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## Review Article

# 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 overturned 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 this 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. Ca may increase NEFA availability although it is not clear whether these effects occur. Ca 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.

**Key words:** Glycaemic index: L-Carnitine: Calcium: Fish oil: Caffeine

Maximum rates of fat oxidation during exercise vary from 0.18 to 1.01 g/min, more than a 5-fold difference<sup>(1)</sup>. Training status, lean body mass (BM), estimated physical activity level, sex 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<sup>(2)</sup>. The limitations to fat oxidation vary depending on the intensity of exercise and have been reviewed extensively elsewhere<sup>(3,4)</sup>. Maximal fat oxidation rates occur during exercise between 45 and 65%  $\text{VO}_{2\text{max}}$  and are drastically reduced to minimal rates above 85%  $\text{VO}_{2\text{max}}$ . Reduced plasma NEFA availability may explain part, but not all<sup>(5)</sup>, of the decrease. Fatty acid transport across muscle and mitochondrial membranes is thought to be

an important rate-limiting step. These restrictive processes are discussed in greater detail later in the 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<sup>(6,7)</sup>. 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<sup>(8)</sup>, makes this an interesting and important topic for research and has led to the construction of some previous reviews on dietary strategies<sup>(3,9)</sup>.

**Abbreviations:** BM, body mass; calcitriol, 1,25-dihydroxyvitamin D<sub>3</sub>; HGI, high glycaemic index; LGI, low glycaemic index; PTH, parathyroid hormone.

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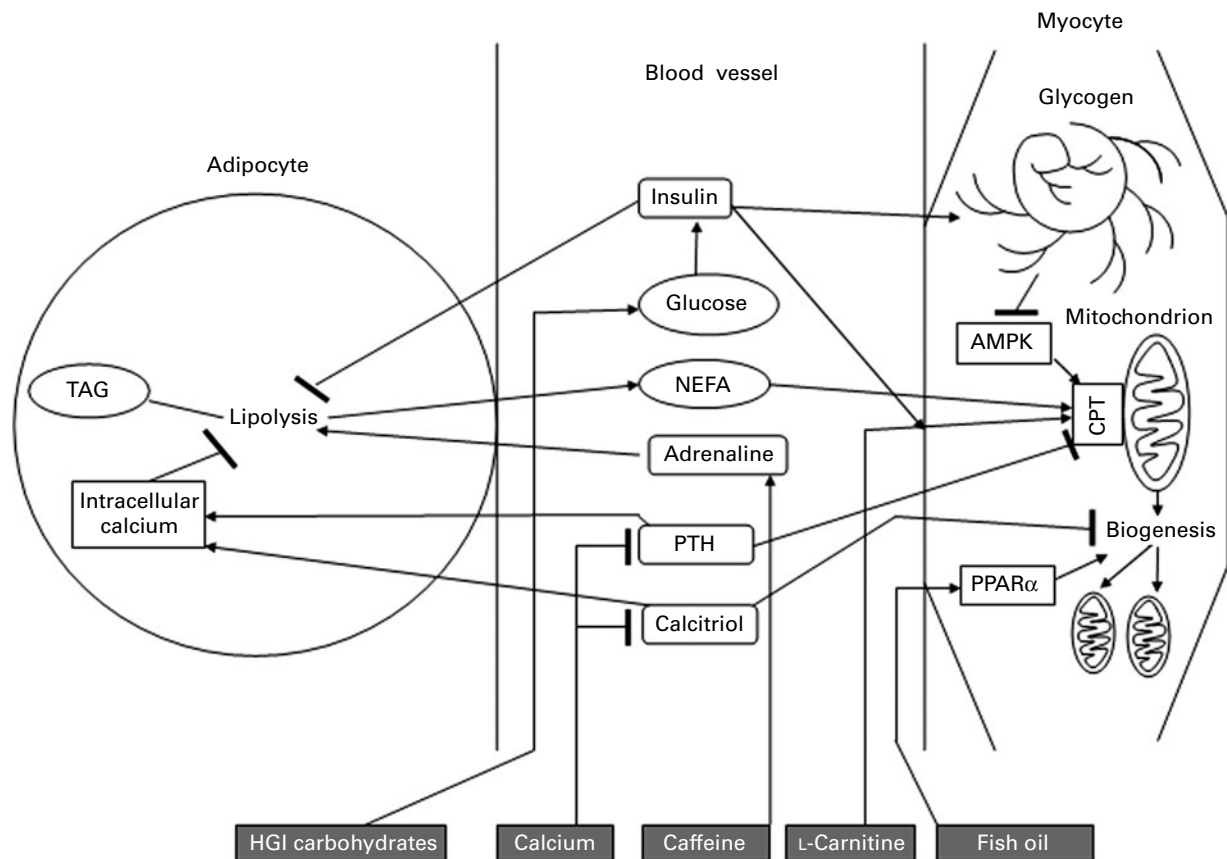
Yet, since the publication of these articles, some exciting advances have occurred in this area. The aim of this review is to provide new insights into the manipulation of lipid oxidation via nutrition in the peri-exercise period and to supply potential mechanisms by which these effects occur (Fig. 1). 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  $\beta$ -cells of the pancreas. The glycaemic index is a method of classifying carbohydrates according to their blood glucose response<sup>(10)</sup>. 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<sup>(11)</sup> and chemical structure of the food<sup>(12)</sup>, the presence of fibre<sup>(13)</sup> and organic acids<sup>(14)</sup>. Insulin is known to inhibit lipolysis and thus reduce NEFA availability<sup>(15)</sup>. 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 Díaz *et al.*<sup>(16)</sup> concluded that metabolic differences between HGI and LGI 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)<sup>(17)</sup> or high relative (5 g/kg BM)<sup>(18)</sup> amounts of carbohydrate. While others used obese subjects<sup>(19)</sup> who, although displayed normal glucose tolerance, may still have exhibited insulin resistance and metabolic inflexibility<sup>(20)</sup>. Therefore, shifts in substrate metabolism from postabsorptive to postprandial states could have been blunted. In others, glucose responses were not evaluated<sup>(21)</sup> and hence the results cannot be attributed to GI with certainty. Differences in resting metabolism have been observed with LGI mixed meals<sup>(22,23)</sup>. 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 *v.* potato only)<sup>(24,25)</sup>, 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



**Fig. 1.** Mechanisms through which nutritional components may influence substrate selection during exercise. —|, Inhibition; —▶, stimulation. HGI, high glycaemic index; AMPK, AMP-activated protein kinase; calcitriol, 1,25-dihydroxyvitamin D<sub>3</sub>; CPT, carnitine palmitoyltransferase; PTH, parathyroid hormone.

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<sup>(23,26)</sup> which is pertinent for real-world application. These mixed-meal design studies have demonstrated up to 2-fold increases in the amount of whole-body fat oxidation during treadmill running<sup>(26)</sup>. This occurred during exercise intensities ranging from 50 to 70%  $\text{VO}_{2\text{max}}$ <sup>(21,24)</sup> in both males<sup>(25)</sup> and females<sup>(21,24)</sup>, differing in activity levels<sup>(21,24)</sup> and differences even occur during the first 15 min of exercise<sup>(24)</sup>. Furthermore, similar findings are seen with carbohydrate intakes ranging from 1 to 2.5 g/kg BM<sup>(23,27)</sup> and when the pre-exercise meal is ingested from 30 min<sup>(28)</sup>, up to 12 h before exercise<sup>(29)</sup>. The intakes of carbohydrate used in the exercise studies (1–2.5 g/kg BM) are within the guidelines for pre-exercise carbohydrate consumption, which strengthens the relevance of these studies for everyday use before exercise training.

Mechanisms explaining the increase in fat oxidation during exercise with LGI meals probably include greater availability of NEFA, and reduced pre-exercise muscle glycogen concentrations compared to HGI meals<sup>(27)</sup>. This leads to greater muscle glycogen utilisation and therefore less of a reliance on fat oxidation via reduced AMP-activated protein kinase<sup>(30)</sup>.

It is appealing to consider that meal timing could be crucial to the effectiveness of GI modulation of substrate metabolism. An LGI meal is known to produce a second-meal phenomenon whereby the glycaemic response to a standard meal is lower following previous consumption of an LGI, compared to a HGI meal. Stevenson *et al.*<sup>(31,32)</sup> examined whether this effect can result in a shift in lipid utilisation during exercise. Participants were given an 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 before exercise, which would optimise the difference in muscle glycogen and therefore influence substrate selection. On the other hand, an LGI diet during recovery from a glycogen-reduction run, led to increased fat usage during fasted exercise the following day<sup>(29)</sup>.

A few explanations provide a more convincing argument:

- (1) Breakfast is the most important time for consuming LGI foods due to its being the first meal consumed after the overnight fast. Exposure to NEFA during fasting can induce insulin resistance<sup>(33)</sup>. 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<sup>(34)</sup>.
- (2) The greater fibre content of the LGI foods may lead to an increase in SCFA via increased gut fermentation<sup>(35)</sup>. 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<sup>(36)</sup>, and may therefore be an integral aspect in the modulation of substrate utilisation from LGI *v.* HGI meals; as the glycaemic response to the standard HGI breakfast (which contained equal amounts of fibre) was different<sup>(32)</sup>. This implies that it may not be the glycaemic response *per se*, but the fibre content inherent in many LGI foods.

- (3) Finally, whole-grain foods contain a variety of bioactive compounds and micronutrients<sup>(37)</sup> 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<sup>(38)</sup>. Although LGI, fructose inhibits fat oxidation during and after exercise to a greater extent than glucose<sup>(39,40)</sup>. This illustrates the powerful influence of GI combined with exercise in these studies<sup>(26,38)</sup>, 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<sup>(41)</sup>, which, it should be noted, also inhibits lipolysis<sup>(42)</sup> via the G protein-coupled receptor GPR81<sup>(43,44)</sup>. This influence of fructose may also provide an explanation for why some of the resting studies showed no effect of GI on fat metabolism<sup>(19)</sup>. High fructose consumption has been linked to *de novo* lipogenesis, TAG accumulation and insulin resistance<sup>(45)</sup>. 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.*<sup>(46)</sup> remarkably displayed increased fat oxidation and NEFA concentrations during exercise following a HGI meal compared to an LGI meal. Explanations for this are not easily forthcoming, although the LGI meal contained a greater quantity of milk. Milk proteins are particularly insulinotropic<sup>(47)</sup>, which may have caused the greater suppression of NEFA 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<sup>(48)</sup>. This latter explanation may also partially clarify why Little *et al.*<sup>(49)</sup> 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.*<sup>(49)</sup>). 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<sup>(50)</sup> also reported no difference in lipid utilisation between HGI and

LGI pre-exercise meals. Yet, the blood glucose response to the 'LGI' meal before 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 an LGI breakfast and lunch are administered, no difference in fat oxidation occurs during exercise after lunch, compared to HGI breakfast and lunch<sup>(38)</sup>. The authors suggested that the intensity of exercise (70%  $\text{VO}_{2\text{max}}$ ) 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%  $\text{VO}_{2\text{max}}$ ) after a single LGI *v.* HGI meal<sup>(27)</sup>, the participants differed in training status ( $\text{VO}_{2\text{max}}$ : 55.1 *v.* 64.6 ml/kg per min). Training status is known to enhance fat utilisation at the same relative intensity<sup>(3)</sup>, 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 have, 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<sup>(51,52)</sup>. However, the relatively short time period (3 and 5 d), combined with the high carbohydrate loads (10 and 7 g/kg BM per 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  $\text{VO}_{2\text{max}}$  and greater reduction in systolic blood pressure with a 7d LGI compared to a HGI diet<sup>(53)</sup>; although no difference in postprandial resting fat oxidation was reported. When the study was extended to 12 weeks, the differences in  $\text{VO}_{2\text{max}}$  and blood pressure were abolished, yet the LGI diet contributed to the improved insulin sensitivity with exercise training<sup>(54)</sup>. 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<sup>(55–57)</sup>. 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<sup>(58–61)</sup>; thus, the application may be limited to endurance events where optimal nutrient intake during competition is prohibited or impractical (e.g. the swim leg of an Ironman triathlon, long-distance open-water swimming). Notwithstanding this, exercise training on an 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 an 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<sup>(62)</sup>, 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 catalyses the esterification of free carnitine, with long-chain acyl-CoA<sup>(63)</sup>. 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 catalyses the transesterification of long-chain acylcarnitine back to free carnitine and long-chain acyl-CoA<sup>(64)</sup>, which is then able to enter the  $\beta$ -oxidation pathway. A thorough review of the role of L-carnitine in fatty-acid transport is provided by Stephens *et al.*<sup>(65)</sup>.

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 carnitine palmitoyltransferase 1-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<sup>(9)</sup>. 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 (approximately 3–5 mmol/l) to plasma (50  $\mu\text{mol/l}$ ). Indeed, even when L-carnitine is administered intravenously, muscle carnitine content is unaffected<sup>(66)</sup> and thus it comes as 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 approximately 14%, probably via an increase in  $\text{Na}^+/\text{K}^+$  pump activity<sup>(66,67)</sup>. 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<sup>(67)</sup>.

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 four boluses of 94 g sugar, resulted in reduced urinary carnitine excretion<sup>(68)</sup>. 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 has performed a 14 d supplemental period with 3 g L-carnitine L-tartrate<sup>(69)</sup>, combined with a fairly high-carbohydrate diet (approximately 7 g/kg BM per d). In contrast to the proposition of Stephens *et al.*, lipid utilisation was not enhanced during cycling at 60%  $\text{VO}_{2\text{max}}$ . In fact, carbohydrate oxidation increased by approximately 20% in male participants, with no significant effects on females (albeit showing a similar trend). A further study by the same group showed similar results<sup>(70)</sup>, 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 human subjects can be elevated by carbohydrate–carnitine co-supplementation, Wall *et al.*<sup>(71)</sup> demonstrate that carnitine's influence on exercise metabolism is dependent upon the intensity. A 24-week 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%  $\text{VO}_{2\text{max}}$ . When the intensity was increased to 80%  $\text{VO}_{2\text{max}}$ , no difference in muscle glycogen utilisation was observed; yet muscle lactate content was 44% lower, and phosphocreatine:ATP ratio was better maintained. Pyruvate dehydrogenase activation status was reduced during cycling at 50%  $\text{VO}_{2\text{max}}$  but increased at 80%  $\text{VO}_{2\text{max}}$  in the supplementation group, versus control. A final, captivating observation was that, although BM increased in the control group (probably due to the 2512 kJ (600 kcal) provided by the carbohydrate supplement), 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 are needed to support intense exercise<sup>(72)</sup>. Glycogen sparing would only be successful if the spared stores are able to be 'tapped into' towards the later 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 in low-intensity exercise, but pyruvate dehydrogenase activity was not down-regulated at higher intensities, which would

in theory enhance performance. In practice, 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 open 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 practice 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<sup>(47,73)</sup>, 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 PUFA. Supplementation with fish oil-derived *n*-3 fatty acids has been shown to reduce postprandial lipaemia<sup>(74)</sup>. The increase in skeletal muscle lipoprotein lipase activity<sup>(75)</sup> 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<sup>(76)</sup>. 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<sup>(77)</sup>.

The major pathways in which *n*-3 fatty acids have been proposed to alter energy metabolism include: providing a substrate for PPAR $\alpha$ <sup>(78)</sup> and increases in skeletal muscle uncoupling protein 3<sup>(79)</sup>. 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 synthetase, and malonyl-CoA<sup>(80)</sup>. Effect on genes can happen in minutes which would support the acute supplementation of fish oil. Fish oil improves endothelial function, and reduces inflammation<sup>(81)</sup>. 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<sup>(4)</sup>. Increases in lipid combustion in resting human subjects have been reported<sup>(82,83)</sup> coinciding with a 40% reduction in the insulin response to an oral glucose load<sup>(82)</sup>. 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<sup>(84,85)</sup>. Hence, exercise may potentiate the effects of fish oil supplementation, thus elucidating changes in metabolism.

A number of studies have investigated the influence of fish oil on energy metabolism during exercise. One of these has shown a clear increase in fat oxidation during exercise after 4 g/d of fish oil supplementation for 3 weeks<sup>(86)</sup>. In this protocol, participants were studied during running at 60%  $\text{VO}_{2\text{max}}$ , under six conditions: (1) in the postabsorptive state; (2) following a high-fat meal; (3) following a high-fat meal supplemented with fish oil; (4–6) as in the first three trials, but after chronic supplementation. The energy contribution from fat, estimated by indirect calorimetry, increased by approximately 10% post-supplementation. Delarue *et al.*<sup>(87)</sup> also found a tendency for enhanced fat oxidation (by approximately 7%) and reduced metabolic glucose disappearance during cycling at 60%  $\text{VO}_{2\text{max}}$ . 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%  $\text{VO}_{2\text{max}}$ <sup>(88)</sup>. 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<sup>(89)</sup>. Exogenous fat oxidation was estimated using labelled isotopes and cycling exercise was, again, performed at 50%  $\text{VO}_{2\text{max}}$ . Whole-body fat oxidation was potentiated to a greater extent (42% *v.* 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 and dairy products

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

Although part of its effects is probably due to reduced absorption of dietary fat<sup>(92)</sup>, a theory has been devised explaining a potential link between Ca intake and substrate utilisation. Zemel<sup>(93)</sup> developed a hypothesis based on animal and cell models that an increase in Ca intake can mediate intracellular Ca concentrations within adipocytes via reductions in 1,25-dihydroxy vitamin D<sub>3</sub> (calcitriol) and parathyroid hormone (PTH) concentrations<sup>(93)</sup>. Intracellular Ca has long been associated with insulin resistance in obesity<sup>(94)</sup>, 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 down-regulate uncoupling protein 2, thereby potentially influencing thermogenesis and  $\beta$ -oxidation. Although the majority of this work has been conducted on adipocytes, a high-Ca 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 carnitine palmitoyltransferase 1 activity, a rate-limiting step discussed in reference to L-carnitine supplementation. Moreover, recent work has demonstrated the calcitriol suppression of mitochondrial biogenesis and increased cytokine production<sup>(95,96)</sup>, both of which are known to alter the energy metabolism.

The manipulation of postprandial fat oxidation by Ca intake in humans has been reviewed elsewhere<sup>(97)</sup>. Thus, only studies involving exercise will be discussed presently.

Both acute and chronic endurance exercise results in shifts in calciotropic hormone concentrations (for a review, see Maimoun & Sultan<sup>(98)</sup>). This is most likely due to sweat Ca losses. Therefore, the metabolic effects of Ca 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 Ca ingestion before exercise<sup>(99)</sup>; 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 Ca and fat oxidation showed an association between acute Ca intake and subsequent fat oxidation<sup>(100)</sup>. This was followed-up by an intervention study where subjects consumed low (500 mg/d) or high (1400 mg/d) dairy Ca, energy-balanced diets<sup>(101)</sup>. These diets were matched for energy, macronutrients, fibre and SFA:MUFA:PUFA ratios. Subjects completed 6 d 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 energy restriction (419 kJ (100 kcal)) with a greater contribution from exercise energy expenditure (approximately 2093 kJ (500 kcal)). The 24 h fat oxidation was 28% greater when subjects consumed the high-Ca diet when in energy deficit versus the low-Ca 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-Ca diet.

The effects of acute ingestion of Ca were studied in a group of trained female runners<sup>(102)</sup>. In the study, 4 h after consumption of a standardised meal containing 3 g carbohydrate/kg BM, the participants consumed a test beverage with a high

(500 mg) or low (80 mg) Ca content. Mean carbohydrate content was 117 and 118 g for the low and high dairy Ca beverages, respectively. Then, 1 h following consumption of the test drink, the subjects ran on a treadmill for 90 min at 70%  $\text{VO}_{2\text{max}}$ . 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<sup>(103)</sup>, thus explaining no effect of Ca. This is supported by the relatively low levels of fat oxidation observed during the exercise bout (approximately 0.2 g/min). Treadmill-based exercise at a similar intensity elicits a rate of fat oxidation of approximately 0.5 g/min in a large group of women<sup>(1)</sup>. Furthermore, resting studies suggest that the availability of NEFA appears to diverge at around 3–4 h after consumption of high- *v.* low-Ca meals<sup>(104)</sup>. As the test drink was consumed 1 h before 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 glycaemic index. It should be acknowledged that resting studies which have demonstrated an increase in fat oxidation have generally compared high-Ca, high-vitamin D meals with low-Ca, low-vitamin D meals<sup>(104–106)</sup>. Whether Ca 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 Ca losses. However, recent evidence suggests that Ca feeding just 20 min before exercise can attenuate the effect of exercise on PTH by approximately 30% relative to placebo<sup>(99)</sup>.

There exists a substantial amount of theory supporting the role of Ca 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 human subjects during exercise, which itself produces shifts in calciotropic hormone concentrations. More research is needed before any recommendations can be made, to establish whether Ca can influence fat oxidation during exercise. Studies should determine whether this occurs under different nutritional states with varying carbohydrate availability, habitual Ca intakes and vitamin D status.

### Caffeine

Former reviews have concluded that there is little evidence of caffeine influencing fat oxidation during exercise<sup>(9,107)</sup>. 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<sup>(108,109)</sup>.

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.*<sup>(110)</sup> attempted to overcome the problems of small sample sizes which have been apparent in muscle biopsy studies involving caffeine ingestion and exercise. 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 BM; a dose which is almost certainly ergogenic<sup>(111)</sup>. 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%  $\text{VO}_{2\text{max}}$ ), 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 NEFA<sup>(8)</sup>; thus the effect of caffeine on 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.*<sup>(112)</sup> aimed to tackle this by providing a high-fat (1.2 g/kg BM) meal combined with an intravenous heparin infusion (to increase NEFA availability). This was compared to the same meal and infusion in addition to caffeine (6 mg/kg BM) ingestion, *v.* a high-carbohydrate (2.6 g/kg BM) meal and high-carbohydrate meal with caffeine ingestion. All meals were consumed 60 min before 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 before exercise; and 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.*<sup>(112)</sup>. Ingestion of caffeine (800 mg) reduced RER and tended to increase fat oxidation by approximately 10% ( $P=0.069$ ) during 2 h of cycling at 50% of maximum power output with carbohydrate ingestion (60 g/kg per h)<sup>(113)</sup>. 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 d of reduced energy intake (approximately 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 BM 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<sup>(114)</sup>. In fact caffeine, co-ingested with carbohydrate after exercise, leads to the highest rates of



glycogen re-synthesis (approximately 60 mmol/kg dry weight per h) ever reported (excluding infusion studies)<sup>(115)</sup>. 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 AMP-activated protein kinase-activated fatty acid oxidation<sup>(116)</sup>. Higher serum NEFA concentrations have been noted with caffeine ingestion following exercise<sup>(109)</sup>, 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<sup>(117)</sup> 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 an LGI 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 of the population who may benefit from increased muscle carnitine content. It is currently not completely clear whether Ca 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 in 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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