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Metabolic fuels: regulating fluxes to select mix

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Summary

Animals must regulate the fluxes of multiple fuels to support changing metabolic rates that result from variation in physiological circumstances. The aim of fuel selection strategies is to exploit the advantages of individual substrates while minimizing the impact of disadvantages. All exercising mammals share a general pattern of fuel selection: at the same %$\dot{V}_{O_2,max}$ they oxidize the same ratio of lipids to carbohydrates. However, highly aerobic species rely more on intramuscular fuels because energy supply from the circulation is constrained by trans-sarcolemmal transfer. Fuel selection is performed by recruiting different muscles, different fibers within the same muscles or different pathways within the same fibers. Electromyographic analyses show that shivering humans can modulate carbohydrate oxidation either through the selective recruitment of type II fibers within the same muscles or by regulating pathway recruitment within type I fibers. The selection patterns of shivering and exercise are different: at the same %$\dot{V}_{O_2,max}$, a muscle producing only heat (shivering) or significant movement (exercise) strikes a different balance between lipid and carbohydrate oxidation. Long-distance migrants provide an excellent model to characterize how to increase maximal substrate fluxes. High lipid fluxes are achieved through the coordinated upregulation of mobilization, transport and oxidation by activating enzymes, lipid-solubilizing proteins and membrane transporters. These endurance athletes support record lipolytic rates in adipocytes, use lipoprotein shuttles to accelerate transport and show increased capacity for lipid oxidation in muscle mitochondria. Some migrant birds use dietary omega-3 fatty acids as performance-enhancing agents to boost their ability to process lipids. These dietary fatty acids become incorporated in membrane phospholipids and bind to peroxisome proliferator-activated receptors to activate membrane proteins and modify gene expression.

Key words: metabolic fuel selection, substrate flux, lipid, carbohydrates, energy metabolism, energetics, exercise, shivering.

Comparing fuels: lipids, carbohydrates and proteins

Animal life depends on the capacity to match metabolic fuel supply to changing rates of energy use. The fluxes of multiple substrates must be modulated to achieve real-time selection of mixtures able to support adequate metabolic rates for variable physiological circumstances: from years of torpor to seconds of sprinting. The regulation of energy metabolism is a complex challenge because the fuels available vary widely in stored quantity, energy density, speed of conversion to ATP and water solubility. Therefore, fuel selection strategies aim to exploit the advantages of individual substrates while minimizing the impact of their disadvantages. The rate of energy expenditure necessary for a particular task determines for how long that task can be maintained. Fig. 1A illustrates the relationship between metabolic intensity and duration by plotting record times of human athletes running over different distances *vs* average speed. This example is used to characterize the shape of the more general relationship between metabolic rate and duration. Fig. 1B–E compares lipids and carbohydrates for the main parameters determining whether these fuels tend to support prolonged low-intensity tasks or intense activities of short duration.

Lipids show unique characteristics that make them ideally suited for long-lasting physiological work because they contain the most energy per unit mass (Fig. 1B) and, therefore, are stored in large amounts (Fig. 1C). Lipids pack more joules per gram because they can be stored dehydrated and, to a lesser extent, because they are more chemically reduced than other fuels. Their highly reduced state also allows them to yield more metabolic water than proteins or carbohydrates when they are oxidized. Therefore, organismal dehydration can be avoided by producing metabolic water through the oxidation of lipid stores: a strategy commonly used by birds lacking access to drinking water during long migrations (Carmi et al., 1992; Klaassen, 1996). The low maximal rate of ATP production from lipids (Fig. 1E) is not a limiting factor for prolonged, low- to moderate-intensity tasks. Similarly, the low solubility of lipids in water (Fig. 1D) would severely restrict the capacity to transport them from storage sites, but this serious handicap is greatly reduced by the solubilizing action of albumin in the plasma (van der Vusse, 2009) and fatty acid binding proteins (FABPs) in the cytosol (Haunerland and Spener, 2004). These specialized transport proteins ensure that lipids can be shuttled between tissues, at least rapidly enough to support low- to moderate-intensity tasks. This requirement for transport proteins may be partly responsible for limiting maximal rates of lipid oxidation.

In contrast to lipids, carbohydrates show high maximal rates of ATP production, especially under anaerobic conditions (Fig. 1E), and high solubility in water (Fig. 1D). These fundamental characteristics make them essential for intense physiological work. For most animals, carbohydrates also provide the benefit of being the only usable substrate when oxygen is absent, and their oxidation produces more ATP per mole of O_2 than lipids or proteins (Hutter et al., 1985; Hochachka et al., 1991). Surprisingly, mammals exercising in hypoxic environments do not exploit this advantage, most likely because carbohydrates only account for a minute portion of total energy stores (McClelland et al., 1998).

Storing large carbohydrate stores would unduly increase body mass and volume because their energy density is very low (Fig. 1B). The small size of carbohydrate reserves (Fig. 1C) is therefore a key handicap that severely limits the duration of their use (Fig. 1A).

Fig. 1 also shows the characteristics of proteins or of their constituent amino acids, with values that generally fall between lipids and carbohydrates (Fig. 1B–E). Here, the inclusion of proteins is for comparison only and does not imply that they are used preferentially at intermediate intensities. In reality, animals usually try to curtail the use of proteins as an oxidative fuel (McCue, 2010) for several reasons: (1) most body proteins play essential, alternative roles such as force generation, structural support, catalysis and membrane transport; (2) very few so-called storage proteins have been characterized in animals, and mostly in insects (see Burmester, 2002); and (3) protein catabolism produces toxic ammonia in addition to CO_2 and water normally obtained through the oxidation of all fuels. To minimize the use of functional proteins and to reduce the energy needed for ammonia excretion or detoxification, the contribution of protein oxidation to total ATP synthesis is usually kept low compared with other fuels. Exceptionally, however, protein oxidation can take over energy metabolism almost exclusively, as in migrating salmon just before spawning (Mommsen et al., 1980) or in some hematophagous insects that prefer proline as a fuel for flight (Olembo and Pearson, 1982). In salmon, the prevailing use of proteins becomes necessary in the late stages of migration, when all other fuels reach depletion. However, this fuel selection tactic is not sustainable and rapidly leads to irreversible tissue damage and death. For insects that feed on blood, like the tsetse fly, the high protein content of their diet and high solubility of proline in water (Fig. 1D) are adaptive forces that have shaped the evolution of their unusual fuel selection pattern. Surprisingly, some migrant birds produce up to 15% of their ATP by oxidizing proteins, even though this fuel is much heavier than lipids (Fig. 1B). This apparent paradox may be explained because it allows them to recruit anaplerotic pathways, whereby metabolites generated by protein breakdown can replenish the Krebs cycle intermediates necessary to sustain lipid oxidation (Jenni and Jenni-Eiermann, 1998; McWilliams et al., 2004).

Fuel selection pattern of exercising mammals

Early work investigating metabolic fuel selection during exercise was carried out on humans and dates back to the 1930s (Edwards et al., 1934; Christensen and Hansen, 1939). By measuring changes in the respiratory exchange ratio, it had already been established at this time that the relative importance of carbohydrates increases with exercise intensity. Over the following years, a simple model of fuel selection predicting the partitioning between lipids and carbohydrates emerged. For mammals, it ignores proteins because their oxidation only accounts for <5% of total ATP during exercise (Rennie et al., 1981; Carraro et al., 1994). The model explains that exercise intensity (or metabolic rate) – expressed as a fraction of the aerobic maximum ($\%\dot{V}_{O_2,max}$) – determines the relative contributions of lipids and carbohydrates to total energy expenditure. Across the complete range of work intensities, from rest to $\dot{V}_{O_2,max}$, the relative importance of carbohydrates increases whereas the relative importance of lipids decreases (see Fig. 2). This pattern has been thoroughly characterized in humans and the exercise intensity for which carbohydrates and lipids contribute equally (for mammals, ~50% $\dot{V}_{O_2,max}$) has been called the 'crossover point' (Brooks and Mercier, 1994). At high exercise intensities, the absolute rate of lipid oxidation and the relative contribution of this fuel to total metabolism are decreased (van Loon et al., 2001). The decline is caused by the inherently low maximal rate of ATP production from lipids (see Fig. 1E) and by a regulated decrease in this maximal rate, mediated by reductions in free carnitine concentration and intracellular pH. Such reductions are thought to slow fatty acid entry across the inner mitochondrial membrane by downregulating carnitine palmitoyl transferase I (van Loon et al., 2001). Endurance

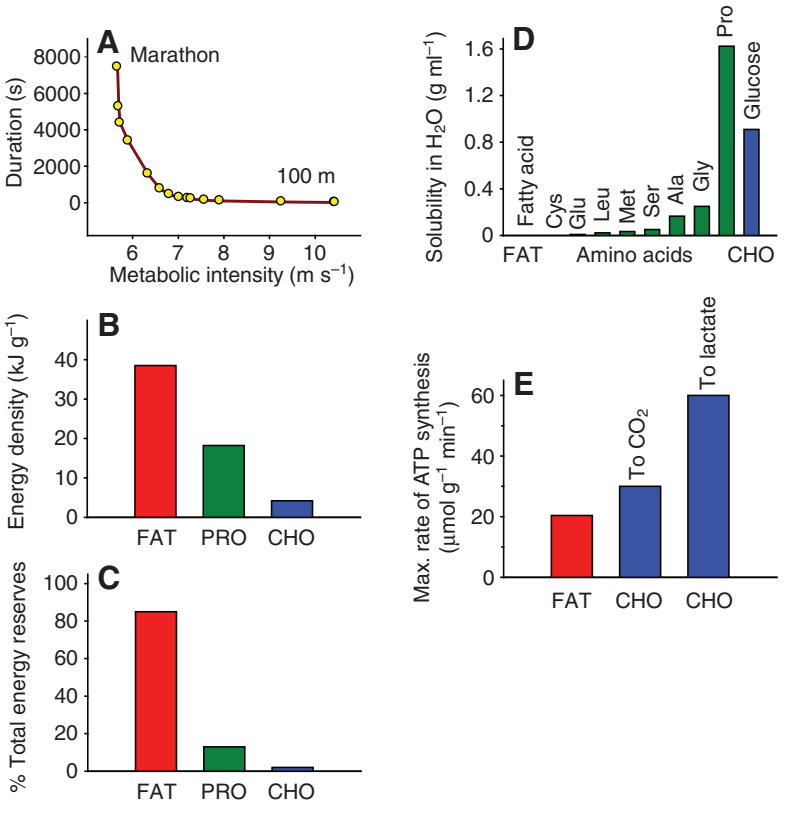

Fig. 1. Metabolic fuel diversity. (A) The relationship between maximum metabolic intensity and duration for different physiological tasks. To illustrate this general relationship, world track records are plotted as running time (s) *vs* average speed (metabolic intensity; m s^{-1}). Running records for men [year 2009 for distances ranging from 100 m to 26.2 miles (marathon)] are indicated. (B–E) Key differences between the fuels available to support physiological tasks of varying intensity and duration [values are adapted from Berg et al. (Berg et al., 2002), Hochachka and Somero (Hochachka and Somero, 2002) and Belitz et al. (Belitz et al., 2009)]. The energy densities of lipids (FAT), proteins (PRO) and carbohydrates (CHO) are indicated in panel B. The relative contributions of the different fuels to total energy reserves of the whole organism are presented in panel C. The solubility of metabolic fuels in water (25°C) is shown in panel D because they must be transported through aqueous fluids (plasma, interstitial fluid and cytosol). Fatty acids are hydrophobic and have extremely low solubility in water, whereas carbohydrates are very soluble. Amino acids cover a wide range of solubilities, from cystein (as low as fatty acids) to proline (higher than glucose). Maximal rates of ATP synthesis from different pathways (μmol ATP g^{-1} wet muscle mass min^{-1}) are indicated in panel E: lipid oxidation, carbohydrate oxidation and anaerobic glycolysis.

training and sympathetic stimulation may influence the crossover pattern of fuel selection, but whether these effects are significant has not been clearly demonstrated (Brooks and Mercier, 1994; Brooks, 1998; Bergman and Brooks, 1999).

To determine the generality of such a fuel selection model, measurements were made on mammals that encompass a wide range of aerobic capacities (Fig. 2). The underlying goal was to vary $\dot{V}_{O_2,max}$ in an attempt to discover exceptional patterns deviating from the model. Measurements in highly aerobic dogs and sedentary goats demonstrated that these animals follow the same fuel selection model as humans, even though the dogs and goats show a 2.2-fold genetic difference in mass-specific $\dot{V}_{O_2,max}$ (Roberts et al., 1996). Small mammals, down to the size of rats, also follow the same model (Brooks and Donovan, 1983) even though, because of allometry, their aerobic capacity is higher than that of large species. Rats with differences in $\dot{V}_{O_2,max}$ caused by either acclimation to sea level or to hypobaric hypoxia simulating high altitude also follow this same fuel selection model (McClelland et al., 1999; McClelland et al., 2001). Therefore, mammals exercising at a specified $\%\dot{V}_{O_2,max}$ use the same relative mixture of carbohydrates and lipids (Fig. 2). The only documented exception to this common pattern has been demonstrated for humans feeding on unusually high-fat, low-carbohydrate diets who are able to increase the relative use of lipids well beyond what the general model predicts (Jansson and Kaijser, 1982; Phinney et al., 1983). The current database on fuel selection in other groups of animals such as fish, birds and some invertebrates, even though much more limited, clearly shows that the mammalian exercise model does not apply generally (Weber, 2009). Unfortunately, the specific reasons for this lack of generality are presently unknown because insufficient comparative information is available. For example, diet, mode of locomotion, environmental factors, morphology, biomechanical design and body size could all play a role in shaping fuel selection in nature.

Sites of energy storage: intra- *vs* extramuscular fuels

The lipid and carbohydrate reserves that support exercise are stored not only within muscles, but in other tissues. Conveniently, all the fuels that power mammalian locomotion fall in a simple 2×2 matrix:

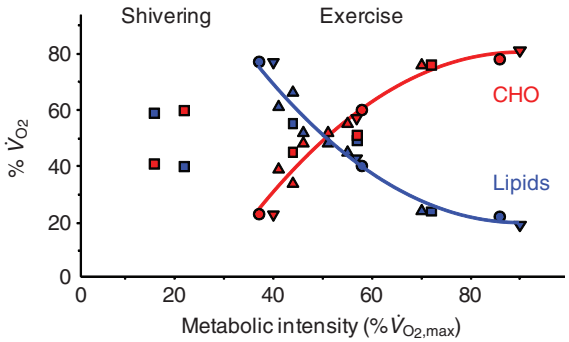

Fig. 2. Fuel selection pattern of exercise and shivering. Relative contribution ($\%\dot{V}_{O_2}$) of lipid and carbohydrate (CHO) oxidation to total energy expenditure as a function of metabolic intensity ($\%\dot{V}_{O_2,max}$) in exercising mammals (right) and shivering humans (left). The fitted curves illustrate the crossover pattern of fuel selection applying to all mammals. Plotted values for exercise are for dogs [circles (Roberts et al., 1996)], goats [inverted triangles (Roberts et al., 1996)], rats [triangles (Brooks and Donovan, 1983; McClelland et al., 2001)] and humans [squares (van Loon et al., 2001; Haman et al., 2005)]. For shivering, all values are for humans (Haman et al., 2002; Haman et al., 2004b).

carbohydrates and lipids stored either within or outside muscles. Here, cellular ATP and creatine phosphate are excluded because they can only support exercise for a few seconds. In mammals, therefore, the complete array of fuels available for aerobic exercise is restricted to: (1) muscle glycogen, (2) circulating glucose derived from liver glycogen and gluconeogenesis, (3) muscle triacylglycerol and (4) circulating nonesterified fatty acids (NEFA) mainly derived from adipose tissue (Romijn et al., 1993). The relative partitioning between total carbohydrates and total lipids is independent of aerobic capacity (previous section), but do aerobic and sedentary species strike the same balance between intra- and extramuscular fuels, be they carbohydrates or lipids? To answer this question, the rates of fuel utilization from the four potential sources available were quantified by indirect calorimetry and tracer methods in dogs and goats running at different intensities (Roberts et al., 1996; Weber et al., 1996a; Weber et al., 1996b). These experiments demonstrated that highly aerobic mammals rely relatively more on intramuscular fuels and relatively less on circulatory fuels than their sedentary counterparts with low aerobic capacity. By definition, animals with a high $\dot{V}_{O_2,max}$ reach higher maximal rates of oxidative fuel supply to their muscle mitochondria. They do so by relying more on intramuscular fuels that reside near their site of oxidation. This conclusion is true for carbohydrates as well as lipids, and supporting evidence is summarized in Fig. 3. Subsequent morphometric analyses of locomotory muscles in dogs and goats have characterized differences in the spatial arrangement of mitochondria and capillaries between the two species (Vock et al., 1996a; Vock et al., 1996b). They reveal that maximal rates of circulatory fuel supply cannot be increased enough to accommodate the high energy fluxes needed by the aerobic species (Weibel et al., 1996). Parallel measurements of functional parameters (maximal substrate fluxes) and structural parameters (three-dimensional arrangement of the vasculature and mitochondria that support these fluxes) have allowed researchers to demonstrate that circulatory fuel supply is constrained at the level of the sarcolemma. For glucose uptake by muscle, the exact nature of the constraint has recently been under careful investigation because of its relevance for the treatment of diabetes (Fueger et al., 2007). Results show that the maximal rate of muscle glucose entry is limited by sarcolemmal glucose transport, but only in the resting state. During exercise, the translocation of glucose transporters (GLUT 4) to the membrane shifts the constraint to glucose phosphorylation by hexokinase II, (see Wasserman et al., 2011). The muscle uptake of NEFA from the circulation also depends on specialized transport proteins [fatty acid translocase (FAT/CD36), plasma membrane FABP (FABPpm) and fatty acid transport proteins (FATP)]. During exercise, NEFA uptake is accelerated by the translocation of FAT/CD36 and FABPpm to the sarcolemma, but this only occurs if muscle contraction is maintained for >30 min (Pelsers et al., 2008). As for glucose, maximal uptake of NEFA by muscle is also constrained, but the exact nature of the limiting step has not been determined. Overall, animals with a high aerobic capacity must boost their reliance on intramuscular stores to compensate for a lower relative use of extramuscular fuels caused by constraints on muscle uptake from the circulation.

Mechanisms of fuel selection

Animals can modulate fuel selection through a variety of mechanisms that operate at different levels of organization. At the whole-organism level, it has long been recognized that different muscles are recruited at different exercise intensities. This selective recruitment of particular muscles has been well characterized in fish because their red oxidative fibers (specialized for lipid metabolism)

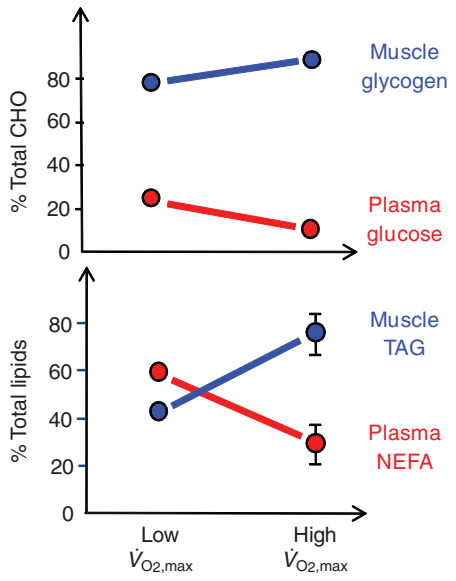

Fig. 3. Relative importance of circulatory and intramuscular fuels. In exercising mammals, all the carbohydrates (CHO) and lipids used to support energy metabolism come either from the circulation [plasma glucose and plasma nonesterified fatty acids (NEFA)] or from intramuscular reserves [muscle glycogen and muscle triacylglycerol (TAG)]. Values were measured in goats (low $\dot{V}_{O2,max}$) and dogs (high $\dot{V}_{O2,max}$) at exercise intensities eliciting near-maximal rates of CHO oxidation (85% $\dot{V}_{O2,max}$) and maximal rates of lipid oxidation (60% $\dot{V}_{O2,max}$). The aerobic capacity of dogs is more than twice that of goats ($\dot{V}_{O2,max}$ dog/$\dot{V}_{O2,max}$ goat=2.2). The relative importance of intramuscular fuels (blue) is increased in highly aerobic mammals compared to their sedentary counterparts. Conversely, the relative importance of circulatory fuels (red) is lower in highly aerobic mammals than in sedentary ones. Values are means ± s.e.m. (s.e.m. are only indicated when larger than the symbols). Adapted from Roberts et al. (Roberts et al., 1996) and Weber et al. (Weber et al., 1996a; Weber et al., 1996b).

and white glycolytic fibers (specialized for carbohydrate metabolism) are spatially separated. Electromyographic (EMG) recordings from this convenient model have shown that red muscle powers low-speed swimming whereas white muscle is only recruited at higher speeds (Johnston, 1980; Johnston, 1981). Therefore, an increase in exercise intensity is accompanied by a progressive change in fuel selection from predominantly lipids to carbohydrates. The same mechanism is used by birds (Marsh et al., 2004) and mammals (Laughlin and Armstrong, 1982), but it has been more difficult to demonstrate it in these other vertebrates because their muscles are composed of mixed fibers. Nevertheless, it is clear that muscles with a high percentage of slow oxidative (type I) fibers are powered by lipids for low exercise intensities whereas those with mostly fast (type II) fibers metabolize carbohydrates and support high work intensities.

At the tissue level, fuel selection can occur within individual mixed muscles *via* regulated recruitment of the different fibers that compose them. Although this mechanism makes intuitive sense, it has been difficult to provide a direct proof for exercise because EMG signals are obscured by background interference from limb movements (Moritani and Muro, 1987; Linnamo et al., 2003). This is not the case during cold exposure, and fuel selection by recruitment of specific fibers within the same muscles has been clearly demonstrated during shivering (Haman et al., 2004b). EMG recordings of shivering muscles show two distinct patterns: continuous, low-intensity shivering at 4–8 Hz (which signals the recruitment of type I motor units) and high-intensity, burst shivering at 0.1–0.2 Hz (from type II motor units) (Petajian and Williams, 1972; Meigal, 2002). During high-intensity shivering, humans are able to modulate their use of carbohydrates by changing the recruitment level of type II fibers (burst shivering) within muscles used for thermogenesis (Haman et al., 2004b). This work has shown for the first time that valuable information about fuel metabolism can be obtained from EMG signatures (Haman et al., 2004b; Weber and Haman, 2005).

Most recent research has focused on the selective recruitment of different metabolic pathways within the same muscle fibers. Following the early observation that exercise causes a decrease in the malonyl-coenzyme A (CoA) concentration of rat muscle (Winder et al., 1989), attention has turned to the central role of AMP-activated protein kinase (AMPK) in the regulation of fuel selection (Hardie et al., 2006; Jorgensen et al., 2006). The activation of AMPK is mediated by increases in the AMP:ATP ratio, and, therefore, can be elicited by a variety of stresses such as hypoxia (which limits ATP synthesis) or exercise (which accelerates ATP use). The modulation of AMPK plays a fundamental role in regulating fuel selection within muscle cells and its main mechanisms of action are summarized in Fig. 4. The stimulation of AMPK promotes the utilization of lipids by increasing trans-sarcolemmal fatty acid uptake *via* FAT/CD36 and by releasing the malonyl-CoA inhibition of carnitine palmitoyl transferase I (CPT I) (Hardie and Sakamoto, 2006). The resulting increase in CPT I activity accelerates the transport of fatty acids across the inner mitochondrial membrane *via* the carnitine shuttle to make them available for β-oxidation. AMPK activation also mediates the movement of GLUT 4 from perinuclear regions to the sarcolemma and stimulates glycolytic flux. When the cellular energy status of the cell is low, AMPK is not only activated to stimulate fuel catabolism, but it inhibits major anabolic processes like the synthesis of lipids, proteins and glycogen, as well as gluconeogenesis. In addition to its direct effect on the activities of various enzymes, AMPK also plays an important role in modulating mitochondrial gene expression *via* the transcription regulator peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α).

Drastic changes in fuel selection within the same muscle fibers have been demonstrated during low-intensity shivering by simultaneous measurement of substrate use and EMG spectra in the same individuals. These experiments have revealed that glycogen-depleted and glycogen-loaded humans can support the same thermogenic rate by using widely different fuel mixtures within the same type I fibers (Haman et al., 2004a; Haman et al., 2004c). This is particularly interesting because it has been recently discovered that AMPK also works as a direct sensor of the size of glycogen reserves (McBride et al., 2009).

Fuel selection during shivering

The effects of cold exposure on energy metabolism have not been studied thoroughly compared with those of exercise, but enough information is available to establish a complete fuel utilization budget for shivering, at least in humans. Rates of heat production from lipids, carbohydrates and proteins have been quantified from gas exchange and nitrogen excretion for low-intensity shivering [2.3 × resting metabolic rate (RMR)] and high-intensity shivering (3.5 × RMR)] (Haman et al., 2002; Haman et al., 2005). Furthermore, the impact of changes in the size of glycogen reserves (depletion or loading) on the thermogenic contribution of the different fuels has been characterized (Haman et al., 2004c). These studies have been reviewed elsewhere (Weber and Haman, 2005)

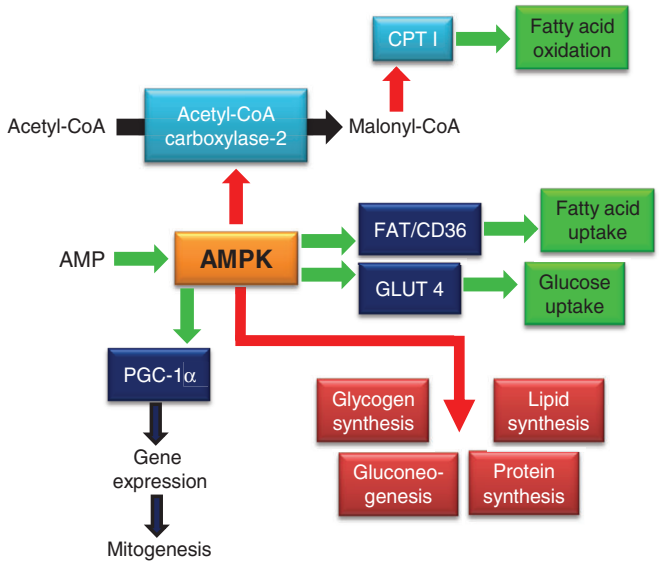

Fig. 4. Regulation of intracellular fuel selection of muscle by AMP-activated protein kinase (AMPK). The enzyme AMPK is regulated by changes in the AMP:ATP ratio and is activated by physiological stresses that limit ATP synthesis or accelerate ATP hydrolysis. It plays a key role in the stimulation of fatty acid catabolism (uptake and oxidation) and in the inhibition of anabolic processes (lipid, carbohydrate and protein synthesis). AMPK stimulates fatty acid oxidation by releasing the malonyl-CoA inhibition of carnitine palmitoyl transferase I (CPT I), the main enzyme catalyzing the transport of fatty acids through the inner mitochondrial membrane. Besides its direct effects on various enzymes, AMPK also acts on gene expression, mainly *via* the transcription regulator peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α). Green arrows/boxes indicate pathways/processes activated by AMPK, whereas red arrows show those inhibited by the enzyme. Adapted from Hardie et al. (Hardie et al., 2006) and Hardie and Sakamoto (Hardie and Sakamoto, 2006).

and are summarized in Fig. 5. As anticipated from what is known on exercise, lipids provide most of the heat during low-intensity shivering, but carbohydrates become dominant when shivering intensifies. Proteins normally play a minor but significant role (~10% of the heat produced). In glycogen-depleted individuals, however, the relative contribution of proteins reaches 19% to help compensate for the lack of carbohydrates. To compare the use of oxidative fuels during shivering and exercise, values for cold-exposed individuals with normal glycogen reserves have been added to Fig. 2. Surprisingly, the fuel selection pattern of shivering humans does not conform to the general crossover pattern of exercising mammals (Fig. 2). At the same relative metabolic rate ($\%\dot{V}_{O_2,max}$), a muscle producing only heat (shivering) or significant movement (exercise) strikes a very different balance between lipid and carbohydrate oxidation (Haman et al., 2005). The underlying mechanism for this intriguing observation is presently unknown. To determine the generality of the difference in fuel selection between shivering and exercise would be premature, as fuel selection during shivering has only been characterized in humans, rats (Vaillancourt et al., 2009) and one species of migratory bird (Vaillancourt et al., 2005). Measurements across the full range of shivering intensities are also needed to establish whether the fuel selection pattern of shivering is simply a left-shifted version of the crossover pattern of exercise (see Fig. 2).

Strategies to increase maximal fluxes of fuels to muscle mitochondria

Endurance-adapted animals must match high O_2 fluxes with equally high fluxes of oxidative fuel. They achieve them through the coordinated upregulation of all the mechanisms involved in substrate mobilization, transport and oxidation. Long-distance migrants are renowned for their capacity to maintain moderate-intensity exercise for several days (Gill et al., 2009), using mainly lipids for locomotion (Jenni and Jenni-Eiermann, 1998; Battley et al., 2001). Therefore, they provide an excellent model to examine adaptations for sustained, high metabolic rates, and will be used here to illustrate fundamental strategies for reaching record substrate fluxes. The main steps of the lipid pathway from adipose reserves to the enzymes that provide ATP to locomotory muscles are summarized in Fig. 6. Contribution from intramuscular lipid reserves are not included in this analysis because the amount of energy needed by long-distance migrants is so high that only a minor portion can be stockpiled directly within muscles. Storing more intramuscular lipids is not a viable option because the machinery for ATP synthesis (mitochondria) and force generation (contractile proteins) would be exceedingly diluted.

As the initial step of the lipid pathway, the triacylglycerol reserves of adipocytes must be mobilized by lipases (mainly hormone-sensitive lipase) to yield NEFA and glycerol. This process of lipolysis can be quantified *in vivo* by monitoring the rate of appearance of glycerol in the circulation ($\dot{R}_a$ glycerol). Measurements of $\dot{R}_a$ glycerol in salmonids (Bernard et al., 1999; Magnoni et al., 2008b) and migratory birds (Vaillancourt and Weber, 2007) reveal that they have the ability to support much higher lipolytic rates than other animals, even in the resting state. Migratory birds preferentially mobilize fatty acids with more double bonds

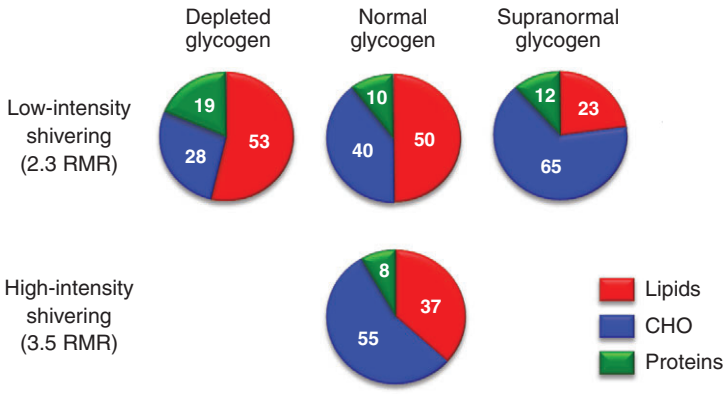

Fig. 5. Fuel selection during shivering. Relative contributions of lipids, carbohydrates (CHO) and proteins to total heat production in adult humans with normal glycogen reserves during low- and high-intensity shivering [2.3 and 3.5× resting metabolic rate (RMR)]. For low-intensity shivering, the effects of glycogen depletion (through exercise preceding cold exposure) and glycogen loading (through changes in exercise and diet) are also indicated. Rates of fuel utilization were obtained by measuring gas exchange (indirect calorimetry) and nitrogen excretion. Adapted from Weber and Haman (Weber and Haman, 2005).

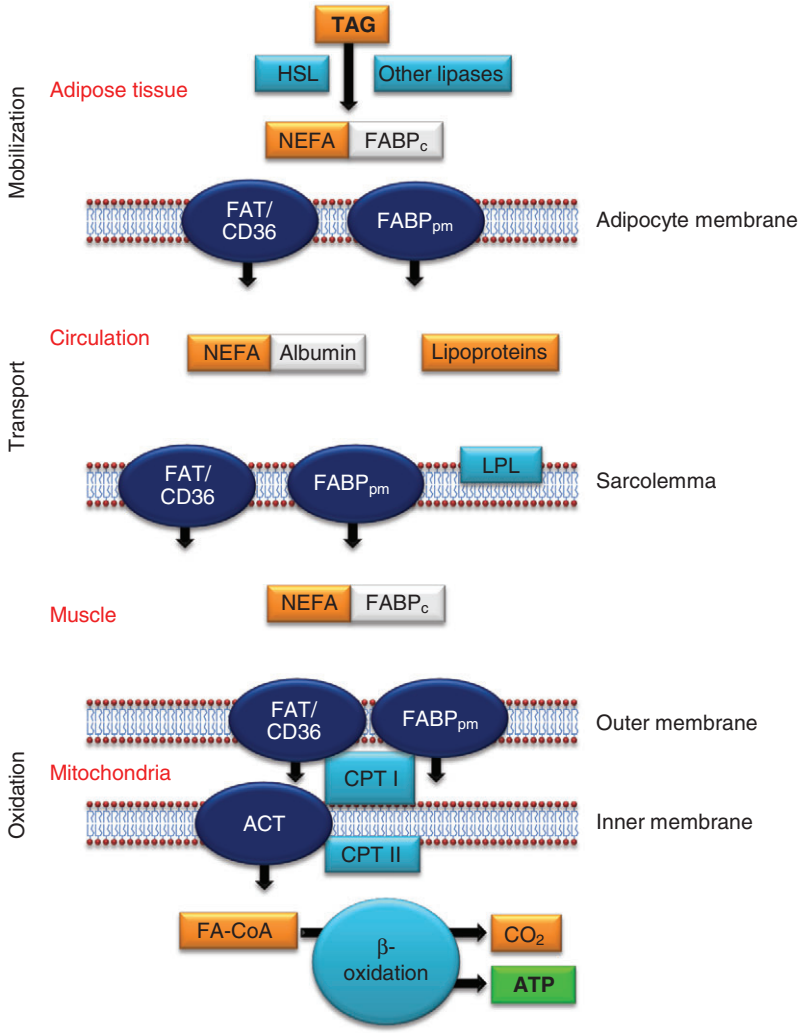

Fig. 6. Pathway for lipid supply from adipose reserves to muscle mitochondria. Metabolites (yellow), enzymes (light blue), transmembrane transporters (dark blue) and solubilizing proteins (gray) of the lipid supply pathway are shown. Most lipids are stored in adipose tissue triacylglycerol (TAG). TAG reserves are mobilized by hormone-sensitive lipase (HSL) and other lipases to yield nonesterified fatty acids (NEFA). Intracellular NEFA transport is mediated by tissue-specific, cytosolic fatty acid binding proteins ($FABP_c$). In the circulatory system, NEFA are transported by lipoproteins or as NEFA-albumin complexes. In muscle capillaries, NEFA transported by lipoproteins are made available for cellular uptake by lipoprotein lipase (LPL). NEFA transport across the cell membrane and the outer mitochondrial membrane is mainly mediated by two proteins: fatty acid translocase (FAT/CD36) and plasma membrane FABP ($FABP_{pm}$). NEFA are transported across the inner mitochondrial membrane by acyl-carnitine-transferase (ACT) before being channelled as fatty-acyl-coenzyme A (FA-CoA) to β-oxidation for ATP production. Adapted from Pelsers et al. (Pelsers et al., 2008) and Glatz et al. (Glatz et al., 2010).

and shorter acyl chains (Price et al., 2008). Long-distance migrants may therefore adjust the fatty acid composition of their lipid reserves to increase lipolytic capacity.

After mobilization, NEFA must be transported to muscle mitochondria – their ultimate site of oxidation. To accelerate transport, many components of the pathway must be upregulated because origin and destination are distant, multiple membranes must be crossed and NEFA are not soluble in aqueous solutions. The transport of NEFA across the adipocyte membrane, the sarcolemma and the outer mitochondrial membrane is mediated mostly by FAT/CD36 and FABPpm (Glatz et al., 2010). FATP also contributes to NEFA transport across these membranes, but it plays a more minor role, restricted to the transfer of very-long-chain fatty acids with 22 or more carbons. In muscle, contraction triggers the translocation of FAT/CD36 and FABPpm from endosomes to the sarcolemma and increases the capacity for transmembrane transport (Pelsers et al., 2008). In migratory birds, gene expression (for both transporters) and protein levels (for FABPpm) show strong seasonal upregulation during migration (McFarlan et al., 2009). CPT I and II and acyl-carnitine transferase (ACT) mediate fatty acid transfer across the inner mitochondrial membrane. A number of recent studies show that FAT/CD36, FABPpm and CPT I are closely associated with each other (Glatz et al., 2010), but the exact nature of this putative functional unit remains to be elucidated. To reach the rates of lipid oxidation needed by migrants, it is clear that all membrane transport proteins must operate at exceptionally high speed.

The lack of solubility of fatty acids in water requires several adaptations for intracellular and circulatory transport. The proteins involved in solubilizing fatty acids include FABP in the cytosol (FABPc) and albumin in plasma. Long-distance migrant birds are known to possess high levels of muscle FABPc that are seasonally upregulated during migration (Pelsers et al., 1999; Guglielmo et al., 2002a; McFarlan et al., 2009). In theory, increasing albumin concentration to accelerate circulatory transport would also make sense. However, albumin plays a crucial role in maintaining oncotic pressure in plasma, and modulating its concentration to accommodate fatty acid transport would interfere with normal osmoregulation. Modifying albumin by increasing its carrying capacity for fatty acids has been observed in some highly aerobic mammals (McClelland et al., 1994), but this alternative approach to accelerate lipid transport through plasma has never been investigated in long-distance migrants. The information presently available on circulatory transport in fish (Magnoni et al., 2006; Magnoni and Weber, 2007; Magnoni et al., 2008a), birds (Jenni-Eiermann and Jenni, 1992; Guglielmo et al., 2002b) and insects (Van der Horst, 2003) clearly shows that, unlike mammals, they do not predominantly use albumin–fatty acid complexes for this purpose. Instead, they rely on lipoproteins to reach the high rates of energy supply necessary for migration (Weber, 2009).

Upregulating lipolysis and lipid transport would be meaningless if the enzymes of lipid oxidation were not stimulated concurrently. Therefore, the flight muscles of migratory birds show exceptionally high oxidative capacity (high activities of β-oxidation and citric acid cycle enzymes) that they upregulate further at the time of migration (Bishop et al., 1995; Guglielmo et al., 2002a; Maillet and Weber, 2007). Some bird species use dietary lipids not just as a source of energy, but as performance-enhancing substances to increase their ability to process oxidative fuel rapidly during long migrations (Maillet and Weber, 2006; Maillet and Weber, 2007). While preparing to cross the Atlantic ocean from the east coast of Canada to South America, the semipalmated sandpiper doubles its body mass by feeding on amphipods loaded with the long-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These fatty acids have dual effects: (1) they become incorporated in membrane phospholipids and (2) they bind to nuclear receptors called peroxisome proliferator-activated receptors (PPARs). Both mechanisms conspire to boost the aerobic capacity of flight muscles in preparation for extreme endurance exercise (Weber, 2009). The incorporation of EPA and DHA in membrane phospholipids is known to affect the local molecular environment of key membrane proteins (transporters, enzymes and receptors) and to modify their function. Therefore, it is easy to see how this unusual omega-3 diet stimulates multiple membrane-related components of the lipid pathway, from adipose stores to the enzymes of β-oxidation and electron transport chain in muscle mitochondria (see multiple membranes in Fig. 6). Similarly, the activation of PPARs by EPA and DHA regulates the expression of many genes controlling crucial aspects of lipid metabolism (CPT, FABP and FAT/CD36, among others) (Feige et al., 2006). Recent experiments have shown that the natural response to dietary omega-3 fatty acids originally observed in wild sandpipers (Maillet and Weber, 2006; Maillet and Weber, 2007) can be replicated in sedentary quails that never migrate (Nagahuedi et al., 2009). Bobwhite quails fed supplements of EPA and DHA for 6 weeks increased the oxidative capacity of their muscles by 58 to 90% (Nagahuedi et al., 2009). Such a strong response is very rare and has only been observed after extreme endurance training in mammals. The quail study demonstrates that dietary omega-3 fatty acids are responsible for the observed metabolic effects because oxidative capacity is strongly stimulated in the absence of training, or even any exercise, and without hormonal signals that could potentially occur seasonally in wild birds.

Conclusions

The diverse characteristics of the substrates available for energy metabolism dictate how fuel selection is coordinated. Lipids are light, abundant, insoluble in water and cannot yield ATP quickly. By contrast, carbohydrates can fuel intense work and dissolve well in aqueous fluids, but they are heavy and scarce. The goal of fuel selection strategies is to exploit the advantages of individual substrates while minimizing their disadvantages. Exercising mammals share a common crossover model of fuel selection: at the same exercise intensity (expressed as $\%\dot{V}_{O_2,max}$), they all consume the same ratio of lipids to carbohydrates. The model applies generally across adaptive (sedentary *vs* highly aerobic species), allometric (small *vs* large body size) and environmental (normoxia *vs* hypoxia) variation in O_2 availability. Mammals with a high aerobic capacity rely proportionately more on intramuscular fuels and less on circulatory fuels than their sedentary counterparts. They must use this strategy because maximal rates of fuel supply from the circulation are constrained by trans-sarcolemmal transport. In humans, the fuel selection patterns of shivering and exercise are different. A muscle producing only heat (shivering) or significant movement (exercise) does not strike the same balance between lipid and carbohydrate oxidation. However, the mechanisms underlying this intriguing observation have not been characterized.

Fuel selection is achieved through different mechanisms at each level of organization. An adequate mix of fuels can be acquired by recruiting different muscles within the organism (e.g. red and white muscles of fish, specialized for lipids or carbohydrates), different fibers within the same muscle (e.g. slow type I *vs* fast type II fibers within mixed avian or mammalian muscles) or different pathways within the same fiber (mainly regulated by AMPK). Combining EMG, indirect calorimetry and tracer methods has allowed determination of fuel selection mechanisms in shivering muscle. It was demonstrated that EMG signatures provide valuable information on oxidative fuel utilization during cold exposure. Shivering humans can modulate carbohydrate oxidation either through the selective recruitment of type II fibers within the same muscles or by regulating pathway recruitment within type I fibers.

Increasing maximal fluxes of energy to muscle mitochondria requires the upregulation of all of the mechanisms involved in fuel mobilization, transport and oxidation. Long-distance migrants rely on lipid metabolism to complete their travels and they provide an excellent model to analyze strategies for achieving record rates of fuel transfer from stores to the machinery for ATP production. Key components of their lipid pathway are modified, including enzymes, solubilizing proteins and trans-membrane transporters. These athletes manage to sustain rapid energy provision through record rates of lipolysis in adipocytes by using FABP and lipoprotein shuttles for cytosolic and circulatory tranport, and by boosting capacity for lipid oxidation in muscle mitochondria. Some migrant birds use the dietary long-chain omega-3 fatty acids EPA and DHA as natural performance-enhancing agents to increase their capacity for lipid metabolism. This omega-3-mediated response has now been replicated in sedentary quails to investigate the exact mechanisms for ‘natural doping’. Dietary EPA and DHA activate essential regulatory proteins through incorporation into membrane phospholipids and by modulating gene expression *via* PPARs.

List of symbols and abbreviations

ACT	acyl-carnitine transferase
AMPK	AMP-activated protein kinase
CoA	coenzyme A
CPT I/II	carnitine palmitoyl transferase I/II
DHA	docosahexaenoic acid
EMG	electromyography or electromyographic
EPA	eicosapentaenoic acid
FABP	fatty acid binding protein
FABPc	cytosolic FABP
FABPpm	plasma membrane FABP
FAT/CD36	fatty acid translocase
FATP	fatty acid transport protein
GLUT 4	glucose tranporter 4
NEFA	nonesterified fatty acids
PGC-1α	peroxisome proliferator-activated receptor gamma coactivator-1α
PPAR	peroxisome proliferator-activated receptor
$\dot{R}_a$	rate of appearance
RMR	resting metabolic rate
$\dot{V}_{O_2,max}$	maximum rate of O_2 uptake

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