THE OBESITY PANDEMIC and its associated comorbidities have been attributed mostly to chronic excess calorie consumption. In healthy subjects, excess calories are directed to adipose tissues where they are stored as triglycerides (TGs). However, when some poorly defined threshold for fat storage is exceeded, as occurs in metabolic syndrome, adipose tissue becomes insulin resistant, suppression of lipolysis is diminished, and the increased lipolysis of adipose TGs elevates circulating free fatty acids (FFAs) (19, 90). This excess circulating fat can be deposited in nonadipose tissues, which can result in cellular dysfunction commonly referred to as lipotoxicity (9, 108). In skeletal muscle, lipid-mediated signaling appears to be involved in insulin resistance (58, 80) through the effects of several molecular species (58, 96). Skeletal muscle insulin resistance is important in the development of type 2 diabetes (19, 21, 86).

Figure 1 summarizes lipid metabolism in skeletal muscle. Lipoprotein-associated lipids are broken down into FFAs by lipoprotein lipase (LPL) on capillary endothelial cells (103). These FFAs, along with circulating FFAs that are derived from adipose lipolysis, are imported into skeletal muscle through multiple pathways involving the multifunctional receptor CD36 (16, 28), fatty acid transport proteins (FATPs), and noncarrier mechanisms (102). Intracellular FFAs are converted into acyl-CoAs by the membrane-associated enzyme acyl-CoA synthases (ACSs) (24, 63) or FATPs (102). ACSs also mediate intramuscular lipolysis by adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoacylglycerol lipase (MGL) (111, 118). FAS is not as abundantly expressed in skeletal muscle as in classically lipogenic tissues such as liver or adipose tissues (K. Funai and C. F. Semenkovich, unpublished data); thus, it is assumed that the contribution of de novo lipogenesis to intramuscular lipid composition is low (85). Acyl-CoAs derived from these distinct pathways may become compartmentalized in separate intramuscular pools. At rest, FFAs that enter muscle cells appear initially to be directed to the intramuscular TG pool, whereas acyl-CoAs destined for mitochondrial β-oxidation appear to originate from TG lipolysis (18, 55). It is unclear whether utilization of these putative acyl-CoA pools is interchangeable when muscle FFA oxidation is accelerated such as during muscle contraction (22, 94, 111), when muscles have defective intramuscular lipolysis (51), or in the setting of diabetes, starvation, or cachectic disease. In the absence of demand for fatty acid oxidation, acyl-CoAs undergo a series of esterification reactions with glycerol initiated by glycerophosphate acyltransferase (GPAT) that ultimately yield TGs or phospholipids (17, 25, 105).

A Case for Lipoexpediency in Skeletal Muscle

Although excess fat deposition in nonadipose tissues may induce pathology, moderate fat deposition in those same tissues is often important for maintaining normal physiology. In skeletal muscle, lipids provide fuel for contractile activity. During exercise, skeletal muscle endurance is highly dependent on its capacity to utilize lipids and spare muscle glycogen (45, 95). Exercise increases circulating FFAs by promoting adipose tissue lipolysis through several mechanisms, including a decrease in insulin and increases in catecholamines (50, 61). In addition to mass action driven by increased FFA availability, lipid uptake by skeletal muscle is also enhanced through

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increased cell surface translocation of CD36 (47). Exercise also stimulates intramuscular TG lipolysis (50, 109, 111). In trained athletes, intramuscular acyl-CoA concentrations are elevated, and ATP synthesis may increase more than 100-fold compared with these variables in the resting state (2). Each exercise bout also appears to be associated with the diversion of a portion of the acyl-CoA pool toward intramuscular TG synthesis (49). Therefore, exercise-induced increases in skeletal muscle lipid flux are an essential component of increasing endurance.

With endurance training, skeletal muscle metabolism shifts so that substrate abundance and enzyme activities promote lipid utilization and storage. Exercise training is known to increase intramuscular TG content, an observation sometimes described as the “athlete’s paradox” (29, 48, 78) but one that might also be considered an “obesity paradox”. TG content alone cannot predict muscle physiology, since elevated muscle TG content in endurance-trained athletes is an adaptive response to increased demand for fuel utilization (45, 93), whereas elevated muscle TG content in obese individuals is a maladaptive response to energy excess (30, 65). In trained muscles, exercise enhances intramuscular TG breakdown, which promotes ATP resynthesis to spare glycogen (the depletion of which probably leads to muscle exhaustion) (8, 32). Exercise training also induces an increase in protein abundance for enzymes that are associated with synthesis, breakdown, and utilization of intramuscular TG (1, 42, 52, 60, 87, 98). Thus, skeletal muscle lipid flux caused by each bout of exercise is further enhanced in endurance-trained athletes compared with untrained individuals. Notably, endurance-trained muscles are exquisitely insulin sensitive (20, 31, 40, 112).

One emerging notion is that it is not the increased content of FFA in skeletal muscle per se but rather the accumulation of lipid intermediates in muscle that may be deleterious. The specific identities of potentially harmful lipid intermediates are controversial, but ceramide, diacylglycerol (DAG), and certain acyl-CoAs are implicated in skeletal muscle insulin resistance, endoplasmic reticulum (ER) stress, mitochondrial stress, and apoptosis (9, 58, 80, 96, 108). Since a single bout of exercise in humans can protect against skeletal muscle insulin resistance induced by an overnight lipid infusion (98), it is likely that in the setting of obesity exercise activates pathways to shunt potentially harmful lipid molecules toward mitochondrial oxidation, TG storage, or phospholipid synthesis. Thus, even with the lipid overload that occurs in obesity, cells have the capacity to alter proportions of different classes of lipid molecules so that lipotoxicity is minimized. We recently coined the term “lipoexpediency” to describe the ability of cells to modify lipid metabolism to promote favorable physiology (72).

Data from transgenic and knockout models support the idea that muscle can protect against lipotoxic agents by producing neutral lipid storage. Increasing net TG synthesis by diacylglycerol acyltransferase-1 (DGAT1) overexpression (70, 106) or ATGL inactivation (34, 59) promoted skeletal muscle insulin sensitivity. Conversely, isolated skeletal muscle from whole body DGAT1 knockout mice exhibited insulin resistance (70). However, insulin sensitivity alone may not reflect improved muscle function, since DGAT1 overexpression and ATGL knockout were accompanied by muscle abnormalities not observed in trained muscles (51, 70, 101). Mechanisms underlying the apparently beneficial increases in intramuscular TG levels observed in trained muscles likely involve a complex set of coordinated physiological responses. A comprehensive understanding of exercise-induced lipid flux will be required if it will be possible to develop a strategy to mimic or enhance exercise and its benefits.

The idea that muscle with higher oxidative demands for ATP synthesis can protect against lipid-induced insulin resistance is
Roles of Specific Lipid Molecules as Ligands for PPARs

A series of novel observations over the past decade have helped clarify some of the mechanisms by which endurance training enhances the capacity of skeletal muscle to metabolize lipids. Several lines of evidence suggest that peroxisome proliferator-activated receptor (PPAR)γ coactivator-1α (PGC-1α) coordinates a broad spectrum of adaptive responses in muscle (3, 37–39, 66, 67, 89, 117). Skeletal muscle PGC-1α coactivates transcription factors such as PPARα and PPARβ/δ to promote expression of proteins critical for lipid metabolism (62, 81, 88). PGC-1α-dependent transcription is induced by multiple signaling events that are known to be activated by exercise. These events include activation of enzymes that serve as energy sensors, including 5′-adenosine monophosphate-activated protein kinase (AMPK) and the sirtuins, as well as calcium sensors, including the Ca2+/calmodulin-dependent kinases (CaMKs) (54, 92, 114, 115). These enzymes are known to activate the skeletal muscle transcriptional machinery involved in lipid metabolism (53, 83, 113, 116). Therefore, it seems plausible that PGC-1α serves as a signaling node for muscle adaptations occurring in response to exercise.

PPARα and PPARβ/δ are members of a nuclear receptor family important for regulating both metabolism and inflammation (23, 33, 74, 110). PPARs form heterodimers with retinoid X receptors (RXRs) and bind to specific DNA sites known as PPAR response elements (PPREs). Transcription is activated by ligand binding to PPARs, and a wide variety of lipid molecules have been implicated as PPAR ligands. Ligand binding induces conformational changes resulting in dissociation of corepressors and recruitment of coactivators such as PGC-1α. It is likely, although not proved, that exercise-induced activation of PPARs requires the binding of a lipid ligand to the receptor. One plausible scenario for skeletal muscle lipid metabolism in response to exercise would thus include the synthesis and binding of lipid ligands to PPARα or PPARβ/δ concurrently with the activation of PGC-1α.

Additional clues as to how synthesis of lipid ligands may participate in exercise-induced activation of the skeletal muscle transcriptional machinery come from studies of altering the lipid milieu without exercise. Raising circulating FFA in the absence of an exercise stimulus is sufficient to increase mitochondrial biogenesis and endurance in skeletal muscle (27, 36, 75, 107), outcomes that might involve PPARα, PPARβ/δ, and/or PGC-1α-dependent transcription (27, 36). These findings suggest that at least a portion of the exercise adaptations that enhance the skeletal muscle’s capacity for lipid metabolism can be induced by simply replicating the increased exposure of that tissue to an appropriate lipid source. However, this lipid intervention also caused skeletal muscle insulin resistance (27, 36), the opposite of the insulin-sensitizing response to an exercise intervention (10, 26, 31, 43, 91). These studies reinforce the idea that enhancing mitochondria mass alone does not increase resting fat oxidation and that increased mitochondrial mass alone is not sufficient to afford skeletal muscle protection from fat-induced insulin resistance (44, 80). These studies did not address effects of chronically increased circulating FFAs on TG stores or lipid enzymes in muscle, so it is possible that the processes of lipid synthesis and breakdown were unaffected (65). Perhaps muscle contractile activity is required to promote synthesis of specific lipid mediators that promote transcriptional programs resulting in protection of skeletal muscle from fat-induced insulin resistance.

Because the identities of physiologically relevant ligands for PPARs in skeletal muscle are unknown, the origin(s) of these putative ligands is also obscure. Multiple lipogenic or lipolytic sites might generate potential ligands. Data from several transgenic and knockout mouse models suggest that such ligands are likely to be derived from branches of the futile cycle of lipid synthesis and breakdown. Upregulation of DGAT1 increased skeletal muscle expression of genes involved in mitochondrial and fatty acid metabolism (68, 106). Conversely, inactivation of DGAT1 decreased the expression of PPAR-dependent genes in skeletal muscle (69). ATGL overexpression increased skeletal muscle oxidative capacity and PPARβ/δ target genes (111). However, skeletal muscle from the whole body ATGL knockout mouse does not have decreased expression of genes relevant to fatty acid oxidation (51), an effect that may be different in cardiac muscle (34). Skeletal muscle CD36 deficiency decreased PPARβ/δ gene expression and mitochondrial fat oxidation (46, 82). In nonmuscle tissues, including liver, brain, and macrophages, the lipogenic enzyme FAS regulates PPARα-dependent gene expression (12, 13, 100). FAS in adipose tissue is probably involved in regulation of PPARγ activity (71, 72, 99). Lipolytic pathways also impact PPAR activation, since ATGL (34, 84, 97) and LPL (6, 7, 14, 119) regulate PPARα at several sites.
What are the physiologically relevant skeletal muscle ligands that activate PPARs and/or PPARβ/δ to coordinate exercise-induced adaptive responses? A distinct phosphatidylcholine species, 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine (16:0/18:1-GPC), was identified as an endogenous PPARα ligand in the liver (11). Phosphatidylcholine synthesis in eukaryotic cells occurs primarily through the Kennedy pathway (56, 57). In this pathway (Fig. 2), CDP-choline is synthesized from the sequential reaction of choline with ATP and CTP; then the phosphocholine group on CDP-choline is transferred to DAG (directly sequestering a potentially toxic lipid intermediate) by choline phosphotransferase-1 (ChPT1) found in the Golgi apparatus or choline/ethanolamine phosphotransferase-1 (CEPT1) found in the nucleus and ER (41). In liver, knockdown of CEPT1, but not ChPT1, resulted in downregulation of PPARα-dependent gene expression, whereas overexpression of liver CEPT1 increased PPARα-dependent gene expression (11), suggesting that generation of GPC in the ER or nucleus is a relevant physiological event in liver.

Do similar phosphatidylcholine species serve as endogenous ligands for PPARs in skeletal muscle? It is unknown whether the ligand for PPARs is the same in liver as in other tissues. CEPT1 protein is abundantly expressed in liver, skeletal muscle and heart (K. Funai and C. F. Semenkovich unpublished results). Abundance of CEPT1 protein varies considerably in skeletal muscle, with slow-twitch muscles such as vastus lateralis and extensor digitorum longus (K. Funai and C. F. Semenkovich unpublished results). Slow-twitch muscles have a higher intramuscular TG content as well as more robust expression of enzymes involved in lipid metabolism (4), so it is possible that CEPT1 contributes to the oxidative phenotype by generating PPAR ligands in skeletal muscle. The role of exercise in modulating expression of CEPT1 or related Kennedy pathways enzymes is unknown, but AMPK, an important mediator of the energy depletion associated with exercise, is known to induce the expression of genes encoding enzymes involved in phospholipid biosynthesis (5).

Exercise training increases phosphatidylcholine in muscle (78), and the effects of exercise on numerous phospholipid species in skeletal muscle have been examined in rats fed a standard chow diet (77) or a high-fat diet (76). With chow feeding, exercise training increased the abundance of two phosphatidylcholine species (77): 16:0/18:1-GPC, the species also identified as an endogenous PPARα ligand in liver, and 16:0/18:2-GPC (1-palmitoyl-2-linoleoyl-sn-glycerol-3-phosphocholine). The latter phosphatidylcholine species was also found to be associated with PPARα in liver, but it was not displaced with the potent PPARα agonist Wy14,643 (11). It is possible that multiple endogenous ligands exist for PPARs. The affinity for phosphatidylcholine may be similar between PPARα and PPARβ/δ (11), so 16:0/18:1-GPC and 16:0/18:2-GPC may be candidates for PPARα and/or PPARβ/δ ligands in skeletal muscle. In addition, exercise training enhanced the abundance of 16:0/18:2-GPA (phosphatidic acid), 18:1/18:2-GPA, 16:0/18:2-plasmeyl-GPE (phosphatidylethanolamine), and 18:0/22:5-GPI (phosphatidylinositol) with the normal chow diet (77). With high-fat feeding (76), exercise training also increased 16:0/18:2-GPC, 18:1/18:2-GPA, and 16:0/18:2-plasmeyl-GPE but additionally resulted in increases in isobaric 18:0/18:2-GPE or 18:1/18:1-GPE, emphasizing the importance of diet in modulating skeletal muscle phospholipid responses to exercise that may be relevant to PPAR signaling.

Conclusion

Exercise is the ideal treatment for chronic conditions associated with individuals in positive energy balance, and this may be due, in part, to its ability to partition skeletal muscle lipid molecules to compartments where they are unlikely to “poison” muscle by disrupting insulin signaling or energy generation. Upregulation of intramuscular TG synthesis or lipid oxidation appears to protect skeletal muscles from fat-induced insulin resistance. Exercise also stimulates phospholipid biosynthesis, a process that might be involved in the production of endogenous ligands for PPARs. Identifying and characterizing physiologically relevant endogenous ligands for PPARs in skeletal muscle as well as the pathways involved in their generation could lead to novel approaches for promoting lipid flux and possibly preventing the poisoning of muscle physiology by fats.

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