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Metabolic Inertia in Contracting Skeletal Muscle: The Expanding Role of the Carnitine Pool

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Summary. The ability of the muscular carnitine pool to accept and temporally donate acetyl groups (from and towards the coenzyme A pool) is an important functional role of carnitine within biological systems that is often overlooked within the scientific literature. The present review will discuss recent research demonstrating the existence of a period of inadequate acetyl-CoA delivery towards the tricarboxylic acid cycle (the so-called 'acetyl group deficit'), which occurs as a consequence of the impaired integration of cytosolic (glycolysis) and mitochondrial energy producing pathways at the onset of muscular contraction; due to a lag in the activation of the pyruvate dehydrogenase complex. During this period of inadequate acetyl-CoA delivery, acetyl groups can be sequestered from the limited muscular acetylcarnitine reserve in an attempt to sustain continued tricarboxylic acid cycle flux. Following on from this, the present review will highlight the metabolic and functional benefits to be gained by overcoming this period of metabolic inertia, through elevating the concentration of acetylcarnitine prior to physical exercise; in the presence and absence of pyruvate dehydrogenase complex activation and through appropriately timed 'warm-up' exercise.

Keywords. Pyruvate dehydrogenase complex; Acetylcarnitine; Metabolic inertia; Acetyl group deficit; Oxidative metabolism.

Introduction

Carnitine, or 3-hydroxy-4-trimethylammonium butyrate, is a naturally occurring compound ubiquitously found within mammalian tissues which marks the 100th anniversary of its original discovery in skeletal muscle extracts this year [1]. *L*-Carnitine, the biologically effective isomer of carnitine, plays a key role within several cellular energy producing pathways [2]. By way of example, carnitine is essential towards the transport of long-chain fatty acids across the inner-mitochondrial membrane towards their oxidative fate inside the mitochondrial matrix [3], is

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important towards the removal of toxic acyl-CoAs from the mitochondria by forming acylcarnitines [4, 5], serves as a temporal acetyl group buffer during the oxidation of carbohydrates during periods of increased pathway flux [6, 7], and, in accordance with the *Hofmeister* series, is an osmoprotectant [8]; thus can impact upon cell volume and osmotic stress responses [9]. There is also expanding evidence indicating that *L*-carnitine has more complex functions within the cell, towards the regulation of gene expression [10, 11] and the blockage of apoptosis at several stages [12–14]. This review article will focus on the physiological role of the carnitine pool as an acceptor and temporal donator of acetyl groups (in the form of acetyl-CoA) at the immediate onset of muscular contraction and will highlight the beneficial effect of acetylating the carnitine pool prior to muscular contraction upon subsequent contractile performance.

Muscular Carnitine Pool and Fluctuations during Physical Exercise

A typical 70 kg omnivore male contains ~21 g of carnitine, maintained through the concerted actions of an active carnitine biosynthetic pathway in the kidney (~30% of daily requirement), efficient renal reuptake of carnitine from the urine, and from the consumption of carnitine dense foods, *i.e.*, red meats (~70% of daily requirement) [15]. Skeletal muscle represents the major storage site of carnitine within the body, accounting for ~90–95% of the total-carnitine reserve, with free-carnitine the major constitute in resting tissue (~80%), with the remainder made up of esterified derivatives (acylcarnitines); products of reactions catalysed by carnitine acyltransferases (Fig. 1). Allied to the dominant distribution of carnitine towards the musculature, there is also a biased distribution of carnitine within the cell, with ~90% of the carnitine pool existing outside of the mitochondria; possibly to aid the carnitine mediated transport of activated long-chain acyl groups across the mitochondrial membranes [16, 17]. The intra and extra-mitochondrial carnitine stores are linked by a reversible carnitine acetyltransferase (Fig. 1), which permits the equilibration of mitochondrial derived acetyl groups with the larger cytosolic carnitine pool which can also lead to the acetylation of the limited cytosolic free-coenzyme A pool [18–20].

The composition of the muscular carnitine pool alters acutely and chronically in response to various external factors, ranging from diabetes [21], dietary composition [22], drug interventions [23], renal dialysis treatment [24], to physical exercise [25]. Nowhere is this alteration more profound than during the transition from rest to exercise where the concentration of acetylcarnitine can increase upto 5-fold and account for ~95% of the total-carnitine pool within the muscle [26]. Indeed, during moderate to intense skeletal muscle contraction acetylcarnitine has been shown to accumulate almost linearly with time [6, 25], with the rate of accumulation being greater with increasing exercise intensity [26] and under conditions of reduced blood flow (ischaemia) [27, 28] and oxygen availability (hypoxia) [29]. The ability of free-carnitine to accept excess acetyl-CoA, when produced by the pyruvate dehydrogenase complex in excess of the demands of the tricarboxylic acid cycle, is critical given that the total store of free-coenzyme A within the muscle is relatively small (~50–60 μmol kg^{-1} dry muscle) [30], with 95% of this store confined to the mitochondria with the remainder existing extra-mitochondrially [31]. Indeed,

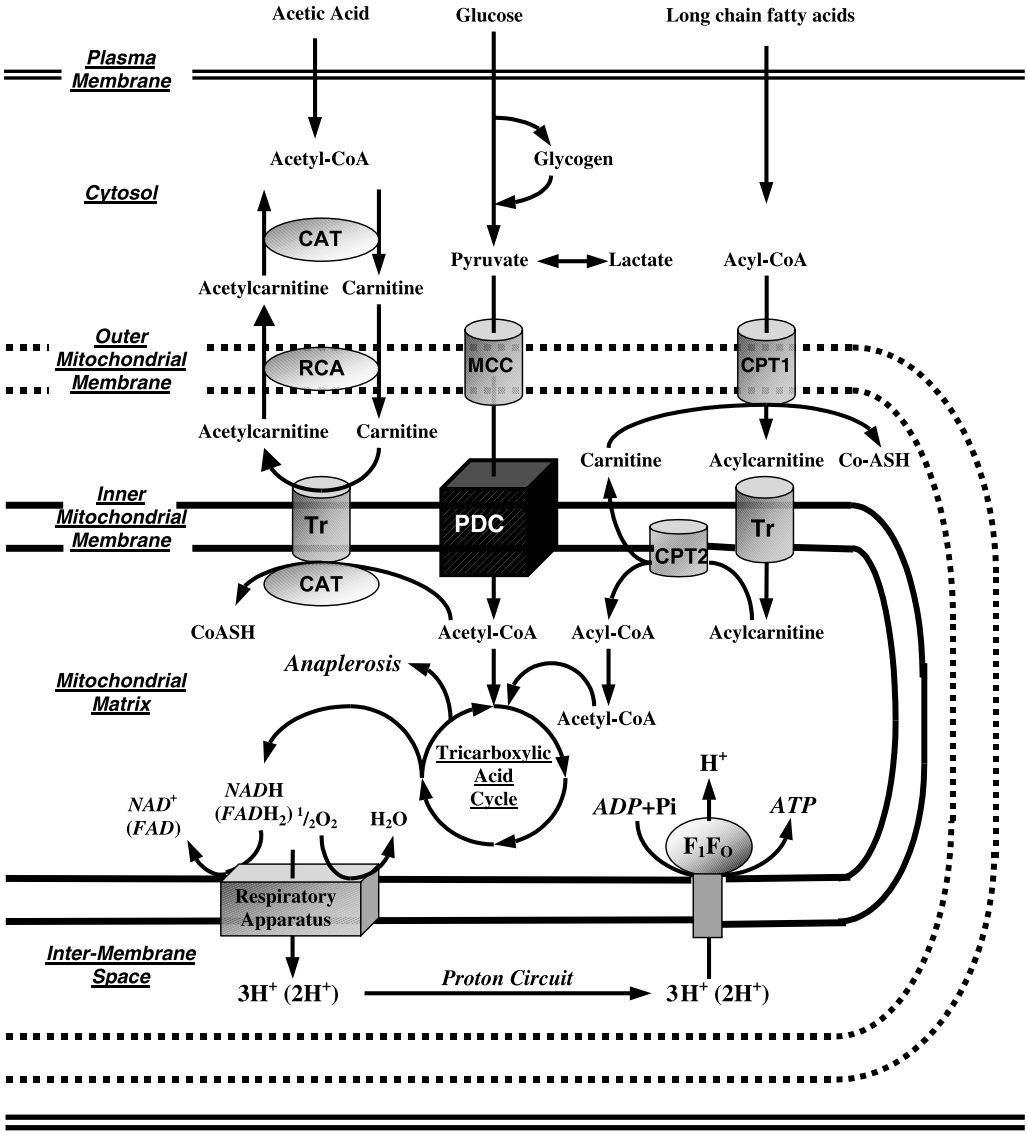


Fig. 1. Carnitine, the pyruvate dehydrogenase complex (PDC), and their location within the major metabolic pathways; the PDC is depicted as a black-box in the centre of the diagram; Key: CAT = carnitine acetyltransferase; RCA = reversible carnitine acetyltransferase; MCC = monocarboxylic carrier; CPT1 = carnitine palmitoyl-transferase 1; CPT2 = carnitine palmitoyl-trasnferase 2; Tr = translocase; $F_1F_0 = F_1F_0$ *ATP*ase

without the creation of this reservoir of 'active acetyl groups' in the form of acetylcarnitine, the increased flux through the pyruvate dehydrogenase complex at the onset of contraction would acetylate the entire free-coenzyme A reserve within just a few seconds, leading to the immediate cessation of flux through the pyruvate dehydrogenase complex. The question arises as to why acetylcarnitine accumulates, and more specifically why does acetyl-CoA (produced *via* the pyruvate dehydrogenase complex reaction) accumulate in excess of the requirements of the tricarboxylic acid cycle? This lack of metabolic efficiency [32] could be

in part attributable to the evolutionary history of the mitochondria [33, 34], due to carnitine acyltransferase competing more successfully for the available acetyl-CoA than citrate synthase at the immediate onset of exercise or possibly exists as a mechanism by which the energy demand of muscular contraction can be repaid following exercise, when pyruvate dehydrogenase complex derived acetyl-CoA delivery to the mitochondria is markedly reduced to preserve carbohydrate reserves. By way of example, following physical exertion the accumulated acetylcarnitine slowly declines back to its basal concentration as acetyl groups are sequestered from this store to produce *ATP* and to replenish the phosphocreatine store. Based on the almost linear increase in acetylcarnitine during exercise it has been inferred that acetyl-CoA availability matches or is in excess of tricarboxylic acid cycle requirements throughout contraction, although recent evidence has cast doubt upon this inference [7]; a point I will return to later in the text.

Recently it has been observed that the concentration of acetylcarnitine can decline during the first minute of contraction under conditions where the muscular carnitine reserve had been near-maximally acetylated to acetylcarnitine through pharmacological intervention [28]. This study raised, for the first time, the possibility that if the availability of acetyl groups (in the form of acetylcarnitine or acetyl-CoA) could be increased prior to muscular exertion, then this extra substrate reserve could be utilised during subsequent contraction [28]. I will return to the subject of acetylcarnitine utilisation during muscular contraction later in this review.

Mitochondrial Energy Production at the Onset of Exercise – 'The Oxygen Deficit'

At the onset of contraction a step-increase in the demand for free-energy, in the form of *ATP*, is put in place by skeletal muscle. As the store of *ATP* within the cell is limited ($\sim$25 mmol kg^{-1} dry muscle), pathways to regenerate *ATP* must be rapidly activated to maintain both contractile function and cellular homeostasis. It is a remarkable feature therefore that during contraction, at all but the highest of intensities, the cellular concentration of *ATP* remains fairly constant. The increase in demand for *ATP* cannot be met solely by oxygen-dependent *ATP* re-synthesis at the onset of contraction, whose activation is delayed and follows an approximately exponential time-course [35].

During this period of latency, the transient shortfall in mitochondrial-*ATP* production, classically termed the 'oxygen deficit' [36, 37], is supplemented by *ATP* re-synthesis from oxygen independent routes (*i.e.*, *ATP* and phosphocreatine breakdown and lactate formation) [38–42]. By way of example, *Bangsbo et al.* [41] observed that phosphocreatine hydrolysis and lactate accumulation collectively contributed $\sim$80% of the total *ATP* generated during the initial 30 s of high-intensity exercise. This figure declined to $\sim$45% from 60–90 s and to $\sim$30% after 120 s of exercise, and appeared to be accompanied by a parallel increase in mitochondrial *ATP* delivery. Although *ATP* production from oxygen-independent routes enables rapid rates of *ATP* turnover to be achieved, it has only a finite capacity and also results in the accumulation of metabolic by-products that are deleterious to muscular contractile function (hydrogen ions, lactate ions, and inorganic

phosphate) [43]. Indeed, without the progressive increase in mitochondrial *ATP* production at the onset of contraction, and thereby the reduction in energy delivery *via* oxygen-independent routes, the onset of muscular fatigue would be markedly accelerated.

Classically, the lag in oxygen-dependent (mitochondrial) *ATP* re-synthesis at the onset of contraction, and the resulting activation of oxygen-independent *ATP* regeneration, has been attributed to an impaired rate of increase, or inertia, in skeletal muscle blood flow and thereby oxygen delivery towards the contracting muscle fibres [44–47]. Indeed, the temporal changes in muscle oxygen utilisation at the onset of exercise closely follow the increase in total limb blood flow during this period; hence the general acceptance of the phrase 'oxygen deficit' within the literature [36, 37]. Over the past decade, however, there has been a growing body of evidence indicating that neither muscle blood flow (bulk oxygen delivery) nor capillary diffusion limit oxygen utilisation at the onset of exercise, such that the true physiological genesis of the lag in oxidative *ATP* resynthesis remains open to debate [35, 48, 49].

Metabolic Inertia – 'The Acetyl Group Deficit'

Work by *Grassi* and colleagues investigating blood flow and oxygen uptake kinetics at the onset of upright cycling exercise in humans noted an increase in venous oxygen content during the first 15 s of contraction at a time when calculated leg oxygen uptake was minimal, indicating that oxygen delivery was in excess of oxygen demand at the very onset of contraction [50]. In a recent series of studies, within an isolated and perfused canine gastrocnemius muscle model, *Grassi* and co-workers demonstrated that elimination of the delay in oxygen delivery at the onset of contraction, by keeping blood flow elevated did not affect the kinetics of muscle oxygen consumption during sub-maximal contraction [51], but did result in faster oxygen uptake by the muscle at a maximal exercise workload and a $\sim$25% reduction in the calculated oxygen-deficit [52]. These findings suggest that mitochondrial *ATP* re-synthesis is not limited by convective blood flow, and thereby oxygen delivery, during transitions from rest to sub-maximal workloads [51]. They concluded that the limitations to the rate of increase in mitochondrial *ATP* re-synthesis at the onset of exercise are likely attributable to heterogeneous microvascular oxygen delivery and/or an intrinsic inertia within mitochondrial energy production of unspecified origin.

Work within the laboratory of Prof. *Paul Greenhaff* (Nottingham, UK) over the past decade has investigated the pyruvate dehydrogenase complex as a potential site of limitation towards mitochondrial *ATP* re-synthesis at the immediate onset of muscular contraction [7, 27, 28, 53–56]. The pyruvate dehydrogenase complex (PDC) is a multi-enzyme complex, located on the mitochondrial inner membrane, which regulates carbohydrate entry into the tricarboxylic acid cycle. The PDC catalyses the physiologically irreversible reaction that commits carbohydrates to their oxidative fate inside the mitochondria through the conversion of the glycolytic product pyruvate into mitochondrial acetyl-CoA (involving NAD^+ and free-coenzyme A as substrates, Fig. 2). Regulation of the rate of formation of acetyl-CoA by the PDC (*i.e.*, flux through the enzyme complex) is achieved by two strategies. The first

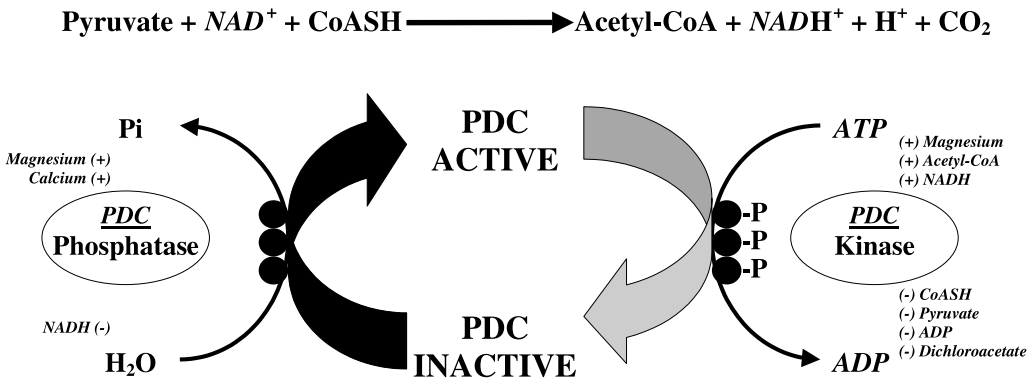


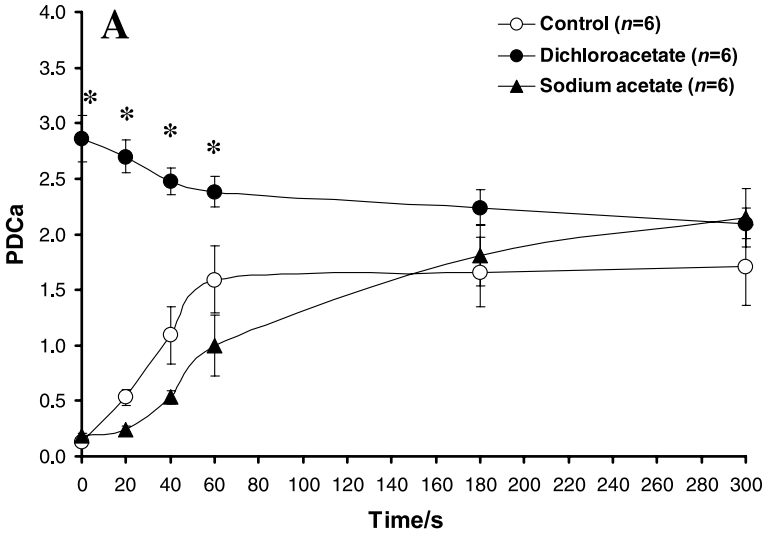
Fig. 2. The pyruvate dehydrogenase complex reaction and covalent regulation of activation status by the intrinsic pyruvate dehydrogenase phosphatase and kinase system; CoASH = free-coenzyme A; Pi = inorganic phosphate; (−) = an inhibitor of the enzyme it is beside; (+) = an activator of the enzyme it is beside; P = phosphorylation of the three specific serine residues upon the haloenzyme core of the pyruvate dehydrogenase complex (PDC); Dichloroacetate = systemic pyruvate dehydrogenase kinase inhibitor sodium dichloroacetate

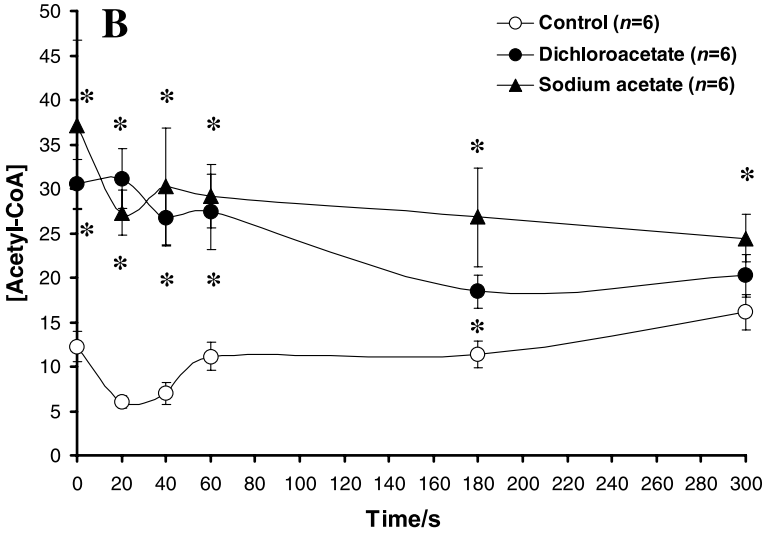
of these is by altering the fraction of PDC that exists in its active form. This is achieved by covalent modification of PDC, either from its inactive (phosphorylated) to active (dephosphorylated) state by loosely associated pyruvate dehydrogenase phosphatases, or *vice versa* by a number of intrinsic and tissue specific pyruvate dehydrogenase kinases (Fig. 2) [57, 58]. These effectors of PDC activation are sensitive to changes in calcium availability, cellular energetics, and substrate/product accumulation [59, 60]. Secondly, the rate of pyruvate oxidation by PDC is regulated by end-product inhibition of flux through the enzyme complex by *NADH* and acetyl-CoA (Fig. 2) [57]. The acetyl groups produced by PDC can be utilised by the tricarboxylic acid cycle or, alternatively, can be stockpiled in the form of acetylcarnitine, presumably when acetyl-CoA re-synthesis exceeds its rate of utilisation by citrate synthase [6]. Therefore, the muscular carnitine pool can be viewed as an indirect stimulator of PDC flux (and thereby carbohydrate metabolism), through the maintenance of a viable pool of free-coenzyme A for sustained tricarboxylic acid cycle flux (Fig. 2).

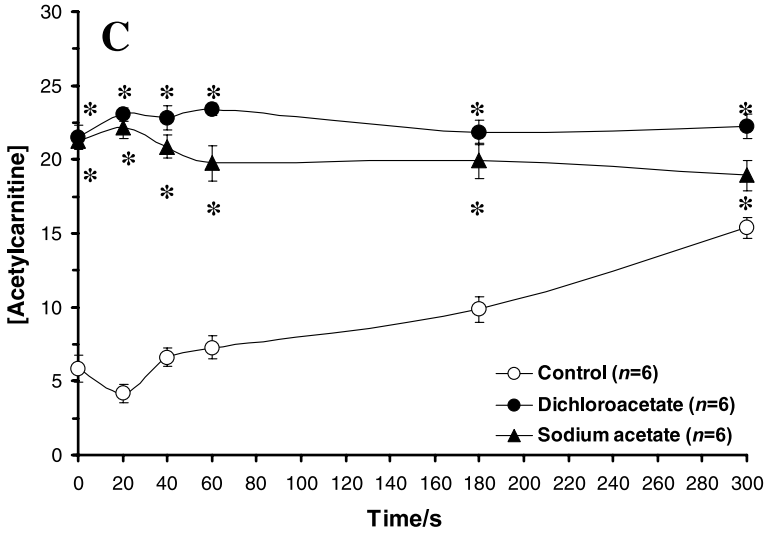
In a landmark study by *Timmons* and co-workers in 1996 [53], pharmacological activation of the PDC, using the systemic PDC kinase (PDK) inhibitor dichloroacetate (Fig. 2) [61, 62], markedly increased acetylcarnitine availability in resting skeletal muscle and appreciably reduced phosphocreatine hydrolysis and lactate accumulation during subsequent intense contraction and under conditions where muscle blood flow and oxygen delivery were fixed at close to resting levels. Following on from this work, the *Greenhaff* group demonstrated in both canine and human skeletal muscle that the rapid hydrolysis of phosphocreatine and accumulation of lactate that occurs at the onset of exercise was at least partially due to an inherent lag in the activation of oxygen-dependent (mitochondrial) *ATP* regeneration [28, 54]. In particular, they were able to show that activation of the PDC at rest, using dichloroacetate, was accompanied by ∼30% reduction in *ATP* re-synthesis from oxygen-independent routes after 1 min of contraction, even though muscle force production was identical to the saline treated (control) group. Following

6 min of contraction, the contribution from oxygen-independent routes to *ATP* re-synthesis had fallen to ~50% of that observed in the control group, while tension development was maintained much better [28]. It also appeared from these studies that some of the acetyl groups that were stockpiled at rest after PDC activation were utilised during contraction, indicating that the mitochondria were able to utilise more acetyl groups at the onset of exercise when provision was increased by dichloroacetate administration [28, 53]. From these investigations, it was concluded that the activation, and thereby flux, through PDC must limit acetyl-CoA availability and consequently mitochondrial *ATP* re-synthesis at the onset of exercise. Moreover, that the activation of PDC and 'priming' of mitochondria with acetyl groups prior to exercise, by administering dichloroacetate, could significantly increase the overall contribution of oxidative pathways to total *ATP* production at the onset of exercise. Another important finding from this series of studies was that the decline in muscle tension development during contraction (*i.e.*, fatigue) was substantially reduced following dichloroacetate administration, most probably due to phosphocreatine hydrolysis and lactate accumulation being reduced at the immediate onset of contraction [28, 53]. Furthermore, this effect was sustainable throughout contraction, at least until the exercise workload was increased to a near maximal intensity [54].

If inertia in the rate of increase in oxygen-dependant *ATP* regeneration at the onset of exercise does indeed reside at the level of PDC, which the work of *Timmons et al*. [28] certainly seems to indicate, then it stands to reason that a period of time must exist at the onset of exercise when acetyl-CoA supply *via* PDC is insufficient to match the demands of the tricarboxylic acid cycle, and the concentration of acetyl-CoA should therefore decline. However, studies to date have shown that acetyl groups appear to accumulate throughout moderate-to-intense muscular contraction [6, 25, 27, 63]. From these findings, it has been inferred that acetyl-CoA production is probably in excess of tricarboxylic acid cycle demands throughout contraction, which contrasts with the generated hypothesis that metabolic inertia resides at the level of PDC. Closer scrutiny of the relevant literature reveals, however, that studies to date have failed to investigate the metabolic events occurring within the initial seconds of contraction, or indeed, at any time-point during contraction prior to significant PDC activation. *Roberts et al.* comprehensively addressed this issue in 2002, in a study designed to conclusively address the issue of acetyl group availability at the immediate onset of contraction [7]. Using a canine hind-limb perfusion model, five muscle biopsy samples were obtained from the gracilis muscle during the first minute (rest, 10, 20, 40, and 60 s) of ischaemic muscle contraction, which was envisaged to give sufficient resolution to elucidate the temporal relationship between PDC activation, acetyl group accumulation, and phosphocreatine hydrolysis and lactate accumulation at the onset of contraction [7]. The results demonstrated that a lag in acetyl group provision (in the form of acetyl-CoA and acetylcarnitine) occurred during the initial 20 s of contraction, which resulted from, and was mirrored by, a lag in PDC activation (Control, Fig. 3). This unequivocally demonstrated the existence of a period of metabolic inertia (the so called 'acetyl group deficit') in skeletal muscle at the onset of contraction [7] and has been supported by further studies [56, 64]. Although the lag in acetyl-CoA and acetylcarnitine accumulation is only temporal


A
Control (n=6)
Dichloroacetate (n=6)
Sodium acetate (n=6)
PDCa
Time/s


B
Control (n=6)
Dichloroacetate (n=6)
Sodium acetate (n=6)
[Acetyl-CoA]
Time/s


C
Control (n=6)
Dichloroacetate (n=6)
Sodium acetate (n=6)
[Acetylcarnitine]
Time/s

and the numerical decline in their respective concentrations is only small, it is important to recognise that the units on the *y*-axis of the acetylcarnitine graph (Fig. 3C) are 1000-fold higher (mmol) than that of the acetyl-CoA (μmol) graph (Fig. 3B). Furthermore, it is important to recognise that 1 mmol of acetyl groups (in the form of acetylcarnitine and acetyl-CoA) is stoichiometrically identical to 12 mmol of *ATP* equivalents being produced by the electron transport machinery; in response to the entrance of a single acetyl-CoA moiety into the tricarboxylic acid cycle and the generation of $1 \times GTP$, $3 \times NAD$H, and $1 \times FAD$H$_2$ with each turn of the cycle. So clearly, a small decline in acetylcarnitine concentration can have a profound effect upon the amount of energy production required from the limited oxygen independent routes.

As dichloroacetate activates the PDC and near maximally acetylates the free-coenzyme A and carnitine pools at rest (Dichloroacetate, Fig. 3), it was not possible to determine in any of our previous studies whether the reduction in oxygen-independent *ATP* re-synthesis at the onset of contraction following dichloroacetate treatment (Dichloroacetate, Fig. 4) was attributable to acetyl-CoA delivery *via* the PDC being increased at the immediate onset of contraction (Dichloroacetate,

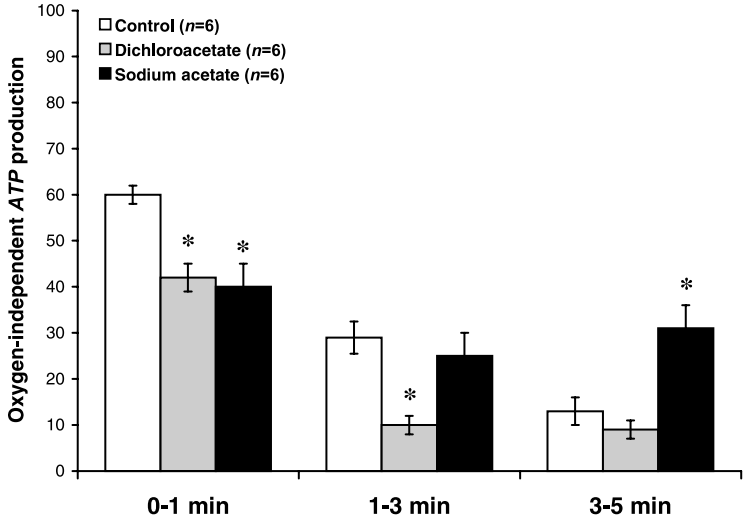


Fig. 4. Rates of *ATP* re-synthesis from oxygen independent routes (phosphocreatine and *ATP* degradation and lactate accumulation) between rest and 1 min, 1 and 3 min, and 3 and 5 min of ischaemic contraction following pre-treatment with control (white), sodium dichloroacetate (grey), and sodium acetate (black); results are expressed as means $\pm$ SEM, with units of mmol of *ATP* equivalents $min^{-1}\,kg^{-1}$ dry muscle; *significantly different from corresponding control value ($p < 0.05$)

Fig. 3. Active form of the pyruvate dehydrogenase complex (PDCa) and acetyl-CoA and acetylcarnitine concentrations at rest and during 5 min of ischaemic contraction following pre-treatment with control (open circles), sodium dichloroacetate (closed circles), and sodium acetate (closed triangles); units are as follows: PDCa, mmol of acetyl-CoA $min^{-1}\,kg^{-1}$ dry muscle (at 37°C); acetyl-CoA, μmol kg^{-1} dry muscle; acetylcarnitine, mmol kg^{-1} dry muscle; results are expressed as means $\pm$ SEM; *significantly different from corresponding control value ($p < 0.05$)

Fig. 3A) and/or was due to the readily available pool of acetyl groups being sequestered by the tricarboxylic acid cycle (Dichloroacetate, Figs. 3B, 3C). With this question in mind, we have recently investigated whether pharmacologically increasing the availability of acetyl-CoA and acetylcarnitine, independent of PDC activation, could overcome the 'acetyl group deficit' at the onset of exercise [56]. We were able to show that administration of sodium acetate increased the availability of acetyl-CoA (Sodium acetate, Fig. 3B) and acetylcarnitine (Sodium acetate, Fig. 3C) in resting skeletal muscle, but did not increase PDC activation (Sodium acetate, Fig. 3A). Furthermore, during the first minute of ischaemic muscle contraction, when the PDC was largely inactive (Sodium acetate, Fig. 3A), treatment with sodium acetate increased the contribution of oxygen-dependent *ATP* regeneration towards the energy demands of the muscle when compared to the saline treated (control) group (Fig. 4) [56]. However, following this first minute, when near maximal activation of PDC had been achieved in both control and acetate groups (Fig. 3A), it appeared that PDC derived acetyl-CoA, rather than stockpiled acetyl groups *per se*, was the principal route of substrate delivery to the tricarboxylic acid cycle. Collectively these investigations have established the activation of the pyruvate dehydrogenase complex as a rate limiting step in the rate of rise in oxygen-dependent *ATP* production in skeletal muscle at the onset of exercise, which in turn will dictate the magnitude of oxygen-independent *ATP* delivery and thereby the rate of fatigue development.

Warm-up Exercise and Carnitine

A period of low-intensity exercise, commonly referred to as 'warm-up' exercise, has been shown to have a range of physiological and biochemical benefits upon performance during a subsequent bout of exercise [65–68]. These benefits have been classically attributed to exercise-induced elevations in muscle temperature and blood (oxygen) delivery prior to the onset of a second bout of more intense exercise. As stated previously, acetylcarnitine accumulates during muscular contraction before gradually returning to its basal concentration upon the cessation of exercise; as the accumulated acetyl groups are sequestered back towards the tricarboxylic acid cycle (*via* the carnitine acyltransferase reaction, Fig. 1), to repay the energy cost of contraction (principally through PCr re-synthesis). Therefore, and in light of the findings discussed above, a period of low-intensity ('warm-up') exercise, of sufficient duration to elevate muscular acetylcarnitine stores, could positively impact upon the rate of onset of oxidative *ATP* re-synthesis and performance within a second bout of more strenuous (prolonged) physical exercise, performed several minutes later. This hypothesis was recently tested by *Campbell-O'Sullivan et al.* [55] where a group of healthy male subjects performed 10 min of exercise at 75% maximal oxygen uptake (VO_2 max) in the presence and absence of 10 min of warm-up exercise (55% VO_2 max), with a 3 min rest between bouts. Warm-up exercise elevated muscle acetylcarnitine by $\sim$4 mmol kg^{-1} dry muscle from basal prior to the start of the second bout of exercise, independent of any alteration in resting blood flow, *ATP*, phosphocreatine, and glycogen concentrations, and was associated with $\sim$40% reduction in the requirement for oxygen-independent *ATP* re-synthesis [55]. Collectively these results suggest that the 'acetyl group deficit' at the immediate onset of contraction can be

overcome by appropriately timed warm-up exercise *via* an envisaged increased onset of mitochondrial *ATP* re-synthesis [55].

Conclusions and Future Perspectives

In conclusion, I hope the present review has provided convincing evidence to support the contention that pyruvate dehydrogenase complex activation, and thereby acetyl-CoA availability, is limiting towards mitochondrial (oxidative) *ATP* re-synthesis at the immediate onset of contraction, resulting in the sequestering of acetyl groups from the limited mitochondrial acetylcarnitine reserve. Increasing the concentration of acetylcarnitine prior to exercise, either directly (with sodium acetate), *via* the activation and increased flux through the pyruvate dehydrogenase complex (dichloroacetate), or through the performance of appropriately timed 'warm-up' exercise can overcome the 'acetyl group deficit' and accelerate the onset of mitochondrial *ATP* re-synthesis. The consequences of this are a marked reduction in *ADP*/*AMP* mediated phosphocreatine and glycogen degradation and lactate formation resulting in a subsequent improvement in the maintenance of contractile function. Based on this evidence, any strategy that can 'prime' the mitochondria or increase their responsiveness towards a step-increase in energy demand upon the initiation of muscular contraction, can be expected to be a novel therapeutic target towards the treatment of clinical conditions where premature fatigue development is prevalent; such as peripheral vascular disease.

In addition, it remains to be established if dietary *L*-carnitine supplementation can elevate the muscular carnitine pool in man and thereby elevate the absolute availability of acetylcarnitine prior to the onset of exercise. This desire is complicated by the fact that the additional acetylcarnitine would need to be readily available towards the tricarboxylic acid cycle (*i.e.*, predominantly located inside the mitochondria) [32] and due to the difficulty in elevating the total-carnitine pool (especially within skeletal muscle) through prolonged carnitine supplementation in healthy (omnivore) humans [69]. Experiments targeted to address this issue will need to wait until technological advancements in tissue specific and organelle specific drug delivery systems have been perfected.

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