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Original article

Maximal-intensity exercise does not fully restore muscle pyruvate dehydrogenase complex activation after 3 days of high-fat dietary intake

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SUMMARY

Background & aims: Exercise activates muscle pyruvate dehydrogenase complex (PDC), but moderate intensity exercise fails to fully activate muscle PDC after high-fat diet [1]. We investigated whether maximal intensity exercise overcomes this inhibition.
Methods: Quadriceps femoris muscle biopsy samples were obtained from healthy males at rest, and after 46 and 92 electrically-evoked maximal intermittent isometric contractions, which were preceded by 3 days of either low- (18%) or high- (69%) isocaloric dietary fat intake (LFD and HFD, respectively).
Results: The ratio of PDCa (active form) to total PDCt (fully activated) at rest was 50% less after HFD (0.32 ± 0.01 vs 0.15 ± 0.01; $P < 0.05$). This ratio increased to 0.77 ± 0.06 after 46 contractions ($P < 0.001$) and to 0.98 ± 0.07 after 92 contractions ($P < 0.001$) in LFD. The corresponding values after HFD were less (0.54 ± 0.06; $P < 0.01$ and 0.70 ± 0.07; $P < 0.01$, respectively). Resting muscle acetyl-CoA and acetylcarnitine content was greater after HFD than LFD (both $P < 0.05$), but their rate of accumulation in the former was reduced during contraction. Muscle lactate content after 92 contractions was 30% greater after HFD ($P < 0.05$). Muscle force generation during contraction was no different between interventions, but HFD lengthened muscle relaxation time ($P < 0.05$). Daily urinary total carnitine excretion after HFD was 2.5-fold greater than after LFD ($P < 0.01$).
Conclusions: A bout of maximal intense exercise did not overcome dietary fat-mediated inhibition of muscle pyruvate dehydrogenase complex activation, and was associated with greater muscle lactate accumulation, as a result of lower PDC flux, and increased muscle relaxation time.

1. Introduction

The mitochondrial membrane-bound enzyme pyruvate dehydrogenase complex (PDC) catalyses the irreversible decarboxylation of pyruvate to acetyl-CoA, and is thought therefore to be the rate limiting step in carbohydrate (CHO) oxidation. Voluntary dynamic and static (isometric) exercise and involuntary (i.e. electrically evoked) muscle contraction have been shown to transform to varying degrees the inactive (phosphorylated) form of PDC to its active (dephosphorylated) form (PDCa) in rodent skeletal muscle and heart [2–4], and in canine [5] and human skeletal muscle [6–9]. The fraction of PDCa to total dephosphorylated PDC (PDCt) is regulated by the activities of PDC phosphatase (two isoforms: PDP1-2) and PDC kinase (four isoforms: PDK1-4). The resulting inter-conversion cycle determines the amount of PDC existing in non-phosphorylated (active) form, i.e. PDCa [8]. It has been suggested that the Ca^{2+} mediated activation of the PDC phosphatase is the main regulator of PDC activity in contracting human muscle [10]. We showed previously that the magnitude of muscle PDC activation correlates to the intensity of exercise performed by human volunteers [7], and that almost complete transformation/activation of muscle PDC to PDCa is achieved within 74 s of intermittent electrically evoked maximal intensity isometric contraction

Abbreviations: PDC, pyruvate dehydrogenase complex; PDCa, active (dephosphorylated) form; PDCt, total (fully dephosphorylated) form; PDK1-4, pyruvate kinase isoforms; LFD, low fat content diet; HFD, high fat content diet.

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[9] or within 10 min of moderate exercise (75%VO_{2max}) [8] under conditions of habitual dietary intake.

High-fat dietary intake (HFD) has been shown to reduce muscle PDC activation and carbohydrate (CHO) oxidation at rest and during moderate intensity exercise [1,11,12], and thereby has been suggested to play a causative role in the induction of dietary mediated skeletal muscle insulin resistance and, presumably under chronic conditions, the development of metabolic syndrome, i.e. central obesity, hypertriglyceridaemia, low HDL-cholesterol and hypertension [13]. We have implicated FOXO1 transcription factor activation in the upregulation of human skeletal muscle PDK4 transcription following an HFD, and consequently the inhibition of PDC and CHO oxidation during submaximal exercise [12], which were overcome by administration of the PDK inhibitor, dichloroacetate (DCA, [12]. Indeed, although DCA administration following an HFD did not increase muscle glycogen use during exercise compared to the HFD alone, it did increase muscle CHO oxidation and reduce muscle lactate and acetylcarnitine accumulation during exercise, demonstrating greater flux through the PDC reaction must have occurred.

The potential of maximum intensity muscle contraction, and therefore maximum calcium release, to overcome HFD-mediated inhibition of PDC has received little attention to date, but is important given the research focus on the utility of high-intensity resistance training at improving skeletal muscle insulin resistance in obese and type 2 diabetic individuals [14,15]. The present study was therefore undertaken to investigate whether maximum intensity involuntary muscle contraction could abolish the inhibitory effect of a 3-day HFD on muscle PDC activation (PDCa) and its catalytic activity.

2. Material and methods

Six Caucasian healthy male volunteers participated in the study (age, height and weight (mean ± SEM) 32 ± 3 yrs, 184 ± 3 cm, and 78 ± 3 kg, respectively). The subjects were physically active, but did not participate in a regular program of physical training. The purpose and nature of the study were explained to the subjects before voluntary consent was obtained. The study which approved by the Ethics Committee of the Karolinska Institute was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

2.1. Experimental protocol

Subjects visited the laboratory on 2 occasions separated by three weeks. Each subject was supplied with a table of foodstuffs and was instructed (what and how much) to consume foods with either a high or low fat content for 3 days. The high fat diet consisted mainly of standard dairy products (mainly cream, full fat milk and hard cheese). Milk fats are comprised of mainly triacylglycerols (97–98% of the total fat), and also diacylglycerides (0.25–0.48%); monoacylglycerides (0.02–0.04%); phospholipids (0.6–1.0%); cholesterol (0.2–0.4%); glycolipids (0.006%); and free fatty acids in milk (0.1–0.4%). Milk fat contains approximately 65% saturated, 30% monounsaturated, and 5% polyunsaturated fatty acids [16]. Dietary records kept by subjects were used the assessed dietary composition and energy intake. Thus, the subjects consumed either a low fat (12% protein, 70% CHO, 18% fat, energy mean 2622 cal day^{-1}) or an isocaloric high fat (21% protein, 10% CHO, 69% fat, energy mean 2647 cal day^{-1}) diet for 3 days before each experimental trial.

All subjects undertook a 24 h urine collection at the end of each diet period using a 2 l container containing 5 ml of a 0.67 mol l^{-1} thymol solution in isopropanol. The 24 h volume of urine was recorded and, following mixing, an aliquot (10 ml) was removed and snap frozen in liquid nitrogen and stored at −20 °C. Experimental interventions were separated by a 3 week washout interval, and the order of dietary manipulation was randomised.

The morning following each 3 days dietary intervention, and having abstained from alcohol intake and strenuous exercise throughout, subjects reported back to the laboratory following an overnight fast. A ~5 ml sample of venous blood was obtained from an antecubital vein and was mixed with lithium heparin. Following centrifugation (15 min at 2500 rpm), plasma was snap frozen in liquid nitrogen and stored at −80 °C. A resting biopsy sample was then obtained from the vastus lateralis of one leg [17], after which the quadriceps muscles of the contralateral limb were stimulated to contract and further biopsy samples were obtained following 46 and 92 contractions. The rest periods between trains of stimulation were elongated to ~3–5 s whilst biopsy sampling occurred. On the second visit, all procedures were repeated, but on this occasion they were preceded by the alternative dietary regimen. All muscle biopsy samples were immediately snap frozen and stored in liquid nitrogen until analysed.

Electrical stimulation was performed with the subjects lying in a bed semi-supine position with both knees flexed at 90° over the end of the bed. Movement of the pelvis was restricted by means of a cushioned band and the knees were fixed to avoid vertical and lateral movement. One leg, chosen at random, was attached by means of an ankle strap to a strain-gauge that was secured to the frame of the bed. Immediately before the start of the study, each subject was asked to perform three maximal voluntary contractions (MVC). The highest of the recordings were used as the maximal isometric force of the knee extensors. Following this, electrical stimulation of the knee extensors was performed as described previously [18]. Briefly, using a stimulation frequency of 20 Hz and square-wave impulses of 0.5 ms duration, the antero-lateral portion of the thigh muscle was stimulated to contract for 1.6 s on 92 occasions, each separated by 1.6 s of rest. Approximately 30% of the musculature that extends the knee is nearly maximally activated when using this procedure, and results in marked phosphocreatine hydrolysis in both type I and type II muscle fibres during contraction demonstrating that both fibre types are recruited [19]. Isometric tension developed was recorded during each contraction as was the muscle relation time following each contraction. Relaxation time (RT) was defined as the time for isometric contraction force to decline from 95 to 50% of the recorded peak tension.

2.2. Analytical methods

2.2.1. Blood plasma and urine analysis

Blood plasma and urine samples were defrosted and aliquots extracted with chloroform/methanol (3/2, v/v). After evaporation, the residue was dissolved in 0.1 mol l^{-1} KOH, incubated at 50 °C for 2 h and subsequent to neutralisation with 0.5 mol l^{-1} HCl used for determination of total carnitine using an enzymatic assay containing radioisotopic substrate, as described previously [20]. Free carnitine was determined by dissolving the residue in water. Acyl carnitine concentration was obtained by subtracting free carnitine from total carnitine concentration. All measurements were performed in duplicate.

2.2.2. Muscle analysis

Upon removal from the muscle, each biopsy sample was immediately frozen and divided into two parts while under liquid nitrogen. One part was freeze-dried, dissected free from visible connective tissue and blood and powdered. Seven to 10 mg of muscle powder was then extracted with 0.5 mol l^{-1} perchloric acid (PCA) containing 1 mmol l^{-1} EDTA and, after centrifugation, the

supernatant was neutralized with 2.2 mol l^{-1} $KHCO_3$. Free carnitine, acetylcarnitine, CoASH and acetyl-CoA were measured in the neutralized extract by enzymatic assays using radioisotopic substrates, as previously described [21]. Briefly, for the determination of CoASH, acetylation was achieved with acetylphosphate in a reaction catalysed by phosphotransacetylase to form acetyl-CoA. In the assay for acetylcarnitine, the acetyl group was transferred to CoASH in a reaction catalysed by carnitine acetyltransferase to form acetyl-CoA. The acetyl-CoA was determined as [^{14}C] citrate after condensation with [^{14}C] oxaloacetate by citrate synthase. Lactate was determined, as described earlier [22]. For the determination of muscle glycogen content, 1.0–2.5 mg of muscle powder were digested in 0.5 mol l^{-1} NaOH and neutralized with HCl-citrate buffer, pH 4.9. The glycogen present in the supernatant was hydrolysed with α-amyloglucosidase and analysed for glucosyl units by an enzymatic method [22].

The remainder of the frozen muscle was used to determine PDC activity, as previously described [23]. Briefly, PDCa was measured with the addition of NaF and dichloroacetate (DCA) to the extraction buffer. PDCt was measured after pre-incubation of muscle homogenates with Ca^{2+}, Mg^{2+}, DCA, glucose and hexokinase to achieve *in vitro* total activation (fully dephosphorylation) of PDC [23].

2.2.3. Statistics

The data were analysed using two-way (diet and time) analysis of variance (ANOVA) for repeated measurements. When the ANOVA resulted in a significant F ratio ($P < 0.05$), the location of significance was determined using Fisher's test. Values are presented as means ± SEM.

3. Results

3.1. Muscle contractile function

Isometric force development and its rate of decline during contraction were no different between interventions (Table 1). After 80 contractions, muscle tension development represented ~60% of the initial peak isometric tension. From 40 contractions onwards, the RT of each twitch contraction increased in the HFD intervention ($P < 0.05$), but not in the LFD intervention (Table 1).

3.2. Blood plasma and urine carnitine

Blood plasma total, free and acylcarnitine concentrations after HFD intervention were significantly greater than after LFD ($P < 0.05$; Table 2). Furthermore, urine total, free and acylcarnitine concentrations after HFD were significantly greater than those after the LFD (all $P < 0.05$; Table 2). No difference in the 24 h urine volume output was observed between diets. In the present study, circulating glucose and NEFA concentrations were not determined at the end each 3-day period of dietary intervention. However, we have previously demonstrated that a 3 day HFD (10% CHO, 65% fat, 25% protein) similar to that used in the present study (10% CHO, 69% fat, 21% protein) resulted in plasma free fatty acid concentration being 2.3-fold greater ($P < 0.05$) and blood glucose concentration being 10% less ($P < 0.05$) compared to a 3 day LFD (66% CHO, 25% fat, 9% protein) similar to that used in the present study [24].

3.3. Muscle PDC and metabolites.

The ratio of active (PDCa) to total dephosphorylated PDC (PDCt) activity at rest after 3 days of HFD intervention was lower than after the LFD (0.15 ± 0.01 and 0.32 ± 0.01, respectively; $P < 0.05$, Fig. 1). Following 46 muscle contractions in the LFD intervention, the ratio PDCa/PDCt increased to 0.77 ± 0.06 and further to 0.98 ± 0.07 after 92 contractions. The corresponding values in the HFD intervention were 0.54 ± 0.06 after 46 contractions and 0.70 ± 0.07 after 92 contractions, which were less than in the LFD intervention ($P < 0.01$ and $P < 0.01$, respectively).

The impact of dietary manipulation on muscle glycogen, lactate, free carnitine, CoASH, and their acetylated forms (i.e. acetylcarnitine and acetyl-CoA) at rest and during muscle contraction is presented in Table 3. Resting muscle glycogen concentration in the LFD intervention was 13% greater than in HFD intervention, but it did not reach statistical significance. Following 92 contractions, the magnitude of glycogen degradation in the LFD intervention was no different from the HFD intervention (Δ90 and Δ100 mmol kg^{-1} dry muscle (dm), respectively). The rate of muscle lactate accumulation in the HFD intervention after 92 isometric contractions was 30% greater than in the LFD intervention (Δ86 and Δ65 mmol kg^{-1} dm, respectively; $P < 0.01$). No differences between diets were found with respect to resting muscle free carnitine and CoASH. However, resting muscle acetyl-CoA and acetylcarnitine concentration after the HFD intervention was greater than after the LFD intervention (both $P < 0.05$). During contraction, acetyl-CoA and acetylcarnitine concentration increased in both treatments, but significantly less rapidly following the HFD (Table 3). However, the sum of muscle free carnitine and acetylcarnitine concentrations, and that of CoASH and acetyl-CoA concentration remained constant throughout contraction in both interventions.

4. Discussion

We previously demonstrated that muscle PDC activation increased with exercise intensity [7], resulting in nearly complete transformation of PDC to PDCa within 74 s of electrically evoked maximal intensity isometric contraction [9] or within 10 min of moderate exercise (75%VO_{2max}) [8]. However, when moderate intensity exercise was preceded by several days of an HFD muscle PDCa was reduced at rest and further activation during exercise was reduced [1,12,25]. Since mitochondrial Ca^{2+} uptake, the primary regulator of muscle PDC activation during exercise [10], is dictated by exercise intensity [26], we aimed to determine whether the HFD-mediated inhibition of PDC activation previously seen during moderate intensity exercise could be overcome by maximal

Table 1
Muscle force and relaxation time after 20, 40, 60 and 80 electrically evoked maximal intermittent isometric contractions when preceded by 3 days of either low- or high-fat feeding.

	Low fat diet				High fat diet			
	20 contr	40 contr	60 contr	80 contr	20 contr	40 contr	60 contr	80 contr
Force[a]	71.8 ± 6.1	49.6 ± 4.6	42.4 ± 3.2	39.0 ± 2.9	76.2 ± 4.5	50.6 ± 3.7	42.4 ± 2.7	42.2 ± 3.3
Relaxation time[b]	71.0 ± 8.7	79.2 ± 7.7	71 ± 3.5	71.6 ± 3.3	73.4 ± 5.5	96.0 ± 10.8	96.2 ± 12.8*	97.8 ± 14.6*

*$P < 0.05$ significantly different from the corresponding diet point (2 way ANOVA).
[a] Newton.
[b] Msec.

Table 2
Plasma and urine total carnitine, free- and acylcarnitine concentrations after 3 days of low- or high-fat dietary intake.

	Low fat diet			High fat diet		
	Total carnitine	Free carnitine	Acylcarnitine	Total carnitine	Free carnitine	Acylcarnitine
Plasma	54 ± 5	50 ± 6	5 ± 1	64 ± 4†	57 ± 5†	9 ± 2†
Urine[a]	327 ± 36	123 ± 16	192 ± 24	809 ± 153††	223 ± 48††	587 ± 142††

Values are mean ± SEM and are expressed as μmol l^{-1}. ††Significantly different between diets ($P < 0.01$).
[a] Urine was collected on the 3rd day of diet over a 24 h period.

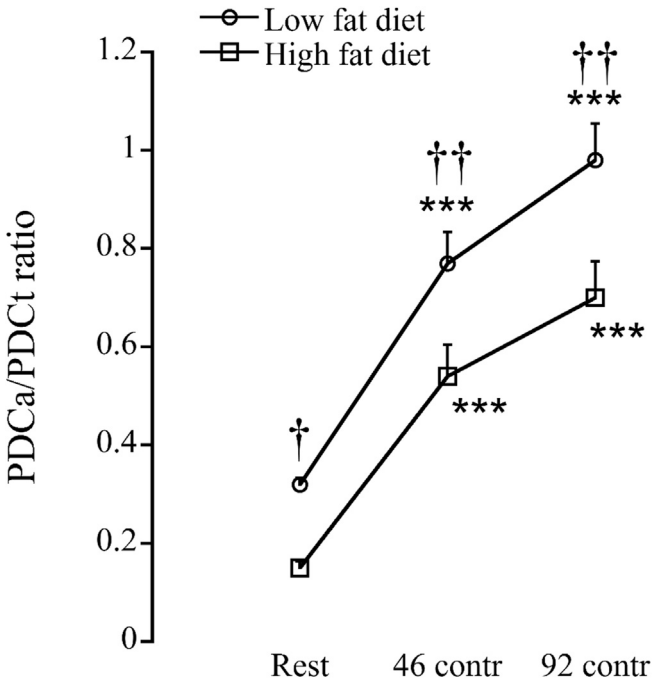

Fig. 1. Human quadriceps PDC activity at rest and following 46 and 92 electrically evoked maximal isometric contractions (20 Hz, 1.6 s stimulation followed by 1.6 s of rest) following 3 days of low and high fat dietary intake. ***Significantly different from rest ($P < 0.001$; one-way ANOVA). †, ††Significantly different between diets ($P < 0.05$, $P < 0.01$; two-way ANOVA).

intensity exercise in human volunteers. This has important implications for the application of resistance exercise training over endurance training to counter muscle insulin resistance in obesity and type 2 diabetes. The results clearly demonstrate that electrically evoked maximal intensity isometric contraction was unable to rescue the impairment of PDC activation seen after 3 days of HFD intervention vs the LFD intervention. Consequently, PDC flux during contraction was impaired after HFD intervention, reflected by muscle lactate and acetylcarnitine accumulation being greater and reduced, respectively, during exercise.

Furthermore, the slowing of muscle relaxation during contraction was increased after HFD intervention, possibly as a consequence of an HFD mediated increase in circulating concentrations of organic acids [27] and/or as demonstrated herein by the inability of muscle to maintain PDC flux during contraction, thus reducing mitochondrial ATP generation, and increasing muscle lactic acid accumulation. Furthermore, the urinary excretion of free and acylcarnitines collected over 24 h following 3 days of HFD was 2.5-fold higher after HFD than after LFD suggestive of a higher carnitine turnover/clearance in the HFD group. It is worth noting that prescribed exercise/physical activity can attenuate the negative effect of 7–14 days of overfeeding on whole-body glycaemic control [28,29]. Whether this would be the case following HFD intake is unknown, but the present findings suggest that the presence of high dietary fat intake is metabolically more deleterious than overconsuming a habitual diet, at least in the context of an acute intense muscle contraction.

The *in vitro* measurement of PDCa activity reflects the maximal possible flux through the PDC reaction for any given level of activation (dephosphorylated form), although this may not be the case *in vivo* since the availability of co-factors is likely to be lower than in the *in vitro* situation. However, when the rates of muscle acetylcarnitine and lactate accumulation during contraction are known, it is possible to estimate the *in vivo* flux through the PDCa reaction. Indeed, by dictating the rate of pyruvate oxidation to acetyl-CoA during muscle contraction, the amount of PDCa not only controls the rate of mitochondrial CHO use, but also appears to play an important role in determining the magnitude of muscle lactate accumulation during exercise. Directly in keeping with this, muscle lactate accumulation during contraction in the present study was greater when PDCa and flux were reduced after HFD. This greater muscle lactate accumulation following a HFD is initially counter-intuitive as previous studies have invariably documented lower muscle and blood lactate concentrations during exercise after 3–5 days of an HFD [1,30], even at workloads as high as 100% VO_{2max} [27]. However, this can be explained by pre-exercise muscle glycogen content being considerably less in previous studies (generally less than 200 mmol kg^{-1} dm vs 362–416 mmol kg^{-1} dm

Table 3
Muscle concentrations of glycogen, lactate, carnitine and CoASH and their acetylated forms at rest and after 46 and 92 electrically evoked maximal intermittent isometric contractions (contr) at 20 Hz (1.6 s stimulation followed by 1.6 s of rest) following 3 days of either low- or high-fat dietary intake.

	Low fat diet			High fat diet		
	Rest	46 contr	92 contr	Rest	46 contr	92 contr
Glycogen[a]	416 ± 25	367 ± 26	326 ± 29*	362 ± 28	303 ± 24	262 ± 22*
Lactate[a]	5.7 ± 0.3	55.2 ± 9.0*	70.8 ± 8.2*	4.8 ± 0.6	72.0 ± 15.9*	90.4 ± 8.7*,† 8.7*,†
Free carnitine[a]	19.2 ± 1.5	15.3 ± 1.5	13.4 ± 1.8*	19.1 ± 1.3	16.9 ± 1.3	14.2 ± 1.8*
Acetylcarnitine[a]	3.2 ± 0.9	8.4 ± 1.3*	10.5 ± 1.6*	5.7 ± 0.6†	7.2 ± 0.9	10.2 ± 1.0*
Total carnitine	22.2 ± 1.3	23.3 ± 1.4	23.5 ± 1.7	24.4 ± 0.9	24.6 ± 1.2	24.2 ± 1.4
CoASH[b]	37.2 ± 7.2	35.3 ± 6.3	32.8 ± 5.7	36.3 ± 5.5	35.3 ± 7.4	32.6 ± 6.6
Acetyl-CoA[b]	8.8 ± 0.8	17.2 ± 2.2*	19.0 ± 3.3*	12.7 ± 3.1†	13.7 ± 1.5	18.3 ± 1.5*

Values are mean ± SE, $n = 6$ subjects. *Significantly different from rest ($P < 0.05$,Two-way ANOVA). †Significantly different between diets ($P < 0.05$, Two-way ANOVA).
[a] mmol kg^{-1} dry mass.
[b] μmol kg^{-1} dry mass.

in the present study) and/or the intensity of exercise employed being considerably lower than that of the present study, which collectively would have reduced rates of glycogenolysis and glycolysis during exercise [31]. Indeed, the magnitude of glycogen degradation during exercise was similar after the two dietary interventions, but muscle lactate accumulation after HFD was 30% greater than after LFD (Table 3). Directly in line with this, the rate of acetyl group accumulation during contraction in HFD intervention was reduced compared with LFD, particularly during the first 46 contractions (Table 3). In short, it is clear that an HFD mediated reduction in PDC activation and flux can increase muscle lactate accumulation during maximal-intensity exercise, which collectively slows the rate of muscle relaxation.

Muscle acetylcarnitine accumulation occurs during contraction when the rate of acetyl group formation by the PDCa reaction is greater than the rate of entry of acetyl groups into the tricarboxylic acid (TCA) cycle. By acting as an acceptor of acetyl groups, carnitine helps to maintain a pool of muscle free CoASH vital for PDC and α-oxoglutarate dehydrogenase reactions [8]. The increase in muscle acetylcarnitine concentration at rest as a result of the HFD intervention and during contraction in both dietary interventions was positively associated with an increase in mitochondrial acetyl-CoA concentration ($r = 0.74$, $P < 0.001$; Fig. 2), albeit of a different order of magnitude, i.e. ~480 mmol of acetylcarnitine formed for every 1 mmol of acetyl-CoA accumulated. A similar numeric relationship between acetyl carnitine and acetyl-CoA accumulation during exercise has previously been reported following the habitual dietary intervention [8,9]. This strongly suggests that provision of acetyl groups from either CHO or fat does not affect the overall conversion of carnitine to acetylcarnitine by carnitine acetyltransferase (CAT), and contradicts the recent contention that skeletal muscle carnitine acetylation is somehow compromised in type 2 diabetes [32]. Rather, acetylcarnitine simply reflects the balance between PDC and TCA cycle flux as we have demonstrated on numerous occasions [7,33–35] and the CAT reaction equilibrium state does not seem to be affected as might have been inferred [36].

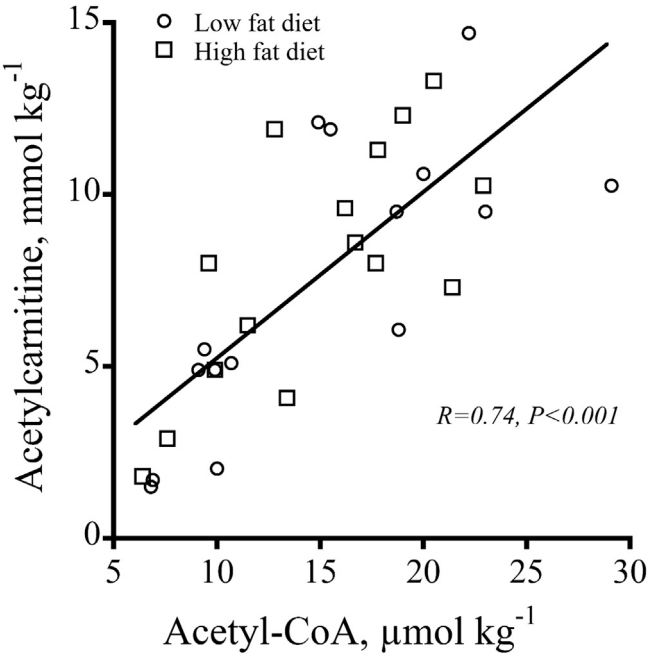

Fig. 2. The Pearson relationship between muscle acetyl-CoA and acetylcarnitine concentrations at rest and following 46 and 92 electrically evoked maximal isometric contractions (20 Hz, 1.6 s stimulation followed by 1.6 s of rest) following 3 days of low and high fat dietary intake.

4.1. Plasma and muscle carnitine metabolism.

Carnitine is present in both plasma and urine in free and esterified forms. Free carnitine concentration represents >80% of the plasma total carnitine content (Table 2). The remaining comprises the esterified fraction, of which about ~70% exists in the acetylated form [37]. However, a redistribution of plasma free carnitine to acyl esterified forms have been reported to occur in disease [38,39] and fasting [40]. In addition to these factors, the present study indicates that 3 days of HFD causes (1) increase in plasma carnitine content probably due to a liver-mediated increase in carnitine biosynthesis aimed at handling increased fat availability and (2) increased urinary excretion of both free and acylcarnitines probably due to increased renal clearance [41].

Presently, we also documented a lengthening of the relaxation time (RT) during contraction in HFD, which is in keeping with a recent observation made in a rodent model [42]. During the relaxation time, Ca^{2+} is pumped back into the sarcoplasmic reticulum while the muscle is stretching back to its original length, thereby preparing the muscle fibre for the next twitch contraction. A possible explanation for the observed increase in RT may be the related to the high-fat dietary intake induced metabolic acidosis [27,43] and/or the additional muscle lactic acid accumulation during contraction in this group.

In conclusion, maximal intense exercise does not overcome a 3-day dietary fat intake-mediated inhibition of muscle pyruvate dehydrogenase complex activation and flux in healthy volunteers. HFD increases muscle RT. HFD is also associated with a greater loss of urinary acylcarnitine compared with LFD.

Conflict of interest

The authors declare there is no conflict of interest to declare.

Contributors

DTC – study design, literature search, data collection, data analysis, data interpretation, figures, writing of the manuscript, and final approval.

MB – data collection, conducting experiments, data analysis, data interpretation, and final approval.

GC – study design, conducting experiments, data analysis, data interpretation, writing of the manuscript, and final approval.

PLG – study design, literature search, data interpretation, writing of the manuscript, and final approval.

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