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SYMPOSIUM

The Fat of the Matter: How Dietary Fatty Acids Can Affect Exercise Performance

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Synopsis Fatty-acid composition of fat stores affects exercise performance in a variety of vertebrates although few such studies focus on flying vertebrates such as migratory birds, which are exceptional exercisers. We first discuss the natural variation in quality of fat available in natural foods eaten by migratory birds and their behavioral preferences for specific fatty acids in these foods. We then outline three proposed hypotheses for how dietary fatty acids can affect exercise performance, and some of the evidence to date that pertains to these hypotheses with special emphasis on the exercise performance of migratory birds. In theory, selectively feeding on certain long-chain unsaturated fatty acids may be advantageous because (1) such fatty acids may be metabolized more quickly and may stimulate key facets of aerobic metabolism (fuel hypothesis); (2) such fatty acids may affect composition and key functions of lipid-rich cell membranes (membrane hypothesis); and (3) such fatty acids may directly act as signaling molecules (signal hypothesis). Testing these hypotheses requires cleverly designed experiments that can distinguish between them by demonstrating that certain fatty acids stimulate oxidative capacity, including gene expression and activity of key oxidative enzymes, and that this stimulation changes during exercise.

Introduction

A fundamental aspect of animal life history and ecology is the ability of an organism to convert available resources into usable nutrients and energy for maintenance, activity, and reproduction (Karasov and Martinez del Rio 2007). In this article, we focus on one large class of nutrients, the fats, and the effect of the quality of fat (i.e., the fatty-acid composition of fats) on animals' performance. Fats are ubiquitous in nature and are crucially important as primary building blocks of cell membranes, as signaling molecules, and as a primary fuel for many animals (Pond 1998). Consequently, the quality of fat affects metabolism, influences disease, aging, and general health and performance (Hulbert et al. 2005; Simopoulos 2007; Weber 2009; Valencak and Azzu 2014). Besides these crucial physiological functions and consequences, two other aspects of fat quality are especially relevant to the performance of animals: First, there is considerable variation in the fatty-acid composition of natural foods; second, the fatty-acid composition of diet can heavily influence that of the consumer's stored fat (Ayre and Hulbert 1996; Huang et al. 2005; Pierce and McWilliams 2005) although less so for membranes (Hulbert et al. 2005; Arnold et al. 2011). Taken together, the implication is that an animal's choice of diet will affect its fatty-acid composition and this in turn can affect performance.

Many studies of heterothermic endotherms and ectotherms document how choice of diet affects fat composition of the consumer, which in turn affects performance in several ways. For example, hibernating yellow-pine chipmunks (*Eutamias amoenus*) fed diets with more polyunsaturated fatty acids (PUFAs) had corresponding changes in the fatty-acid composition of their membranes and this positively affected their performance during torpor

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(Geiser and Kenagy 1987). Golden-mantled ground-squirrels (*Spermophilus lateralis*) consumed diets rich in PUFA in the late summer and fall with subsequent positive effects on their performance during hibernation (Frank 1992, 1994, 2002; Frank and Storey 1995). In fact, certain amounts and types of dietary PUFAs, particularly the essential omega-3 (ω3; first double-bond occurs at the third carbon position from the methyl [ω] end) and omega-6 (ω6; first double-bond occurs at the sixth carbon position from the methyl [ω] end) fatty acids, seem especially important for such heterotherms. For example, a moderate amount of dietary PUFA was optimal for enhancing torpor and decreasing the overall energy cost of hibernation for captive ground-squirrels (Frank 1992, 1994, 2002; Frank and Storey 1995, 1996; Florant 1998). Dietary fatty-acid composition also affects composition of tissues and energetic performance in ectotherms such as fish and reptiles. For example, fatty-acid profiles of tissue fat in soft-shelled turtles (*Pelodiscus sinensis*) were similar to that of their diet (Huang et al. 2005). Atlantic salmon (*Salmo salar*) and Atlantic charr (*Salvelinus alpinus*) increased swimming efficiency when fed diets containing either more 18:2ω6 (nomenclature is the number of carbons in chain:the number of double-bonds) or a higher ratio of ω3 PUFA to saturated fatty acids (SFA) (McKenzie et al. 1998; Wagner et al. 2004; Pettersson et al. 2010). Thus, the quality of fat, and especially the amount or ratio of essential ω3 and ω6 PUFAs, influences energetic performance in hibernating mammals as well as fish and choice of diet can mediate these effects.

The effect of fatty-acid composition on performance of homeothermic endotherms such as migratory birds is less studied, although recent work, including some of our own, provides intriguing evidence for such an effect on exercise performance (Pierce and McWilliams 2005; Price and Guglielmo 2009; Price 2010). Migrating birds are remarkable endurance athletes, in part because they must exercise at very high intensities (>90% VO2max) to achieve and sustain flight, and they rely primarily on fats to fuel such intense exercise (McWilliams et al. 2004; Guglielmo 2010). Below we discuss recent work examining dietary preferences of birds for certain fatty acids, the availability of dietary fatty acids in natural diets of birds, and the impact of those preferences for the fatty-acid composition of birds and for birds' expenditure of energy. We then briefly review three current hypotheses that can explain how certain fatty acids may affect exercise performance, and the most relevant evidence for each of the three proposed hypotheses, with special emphasis on exercise performance of migratory birds.

Quality of dietary fat, preferences for certain fats, and effects of fat on performance

Fatty-acid composition of foods eaten by humans is well studied and demonstrates that there is impressive variation in the quality of fats of natural foods, including oils and fish, the two predominant sources of essential PUFAs in our diet, and this affects our consumption of fatty acids and in turn our health (Kris-Etherton et al. 2000). Few studies have examined the fatty-acid composition of natural foods eaten by wild vertebrates. Boyles (2011) determined the fatty-acid composition of seven species of fruit available to songbirds during fall migration on Block Island, RI, USA (Table 1). These seven fruits were among the most commonly eaten fruits by birds during fall migration in this region (Parrish 1997; Smith et al. 2007; Bolser et al. 2013) and they clearly varied in their fatty-acid composition (Table 1). When given cafeteria-style choices of diets that differed only in fatty-acid composition, several species of migratory songbird consistently preferred diets with certain types of fatty acids, specifically diets with more unsaturated fatty acids over those with more saturated fatty acids, and diets with more monounsaturated fatty acids (MUFAs) over those with more PUFAs (Bairlein 1991; McWilliams et al. 2002; Pierce et al. 2005, 2004; Table 2). Furthermore, Boyles (2011) found that birds preferred a 2:1 ratio of MUFA:PUFA (Fig. 1). These behavioral preferences for certain fatty acids, along with information about the fatty-acid composition of their primary foods, have important ecological implications; for example, if birds select fruits to achieve the preferred 2:1 ratio of MUFA:PUFA, then there is no single fruit that satisfies this preference and so they must eat a combination of fruits that includes northern arrowwood and Virginia creeper. In fact, these two kinds of fruit were found to be among the most readily eaten by migrating songbirds during fall migration on Block Island (Parrish 1997; Smith et al., 2007; Boyles 2011; Bolser et al. 2013). What remains to be demonstrated is whether birds during migration select among foods with different compositions of fatty acids to achieve a body-fat composition that maximizes performance.

Migratory birds change the fatty-acid composition of their fat stores between non-migratory and migratory seasons (Pierce and McWilliams 2005). MUFA consistently comprises more than 50% of the fatty

Table 1 Percent of fatty acids in seven kinds of fruit commonly eaten by migrating birds while on Block Island, RI

Fatty acid[a]	Arrowwood (*Viburnum recognitum*)	Chokeberry (*Photinia floribunda*)	Virginia creeper (*Parthenocissus quinquefolia*)	Multiflora rose (*Rosa multiflora*)	Pokeweed (*Phytolacca americana*)	Bayberry (*Myrica pensylvanica*)	Oriental bittersweet (*Celastrus orbiculatus*)
	Percent of fatty acids in seven kinds of fruit eaten by songbirds						
n:	12	15	3	3	1	1	2
12:0	–	–	–	1.2 ± 0.20	–	–	–
14:0	–	1.1 ± 1.50	–	–	1.5	14.2	6.8
16:0	**17.3 ± 2.42**	**19.2 ± 5.88**	**25.3 ± 2.86**	**18.5 ± 1.83**	**19.0**	**55.3**	**21.8**
16:1ω7	**31.1 ± 23.16**	3.1 ± 5.47	1.2 ± 0.25	3.8 ± 1.54	–	–	–
18:0	1.6 ± 1.18	4.3 ± 2.24	2.4 ± 0.05	3.2 ± 0.35	2.2	**30.5**	4.9
18:1ω9	**40.4 ± 19.07**	12.2 ± 6.39	**29.2 ± 1.51**	**28.8 ± 9.93**	**27.5**	–	12.2
18:2ω6	6.4 ± 2.63	**34.9 ± 10.15**	**40.9 ± 1.41**	**19.7 ± 4.89**	**32.2**	–	**30.2**
18:3ω3	–	12.0 ± 5.05	–	**23.5 ± 4.49**	13.3	–	**15.4**
22:2ω6	–	8.1 ± 4.28	–	–	–	–	–

Notes: All fruits have fat comprised mostly of a few fatty acids (i.e., 16-carbon and 18-carbon series) although the proportions of these few fatty acids differ appreciably between species of fruit (after Boyles [2011]). Primary fatty acids (>15%) are in bold.

[a] Nomenclature is the number of carbons:the number of double-bonds in the fatty acid (e.g., 18:0 has 18 carbons with no double-bonds); nomenclature for unsaturated fatty acids designates the carbon atom at which the double-bond occurs with an "ω" (e.g., the double-bond for 18:1 ω9 is at the ninth carbon position from the methyl [ω] end).

Table 2 Preferences of passerines for MUFAs over polyunsaturated and/or saturated fatty acids

Species	MUFA (18:1) over PUFA (18:2 or 18:3)	Unsaturated (18:1 or 18:2) over saturated (mostly 18:0)	Short-chain saturate over long-chain saturate	1:2 ratio of 18:2/18:1	Source
Garden warbler (*Sylvia borin*)	√	√	√		Bairlein (1991)
Wood thrush (*Hylocichla mustelina*)	√	√			Zurovchak (1997)
American robin (*Turdus migratorius*)		√			Zurovchak (1997)
Yellow-rumped warbler (*Setophaga coronata*)		√			McWilliams et al. (2002)
Red-eyed vireo (*Vireo olivaceous*)	X	√	√		Pierce et al. (2004)
Hermit thrush (*Catharus guttatus*)	√			√	Boyles (2011)
European starling (*Sturnus vulgaris*)	√			X	Boyles (2011)

Notes: Species with a check mark had the stated preference (e.g., Garden Warbler and Wood Thrush chose MUFA over PUFA), those with an "X" mark did not show the stated preference, and those with no mark were not given this particular choice of fatty acids.

acids in the depot fat of birds during both seasons (Fig. 2) although the ratio of MUFA to 18:2 ω6 increased during migratory seasons in all four species of passerine; interestingly, the two non-passerine species showed the opposite trend (Fig. 2). Such seasonal changes in the fatty-acid composition of fat stores in birds seem primarily caused by changes in diet with selective metabolism of specific fatty acids playing a possibly important, but minor, role (Pierce et al. 2004, Price et al. 2008; McWilliams et al. 2004; Pierce and McWilliams 2005; Price 2010). These studies demonstrated that diet primarily affects the mid-chain-length fatty acids (e.g., 16:1 and 18:1) as well as the essential fatty acids (e.g., 18:2) that predominate in the fat stores of free-living migratory birds. Given that fat stores in songbirds during migration had a consistently higher ratio of MUFA to 18:2 ω6, this provides the ecological basis for the hypothesis that songbirds with such a ratio of MUFA:PUFA have enhanced exercise performance.

Two recent studies demonstrated for the first time that migratory songbirds with fat depots mostly composed unsaturated ω6 fatty acids, in general, or a certain essential fatty acid (i.e., 18:2), in particular, have enhanced exercise performance as measured during short-term intense exercise in a flight wheel

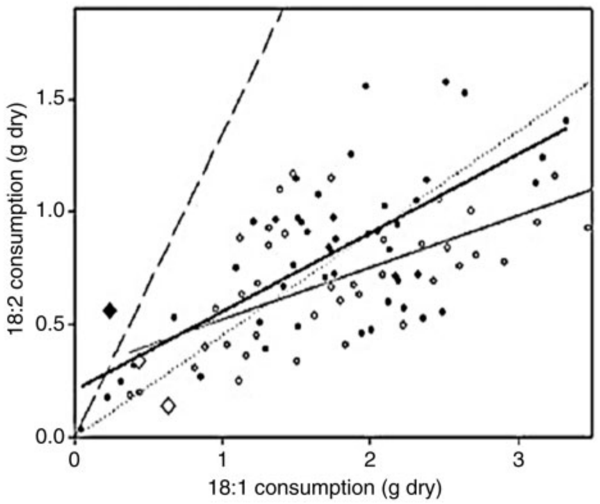

Fig. 1 Ratio of fatty acids consumed when hermit thrushes (*Catharus guttatus*) were given paired choices of three diets that differed only in certain fatty acids. Large diamonds show the ratio of the two primary fatty acids (18:1 and 18:2) in each of the three diets: Low 18:2 (bottom), medium 18:2 (middle), and high 18:2 (top). If thrushes had no dietary preference, then they would eat equal amounts of each paired choice as shown by the gray dashed line (low and medium choices of 18:2) and black dashed line (medium and high choices of 18:2). However, total 18:1 versus 18:2 consumed (grams of dry mass day^{-1} $\pm$ SD) by thrushes when given either paired-diet choice (two solid lines) suggested a consistent preference for a 1:2 ratio of 18:2 to 18:1 (data from Boyles [2011]).

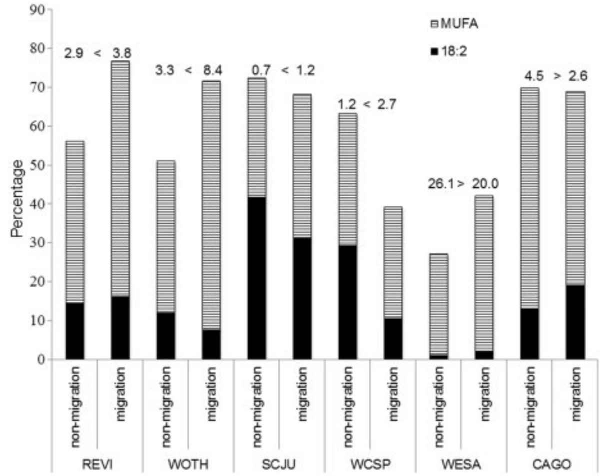

Fig. 2 Seasonal changes in the percent of MUFAs and linoleic acid (18:2) in subcutaneous fat stores or whole-body fat for several bird species during non-migratory and migratory periods. Numbers above bars denote the ratio of MUFAs to 18:2 in the fat stores or body fat for the four passerine species and two non-passerine species studied to date. Original data sources: REVI (*Vireo olivaceus*) (Pierce and McWilliams 2005), WOTH (*Hylocichla mustelina*) (Conway et al. 1994), SCJU (*Junco hyemalis*) (Bower and Helms 1968), WCSP (*Zonotrichia leucophyrs*) (Morton and Liebman 1974), WESA (*Calidris mauri*) (Egeler and Williams 2000), and CAGO (*Branta canadensis*) (Thomas and George 1975).

(Pierce et al. 2005; Price and Guglielmo 2009). Specifically, red-eyed vireos (*Vireo olivaceous*) with fat stores composed of more 18:2 had higher peak metabolic rates (PMRs) and greater metabolic scope than did vireos with more MUFAs (Pierce et al. 2005; Fig. 3a), and white-throated sparrows (*Zonotrichia albicollis*) with fat stores composed of more ω6 fatty acids had higher PMR than did sparrows with fat stores composed of mostly ω3 fatty acids (Price and Guglielmo 2009; Fig. 3b). These results are not consistent with the hypothesis that songbirds with a higher ratio of MUFA to 18:2 ω6 have enhanced exercise performance. However, close inspection of the seasonal changes in songbirds during migratory periods, compared with non-migratory periods (Fig. 2), reveals that in three of the four songbird species (the exception is the red-eyed vireo) the overall amount of 18:2 ω6 in fat stores increased during migration. Thus, the fatty-acid composition of fat stores affected exercise performance in the two songbird species studied thus far, and the enhanced exercise performance may be related to having more 18:2 ω6 rather than having a certain ratio of MUFA:PUFA. Clearly, more studies are needed that measure the quality of fat of a variety of natural foods eaten by free-living birds and that directly measure the effect of fatty-acid composition of tissues on exercise performance.

Possible mechanisms explaining enhanced exercise performance

In theory, selectively eating, and hence storing, certain long-chain unsaturated fatty acids may be advantageous because (1) such fatty acids may be preferentially mobilized and metabolized more quickly (fuel hypothesis); (2) such fatty acids may affect composition and key functions of lipid-rich cell membranes (membrane hypothesis); and (3) such fatty acids may stimulate key facets of aerobic metabolism, for example, stimulating expression of genes involved in the oxidation of fatty acids (signal hypothesis) (Fig. 4). Below we discuss aspects of each of these hypotheses and how they may relate to the observed effect on exercise performance of migratory birds. Although we separately describe these three hypotheses for convenience, they are not mutually exclusive.

Fuel hypothesis

The fuel hypothesis states that enhanced exercise performance occurs because certain fatty acids are more quickly mobilized and metabolized, and this can

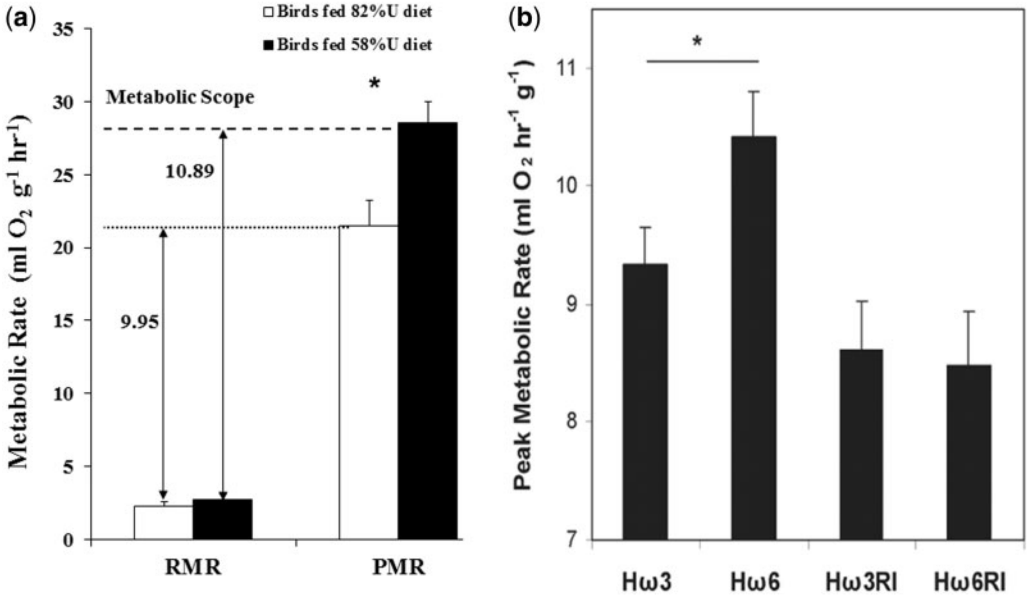

Fig. 3 (**a**) Resting metabolic rate (RMR) and PMR for two groups of red-eyed vireos fed diets with different compositions of fatty acids. Metabolic scope is PMR divided by RMR. Asterisk denotes significant difference in PMR between the two dietary groups (after Pierce et al. [2005]). (**b**) PMR in white-throated sparrows. Hω3 and Hω6 birds were fed high-omega-3 (ω3) and high-ω6 PUFA diets. Hω3RI and Hω6RI birds were fed the same diets, lost mass through restricted intake, and then were switched to an intermediate diet. There was a significant difference in PMR (*$P = 0.037$) between the Hω3 and Hω6 groups. Data expressed as mean + SE (after Price and Guglielmo [2009]).

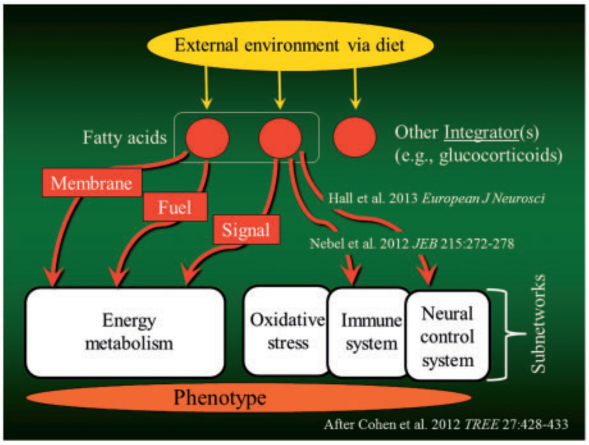

Fig. 4 A simplified schematic of a physiological regulatory network that indicates how certain dietary fatty acids serve as key "integrators" that interact with multiple systems (subnetworks) and each other, thereby ensuring an appropriate match between phenotype and environmental conditions (after Cohen et al. [2012]). Dietary fatty acids have been shown to influence the immune system (Nebel et al. 2012) and neurogenesis (Hall et al. 2013). The three hypotheses (membrane, fuel, and signal) whereby certain fatty acids affect exercise performance are described in the text.

occur in several ways: Selective mobilization from adipocytes, enhanced transport to target tissues, and/or selective uptake by, and intracellular transport in, muscles cells (McWilliams et al. 2004; reviewed by Guglielmo [2010] and Price [2010]). Several studies across taxa have shown that mobilization of shorter-chain SFAs and fatty acids with more double-bonds (e.g., 18:2 ω6) was more rapid from fat stores in rats, fish, and birds (Raclot and Groscolas 1995; Sidell et al. 1995; Hulbert et al. 2005; Price et al. 2008). This pattern of selective mobilization appears to be unaffected by season or physiological state (e.g., migratory-state, fasted, energy-depleted, and exercised) in rats and birds (Raclot and Groscolas 1995; Price et al. 2008; Price 2010). Once mobilized from fat stores, fatty acids require solubilization and intracellular translocation by the family of fatty-acid binding proteins (FABPs) such that an increase in the number and action of FABPs present in the cell membranes and in cytosols of muscle cells increases oxidative capacity (Guglielmo et al. 1998, 2002; McFarlan et al. 2009; Guglielmo 2010).

The hypothesis of preferential mobilization and utilization of certain fatty acids has been invoked to explain how fatty-acid composition affects performance in rats, lizards, fish, and more recently migratory birds (Leyton et al. 1987; Geiser and Learmonth 1994; Raclot and Groscolas 1995; McKenzie et al. 1998; Wagner et al. 2004; Pierce et al. 2005, Price et al. 2008; Price and Guglielmo 2009; Pettersson et al. 2010). The work of Price and Guglielmo (2009) is worth highlighting because they used a cleverly designed sequence of feeding and fasting protocols to produce white-throated sparrows with fat stores and muscle membranes composed of different fatty acids. Consequently, they were able to demonstrate for the first time that the enhanced exercise performance of sparrows (Fig. 3) was associated with the fatty-acid composition of fat stores rather than with that of muscle membranes. The implied mechanism for the enhanced performance included selective uptake of fatty acids into muscle cells and selective intramyocyte transport (Price and Guglielmo 2009; Price 2010). Although many of these studies show support for the fuel hypothesis in that certain fatty acids, such as 18:2 ω6, are metabolized more quickly at many steps of fatty-acid oxidation, and selective uptake and processing are possible, they do not prove that this is the primary mechanism responsible for enhanced exercise performance in migratory birds with certain compositions of fatty acids (Guglielmo 2010; Price 2010). *In vivo* studies that examine selective uptake and intracellular transport of certain fatty acids in muscle cells during exercise would be quite informative, especially if combined with well-designed manipulations of diets and feeding regimes.

Membrane hypothesis

The membrane hypothesis states that the fatty-acid composition of membrane phospholipids can affect key aspects of the structure and function of membranes (2000; Hulbert et al. 2005), and thus the exercise performance of an organism (Valencak et al. 2003; Maillet and Weber 2006, 2007; Weber 2009; Price 2010). The fatty-acid composition of the diet influences the phospholipid composition of muscle membranes in vertebrates, and this effect of diet on the composition of membranes is especially strong for dietary ω3 and ω6 compared with other fatty acids (e.g., shorter-chained saturated fatty acids [Hulbert et al. 2005; Maillet and Weber 2006, 2007]). Accordingly, the fatty-acid composition of cellular and sub-cellular membrane phospholipids converged with that of diet in migrating shorebirds that consumed marine invertebrates laden with ω3 PUFAs (Maillet and Weber 2006, 2007). An increase in membrane PUFAs has potential functional importance for animals undergoing exercise in that (1) ω3 and/or ω6 PUFAs increase the fluidity and permeability of cell membranes (Stillwell and Wassall 2003; Weber 2009) and (2) ω3 and/or ω6 PUFAs are known to influence the activity of membrane-bound proteins and enzymes (e.g., UCP and -ATPases) that could affect efficiency of aerobic respiration (Hulbert and Else 2000; Infante et al. 2001; Hulbert et al. 2005; Maillet and Weber 2006, 2007; Gerson et al. 2008).

Several lines of evidence suggest a link between the ω3 and/or ω6 PUFA content in membrane phospholipids and exercise performance. For example, maximal running speed in 36 species of mammals was strongly related to the ω6 PUFA content of the phospholipids in their muscle membranes (Ruf et al. 2006), and regular exercise increased PUFA content of muscle membranes in humans (Andersson et al. 2000). Also, PUFA-rich membranes have been found in various muscle types having high aerobic capacity, such as the *pectoralis* muscle in hummingbirds and the shaker muscle in rattlesnakes (Infante et al. 2001). Along with high levels of ω3 PUFAs, these muscle membranes had increased Ca^{2+}-ATPase activity, which may play a significant role in metabolism during exercise (Infante et al. 2001; Ruf et al. 2006). Furthermore, the activity of Na+/K+-ATPase enzyme in the tissue membranes of birds and mammals has been correlated with the amount of 22:6ω3 in the membrane, suggesting a causal link between certain types of PUFA and metabolic rate (Wu et al. 2001, 2004; Turner et al. 2003).

Signal hypothesis

The signal hypothesis states that certain dietary fatty acids (most notably the ω3 and ω6 PUFAs) directly act as natural ligands for receptors that regulate the expression of genes associated with lipid metabolism (McClelland 2004; Bordoni et al. 2006; Maillet and Weber 2006, 2007; Weber 2009). In particular, PUFA directly bind to, and regulate, the activity of peroxisome proliferator-activated nuclear receptors (PPARs) which are well known to stimulate expression of genes involved in the oxidation of fatty acids (Hochachka and Somero 2002; Pawar et al. 2002; Zhang et al. 2004; Narkar et al. 2008; Weber 2009), although the specific mechanism of action depends on the type of PPAR (Feige et al. 2006). PPAR α and β stimulate the oxidation and transport of fatty acids, whereas PPAR γ is involved in the storage of lipids and in the differentiation of adipocytes (Desvergne and Wahli 1999; Nagahuedi et al. 2009). The effects of PUFAs on gene expression depend largely on the cellular concentration of PUFA, although it is not yet clear whether a specific amount, or ratio, of dietary ω3 and ω6 fatty acids is optimal (Bordoni et al. 2006). The direct binding of certain dietary PUFAs to PPARs that then activate the genes regulating lipid metabolism provides an example of "natural doping" whereby exercising animals can eat certain PUFAs, upregulate key aspects of lipid metabolism, and so enhance their exercise performance (Maillet and Weber 2006, 2007; Weber 2009). Such natural doping on ω3 PUFAs has been proposed for semipalmated sandpipers (*Calidris pusilla*) as they pause at key stopover sites during fall migration (Maillet and Weber 2007). Whether this is a most interesting but unusual case in migratory birds deserves further investigation.

PUFAs and exercise performance of migratory birds: an integrative perspective

A fundamental aspect of the interactions between organism and environment is the integration of physiological and biochemical processes across organizational levels to understand whole-organism performance within an ecological context. Migratory birds are excellent model systems for investigating such integrative questions because migratory birds are impressive endurance athletes that use primarily fats to fuel their flights, and diet strongly influences the fatty-acid composition of stored fat that in turn affects whole-animal energetics during exercise. Evidence to date from migratory birds suggests that the fatty-acid composition of diet can influence performance during flight through mechanisms that control energy metabolism and the peroxidation of lipid. In this article, we have emphasized a few other important aspects of this interaction between diet and performance: (1) The fatty-acid composition of natural foods is quite variable; (2) migratory birds, as well as other vertebrates, discriminate between foods based on fatty-acid composition; and (3) diet primarily affects the mid-chain-length fatty acids (e.g., 16:1 and 18:1) that predominate in free-living migratory birds, as well as the essential fatty acids (e.g., 18:2). The implication is that migratory birds, as well as other vertebrates, can select diets so as to achieve a certain fatty-acid composition and thereby influence their exercise performance.

The three proposed hypotheses (fuel, membrane, and signal) for how dietary fatty acids affect exercise performance implicate the amount and/or ratio of ω3 and ω6 PUFAs as being most important. Selective uptake of these PUFAs by myocytes directly influences their intracellular concentration that, in turn, impacts multiple aspects of lipid metabolism. Intracellular concentrations determine the availability of PUFAs as fuel, and permit the incorporation of PUFAs into the phospholipids of cellular membranes and affect the activity of key membrane proteins responsible for energy metabolism. In addition, the intracellular concentration of ω3 PUFAs is directly related to their degree of nuclear action in regulating genes involved in lipid metabolism. Thus, discriminating between these three hypotheses requires well-designed experiments that elucidate how certain fatty acids (e.g., 18:2 ω6) stimulate oxidative capacity, including PPAR expression and the activity of key oxidative enzymes and transport proteins, and how this stimulation of oxidative capacity changes during exercise. Furthermore, the reliance on PUFAs to fuel exercise is physiologically challenging because PUFAs are highly prone to oxidation and generally produce increased levels of oxidative stress compared with other shorter-chained and/or saturated fatty acids. How the antioxidant defense system in birds responds to the increased oxidative stress associated with metabolism of certain fatty acids, for example, PUFAs, during exercise remains largely unexplored. Unraveling such basic determinants of whole-animal performance, in general, and exercise performance in migratory birds, in particular, is a fundamental yet largely unexplored aspect of organism–environment interactions that has broad implications for animal ecology (e.g., diet selection, habitat requirements, and competition) and, in turn, the conservation and management of migratory birds.

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