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Review

Skeletal muscle lipid metabolism: A frontier for new insights into fuel homeostasis

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Although skeletal muscle is recognized as a primary site of lipid utilization, the study of muscle bioenergetics has focused mainly on carbohydrate, and consequently our understanding of the variables that regulate muscle lipid metabolism is comparably poor. This review focuses on the significance of muscle lipid metabolism in regulating whole-body energy homeostasis. Multiple pathways involved in controlling muscle lipid biochemistry are discussed, and comparisons with other tissues are described. Considerable evidence indicates that muscle lipid biochemistry is altered in disease states, and a number of metabolic disorders may be explained by dysregulation of muscle lipid metabolism. An understanding of the factors accounting for dysregulated muscle metabolism is a necessity in light of the increase in the incidence of disease syndromes such as obesity and diabetes, which collectively account for a high incidence of morbidity and mortality in the western society. Therefore, the purpose of this review is to describe the biochemical events involved in the regulation and dysregulation of skeletal muscle lipid metabolism and to encourage new investigation in muscle lipid research. (J. Nutr. Biochem. 8:228–245, 1997) © *Elsevier Science Inc. 1997*

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Introduction

Lipids are well recognized as important substrates for fueling the energy demands of skeletal muscle. However, the study of muscle lipid utilization has long been overshadowed by research focused on muscle carbohydrate metabolism. Consequently, muscle lipid metabolism has been examined primarily within a "glucocentric" model of muscle physiology committed to an understanding of lipid metabolism only within the context of glucose-fatty-acid interactions. Accordingly, whereas the regulation of muscle glucose metabolism is well understood within a variety of physiological and pathophysiological paradigms, comparatively little is known regarding the mechanisms that regulate skeletal muscle lipid metabolism.

The objective of this review is to approach muscle biochemistry from a more "lipocentric" perspective in which muscle lipid metabolism will be presented as: (1) a key parameter in determining whole-body energy homeostasis and (2) a factor that may play an underlying role in the pathogenesis of metabolic disorders such as obesity and diabetes. This discussion will evaluate the pathways of muscle lipid metabolism, highlighting the biochemical targets that represent potentially critical sites of muscle lipid regulation and/or dysregulation. The purpose of such a discussion is to stimulate new thinking about muscle lipid metabolism and to encourage investigation in areas of muscle research that have been thus far overlooked.

Regulation of skeletal muscle lipid metabolism

Skeletal muscle is responsible for a large proportion of whole-body lipid oxidation, and the primary fate of lipid

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delivered to muscle is for use as an oxidative fuel. This section will therefore focus on the regulatory aspects of muscle lipid oxidation, but will also discuss the channeling of free fatty acids (FFA) into the muscle acylglycerol pool, because intramuscular triacylglycerols (TAG) provide an important endogenous source of lipid substrate. The relevant parameters to consider in the regulation of muscle lipid metabolism can be categorized under the criterion of (1) substrate or FFA availability, which depends on mobilization and transport of FFA into muscle and (2) subsequent trafficking of FFA within the muscle cell, which is orchestrated by proteins that facilitate translocation across cellular and subcellular membranes, cytosolic carrier proteins, and key enzymes that direct FFA toward oxidation or storage. Delivery, transport, and intracellular handling of FFA differs markedly between long-chain and medium-chain fatty acids. The present review will discuss only the metabolism of long-chain fatty acids, which represent the principal lipid substrate metabolized by skeletal muscle.

Substrate availability—the role of muscle lipases

Delivery of FFA substrate to the muscle depends on the mobilization and transport of FFA that are originally esterified in the form of TAGs, and that are hydrolyzed from a glycerol backbone by specific lipases. Sources of FFA include plasma nonesterified fatty acids released from adipose TAGs by hormone-sensitive lipase (HSL), circulating triacylglycerol fatty acids (TAGFA) made available to the muscle via the action of muscle lipoprotein lipase (mLPL), and FFA released from endogenously stored intramuscular TAG through the action of a muscle-localized HSL. Plasma nonesterified fatty acids account for a large majority (approximately 80 to 90%) of the muscle's lipid fuel needs during fasting and mild exercise,[1] because under these conditions, increased adipose tissue lipolysis and a corresponding elevation in circulating concentrations of FFA lead to increased muscle uptake and turnover of plasma FFA. Mobilization of FFA from adipose tissue occurs through a well described, neurohormonally mediated mechanism that has been discussed at length elsewhere and will not be addressed here.[2] Alternatively, after feeding or during moderate exercise, circulating TAGFA or intramuscularly derived TAGFA, respectively, can account for a significant proportion of total muscle lipid oxidation. Under these circumstances, the lipase enzymes that release esterified fatty acids from TAG play an important role in determining muscle lipid uptake and utilization.

Lipoprotein lipase. Delivery of circulating TAGFA to the muscle is accomplished via the action of mLPL, which hydrolyzes fatty acids from TAG carried in circulating lipoproteins. Although plasma lipoproteins appear to contribute only 5 to 15% of the fatty acids used by muscle during exercise, they are of considerable importance in delivering lipid to muscle during rest and after exercise.[3–6] An investigation of fatty acid turnover from circulating TAG and plasma FFA in resting-fed humans, dogs and rats, revealed that circulating TAG provide approximately 50 to 80% of the fatty acids taken up by skeletal muscle.[7] The majority of TAGFA sequestered by muscle (under resting conditions) is diverted immediately to the muscle acylglycerol pool where it is available for oxidization on demand.[4,6] Whereas conventional wisdom depicts adipose tissue as the primary destiny of circulating TAGFA, alternative viewpoints now consider muscle a critical site for lipoprotein TAGFA clearance. This view is supported by recent data demonstrating that muscle tissue was the primary site of TAGFA clearance in rats fed ^{14}C-labeled triolein.[8] Investigators followed the metabolic fate of lipid starting at 20 min after ingestion and throughout 30 days. Two hours after ingestion, 3.5 times more label was detected in skeletal muscle and as CO_2, than in adipose tissue. Intramuscular TAG was the muscle lipid class most extensively labeled and the total amount of labeled lipid in muscle declined to negligible levels between days 2 and 10 after ingestion, suggesting that the TAGFA were most likely oxidized during that time. The interpretation of this data must be approached cautiously, because the animals were fed the labeled lipid after an overnight (14 hr) fast. However, the results suggest that, at least subsequent to fasting conditions, skeletal muscle and mLPL strongly influence the degree to which dietary lipids are either stored or oxidized.

Although much research has been devoted to examining the hormonal and nutritional regulation of adipose LPL at multiple levels of cellular and molecular control, comparatively little attention has been focused on understanding the mechanisms involved in the regulation of mLPL. The activity of mLPL is often reciprocally regulated with adipose tissue LPL and therefore demonstrates inverse responsiveness to a variety of physiological stimuli. For example, most investigators report increases in mLPL activity after endurance training,[5,9] starvation,[10] high-fat feedings,[11,12] and exposure to cold temperatures;[13] whereas, these same stimuli have been shown to reduce adipose LPL activity.[10,12,14] In addition to demonstrating tissue-specific regulation, LPL also exhibits muscle fiber type-specific regulation. Both mLPL activity and the rate of uptake of circulating TAGFA is greater (by five fold) in muscle composed mainly of highly oxidative slow-twitch or "red" muscle fibers, as compared with less oxidative fast-twitch or "white" fibers.[3] Muscle LPL plays a critical role in replenishing intramuscular TAG stores that have been depleted during contractile activity.[15–17] Thus, the fact that mLPL activity is greater in red than in white muscle can be explained by the respective differences between the two fiber types in the storage levels and use of intramuscular TAG (discussed below). The observation that LPL is regulated, not only in a tissue-specific, but also in a fiber type-specific manner, provides further evidence of its putative role in directing the partitioning of lipid fuel between adipose and muscle and between storage and oxidation.

The activity of LPL depends on its site-specific synthesis by parenchymal cells, followed by glycosylation within the Golgi complex, and subsequent transport and binding of the enzyme to heparin sulfate proteoglycans on the cell surface of the capillary endothelium supplying the tissue. LPL activity can therefore be controlled at multiple levels. The measurement of post-transcriptional regulation of LPL is complicated because of the presence of intracellular, membrane-bound, and heparin-releasable compartments of enzyme activity. Thus, an increase in mRNA and protein

levels does not necessarily indicate an increase in functional LPL activity, which is commonly measured by and referred to as heparin-releasable LPL activity. Like adipose LPL, hormones play an important role in modulating mLPL activity. However, in contrast to adipose LPL, insulin decreases mLPL activity,[18,19] whereas glucocorticoids and catecholamines increase mLPL activity.[5,20] The effects of these hormones on LPL activity is at least partially mediated by intracellular changes in cAMP levels, demonstrated by the observation that β-adrenergic agonists and other agents that elevate cAMP levels also produce increases in mLPL.[21,22] In cardiac myocytes, adrenaline increases heparin releasable LPL activity, the level of LPL glycosylation as well as the synthesis of LPL, suggesting that cAMP regulates LPL activity at both the transcriptional and post-transcriptional levels.[23] Other hormones that are involved in regulating adipose LPL synthesis and/or activity include thyroid hormone, prolactin, growth hormone, and proinflammatory cytokines such as TNF, IL-1, IL-6, and LIF (reviewed in Ref. 14). Interestingly, these cytokines have been implicated in mediating a number of pathologies that are associated with alterations in muscle lipid metabolism.

The LPL gene has been cloned and sequenced for a variety of species including human, mouse, and rat.[24,25] Analysis of the 5′ flanking region has revealed several *cis*-acting regulatory elements that respond to hormone- and nutrient-specific transcription factors.[14,26] Most studies on transcriptional control of LPL have been conducted in adipocyte and pre-adipocyte cell lines. Investigations examining LPL gene regulation in muscle tissue indicate that mLPL expression is reciprocal to that of adipose[10,12] and is less acutely responsive to physiologic regulators such as hormones, nutritional status, and exercise.[14,27] Therefore, compared with adipose LPL, changes in mLPL activity are less often associated with corresponding changes in mLPL mRNA levels, suggesting that post-transcriptional events are primarily responsible for regulating mLPL activity. Whether such regulation occurs at the levels of translation, glycosylation, transport, or heparin-binding activity remains to be elucidated. LPL of various tissues is the same gene product and to date, there is no evidence of more than one isoform of the protein. Thus tissue-specific responsiveness to various hormonal and physiological stimuli is likely regulated at the level of the gene promoter region in accordance with tissue-specific expression of *trans*-acting enhancing or suppressing factors.[28,29] Although unexplored, differences between tissues in the LPL processing pathway may also contribute to tissue-specific control of LPL activity. For a more detailed description of LPL regulation the reader is referred to an excellent review by Enerback and Gimble.[14]

Hormone-sensitive lipase. In addition to plasma sources, FFA can also be derived from endogenously stored TAG. In contrast to glycogen, TAG in muscle is stored heterogenously in the fiber and the TAG content of muscle varies considerably according to fiber type, with the greatest stores apparent in muscles comprised predominately of slow-twitch, oxidative fibers and the lowest stores seen in muscles characterized by fast-twitch, glycolytic type fibers.[30] Muscle TAGs provide a significant source of FFA for oxidation during moderate-intensity exercise,[17,30,31] and fatty acids derived from muscle TAG account almost entirely for the training-induced increase in muscle lipid oxidation during exercise.[15] Evidence pointing to the importance of intramuscular TAG as an oxidative fuel source for muscle comes largely from several reports of a sizable discrepancy between total fat and plasma FFA oxidation during moderately strenuous exercise.[17] However, an often neglected limitation to most, if not all, studies that have estimated the relative contribution of muscle TAG to total muscle fatty acid oxidation is the inability to account for the role of fat deposits interspersed among muscle fibers. Fatty acids released by adipocytes present in the perimyseum are generally assumed to contribute to skeletal muscle lipid utilization, but at a physiologically insignificant rate, considering the low interstitial concentrations of albumin that is necessary for binding FFA in an aqueous environment.[17] Because skeletal muscle uptake and oxidation of FFA derived from adipose tissue marbled within muscle is methodologically difficult to quantify, the above assumptions have not been validated.

The mechanisms controlling muscle TAG hydrolysis appear to parallel those implicated in adipose tissue lipolysis and involve activation of a classical cAMP cascade with subsequent phosphorylation and activation of a muscle HSL. Using antibody raised to purified rat adipose HSL, Holm et al.[32] evidenced the existence of an antigenic protein in muscle extract that corresponds in size with adipose HSL. Expression of HSL in muscle was confirmed using a cDNA clone from rat adipose HSL to perform Northern analyses that revealed HSL mRNA in both heart and skeletal muscle at a level approximately 2% of that in adipose.[33] Three mRNA species of HSL were identified (3.3, 3.5, and 3.9 kb) that mapped to the same chromosomal locus and showed tissue-specific expression (the 3.5 kb species is expressed in heart and skeletal muscle). The nature or significance of the mRNA size variations has not been determined, but may represent a mechanism for tissue-specific regulation of HSL activity. The amino acid sequence predicted from a cDNA clone revealed an activity-controlling phosphorylation site. This was confirmed by Small et al.[34] who used HSL purified from bovine heart and cardiac myocyte preparations to demonstrate that muscle HSL is activated acutely through reversible phosphorylation by a cAMP dependent protein kinase.

Because the hydrolysis of muscle TAG has been reported to be inhibited completely by nonselective β-adrenergic antagonists, whereas only partial inhibition is observed in adipose tissue,[35] muscle TAG hydrolysis is considered to be more sensitive to sympathetic stimulation than adipose tissue. Additionally, because a nonselective but not a β_1-selective adrenergic blockade inhibits skeletal muscle lipolysis, the hydrolysis of muscle TAG is thought to be mediated by activation of β_2-adrenergic receptors.[36] Radioligand binding analyses have confirmed the presence of β_2-receptors in skeletal muscle.[37] The degree to which intramuscular TAG are used as a fuel is greater in oxidative muscle-types and also increases in response to exercise training.[15,30] These variations in muscle TAG utilization among fiber types and in response to training stimuli may be partly explained by tissue-specific and exercise-induced

increases in β-adrenergic receptor density.[38] Alternatively, fiber specific expression and/or training-induced effects on muscle HSL must also be considered, but have yet to be explored. Data from Miller and Oscai[39] demonstrating that an increase in mLPL activity occurs concomitant to an increase in muscle TAG hydrolysis in response to fasting, cold exposure, and fat feedings, suggest that muscle HSL is simultaneously activated with mLPL under a variety of physiological conditions. The effects of these stimuli on muscle TAG metabolism are likely mediated by sympathetic hormones because a similar relationship between LPL activity and muscle TAG levels is observed during muscle perfusions with epinephrine.[22] Investigators have proposed that the two muscle lipases may function as a coordinated unit in meeting the energy demands of the muscle. The development of advanced molecular technologies now offers new approaches to evaluate the co-expression and coregulation of these muscle lipases. Evaluating the gene promoter regions and identifying potential enhancer or repressor elements will provide insight into muscle specific control and coordination of these enzymes.

Cellular trafficking of muscle lipids

Fatty acid binding proteins. Once FFA are mobilized and/or delivered to the muscle, maximal fluxes of FFA through metabolic pathways may be limited by the muscle's ability to direct FFA to the appropriate sites for intracellular processing. A family of low molecular weight proteins referred to as fatty acid binding proteins (FABP) have been identified and characterized in cell membranes and cytoplasm. These proteins have been shown to bind fatty acids[40] and are hypothesized to mediate cellular fatty acid transport and trafficking. Other proposed functions include compartmentalization of cellular fatty acids, modulation of the activity of lipid regulatory enzymes, and protection of cellular enzymes and membranes from the potentially adverse effects of fatty acids. Because fatty acids are a major source of energy for skeletal muscle, it follows that the translocation of fatty acids within the myocyte from the sarcolemma to the mitochondria is critical. Although the evidence is still only suggestive, fatty acid transport proteins and FABPs may play an integral role in controlling muscle fatty acid metabolism.

Fatty acids delivered to muscle from extracellular sources must be transported from the capillary bed across the cellular membrane into the cytosol. Because protonated fatty acids are soluble in membranes, and in light of the tight relationship that is observed between arterial inflow of FFA and their uptake by muscle,[41] FFA were long thought to cross the sarcolemma freely in an unassisted manner. More recently, however, FFA movement across muscle membrane was shown to be a saturable process that occurs at a greater rate in endurance exercise trained versus untrained muscle,[41,42] suggesting the presence of a regulated FFA transporter. Multiple membrane-bound fatty acid binding proteins referred to as fatty acid translocase or fatty acid transport proteins (FATP) have been identified in a variety of tissues, with high expression levels observed in adipose tissue, heart, and skeletal muscle.[43,44] When expressed in cultured cells, a 71 kDa FATP, that was localized to the plasma membrane of 3T3-L1 adipocytes, augmented uptake of long-chain fatty acids.[45] The observation that this protein is highly expressed in heart and skeletal muscle supports the proposed role for FATPs in mediating muscle FFA uptake. The expression level of FATPs in muscle seems to depend on the metabolic nature of muscle. For example, the expression level of an 88 kDa FATP in rat skeletal muscle relative to cardiac muscle (100%) was 72% in soleus and 54% in extensor digitorum longus.[44] In addition, this FATP is apparently co-expressed in muscle with a cytosolic muscle FABP (discussed below), which is also expressed in a fiber-type specific manner.[43] During development, heart mRNA levels for both the membrane-bound and the cytosolic proteins are higher in adult versus fetal cardiomyocytes,[43] which is consistent with the reported developmental increase in heart FFA utilization. These observations, coupled with the finding that expression of both FATP and muscle FABP is greater in the oxidative soleus muscle as compared with the more glycolytic extensor digitorum longus, not only support the predicted role for FATPs in the sequestration and transport of long-chain fatty acids, but furthermore, suggest a link between FATP and muscle FABP in coordinating oxidative metabolism in muscle. Details on exact positioning in the muscle membrane, protein density, transport kinetics, and movement between subcellular locations are under current investigation.

Because FFA are insoluble in aqueous media, upon their entry or release into the cytosol, they must be transported via cytosolic proteins. Unique FABP types (liver, intestinal, adipose, myelin, and heart) have been identified in a variety of mammalian tissues at both the protein and cDNA levels.[46] The FABP subtype expressed in skeletal muscle is identical to the heart FABP. Muscle FABP is widely distributed among tissues but is found in the highest concentration in heart and skeletal muscle.[43] Based on in vitro binding assays and competition studies, muscle FABP shows a preference for unsaturated fatty acids, with highest affinity for those with 18 carbons.[40] Although each distinct FABP differs in structure, tissue distribution, and ligand specificity, all except for the liver type demonstrate a binding specificity for FFA.[46] The cytoplasmic FABPs are unique from other cellular proteins in that they are among the most abundant cytoplasmic proteins in cells of tissues that are characterized by higher rates of FFA utilization (intestine, heart, skeletal muscle, liver, and adipose).[47]

Despite considerable investigation, the precise physiologic function of FABPs is still unclear. Although conclusive evidence is still lacking, data from in vitro experiments suggest involvement of FABPs in delivering fatty acids to mitochondria and peroxisomes for oxidation. Using cell model systems, FABPs have been shown to take up fatty acids from vesicles, liposomes, or from mitochondrial membranes,[48,49] to also release fatty acids to membranes,[48] and to donate fatty acids to mitochondria or peroxisomes for oxidation.[48,50] The expression of liver FABP in transfected fibroblasts resulted in increased fatty acid uptake into cells and also stimulated incorporation of fatty acid into specific esterified lipid pools.[51] In addition, Glatz et al.[17] calculated that the presence of muscle FABP in cardiomyocytes increases the solubility of FFA in the cytoplasm by more than 1000 fold (from .002 to 2.5 μM), and may thus assist

in maintaining diffusional flux of unbound FFA into the mitochondria. Still, predictions of the biological significance of these cytosolic proteins stem largely from apparent relationships between changes in cellular FABP content and concomitant alterations in cellular or tissue lipid metabolism. Investigations to examine muscle FABP expression among various muscle fiber types indicate that both the protein as well as mRNA levels are 4 to 6 fold higher in soleus muscle than in extensor digitorum longus,[43] and that a strong correlation exists between muscle FABP content of muscle and the oxidative capacity of the muscle.[43,46] These observations suggest that FABPs may support tissue-specific requirements for higher maximal FFA fluxes, and that the maximal flux of FFA, fatty acyl-CoA, and acylcarnitine esters through the cytoplasm and into oxidative pathways may be limited by the availability of cytosolic binding proteins. Indirect data to support such a limitation comes not only from the observation that the ability of different tissue types, as well as muscles comprised of different fiber types, to oxidize fatty acids is strongly correlated with the fatty acid binding capacity of their respective cytosolic FABPs,[52] but also from finding that expression and/or content of muscle FABP is increased in response to starvation, electrical stimulation, and endurance training,[42,43] all representing physiological states in which the need for fatty acid oxidation is enhanced.

Acyl-CoA synthetase and Acyl-CoA binding protein. Before being directed into storage or oxidation, fatty acids are first converted to fatty acyl-CoA (acyl-CoA) by acyl-CoA synthetase (ACS). ACS is present in mitochondria, endoplasmic reticulum, and peroxisomes and is membrane-bound in all three organelles. After activation by ACS, acyl-CoA can be committed to glycerolipid formation by the action of GPAT (glycerol-3-phosphate acyltransferase) or can be channeled toward β-oxidation by CPT 1 (carnitine palmitoyltransferase I). Although it is generally assumed that these enzymes compete for the same substrate (acyl-CoA), the existence of functionally specific ACS isoforms that direct acyl-CoA moieties into distinct pools has been hypothesized.[53] Because fatty acids must be activated by ACS before coming available for β-oxidation, this enzyme, along with the availability of coenzyme A, may represent another biochemical bottleneck that restricts maximal oxidation rates. Studies on the regulation of ACS have been conducted primarily in liver and adipose tissue where ACS is considered a key enzyme in controlling lipid accumulation. Limited data on ACS regulation in muscle indicate that the enzyme activity, but not mRNA, increases by more than 2 fold in response to exercise training.[54] This finding is consistent with the prediction that ACS can contribute to the regulation of muscle lipid oxidation, but the biological significance and mechanisms responsible for these observations need to be studied.

It is now hypothesized that acyl-CoA moieties synthesized by ACS can subsequently bind to a cytosolic acyl-CoA binding protein (ACBP). The foundation for this hypothesis comes mostly from the observation that ACBP exhibits a high binding affinity for acyl-CoA esters with chain lengths of C_{14}–C_{22}.[55] Like FABP, the exact function of ACBP is still only speculative and its putative participation in acyl-CoA metabolism requires further investigation. There is in vitro data, however, to suggest that ACBP may have a function in modulating processes that depend on acyl-CoA availability. In rat mitochondrial membrane preparations, recombinant ACBP stimulated the synthesis of long-chain acyl-CoA esters by binding to acyl-CoA moieties and thereby opposing product feedback inhibition.[56] In addition, a high ACBP/acyl-CoA ratio attenuated long-chain acyl-CoA inhibition of acetyl-CoA carboxylase and mitochondrial adenine nucleotide translocase. The authors proposed that ACBP serves dual functions (1) in maintaining a large intracellular pool of acyl-CoA to be used in processes such as β-oxidation or glycerolipid synthesis and (2) in protecting acyl-CoA sensitive enzymes from the regulatory effects of acyl-CoA esters. In vivo data to support an important regulatory role for ACBP comes from the observation that changes in nutritional status of rats affected ACBP levels in liver, kidney, and heart.[57] In general, fasting caused a decrease in ACBP, whereas a high-fat diet produced an increase in ACBP. The biological significance of ACBP in skeletal muscle has not been addressed, but the data described above present potential implications for the interaction of ACBP on muscle lipid oxidation, especially as it may be related to the control of CPT-1 and acetyl-CoA carboxylase (discussed below).

Fatty acid oxidation and the CPT system. After being activated by ACS, acyl-CoA must first be translocated into the mitochondria before undergoing β-oxidation. CPT 1 is the enzyme that catalyzes the rate-limiting step for the transport of acyl-CoA across the mitochondrial membrane, and is widely considered the most important regulatory enzyme in determining the cellular rate of FFA oxidation. CPT 1 is expressed as at least two isoforms; the liver enzyme and its counterpart in skeletal muscle, each having different kinetic properties.[58] Because the literature on this topic is voluminous and has been reviewed previously,[58–60] the scope of the current discussion will focus on muscle CPT 1 and its interaction with malonyl-CoA in a newly described model of a malonyl-CoA fuel-sensing mechanism in muscle.[61]

Malonyl-CoA is a precursor for de novo fatty acid synthesis and acts as the key regulator of acyl-CoA entry into mitochondria by inhibiting the activity of CPT 1. In muscle, the K_i of malonyl-CoA for CPT 1 is approximately 100 times lower than for CPT 1 in liver (0.03 vs. 2.7 μmol/gww).[62] Although not considered a lipogenic tissue, skeletal muscle content of malonyl-CoA ranges from 0.4 to 2.1 nmol per gram wet weight, which is approximately one third the content in liver, which ranges from 1.7 to 7.5 nmol/gww.[62] Unanswered is the paradoxical question of how β-oxidation can occur in muscle given that even at its lowest physiological range, the measured malonyl-CoA content of muscle should completely inhibit CPT 1, given a IC_{50} value for muscle CPT 1 of 0.03 μmol. In his review, McGarry suggests that most of the malonyl-CoA present in muscle may be in a bound form, inaccessible to CPT 1.[59] In liver, starvation, hypoinsulinemic, or insulin-resistant states result in decreased sensitivity of CPT 1 to inhibition by malonyl-CoA, thereby promoting β-oxidation and ketogenesis.[63] This desensitization does not occur in heart, and

because the muscle and heart CPT 1 isoforms are similar, it is likely that CPT 1 sensitivity to malonyl-CoA in skeletal muscle is also unresponsive to physiological conditions. Control of fatty acid oxidation in these tissues is thus achieved primarily through changes in cytosolic malonyl-CoA concentrations.

An integral role for malonyl-CoA in regulating muscle CPT 1 and β-oxidation is evidenced by a number of studies showing that tissue concentrations of malonyl-CoA change in response to various physiological stimuli known to produce effects on lipid oxidation. The concentration of malonyl-CoA decreases in rodent muscle after exercise (by 65%)[64,65] or electrical stimulation (by 35%),[61] and after starvation.[66] In contrast, muscle levels of malonyl-CoA are increased by 2 to 3 fold in response to muscle denervation[61] and to a lesser degree by refeeding.[66] These data are consistent with the directional changes in fatty acid oxidation and glycerolipid synthesis that occur under the respective physiological conditions. In addition, Saha et al.[61] reported that in the presence of either glucose or insulin, malonyl-CoA levels in isolated soleus rise acutely in a dose dependent manner, and the greatest effect occurs when muscles are incubated in the presence of both insulin and glucose. Because de novo synthesis of fatty acids is not a predominant pathway in skeletal muscle, the authors suggest that malonyl-CoA is generated strictly as a regulatory signal and that glucose and insulin might stimulate malonyl-CoA production via activation of acetyl-CoA carboxylase (ACC), the enzyme that synthesizes malonyl-CoA from acetyl CoA.

A unique muscle isoform of ACC appears to be regulated differently than the liver isoform. ACC in muscle has a higher K_m for acetyl-CoA and higher K_i for malonyl-CoA.[67] Consequently, the predominant ACC isoform in muscle is more dependent on substrate supply and less sensitive to product inhibition. This observation has important implications in the development of metabolic dysregulations that may present pathological consequences (discussed in section II). In liver, where ACC production of malonyl-CoA is the first step in fatty acid biosynthesis, insulin causes rapid dephosphorylation and consequent activation of ACC. This is consistent with insulin's role as a lipogenic hormone. The report by Saha et al.[61] implies that insulin may also regulate muscle ACC by a phosphorylation-dephosphorylation-dependent mechanism. Furthermore, Winder and Hardie[65] recently reported that the activation of an AMP-activated protein kinase resulted in the concomitant phosphorylation and inactivation of the muscle ACC isoform both in vivo and in vitro. Treadmill running resulted in the activation of an AMP-activated protein kinase, which occurred concurrently with a marked decrease in muscle ACC activity. These data, together with observations of marked decreases in muscle malonyl-CoA levels in response to fasting, exercise, and electrical stimulation, suggest that muscle ACC is modulated by the energy milieu of the muscle.

A newly proposed model of a multitissue fuel sensing mechanism describes malonyl-CoA as an intracellular "signal of plenty" that regulates fuel partitioning by directing the trafficking of acyl-CoA between fates of oxidation or storage.[68] ACC is presented as the fuel sensor that integrates the concentrations of all cellular fuels and responds appropriately by altering the synthesis of malonyl-CoA. Accordingly, during states of energy deficit, hormones, and/or factors associated with muscle contraction mediate the phosphorylation and inactivation of ACC, resulting in a concomitant decrease in muscle malonyl-CoA production. The reduction in cellular malonyl-CoA level is postulated to relieve inhibition of CPT 1, thereby promoting fatty acid oxidation. In contrast, hyperglycemic, hyperinsulinemic states result in activation of ACC and production of malonyl-CoA, leading to a shift toward increased glucose oxidation and decreased fatty acid oxidation. Inhibition of muscle fatty acid oxidation would also presumably lead to an increase in muscle acylglycerol biosynthesis and increased intramuscular TAG storage.

The interaction between glucose and FFA metabolism in controlling muscle substrate utilization has been debated in the literature. This issue is the focal point of the "glucose-fatty acid cycle," first proposed by Randle et al. in 1963, which predicts that an increase in fatty acid oxidation inhibits glycolysis and glucose transport (for review, see Ref. 61). For years the so-called Randle cycle has provided a theoretical basis for explaining the metabolic abnormalities associated with insulin-resistant states (i.e., elevated serum lipids, hyperglycemia, and decreased glucose disposal). Although a widely accepted theory, its validity has been the subject of much controversy and has been recently challenged by data suggesting that glucose availability controls fatty acid oxidation rather than the reverse.[69] Investigators demonstrated that in normal-weight humans during a hyperglycemic (7.7 mM), hyperinsulinemic clamp in which serum FFA and TAG were held constant at basal levels, whole-body FFA oxidation decreased markedly. Using stable isotope techniques, researchers determined that plasma FFA oxidation decreased by almost 50%, whereas total FFA oxidation decreased by 84%, indicating that oxidation of endogenously derived FFA was also reduced. The authors speculated that the mechanism by which accelerated glucose metabolism inhibited FFA oxidation involved glucose and insulin stimulated production of malonyl-CoA in both liver and muscle. These findings suggest that glucose availability primarily determines whole-body substrate selection. With respect to fuel homeostasis, these data imply that prolonged states of elevated serum glucose and insulin levels (i.e. overfeeding) may chronically inhibit muscle FFA oxidation, thereby leading to accumulation of intramuscular acyl-CoA and TAG.[70]

Channeling of fatty acids into the acylglycerol pool. A widely accepted model of muscle FFA metabolism proposes that FFA entering the muscle are rapidly esterified and incorporated into the muscle TAG pool before being oxidized, and FFA released from intracellular lipolysis provide the majority of substrate for mitochondrial β-oxidation. This model is founded largely on indirect evidence from research conducted in the 1970's to 1980's, indicating that in either fasted or fed rats, 65 to 90% of FFA taken into muscle from chylomicron TAG are detected in the muscle TAG pool.[3,4,6] The most convincing evidence was provided by Zierler[71] who infused [^{14}C]oleate into the brachial artery of humans subjects and measured uptake of label and production of $^{14}CO_2$ by forearm muscles. Data revealed that

although uptake of [^{14}C]oleate reached steady state within 10 min, $^{14}CO_2$ released into venous blood was not detected until 30 min, and the delay was not because of mixing of $^{14}CO_2$ in a large CO_2 pool. Researchers concluded that FFA taken into muscle are channeled preferentially into TAG, and that FFA released by lipolysis of intramuscular TAG are the major immediate substrate for β-oxidation. Studies to discern a mechanism by which incoming muscle FFA may be preferentially directed into the TAG pool have not been conducted.

The biosynthetic pathways responsible for muscle TAG synthesis involve multiple enzymes and therefore present a number of potential regulatory points for determining the fate of long chain acyl-CoA. Because these pathways are poorly studied in muscle, only GPAT, the enzyme that converts glycerol-3-phosphate and acyl-CoA to lysophosphatidic acid, will be considered. GPAT represents a primary candidate for controlling lipid partitioning because it catalyzes the first committed step in TAG synthesis. The lysophosphatidic acid that is produced by GPAT serves as a precursor for the production of diacylgercerol (DAG), which is the immediate substrate for both TAG and phospholipid synthesis. GPAT activity has been demonstrated in skeletal muscle[72] and in BC3H-1 myocytes[73] and therefore represents a potential target for controlling muscle lipid storage and TAG cycling. GPAT is thought to exist as two isozymes, one located in the outer mitochondrial membrane and the other in the endoplasmic reticulum.[74] The two isoforms seem to be regulated differently and it is the microsomal fraction that predominates in muscle, accounting for approximately 85% of total muscle GPAT activity.[73] The control of GPAT likely involves insulin, which seems to regulate GPAT at multiple levels. In vivo, hypoinsulinemic states (diabetes, starvation) result in decreased mitochondrial GPAT expression, and insulin injections increase GPAT mRNA by 2 fold within an hour and by 19 fold 6 hr after injections.[75] Administration of cAMP abolished the insulin-stimulated increase in GPAT transcription. In perfused rat liver, cardiomyocytes, and in adipocytes, insulin causes acute activation of mitochondrial GPAT,[76,77] whereas in BC3H-1 myocytes insulin stimulates rapid activation of microsomal GPAT.[73,78] These data are consistent with the observation that insulin increases muscle TAG and DAG de novo biosynthesis.[79] Evidence in both myocytes and adipocytes indicates that acute insulin-induced stimulation of GPAT occurs through a glycosyl-phosphatidylinositol derived mediator that is released by G-protein coupled activation of phospholipase C.[73] The physiological significance of this non-tyrosine-kinase insulin-activated signaling pathway is still unclear, but available data suggest that it not only plays a role in mediating insulin's effects on muscle lipid metabolism, but that there may be "cross-talk" between this pathway and the tyrosine-kinase-dependent pathways involved in regulating muscle glucose homeostasis.[80] Elevated levels of both muscle TAG and DAG are strongly correlated with defects in insulin signaling and cellular glucose regulation, and therefore a cause-effect relationship between muscle lipid dysregulation and insulin resistance has been proposed (discussed below).

Dysregulation of skeletal muscle lipid metabolism

Insights into skeletal muscle lipid metabolism can also be gained from studying conditions of metabolic dysregulation. Obesity and diabetes are extremely important areas of research because of their prevalence in Western societies. Although both may be viewed as a disorder in glucose metabolism, they also represent diseases of dysregulated lipid metabolism. This section of the review will therefore present a discussion of the relationship(s) between obesity and non-insulin-dependent diabetes mellitus (NIDDM) and skeletal muscle lipid metabolism.

Impaired skeletal muscle lipid utilization

Obesity. Obesity is the most common disorder of metabolism in man in the Western World[81] and is strongly associated with morbidity and mortality.[82] Obesity is characterized by increased adipocyte mass consequent to both genetic and environmental factors. Whereas genetic variability predisposes a person to weight gain rather than determining the disease with certainty, feeding studies emphasize the influence that environmental variability plays on the manifestation of the disease.

With regard to systemic lipid dynamics, obesity is associated with adipocyte resistance to insulin's antilipolytic effects resulting in increased lipolysis and elevated serum FFA.[83] Obesity associated alterations in skeletal muscle lipid uptake and oxidation is less clearly understood. In healthy, lean individuals, skeletal muscle lipid oxidation is elevated in association with high rates of FFA uptake across the limb.[84,85] In contrast, other investigations suggest that lipid oxidation is impaired in individuals at risk for obesity.[86–89] In a study by Colberg et al.,[87] women with increased visceral fat content demonstrated reduced postabsoptive rates of FFA uptake across the leg. Muscle CPT 1 activity was positively correlated with postabsorptive rates of FFA uptake by muscle, whereas visceral adipocity was negatively correlated with activities of muscle CPT 1 and citrate synthetase (marker of oxidative capacity). Because neither fasting arterial nor systemic appearance of FFA were diminished in these obese women, it was concluded that individuals with increased visceral fat have a diminished capacity for skeletal muscle FFA utilization during fasting conditions.

Among the adverse effects expected from impaired FFA oxidation by muscle is a diversion of FFA towards increased TAG synthesis, leading to increased lipoprotein synthesis in the liver and increased fat storage in other tissues.[90] This is consistent with the findings that visceral adipocity is linked to diminished muscle oxidative enzyme capacity,[86] obesity is associated with increased reesterification of FFA,[91] and that elevated muscle TAG content is highly correlated with obesity and insulin resistance[92] (discussed below). Further evidence to indicate the importance of skeletal muscle in controlling whole-body lipid metabolism was provided by Bessesen et al.[93] To examine the partitioning of TAGFA in the obese state, these investigators followed dietary ^{14}C-labeled oleic acid in lean and obese Zucker rats. The amount of ^{14}C recovered in CO_2 over 6 hr after ingestion was significantly less in obese

compared with lean animals, whereas the quantity of label found in adipose tissue was greater in the obese rats. Additionally, although at 2.2 hr after ingestion, more label was detected in skeletal muscle of lean compared with obese rats, the differences disappeared at 6 hr, suggesting that in lean animals the fat was oxidized, whereas in obese animals the fat was shunted toward storage.

NIDDM. NIDDM is associated with a number of lipid abnormalities including increased plasma FFA,[94] increased fasting rates of lipolysis,[95] decreased insulin-induced suppression of lipolysis,[96] and impaired clearance of plasma FFA in relation to hyperglycemia.[97] Recent reports indicate that during postabsorptive conditions (fasting hyperglycemia), the respiratory quotient (RQ) across the leg is increased,[98] and the fractional extraction of [^{3}H] oleate is reduced[96] in subjects with NIDDM. These data suggest that postabsorptive skeletal muscle FFA utilization is impaired in these subjects. In addition, investigators also report that postprandial suppression of FFA uptake across the leg was diminished in NIDDM, yet impaired FFA oxidation persisted. Therefore, it seems that during both fasting and postprandial conditions, NIDDM subjects have a reduced rate of muscle lipid oxidation. However, although lipid oxidation is reduced in these subjects, muscle uptake of FFA is elevated during postprandial conditions, suggesting that FFA taken into muscle are being stored as TAG.

A relative impairment of muscle FFA utilization during postabsorptive conditions would contribute to several of the metabolic abnormalities associated with NIDDM. Plasma FFA not utilized by muscle would provide additional substrate for hepatic FFA oxidation and esterification. Respectively, these could contribute to gluconeogenesis and synthesis of VLDL lipoproteins, processes that are increased in NIDDM. Impaired oxidation of FFA by muscle would also contribute to fat deposition within muscle, which is consistent with the observation that skeletal muscle TAG content is increased in obese and obese-diabetic patients, and an increased fat content within muscle is a marker of insulin resistance.[92,99] Thus, in addition to the well described impairment of insulin-stimulated glucose metabolism in patients with NIDDM, muscle lipid partitioning is also dysregulated, evidenced by reduced oxidation and increased storage. Interestingly, this scenario is in contrast to the known effects of endurance exercise training on muscle lipid storage. Endurance training is also associated with elevations in intramuscular TAG storage, but because lipid oxidation is also enhanced,[17] the increase in stored lipids is lower than in the diabetic condition[15,100,101] and thus would not be expected to be associated with impaired insulin action (muscle insulin sensitivity is actually improved with exercise training).[102] Muscle TAGs are an important source of FFA for oxidation during moderate-intensity exercise,[17,30,31] and fatty acids derived from muscle TAG account almost entirely for the training-induced increase in muscle lipid oxidation during exercise.[15] Accumulation of intramuscular lipid in the trained state therefore represents a healthy, physiological response to the exercise stimulus rather than a consequence of a pathological condition as in the obese or diabetic state.

Dysregulation of skeletal muscle lipoprotein lipase

Human studies. As discussed above, skeletal muscle lipoprotein lipase (mLPL) clears TAGFA from serum lipoproteins and provides fatty acids to myocytes for oxidation. Therefore, dysregulation of mLPL activity may account for changes in lipid storage and oxidation. Yost[103] determined the effect of obesity on fasting mLPL and on the change of mLPL activity in response to an insulin/glucose infusion. Measurements of mLPL from biopsies of vastus lateralis before and after 6 hr of insulin/glucose infusion under a euglycemic clamp revealed that mLPL levels in the fasted state were significantly lower in obese individuals. However, mLPL decreased significantly after 6 hr of insulin/glucose infusion in the lean, but increased in the obese group. This study supports the hypothesis that dysregulation of muscle lipoprotein lipase contributes to an increased FFA accumulation in adipose tissue under fasting conditions and increased FFA accumulation in skeletal muscle under fed conditions. Furthermore, these results indicate that the insulin-suppressive effect on lipid uptake in muscle is diminished in the obese condition.

Similar findings have been reported in Pima Indians, a group known for their predisposition toward obesity and NIDDM.[89] In 16 nondiabetic Pima males, muscle biopsies were taken from the vastus lateralis at basal and after 3 and 6 hr of euglycemic, hyperinsulinemic clamp. Results revealed that mLPL activity did not change after 3 hr, but unlike lean subjects,[19] mLPL activity was increased above basal after 6 hr. These findings reinforce the presence of dysregulated lipid partitioning (in the fed condition) in obese/NIDDM-prone individuals, attributable in part to an insensitivity of mLPL to insulin, favoring increased lipid deposition in muscle.

Dysregulated LPL function has been presumed to contribute to resumption of the obese state after weight loss. After weight reduction by obese individuals, there is an increase in fasting adipose tissue lipoprotein lipase activity,[104,105] but the effects on mLPL are less understood. Eckel et al.[106] tested whether changes in mLPL and its response to insulin/glucose after sustained weight loss would indicate an alteration in the partitioning of lipid fuels away from oxidative pathways in muscle to storage in adipose tissue. Biopsies of vastus lateralis muscle were made on premenopausal obese women before and after a 900 kcal/day diet for 3 months and after 3 months of isocaloric maintenance of the reduced weight. Fasting mLPL was similar in obese versus normal weight controls before weight loss, but obese women had an increase mLPL response after 6 hr of insulin/glucose infusion. In contrast, after sustained weight loss, fasting mLPL activity was reduced in the obese individuals. These results indicate that a decrease in mLPL after weight loss may help to explain the difficulties in maintaining weight reduction in obese subjects.

In summary, obesity and NIDDM are associated with elevated plasma FFA levels, increased lipid content in both adipose and muscle, and reduced FFA oxidation by muscle. These changes in lipid homeostasis are, to a large extent, a consequence of a breakdown in the normal orchestration of storage and oxidation of lipid in skeletal muscle. In healthy individuals, insulin in the fed state reduces lipid uptake into

muscle through its suppressive effect on mLPL and stimulates lipid uptake and storage in the adipocyte. In contrast, in the postabsorptive state, lipids are mobilized from adipose tissue to the muscle for energy via oxidative/phosphorylation-coupled events and support the limited storage of lipid (TAG) at this site. In obesity and NIDDM however, the insulin-suppressive effect on adipocyte lipolysis and muscle lipid uptake is attenuated, both of which are coupled with an impaired skeletal muscle oxidative capacity. This dysregulation in lipid mobilization and utilization contributes to excessive fat gain on the whole-body level as well as lipid accumulation in skeletal muscle. Furthermore, dysregulated muscle lipid metabolism has been linked to the development of insulin resistance, diabetes, and associated physiological complications. In addition, perturbations of mLPL may also explain the inability of obese individuals to lose weight.

Animal models of disordered skeletal muscle lipoprotein lipase. To study the effects of increased FFA uptake in muscle tissue, Levak-Frank et al.[107] generated a transgenic mouse line with a human LPL minigene expressed exclusively in skeletal and heart muscle. The increase in lipolytic activity in muscle led to increased intracellular FFA, which was associated with weight loss and death. The animals developed severe myopathy characterized by proliferation of mitochondria and peroxisomes, glycogen storage, lymphocyte infiltration, fiber atrophy, fiber degeneration, and centralized nuclei. The results were dependent on the level and duration of LPL overexpression in muscle. In the plasma, LPL overexpression resulted in a dose-dependent reduction of TAG concentration, attributable mostly to reduced VLDL. Surprisingly, the plasma FFA levels were not increased, and were reflected by unaffected ketone levels. Thus, the majority of the FFA must have been taken up directly by skeletal muscle. This study suggests that FFA transport through the endothelial cell layer and the sarcolemal membrane does not represent an important regulatory step because LPL-driven FFA uptake can cause increased intracellular FFA levels to the extent that severe myopathy and premature death result. The increase of intracellular FFA levels without an increase in the plasma levels suggest that triglyceride hydrolysis and FFA uptake are tightly coupled in muscle. Furthermore, LPL may represent the rate limiting step in the cellular uptake of TAG derived FFA fulfilling a "gatekeeping function" in muscle.

Interestingly, the excess intramuscular supply of FFA was associated with a large increase in peroxisomal number and activity of the marker enzyme catalase. This in accordance with earlier observations that FFA can activate the peroxisome proliferator activated receptor (PPAR) by interacting with a PPAR retinoid X receptor dimer and thereby induce peroxisome proliferation/activation. This seems to preceed mitochondrial proliferation and may be a first response of muscle cells to increased FFA supply. Indirect evidence that peroxisome proliferation might occur in connection with myopathy is provided in rat and human studies demonstrating that peroxisome proliferation is associated with the induction of myopathy.[108,109]

Although the molecular abnormalities of many human myopathies are unknown, several defects have been characterized that affect FFA and carbohydrate catabolism such as deficiencies in carnitine, CPT 1 and 2, short, medium, and long acyl CoA dehydrogenases, and defects in oxidative phosphorylation. Furthermore, although the myopathic changes observed in these transgenic mice do not completely match the pathology identified in human myopathies, excessive FFA and neutral lipid accumulation seems to be a common finding in the pathogenesis of each and represents a focal point of research for identifying the molecular defects of these diseases.

In contrast to the transgenic model of mLPL overexpression, Blanchette-Mackie et al.[110] studied the dysregulation of muscle lipid metabolism in mice born with genetic combined lipase deficiency (cld/cld). Mice with this defect develop lethal hyperchylomicronemia within 2 days postpartum as a consequence of nursing, and plasma TAG values in affected animals often reach 20,000 mg/dl (100 times higher than in normal litermates). Of further interest was the finding that myocytes of heart and diaphragm from suckled cld/cld mice contained no lipid droplets, whereas those from suckled, unaffected mice contained numerous lipid droplets. Also, the large amount of chylomicrons in the capillaries and the small amount of lipid droplets in cells of suckled cld/cld mice reflect the very low level of LPL activity in these animals. These observations occurred in conjunction with the finding that the chylomicrons were readily hydrolizable by bovine lipoprotein lipase in vitro indicating that in the lipase-deficient mice, chylomicrons contained ample apoprotein C 2 for activation of lipoprotein lipase. Thus, LPL seems to have an important role in providing fatty acids for intracellular TAG synthesis in muscle.

In summary, changes in muscle LPL activity have profound effects on skeletal muscle structure and function and may contribute to the pathogenesis of skeletal muscle myopathy. Excessive FFA uptake into muscle by increased LPL activity can cause pathological changes including the proliferation of mitochondria and peroxisomes and the development of a lipolysis-induced myopathy. Findings in mutant, lipase-deficient mice emphasize the importance of mLPL in skeletal muscle lipid metabolism.

Obesity and skeletal muscle lipid composition

Membrane fatty acid composition. Pan et al.[111] have reviewed the role of the dietary fatty acid profile in altering the composition of membrane phospholipids and have suggested that changes in the ratio of specific membrane fatty acids can influence cell lipid storage and oxidation thus contributing to obesity. In cell culture (hepatocytes) and whole-body perfusion studies,[112,113] enrichment of plasma, microsomal, and mitochondrial membranes with n-6 (ω-6) polyunsaturates (linoleic acid) results in a dramatic increase in cellular TAG content,[114] whereas enrichment with n-3 polyunsaturates (α-linolenic acid) produces no change or a decrease in TAG production.[115] Likewise, in the whole-animal, a significant and positive correlation between muscle phospholipid n-3 content and insulin action in rat skeletal muscle has been observed, and these findings are associated with an inverse relationship between insulin action and TAG content.[99]

As with TAG biosynthesis, there is evidence that the rate of lipid oxidation differs markedly with the composition of the dietary fat ingested. Leyton et al.[116] evaluated the in vivo oxidation of fatty acids of differing chain length and saturation and found that oleic (18:1, n-9) and α-linolenic (18:3, n-3) were preferentially oxidized, followed by linoleic (18:2, n-6) and stearic (18:0)/palmitic (16:0) acids. Consistent with these data (because storage rates should be inversely related to oxidation), the rate of weight gain in genetically obese (ob/ob) mice[117] and adult rats[118] is attenuated when a diet lipid profile high in n-3 fatty acids is fed.

Similar to the cell culture and animal studies, results in humans demonstrate that the greater the saturated FFA composition in the diet, the lower the BMR, thereby favoring weight gain.[119] In contrast, body mass index (BMI) is correlated negatively with C20–C22 polyunsaturated fatty acids (PUFAs)[120] and there exists an inverse relationship between the proportion of body fat and the percentage of long-chain PUFA in muscle.[121]

Reductions in the proportion of arachidonate (20:4- n-6) in skeletal muscle has been linked to obesity.[122,123] Phinney et al.[124] have demonstrated that variations in the distribution of arachidonate (20:4- n-6) between phospholipid and cholesteryl ester fractions in various tissues participate in the abnormal fuel partitioning is associated with obesity. In addition, Borkman et al.[120] reported that a reduced 20:4 n-6 level in human skeletal muscle is associated with insulin resistance. Accordingly, insulin resistance could make glucose available for liver lipogenesis and potentially result in increased lipid storage. Observations in the obese Zucker rat have led to the proposal that a metabolic shift (maldistribution) of 20:4 n-6 from phospholipids to cholesteryl esters may occur in the obese. This coincides with the finding that a strong positive relationship between redistribution of phospholipid 20:4 n-6 to liver cholesteryl ester 20:4 n-6 exists in the obese Zucker (single gene defect) rat and other multigenetic models of obesity (e.g., humans). Thus, it may be that the dynamics of 20:4 n-6 production and distribution are important in the regulation of lipogenesis, insulin action, and obesity in animal models and humans.

It has been proposed that the phospholipid fatty acid composition of membranes might directly influence the metabolic rate in mammals. This is supported by the finding that higher levels of saturated fatty acids in the diet affect membrane fatty acid composition, leading to a decreased metabolic rate and increased susceptibility to obesity.[121] In animal studies comparing isocaloric high fat diets containing either saturated (beef tallow) or (n-6) unsaturated fat (safflower oil), reduced oxygen consumption and increased body fat accumulation was observed in animals fed a diet higher in saturated fat content.[125] Similarly, using open-circuit respirometry, rats consuming a highly unsaturated fat diet [saturated + (n-3)] had significantly higher metabolic rates than those consuming isocaloric highly saturated fat diets.[111,118] Skeletal muscle is likely the major contributor to this association because muscle metabolism constitutes at least 20% of 24-hr resting energy expenditure[126] and is the major metabolic tissue during elevated activity.

Interestingly, weight loss in obese human subjects is associated with a decrease in α-linolenic acid [18:9 (n-3)] in adipose tissue.[127] The mobilization of n-3 polyunsaturated fats would result in an increased oxidation rate of these fatty acids (presumably by skeletal muscle) and therefore may account for reduced fat mass with decreased energy intake. Although the fat mass loss is of obvious benefit to the obese, the body cannot synthesize n-3 fatty acids, and thus an enhancement of n-3 mobilization/oxidation would lead to an increased saturated/PUFA ratio leading to a decline in metabolic rate and the promotion of obesity. According to Pan et al.,[111] maintenance of weight loss would be best achieved via the replacement of n-3 fats for both saturates and n-6 fats into a reduced total fat diet.

Each of the dietary fat classes competes for the same elongation and desaturation enzymes[128] and both genetic and diet-induced changes in desaturase activity have been shown.[129,130] Δ9 desaturase activity [converts 16:0 palmitic and 18:0 steric acid to 16:1 (n-7) palmitoleic and 18:1 (n-9) oleic acid respectively] and its content in skeletal muscle has been shown to have a positive correlation with the percentage of body fat.[111] In addition, stearoyl-CoA desaturase mRNA levels are elevated in obese Zucker rats.[131] In contrast, a decrease in Δ6 and Δ5 reductase activity (required for PUFA biosynthesis) has been associated with an increased fat mass in the rat[132,133] and has been implicated to decrease in obese humans.[111] In support, measured precursor/product ratios in the muscle phospholipids (used as an indirect measure of enzyme activity) associate a reduced phospholipid 20:4 n-6 with impaired Δ5-desaturase enzyme.[120] Thus, a dietary modification of desaturase activity, resulting in a reduction of long chain PUFA in the phospholipid component of the cell membrane, could promote adiposity by altering the partitioning of fat toward storage.

Dietary lipid-induced variations in the activity of the membrane Na^+-K^+ ATPase pump has been proposed by Pan et al.[111] to account for lipid-induced variations in metabolic rate. A higher long-chain PUFA to saturated fatty acid ratio is associated with a "leakier" membrane requiring a higher Na^+-K^+ pump activity and/or more pumps, thereby raising the energy requirement of the cell. In agreement, red blood cells in the obese have been reported to have a reduced number of pumps.[134,135] Investigation of a similar relationship in skeletal muscle (or other organs) merits further investigation,[136] and although currently speculative, may account for a lower metabolic rate and propensity to gain fat mass with obesity. Potential differences in membrane fatty acids and their effect on altered ion permeability at other cellular sites (e.g., the mitochondria) also requires attention.

In summary it has been suggested[111] that much of the difference between rates of oxidation and partitioning for storage in adipose and skeletal muscle tissue may depend on the competition between fatty acid classes and associated biosynthetic enzymes of elongation and desaturation in the skeletal muscle plasma membrane. Studies in both humans and animals have shown that increased adiposity is associated with increased Δ9 desaturase activity and reduced Δ5/Δ6 desaturase activity. The desaturase enzymes themselves may exert their effects at the nuclear level (i.e., directly on gene transcription) or via membrane-associated events. It has also been hypothesized that the fatty acid composition of membranes may control the metabolism of

the cell via increased polyunsaturated fatty acids, and thereby increase the "leakiness" of either the plasma membrane to sodium and potassium, or mitochondrial membranes to protons, or both. According to Pan et al.,[111] membrane lipids may have a "pacemaker" role in the overall metabolic activity of the body. These findings have important implications for the treatment of obesity because the available evidence suggests that the dietary lipid profile may favorably alter muscle membrane lipid composition to positively affect metabolic activity.

Implications: protein kinase C in cell function. Protein kinase C (PKC) is a membrane associated signaling protein shown to phosphorylate serine and threonine residues on a wide range of proteins.[137] Boneh[138] has proposed that the pathological accumulation of naturally occurring lipid metabolites may affect PKC activity and lead to dysregulated substrate phosphorylation and cellular function. Fatty acids of various chain lengths, and their CoA esters have been shown to alter PKC function.[139–141] El Touny et al.[141] demonstrated that oleic acid preferentially activates PKC in the soluble fraction of platelets, whereas sphingosine was more potent than oleate in inhibiting PKC activation by phosphtidylserine/DAG. It was suggested that soluble PKC would be a target for *cis*-unsaturated fatty acids, whereas membrane-bound PKC would be targeted by phosphatidylserine/DAG. Another fatty acid-activating mechanism offered is through the accumulation of fatty acid acyl-CoA esters diverted into the synthesis of diacyl/triacyl-glycerols by phosphatidate phosphohydrolase, thereby activating PKC.[142] Although the acyl groups of most physiological DAG consist of stearate and arachidonate, modifications in the fatty acyl substitutes of these DAG have been shown to contribute to alterations in PKC activity.[143]

By contrast, several studies have shown that unesterified fatty acids (arachidonic, oleic, linoleic, and linolenic) activate PKC synergistically with DAG[141,144] and it has been suggested that *cis*-unsaturated fatty acids act as enhancer molecules, with DAG required for the fatty acid activation of PKC.[144] However, in the absence of DAG, palmitoyl- oleoyl- and to a lesser extent octanoyl-CoA can enhance PKC activity. The phosphorylation pattern of endogenous cytosolic and particulate proteins have been shown to be altered by palmitoyl- and oleoyl-CoA[138] although these events may not be assigned solely to PKC activation. Thus, in addition to PKC, the activation or inactivation of other kinases and phosphatases by fatty acids can not be excluded, and further work is needed to clarify whether these fatty acid induced events coincide or are independent of PKC activation.

In summary, evidence for lipid induction of PKC activity in the pathogenesis of fatty acid oxidation disorders are numerous. Further studies will elucidate the direct or indirect role that the dysregulation of muscle lipid metabolism and PKC play in the pathogenesis of inborn errors of metabolism, and in a wider view, signal transduction mechanisms. The potential role for PKC in insulin resistance is presented below.

Insulin resistance and skeletal muscle lipid composition

NIDDM and muscle lipid profiles. Elevation in circulating lipids is a common finding in persons with obesity[145] and many in vivo studies have demonstrated that abnormally high plasma fatty acids and TAG are associated with a decrease in insulin mediated glucose disposal in humans.[146] In association with these changes, skeletal muscle of type II diabetics has been shown to contain an elevated intracellular lipid pool, which has been associated with muscle insulin resistance.[100,101] Thus, abnormalities in lipid metabolism found in the obese may represent a potential causative factor leading to muscle insulin resistance. Because the majority of the whole-body glucose disposal in the fed state is accounted for by skeletal muscle (approximately 85%),[147] diabetes may be a natural progression stemming from insulin resistance induced by a dysregulation of skeletal muscle lipid metabolism. Recent findings have also implicated changes in the dietary lipid profile with alterations in the skeletal muscle membrane lipid composition, insulin resistance, and diabetes.[148]

Impaired insulin sensitivity has been induced in vivo in rats fed high fat diets[149] and in humans after infusion of free fatty acids.[150,151] For example, Roden et al.[152] demonstrated (in humans), that under euglycemic/hyperinsulinemic clamp conditions, elevated plasma FFA levels (6 hr infusion of 2 mM oleate) are accompanied by a reduction in whole-body glucose uptake and oxidative glucose metabolism. Furthermore, reduced skeletal muscle glycogen synthesis, which was preceded by a fall in glucose-6-phosphate concentrations, was also reported. These findings demonstrate that elevated FFA concentrations can induce insulin resistance, apparently by an inhibition of skeletal muscle glucose transport and/or phosphorylation. These results are consistent with data from cell culture. Shillabeer et al.[153] exposed L6 myocytes to triacylglycerol for 4 hr and demonstrated a significant transfer of lipid into the cells, which was associated with a 60% reduction in insulin stimulated 2-deoxyglucose uptake.

In contrast, weight loss is associated with improved insulin action. Friedman et al.[154] observed in insulin resistant, morbidly obese patients that in vivo glucose disposal improved to within 78% of normal after weight loss (43 kg). In addition, maximal insulin-stimulated in vitro glucose transport activity in incubated muscle strips increased 2 fold (88% of normal) after weight reduction. Similarly, antilipolytic drugs in humans[155] and rats[156] have demonstrated that acute decreases in lipids result in improved glycemic control. Collectively, these studies are important because they provide support for the hypothesis that dysregulated lipid metabolism may be associated with changes in insulin action at the level of the skeletal muscle.

As in the obese state, there exists a close association between insulin action and the fatty acid composition of phospholipids in muscle cell membranes.[148] Wong et al.[112] identified that a diet high in n-3 PUFAs is associated with hypotriglyceridemia and that with the addition of n-3 fatty acids into a high-fat diet, muscle insulin resistance could be prevented. Storlein et al.[157] assessed insulin action with the euglycemic clamp in rodents on a diet high in various

specific fatty acids. Diets held constant for total lipid content, but high in saturated, monounsaturated (n-9), or polyunsaturated (n-6) fatty acids led to severe insulin resistance as determined by differences in the glucose infusion rate required to maintain eulgycemia. Substituting fatty acids in the polyunsaturated fat diet with long-chain n-3 fatty acids (fish oils) normalized insulin action. Furthermore, a close relationship between long-chain n-3 fatty acids in skeletal muscle phospholipid and insulin-stimulated glucose metabolism also was evident, suggesting a role for this class of fatty acids in insulin action. In addition, a fat diet with a high n-6/n-3 ratio was also shown to be detrimental to insulin potency. With regard to insulin resistance, a high n-6/n-3 ratio would mean lower incorporation and less elongation and desaturation of dietary n-3 into muscle membranes. Under this scenario, a reduction in n-3 fatty acids from metabolically important structural lipids may predispose to diabetes. It should be noted, however, that other studies in individuals with NIDDM have shown no efficacy or adverse effects of n-3 fatty acid supplementation[158,159] and thus further research is necessary to identify the variables underlying the observed effects of n-3 fatty acid supplementation in rats.

Regardless, a similar association between muscle membrane lipid composition and insulin action can be found in the human.[120,160,161] Borkman et al.[120] determined the relationship between the fatty-acid composition of skeletal muscle phospholipids and insulin sensitivity. Phospholipid fatty acid composition of rectus abdominus was compared with fasting insulin levels and insulin sensitivity assessed by euglycemic-clamp technique. Results indicated that fasting serum insulin concentrations and insulin sensitivity were negatively correlated with the percentage of individual PUFAs (particularly arachidonic acid) in the phospholipid fraction of muscle. It was proposed that PUFAs may modulate the function of membrane proteins mediating the action of insulin (e.g., insulin receptor, subsequent signaling proteins) through effects on the physical properties of the surrounding lipid environment.[162] Alternatively, PUFAs might influence the action of insulin by acting as precursors for the generation of second messengers such as eicosanoids[163] or DAG.[164] The diverse biological action of eicosanoids as second messengers (e.g., prostaglandins, thromboxanes, and leukotrienes) are partly dependent on the nature of the fatty acids within the precursor phospholipid pool. Thus, the strong positive correlation between arachidonic acid and the indexes of insulin sensitivity in these studies may represent an effect on insulin action of eicosanoids specifically derived from this parent fatty acid. The potential for DAG to influence insulin action via protein kinase C is discussed below.

In summary, a range of evidence in humans and experimental animals demonstrate a strong relationship between the intramuscular lipid content and the fatty acid composition of structural membrane lipids and insulin action. With respect to the latter, several mechanisms involved include: (1) alterations in one or more of the enzymes of fatty acid elongation and desaturation necessary to convert the major dietary fats into longer-chain, more highly unsaturated fatty acids (2) a more saturated membrane lipid profile that may impair insulin action by altering physical properties and (3) hydrolysis of phospholipids in membranes yielding second messengers (DAG with differing fatty acid groups, various eicosanoids) that under conditions of dysregulated skeletal muscle lipid metabolism may impair insulin action.

Speculative role for PKC in skeletal muscle insulin resistance

As described above, the literature supports an association between dysregulated skeletal muscle lipid metabolism and impaired insulin action. Consistent with this hypothesis, Goodyear et al.[165] have shown insulin to be less effective in stimulating IRS-1 tyrosine phosphorylation, PI3-kinase activity, and glucose transport in human muscle from obese versus lean control subjects. However, the identification of a link for the putative association between elevated lipids and impaired insulin action remains unresolved. Recent studies however, have implicated lipid activated PKC in this role. According to Boneh et al.,[138] accumulation of lipids and/or their metabolites could inadvertently modulate PKC activity. Dysregulation of PKC activity would lead to perturbation of phosphorylation of endogenous substrates and signal transduction. These alterations could result in derangement of various short- and long-term cellular functions mediated by PKC.

PKC has been shown to be elevated significantly in liver[166] and skeletal muscle[167] of diabetic rats. PKC-mediated phosphorylation has also been shown to inactivate the insulin receptor tyrosine kinase.[168] The finding that DAG mass is associated with insulin resistance is also important because it may provide a connection between elevated intracellular lipids, PKC activation, and skeletal muscle insulin resistance. DAG is both an intermediate in the synthesis of triglycerides and phospholipids and an activator of PKC. Increases in DAG content have been observed in the insulin-resistant skeletal muscle of genetically obese, hyperinsulinemic rat[169] and insulin resistant mice.[170] In addition, in denervated insulin resistant muscle, a three fold increase in insulin-stimulated glucose incorporation into DAG is associated with an increase in membrane-associated PKC activity and inhibition of insulin-stimulated glycogen synthesis.[171] Recently, reductions in DAG and nonesterified fatty acid mass by the antilipolytic drug BRL49653 in rats made insulin resistant by high fat feeding has been associated with improved insulin sensitivity.[172] Therefore, studies demonstrating DAG (or other lipids) ability to impair insulin action through activation of PKC may be a fruitful, future experimental pursuit.

Conclusion and future directions

Skeletal muscle accounts for large proportions of clearance of circulating FFA and TAGFA and whole-body lipid oxidation, yet skeletal muscle lipid metabolism remains relatively underinvestigated. Emerging evidence suggests that insulin plays an important role in muscle lipid regulation and dysregulation. Insulin action, lipid metabolism, peripheral insulin resistance, and systemic lipid abnormalities seem to share several common physiological and pathophysiological pathways. Whereas much is known regarding the complex insulin signaling pathway leading to

insulin regulation of glucose metabolism, the signaling cascade(s) connecting insulin to lipid metabolism is essentially undefined and is a likely target for future research. The enzymes and proteins that regulate lipid metabolism in skeletal muscle generally parallel those identified in adipose tissue and liver. In many instances, however, these proteins are regulated in a tissue-specific manner through the expression of unique protein isoforms resulting in distinct biochemical and kinetic properties, as is the case with several of the enzymes and/or proteins associated with controlling fatty acid oxidation. Muscle-specific regulation of lipid metabolizing proteins is also observed in the absence of multiple protein isoforms, best illustrated by the reciprocal regulation of adipose compared with muscle LPL, indicating that unique molecular mechanisms of gene regulation exist between tissue types. Research on the regulation of gene expression by alternative promoters and tissue-specific expression of transcription factors has revealed a number of potential mechanisms for explaining muscle-specific regulation of proteins. Fatty acids and fatty acid metabolites have recently been implicated in regulating expression of the genes and proteins involved in lipid metabolism (for review, see Clark and Jump).[173] Although most of this work has not been performed using skeletal muscle, many potential parallels between tissue and cell types can be speculated. Particularly, one can envision a role for tissue-specifically expressed PPAR's (a family of lipid-activated transcription factors) in mediating gene regulation of the muscle-localized enzymes and proteins that are addressed in this review.[174,175,176]

The observation of tissue-dependent control of lipid metabolizing proteins implies that coordinate regulation of lipid pathways between tissue types confers a biological scheme for controlling whole-body lipid partitioning. The recent discovery of several adipocyte-secreted bioactive peptides (e.g. leptin, TNFα), that act through both centrally and peripherally mediated mechanisms to regulate energy metabolism, has vastly expanded our current view of fuel and lipid homeostasis.[177] Critical directions for future research will be not only to clarify the role of such peptides in altering muscle energy metabolism, but to further decipher the communication networks between muscle and other tissues, which may prove to be integral in directing the partitioning of lipid fuels between fates of oxidation or storage.

The central role that skeletal muscle plays in lipid metabolism becomes evident during instances of dysregulation such as obesity and diabetes, which are characterized by peripheral tissue insulin resistance. Adipocyte lipolysis is accelerated and accounts for the large increase in plasma FFA levels evident in these disease states. Despite the increased availability of substrate however, FFA uptake and utilization is impaired. Because of an insensitivity to insulin, regulation of muscle mLPL is compromised and thus uptake of FFA by muscle is accelerated in the postprandial, but attenuated in the fasted condition. Combined with a decrease in muscle oxidation, the net result is a substantial increase in intramuscular as well as adipocyte TAG storage.

The composition of the cell membrane, its physical properties, and hence tissue physiology are regulated by environmental as well as by genetic factors. Accordingly, it has been proposed that differences in the rates of muscle lipid oxidation and partitioning of lipid storage in obese and diabetic individuals may be attributed to differences in the dietary fatty acid profile (environmental influence). These differences may result in alterations in muscle membrane structure and competition between fatty acid classes and associated biosynthetic enzymes of elongation and desaturation. Studies in humans and animals have shown that increased adiposity is associated with increased Δ9 desaturation and reduced Δ5 desaturase activity. The desaturase enzymes themselves may exert their effects on gene expression or via membrane-associated events. Similarly, there may also be an association between insulin action and the fatty acid composition of the major structural lipids in the cell membrane. Particularly, a diet higher in proportion of saturated fatty acids has been linked to insulin resistance, whereas n-3 PUFAs may play a role in improving insulin action. As above, cellular membrane lipid composition may also reflect a genetic defect in one or more of the enzymes of fatty acid elongation and desaturation that are necessary to turn the major dietary fatty acids into longer-chain, more highly unsaturated fatty acids in membranes, which is linked to good insulin action.

The above findings suggest an association between the quality/quantity of dietary fatty acid intake and elevated plasma lipid levels with dysregulated skeletal muscle lipid metabolism, obesity, and insulin resistance. However, it should be cautioned that these data do not unequivocally support a cause and effect relationship. The identification of a specific lipid culprit or metabolite that can induce these pathologies awaits further experimentation. Interestingly, however, PKC has been implicated as a possible link for the putative association between dysregulated muscle lipid metabolism, the pathogenesis of fatty acid oxidation disorders, and insulin resistance. Recent studies suggest that the dysregulation of PKC activity could lead to perturbations in phosphorylation of endogenous substrates and signal transduction. These alterations could result in derangement of various short- and long-term cellular functions mediated by PKC, and might include effects on insulin action and metabolic activity. Conclusions on the role of PKC and obesity/diabetes are speculative at this time, but certainly merit further research.

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