

Jacques Décombaz

Nutrition Department, Nestlé Research Centre, Lausanne, Switzerland

# Nutrition and recovery of muscle energy stores after exercise

## Summary

In the process of recovery after exercise, nutrition is a central consideration for rehydrating and refueling the body. Nutritional intervention also supports the anabolic events triggered by muscle contraction and leads to training adaptations. When recovery time is short between physical challenges, it is even more important to adopt an adequate nutrient intake proactively and in good time.

Restoration of muscle glycogen is faster when rapidly available carbohydrates are ingested at a rate of about  $1 \text{ g}\cdot\text{kg body mass}^{-1}\cdot\text{h}^{-1}$  immediately after the end of exercise, preferably as a drink. In optimal conditions replenishment is achieved within 24 h. Proteins, when co-ingested with carbohydrates play a subsidiary role on glycogen resynthesis. On the other hand, ingestion of proteins in the time frame of exercise is important for rapid tissue repair and the initiation of muscle building by, in particular, their essential amino acids. This consideration finds application for muscle activities involving intermittent resistance work. The restoration of intramuscular triacylglycerol (mTAG, IMCL) is achieved within 24 h by the ingestion of about 2 g dietary lipids·kg body mass<sup>-1</sup>·day<sup>-1</sup>. Extremely high-carbohydrate, glycogen-loading diets consumed for several days after endurance exercise will prevent or inhibit the rapid restoration of IMCL stores. Glycogen resynthesis proceeds rapidly in the first hours whereas IMCL start accumulating after a lag time of a few hours. Therefore when both muscle glycogen and lipid replenishment is required, as is presumably the case before very prolonged endurance exercise, it is suggested to initiate recovery with rapidly absorbed carbohydrates followed by addition of lipids in a second phase.

### Key words:

Exercise, recovery, diet, muscle glycogen, muscle triacylglycerol, intramyocellular lipids (IMCL), protein

## Zusammenfassung

*Ernährung und der Wiederaufbau von Energiespeichern im Muskel nach einer physischen Aktivität*

Während der Erholung nach einer physischen Aktivität spielt die Ernährung im Rahmen der Rehydratation und des Wiederauffüllens von Energiespeichern im Körper eine zentrale Rolle. Ernährungsmaßnahmen unterstützen zudem auch die anabole Stoffwechsellage, die durch Muskelkontraktionen hervorgerufen wird und zu einer belastungsbedingten Adaptation führt. Wenn die Erholungsphase bis zur nächsten physischen Aktivität kurz ist, ist es umso wichtiger, die Ernährung auf eine aktive Art und Weise und zu geeignetem Zeitpunkt durchzuführen.

Die Wiederauffüllung des Muskelglykogens ist effizienter, wenn schnell verfügbare Kohlenhydrate sofort nach Ende der physischen Aktivität in ausreichender Menge (ca.  $1 \text{ g}\cdot\text{kg Körpermasse}^{-1}\cdot\text{h}^{-1}$ ), bevorzugt als Getränk, aufgenommen werden. Unter optimalen Bedingungen ist das Wiederauffüllen der Speicher in 24 Std. erreicht. Proteine spielen, wenn gemeinsam mit Kohlenhydraten aufgenommen, eine untergeordnete Rolle in der Glykogenresynthese. Die Aufnahme von Proteinen, besonders von essenziellen Aminosäuren, während oder unmittelbar nach einer physischen Belastung ist jedoch zur Wiederherstellung von Gewebe und der Stimulierung von Muskelbildung wichtig. Dieser Ansatz findet Anwendung für physische Aktivitäten, die intermittierende Muskelarbeit einbeziehen. Der Aufbau von intrazellulären Triacylglycerole wird innerhalb 24 Std. durch Aufnahme von 2 g Fett·kg Körpermasse<sup>-1</sup>·Tag<sup>-1</sup> erreicht, wobei der Wiederaufbau intramuskulärer Triacylglycerole erst mit einer Verzögerung von einigen Stunden beginnt. Extrem kohlenhydratreiche, Glykogen aufbauende Ernährungsweisen hemmen den Wiederaufbau von intramuskulären Triacylglycerolen über mehrere Tage. Nach langen Ausdauerleistungen und als Vorbereitung einer weiteren lang andauernden Belastung ist ein Auffüllen von Muskelglykogen, gemeinsam mit einer Regeneration der Lipidspeicher, erwünscht. Deshalb wird geraten, den Aufbau mit Kohlenhydraten zu beginnen und in einem zweiten Schritt Lipide hinzuzufügen.

Schweizerische Zeitschrift für «Sportmedizin und Sporttraumatologie» 51 (1), 31–38, 2003

## Introduction

Intense or prolonged physical exercise leads to fatigue, which is the inability to immediately perform exercise at the same levels of intensity and duration. This condition involves the loss of body water, the depletion and exhaustion of fuel stores, imbalances in the status of nutrients (including sodium, lipids and amino acids) and various homeostatic disturbances of hormones and the immune system. Recovery following exercise is the physiological process of reassembling the energy and physiological resources until one is able to deliver maximal performance once more. Food and drinks, rest, sleep and time are all elements necessary for the

successful restoration of the athletic functions of the body and mind. Nutrition is only one part of the recovery process, but it is of vital importance for rehydration, refueling the body and replacing tissue proteins that have suffered wear and tear during the exercise.

The main focus of this review is the restoration of muscle glycogen and lipid stores after exercise, and the nutritional recommendations that can be derived from applied scientific research to optimize this process. Nutritional facts that have been established during the period of recovery will be recalled, but only the more recent findings will be developed and references to many of them will be given. This choice should not mislead the reader into believing that this topic is just being discovered. However,

recent developments in proton magnetic resonance spectroscopy ( $^1\text{H-MRS}$ ) have provided new insights into the uniqueness of the lipids located in muscle fibers and their relation to diet and exercise, and will be discussed in more detail.

Recovery is not only a period needed to offset the disturbances caused by exercise. It is also an active phase during which the anabolic stimuli triggered by muscle contraction start developing their effects. Although this review does not address long-term training changes such as quantitative muscle gain, dietary proteins will be discussed in as much as they participate in determining a favorable state to initiate training adaptations.

The subject will be introduced by a short summary of the substrates used by the muscle during exercise. For a broader recognition of the principles of nutritional recovery after exercise as established by research, the reader is referred to [10] or [31] depending on his preferred language.

## Use of muscle energy stores during exercise

### Glycogen

Glycogen is the storage form of carbohydrates in the body. Its concentration in muscle of someone consuming a mixed diet averages  $15 \text{ g}\cdot\text{kg wet mass}^{-1}$ . Glycogen is used during exercise in proportion to the work intensity. Its stores in the muscle cells (about  $0.5 \text{ kg}$ ) may easily be reduced to 20%, even concurrently with the ingestion of sports drinks during the exercise. Higher glycogen contents at the onset of exercise result in higher rates of use. Regeneration of muscle glycogen is crucial before another hard bout of exercise can be performed.

### Lipids

During prolonged exercise, body lipids can provide approximately as much energy as body glycogen, such that  $0.2$  to  $0.3 \text{ kg}$  of body fat may be utilized. If this fat came from adipose tissue lipids, which amount to some  $10 \text{ kg}$ , even in a lean male, this would be of little consequence. However, up to half of the lipids that serve as fuels for exercise are stored locally as droplets in the muscle cells. Their content in muscle averages  $5 \text{ g}\cdot\text{kg wet mass}^{-1}$ . Intramyocellular lipids (IMCL<sup>1</sup>), the mass of which is composed of triacylglycerol, may decrease to 20% of their resting level during endurance exercise. Higher muscle lipid contents at the onset of exercise seem to result in higher rates of use [43]. The issue of whether females consume more myocellular triacylglycerol, as reported using biochemical determination [36] is still debatable, since a preliminary report using  $^1\text{H-MRS}$  indicates the contrary [51]. So far, a direct link between IMCL levels and endurance performance has not been established, but it is less than 10 years since non-invasive technology has permitted investigations into the causes and factors involved in IMCL fluctuations. In contrast, the relationship of glycogen concentration in muscle with diet and performance is 35 years old.

Glycogen is used for the benefit of the muscle where it is stored. Muscles that remain inactive during work are not depleted. This has implications on the need for replenishment of glycogen, depending on whether the same or other muscles are going to be recruited in the follow-up exercise. The same property may also apply to lipids since mTAG data obtained in *extensor* muscle after one-legged knee extension exercise [38] and IMCL data obtained in *soleus* muscle [26] have shown lower lipid concentrations in the exercised versus the inactive muscle.

### Proteins

Proteins are subject to constant remodeling mediated by variable rates of synthesis and breakdown. They are not used quantitatively

to any great extent during the exercise itself unless the glycogen stores are depleted, but many of their component amino acids – the building blocks of proteins – are subject to considerable conversions and inter-organ trafficking. For example, the branched-chain amino acids (BCAA: leucine, isoleucine, valine) and glutamate are increasingly metabolized in muscles during exercise. Glutamine and alanine are exported from muscle to liver and kidney where glucose can be produced from them *de novo*. The increased diversion of energy and amino acids towards events of muscle contraction during exercise suggests that the availability of substrates may limit postexercise muscle protein repair.

## Recovery and the option to let nature follow its course

There is only a weak relationship between the energy and nutrient deficits induced by physical exercise and subsequent spontaneous dietary intake. Contrary to a commonly held view, there is usually no automatic increase in hunger or energy intake as a result of exercise-induced energy deficit. If exercise is intense, appetite may even be suppressed. The drive to restore energy balance is slow to operate, certainly slower than what is recommended for a fast recovery to be able to perform once again.

It is entirely possible to neglect any intentional action to promote recovery, because recovery will be achieved slowly by a spontaneous eating and drinking behavior guided by thirst and appetite. This natural course is sufficient for someone who has no particular athletic ambition and who has several days in front of him to fully recover until the next challenge. Someone whose primary motivation for practising sports is to control body weight and reduce body fat will not have to rush to restore his energy balance. On the other hand, those wishing to train often and hard for a rapid and injury-free progression, or those considering two or more demanding physical workouts with little interval between, will be aware of the need to shorten recovery and will want to take appropriate dietetic measures. Only active feeding practices, sometimes against one's «gut feelings» will yield optimal results.

## Restoration of muscle glycogen

Restoration of muscle glycogen proceeds faster when the stores are depleted than when they are not. It is believed that activation of the enzyme glycogen synthase and facilitation of glucose transport across the muscle fiber membrane act in concert to speed up glycogen resynthesis after glycogen has been depleted to very low levels. For instance, Price et al. [33] compared the rate of muscle glycogen recovery after a depletion of equal magnitude starting from a normal or from a glycogen-loaded situation. They found that the rate of glycogen resynthesis was faster when the glycogen remaining after the exercise was lower, and concluded that the absolute level remaining was a more decisive factor than the degree of depletion. Furthermore, two distinct pools of glycogen in muscle with different granular structures (proglycogen and macroglycogen) appear to be resynthesized at different rates in response to carbohydrate intake [1].

Total muscle glycogen resynthesis occurs in a biphasic pattern. The first phase is short (less than 1–2 hours), rapid and insulin independent. The second phase is insulin dependent, and although it can proceed in the absence of carbohydrate intake glycogen resynthesis will be very slow. When carbohydrates are ingested, blood glucose and insulin are raised and glycogen resynthesis is accelerated. There are several factors related to the ingestion of carbohydrates that have been described to influence glycogen storage. The quantity of carbohydrate and the timing of ingestion at the end of the exercise are the most important ones. Other factors that play a role in glycogen storage are the type of carbohydrate, the form of ingestion (liquid or solid) and finally whether the carbohydrate supplement is taken alone or is co-ingested with protein.

<sup>1</sup> Other acronyms in use include mTG or mTAG (muscle triacylglycerol), MCTG (myo-cellular TG), IMTG and imcTG, but they are not strictly interchangeable with IMCL [18]. IMCL is used in the text for values determined by  $^1\text{H-MRS}$  or stereological methods.

### *Amount of carbohydrate*

The primary factor required to replenish liver and muscle glycogen is the amount of carbohydrate ingested. A direct relationship between carbohydrate intake and the rate at which glycogen is resynthesized is well established, with a maximal postexercise storage rate over a day achieved at intakes of about 7–10 g carbohydrate per kg body mass ( $\text{g}\cdot\text{kg}^{-1}$ ). A recent confirmation of this notion was provided by Fairchild et al. [15], who found that 10 g carbohydrate $\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  led to supranormal amounts of muscle glycogen within only 24 h after short bouts of near-maximal exercise. Such a brief and intense glycogen depletion technique occurs with little total energy expenditure, which might facilitate the complete restoration of muscle reserves during the subsequent day.

### *Timing of carbohydrate ingestion*

The second factor required to replenish body glycogen effectively, is timing. If the next exercise session takes place less than half a day after the first one, carbohydrate feedings should be initiated immediately at the end of the first session. Thus, one takes advantage of the fast storage phase that follows glycogen depletion and which only lasts a couple of hours. If carbohydrate ingestion is delayed after the end of exercise for any reason (such as priority being given to fluid intake, lack of appetite or simple negligence), the rate of glycogen accretion may be slowed by half. For instance, Levenhagen et al. [29] found that leg glucose uptake, which is related to glycogen resynthesis in leg muscles, was increased 3-fold above basal when supplemented immediately post exercise, but only 40% above basal when supplemented 3 hours after. The amount of carbohydrates required for an optimal rate of glycogen resynthesis during the first 1 to 4 hours of recovery approximates 1.2 to 1.5  $\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$  [19]. A recommended strategy is to ingest a bolus drink as soon as possible, then follow with smaller quantities every 30 min or every hour. Because a large volume of liquid in the stomach is a key determinant for fast gastric emptying, this will ensure the immediate delivery of substantial amounts of carbohydrate to the small intestine, from which glucose can pass to the blood without delay at a rate that remains high for several hours.

### *Type of carbohydrate*

Another factor to consider is the type of carbohydrate. Glycogen is made of glucose molecules, so that dietary carbohydrates made of glucose (starch, maltodextrins, dextrose) are its most natural dietary precursors. Fructose is a sugar that seems to be easily stored in liver glycogen. However, fructose and fructose-containing sugars (e.g. sucrose – table sugar) are not optimal for muscle glycogen storage because they are less insulinogenic than glucose, and because fructose needs to first be transformed in the liver before it can be released into the circulation as glucose and taken up by muscle. For instance, Bowtell et al. [9] compared glucose and sucrose drinks and found that a glucose polymer drink (330 ml, concentration 18.5%) promoted a slightly more rapid storage of glycogen in skeletal muscle during the 4-h period of recovery than an isoenergetic sucrose drink. This was supported by the lower plasma glucose concentration produced by the sucrose drink, which was attributed to the fructose moiety of the sucrose. Part of this effect may be related to the lower osmolality of the polymer drink facilitating gastric transit, however the calculated rates of gastric emptying of both drinks were similar. It should be noted that the amount of carbohydrate ingested, expressed per hour, was small ( $0.2 \text{ g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ). In another study, Piehl Aulin et al. [32] compared two energy equivalent, concentrated carbohydrate drinks (150  $\text{g}\cdot\text{l}^{-1}$ , 300 g carbohydrate over 90 min), one containing a polyglucoside (osmolality 84  $\text{mosmol}\cdot\text{l}^{-1}$ ), and one containing monomers and oligomers of glucose (350  $\text{mosmol}\cdot\text{l}^{-1}$ ) on muscle glycogen resynthesis. Mean glycogen synthesis rate was significantly higher during the initial 2 h after the polyglucoside drink compared with the glucose drink (10 vs. 6  $\text{mmol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ). These data indicate that the osmolality of the carbohydrate drink

may influence the rate of resynthesis of glycogen in muscle. It is tempting to speculate that the transit of the hypotonic polymer drink through the stomach was faster than that of the hypertonic glucose drink, however mean blood glucose and insulin concentrations did not differ between the two drinks, which argues against this explanation. Of the two chief physical factors defining the concentration of carbohydrate solutions (mass concentration and molar concentration), it is clear that osmolality plays a weaker role on the speed of gastric emptying than energy density, or the weight of the carbohydrate dissolved. This was again recently confirmed (preliminary data) when gastric osmolality was found to have little effect on the gastric emptying rate, at least of dilute carbohydrate solutions [39]. The reason why, despite this, more energy dense hypotonic carbohydrate drinks may promote faster glycogen storage than hypertonic ones is unclear.

### *Glycemic index*

The glycemic index of a carbohydrate or a carbohydrate-rich food is a normalized score of the magnitude of the rise in blood glucose induced by its ingestion. As a fast, meaningful, and prolonged elevation in blood sugar concentration over a few hours is required for optimal glycogen resynthesis, high glycemic index carbohydrates ingested at frequent intervals following exercise are the best choice. This is not difficult to achieve with beverages, as most of them are composed of easily available sugars with a high glycemic index. If the supplement is a food (e.g. an energy bar), some critical sense is occasionally needed to select the one with an appropriate formulation. Combinations of different nutrients in a food, such as lipids or fiber, can reduce the glycemic index of the carbohydrate component.

### *Liquid and solid carbohydrate supplements*

As the immediate postexercise phase usually overlaps with the need to achieve complete rehydration, the most practical form of carbohydrate at this time is a sports drink. Furthermore, following strenuous exercise a drink is often more easily tolerated by the gut than a solid food and it may be the only practical way to deliver carbohydrate energy effectively. Another occasion when beverages are preferred is when the recovery time before the next exercise is short: carbohydrates in solution transit through the stomach with less delay, they increase blood glucose and insulin earlier than solids [e.g. 35] because of the time needed for digestion of the solid food mass. With a food, there may be further delay due to fat or dietary fiber components. The exact formulation of a post-exercise carbohydrate drink, although important, is probably less critical than that of a drink consumed during exercise, when gastrointestinal function is compromised and concurrent needs compete with each other (e.g. optimal rehydration competing with optimal energy supply). Overall, the consumption of concentrated carbohydrate solutions (up to 30% by weight) enhances carbohydrate delivery and glycogen synthesis, despite impaired gastric emptying and fluid replacement.

When there is plenty of time for recovery (one day or more), the form of ingestion, the type and glycemic index of the carbohydrates, are much less important. The consumption of regular carbohydrate-rich foods and more balanced meals can be resumed promptly after the first hours.

### *Additional role of proteins*

The efficacy of a particular carbohydrate in promoting resynthesis of glycogen stores is largely dependent on the glucose and insulin responses to the carbohydrate load. Attempts have been made to boost the insulin response of carbohydrates through co-ingestion of proteins or amino acids, because this may increase insulin concentration above that reached with carbohydrates alone. Early investigations suggested higher rates of glycogen resynthesis with, rather than without co-ingestion of proteins. Subsequently, the addition of arginine ( $0.08 \text{ g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ), one of the most effective insulin secretagogues, to carbohydrate (maltodextrin,  $1.0 \text{ g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ )

did not increase the rate of glycogen storage, although significance was approached [50]. These results were difficult to interpret because, due to the addition of the nitrogenous component to that of carbohydrate, the diets were no longer isoenergetic. Moreover, as found later, the carbohydrate supply was suboptimal, leading perhaps to overstating the benefit of the amino acid component. Another study investigated the effect of incorporating whey protein and arginine, both of which have specific stimulating effects on insulin secretion, into a high carbohydrate drink [37]. A preparatory trial confirmed that plasma insulin concentrations were significantly higher after ingestion of a solution of carbohydrate based on fermented cereals associated with protein plus arginine, compared with ingestion of carbohydrate alone. The drinks in the study were isoenergetic and provided carbohydrates at a rate presumed to saturate digestion and glycogen synthesis. The carbohydrate drink ( $1.7 \text{ g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ), and the protein-carbohydrate drink ( $0.5 \text{ g protein}\cdot\text{kg}^{-1}\cdot\text{h}^{-1} + 1.2 \text{ g carbohydrate}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ) were ingested after a glycogen depleting cycle exercise. This resulted in equal glycogen resynthesis in the quadriceps muscles over the next 4 hours [37]. Carrithers et al. [12] also investigated the effect of carbohydrate (glucose  $1.0 \text{ g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ) with the isoenergetic substitution (25%) of either protein from milk or a mixture of essential free amino acids. They found no difference in glycogen resynthesis in the first 4 h of recovery from a glycogen depleting exercise. In a protocol identical to that used to study different carbohydrates [9], Bowtell et al. found that ingestion of glutamine (8 g) promoted the storage of muscle glycogen to an extent similar to that after ingestion of glucose polymer [8], but the amount of carbohydrate ingested was modest ( $0.2 \text{ g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ) and there was no control group without supplement, which would have been useful in this design. In a systematic series of studies, van Loon et al. first identified leucine, phenylalanine and tyrosine as having strong correlations with the insulin response when co-ingested with carbohydrates in resting conditions [49]. In a second step, they addressed the postexercise period and found that a mixture of leucine, phenylalanine and arginine (alone or together with a wheat protein hydrolysate) produced a large insulinotropic effect when ingested in combination with carbohydrates [47]. Having identified compositions that markedly elevate insulin levels and plasma amino acid availability, they then examined the effect of a dietary manipulation in which the nitrogenous component was either included in exchange for carbohydrate (isoenergetic supplement), or added to the carbohydrate content (isocarbohydrate supplement) on glycogen storage [48]. When  $0.4 \text{ g hydrolysate per kg body mass per hour}$  was added to  $0.8 \text{ g carbohydrate}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$  (i.e. isocarbohydrate), the insulin response and muscle glycogen synthesis were elevated compared with the carbohydrate alone, however  $1.2 \text{ g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$  carbohydrate without amino acids (i.e. isoenergetic) was as efficient, if not more, as carbohydrate plus amino acids in increasing glycogen synthesis. Subsequently, this group reassessed the issue of the upper limit of carbohydrate that would show an effect [23]. They added proteins to a larger intake of carbohydrate ( $1.2 \text{ g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ). Although the addition of  $0.4 \text{ g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$  protein and amino acids to carbohydrate during recovery from exercise increased insulin levels, there was no difference in plasma glucose nor muscle glycogen synthesis. Similarly, van Hall et al. [46] compared the intake of carbohydrate (sucrose  $1.25 \text{ g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ) with that of the same amount of carbohydrate plus a whey protein hydrolysate ( $0.30 \text{ g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ). Because they used sucrose, which is not as insulinogenic as glucose or maltodextrin, and selected whey protein hydrolysates, which are known to be easily digestible and to induce a high insulin stimulation, the conditions were such as to maximize the difference in insulin stimulation between treatments, despite the high level of carbohydrate intake. Nevertheless, although the carbohydrate+whey supplement resulted in higher circulating insulin concentrations than the carbohydrate alone, these authors found equal rates of glucose uptake and glycogen storage in leg muscles over the next 4 hours. They suggested that the difference might have been caused by reduced glucose appearance from the gastrointestinal tract in relation to the higher energy load of the protein-carbohydrate drink. Finally, Ivy

et al. [20] reassessed the issue of isoenergetic versus isocarbohydrate carbohydrate-protein supplements. In their study, glycogen resynthesis was followed by  $^{13}\text{C}$ -NMR during 4 hours of recovery with a better time resolution than is permitted by the invasive biopsy method. The carbohydrate-protein supplement (carbohydrate  $0.55 \text{ g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ , protein  $0.2 \text{ g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ) ingested early after and 2 hours after the end of exercise resulted in greater glycogen resynthesis than with carbohydrate alone, both at equal carbohydrate content and at equal energy content. The observed difference was due to higher rates of glycogen synthesis only occurring in the first 40 min of recovery, not later.

Together, these data underscore the importance of early postexercise refeeding and mitigate the role of high circulating insulin *per se*, as well as the relevance of proteins and insulinogenic amino acids, on glycogen storage. To maximize the rate of muscle glycogen storage during short-term recovery, it is concluded that a large carbohydrate supplement ingested as soon after exercise as possible is the most effective option. If less carbohydrate consumption is desired, a similar rate of glycogen storage can be achieved with the addition of protein and amino acid supplements. Furthermore, if the results of the study by Ivy et al. [20] can be confirmed, they suggest that a carbohydrate-protein supplement would be advantageous when the recovery time is extremely limited.

### Protein and tissue repair

The ingestion of protein together with carbohydrate would be of moderate interest if glycogen restoration was the only of one's concerns. However, the addition of protein with carbohydrate may stimulate amino acid uptake and protein accretion. This could be important for rapid tissue repair and initiation of muscle building. The coincidence of three factors is important to activate net protein synthesis: the stimulus of muscle contraction, the availability of insulin, and the availability of amino acids. Presumably this is best achieved with carbohydrate-protein ingestion during recovery. Recently, Levenhagen et al. [29] reported that, similar to carbohydrate homeostasis, ingestion of a nutrient supplement early after 60 min of moderate-intensity cycling exercise (rather than 3 hours later) enhanced accretion of whole body and leg protein, suggesting a common mechanism of exercise-induced insulin action. In an extension of this study, the authors compared the effect of a protein + carbohydrate ( $10 \text{ g} + 8 \text{ g}$ ) supplement with that of carbohydrate alone on protein homeostasis [28]. The combined supplement resulted in a net leg uptake of essential amino acids and net whole-body and leg protein gain. In contrast, carbohydrate alone resulted in a net loss of protein. Larger amounts of carbohydrates, such as those needed for glycogen replenishment ( $\sim 200 \text{ g}$ ), may be more anabolic than the small quantity given here ( $8 \text{ g}$ ) over 3 hours, but the findings suggest that the availability of amino acids is more important than the availability of energy for post exercise repair and synthesis of muscle proteins.

Essential amino acids, including the branched-chain amino acids and particularly leucine, seem to be the most effective. In a recent study,  $8 \text{ g}$  of BCAA or a placebo was given to subjects during ergometer cycle exercise and a 2-h recovery period [3]. The results suggested that BCAA had a protein-sparing effect, either protein synthesis had been stimulated and/or protein degradation had decreased. The effect did not seem to be mediated by insulin. Together with other data [7], this suggests that relatively small amounts of essential amino acids stimulate protein synthesis during recovery. Milk proteins are good dietary sources of essential amino acids and preference should be given to fractions that are digested and released faster from the stomach, such as the proteins from whey (in contrast to casein).

### Timing of protein ingestion

The ingestion of an amino acid-carbohydrate solution ( $6 \text{ g}$  essential amino acids,  $35 \text{ g}$  sucrose in  $500 \text{ ml}$ ) before resistance exercise

was recently shown to result in a greater response of net protein synthesis than that obtained when the solution was consumed after the exercise [45]. This may be due to a greater delivery of amino acids to the muscles when blood flow is elevated, resulting in better permeation and higher tissue concentration of precursor molecules at the end of exercise, when energy can again be released for anabolic purposes. Furthermore, this effect observed acutely in the first 2 hours of recovery seems to be reflected in a positive nitrogen balance over the full 24-h period [44], suggesting that muscle mass could increase if the intervention was carried out over longer periods of time, in comparison with sedentary activities without supplement. The latter result obtained over 24 hours is not quite unexpected, since the term of comparison was a 24-h period of very sedentary activities, a situation which tends to be catabolic. It remains to be demonstrated whether exercise plus the solution promotes a more positive 24-h protein balance, considering potential influences of physical exercise on protein metabolism during the rest of the day (meals, night period). The concept of pre-exercise ingestion for a postexercise outcome is appealing for muscle activities involving intermittent resistance work. It would not be advised in relation with long-lasting aerobic exercise, where cardiocirculatory and gastrointestinal limitations are predominant and the nutritional priority must be given to maintaining hydration and blood glucose. Flooding the body with urea-producing proteins before or during endurance exercise would only add a further load to the already metabolically stressed kidneys.

### Restoration of muscle triacylglycerol

Until recently, changes occurring in myocellular triacylglycerol concentrations during recovery from exercise were not well documented, although it has been supposed for a long time that variations of the lipid intake could be a major factor contributing to the fluctuation of TAG stores in skeletal muscles. Using  $^1\text{H-MR}$  spectroscopy (see footnote 1), Boesch et al. [6] reported that the time constant of IMCL recovery was ~40 h in one male subject who experienced a 40% exercise-induced fall in IMCL, but dietary intake during that time was self-selected and not controlled.

#### Low lipid intake

Stereological [42], biochemical [25, 41] as well as magnetic resonance spectroscopy [5, 13, 14] studies indicate that when a low fat diet (<20–25% by energy) or, in other words, a very high carbohydrate diet, is consumed after exercise to speed up glycogen reloading, IMCL fail to return to their pre-exercise concentrations until three [27] or even five to seven days [5, 42] of recovery. It was also repeatedly observed that IMCL continue to decrease for at least one day after the end of exercise on such diets, in line with the fact that whole body fat oxidation remains elevated after exercise. In studies where mTAG concentrations did not decrease during exercise, they did afterwards if low-fat recovery diets were consumed [25, 41]. This led Kiens et al. to suggest [25] that muscle glycogen resynthesis has such a high metabolic priority during recovery that combustion of lipids is necessary to cover energy expenditure in muscle and that mTAG provides a substantial part of it. However, their hypothesis is difficult to reconcile with the view that a low fat (high carbohydrate) diet increases glucose availability, thereby increasing muscle malonyl coenzyme A, which in turn suppresses the transfer of fatty acids into the mitochondria for subsequent oxidation. On the other hand, physical activity inhibits malonyl-CoA during both exercise and the recovery period [34].

#### High lipid intake

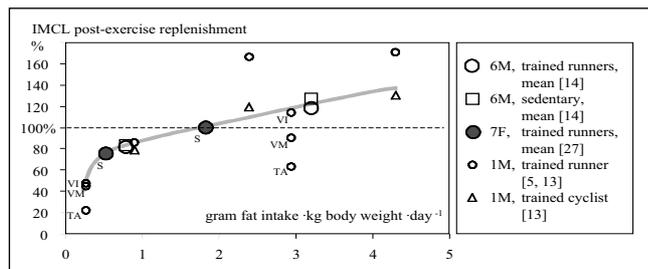
In contrast, Jansson and Kaijser [21] found that feeding a high-fat diet for five days resulted in 80% higher mTAG concentrations than feeding a low-fat diet. The investigation of Starling et al. [41] was the first, to the author's knowledge, to examine the effect of different dietary compositions on mTAG concentration during an

acute period after prolonged exercise. Subjects ingested an isoenergetic high-fat (68% of energy) or low-fat (5%) diet following a 2-h cycling exercise. After a day of recovery, total mTAG concentration was higher (121% of resting levels) for the high-fat compared with the low-fat trial (83%). Expectedly, the effect of high-fat diets on the rate of postexercise IMCL recovery is not identical in different muscles. This is illustrated by the case of a marathon runner, whose IMCL content in *tibialis anterior* (TA), *vastus medialis* (VM) and *vastus intermedius* (VI) muscles after 2 days of recovery on a 63% fat energy diet each reached a different value relative to the pre-exercise concentration (TA 70%, VM 109% and VI 193%) [5]. Concentrations after a low-fat diet (6% energy) in the same muscles were then only 47–48% of initial in all three leg muscles. A practical question is to understand how IMCL content responds to different amounts of dietary lipids. In a pilot study with two subjects, three widely different levels of lipids were fed in isoenergetic diets for 32 hours after running for 2 h [13]. The degree of IMCL storage in TA muscle was minimal with the lower-fat diet (15% energy), whereas with both the intermediate (40%) and the higher-fat (70%) diets, its concentration reached similarly high values, indicating that a maximal effect on 24-h storage was to be expected already at the intermediate fat intake. Interestingly, the absolute storage rate with both intermediate and high-fat diets was much greater in the subject who was a trained runner (3.1 and 3.2 mmol·kg muscle wet weight<sup>-1</sup>·24 h<sup>-1</sup>) than in the subject who was a cyclist of equal  $\text{VO}_2\text{max}$  (0.7 and 0.9 mmol·kg ww<sup>-1</sup>·24 h<sup>-1</sup>) [13]. The large variation in IMCL storage observed between muscles and individuals involves a number of factors such as muscle type, training status and specificity, effective recruitment in the work, initial content, degree of depletion by exercise, and possibly uncontrolled physical activity during the replenishment period. The exact role of each needs clarification.

One of the major physiological adaptations to endurance training is to enhance the capacity for fat metabolism (fatty acid extraction by muscle, transport protein capacity, activities of carnitine palmitoyltransferase I and oxidative enzymes, larger IMCL stores). We investigated if there was evidence of a similar metabolic adaptation to training in the ability to speed up IMCL storage [14]. Trained and sedentary men underwent ~25% IMCL depletion by running for two hours at ~50%  $\text{VO}_2\text{peak}$ . When they consumed a 55% fat energy diet during recovery, IMCL concentrations in the TA muscle returned fully to resting levels after 15 h in both groups. In line with this observation, no difference between resting trained and untrained muscles was found in the expression of diacylglycerol acyltransferase, an intrinsic membrane protein providing the crucial final step in IMCL synthesis (Schmitt et al., unpublished observation). Independently, trained females who were depleted to the same extent as in the above study recovered in 22 h on a diet containing 35% fat energy [27], the proportion found in a typical normal diet. Clearly present literature indicates that with suitable amounts of lipids in the diet, postexercise IMCL stores can be completely replenished in less than a day, in both athletes and sedentary individuals.

#### Summary of amount of dietary lipids

Evidence has been provided that IMCL replenishment is strongly and positively dependent on the intake of dietary fat, like glycogen is on the intake of carbohydrate. Minimal lipid intake during recovery leads to no change or a further depression in IMCL concentrations. Very high fat intake for as little as one day may cause an overshoot of IMCL concentrations to between 120 and 170% of their initial levels. The idea that muscle triacylglycerol could be supercompensated, like glycogen, is not new. In 1989 for example, the question was raised if it was also possible to «lipid load» [42]. At this point in time, no recommendation can be made to target such high lipid storage, neither from the standpoint of performance nor of safety. Conversely, it would be wise to avoid emptying IMCL stores prior to undertaking a prolonged competitive exercise. Consequently a balance should be found such that average IMCL concentrations are maintained. A compilation of



**Figure 1:** Degree of IMCL replenishment (expressed as % of pre-exercise concentrations) reached one day after recovery from exercise at different levels of lipid intake. IMCL concentrations decreased about 30% during exercise (running for 2–3 h at intensities of 70–50%  $\text{VO}_2\text{peak}$ ). On average, 2 g dietary lipids·kg body mass·day<sup>-1</sup> are needed to achieve repletion (= 100%) in 24 h. <sup>1</sup>H-MRS data are from four studies [5, 13, 14, 27] in different muscles from the calf (TA, *m. tibialis anterior*; S, *m. soleus*) and the thigh (VM, *m. vastus medialis*; VI, *m. vastus intermedius*). Measurements are made in TA if not indicated otherwise. Trendline is in gray.

available IMCL data in relation to the amount of lipid ingested (*Figure 1*) shows that about 2 g dietary lipids·kg<sup>-1</sup>·day<sup>-1</sup> will achieve full replenishment of IMCL to pre-exercise levels in 24 hours. Therefore, a 65-kg athlete on a daily energy intake of 12.6 MJ (3200 kcal, protein 13%E, carbohydrate 50%E) will reach this goal with 37% of his diet energy as lipids.

*Table 1* compares the rate of storage of both major muscle energy substrates (glycogen, IMCL) in relation with the provision of their respective dietary substrate (carbohydrates, lipids). The only study with biochemical determination of mTAG on which similar calculations could be made is that of Starling et al. [41]. Recalculation of these data indicates rates of storage of ~2.4 mmol·kg ww<sup>-1</sup>·d<sup>-1</sup> with the high lipid intake (3.6 g·kg<sup>-1</sup>·d<sup>-1</sup>) and -0.7 mmol·kg ww<sup>-1</sup>·d<sup>-1</sup> with the low lipid intake (0.3 g·kg<sup>-1</sup>·d<sup>-1</sup>), which is coherent with the IMCL values in *Table 1b* obtained by MRS. In terms of rate of energy storage during the first 24 h of recovery, the energy that is stored in muscle glycogen at high carbohydrate intake is approximately 2.5-fold the energy that is stored in IMCL at high lipid intake (*Table 1c*). It is unknown whether this difference is related to the relatively smaller degree of depletion of the IMCL (by ~30%), prior to the present repletion data, compared with that of the glycogen (depletion by >50%) in the majority of the studies used to derive glycogen storage rates.

*a.* Hourly glycogen storage rates at high and low intakes of carbohydrates

Dietary carbohydrate intake g·kg body mass <sup>-1</sup> ·h <sup>-1</sup>	Glycogen storage rate mmol·kg ww <sup>-1</sup> ·h <sup>-1</sup> (a)
<b>High intake</b>	fast phase (up to 4 hours) <sup>(b)</sup> 4 to 10
0.7 to 1.5	
<b>Low intake</b>	1 to 2
0.0 to 0.1	

*b.* Daily IMCL storage rates at high and low intakes of lipids

Dietary lipid intake g·kg body mass <sup>-1</sup> ·day <sup>-1</sup>	IMCL storage rate mmol·kg ww <sup>-1</sup> ·day <sup>-1</sup> (a)
<b>High intake</b>	1 to 3
2 to 4	
<b>Low intake</b>	-1 to 1
0.3 to 0.9	

*c.* Comparative rates of storage of muscle energy substrates over the first 24 hours

Dietary intake g·kg body mass <sup>-1</sup> ·day <sup>-1</sup>	Storage rates	mmol·kg ww <sup>-1</sup> ·day <sup>-1</sup>	kJ·kg ww <sup>-1</sup> ·day <sup>-1</sup>
<b>High 24-h carbohydrate</b>	<b>Glycogen</b>	85	240
<b>High 24-h lipid</b>	<b>IMCL</b>	3	100

**Table 1:** Rates of storage of energy substrates in muscle during recovery from exercise.

(a) «kg ww» is for «kg of muscle wet weight»

(b) carbohydrates with or without up to 30% protein

*Precursor molecules for IMCL formation*

At present, it is unclear which metabolic factors determine IMCL formation. A high fat meal is followed by an elevation of plasma chylomicron TAG lasting 3–5 h, the clearance of which is improved by prior exercise. With fasting, and presumably postexercise, muscle lipoprotein lipase (LPL), a key enzyme in the capillary endothelium regulating the disposal of plasma TAG, is upregulated. It is reasonable to assume an increased uptake of TAG-derived fatty acids by IMCL depleted muscles. However, although exercise reduces postprandial lipemia substantially, direct measurements of substrate extraction across the leg showed no increase in the absolute amount of TAG captured by muscles after a fat meal [30]. This suggests that other mechanisms such as decreased hepatic TAG secretion must contribute to the lower postprandial lipemia after exercise. Alternatively, other studies have investigated the role of plasma free fatty acids as plausible precursors of IMCL. In resting conditions, hyperinsulinemic-euglycemic clamps were performed in combination with lipid and heparin infusions to study the influence of short-term increases of FFAs on IMCL concentrations. Four hours after a rise in FFA to 1.8 mmol·l<sup>-1</sup>, IMCL levels increased by 33% [4]. Similarly when FFA concentrations were raised to >3 mmol·l<sup>-1</sup> the IMCL pools also increased, by 20% in *soleus* (S) and 60% in TA, 2–3 hours after the start of the FFA infusion [2]. A fasting experiment in which the subjects only received water for three days led to a sustained elevation of FFA and beta-hydroxybutyrate concentrations (both at ~1.5 mmol·l<sup>-1</sup> for more than 30 hours), decreased plasma glucose and insulin, and a doubling of IMCL levels in the VL muscle [40]. In contrast, there are also data indicating that fasting gradually reduces the triacylglycerol content in different types of skeletal muscle [22].

Fatty acids may play a role in postexercise recovery as well. Krssak et al. [26] studied changes in IMCL concentrations in the S muscle following an exercise that decreased their concentrations by 33%. Restoration to pre-exercise values was achieved between 4 and 17 h postexercise, while the subjects were still fasting. During the first four hours of recovery, FFA plasma concentrations were markedly elevated (>1 mmol·l<sup>-1</sup>) whereas plasma glucose remained relatively low (<4.5 mmol). In this study, however, IMCL content was normalized with reference to the water peak, which makes it difficult to correctly interpret these values, because of potential fluid shifts and muscle swelling that can occur after exercise.

Overall, these data suggest that plasma FFAs have a precursor role for the synthesis of IMCL and that elevated insulin is not a prerequisite, but we are far from a clear view of the precursor-substrate regulation of IMCL accumulation in different metabolic states and postexercise. Furthermore, a role of different fatty acid compositions on IMCL modulation is still unknown.

*Rate of IMCL resynthesis*

Lipid infusion studies suggest that 2 to 4 hours is the shortest time over which some degree of IMCL resynthesis may be observed [4, 2]. From investigations with postexercise lipid ingestion, the storage rate might appear linear at high intakes for up to 32 hours into recovery [13, 14, 27], but the evidence is still too limited for a definite statement. What appears persuasively, however, is a contrast between the dynamics of IMCL storage and that of glycogen storage, which is initiated immediately after exercise at a high rate and gradually slows down with time.

*High fat diets for exercise: metabolic adaptation or filling-up a reservoir?*

Renewed attention in recent years for a role of high fat diets on endurance performance has been extensively reviewed [10, 16, 24] and has focused specifically on metabolic adaptations during chronic exposure to dietary fats, a paradigm underlying the design of all investigations. The effect on exercise metabolism and performance in most of these studies has been disappointing.

In contrast, the ability of lipids to serve as a fuel, because of preferential storage inside muscle cells for on site utilization, has been largely ignored. The main reason for this neglect lies in the invasiveness and the technical difficulty of quantifying IMCL accurately with the muscle biopsy technique. Another reason may be that circulating fatty acids increase continuously during the course of prolonged exercise, suggesting that one should focus attention on resolving the metabolic bottlenecks that limit FA access to muscle cells and their mitochondria. There is also the opinion that the body possesses adipose reserves so much in excess of any exercise requirement that the size of the small TAG storehouse localized in muscles is of little or no concern. However, it appears that IMCL are a plastic and dynamic fuel reserve for the muscle just like glycogen. Both stores have approximately the same size (0.1–0.3 MJ per kg muscle), are lowered by exercise, and are replenished (even supercompensated) within a day when an appropriate diet is consumed.

Experimental design will depend very much upon how one interprets the effect of high fat diets on exercise performance. They can be considered to trigger a gradual metabolic adaptation or simply a means of filling a localized and fluctuating energy store. Metabolic adaptation requires imposing a high fat diet for several days or weeks, in combination with a training program. Negative consequences on exercise capacity due to carbohydrate deprivation during fat overfeeding can be avoided by using different modes of dietary intervention. The most common intervention involved consuming a high-carbohydrate diet for one or more days after adaptation to the high-fat diet, to ensure optimal glycogen loading prior to exercise [11].

In contrast, if dietary fat is considered to be simply a substrate to replenish the IMCL stores, an alternative raised by the MRS data, then an understanding of the relationship between provision and storage implies a different approach.

- Since IMCL replenishment can be achieved in one day, the period of lipid supplementation can be as short as one day.
- Since the dynamics of glycogen storage are rapid and those of IMCL are slow, feeding carbohydrate as soon as possible post exercise is critical. Consequently the optimal starting diet should be rich in carbohydrate.
- There is no reason to reduce carbohydrate intake before the next exercise and run the risk of lowering the body glycogen stores. Instead of exchanging lipids isoenergetically for carbohydrates, they should be added to supplement the high-carbohydrate diet on the day before the next bout of exercise.

However, this strategy is still theoretical and needs to be investigated further. In particular, optimal muscle glycogen concentrations must be assured while the lipid supplement is consumed. Preliminary evidence suggests that the proposed dietary scheme results in high glycogen as well as high IMCL stores (Zehnder et al., unpublished observations). It would also be necessary that glycogen utilization during exercise is not impaired as seems to be the case after chronic high-fat feeding.

#### Functions of IMCL

The positive association between muscle TAG levels and insulin resistance (with the noteworthy exception of athletes) suggests caution when proposing recommendations. Excess mTAG stores could be implicated in the pathogenesis of diabetes in sedentary individuals. Muscle triacylglycerol has non-energetic functions which in the end may prove to be more crucial than their role of distilling lipid energy from inside the muscle fiber for aerobic metabolism. These functions include the sequestration of bioactive lipids released from membranes (diacylglycerol and fatty acids) and the provision of building blocks for the formation of membrane phospholipids. The latter participate in the events of cell growth and differentiation and could be important for the regeneration of damaged cells and organelles after exercise.

Today there are indications [17] but no proof that IMCL stores are related to performance. Strong arguments may be put forward

to back up the notion of a possible link: (1) evidence of utilization during prolonged exercise, (2) higher concentrations in muscles of endurance-trained individuals, and (3) close contact areas between IMCL droplets and mitochondria, presumably to facilitate fatty acid transfer and oxidation. However, a conclusion on the role of IMCL for exercise performance must be left for future studies. Overall, it is premature to qualify IMCL as the next «magic bullet» for the endurance performer, but this remains a plausible possibility.

#### Practical conclusions for athletes

- When replenishment of muscle glycogen after exercise is required to be complete within a day, athletes should ingest 1 to 1.5 g carbohydrates per kg body mass as soon as possible after the end of exercise, preferably as a drink. They should then continue to consume carbohydrates at regular intervals for at least 4 hours.
- If they find such high amounts of carbohydrates difficult to tolerate, they may ingest less carbohydrate in a nutrient combination containing 10–20% protein from an easily digestible source such as whey.
- They should then resume eating regular meals with high carbohydrate content. If they have a prolonged physical challenge ahead, the meals should also supply normal amounts of dietary lipids (a «carbonara party» type of meal instead of the «pasta party»).
- For rapid tissue repair and initiation of muscle building, resistance athletes and fitness fans may wish to consume protein-carbohydrate combinations after their exercise session. They could also choose to ingest this type of supplement before the session.

#### Acknowledgment

The author is grateful to K. Acheson and M. Zehnder for reviewing the manuscript.

Address for correspondence:

Jacques Décombaz, Lic. Sc., Dipl. Nutr. (Cantab), Nestlé Research Centre, Nestec Ltd, Vers-chez-les-Blanc, CH-1000 Lausanne 26, Switzerland

#### References

- 1 Adamo K.B., Tarnopolsky M.A., Graham T.E.: Dietary carbohydrate and postexercise synthesis of proglycogen and macroglycogen in human skeletal muscle. *Am. J. Physiol.* 275: E229–E234, 1998.
- 2 Bachmann O.P., Dahl D.B., Brechtel K., Machann J., Haap M., Maier T., Loviscach M., Stumvoll M., Claussen C.D., Schick F., Haring H.U., Jacob S.: Effects of intravenous and dietary lipid challenge on intramyocellular lipid content and the relation with insulin sensitivity in humans. *Diabetes* 50: 2579–2584, 2001.
- 3 Bloemstrand E., Saltin B.: BCAA intake affects protein metabolism in muscle after but not during exercise in humans. *Am. J. Physiol. Endocrinol. Metab.* 281: E365–E374, 2001.
- 4 Boden G., Lebed B., Schatz M., Homko C., Lemieux S.: Effects of acute changes of plasma free fatty acids on intramyocellular fat content and insulin resistance in healthy subjects. *Diabetes* 50: 1612–1617, 2001.
- 5 Boesch C., Décombaz J., Slotboom J., Kreis R.: Observation of intramyocellular lipids by means of <sup>1</sup>H magnetic resonance spectroscopy. *Proc. Nutr. Soc.* 58: 841–850, 1999.
- 6 Boesch C., Slotboom J., Hoppeler H., Kreis R.: In vivo determination of intra-myocellular lipids in human muscle by means of localized <sup>1</sup>H-MR-spectroscopy. *Magn. Reson. Med.* 37: 484–493, 1997.
- 7 Borsheim E., Tipton K.D., Wolf S.E., Wolfe R.R.: Essential amino acids and muscle protein recovery from resistance exercise. *Am. J. Physiol. Endocrinol. Metab.* 283: E648–E657, 2002.
- 8 Bowtell J.L., Gelly K., Jackman M.L., Patel A., Simeoni M., Rennie M.J.: Effect of oral glutamine on whole body carbohydrate storage

- during recovery from exhaustive exercise. *J. Appl. Physiol.* 86: 1770–1777, 1999.
- 9 *Bowtell J.L., Gelly K., Jackman M.L., Patel A., Simeoni M., Rennie M.J.*: Effect of different carbohydrate drinks on whole body carbohydrate storage after exhaustive exercise. *J. Appl. Physiol.* 88: 1529–1536, 2000.
  - 10 *Burke L.M.*: Nutrition for recovery after competition and training. In: *Clinical Sports Nutrition* (eds. L. Burke & V. Deakin), 2<sup>nd</sup> edn., 396–427, 2000. McGraw-Hill, Sydney, Australia.
  - 11 *Burke L.M., Hawley J.A.*: Effect of short-term fat adaptation on metabolism and performance of prolonged exercise. *Med. Sci. Sports Exerc.* 34: 1492–1498, 2002.
  - 12 *Carrithers J.A., Williamson D.L., Gallagher P.M., Godard M.P., Schulze K.E., Trappe S.W.*: Effects of postexercise carbohydrate-protein feedings on muscle glycogen restoration. *J. Appl. Physiol.* 88: 1976–1982, 2000.
  - 13 *Décombaz J., Fleith M., Hoppeler H., Kreis R., Boesch C.*: Effect of diet on the replenishment of intramyocellular lipids after exercise. *Eur. J. Nutr.* 39: 244–247, 2000.
  - 14 *Décombaz J., Schmitt B., Ith M., Decarli B., Diem P., Kreis R., Hoppeler H., Boesch C.*: Postexercise fat intake repletes intramyocellular lipids but no faster in trained than in sedentary subjects. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 281: R760–R769, 2001.
  - 15 *Fairchild T.J., Fletcher S., Steele P., Goodman C., Dawson B., Fournier P.A.*: Rapid carbohydrate loading after a short bout of near maximal-intensity exercise. *Med. Sci. Sports Exerc.* 34: 980–986, 2002.
  - 16 *Helge J.W.*: Long-term fat diet adaptation effects on performance, training capacity, and fat utilization. *Med. Sci. Sports Exerc.* 34: 1499–1504, 2002.
  - 17 *Hoppeler H., Billeter R., Horvath P.J., Leddy J.J., Pendergast D.R.*: Muscle structure with low- and high-fat diets in well-trained male runners. *Int. J. Sports Med.* 20: 522–526, 1999.
  - 18 *Howald H., Boesch C., Kreis R., Matter S., Billeter R., Essen-Gustavsson B., Hoppeler H.*: Content of intramyocellular lipids derived by electron microscopy, biochemical assays, and <sup>1</sup>H-MR spectroscopy. *J. Appl. Physiol.* 92: 2264–2272, 2002.
  - 19 *Ivy J.L.*: Dietary strategies to promote glycogen synthesis after exercise. *Can. J. Appl. Physiol.* 26: S236–S245, 2001.
  - 20 *Ivy J.L., Goforth H.W. Jr, Damon B.M., McCauley T.R., Parsons E.C., Price T.B.*: Early postexercise muscle glycogen recovery is enhanced with a carbohydrate-protein supplement. *J. Appl. Physiol.* 93: 1337–1344, 2002.
  - 21 *Jansson E., Kaijser L.*: Effect of diet on the utilization of blood-borne and intramuscular substrates during exercise in man. *Acta Physiol. Scand.* 115: 19–30, 1982.
  - 22 *Jaromowska M., Gorski J.*: Effect of fasting on skeletal muscle triglyceride content. *Experientia.* 41: 357–358, 1985.
  - 23 *Jentjens R.L., van Loon L.J., Mann C.H., Wagenmakers A.J., Jeukendrup A.E.*: Addition of protein and amino acids to carbohydrates does not enhance postexercise muscle glycogen synthesis. *J. Appl. Physiol.* 91: 839–846, 2001.
  - 24 *Jeukendrup A.E.*: High-carbohydrate versus high-fat diets in endurance sports. *Schweiz. Ztschr. Sportmed. Sporttraum.* 51: 17–23, 2003.
  - 25 *Kiens B., Richter E.A.*: Utilization of skeletal muscle triacylglycerol during postexercise recovery in humans. *Am. J. Physiol. Endocrinol. Metab.* 275: E332–E337, 1998.
  - 26 *Krssak M., Petersen K.F., Bergeron R., Price T., Laurent D., Rothman D.L., Roden M., Shulman G.I.*: Intramuscular glycogen and intramyocellular lipid utilization during prolonged exercise and recovery in man: a <sup>13</sup>C and <sup>1</sup>H nuclear magnetic resonance spectroscopy study. *J. Clin. Endocrinol. Metab.* 85: 748–754, 2000.
  - 27 *Larson-Meyer D.E., Newcomer B.R., Hunter G.R.*: Influence of endurance running and recovery diet on intramyocellular lipid content in women: a <sup>1</sup>H NMR study. *Am. J. Physiol. Endocrinol. Metab.* 282: E95–E106, 2002.
  - 28 *Levenhagen D.K., Carr C., Carlson M.G., Maron D.J., Borel M.J., Flakoll P.J.*: Postexercise protein intake enhances whole-body and leg protein accretion in humans. *Med. Sci. Sports Exerc.* 34: 828–37, 2002.
  - 29 *Levenhagen D.K., Gresham J.D., Carlson M.G., Maron D.J., Borel M.J., Flakoll P.J.*: Postexercise nutrient intake timing in humans is critical to recovery of leg glucose and protein homeostasis. *Am. J. Physiol. Endocrinol. Metab.* 280: E982–E993, 2001.
  - 30 *Malkova D., Evans R.D., Frayn K.N., Humphreys S.M., Jones P.R., Hardman A.E.*: Prior exercise and postprandial substrate extraction across the human leg. *Am. J. Physiol. Endocrinol. Metab.* 279: E1020–E1028, 2000.
  - 31 *Mannhart C., Colombani P.*: Grundlagen der Sporternährung – die elementare Bedeutung der Energie-, Makronährstoff- und Flüssigkeitszufuhr. *Schweiz. Ztschr. Sportmed. Sporttraum.* 50: 125–130, 2002.
  - 32 *Piehl Aulin K., Soderlund K., Hultman E.*: Muscle glycogen resynthesis rate in humans after supplementation of drinks containing carbohydrates with low and high molecular masses. *Eur. J. Appl. Physiol.* 81: 346–351, 2000.
  - 33 *Price T.B., Laurent D., Petersen K.F., Rothman D.L., Shulman G.I.*: Glycogen loading alters muscle glycogen resynthesis after exercise. *J. Appl. Physiol.* 88: 698–704, 2000.
  - 34 *Rasmussen B.B., Hancock C.R., Winder W.W.*: Postexercise recovery of skeletal muscle malonyl-CoA, acetyl-CoA carboxylase, and AMP-activated protein kinase. *J. Appl. Physiol.* 85: 1629–1634, 1998.
  - 35 *Ribordy V., Gremaud G., Meirim I., Piguët-Welsch C., Décombaz J.*: Effet de l'ingestion avant l'effort d'une barre céréale riche en son d'avoine sur le transit gastro-intestinal et sur la glycémie. *Schweiz. Z. für Sportmed. u. Sporttraumatol.* 45: 174–178, 1997.
  - 36 *Roepstorff C., Steffensen C.H., Madsen M., Stallknecht B., Kanstrup I.L., Richter E.A., Kiens B.*: Gender differences in substrate utilization during submaximal exercise in endurance-trained subjects. *Am. J. Physiol. Endocrinol. Metab.* 282: E435–E447, 2002.
  - 37 *Rotman S., Slotboom J., Kreis R., Boesch C., Jéquier E.*: Muscle glycogen recovery after exercise measured by <sup>13</sup>C-magnetic resonance spectroscopy in humans: effect of nutritional solutions. *MAGMA* 11: 114–121, 2000.
  - 38 *Sacchetti M., Saltin B., Osada T., van Hall G.*: Intramuscular fatty acid metabolism in contracting and non-contracting human skeletal muscle. *J. Physiol.* 540: 387–395, 2002.
  - 39 *Simpson C.H., Shirreffs S.M., Leiper J.B., Maughan R.J.*: Effect of osmolality on gastric emptying of carbohydrate solutions. *Proc. Nutr. Soc.* 61: 113A, 2002.
  - 40 *Stannard S.R., Thompson M.W., Fairbairn K., Huard B., Sachinwalla T., Thompson C.H.*: Fasting for 72 h increases intramyocellular lipid content in nondiabetic, physically fit men. *Am. J. Physiol. Endocrinol. Metab.* 283: E1185–E1191, 2002.
  - 41 *Starling R.D., Trappe T.A., Parcell A.C., Kerr C.G., Fink W.J., Costill D.L.*: Effects of diet on muscle triglyceride and endurance performance. *J. Appl. Physiol.* 82: 1185–1189, 1997.
  - 42 *Starron R.S., Hikida R.S., Murray T.F., Hagerman F.C., Hagerman M.T.*: Lipid depletion and repletion in skeletal muscle following a marathon. *J. Neurol. Sci.* 94: 29–40, 1989.
  - 43 *Steffensen C.H., Roepstorff C., Madsen M., Kiens B.*: Myocellular triacylglycerol breakdown in females but not in males during exercise. *Am. J. Physiol. Endocrinol. Metab.* 282: E634–E642, 2002.
  - 44 *Tipton K.D., Borsheim E., Sanford A.P., Wolf S.E., Wolfe R.R.*: Acute response of net muscle protein balance reflects 24-h balance after exercise and amino acid ingestion. *Am. J. Physiol. Endocrinol. Metab.* 284: E76–E89, 2003.
  - 45 *Tipton K.D., Rasmussen B.B., Miller S.L., Wolf S.E., Owens-Stovall S.K., Petrini B.E., Wolfe R.R.*: Timing of amino acid-carbohydrate ingestion alters response of muscle to resistance exercise. *Am. J. Physiol. Endocrinol. Metab.* 281: E197–E206, 2001.
  - 46 *van Hall G., Shirreffs S.M., Calbet J.A.*: Muscle glycogen resynthesis during recovery from cycle exercise: no effect of additional protein ingestion. *J. Appl. Physiol.* 88: 1631–1636, 2000.
  - 47 *van Loon L.J.C., Kruijshoop M., Verhagen H., Saris W.H.M., Wagenmakers A.J.M.*: Ingestion of protein hydrolysate and amino acid-carbohydrate mixtures increases postexercise plasma insulin responses in men. *J. Nutr.* 130: 2508–2513, 2000.
  - 48 *van Loon L.J.C., Saris W.H.M., Kruijshoop M., Wagenmakers A.J.M.*: Maximizing muscle glycogen synthesis: carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures. *Am. J. Clin. Nutr.* 72: 106–111, 2000.
  - 49 *van Loon L.J.C., Saris W.H.M., Verhagen H., Wagenmakers A.J.M.*: Plasma insulin responses after ingestion of different amino acid or protein mixtures with carbohydrate. *Am. J. Clin. Nutr.* 72: 96–105, 2000.
  - 50 *Yaspelkis B.B.III, Ivy J.*: The effect of a carbohydrate-arginine supplement on postexercise carbohydrate metabolism. *Int. J. Sports Nutr.* 9: 241–250, 1999.
  - 51 *Zehnder M., Ith M., Boesch C., Saris W., Boutellier U.*: Gender differences in the consumption of intramyocellular lipids during endurance exercise measured by <sup>1</sup>H magnetic resonance spectroscopy. *Can. J. Appl. Physiol.* 26: S269 (abstract), 2001.