Title:

New perspectives on nutritional interventions to augment lipid utilisation during exercise

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Abstract:

The enhancement of fat oxidation during exercise is an aim for both recreational exercising individuals and endurance athletes. Nutritional status may explain a large part of the variation in maximal rates of fat oxidation during exercise. This review reveals novel insights into nutritional manipulation of substrate selection during exercise, explaining putative mechanisms of action and evaluating the current evidence. Lowering the glycaemic index of the pre-exercise meal can enhance lipid utilisation by up to 100% through reduced insulin concentrations, although its application may be restricted to specific training sessions rather than competition. Chronic effects of dietary glycaemic index are less clear and warrant future study before firm recommendations can be made. A flurry of recent advances has overthrown the conventional view of L-carnitine supplementation, with skeletal muscle uptake possible under certain dietary conditions and providing a strategy to influence energy metabolism in an exercise intensity-dependent manner. Use of non-carbohydrate nutrients to stimulate muscle L-carnitine uptake may prove more beneficial for optimising lipid utilisation but requires more research. Studies investigating fish oil supplementation on fat oxidation during exercise are conflicting. In spite of some strong putative mechanisms, the only crossover trial showed no significant effect on lipid use during exercise. Calcium may increase NEFA availability although it is not clear whether these effects occur Calcium and caffeine can increase NEFA availability under certain circumstances which could theoretically enhance fat oxidation, yet strong experimental evidence for this effect during exercise is lacking. Co-administration of nutrients to maximise their effectiveness needs further investigation.

Maximum rates of fat oxidation during exercise vary from 0.18 to 1.01 $g \cdot min^{-1}$, more than a 5-fold difference[1]. Training status, lean body mass, estimated physical activity level, gender and fat mass account for only 34% of the variance. Hence, along with heritability, nutrition has the potential to largely influence fat oxidation. Indeed, acute pre-exercise nutrition may in fact override some of the training-induced adaptations in gene transcription related to substrate metabolism[2]. The limitations to fat oxidation vary depending on the intensity of exercise and have been reviewed extensively elsewhere[3; 4]. Maximal fat oxidation rates occur during exercise between 45 and 65% VO_{2max} and are drastically reduced to minimal rates above 85% VO_{2max}. Reduced plasma NEFA availability may explain part, but not all[5], of the decrease. Fatty acid transport across muscle and mitochondrial membranes are thought to be important rate limiting steps. These restrictive processes are discussed in more detail later in context of the nutritional manipulation involved. The rate of fatty acid flux may modulate the improvements in insulin sensitivity and glucose tolerance seen with an exercise training programme[6; 7]. Thus, enhanced fat oxidation could be seen as a goal for both athletes and the general population for enhancing adaptation to exercise which can potentially improve performance for the former, and health for the latter.

The broad-ranging relevance of enhanced fat oxidation, combined with the complex integration of biological systems and limiting step behind its regulation[8], makes this an interesting and important topic for research and has led to the construction of some previous reviews on dietary strategies[9; 3]. Yet, since the publication of these articles, some exciting advances have occurred in this area. The aim of this review is to provide new insights into the manipulation of lipid oxidation via nutrition in the peri-exercise period and to supply potential mechanisms by which these effects occur (Figure 1). Further intentions include the emphasis of crucial gaps in knowledge such as the optimal timing and potential co-ingestion of nutrients. If these points are addressed, the external validity of nutritional manipulations can be extended to athletes and recreational exercisers.

Glycaemic index

Consumption of carbohydrate containing foods results in an increase in blood glucose concentration, in turn stimulating insulin release by the β-cells of the pancreas. The glycaemic index is a method of classifying carbohydrates according to their blood glucose response[10]. Consumption of low glycaemic index (LGI) carbohydrates results in a smaller glycaemic response than an equal quantity of high glycaemic index (HGI) carbohydrates. This can be due to a variety of factors, including the physical texture[11] and chemical structure of the food[12], the presence of fibre[13] and organic acids[14]. Insulin is known to inhibit lipolysis and thus reduce NEFA

availability[15]. This led to the hypothesis that LGI foods can attenuate the suppression of fat oxidation compared to HGI foods.

A review of studies where lipid oxidation was determined under sedentary conditions by Diaz *et al.*[16] concluded that metabolic differences between high and low GI foods were too small to influence fat metabolism. Although outside of the scope of this review of exercise metabolism, a number of useful points can be taken which may explain why no difference was detected with these studies. Of the acute studies reviewed, some used either fairly low absolute (50 g)[17] or high relative (5 g/kg body mass)[18] amounts of carbohydrate. While others used obese subjects[19] who, although displayed normal glucose tolerance, may still have exhibited insulin resistance and metabolic inflexibility[20]. Therefore, shifts in substrate metabolism from postabsorptive to postprandial states could have been blunted. In others, glucose responses were not evaluated[21] and hence the results cannot be attributed to GI with certainty. Differences in resting metabolism have been observed with LGI mixed meals[22; 23]. Yet, the increased metabolic flux seen during exercise may accentuate any differences, thus illuminating a change in fat utilisation not apparent at rest.

Early exercise studies showed promising results for augmented fat oxidation, but employed isolated, carbohydrate rich foods (such as lentils only vs potato only)[24; 25], which, are unlikely to be consumed by the general population in isolation due to low palatability. Therefore, a series of subsequent studies used high carbohydrate mixed-meals containing carbohydrate, protein and fat (contributing 76%, 12% and 12% to total energy content, respectively) from typical breakfast foods such as cereal, fruit and bread[26; 23] which is pertinent for real-world application. These mixed-meal design studies have demonstrated up to two-fold increases in the amount of whole body fat oxidation during treadmill running[26]. This occurred during exercise intensities ranging from 50 – 70% VO_{2max}[21; 24] in both males[25] and females[21; 24], differing in activity levels[21; 24] and differences even occur during the first 15 min of exercise[24]. Furthermore, similar findings are seen with carbohydrate intakes ranging from 1 to 2.5 g/kg body mass[27; 23] and when the pre-exercise meal is ingested from 30 min[28], up to 12 h before exercise[29]. The intakes of carbohydrate used in the exercise studies (1-2.5 g/kg body mass) are within the guidelines for pre-exercise carbohydrate consumption which strengthens the relevance of these studies for everyday use prior to exercise-training.

Mechanisms explaining the increase in fat oxidation during exercise with LGI meals probably include greater availability of NEFAs, and reduced pre-exercise muscle glycogen concentrations

compared to HGI meals[27]. This leads to greater muscle glycogen utilisation and therefore less of a reliance on fat oxidation via reduced AMP-activated protein kinase (AMPK)[30].

It is appealing to consider that meal timing could be crucial to the effectiveness of GI modulation of substrate metabolism. A low GI meal is known to produce a second-meal phenomena whereby, the glycaemic response to a standard meal is lower following previous consumption of a LGI, compared to a HGI meal. Stevenson *et al.*[31; 32] examined whether this effect can result in a shift in lipid utilisation during exercise. Participants were given a LGI or HGI evening meal, followed by a standard HGI breakfast the morning after. In spite of a lower glycaemic response to breakfast, no differences in NEFA availability or fat oxidation were observed during exercise. Reasons for this discrepancy are unclear, although there are a number of possible explanations. It could be hypothesised from these studies that LGI foods need to be consumed within the 3 h prior to exercise which would optimise the difference in muscle glycogen and therefore influence substrate selection. On the other hand a LGI diet during recovery from a glycogen-reduction run, led to increased fat usage during fasted exercise the following day[29].

A few explanations provide a more convincing argument;

1) Breakfast is the most important time for consuming LGI foods due to being the first meal consumed after the overnight fast. Exposure to NEFAs during fasting can induce insulin resistance[33]. Therefore, changes in the insulin response could be enhanced and may provide an opportunity to exploit the difference between the GI of meals along with the allied metabolic consequences. Evidence supporting this is provided by the attenuated disparity in glycaemic response to LGI and HGI meals when consumed at lunch as opposed to breakfast[34].

2) The greater fibre content of the LGI foods may lead to an increase in SCFAs via increased gut fermentation[35]. Indeed, this is probably a major contributor to the second-meal phenomenon. In an animal model, the SCFA butyrate has been shown to increase lipid utilisation[36], and may therefore be an integral aspect in the modulation of substrate utilisation from LGI vs HGI meals. As the glycaemic response to the standard HGI breakfast (which contained equal amounts of fibre) was different[32]. This implies that it may not be the glycaemic response *per se*, but the fibre content inherent of many LGI foods.

3) Finally, whole-grain foods contain a variety of bioactive compounds and micronutrients[37] which should not be disregarded in terms of their potential to influence metabolism.

Interestingly, the fructose load of the LGI breakfasts used in some of these studies was 25 g for a 70 kg participant[38]. Although LGI, fructose inhibits fat oxidation during and after exercise to a greater extent than glucose[39; 40]. This illustrates the powerful influence of GI combined with exercise in these studies[38; 26], as substrate metabolism was greatly affected, in spite of the large fructose load of the LGI breakfast. In fact, fructose was probably the reason for the greater postprandial blood lactate concentrations[41], which, it should be noted, also inhibits lipolysis[42] via the G protein-coupled receptor GPR81[43; 44]. This influence of fructose may also provide an explanation for why some of the resting studies showed no effect of GI on fat metabolism[19]. High fructose consumption has been linked to de novo lipogenesis, TAG accumulation and insulin resistance[45]. Therefore, the use of fructose to reduce the GI of a meal may be counterproductive to lipid metabolism not only acutely, but also in the long-term.

A few recent studies have found results which, at a glance, conflict with the majority of previous findings. Moore *et al.*[46] remarkably displayed increased fat oxidation and NEFA concentrations during exercise following a HGI meal compared to a LGI meal. Explanations for this are not easily forthcoming, although, the LGI meal contained a greater quantity of milk. Milk proteins are particularly insulinotropic[47], which may have caused the greater suppression of NEFAs and therefore fat oxidation. However, this is merely speculation, as insulin concentrations were not measured. Furthermore, the validity of indirect calorimetry to estimate substrate utilisation is dependent upon exercise being "steady-state". As the exercise in the study by Moore *et al.* was a time trial, power output is likely to fluctuate and hence invalidate the assumptions of indirect calorimetry[48]. This latter explanation may also partially clarify why Little *et al.*[49] found no difference between HGI and LGI pre-exercise meals on substrate metabolism during high-intensity, intermittent running estimated by indirect calorimetry. Moreover, muscle glycogen was approximately 20% lower in the HGI compared to the LGI trial after 75 min of the protocol (as estimated from the figure in Little *et al.* 2010). Albeit not statistically significant, this is a considerable difference. As it was reported that muscle glycogen was similar pre-exercise (values not given), it could be assumed that the LGI meal did in fact reduce muscle glycogen utilisation, and hence increase lipid oxidation that was not detected by indirect calorimetry. Bennard & Doucet also reported no difference in lipid utilisation between HGI and LGI pre-exercise meals[50]. Yet, the blood glucose response to the "LGI" meal prior to exercise tended to be *greater* than that of the "HGI" meal. Thus, by definition, the LGI meal was not lower in glycaemic index than the HGI meal. It appears that the glycaemic index of the pre-exercise meal can significantly influence substrate selection during exercise, with LGI meals producing a lower insulin-induced suppression

of lipolysis and NEFA availability, along with reduced muscle glycogen content compared with HGI pre-exercise meals.

When a LGI breakfast and lunch are administered, no difference in fat oxidation occurs during exercise after lunch, compared to HGI breakfast and lunch[38]. The authors suggested that the intensity of exercise (70% VO_{2max}) may have been too high for a difference in fat oxidation to occur. Although another study has demonstrated a difference in fat oxidation during similar intensity exercise (71% VO_{2max}) after a single LGI vs. HGI meal[27], the participants differed in training status (VO_{2max}: 55.1 vs 64.6 ml/kg/min). Training status is known to enhance fat utilisation at the same relative intensity[3], which may explain this incongruity. Increased fat oxidation in the postprandial period following lunch makes it tempting to speculate that a lower intensity of exercise for this population would have led to differences in substrate selection to become apparent.

In contrast to acute feedings, dietary glycaemic index and fat oxidation during exercise has, to date, received relatively little research interest. To our knowledge, two studies have investigated the effects of dietary GI on whole body fat oxidation during exercise and both found no effect of diet[51; 52]. However, the relatively short time period (3 & 5 d), combined with the high carbohydrate loads (10 and 7 g/kg body mass/d) may have restricted the ability to identify differences between diets. The only study to date, in which LGI and HGI diets were provided, alongside an aerobic exercise programme found a greater increase in VO_{2max} and greater reduction in systolic blood pressure with a 7-d LGI compared to a HGI diet[53]. Although no difference in postprandial resting fat oxidation was reported. When the study was extended to 12 weeks, the differences in VO_{2max} and blood pressure were abolished, yet the LGI diet contributed to the improved insulin sensitivity with exercise training[54]. It would be interesting to explore this further and investigate whether GI can influence adaptations to an exercise programme. A plausible rationale exists; increasing fat oxidation during an exercise programme, via reduced carbohydrate availability and/or increased NEFA availability, could enhance the increase in mitochondrial enzyme activity[55; 56; 57]. If the glycaemic index of the pre-exercise meal can be used to alter substrate availability, then this may be a novel method of maximising the benefits of an exercise programme for the general public.

It is apparent that pre-exercise LGI meals do lead to a greater rate of fat oxidation during sub-maximal, continuous exercise. Although questions remain unanswered: is the effect only observed with the first meal after an overnight fast, or the last meal before exercise? The acute applications of glycaemic index in terms of sports nutrition are probably limited as, in most sports, there are no

limitations on nutritional intake during exercise. Carbohydrate consumption during exercise nullifies the influence of a pre-exercise meal GI on substrate metabolism[58; 59; 60; 61], thus, the application may be limited to endurance events where optimal nutrient intake during competition is prohibited or impractical (eg. the swim leg of an Ironman triathlon, long-distance open-water swimming). Notwithstanding this, exercise training on a LGI diet may still be pertinent for enhancing endurance adaptations and for improving health of the general public and chronic trials are needed to confirm the effect of consuming a LGI diet in combination with exercise.

L-carnitine

The amino acid carnitine exists in two isoforms. While D-carnitine is thought to be biologically inactive[62], L-carnitine plays an important role in enabling the transport of long-chain acyl-CoA across the otherwise impermeable inner mitochondrial membrane. Carnitine palmitoyltransferase 1 (CPT1) catalyses the esterification of free carnitine, with long-chain acyl-CoA[63]. The long-chain acylcarnitine is subsequently transported across the mitochondrial membrane, in concurrent substitution with free carnitine from inside the mitochondrial matrix. Once inside the mitochondrion, carnitine palmitoyltransferase 2 (CPT2) catalyses the transesterification of long-chain acylcarnitine back to free carnitine and long-chain acyl-CoA[64], which is then able to enter the β–oxidation pathway. A thorough review of the role of L-carnitine in fatty-acid transport is provided by Stephens *et al.* [65].

Muscle free carnitine content is reduced during high-intensity exercise and when muscle glycogen content is elevated. This has led to suggestions that free carnitine content may limit the rate of fat oxidation via reduced CPT1-mediated mitochondrial membrane transport. This has been demonstrated in vitro, where elevating the free carnitine pool raises long-chain fatty acid oxidation by mitochondria.

In vivo studies have had less success. A previous review concluded that L-carnitine supplementation is not effective[9]. This is due to the fact that oral L-carnitine supplementation *per se* is unable to increase intramuscular carnitine content, partly due to the high concentration gradient from muscle (~3.5 $mmol \cdot L^{-1}$) to plasma (50 $\mu mol \cdot L^{-1}$). Indeed, even when L-carnitine is administered intravenously, muscle carnitine content is unaffected[66] and thus it is no surprise when differences in lipid utilisation are absent.

Intriguingly, recent studies have demonstrated that hyperinsulinaemia, in the presence of hypercarnitinaemia can augment muscle carnitine content by ~14%, probably via an increase in NA+/K+ pump activity[66; 67]. In addition, the increase in muscle carnitine content resulted in a 30% increase in muscle glycogen content, thus resulting in the exciting proposition that lipid oxidation was indeed enhanced, leading to reduced carbohydrate utilisation[67].

These studies however, used intravenous infusions of L-carnitine and insulin, with plasma carnitine concentrations reaching supraphysiological levels. Thus, the logical progression was to investigate whether oral carnitine supplementation can result in raised muscle carnitine content with dietary carbohydrate used to stimulate insulin concentrations. The same group found that 3 g of L-carnitine supplementation, with 4 boluses of 94 g sugar, resulted in reduced urinary carnitine excretion[68]. The authors hypothesised that, if the regimen were maintained for 100 d, then the result would be a 10% increase in muscle carnitine content.

Another group have performed a 14 d supplemental period with 3 g L-carnitine L-tartrate[69], combined with a fairly high carbohydrate diet (approx 7 g/kg body mass/d). In contrast to the proposition of Stephens *et al.*, lipid utilisation was not enhanced during cycling at 60% VO_{2max}. In fact, carbohydrate oxidation increased by ~20% in male participants with no significant effects of females (albeit showing a similar trend). A further study by the same group showed similar results[70], with fat oxidation tending to be reduced after 2 weeks of L-carnitine L-tartrate supplementation, compared to placebo.

An explanation for these discordant results may be provided by the exercise intensities employed. In the first study to show that muscle carnitine content in humans can be elevated by carbohydrate-carnitine co-supplementation, Wall *et al.* demonstrate that carnitine's influence on exercise metabolism is dependent upon the intensity[71]. 24 weeks of supplementation with 2 g of L-carnitine L-tartrate and 80 g of carbohydrate, consumed twice daily, increased muscle total carnitine content by 21%. A control group consuming the same quantity of carbohydrate, with no carnitine supplementation, showed no effect. A 55% reduction in muscle glycogen utilisation was found with carnitine supplementation during cycling at 50% VO_{2max}. When the intensity was increased to 80% VO_{2max}, no difference in muscle glycogen utilisation was observed, yet muscle lactate content was 44% lower, PCr/ATP ratio was better maintained. Pyruvate dehydrogenase activation status was reduced during cycling at 50% VO_{2max} but increased at 80% VO_{2max} in the supplementation group, versus control. A final, captivating observation was that, although body mass increased in the control group (probably due to the 2512 kJ provided by the carbohydrate supplement; 600 kcal),

this was not evident in the carnitine group. Could the increase in fat oxidation have been apparent in training and consequently account for the attenuation of weight gain? Determination of energy intake would help to clarify this and could be an important aspect for future studies.

The implications of this for endurance performance are stirring. Previous attempts to spare muscle glycogen via fat adaptation strategies have failed to enhance endurance performance, probably due to a reduction in pyruvate dehydrogenase activation, thus impairing glycogenolysis when high rates of carbohydrate flux needed to support intense exercise[72]. Glycogen sparing would only be successful if the spared stores are able to be "tapped into" towards the latter stages of a race. What is the rationale of having more fuel if it is able to be used? This makes the findings of Wall *et al.* all the more captivating, as carbohydrate utilisation is spared at low intensity exercise, but pyruvate dehydrogenase activity was not downregulated at higher intensities, which would in theory enhance performance. In practise, total work completed in 30 min increased by 11% after carnitine supplementation, which confirms the idea that endurance performance can be enhanced. Further studies must now investigate whether it is the glycogen sparing, acetyl group buffering, or any other mechanisms at play which improve performance.

These findings shed new light on L-carnitine supplementation and opens up an exciting avenue for future research. Notwithstanding this, as formerly mentioned, both the carbohydrate *per se*, and the concomitant rise in insulin may counter the influence of the increased free carnitine pool, through the increase in muscle glycogen and reduction in lipolysis. Therefore, although this practise may be applicable to athletes who readily consume a high carbohydrate diet, it may not be advisable for those with insulin resistance or diabetes to consume such a large amount of rapidly digestible carbohydrates. A potentially suitable alternative could be by providing certain amino acids such as glycine or leucine which are potent stimulators of insulin[73; 47] , thereby achieving the insulin-stimulated uptake of l-carnitine with a lower carbohydrate load.

Fish Oil

Fish oils are rich in long-chain *n*-3 PUFAs. Supplementation with fish oil derived *n*-3 fatty acids has been shown to reduce postprandial lipaemia[74]. The increase in skeletal muscle lipoprotein lipase activity[75] is probably the major mechanism behind this. Moreover, rodent models have also demonstrated a protective effect of fish oil on high fat diet induced obesity, despite no differences in energy intake[76]. Taken together, these findings indicate that lipid utilisation is increased. In

addition, some have proposed that *n*-3 induced insulin sensitivity would enhance glycogen storage, thus shifting the oxidation of carbohydrate onto lipids[77].

Major pathways in which *n*-3 fatty acids have been proposed to alter energy metabolism include; providing a substrate for PPARα[78] and increases in skeletal muscle uncoupling protein 3[79]. Transcription of several genes involved in metabolism are also influenced by fish oil ingestion such as carnitine palmitoyltransferase, fatty acid binding proteins, fatty acid transporter and fatty acyl-CoA sythetase, and malonyl-CoA[80]. Effect on genes can happen in minutes which would support the acute supplementation of fish oil. Fish oil improves endothelial function, and reduces inflammation[81]. Thus, another putative pathway for enhancing lipid utilisation could be via increased adipose tissue blood flow which may be a limiting factor for fatty acid availability during exercise[4]. Increases in lipid combustion in resting humans have been reported[82; 83] coinciding with a 40% reduction in the insulin response to an oral glucose load[82]. Exercise studies show an additive relationship between exercise and fish oil, with the combination of both showing the most preferential changes in body composition and the greatest improvements in postprandial lipaemia[84; 85]. Thus exercise may potentiate the effects of fish oil supplementation, thus elucidating changes in metabolism.

A number studies have investigated the influence of fish oil on energy metabolism during exercise. One has shown a clear increase in fat oxidation during exercise after 4 g/d of fish oil supplementation for 3 weeks[86]. In this protocol, participants were studied during running at 60% VO_{2max}, under 6 conditions; 1) in the postabsorptive state, 2) following a high fat meal, 3) following a high fat meal supplemented with fish oil, 4) 5) and 6) as the first 3 trials, but after chronic supplementation. The energy contribution from fat, estimated by indirect calorimetry, increased by ~10%, post-supplementation. Delarue *et al.* also found a tendency for enhanced fat oxidation (by ~7%) and reduced metabolic glucose disappearance during cycling at 60% VO_{2max} [87]. These results prove promising, yet, controlled studies would provide more tangible evidence, as a number of factors could influence fat usage over the 3-week periods used between these tests.

A crossover study found no significant difference in fat oxidation during cycling at 50% VO_{2max}[88]. The participants in this study were examined with and without 7.2 g/d of fish oil supplementation for 2 weeks, with a 6 week washout period. This was followed up with a parallel study by the same group, with a fish and olive oil supplemented diet compared to an isoenergetic control diet[89]. Exogenous fat oxidation was estimated using labelled isotopes and cycling exercise was, again, performed at 50% VO_{2max}. Whole-body fat oxidation was potentiated to a greater extent (42% vs

4%) in the intervention group, but from a lower baseline. Thus post-intervention, both groups showed similar rates of fat utilisation. Exogenous fat oxidation on the other hand, showed a trend to be increased in the intervention group compared to the control group, post supplementation.

Fish oil supplementation has a plausible rationale for influencing substrate metabolism. A handful of studies have shown trends and/or significant increases in lipid utilisation during exercise with fish oil supplementation, yet evidence from randomised, crossover studies are still lacking to provide the most concrete evidence. Until this clarity is achieved, it is unlikely that increasing fish oil intake modestly (to around 4 g/d) is of detriment to athletes (recreational or elite) and may provide some benefits to fat metabolism alongside other health outcomes. Therefore on a cost-benefit decision fish oil could be taken as a dietary supplement.

Calcium & Dairy

The theoretical role of calcium and dairy products in modulating energy metabolism stemmed from an anti-hypertensive study in obese African-Americans. An unexpected result was found, that increasing calcium intake from 400 to 1000 mg/d through yoghurt consumption led to a reduction in body fat of 4.9 kg over a 1 yr period. The link between calcium and fat mass has been confirmed by a variety of both epidemiological and intervention studies (For review see ref.[90]). Calcium intake has also been associated with reduced risk of hypertension and insulin resistance. Of interest is that dairy calcium appears to be 50-100% more effective for body fat loss than calcium alone[91].

Although part of its effects are probably due to reduced absorption of dietary fat[92], a theory has been devised explaining a potential link between calcium intake and substrate utilisation. Zemel[93] developed a hypothesis based on animal and cell models that an increase in calcium intake can mediate intracellular calcium concentrations within adipocytes via reductions in 1,25-dihydroxyvitamin D_3 (calcitriol) and parathyroid hormone (PTH) concentrations[93]. Intracelllular calcium has long been associated with insulin resistance in obesity[94], but is also thought to be a key regulator in adipocyte lipolysis and lipogenesis. Calcitriol may also act via other pathways, including the nuclear Vitamin D receptor to downregulate uncoupling protein 2 thereby potentially influencing thermogenesis and b-oxidation. Although the majority of this work has been conducted on adipocytes, a high calcium diet has also been shown to increase uncoupling protein 3 expression in the skeletal muscle of mice, also acting via calcitriol suppression. PTH can also inhibit CPT1 activity, a rate limiting step discussed in reference to L-carnitine supplementation. Moreover, recent work has demonstrated calcitriol suppression of mitochondrial biogenesis and increased cytokine production[95; 96]. Both of which are known to alter energy metabolism.

The manipulation of postprandial fat oxidation by calcium intake in humans, has been reviewed elsewhere[97]. Thus, only studies involving exercise will be discussed presently.

Both acute and chronic endurance exercise results in shifts in calciotropic hormone concentrations (For review see Maimoun & Sultan[98]). This is most likely due to sweat calcium losses. Therefore, the metabolic effects of calcium intake may alter during exercise compared to rest and warrants research in this area. Indeed, it is possible to attenuate the effect of exercise on calciotropic hormones with calcium ingestion prior to exercise[99], yet substrate utilisation was not measured, as bone turnover was the main outcome in this study.

The earliest human study to identify a link between calcium and fat oxidation showed an association between acute calcium intake and subsequent fat oxidation[100]. This was followed up by an intervention study where subjects consumed low (500 mg/d) or high (1400 mg/d) dairy calcium, energy-balanced diets[101]. These diets were matched for energy, macronutrients, fibre, and saturated:monounsaturated:polyunsaturated fatty acid ratios. Subjects completed 6 days of each diet, twice in a randomised, crossover design. On day 7 of each trial, a room calorimeter was used to study energy expenditure and substrate utilisation under energy balance and energy deficit conditions. The energy deficit was achieved by modest calorie restriction (419 kJ; 100 kcal) with a greater contribution from exercise energy expenditure (~2093 kJ; ~500 kcal). 24 h fat oxidation was 28% greater when subjects consumed the high calcium diet when in energy deficit versus the low calcium diet in energy deficit. However, under energy balance, no differences were seen between diets. Calcitriol concentrations were suppressed to a slightly greater extent in energy deficit by the high calcium diet.

The effects of acute ingestion of calcium were studied in a group of trained female runners[102]. Four hours after consumption of a standardised meal containing 3 g carbohydrate/kg body mass, the participants consumed a test beverage with a high (500 mg) or low (80 mg) calcium content. Mean carbohydrate content was 117 and 118 g for the low and high dairy calcium beverages, respectively. One hour following consumption of the test drink subjects ran on a treadmill for 90 min at 70% VO_{2max}. This was followed by a 5-min break and a subsequent 10 km time trial. Neither RER, nor fat oxidation differed between trials. Yet, fat oxidation was in minus figures at rest. This would suggest that the high carbohydrate content of the prior meals was inhibiting fat oxidation to such an extent that it induced net, whole body de novo lipogenesis[103], thus explaining no effect of calcium. This is supported by the relatively low levels of fat oxidation observed during the exercise bout (~0.2 g/min). Treadmill based exercise at a similar intensity elicits a rate of fat oxidation of ~0.5

g/min in a large group of women[1]. Furthermore, resting studies suggest the availability of NEFAs appears to diverge at around 3-4 h after consumption of high vs low calcium meals[104]. As the test drink was consumed 1 h prior to exercise, this may have been too short a time period for the effects to be apparent. A further limitation may have been the fairly high exercise intensity as mentioned earlier in the section covering GI. It should be acknowledged that resting studies which have demonstrated an increase in fat oxidation have generally compared high calcium, high vitamin D meals with low calcium low vitamin D meals[105; 104; 106]. Whether calcium alone is capable of augmenting fat oxidation, or whether the presence of vitamin D is required needs elucidating.

The authors proposed that another reason for no effect on fat oxidation was due to the exercise-induced increase in PTH, which is probably due to dermal sweat calcium losses. However, recent evidence suggests calcium feeding just 20 min before exercise can attenuate the effect of exercise on PTH by ~30% relative to placebo[99].

There exists a substantial amount of theory supporting the role of calcium intake in both muscle and adipocyte fat metabolism. Acute effects may result from an increase in adipocyte lipolysis, raising plasma NEFA availability and reduced inhibition of carnitine and long-chain fatty acid esterification. Chronic intake may result in increased mitochondrial density, although all these effects are yet to be seen in humans during exercise, which itself produces shifts in calciotropic hormone concentrations. More research is needed before any recommendations can be made, to establish whether calcium can influence fat oxidation during exercise. Studies should determine whether this occurs under different nutritional states with varying carbohydrate availability, habitual calcium intakes and vitamin D status.

Caffeine

Former reviews have concluded that there is little evidence of caffeine influencing fat oxidation during exercise[9; 107]. This is surprising, given the vast metabolic changes that caffeine consumption can bring about. These include decreased insulin sensitivity, glucose tolerance, and muscle glucose uptake, and increases in catecholamines and NEFA availability[108; 109].

Enhanced fat oxidation, with subsequent glycogen sparing was originally thought to be the major mechanism behind the ergogenic potential of pre-exercise caffeine ingestion. Although the results of these early studies have not been recently replicated. Graham *et al*. attempted to overcome the problems of small sample sizes which have been apparent in muscle biopsy studies involving

caffeine ingestion and exercise[110]. Data were pooled from a couple of studies which increased the sample size to 37. The caffeine intake was between 5 and 9 mg/kg body mass; a dose which is almost certainly ergogenic[111]. They found a tendency for reduced glycogen utilisation, but this was not significant (P=0.22). This may be due to the high intensities of exercise used (70-85% VO_{2max}), combined with the time point of the sample being only 10 and 15 min after exercise initiation. The lower the intensity and the more prolonged the duration of exercise, the greater the reliance on NEFAs[8], thus the effect of caffeine of fat oxidation via lipolysis may be blocked at high intensities of exercise. Moreover, it would be expected that any differences would only be augmented as exercise continued, thereby potentiating the chances of evidencing a significant change.

Jacobson *et al.*[112] aimed to tackle this by providing a high fat (1.2g/kgBM) meal combined with an IV hepain infusion (to increase NEFA availability). This was compared to the same meal and infusion in addition to caffeine (6mg/kgBM) ingestion, vs a high carbohydrate (2.6g/kgBM) meal and high carbohydrate meal with caffeine ingestion. All meals were consumed 60 min prior to a bout of 120 min cycling at 63% of peak power output. Although caffeine consumed with the high fat meal increased circulating NEFA concentrations compared to the high fat meal alone. This increase was only significant in the period prior to exercise, after this, there was a tendency for NEFA concentrations to remain elevated but fat oxidation was unaffected.

Findings from a more recent study are at slight discord with that of Jacobson *et al.*[112]. Ingestion of caffeine (800 mg) reduced RER and tended to increase fat oxidation by ~10% (P=0.069) during 2 h of cycling at 50% of maximum power output with carbohydrate ingestion (60g/kg/h)[113]. When carbohydrate intake during exercise was prevented, there was no difference in RER when caffeine or placebo was consumed. Furthermore, muscle glycogen use was similar between all trials. Hence, the support for augmented fat oxidation is not robust. That this study was conducted after 2 days of reduced energy intake (~5103 kJ/d; ~1219 kcal/d) combined with exercise (2 h at 50% of maximum power output) is a strength, as the results can be applicable to those who are looking to achieve a negative energy balance to reduce body mass through dietary restriction and exercise.

Although the many metabolic effects of caffeine such as insulin resistance and reduced glucose tolerance have been demonstrated various times at rest, exercise may influence the outcome of caffeine ingestion. A single bout of exercise has been shown to attenuate the insulin resistance caused by caffeine ingestion[114]. In fact caffeine, co ingested with carbohydrate after exercise, leads to the highest rates of glycogen resynthesis (~60 mmol/kg d/w/h) ever reported (excluding infusion studies)[115]. If more carbohydrate is being stored as glycogen rather than being oxidised,

then presumably more lipid would be used to fuel metabolism during this period, yet the rapid replenishment of muscle glycogen would lead to a shorter period of AMPK-activated fatty acid oxidation[116]. Higher serum NEFA concentrations have been noted with caffeine ingestion following exercise[109], providing support for potentially enhanced fat oxidation. Unfortunately, measures of substrate metabolism have not been noted in the post-exercise recovery period to date.

The effect of caffeine on fat oxidation may only become apparent at lower exercise intensities than those used in studies to date. As previously mentioned, increased NEFA availability may only be an important regulator in substrate metabolism at moderate exercise intensities. Thus, the application of energy metabolism for endurance performance may be limited, although, for certain training sessions performed at a lower intensity or for the recreationally active population, caffeine may be able to enhance fat oxidation. Looking to the future, it appears reasonable to investigate caffeine in combination with the polyphenols that naturally occur in beverages such as green tea. Not only does this increase the applicability for those who obtain caffeine from drinking green tea, but a recent meta-analysis[117] has discovered that the caffeine-catechin mixtures can significantly increase fat oxidation by 16% relative to placebo, whereas caffeine alone elicits a non-significant increase of 12%. Hence, exercise studies using this combination are justified.

Conclusion

Recent developments have transformed some of the previous conceptions regarding nutritional modulation of substrate metabolism during and after exercise. Lowering the glycaemic index of the pre-exercise meal can increase fat oxidation during exercise, although information regarding the long-term effects of a low glycaemic index diet in combination with exercise are lacking. L-carnitine supplementation can affect metabolism in an exercise-intensity dependent fashion, but only when combined with carbohydrate ingestion to enable uptake into skeletal muscle which may not be suitable for some population who may benefit from increased muscle carnitine content. It is currently not completely clear whether calcium can affect fat utilisation in isolation and future work should aim to tease out potential integrative effects with vitamin D. Fish oil-derived n-3 fatty acids have credible theory and some evidence of increased exogenous lipid oxidation during exercise yet conclusive evidence of increase whole-body fat oxidation is not yet present. Similarly, caffeine has been shown to stimulate NEFA availability and fat oxidation under certain dietary conditions, and co-ingestion with catechins may modulate or potentiate the effectiveness of caffeine.

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References

1. Venables MC, Achten J & Jeukendrup AE (2005) Determinants of fat oxidation during exercise in healthy men and women: a cross-sectional study. *J Appl Physiol* **98**, 160-167.
2. De Bock K, Derave W, Eijnde BO*, et al.* (2008) Effect of training in the fasted state on metabolic responses during exercise with carbohydrate intake. *J Appl Physiol* **104**, 1045-1055.
3. Achten J & Jeukendrup AE (2001) Optimizing fat oxidation through exercise and diet. *Nutrition* **20**, 716-727.
4. Frayn KN (2010) Fat as a fuel: emerging understanding of the adipose tissue–skeletal muscle axis. *Acta Physiol* **199**, 509-518.
5. Romijn JA, Coyle EF, Sidossis LS*, et al.* (1995) Relationship between fatty acid delivery and fatty acid oxidation during strenuous exercise. *J Appl Physiol* **79**, 1939-1945.
6. Bruce CR & Hawley JA (2004) Improvements in insulin resistance with aerobic exercise training: a lipocentric approach. *Med Sci Sports Exerc* **36**, 1196-1201.
7. Van Proeyen K, Szlufcik K, Nielens H*, et al.* (2010) Training in the fasted state improves glucose tolerance during fat-rich diet. *J Physiol* **588**, 4289-4302.
8. Frayn KN (2010) Fat as a fuel: emerging understanding of the adipose tissue-skeletal muscle axis. *Acta Physiol (Oxf)* **199**, 509-518.
9. Hawley JA, Brouns F & Jeukendrup A (1998) Strategies to enhance fat utilisation during exercise. *Sports Med* **25**, 241-257.
10. Jenkins D, Wolever T, Taylor R*, et al.* (1981) Glycemic index of foods: a physiological basis for carbohydrate exchange. *Am J Clin Nutr* **34**, 362-366.
11. Granfeldt Y, Hagander B & Bjorck I (1995) Metabolic responses to starch in oat and wheat products. On the importance of food structure, incomplete gelatinization or presence of viscous dietary fibre. *Eur J Clin Nutr* **49**, 189-199.
12. Goddard M, Young G & Marcus R (1984) The effect of amylose content on insulin and glucose responses to ingested rice. *Am J Clin Nutr* **39**, 388-392.

519 13. Haber G, Heaton K, Murphy D*, et al.* (1977) Depletion and disruption of dietary fibre: effects
520 on satiety, plasma-glucose, and serum-insulin. *Lancet* **310**, 679-682.
521 14. Todesco T, Rao A, Bosello O*, et al.* (1991) Propionate lowers blood glucose and alters lipid
522 metabolism in healthy subjects. *Am J Clin Nutr* **54**, 860-865.
523 15. Frayn KN (2003) The glucose-fatty acid cycle: a physiological perspective. *Biochem Soc Trans*
524 **31**, 1115-1119.
525 16. Díaz EO, Galgani JE & Aguirre CA (2006) Glycaemic index effects on fuel partitioning in
526 humans. *Obes Rev* **7**, 219-226.
527 17. Ritz P, Krempf M, Cloarec D*, et al.* (1991) Comparative continuous-indirect-calorimetry study
528 of two carbohydrates with different glycemic indices. *Am J Clin Nutr* **54**, 855-859.
529 18. Korach-Andre M, Roth H, Barnoud D*, et al.* (2004) Glucose appearance in the peripheral
530 circulation and liver glucose output in men after a large 13C starch meal. *Am J Clin Nutr* **80**,
531 881-886.
532 19. Diaz EO, Galgani JE, Aguirre CA*, et al.* (2005) Effect of glycemic index on whole-body
533 substrate oxidation in obese women. *Int J Obes Relat Metab Disord* **29**, 108-114.
534 20. Vaitheesvaran B, LeRoith D & Kurland I (2010) MKR mice have increased dynamic glucose
535 disposal despite metabolic inflexibility, and hepatic and peripheral insulin insensitivity.
536 *Diabetologia* **53**, 2224-2232.
537 21. Sparti A, Milon H, Di Vetta Vr*, et al.* (2000) Effect of diets high or low in unavailable and
538 slowly digestible carbohydrates on the pattern of 24-h substrate oxidation and feelings of
539 hunger in humans. *Am J Clin Nutr* **72**, 1461-1468.
540 22. Tappy L, Wursch P, Randin J*, et al.* (1986) Metabolic effect of pre-cooked instant preparations
541 of bean and potato in normal and in diabetic subjects. *Am J Clin Nutr* **43**, 30-36.
542 23. Stevenson EJ, Astbury NM, Simpson EJ*, et al.* (2009) Fat oxidation during exercise and satiety
543 during recovery are increased following a low-glycemic index breakfast in sedentary
544 women. *J Nutr* **139**, 890-897.
545 24. Thomas DE, Brotherhood JR & Brand JC (1991) Carbohydrate Feeding before Exercise: Effect
546 of Glycemic Index. *Int J Sports Med* **12**, 180,186.
547 25. Sparks M, Selig S & Febbraio M (1998) Pre-exercise carbohydrate ingestion: effect of the
548 glycemic index on endurance exercise performance.
549 . *Med Sci Sports Exerc* **30**, 844-849
550

551 26. Stevenson EJ, Williams C, Mash LE*, et al.* (2006) Influence of high-carbohydrate mixed meals
552 with different glycemic indexes on substrate utilization during subsequent exercise in
553 women. *Am J Clin Nutr* **84**, 354-360.
554 27. Wee SL, Williams C, Tsintzas K*, et al.* (2005) Ingestion of a high-glycemic index meal
555 increases muscle glycogen storage at rest but augments its utilization during subsequent
556 exercise. *J Appl Physiol* **99**, 707-714.
557 28. DeMarco HM, Sucher KP, Cisar CJ*, et al.* (1999) Pre-exercise carbohydrate meals: application
558 of glycemic index. *Med Sci Sports Exerc* **31**, 164-170.
559 29. Stevenson E, Williams C, McComb G*, et al.* (2005) Improved recovery from prolonged exercise
560 following the consumption of low glycemic index carbohydrate meals. *Int J Sport Nutr*
561 *Exerc Metab* **15**, 333-349.
562 30. Steinberg GR (2009) Role of the AMP-activated protein kinase in regulating fatty acid
563 metabolism during exercise. *Appl Physiol Nutr Metab* **34**, 315-322.
564 31. Stevenson E, Williams C, Nute M*, et al.* (2005) The effect of the glycemic index of an evening
565 meal on the metabolic responses to a standard high glycemic index breakfast and subsequent
566 exercise in men. *Int J Sport Nutr Exerc Metab* **15**, 308-322.
567 32. Stevenson E, Williams C, Nute M*, et al.* (2008) Influence of the glycaemic index of an evening
568 meal on substrate oxidation following breakfast and during exercise the next day in healthy
569 women. *Eur J Clin Nutr* **62**, 608-616.
570 33. Grill V & Qvigstad E (2000) Fatty acids and insulin secretion. *Br J Nutr* **83**, S79-S84.
571 34. Wolever T & Bolognesi C (1996) Time of day influences relative glycaemic effect of foods.
572 *Nutr Res* **16**, 381-384.
573 35. Nilsson A, Granfeldt Y, Ostman E*, et al.* (2006) Effects of GI and content of indigestible
574 carbohydrates of cereal-based evening meals on glucose tolerance at a subsequent
575 standardised breakfast. *Eur J Clin Nutr* **60**, 1092-1099.
576 36. Gao Z, Yin J, Zhang J*, et al.* (2009) Butyrate Improves Insulin Sensitivity and Increases Energy
577 Expenditure in Mice. *Diabetes* **58**, 1509-1517.
578 37. Fardet A (2010) New hypotheses for the health-protective mechanisms of whole-grain cereals:
579 what is beyond fibre? *Nutr Res Rev* **23**, 65-134.
580 38. Stevenson E, Williams C & Nute M (2005) The influence of the glycaemic index of breakfast
581 and lunch on substrate utilisation during the postprandial periods and subsequent exercise.
582 *Br J Nutr* **93**, 885-893.

583 39. Tittelbach TJ, Mattes RD & Gretebeck RJ (2000) Post-exercise substrate utilization after a high
584 glucose vs. high fructose meal during negative energy balance in the obese. *Obes Res* **8**,
585 496-505.
586 40. Burelle Y, Lamoureux MC, Peronnet F*, et al.* (2006) Comparison of exogenous glucose,
587 fructose and galactose oxidation during exercise using 13C-labelling. *Br J Nutr* **96**, 56-61.
588 41. Henry RR, Crapo PA & Thorburn AW (1991) Current issues in fructose metabolism. *Annu Rev*
589 *Nutr* **11**, 21-39.
590 42. Fredholm BB (1971) The effect of lactate in canine subcutaneous adipose tissue in situ. *Acta*
591 *Physiol Scand* **81**, 110-123.
592 43. Liu C, Wu J, Zhu J*, et al.* (2009) Lactate Inhibits Lipolysis in Fat Cells through Activation of an
593 Orphan G-protein-coupled Receptor, GPR81. *J Biol Chem* **284**, 2811-2822.
594 44. Rooney K & Trayhurn P (2011) Lactate and the GPR81 receptor in metabolic regulation:
595 implications for adipose tissue function and fatty acid utilisation by muscle during exercise.
596 *Br J Nutr*, 1-7.
597 45. Tappy L & Le K-A (2010) Metabolic Effects of Fructose and the Worldwide Increase in
598 Obesity. *Physiol Rev* **90**, 23-46.
599 46. Moore LJS, Midgley AW, Thomas G*, et al.* (2009) The effects of low- and high-glycemic index
600 meals on time trial performance. *Int J Sports Physiol Perform* **4**, 331-344.
601 47. Nilsson M, Stenberg M, Frid AH*, et al.* (2004) Glycemia and insulinemia in healthy subjects
602 after lactose-equivalent meals of milk and other food proteins: the role of plasma amino
603 acids and incretins. *Am J Clin Nutr* **80**, 1246-1253.
604 48. Jeukendrup AE & Wallis GA (2005) Measurement of Substrate Oxidation During Exercise by
605 Means of Gas Exchange Measurements. *Int J Sports Med* **26**, S28,S37.
606 49. Little JP, Chilibeck PD, Ciona D*, et al.* (2010) Effect of low- and high-glycemic-index meals on
607 metabolism and performance during high-intensity, intermittent exercise. *Int J Sports Nutr*
608 *Exerc Metab* **20**, 447-456.
609 50. Bennard P & Doucet E (2006) Acute effects of exercise timing and breakfast meal glycemic
610 index on exercise-induced fat oxidation. *Appl Physiol Nutr Metab* **31**, 502-511.
611 51. Cheong SH, McCargar LJ, Paty BW*, et al.* (2009) The First Step First Bite Program: Guidance
612 to Increase Physical Activity and Daily Intake of Low-Glycemic Index Foods. *J Am Diet*
613 *Assoc* **109**, 1411-1416.
614 52. Hamzah S, Higgins S, Abraham T*, et al.* (2009) The effect of glycaemic index of high
615 carbohydrate diets consumed over 5 days on exercise energy metabolism and running
616 capacity in males. *J Sports Sci* **27**, 1545 - 1554.

617 53. Solomon TP, Haus JM, Kelly KR*, et al.* (2009) Randomized trial on the effects of a 7-d low-
618 glycemic diet and exercise intervention on insulin resistance in older obese humans. *Am J*
619 *Clin Nutr* **90**, 1222-1229.
620 54. Solomon TP, Haus JM, Kelly KR*, et al.* (2010) A low glycemic index diet combined with
621 exercise reduces insulin resistance, postprandial hyperinsulinemia, and glucose-dependent
622 insulinotropic polypeptide responses in obese, prediabetic humans. *Am J Clin Nutr* **92**,
623 1359-1368.
624 55. Garcia-Roves P, Huss JM, Han D-H*, et al.* (2007) Raising plasma fatty acid concentration
625 induces increased biogenesis of mitochondria in skeletal muscle. *Proc Natl Acad Sci* **104**,
626 10709-10713.
627 56. Yeo WK, Paton CD, Garnham AP*, et al.* (2008) Skeletal muscle adaptation and performance
628 responses to once a day versus twice every second day endurance training regimens. *J Appl*
629 *Physiol* **105**, 1462-1470.
630 57. Morton JP, Croft L, Bartlett JD*, et al.* (2009) Reduced carbohydrate availability does not
631 modulate training-induced heat shock protein adaptations but does upregulate oxidative
632 enzyme activity in human skeletal muscle. *J Appl Physiol* **106**, 1513-1521.
633 58. Burke LM, Claassen A, Hawley JA*, et al.* (1998) Carbohydrate intake during prolonged cycling
634 minimizes effect of glycemic index of preexercise meal. *J Appl Physiol* **85**, 2220-2226.
635 59. Chen YJ, Wong SH, Wong CK*, et al.* (2008) Effect of preexercise meals with different
636 glycemic indices and loads on metabolic responses and endurance running. *Int J Sport Nutr*
637 *Exerc Metab* **18**, 281-300.
638 60. Chen YJ, Wong SH, Chan CO*, et al.* (2009) Effects of glycemic index meal and CHO-
639 electrolyte drink on cytokine response and run performance in endurance athletes. *J Sci Med*
640 *Sport* **12**, 697-703.
641 61. Wong SH, Chan OW, Chen YJ*, et al.* (2009) Effect of preexercise glycemic-index meal on
642 running when CHO-electrolyte solution is consumed during exercise. *Int J Sport Nutr Exerc*
643 *Metab* **19**, 222-242.
644 62. Liedtke AJ, Nellis SH, Whitesell LF*, et al.* (1982) Metabolic and mechanical effects using L-
645 and D-carnitine in working swine hearts. *Am J Physiol* **243**, H691-697.
646 63. Murthy MS & Pande SV (1987) Malonyl-CoA binding site and the overt carnitine
647 palmitoyltransferase activity reside on the opposite sides of the outer mitochondrial
648 membrane. *Proc Natl Acad Sci U S A* **84**, 378-382.
649 64. McGarry JD & Brown NF (1997) The mitochondrial carnitine palmitoyltransferase system.
650 From concept to molecular analysis. *Eur J Biochem* **244**, 1-14.

651 65. Stephens FB, Constantin-Teodosiu D & Greenhaff PL (2007) New insights concerning the role
652 of carnitine in the regulation of fuel metabolism in skeletal muscle. *J Physiol* **581**, 431-444.
653 66. Stephens FB, Constantin-Teodosiu D, Laithwaite D*, et al.* (2006) Insulin stimulates L-carnitine
654 accumulation in human skeletal muscle. *FASEB J* **20**, 377--.
655 67. Stephens FB, Constantin-Teodosiu D, Laithwaite D*, et al.* (2006) An Acute Increase in Skeletal
656 Muscle Carnitine Content Alters Fuel Metabolism in Resting Human Skeletal Muscle. *J*
657 *Clin Endocrinol Metab* **91**, 5013-5018.
658 68. Stephens FB, Evans CE, Constantin-Teodosiu D*, et al.* (2007) Carbohydrate ingestion augments
659 L-carnitine retention in humans. *J Appl Physiol* **102**, 1065-1070.
660 69. Abramowicz W & Galloway S (2005) Effects of acute versus chronic L-carnitine L-tartrate
661 supplementation on metabolic responses to steady state exercise in males and females. *Int J*
662 *Sports Nutr Exerc Metab* **15**, 386-400.
663 70. Broad EM, Maughan RJ & Galloway SD (2005) Effects of four weeks L-carnitine L-tartrate
664 ingestion on substrate utilization during prolonged exercise. *Int J Sport Nutr Exerc Metab*
665 **15**, 665-679.
666 71. Wall BT, Stephens FB, Constantin-Teodosiu D*, et al.* (2011) Chronic oral ingestion of l-
667 carnitine and carbohydrate increases muscle carnitine content and alters muscle fuel
668 metabolism during exercise in humans. *J Physiol* **589**, 963-973.
669 72. Burke LM & Kiens B (2006) "Fat adaptation" for athletic performance: the nail in the coffin? *J*
670 *Appl Physiol* **100**, 7-8.
671 73. Gannon MC, Nuttall JA & Nuttall FQ (2002) The metabolic response to ingested glycine. *Am J*
672 *Clin Nutr* **76**, 1302-1307.
673 74. Harris WS, Connor WE, Alam N*, et al.* (1988) Reduction of postprandial triglyceridemia in
674 humans by dietary n-3 fatty acids. *J Lipid Res* **29**, 1451-1460.
675 75. Herzberg GR & Rogerson M (1989) The effect of dietary fish oil on muscle and adipose tissue
676 lipoprotein lipase. *Lipids* **24**, 351-353.
677 76. Jang IS, Hwang DY, Chae KR*, et al.* (2003) Role of dietary fat type in the development of
678 adiposity from dietary obesity-susceptible Sprague-Dawley rats. *Br J Nutr* **89**, 429-438.
679 77. Clarke SD (2000) Polyunsaturated fatty acid regulation of gene transcription: a mechanism to
680 improve energy balance and insulin resistance. *Br J Nutr* **83 Suppl 1**, S59-66.
681 78. Price PT, Nelson CM & Clarke SD (2000) Omega-3 polyunsaturated fatty acid regulation of
682 gene expression. *Curr Opin Lipidol* **11**, 3-7.

79. Baillie RA, Takada R, Nakamura M, *et al.* (1999) Coordinate induction of peroxisomal acyl-CoA oxidase and UCP-3 by dietary fish oil: a mechanism for decreased body fat deposition. *Prostaglandins Leukot Essent Fatty Acids* **60**, 351-356.
80. Clarke SD (2001) Polyunsaturated fatty acid regulation of gene transcription: a molecular mechanism to improve the metabolic syndrome. *J Nutr* **131**, 1129-1132.
81. Dangardt F, Osika W, Chen Y, *et al.* (2010) Omega-3 fatty acid supplementation improves vascular function and reduces inflammation in obese adolescents. *Atherosclerosis* **212**, 580-585.
82. Delarue J, Couet C, Cohen R, *et al.* (1996) Effects of fish oil on metabolic responses to oral fructose and glucose loads in healthy humans. *Am J Physiol Endocrinol Metab* **270**, E353-E362.
83. Couet C, Delarue J, Ritz P, *et al.* (1997) Effect of dietary fish oil on body fat mass and basal fat oxidation in healthy adults. *Int J Obes Relat Metab Disord* **21**, 637-643.
84. Smith BK, Sun GY, Donahue OM, *et al.* (2004) Exercise plus n-3 fatty acids: additive effect on postprandial lipemia. *Metabolism* **53**, 1365-1371.
85. Hill AM, Buckley JD, Murphy KJ, *et al.* (2007) Combining fish-oil supplements with regular aerobic exercise improves body composition and cardiovascular disease risk factors. *Am J Clin Nutr* **85**, 1267-1274.
86. Huffman DM, Michaelson JL & Thomas TR (2004) Chronic supplementation with fish oil increases fat oxidation during exercise in young men. *J Exerc Physiol online* **7**, 48-56.
87. Delarue J, Labarthe F & Cohen R (2003) Fish-oil supplementation reduces stimulation of plasma glucose fluxes during exercise in untrained males. *Br J Nutr* **90**, 777-786.
88. Bortolotti M, Tappy L & Schneiter P (2007) Fish oil supplementation does not alter energy efficiency in healthy males. *Clin Nutr* **26**, 225-230.
89. Boss A, Lecoultre V, Ruffieux C, *et al.* (2010) Combined effects of endurance training and dietary unsaturated fatty acids on physical performance, fat oxidation and insulin sensitivity. *Br J Nutr* **103**, 1151-1159.
90. Schrager S (2005) Dietary Calcium Intake and Obesity. *J Am Board Fam Pract* **18**, 205-210.
91. Dougkas A, Reynolds CK, Givens ID, *et al.* (2011) Associations between dairy consumption and body weight: a review of the evidence and underlying mechanisms. *Nutr Res Rev* **15**, 1-24.
92. Christensen R, Lorenzen JK, Svith CR, *et al.* (2009) Effect of calcium from dairy and dietary supplements on faecal fat excretion: a meta-analysis of randomized controlled trials. *Obes Rev* **10**, 475-486.

717 93. Zemel MB (2004) Role of calcium and dairy products in energy partitioning and weight
718 management. *Am J Clin Nutr* **79**, 907S-912S.
719 94. Draznin B (1993) Cytosolic calcium and insulin resistance. *Am J Kidney Dis* **21**, 32-38.
720 95. Sun X & Zemel MB (2008) Calcitriol and calcium regulate cytokine production and adipocyte-
721 macrophage cross-talk. *J Nutr Biochem* **19**, 392-399.
722 96. Sun X & Zemel MB (2009) Leucine modulation of mitochondrial mass and oxygen
723 consumption in skeletal muscle cells and adipocytes. *Nutr Metab (Lond)* **6**, 26.
724 97. Soares MJ & Chan She-Ping-Delfos WL (2010) Postprandial energy metabolism in the
725 regulation of body weight: is there a mechanistic role for dietary calcium? *Nutrients* **2**, 586-
726 598.
727 98. Maimoun L & Sultan C (2009) Effect of physical activity on calcium homeostasis and
728 calciotropic hormones: a review. *Calcif Tissue Int* **85**, 277-286.
729 99. Barry DW, Hansen KC, Van Pelt RE*, et al.* (2011) Acute Calcium Ingestion Attenuates
730 Exercise-induced Disruption of Calcium Homeostasis. *Med Sci Sports Exerc* **43**, 617-623.
731 100. Melanson EL, Sharp TA, Schneider J*, et al.* (2003) Relation between calcium intake and fat
732 oxidation in adult humans. *Int J Obes Relat Metab Disord* **27**, 196-203.
733 101. Melanson EL, Donahoo WT, Dong F*, et al.* (2005) Effect of low- and high-calcium dairy-
734 based diets on macronutrient oxidation in humans. *Obes Res* **13**, 2102-2112.
735 102. White KM, Lyle RM, Flynn MG*, et al.* (2006) The acute effects of dairy calcium intake on fat
736 metabolism during exercise and endurance exercise performance. *Int J Sport Nutr Exerc*
737 *Metab* **16**, 565-579.
738 103. Schutz Y (2004) Concept of fat balance in human obesity revisited with particular reference to
739 de novo lipogenesis. *Int J Obes Relat Metab Disord* **28 Suppl 4**, S3-S11.
740 104. Soares MJ & Chan She Ping-Delfos W (2008) Second meal effects of dietary calcium and
741 vitamin D. *Eur J Clin Nutr* **62**, 872-878.
742 105. Cummings NK, James AP & Soares MJ (2006) The acute effects of different sources of dietary
743 calcium on postprandial energy metabolism. *Br J Nutr* **96**, 138-144.
744 106. Ping-Delfos WC & Soares M (2011) Diet induced thermogenesis, fat oxidation and food
745 intake following sequential meals: influence of calcium and vitamin D. *Clin Nutr* **30**, 376-
746 383.
747 107. Graham TE, Battram DS, Dela F*, et al.* (2008) Does caffeine alter muscle carbohydrate and fat
748 metabolism during exercise? *Appl Physiol Nutr Metab* **33**, 1311-1318.

749 108. Greer F, Hudson R, Ross R*, et al.* (2001) Caffeine Ingestion Decreases Glucose Disposal
750 During a Hyperinsulinemic-Euglycemic Clamp in Sedentary Humans. *Diabetes* **50**, 2349-
751 2354.
752 109. Battram DS, Shearer J, Robinson D*, et al.* (2004) Caffeine ingestion does not impede the
753 resynthesis of proglycogen and macroglycogen after prolonged exercise and carbohydrate
754 supplementation in humans. *J Appl Physiol* **96**, 943-950.
755 110. Graham TE (2001) Caffeine, coffee and ephedrine: impact on exercise performance and
756 metabolism. *Can J Appl Physiol* **26 Suppl**, S103-119.
757 111. Graham TE (2001) Caffeine and exercise: metabolism, endurance and performance. *Sports*
758 *Med* **31**, 785-807.
759 112. Jacobson TL, Febbraio MA, Arkinstall MJ*, et al.* (2001) Effect of caffeine co-ingested with
760 carbohydrate or fat on metabolism and performance in endurance-trained men. *Exp Physiol*
761 **86**, 137-144.
762 113. Slivka D, Hailes W, Cuddy J*, et al.* (2008) Caffeine and carbohydrate supplementation during
763 exercise when in negative energy balance: effects on performance, metabolism, and salivary
764 cortisol. *Appl Physiol Nutr Metab* **33**, 1079-1085.
765 114. Thong FS, Derave W, Kiens B*, et al.* (2002) Caffeine-induced impairment of insulin action but
766 not insulin signaling in human skeletal muscle is reduced by exercise. *Diabetes* **51**, 583-590.
767 115. Pedersen DJ, Lessard SJ, Coffey VG*, et al.* (2008) High rates of muscle glycogen resynthesis
768 after exhaustive exercise when carbohydrate is coingested with caffeine. *J Appl Physiol* **105**,
769 7-13.
770 116. Aschenbach WG, Sakamoto K & Goodyear LJ (2004) 5' adenosine monophosphate-activated
771 protein kinase, metabolism and exercise. *Sports Med* **34**, 91-103.
772 117. Hursel R, Viechtbauer W, Dulloo AG*, et al.* (2011) The effects of catechin rich teas and
773 caffeine on energy expenditure and fat oxidation: a meta-analysis. *Obes Rev* **12**, e573-581.
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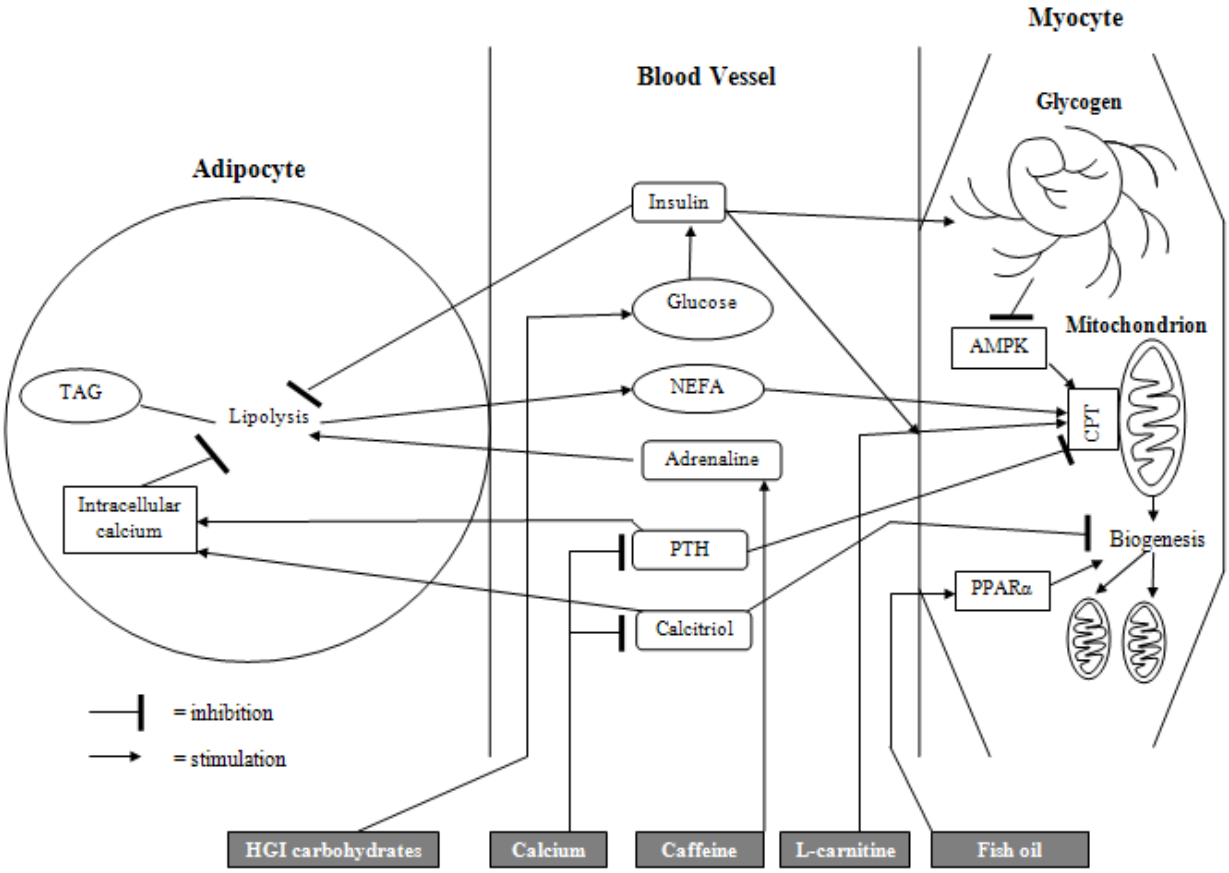

Figure 1. Mechanisms through which nutritional components may influence substrate selection during exercise. AMPK, AMP-activated protein kinase; Calcitriol, 1,25-dihydroxyvitamin D_3; CPT, carnitine palmitoyltransferase; PTH, parathyroid hormone.