, blood lactate concentration increased in all treatment groups, but the magnitude of increase in HFD+DCA was significantly less than in CD ($P < 0.05$; treatment effect) (Fig. 3A). Plasma FFA concentration was significantly lower in CD during resting infusion and throughout exercise compared with HFD and HFD+DCA ($P < 0.05$; treatment effect) (Fig. 3B).

Muscle metabolites and PDC activity. Resting, pre-exercise muscle glycogen content was significantly greater

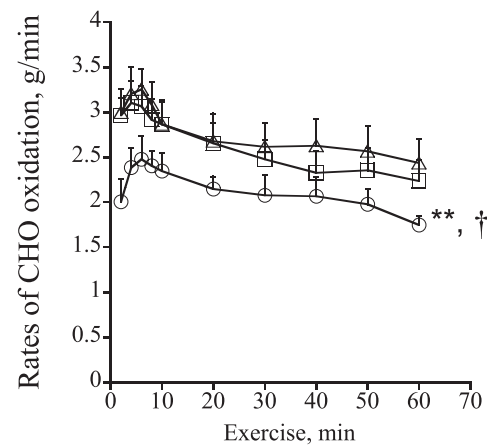


FIG. 2. Rates of CHO oxidation (g/min) during 60 min of exercise at 75% Vo_{2max} . Six healthy male subjects consumed a prescribed CD for 3 days, and on two further occasions a prescribed isocaloric HFD, each for 3 days. The morning after each period of dietary intervention, subjects cycled at 75% Vo_{2max} for 60 min after intravenous infusion with either saline (CD+saline, Δ), HFD+saline, (\circ) or DCA (HFD+DCA, \square). **Significantly different from the CD trial ($P < 0.01$). †Significantly different from the HFD+DCA trial ($P < 0.05$).

in CD compared with HFD and HFD+DCA (550 ± 31 vs. 402 ± 27 and 368 ± 24 mmol/kg dry muscle (dm), respectively; $P < 0.05$ and $P < 0.01$). Muscle glycogen utilization during exercise is shown in Fig. 4A. Glycogen breakdown over 60 min of exercise was significantly lower in the HFD and HFD+DCA than in CD ($P < 0.05$ and $P < 0.01$, respectively). There was no difference in muscle glycogen utilization between HFD and HFD+DCA. These between-group differences were mainly accounted for by events that occurred between 10 and 60 min of exercise (Fig. 4A).

Resting muscle lactate content was lower in HFD+DCA compared with CD ($P < 0.05$) and HFD ($P < 0.05$) (5.4 ± 0.8 , 7.7 ± 0.8 , and 7.5 ± 0.6 mmol/kg dm, respectively). Muscle lactate content increased during the first 10 min of exercise in all groups, although at different rates. Thus, although muscle lactate accumulation during exercise in CD was significantly greater than in HFD and HFD+DCA ($P < 0.05$ and $P < 0.01$, respectively), lactate accumulation in HFD+DCA was less than in HFD ($P < 0.05$). Muscle lactate content declined between 10 and 60 min of exercise in all groups (Fig. 4B), although the decline in HFD+DCA was significantly less than in CD ($P < 0.05$).

Muscle PDC activity (expressed as amount of active form, PDCa) at rest and during exercise is shown in Fig. 5A. HFD significantly reduced resting muscle PDCa compared with CD ($P < 0.05$) (Fig. 5A). However, HFD+DCA resulted in PDCa being markedly increased above CD and HFD at rest ($P < 0.01$ and $P < 0.01$, respectively). The magnitude of increase in PDCa after 10 min of exercise was significantly less in HFD compared with CD and HFD+DCA ($P < 0.05$ and $P < 0.01$, respectively). A further increase in PDCa was observed in CD over the remaining 50 min of exercise, but PDCa remained unchanged over this period in HFD and HFD+DCA, resulting in PDCa remaining significantly lower in HFD compared with CD and HFD+DCA by the end of exercise ($P < 0.01$ and $P < 0.01$, respectively).

Resting muscle acetylcarnitine content in HFD and HFD+DCA was significantly greater than in CD (3.9 ± 0.4 ,

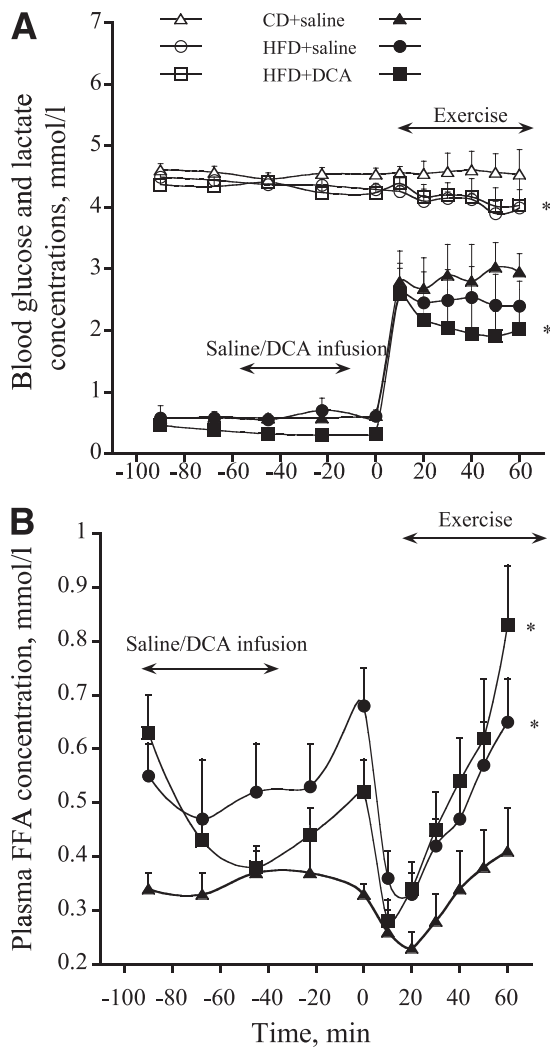


FIG. 3. A: Whole-blood glucose (white symbols) and lactate (black symbols) concentrations at rest during intravenous infusion of either saline or DCA and during a subsequent 60-min bout of exercise at 75% $\dot{V}O_{2max}$. Six healthy male subjects consumed a prescribed CD for 3 days, and on two further occasions a prescribed isocaloric HFD, each for 3 days. The morning after each period of dietary intervention, subjects cycled at 75% $\dot{V}O_{2max}$ for 60 min after intravenous infusion with either saline (CD+saline and HFD+saline) or DCA (HFD+DCA). *Significantly different from the CD trial ($P < 0.05$). **B:** Plasma FFA concentrations during 45 min of intravenous infusion of a saline or DCA solution followed by 60 min of exercise at 75% $\dot{V}O_{2max}$. Six healthy male subjects consumed a prescribed CD for 3 days, and on two further occasions a prescribed isocaloric HFD, each for 3 days. The morning after each period of dietary intervention, subjects cycled at 75% $\dot{V}O_{2max}$ for 60 min after intravenous infusion with either saline (CD+saline and HFD+saline) or DCA (HFD+DCA). *Significantly different from the CD trial ($P < 0.05$).

10.8 ± 0.5 , and 2.0 ± 0.5 mmol/kg dm, respectively; $P < 0.05$ and $P < 0.01$). Acetylcarnitine accumulation during exercise is presented in Fig. 5B. Although DCA infusion is likely to have acetylated a large portion of cellular free carnitine pool at rest prior to the exercise (32), the magnitude of muscle acetylcarnitine accumulation during exercise in HFD+DCA was significantly less than in CD and HFD ($P < 0.01$ and $P < 0.05$, respectively). The exercise-induced increase in muscle acetylcarnitine in CD occurred mainly over the first 10 min of exercise, which was not the case in HFD+saline and HFD+DCA, where the magnitude of accumulation was approximately the same between

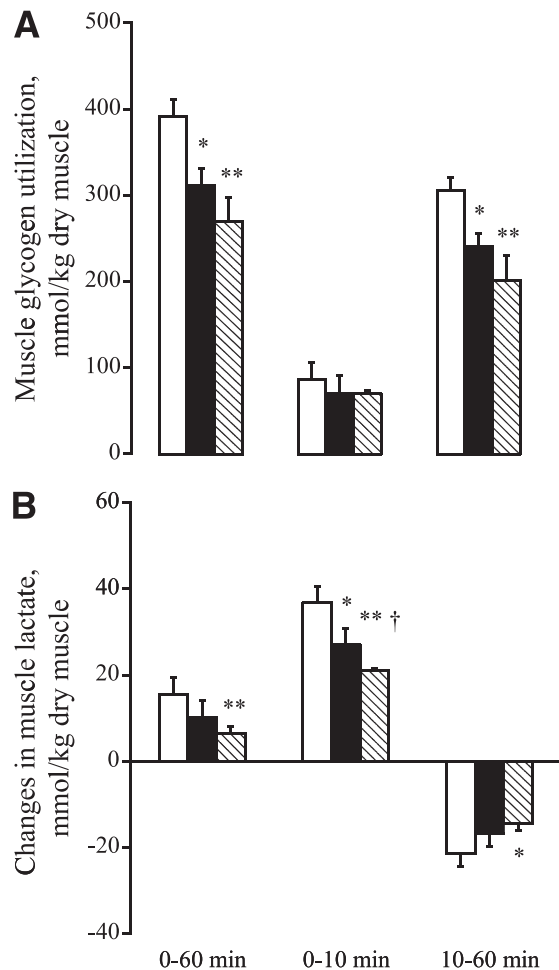


FIG. 4. A: Muscle glycogen use (mmol/kg dm) during 0–60, 0–10, and 10–60 min of exercise at 75% $\dot{V}O_{2max}$. Six healthy male subjects consumed a prescribed CD, and on two further occasions a prescribed isocaloric HFD, each for 3 days. The morning after each period of dietary intervention, subjects cycled at 75% $\dot{V}O_{2max}$ for 60 min after intravenous infusion with either saline (CD+saline, □; HFD+saline, ■) or DCA (HFD+DCA, hatched bars). **Significantly different from CD trial ($P < 0.05$ and $P < 0.01$). **B:** Rates of lactate accumulation (mmol/kg dm) in human vastus lateralis muscle during 0–60, 0–10, and 10–60 min of exercise at 75% $\dot{V}O_{2max}$. Six healthy male subjects consumed a prescribed CD, and on two further occasions a prescribed isocaloric HFD, each for 3 days. The morning after each period of dietary intervention, subjects cycled at 75% $\dot{V}O_{2max}$ for 60 min after intravenous infusion with either saline (CD+saline, □; HFD+saline, ■) or DCA (HFD+DCA, hatched bars). **Significantly different from resting value ($P < 0.05$ and $P < 0.01$). †Significantly different from the corresponding time point in the CD trial ($P < 0.05$).

0–10 and 10–60 min, albeit approximately twice as high in HFD+saline as in HFD+DCA ($P < 0.05$).

Muscle mRNA expression. mRNA expression levels of selected signaling proteins from pathways thought to regulate muscle fat and CHO metabolism (*PDK2* and *4*, *PPAR α* , δ , and γ , *Akt1*, *IRS1*, *FOXO1* and *3*, *PDP1* and *2*, and *PGC-1 α*) are presented in Table 1. The abundance of *PDK4* and *FOXO1* mRNA transcripts was significantly increased in muscle at rest after 3 days of HFD ($P < 0.01$ and $P < 0.01$, respectively) and HFD+DCA ($P < 0.01$ and $P < 0.01$, respectively) compared with CD. Although of lower magnitude, muscle *PDK2* ($P < 0.05$), *PPAR α* ($P < 0.01$), and *PPAR γ* ($P < 0.05$) mRNA expression were also significantly increased in HFD compared with CD at rest, but this was not the case for HFD+DCA. Furthermore, the

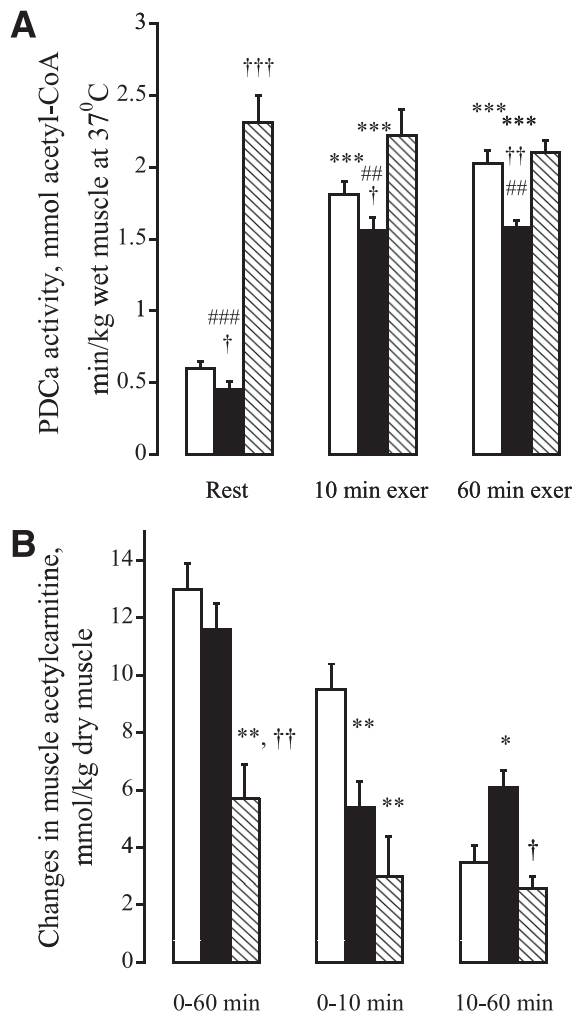


FIG. 5. A: Activity of PDCa (mmol/kg dm) in human vastus lateralis muscle at rest, and after 10 and 60 min of exercise at 75% $V_{O_{2max}}$. Six healthy male subjects consumed a prescribed CD for 3 days, and on two further occasions a prescribed isocaloric HFD, each for 3 days. The morning after each period of dietary intervention, subjects cycled at 75% $V_{O_{2max}}$ for 60 min after intravenous infusion with either saline (CD+saline, □; HFD+saline, ■) or DCA (HFD+DCA, hatched bars). ***Significantly different from the resting value within the trial ($P < 0.01$). †, ††, ††† Significantly different from the corresponding time point in the CD trial ($P < 0.05$, $P < 0.01$, and $P < 0.001$). ##, ### Significantly different from the corresponding time point in the HFD+DCA trial ($P < 0.05$, $P < 0.01$, and $P < 0.001$). **B:** Rates of acetyl-carnitine accumulation (mmol/kg dm) in human vastus lateralis muscle during 60 min (0–10 and 10–60 min) of exercise at 75% $V_{O_{2max}}$. Six healthy male subjects consumed a prescribed CD for 3 days, and on two further occasions a prescribed isocaloric HFD, each for 3 days. The morning after each period of dietary intervention, subjects cycled at 75% $V_{O_{2max}}$ for 60 min after intravenous infusion with either saline (CD+saline, □; HFD+saline, ■) or DCA (HFD+DCA, hatched bars). *, ** Significantly different from the CD trial ($P < 0.05$ and $P < 0.01$). †, †† Significantly different from the HFD+saline trial ($P < 0.05$).

expression of *PPAR α* in resting muscle was significantly lower in HFD+DCA compared with HFD ($P < 0.05$). After 60 min of exercise, muscle *PDK4* and *FOXO1*, but not *PDK2*, mRNA expressions were still greater in HFD ($P < 0.01$ and $P < 0.01$, respectively) and HFD+DCA ($P < 0.01$ and $P < 0.01$, respectively) compared with CD at 0 min. Even when compared with CD at 60 min of exercise, muscle *PDK4* and *FOXO1* mRNA expressions were still greater in HFD ($P < 0.05$ and $P < 0.05$, respectively) and HFD+DCA ($P < 0.05$ and $P < 0.05$, respectively). The

exercise per se did increase *PDK4*, but not *PDK2* and *FOXO1*, mRNA expression in CD ($P < 0.01$). Conversely, mRNA expression levels of all three *PPAR* isoforms were no different between treatment groups after 60 min of exercise.

DISCUSSION

The current study demonstrated for the first time that consumption of an HFD for 3 days concurrently increased *FOXO1* (threefold), *PPAR α* (1.6-fold), *PPAR γ* (1.6-fold), *PDK2* (1.5-fold), and *PDK4* (threefold) mRNA expression in human skeletal muscle in the resting state compared with an isocaloric CD, while simultaneously reducing muscle PDC activity. In line with these observations, the magnitude of PDC activation and the extent of whole-body CHO oxidation and muscle glycogen use were reduced by the HFD during subsequent exercise at 75% of $V_{O_{2max}}$. However, although the HFD-mediated upregulation of *FOXO1* and *PDK4* mRNA expression seen at rest was maintained during exercise, this was not the case for *PPAR α* and γ expression. Finally, DCA infusion abolished this HFD-mediated inhibition of muscle PDC activation seen at rest and during exercise, and increased the rate of whole-body CHO oxidation during exercise toward that seen after the CD, but did not affect the HFD-mediated changes in muscle gene expression, with the exception of a small, but significant, reduction in *PPAR α* at rest. Jointly, these data point to a role for *FOXO1* in HFD-mediated upregulation of muscle *PDK4*, and thereby the inhibition of PDC activation and CHO oxidation during exercise in humans. Furthermore, pharmacological activation of PDC restores HFD-mediated inhibition of CHO oxidation during exercise, and independently of diet-mediated changes in muscle gene expression.

Previous human volunteer studies have shown that the increase in circulating FFA concentration after short-term (days) HFD intake is associated with increased whole-body fat oxidation and reduced CHO use during subsequent submaximal intensity exercise (1,2). However, despite these changes, consumption of an HFD did not increase exercise performance nor did it have any apparent health benefit because it decreased resting muscle and liver glycogen content compared with a CD (33,34), and increased muscle *PDK4* mRNA and protein expression (3), which has been implicated as being a causative factor in muscle insulin resistance and the metabolic syndrome (4). Although we did not measure insulin resistance directly, consumption of an HFD for 3 days has been shown to induce whole-body insulin resistance (3,35). Furthermore, in the current study, we observed changes in CHO oxidation rates (Fig. 2), muscle glycogen use, and PDC activation during exercise (Fig. 4A and Fig. 5A), consistent with the induction of at least peripheral (muscle) insulin resistance.

Short-term increases in circulating insulin concentration under “normal” dietary conditions, together with an increase in muscle Ca^{2+} and pyruvate availability during exercise, appear to be the main physiological activators of muscle PDC in humans, and they act by controlling PDH kinase and phosphatase activity, respectively. Chronically, they may also control PDC by suppressing muscle *PDK4* mRNA and protein expression (6,22,36). Conversely, an increase in systemic circulating FFA availability, as seen after HFD intake and in type 2 diabetes, is associated with an increase in muscle *PDK4* mRNA and protein expression, thereby inhibiting activity of PDC. The latter effect

TABLE 1
Human skeletal muscle mRNA expression

Gene	CD+saline 0 min	CD+saline 60 min	HFD+saline 0 min	HFD+saline 60 min	HFD+DCA 0 min	HFD+DCA 60 min
<i>Akt1</i>	1	1.27 ± 0.26	1.20 ± 0.15	1.06 ± 0.15	0.97 ± 0.13	0.93 ± 0.17
<i>PDK2</i>	1	1.13 ± 0.15	1.53 ± 0.19*	1.32 ± 0.22	1.23 ± 0.15	1.31 ± 0.20
<i>PDK4</i>	1	1.67 ± 0.16**	3.03 ± 0.68**	2.59 ± 0.25**†	2.28 ± 0.43**	2.73 ± 0.25**†
<i>PPARα</i>	1	1.18 ± 0.15	1.60 ± 0.13**	1.27 ± 0.16	1.17 ± 0.09#	1.17 ± 0.07
<i>PPARδ</i>	1	1.15 ± 0.18	1.14 ± 0.14	1.07 ± 0.10	1.14 ± 0.12	1.11 ± 0.19
<i>PPARγ</i>	1	1.38 ± 0.32	1.59 ± 0.27*	1.25 ± 0.10	1.12 ± 0.19	1.63 ± 0.32
<i>FOXO1</i>	1	1.34 ± 0.25	2.66 ± 0.51**	2.14 ± 0.08**†	1.91 ± 0.24**	2.11 ± 0.24**†
<i>FOXO3</i>	1	1.23 ± 0.23	0.95 ± 0.24	1.41 ± 0.25	1.08 ± 0.11	1.33 ± 0.22
<i>IRS1</i>	1	0.89 ± 0.10	1.19 ± 0.13	1.16 ± 0.18	1.26 ± 0.07	1.37 ± 0.24
<i>PPM2C</i>	1	0.86 ± 0.20	0.67 ± 0.11	0.74 ± 0.16	0.84 ± 0.20	0.73 ± 0.12
<i>PDP2</i>	1	1.15 ± 0.24	0.99 ± 0.14	1.13 ± 0.17	1.18 ± 0.22	1.47 ± 0.22
<i>PGC1α</i>	1	0.90 ± 0.08	1.34 ± 0.39	1.17 ± 0.23	1.23 ± 0.16	1.31 ± 0.25

Human skeletal muscle *Akt1*, *PDK4*, *PPARs* (α , δ , and γ), *FOXO1* and *3*, *IRS1*, *PPM2C*, *PDP2*, and *PGC1α* mRNA expression at rest and after 60 min of exercise at 75% $\dot{V}O_{2max}$ after consumption of a CD, and on two further occasions a prescribed isocaloric HFD, for 3 days each. The morning after each period of dietary intervention, just before the start of cycling, subjects were intravenously infused with either saline (CD and HFD) or the PDC activator DCA (HFD+DCA). Ct values of the target gene were normalized to Ct values of the internal control porphobilinogen deaminase, and final results were calculated according to the $2^{-\Delta\Delta Ct}$ method. The CD 0 min was used as calibrator with a value of 1 within each line. **, **Significantly different from the resting CD (0 min) trial ($P < 0.05$ and $P < 0.01$). †Significantly different from the corresponding time point in the CD trial ($P < 0.05$). #Significantly different from the corresponding time point in the HFD trial ($P < 0.05$).

could be attributable to either a direct FFA-mediated activation of *PPARα*, δ , or γ receptors (14,17,37) or an indirect FFA-mediated activation of the forkhead/winged helix box family gene-coded transcription factors, *FOXO1* or *FOXO3* (20,38), followed by direct binding to the promoter region of the *PDK4* gene (20).

Evidence from cell and animal work suggest that molecular responses to increased circulating FFA availability include activation of *PPARs* and *PGC-1*, especially *PGC-1α* (14,39,40). *PPARs* are members of the nuclear hormone receptor superfamily of ligand-activated transcription factors that interact with FFA and regulate the expression of genes involved in the transport, metabolism, and handling of FFAs within the cells (13). Of the three distinct *PPAR* subtypes that have been identified so far, i.e., *PPARα*, *PPARδ* (also known as *PPARβ*), and *PPARγ* (41), the signaling pathways via *PPARα* and δ have been suggested to be a component of the mechanism that increases *PDK4* mRNA and protein expression during starvation and type 2 diabetes (10), and after pharmacological intervention with *PPARα* and δ agonists (14,17). Nevertheless, based on mRNA data at least, the present results appear to speak against a major involvement of the muscle *PPARs* in increasing muscle *PDK4* mRNA expression, because the HFD was only associated with a modest increase in muscle *PPARα* and γ expression at rest, which further waned during exercise (Table 1). In line with our finding, Spriet et al. (19) did not see any change in *PPARα* mRNA and protein expression in human skeletal muscle over 40 h of starvation despite expression of *PDK4* mRNA being 14-fold upregulated. However, in the absence of protein measurements in the present experiment, our interpretation cannot be fully substantiated.

Alternatively, rather than being a direct effect of increased circulating FFA availability per se, an increase in muscle *PDK4* mRNA expression could also be accounted for by a reduction/impairment of *IRS1* signaling after an HFD-induced decrease in insulin availability/sensitivity (42). *FOXO1* transcription factor is known to be regulated by the phosphatidylinositol 3-kinase/*Akt1* signaling pathway (43), which is a pathway known to be sensitive to circulating

insulin and FFA availability in humans (44). Because *FOXO1* can sense changes in availability of FFAs or insulin and relay the message downstream by modulating transcription of many skeletal muscle genes, including that of *PDK4* (20), it could therefore inhibit PDC-controlled CHO oxidation. Indeed, recent rodent-based evidence suggests a causative link between changes in the expression of muscle *PDK4* and levels of *FOXO1* protein dephosphorylation in acute insulin-resistant states (45). The current study goes further by offering evidence for an increase in the transcriptional drive of *FOXO1*, but not *FOXO3*, and presumably of total protein after 3 days of HFD intake in humans. It is worth noting that when *FOXO1* and *3* were transfected in HepG2 cells, marked h*PDK4* induction (measured as relative luciferase activity) occurred, and mainly when the cells were transfected with *FOXO3* (46).

In the current study, consumption of an isocaloric HFD for 3 days reduced the rate of muscle glycogen use (Fig. 4A) and the rate of whole-body CHO oxidation (Fig. 2) during exercise, which, in keeping with previous work (1–3), can be attributed to a reduction in exercise-induced PDC activation (Fig. 5A). However, infusion of the PDC inhibitor DCA abolished this HFD-mediated inhibition of PDC activity at rest and during exercise (Fig. 5A). It is worth noting that the marked activation of muscle PDC by DCA (Fig. 5A) occurred independently of diet or exercise-induced changes in muscle *PDK4* mRNA expression (Table 1), and probably also protein expression (17). Although DCA infusion after the HFD did not increase muscle glycogen use during exercise compared with the HFD alone, it did reduce blood lactate concentration (Fig. 3A) and muscle lactate and acetylcarnitine accumulation during exercise (Fig. 4B and Fig. 5B) more than in any other group, suggesting more efficient oxidative CHO use, i.e., higher flux through the PDC reaction. Additionally, because muscle glycogen use was not altered by DCA compared with HFD alone (Fig. 4A), despite an increase in whole-body CHO oxidation, this suggests that DCA may have increasing PDC-mediated blood glucose extraction and oxidation (47). Alternatively, DCA might have also concurrently inhibited glycolysis (48), thereby resulting in

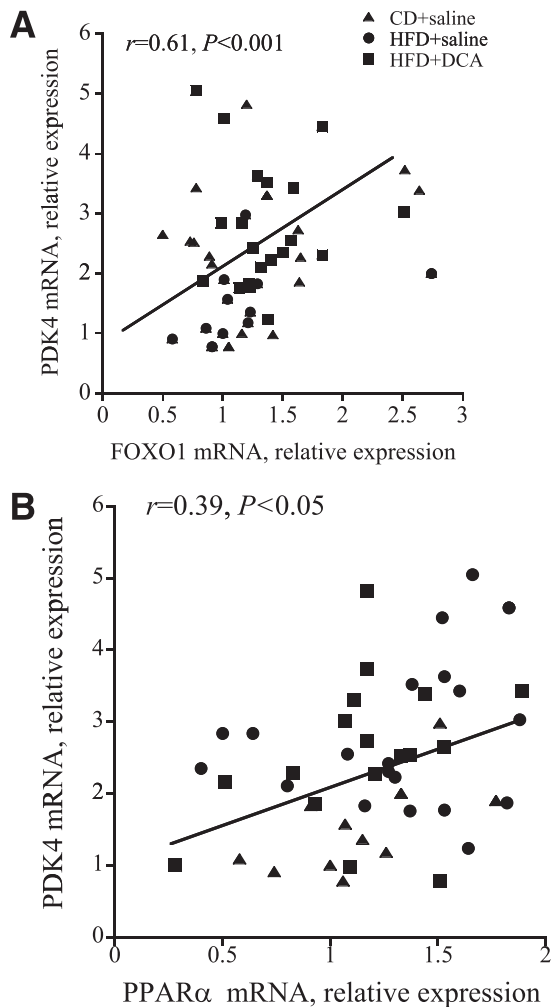


FIG. 6. Pearson correlation between *PDK4* and *FOXO1* mRNA expression (A), and *PDK4* and *PPAR α* mRNA expression (B) in human vastus lateralis muscle at rest, and after 10 and 60 min of exercise at 75% $\dot{V}O_{2max}$. Six healthy male subjects consumed a prescribed CD for 3 days, and on two further occasions a prescribed isocaloric HFD, each for 3 days. The morning after each period of dietary intervention, subjects cycled at 75% $\dot{V}O_{2max}$ for 60 min after intravenous infusion with either saline (CD+saline and HFD+saline) or DCA (HFD+DCA).

a better matching of glycolytic flux and PDC flux. Finally, DCA may have also increased hepatic glucose oxidation (49). Overall, these events may have accounted for the observed improvement in whole-body CHO use during exercise in the HFD+DCA trial (Fig. 2) that occurred in the face of similar muscle glycogen use during exercise compared with the HFD+saline trial (Fig. 4A). Therefore, what appears to be novel here is that in human skeletal muscle, DCA has rescued the negative effects of HFD intake on CHO oxidation during submaximal exercise. This supports the stance that PDC plays a central role in the regulation of muscle fuel selection, particularly during exercise when fat and CHO oxidation rates are increased. Although, initially, DCA was presented as a potentially novel oral antidiabetic agent that could reduce blood glucose and lactate by inhibiting hepatic glucose synthesis and stimulating glucose clearance (12) and use by peripheral tissues (25), including skeletal muscle, concerns about its lack of tissue specificity and long-term safety has hampered its therapeutic use as an antidiabetic agent.

Although the current study cannot offer direct evidence of a causative relationship between changes in either *FOXO1* or *PPAR α* and *PDK4* mRNA expression in human skeletal muscle, the strong relationship between *FOXO1* and *PDK4* mRNA expression at rest and during exercise ($r = 0.61, P < 0.001$) (Fig. 6A), together with the less robust association between *PPAR α* and *PDK4* mRNA expression ($r = 0.39, P < 0.05$) (Fig. 6B), supports the notion that FOXO1 played a more significant role than PPAR α in the control of PDK4 expression and muscle CHO oxidation under the present experimental conditions. However, because the amount of FOXO1 protein and its phosphorylation status (covalent modification) are also likely to be important to any association between FOXO1 and PDK4, and also that the message levels of either FOXOs or PPARs will be subject to regulation by more than one mechanism, the present findings warrant further scrutiny by performing measurements at the protein level.

In conclusion, these data point to FOXO1 as an important metabolic regulator of muscle CHO and fat oxidation after HFD in humans by regulating muscle PDK4 expression and consequently PDC activity. The finding that DCA infusion prior to exercise overrode this HFD-mediated inhibition of PDC activation and CHO oxidation during exercise points to PDK4 inhibition as a viable pharmacological strategy for treating muscle insulin resistance in obesity and type 2 diabetes.

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D.C.-T. generated the experimental hypothesis and study design, researched data, and wrote and edited the manuscript. D.C. and F.S. researched data and reviewed and edited the manuscript. D.L. researched data. P.L.G. generated the experimental hypothesis and study design and wrote and edited the manuscript. D.C.-T. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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