

Skeletal muscle lipid deposition and insulin resistance: effect of dietary fatty acids and exercise¹⁻³

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ABSTRACT

Mounting evidence indicates that elevated intramyocellular triacylglycerol concentrations are associated with diminished insulin sensitivity in skeletal muscle. This lipid accumulation is most likely due to enhanced fatty acid uptake into the muscle coupled with diminished mitochondrial lipid oxidation. The excess fatty acids are esterified and either stored or metabolized to various molecules that may participate or interfere with normal cellular signaling, particularly insulin-mediated signal transduction, thus altering cellular and, subsequently, whole-body glucose metabolism. Impaired insulin responsiveness, if not managed, can further progress to type 2 diabetes mellitus, an all too common condition. For most of the human population this is avoidable, given that causes of intramyocellular lipid deposition are predominantly lifestyle-mediated. Chronic overconsumption of calories coupled with deleterious intakes of saturated or *trans*-unsaturated fatty acids inconsistent with the recommendations outlined in the Dietary Guidelines for Americans have been shown to increase the risk of insulin resistance. Furthermore, lack of exercise, which can have a profound effect on skeletal muscle lipid turnover, is implicated in this lipid-induced insulin resistance. This review summarizes the current understanding of the effects of elevated intramyocellular lipids on insulin signaling and how these effects may be altered by varying dietary fat composition and exercise. *Am J Clin Nutr* 2007;85:662–77.

KEY WORDS Insulin resistance, skeletal muscle, intramyocellular triacylglycerol, dietary fat, exercise

INTRODUCTION

With the substantial increase in the rate of obesity in both children and adults during the past few decades, concern has risen over the increasing prevalence of morbidity and mortality affiliated with this condition. The incidence of obesity is currently of epidemic proportion, and there are no signs that it will decrease, given the current trend. Obesity, a condition characterized by excess body fat, is defined as a body mass index (in kg/m²) ≥ 30 (a body mass index of 25–29 indicates overweight status). Currently, 30% of American adults are classified as obese and 1 of every 6 children are overweight according to the National Health and Nutrition Examination Survey (1). The likelihood of becoming obese does not depend on sex, age, or ethnicity, yet disparities do exist in their prevalence, and children who are overweight have an increased likelihood of becoming obese adults (2, 3). This increase in the incidence of obesity is undoubtedly an important contributor to the increase in insulin resistance (4) and the

metabolic syndrome (5), as well as in type 2 diabetes mellitus (T2DM) among both children and adults (4).

Despite the fact that the epidemiologic correlations are well established, the pathophysiology of obesity, particularly with regard to insulin resistance, has yet to be clearly defined. Insulin resistance, a fundamental feature of T2DM, is characterized as the tissues' inability to take up glucose in response to the pancreatic hormone insulin. Skeletal muscle has been identified as the major tissue in glucose metabolism, accounting for $\approx 75\%$ of whole-body insulin-stimulated glucose uptake (6, 7), and insulin resistance has been associated with accumulation of body fat (8), particularly intramyocellularly in both animals (9, 10) and humans (11, 12). This suggests a possible causative role for skeletal muscle lipid oversupply associated with chronic obesity in the development of insulin resistance (13). However, this assumption has not always been validated, because studies have also shown 1) improvements in skeletal muscle insulin sensitivity with little to no change in intramyocellular lipid concentrations (14, 15) and 2) improvements in skeletal muscle insulin sensitivity coinciding with actual increases in intramyocellular lipid concentrations (16). Furthermore, elite endurance athletes have extremely high concentrations of muscle lipid, yet are also quite insulin sensitive (17). The nature of this metabolic paradox seems to indicate that it is not the size of the intramyocellular triacylglycerol (IMTG) pool, but rather the balance between fatty acid availability, cellular uptake, and oxidation (ie, lipid turnover). Thus, the cellular and molecular mechanisms linking obesity to lipid-induced insulin resistance are currently a topic of intense investigation, and prevailing theories speculate that lipotoxic effects are mainly due to metabolites derived from intramyocellular lipid metabolism in addition to alterations in membrane function through changes in sarcolemma fluidity (13, 18, 19).

To further elucidate how elevated plasma and intramyocellular free fatty acids (FFAs) affect insulin signaling on a mechanistic level and how these defects can potentially be normalized

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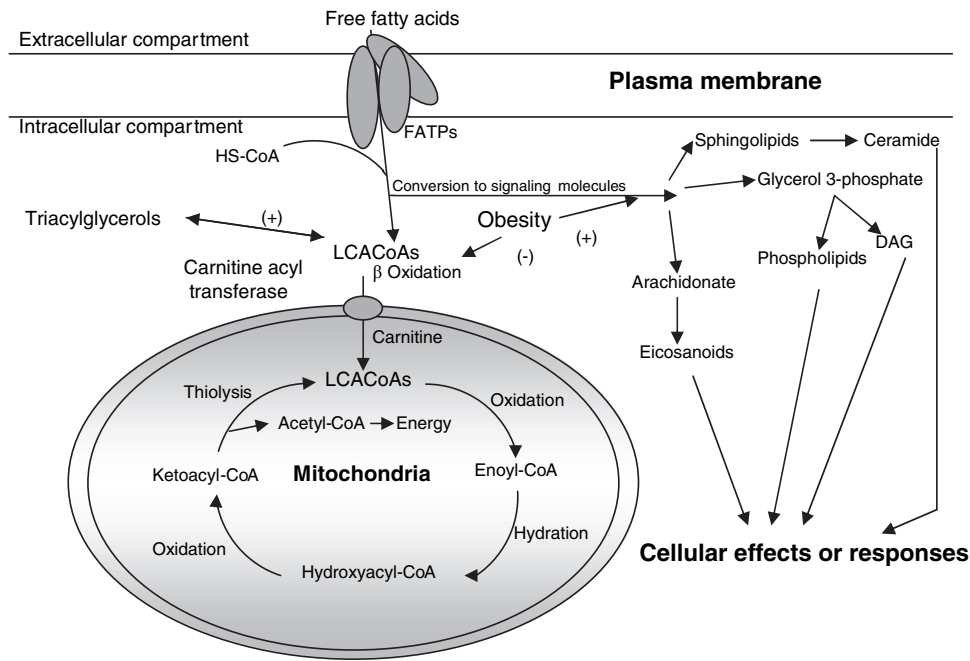


FIGURE 1. Metabolism of free fatty acids to long-chain fatty acyl-CoAs (LCACoAs). LCACoAs can either be used for energy production through β oxidation or undergo conversion to various signaling molecules, such as ceramide and diacylglycerol (DAG). Because obese persons have higher concentrations of intramyocellular fatty acids than do lean persons, the abundant supply of LCACoAs in skeletal muscle is favored toward signal molecule production. FATP, fatty acid transport protein; HS-CoA, coenzyme A.

by lifestyle modifications, a detailed understanding of the extent to which increased muscle lipids act in the insulin signaling pathway needs to be determined. In the present review, current knowledge on the implications of intramyocellular lipid deposition on skeletal muscle metabolism, particularly in regard to insulin resistance, is presented. First, mechanisms of lipid accumulation and fat distribution in muscle will be discussed, followed by a general overview of the alterations in insulin signaling that have been shown to occur, including possible lipid mediators linked to insulin resistance. Finally, the effect of exercise and of manipulating dietary fat composition will be addressed.

MECHANISMS OF INTRAMYOCYLLULAR LIPID DEPOSITION

Skeletal muscle lipid metabolism

Plasma lipid concentrations play a role in determining the rate of uptake of FFA into the muscle, particularly during conditions of hyperinsulinemia (20), which is often present with insulin resistance. Interestingly, circulating FFA concentrations are usually elevated in obese persons. Fasting plasma FFA concentrations in obese and T2DM patients typically range from 600 to 800 $\mu\text{mol/L}$ compared with 300–400 $\mu\text{mol/L}$ in lean healthy persons (21). This, coupled with reduced lipid oxidation often exhibited in obese skeletal muscle (22), results in excessive intramyocellular lipid deposition. Consequently, the excess muscle FFAs are either stored in lipid droplets or converted to various signaling molecules. This FFA conversion “spill over” is predominately due to increased availability of fatty-acyl-CoA substrates for enzymes involved in synthesis of sphingolipids, eicosanoids, phospholipids, etc, and results in abnormal concentrations of

these respective molecules, which may play a significant role in lipid-mediated insulin desensitization.

In order for lipids to be used as fuel by skeletal muscle, FFAs must be taken up and converted intracellularly to long-chain fatty acyl-CoAs (LCACoAs), imported into the mitochondria by carnitine acyltransferases, and subjected to β -oxidation. However, LCACoAs also serve as a source of second messengers, such as diacylglycerol (DAG) or ceramide, either by de novo synthesis or through phospholipase C activation or phospholipid hydrolysis, as shown in **Figure 1**.

Lipid transport and tissue delivery

Fatty acids are transported in the blood bound to albumin as nonesterified fatty acids or as part of triacylglycerols in lipoprotein complexes, which typically require triacylglycerol hydrolysis via lipoprotein lipase (LPL) to deliver the fatty acids to the tissues. Overexpression of muscle LPL has been associated with insulin resistance (23, 24), possibly because of the effect LPL has in increasing intramyocellular triacylglycerol concentrations (23, 25), though this effect has not always been consistent. For example, Voshol et al (25) showed no effect on insulin-stimulated whole-body or muscle-specific glucose uptake using mice overexpressing LPL. This argues against a simple causal relation between intramyocellular triacylglycerol content and insulin resistance. The discrepancy may be explained by differences in experimental conditions, such as the genetic background of the mouse models used, dietary fat content, body weight, muscle and liver triacylglycerol content, and insulin concentrations during the hyperinsulinemic-euglycemic clamp. Regardless, the authors did show that elevated LPL caused alterations in intracellular glucose metabolism, including decreased glycolysis, glucose oxidation, and glycogen synthesis (25), indicating possible abnormalities downstream of

insulin interaction with its receptor independent of GLUT4 trafficking (*see* "Insulin-mediated signaling transduction and glucose uptake" section).

Cell-mediated lipid uptake

Fatty acid transport proteins (FATPs) are implicated in the facilitated cellular uptake of lipids and their activation via ligation to acetyl-CoA. Lipid uptake by muscle tissue occurs mainly via the fatty acid transport protein 1 (FATP-1), a 646 amino acid integral plasma membrane protein that is expressed in all cells requiring high levels of fatty acid uptake for storage or metabolism (26–28). In a recent study, FATP-1 knockout mice exhibited protection from fat-induced accumulation of intramyocellular fatty acyl-CoA and insulin resistance in skeletal muscle compared with wild type mice, despite lipid infusion or after a high-fat diet (29). Therefore, FATP-1-mediated lipid uptake is linked with lipid storage. Cellular exposure to insulin results in a rapid translocation of FATP-1 from an intracellular perinuclear compartment to the plasma membrane, which parallels LCACoA uptake (30, 31). This suggests that FATP-1 may be involved in insulin-mediated regulation of fatty acid uptake, particularly in obese non-insulin resistant conditions.

Mitochondrial abnormalities

One consistent finding with obesity is the reduced capacity for lipid oxidation through lowered activity of key mitochondrial enzymes (22, 32). Carnitine palmitoyl transferase is a particularly important enzyme responsible for fatty acid transport into the mitochondria. Reduced carnitine palmitoyl transferase activity has been consistently observed in obese volunteers (22, 33). Diminished activity of mitochondrial NAD (NADH) oxidoreductase, an enzyme that reflects the overall activity of the respiratory chain, has also been shown to occur in obese nondiabetic and T2DM patients relative to lean subjects (34). This is rather significant, because normal mitochondrial function is required for adequate cellular glucose and fatty acid metabolism and homeostasis. Petersen et al (35) found that diminished muscle insulin sensitivity associated with elevated intramyocellular triacylglycerols in elderly individuals corresponded with decreases in both mitochondrial oxidative capacity and mitochondrial ATP synthesis. The influence that skeletal muscle mitochondria plays in lipid turnover may in part explain why athletes have such high IMTG concentrations and yet are quite insulin sensitive, a finding vastly different from obese and diabetic patients. Endurance training in particular is known to increase both mitochondrial quantity and quality in skeletal muscle (*see* "Exercise modulation of skeletal muscle insulin sensitivity and lipid metabolism" section). Fatty acids in trained skeletal muscle are in a higher state of flux, and greater IMTG concentrations here may represent an adaptive response to training that is associated with greater insulin sensitivity. In contrast, obese persons and persons with T2DM exhibit reduced mitochondrial efficiency and lipid turnover, which may facilitate the build up of deleterious lipid metabolites and encourage lipid peroxidation, which in turn can affect both insulin signal transduction and mitochondrial function (34, 36). Overall, reduced lipid turnover is a necessary component to any apparent lipotoxic effects on insulin signaling that may arise from IMTG accumulation. Under conditions of reduced lipid oxidation, there is an increased load of fatty acids on the mitochondrial membrane facilitating the entrance of neutral fatty acids into the mitochondrial matrix (37), where they are

prone to lipid peroxidation. Studies have shown that there is a higher degree of lipid peroxidation within skeletal muscle of obese insulin-resistant persons (36). The peroxide products are highly reactive cytotoxic metabolites that damage DNA and proteins and further hinder mitochondrial oxidative capacity. This constitutes a vicious cycle, and it is currently unclear whether mitochondrial defects lead to IMTG accumulation or whether IMTG accumulation leads to mitochondrial defects. Regardless, it seems apparent that each can affect the other.

LIPID DISTRIBUTION IN SKELETAL MUSCLE

One of the difficulties when working with skeletal muscle is the challenge of completely separating out the extraneous fatty and fibrous tissue that tends to accompany the muscle specimen and, therefore, minimize contamination that can affect sample analysis. In this section, we briefly discuss lipid distribution within the muscle fiber (intramyocellular) as opposed to between these fibers (intermuscular) and place particular emphasis on the heterogenous nature of skeletal muscle. Additionally, we define total fiber area as the volume of a muscle fiber, which is a cylindrical cell $\approx 10\text{--}100\ \mu\text{m}$ in diameter and 1–400 mm in length (38).

Early evidence for *in vivo* intramyocellular lipid distribution and insulin resistance relied on histologic and biochemical analysis of skeletal muscle biopsies. These techniques have a fundamental flaw in that they do not discriminate adequately between intramyocellular and intermuscular lipid deposition. Additionally, repeated measurements are difficult because of the invasive nature of muscle biopsies especially when examining children. More recently, noninvasive techniques, such as proton magnetic resonance spectroscopy (^1H MRS), have enabled a detailed analysis by their ability to distinguish between protons of lipids outside the myofiber and those of intracellular lipids in humans (39–41).

Lipid stores and muscle fiber type

Previous studies have indicated that in obese skeletal muscle, intracellular lipid comprises $\approx 3\text{--}4\%$ of total fiber area, whereas in the muscle of lean persons, this value decreases to $\approx 1\text{--}2\%$ (17, 42). This represents a substantial difference in total body lipid distribution, given the percentage of whole-body mass that skeletal muscle makes up. For reference, skeletal muscle mass tends to make up $\approx 36.5\%$ of body weight (43), and, therefore, if one were to make a comparison between an obese man (100 kg) and a lean man (68 kg), this could amount, on average, to a 0.9 kg difference in myocellular lipid content between the two. Additionally, this difference may also be dependent on muscle fiber type (44–46). Skeletal muscle is a heterogenous tissue composed of 2 main distinct fiber categories, each with slightly different metabolic capabilities. Type I, or slow twitch, fibers are predominantly oxidative and contain more mitochondria than do type II, or fast twitch, glycolytic fibers. Therefore, type I fibers are more efficient "fat burning" fibers. Type I fibers also seem to be more responsive to insulin, exhibiting greater insulin binding capacity and increased insulin receptor kinase activity and autophosphorylation compared with type II fibers (44, 47–49). Additionally, whole-body glucose uptake and muscle glucose transport are positively associated with type I fibers (44–46). This is a particularly significant fact when examining muscle fiber composition and obesity. Obese persons tend to exhibit fewer type I



fibers and an increased percentage of type II fibers than do lean subjects (45). Studies have reported a negative association between adiposity and the relative percentage of type I fibers (47, 50, 51). Given these observations, it is likely that there is a relation between muscle fiber composition and obesity, a notion supported by further studies (52).

INSULIN-MEDIATED SIGNALING TRANSDUCTION AND GLUCOSE UPTAKE

Recent reviews have discussed our current understanding of insulin signal transduction, particularly in resistant states such as T2DM (53). Therefore, it will be discussed only briefly to illustrate insulin mediated glucose metabolism and to provide clarity when discussing signaling defects affiliated with lipid oversupply.

The insulin receptor (IR) is a heterotetrameric tyrosine kinase receptor composed of two α and two β chains and belongs to a family of growth factor receptors (54). Insulin binding triggers autophosphorylation of the receptor, which creates a recognition motif for the binding domain of insulin receptor substrates (IRSs) (55). There are ≥ 13 different IRSs (IRS 1–6, Gab-1, Shc 1–3, p62^{dok}, APS, and Cbl/CAP) (56–58), which show little sequence homology yet are functionally linked (59). These proteins, particularly IRS-1 and -2 and Shc, are tyrosine phosphorylated upon binding to the activated IR, which leads to further recruitment of *src* homology 2 domain-containing proteins. IRS-1, and to a lesser extent IRS-2, recruit the *src* homology 2 protein phosphoinositide 3-kinase (PI3K) (60), which then catalyzes the formation of phosphoinositol lipids such as PI(3,4,5)P₃, which activates 3-phosphoinositide-dependent protein kinase (PDK) 1. This then phosphorylates and activates other kinases, such as atypical protein kinase C (aPKC) and Akt (also known as protein kinase B) that mediate the translocation of the skeletal muscle glucose transporter GLUT4 to the cell membrane. The molecular details linking aPKC and Akt with GLUT4 translocation are currently unknown; therefore, the mechanisms underlying the control of insulin metabolism are not yet completely understood. What is known, however, is that Akt phosphorylates glycogen synthase kinase 3, the enzyme that inactivates glycogen synthase via phosphorylation. Phosphorylation of glycogen synthase kinase 3 by Akt inactivates the enzyme and therefore promotes glycogen synthesis.

Furthermore, insulin is known to mediate gene regulatory events through Shc and the activation of the ras–mitogen-activated protein kinase pathway. The protein Grb-2 is bound by both IRS-1 and Shc upon insulin receptor binding and subsequent receptor substrate phosphorylation. This binding facilitates ras activation and the extracellular-regulated kinase (ERK) mitogen-activated protein kinase cascade, which in turn affects expression of genes involved in the metabolic and growth-promoting effects of insulin (61). The whole pathway is illustrated in **Figure 2**.

ALTERATIONS IN SKELETAL MUSCLE INSULIN SIGNALING ASSOCIATED WITH EXCESS LIPID ACCUMULATION

Serine/threonine phosphorylation of insulin receptor 1

Pan et al (12) first reported in 1997 that the IMTG concentration is associated with insulin resistance in humans. Since then,

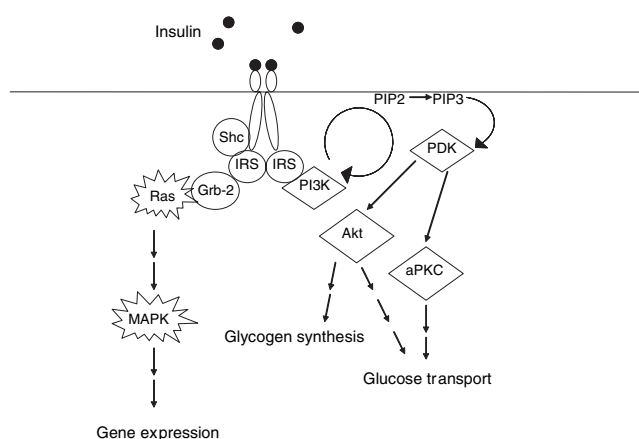


FIGURE 2. Metabolic effects associated with the insulin signaling pathway in skeletal muscle. The 2 main effects seen on insulin binding to the insulin receptor in a healthy individual are GLUT4 translocation to the membrane surface and increased glycogen synthesis. IRS, insulin receptor substrate; PI3K, phosphoinositide 3 kinase; PIP2, phosphoinositol biphosphate; PIP3, phosphoinositol triphosphate; PDK, 3-phosphoinositide-dependent protein kinase; aPKC, atypical protein kinase C; MAPK, mitogen-activated protein kinase; Shc, *src* homology 2 and collagen homology domain-containing protein; Grb-2, growth factor receptor-bound protein 2; Akt, protein kinase B.

other studies have shown similar associations (8, 62–64). Note that the bulk of these studies examined sedentary populations that were either overweight or obese, diabetic, or had a family history of T2DM. To understand the defects in skeletal muscle insulin signaling that are known to occur in these populations and mechanistically link IMTG deposition to these defects, a molecular understanding of insulin resistance is needed. Insulin resistance in general has been associated with reduced tyrosine phosphorylation of IRS-1, leading to diminished activity of PI3K (60, 65). As described previously, when IRS-1 becomes tyrosine phosphorylated, it recruits a number of SH2-containing signal transducers such as PI3K. Although the mechanisms leading to diminished phosphorylation have not as of yet been determined, it is important to realize that IRS-1 contains numerous potential serine/threonine phosphorylation sites as well. Serine/threonine phosphorylation of IRS-1 has been implicated in diminished insulin action (60, 66). Specifically, the structural mechanism appears to involve IRS protein dissociation from the IR by inducing conformational changes, thereby impeding access to tyrosine phosphorylation sites (66–68). This may further facilitate IRS release from the intracellular complexes that maintain the proteins in close proximity to the IR (69). The result is reduced IRS-1 activation of PI3K and, consequently, diminished GLUT4 translocation to the membrane surface.

To decipher whether elevated plasma triacylglycerol concentrations are specifically implicated in altered IRS-1 signaling, Belfort et al (21) examined a dose-response effect of elevated plasma FFA concentrations, comparable to concentrations observed in obese and T2DM subjects, on insulin-mediated glucose disposal in lean, healthy subjects. The authors observed a significant reduction in IRS-1 tyrosine phosphorylation and PI3K activity, associated, in part, with increased IRS-1 serine phosphorylation in muscle biopsy samples (21). Similar effects were also seen in additional human and animal studies (70–72). Furthermore, elevated FFA concentrations within the muscle itself have been linked with increased serine phosphorylation of IRS-1 (73),

indicating one specific mechanism of fatty acid-induced insulin resistance.

Serine phosphorylation of IRS-1 occurs at specific serine residues (68, 74) and seems to be the result of increased activation of particular isoforms of PKC such as PKC θ (71), a novel DAG-dependent PKC (19). DAG is a fatty acid metabolite (*see* "Possible lipid mediators involved in insulin resistance" section), and because chronically elevated plasma FFA concentrations correspond to increased lipid deposition within the muscle, the concentration of DAG intracellularly is expected to increase. This increase in DAG concentrations is associated with the blunting of insulin signaling (72) and, therefore, may offer a very plausible mechanism to lipid-alteration of IRS-1 activity.

PKC isoforms and lipid-induced insulin resistance

The metabolic effects caused by insulin are predominantly mediated by effectors downstream of PI3K, with the most notable among these being PKCs. The PKC family is composed of >10 isoforms, grouped into atypical, classical, and novel PKCs (nPKCs). The role of these various PKC isoforms in insulin resistance has been studied extensively because of their lipid mediated regulation [reviewed in Schmitz-Peiffer (19)]. Atypical PKCs (aPKC ζ , ι , and λ) are both DAG- and calcium-independent and are strongly activated by PDK1. nPKCs (nPKC δ , ϵ , θ , μ , and η) are DAG-dependent and calcium-independent and also require PDK phosphorylation for full activity, although this may occur through posttranslational modification (75). Finally, classical PKCs (cPKC α , β , and γ) are both calcium- and DAG-dependent.

Animal studies provide compelling evidence that PKC isoforms display distinct tissue, cellular, and subcellular distributions and that their localization is developmentally regulated (76). For example, PKC α , PKC ϵ , PKC δ , PKC ζ , and PKC λ are expressed almost ubiquitously in the mature animal (77–79). In contrast, PKC η is localized primarily to the skin and lung (80), PKC γ is abundant in the brain (79), and PKC θ is abundant in skeletal muscle (81). Regardless of PKC tissue and cellular distribution, numerous isoforms have been implicated in insulin resistance and intramyocellular fat deposition. The aPKCs appear to play a positive role in glucose transport, whereas abnormally elevated cPKC and nPKC activation seem to be highly associated with resistance.

Atypical PKCs

Studies conducted in diabetic patients, obese human subjects, or both have shown defective activation of aPKCs due to impaired activation of PI3K (82–84). Activation of aPKCs is required for insulin-mediated GLUT4 translocation to the plasma membrane, which enables glucose uptake in skeletal muscle (85). Therefore, by inhibiting appropriate phosphorylation and subsequent activation of these aPKCs by PI3K, insulin-mediated glucose uptake is impaired. Despite impaired aPKC activation, phosphorylation of Akt has shown to be relatively unaffected under diabetic conditions (82–84, 86–88), which presents a paradox. One plausible explanation may involve PI3K-independent factors that may also facilitate Akt activation either by direct protein-protein interaction, phosphorylation via another kinase, or through indirect means (slowing degradation or dephosphorylation, which would otherwise lead to inactivation). Regardless, defective activation of aPKC in insulin resistance

may be the result of impaired IRS-1–dependent PI3K activation or through poor responsiveness of PDK to PI3K-mediated PI(3,4,5)P₃ production. Interestingly, impaired activation of aPKC by PI(3,4,5)P₃ has been shown to occur in diabetic rats (89), humans with T2DM (83), and muscle culture preparations of obese subjects (90).

Novel mechanisms facilitating GLUT4 translocation by aPKC activation independent of PI3K have been found in both adipocytes and skeletal muscle cells of rodents *in vitro* (91, 92). Because glucose serves not only as a source of energy, but also as a regulator of physiologic processes, its role as a metabolic regulator, particularly in the pathology of insulin resistance with respect to ERKs, has been of interest. Bandyopadhyay et al (93) reported previously that glucose activates ERKs in adipocytes by a mechanism that is independent of glucose uptake and metabolism yet dependent on Grb2. PI3K-independent aPKC activation seems to depend on ERK activation as well as phospholipase D (PLD), a membrane associated enzyme that can generate phosphatidic acid (PA) from phosphatidylcholine (PC) (91). PA is a known direct activator of aPKCs (94, 95), and evidence has linked PLD-generated PA with GLUT4 translocation (92, 96, 97); however, this remains controversial. Millar et al (98) reported that inhibition of PA production by butanol did not affect insulin-mediated glucose uptake in 3T3-L1 adipocytes. In contrast, the concentration of butanol typically used for experiments such as this may not have been enough to effectively block PA production, and, therefore, PLD-dependent GLUT4 translocation would not have been effectively inhibited (99).

Regardless, insulin activation of PLD has been suggested to be mediated by ADP ribosylation factor (ARF) proteins, which are thought to regulate the synthesis of PLD products (100). ARF activation seems to be facilitated by specific guanine nucleotide exchange factors, in particular members of the cytohesin/ARF nucleotide-binding site opener (ARNO) family. ARNO-mediated recruitment of ARF proteins to the plasma membrane with insulin stimulation has been shown, suggesting a general model of PLD activation (100). On insulin binding, ARNO is translocated to the plasma membrane and interacts directly with the IR. The particular intricacies leading up to this interaction possibly involve direct interaction with specific protein binding domains on ARNO or through as of yet unidentified targets. More research is needed to clearly elucidate the role of ARNO and PLD activation of aPKC in response to insulin stimulation, especially with regard to high intracellular lipid concentrations.

In summary, defective activation of aPKCs appears to be implicated in insulin resistance, yet whether elevated intramyocellular triacylglycerol concentrations directly contribute to this impairment remains to be clarified. The insulin signaling pathway itself is a complex myriad of reactions and, therefore, factors that affect upstream effectors, such as the insulin receptor or IRS, will most likely translate to impaired activation of downstream targets. Indeed, defective aPKC activation may, in part, be the result of impaired IRS-1–dependent PI3K activation, which is itself affected by fatty acid metabolites.

Novel and classical PKCs

Several studies have shown that abnormal activation of nPKCs and cPKCs result in diminished insulin responsiveness, particularly in acute lipid accumulation (71, 101–106). Most studies have shown a positive correlation between intramyocellular lipid deposition and nPKC activity, particularly the serine kinases



PKC θ and PKC ϵ . In rodent skeletal muscle, chronic activation of the nPKCs δ , ϵ , and θ was shown with high-fat feeding and was correlated with an increase in both intramyocellular lipid accumulation and DAG concentration (106). In humans, PKC θ translocation was associated with insulin resistance in muscle after acute FFA infusion (71). PKC ϵ was shown to mediate IR degradation and signal attenuation in vitro, whereas overexpression in skeletal muscle may be linked with insulin resistance in the diabetic sand-rat *Psammomys obesus* upon high energy intake (101).

Because PKC θ and PKC ϵ are serine kinases, their heightened activation may lead to enhanced serine phosphorylation of IRS-1, which may interfere with IRS-1 tyrosine phosphorylation, thereby inhibiting PI3K activation (107). Yu et al (72) observed a 30% reduction in insulin activation of IRS-1 tyrosine phosphorylation and approximately a 50% reduction in IRS-1-associated PI3K activity after lipid infusion in rats coinciding with activation of PKC θ activity. It was proposed that this was due to IRS-1 phosphorylation of serine³⁰⁷, a critical residue in IRS-1 inactivation as shown by Aguirre et al (108); they showed that mutation to alanine³⁰⁷ resulted in protection from tumor necrosis factor α (TNF- α)-induced insulin resistance. An increase in membrane-associated PKC θ accompanied by a decrease in the inactive cytosolic pool has been observed in lipid-induced insulin resistance (106, 109), possibly indicating increased mobilization of the kinase and subsequent phosphorylation of membrane-bound substrates. Alternatively, PKC ϵ has been shown to be relatively resistant to proteolysis after long-term chronic activation under similar conditions (105, 106, 109, 110). Therefore, one may suspect that the role of both PKCs in lipid-mediated insulin desensitization are dependent on time: resistance seen with acute lipid infusion may be PKC θ mediated, whereas, over the long term such as that seen with chronic high-fat feeding, PKC ϵ may be implicated. Because obesity is a chronic disease associated with elevated plasma FFA and potentially with significant lipid deposition in the muscle, both PKC isoforms may play a prominent role in the pathology of intramyocellular lipid deposition leading to insulin resistance. This is perhaps debatable, however, given that Kim et al (111) showed no change in skeletal muscle PKC ϵ expression in a PKC θ null mouse model that showed protective effects from lipid-induced insulin resistance. This suggests that it may be unlikely that other PKC isoforms significantly contribute to the protection of lipid-induced insulin resistance caused by the PKC θ knockout.

Therefore, the role nPKCs play in lipid-mediated insulin resistance needs to be further examined. PKC θ inactivation has led to mixed results. Kim et al (111) showed that 3–4 mo-old PKC θ knockout mice had normal glucose uptake and insulin-associated IRS-1 tyrosine phosphorylation and subsequent PI3K activation after a 5-h lipid-heparin infusion compared with abnormal levels of these variables observed in wild type mice. In contrast, a prior study by Serra et al (112) showed PKC θ dominant negative mice had an age-associated reduction in insulin sensitivity and developed obesity at 6–7 mo of age. The discrepancy may be due to differences between PKC θ deletion and dominant negative expression or it may be an issue of time. At 3–4 mo of age, obesity was not present, therefore providing the possibility that the reduced glucose tolerance and skeletal muscle insulin signaling seen in the dominant negative PKC θ by age 6–7 mo may be secondary to obesity.

Unfortunately, little has been done to closely examine the effects of a PKC ϵ knockout model specifically on lipid-mediated insulin resistance. However, other PKC knockout models have been examined. In particular, Standaert et al (110) examined cPKC α and β knockout mice. Overall, glucose homeostasis in vivo was not impaired in PKC β knockout mice and although glucose transport did increase moderately in some tissues, PKC β was not considered essential to insulin-stimulated glucose transport (113). Similar findings were also reported for PKC α . PKC α was not required for insulin-stimulated glucose transport, yet activation of the kinase resulted in significant increases in this transport most likely due to insulin-induced activation of PI3K (114). A general picture of the PKC-mediated effects on insulin signaling is shown in **Figure 3**.

POSSIBLE LIPID MEDIATORS INVOLVED IN INSULIN RESISTANCE

Intracellular triacylglycerols are relatively inert molecules, and, as such, IMTG concentrations may merely represent a surrogate marker for the potential build up of other lipid species within the muscle. In particular, metabolically active cellular LCACoAs are seen as better predictors of insulin sensitivity than triacylglycerols (115, 116). They are the activated form of intracellular FFAs produced by the action of acyl-CoA synthase and are recognized as signaling molecules that participate in a variety of cellular processes and through these processes possibly influence skeletal muscle insulin action.

Diacylglycerol and ceramide

DAG and ceramide are intracellular fatty acid metabolites that have been suggested to play roles as primary mediators in lipid-induced insulin resistance (72, 117–119). Both are elevated in obese skeletal muscle with increased myocellular lipid content and have been shown to accumulate in insulin-resistant tissues (120). Therefore, considerable attention has been given to the effects of each molecule on insulin signaling.

DAG is an intermediate of both triacylglycerol and phospholipid metabolism that has been shown to accumulate in many human and rodent models of insulin resistance, including lipid-induced insulin resistance (105, 106, 109, 121–124). DAG can be generated by the breakdown of phospholipids via phospholipases or through de novo synthesis via the esterification of LCACoA to glycerol-3-phosphate (**Figure 4**). It acts as an important second messenger involved in intracellular signaling and, because of its role in cPKC- and nPKC-mediated activation (125, 126), is a prime candidate in lipid-induced insulin resistance. High DAG concentrations seem to correspond to greater IR and IRS-1 inhibition in animals (101), and chronic activation of nPKC θ and ϵ have been observed concomitant with elevated DAG concentrations in high-fat fed rats. This activation was associated with insulin resistance (106). Furthermore, muscle cell studies have directly shown that DAG reduces insulin-stimulated glucose uptake by a PKC-dependent mechanism (118). Thus, it is possible that normalization of DAG concentrations ameliorate this aberrant PKC activity, potentially improving skeletal muscle insulin sensitivity through enhanced IRS activity.

Ceramide is a derivative of sphingomyelin, a phospholipid component of cell membranes, and is generated either by sphingomyelinase or via de novo synthesis with palmitoyl-CoA as the



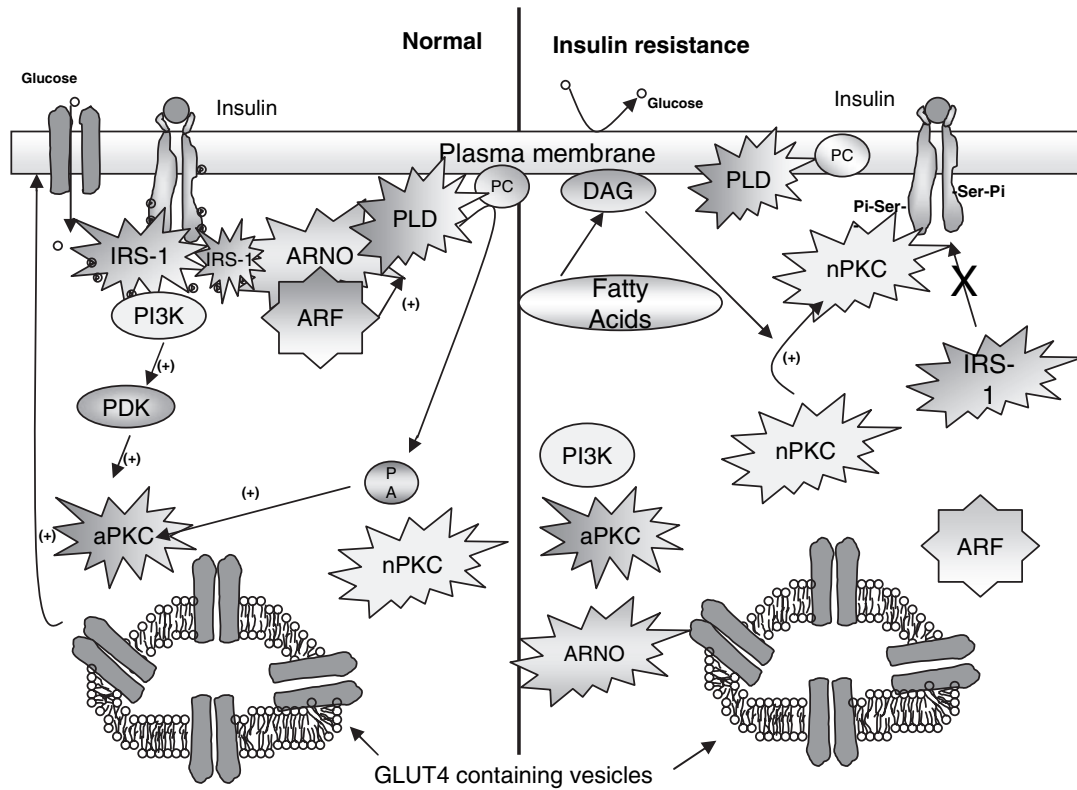


FIGURE 3. Protein kinase C (PKC)-mediated effects on insulin signaling. The left panel symbolizes normal insulin-mediated GLUT4 transport via activation of atypical PKC (aPKC). The right panel symbolizes insulin resistance due to aberrant activation of novel PKCs (nPKCs), which serine-phosphorylate the insulin receptor, thereby inhibiting the tyrosine autophosphorylation required for insulin receptor substrate (IRS) docking. Incidentally, IRSs are also serine phosphorylated by nPKCs (not shown). ARNO, cytohesin/ARF nucleotide-binding site opener; PLD, phospholipase D; PC, phosphatidylcholine; PA, phosphatidic acid; ARF, ADP ribosylation factor; PI3K, phosphoinositide 3 kinase; DAG, diacylglycerol; PDK, 3-phosphoinositide-dependent protein kinase.

precursor (Figure 4). Similar to DAG, ceramide can act as a second messenger either by altering the activity of kinases, phosphatases, or transcription factors and has been shown to play a role in cell proliferation, differentiation, and apoptosis (127). Insulin action has been shown to be inhibited by ceramide through inhibition of insulin signal transduction in vitro (119, 128–130). This may be due to the fact that Akt activation has been shown to be reduced in the presence of ceramide (129, 131), which in turn may lead to both reduced GLUT4 translocation to the plasma membrane and diminished glycogen synthase activity. In addition, overexpression of acid ceramidase, which catalyzes the lysosomal hydrolysis of ceramide to sphingosine and FFA, reversed the inhibitory effects that saturated FFAs have on insulin signaling by blocking their stimulation of ceramide accumulation (132).

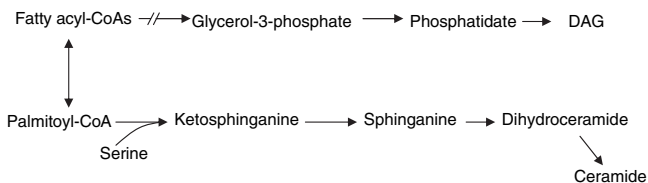


FIGURE 4. De novo synthesis of diacylglycerol (DAG) and ceramide from fatty acids.

Peroxisome proliferator activated receptors

Peroxisome proliferator activated receptors (PPARs) α , δ , and γ belong to a family of nuclear hormone receptors that regulate the expression of genes involved in glucose and lipid metabolism. They are bound and activated by fatty acids, their derivatives, or both. Elevated intramyocellular lipids are known to affect PPAR gene expression and, in turn, alter cellular metabolism [reviewed by Ferre (133)]. Furthermore, PPAR agonists have emerged as important pharmacologic treatments to improve hyperlipidemia and insulin action (134). Because PPARs regulate skeletal muscle fatty acid utilization, they merit further investigation.

PPAR- α is expressed in skeletal muscle and in other tissues such as liver, whereas PPAR- γ is mainly localized to adipose tissue and immune cells. PPAR- δ is ubiquitously expressed in all tissues. Insulin sensitivity seems to be significantly improved on PPAR- α activation in genetic (obese Zucker *fa/fa* rats), nutritional (high-fat diet), or lipotrophic (A-ZIP/F-1) models of insulin resistance (135–137). PPAR- α activation increases lipid oxidation, thus reducing fatty acid content in tissue and minimizing lipotoxicity. The role of PPAR- γ in regulation of insulin resistance in skeletal muscle is not fully known. Pharmacologic ligands of PPAR- γ such as thiazolidinediones cause enhanced glucose disposal. Although PPAR- γ is localized mainly to white and brown adipose tissue and, to a lesser extent, immune cells, it

may play an indirect whole-body role in lipid-mediated insulin resistance. Expression of PPAR- γ in C2C12 skeletal muscle cells affects insulin sensitivity, which indicates the possibility of cross-talk between PPAR- γ and insulin in skeletal muscle cells (138). The exact connection remains in question, but it is possible that PPAR- γ activation encourages fatty acid channeling to adipose tissue, thereby reducing their availability to muscle by decreasing circulating FFAs. PPAR- δ is the predominant isoform in rodent skeletal muscle and, similar to PPAR- α , promotes fatty acid oxidation and utilization. Furthermore, PPAR- δ may be the main isoform mediating the response to increased fatty acid availability in muscle cells. Agonists of PPAR- δ seem to normalize blood lipids and reduce insulin resistance and adiposity in both rodents and primates. The PPAR- δ agonist GW501516 significantly increased fatty acid oxidation in C2C12 myotubes (139). It should be noted, however, that a recent study examining the effects of GW501516 in skeletal muscle tissue showed that, in contrast to cultured myotubes, no effect was seen with respect to glucose transport or enhanced insulin action (140). Therefore, although these PPAR- δ agonists show promise, more research is needed in vivo to determine their true effectiveness.

Inflammatory mediators

Subacute low-grade inflammation is associated with insulin resistance and T2DM, and various inflammatory mediators seem to be involved in lipid-mediated insulin desensitization. For example, abnormal activity of I κ B kinase- β (IKK- β), a serine kinase of IRS-1, signifies a subacute inflammatory condition and has been clearly shown in insulin resistant states. Inhibition or normalization of IKK- β can prevent fat-induced insulin resistance [reviewed by Perseghin et al (141)]. Additionally, mice expressing constitutively active IKK- β in hepatocytes have a T2DM phenotype, including effects in the muscle that parallel those of high-fat fed wild-type mice (142). This insulin resistance associated with subacute inflammation from hepatic and muscle activation of IKK- β was reversed by inhibition of IKK- β (142). TNF- α has been shown to decrease insulin responsiveness in skeletal muscle by reducing IRS-1 and subsequent PI3K activity (143, 144) and by downregulating GLUT4 (145). Additionally, concentrations of skeletal muscle TNF- α in insulin-resistant obese patients have been shown to be 4-fold those of healthy volunteers (146). The inhibitory effects that TNF- α has on insulin signaling may also be attributed in part to the activation of sphingomyelinase, which leads to the release of ceramide (147). Therefore, inhibition of IKK- β may hold potential for future pharmacologic treatments in obesity-induced insulin resistance.

One of the most prominent cytokines to be examined is interleukin 6 (IL-6). Its role in insulin resistance is controversial. Animal studies suggest that IL-6 can induce insulin resistance (148), and, in humans, circulating IL-6 may (149, 150) or may not (151, 152) be associated with diminished insulin sensitivity. Obese diabetic and nondiabetic patients show increased circulating IL-6 concentrations that correspond with reduced insulin sensitivity (153), yet it should be noted that, during resting conditions, 10–35% of the body's IL-6 is produced by adipocytes (154). Within adipocytes, IL-6 production is linked with reduced insulin sensitivity, and its release can be triggered by TNF- α (155, 156). Its regulation in skeletal muscle is complex and not completely understood. A marked increase in circulating IL-6

concentrations has been shown to occur after exercise; this increase was mostly mediated by skeletal muscle (157–159). The contracting muscle fibers seem to produce and release IL-6, which induces several metabolic effects. IL-6 here induces lipolysis and fat oxidation and plays a role in glucose homeostasis during exercise (160, 161). This, therefore, suggests that IL-6 may play multiple roles in skeletal muscle.

THE INFLUENCE OF DIETARY FATTY ACID COMPOSITION ON SKELETAL MUSCLE LIPID DISTRIBUTION AND INSULIN SENSITIVITY

Although development of T2DM is linked to genetic predisposition, diet is a major contributor. The fatty acid composition of the diet and the relation it has with insulin resistance is currently a topic of intense investigation. Most observational studies suggest that certain fatty acid types promote insulin resistance, whereas other fatty acid types may protect against it. For example, high dietary intake of the monounsaturated fatty acid oleic acid, which is abundant in olive oil, has been associated with improved insulin sensitivity in the general population, whereas saturated fatty acids promote the opposite (162, 163). However, in observational studies such as these, it is difficult to distinguish between the effects of fat composition and the effects of energy density. Furthermore, no method of measuring dietary intake is completely reliable. Therefore, definitive evidence linking fat quality with insulin sensitivity and, additionally, IMTG accumulation can only truly be determined by intervention trials. Unfortunately, most trials (164–166) have been short term and have had a small number of subjects, and their results are inconclusive. Despite the lack of conclusive data from human studies, a substantial body of literature of studies that used animal models clearly suggests that certain fats promote skeletal muscle insulin resistance, though the effect specific fatty acids have on IMTG quantity is not clear. It is possible that high intakes of saturated fatty acids encourage IMTG accumulation compared with unsaturated fatty acid-rich diets, given that these latter fatty acid types are preferentially oxidized over the former (167). The overall significance of this, however, is speculative at the moment. Regardless, these animal studies suggest 2 possible ways in which fatty acid quality may affect insulin sensitivity. The first is with regard to sarcolemma fatty acid composition. In humans, as in other species, the body is particularly efficient at regulating the components of cell membranes such as the sarcolemma. However, the fatty acid composition of cellular membranes can be influenced by diet (168). This is especially of note, because the fatty acid types taken in by the human diet—saturated, monounsaturated, polyunsaturated, and *trans*-unsaturated fatty acids—differ by spatial configuration and thus chemical property, which in turn can affect cell membrane fluidity and rigidity. Overall, most animal and cell studies seem to indicate that saturated and *trans*-unsaturated fatty acids significantly increase insulin resistance, whereas polyunsaturated n-3 fatty acids improve it (169). The effects on insulin sensitivity of n-6 polyunsaturated fatty acids appear to range somewhere between the saturated and n-3 fatty acids (170). Because cellular membranes are complex networks in and of themselves, whereby the efficiency of molecular signal transduction is highly dependent on the orientation and positioning of various proteins within the membrane, the fatty acid composition of cellular membranes may play a pivotal role in an adequate insulin response. Cross-sectional studies conducted in humans suggest that the fatty acid composition of



phospholipids in the sarcolemma may modulate insulin sensitivity (171, 172), and, interestingly, obese patients or those with T2DM display a different fatty acid composition of serum lipids compared with lean subjects, with a higher proportion of the saturated fat palmitate and lower concentrations of linoleic acid (an n-6 fatty acid) (173). This may be due to differences in the quality of fat that each group tends to consume on a daily basis. Animal studies seem to directly show that saturated fat-laden membranes promote insulin resistance, whereas more unsaturated membranes protect against it, a finding also noted in humans (174).

Along with diet-induced changes in membrane fatty acid composition, the type of fat consumed seems to determine the fatty acid composition of the IMTG pool. In humans, insulin resistance directly correlates with increased saturated fatty acids in skeletal muscle triacylglycerols (175). Repeatedly, the quantity of IMTG seems not to be an entirely accurate maker for insulin sensitivity. Rather, IMTG accumulation in obese and T2DM skeletal muscle may represent more of a potential for the accumulation of specific lipid metabolites that in turn may negatively affect insulin sensitivity. The identity of these lipid metabolites may, to a certain extent, be influenced by the dietary fat composition of an individual's diet. Animal studies have linked high saturated fatty acid intake with elevated concentrations of specific lipid messengers in muscle (176), and cell culture studies have been particularly insightful in directly linking a particular fatty acid type with a specific second messenger. For example, saturated FFAs, such as palmitate (16:0), stearate (18:0), or arachidate (20:0), effectively induce DAG and ceramide synthesis as well as inhibit Akt activation (117, 119, 177). Saturated FFAs with hydrocarbon chains shorter than those of palmitate do not produce these results (177). Because ceramide, for the most part, is derived from long-chain saturated fats, this may partially explain these findings. Palmitate is often used as the FFA of choice in these studies, because it is one of the most prevalent FFAs in plasma (in addition to the monounsaturated fat oleate). Saturated fatty acids, which accumulate in the form of DAG and activate PKC, have been shown to reduce glucose uptake via desensitization of insulin stimulation in cultured human skeletal muscle cells (118), and, as indicated previously, palmitoyl-CoA is a direct precursor to de novo ceramide synthesis. However, palmitate does not necessarily have to be converted to an intracellular lipid second messenger such as ceramide to affect intracellular signaling. Elevated palmitate concentrations can affect cellular signaling by inhibiting IR or IRS-1 phosphorylation (178) and Akt activation (117, 119, 177). This fatty acid has also been shown to induce cytokine expression (179) and result in downregulation of GLUT4 by an NF- κ B-dependent mechanism (180). Lastly, palmitate, but not oleate, effectively blocked insulin-stimulated phosphorylation of glycogen synthase kinase in C2C12 myotubes (177).

In contrast to the deleterious effect that saturated fatty acids have on skeletal muscle insulin sensitivity, n-3 polyunsaturated fatty acids (n-3 PUFAs) may ameliorate insulin resistance. Intake of n-3 PUFAs has a protective effect in rodents in vivo against a high fat diet that induces insulin resistance (9, 181, 182). This can be explained on a molecular level on the basis of studies in which n-3 PUFAs prevented some of the aberrations seen with high intramyocellular lipid deposition. For example, one study showed that rats fed a high-fat diet enriched with n-3 fatty acids maintained the activity of IR, IRS-1, and PI3K activity, as

well as total GLUT4 content in skeletal muscle (183). Additionally, n-3 PUFAs, specifically eicosapentaenoic acid and docosahexaenoic acid, which are found mainly in fatty fish, may reduce the rate at which insulin resistance progresses to T2DM (184, 185). Higher concentrations of n-3 PUFAs in the membrane of skeletal muscle are associated with lower fasting plasma glucose concentrations in rodents and humans [reviewed by Lombardo and Chicco (186)]. Furthermore, dietary intake of n-3 PUFAs in rats has been shown to induce an increase in the glucose-6-phosphate pool that is accompanied by an increase in glycogen synthesis, signifying enhanced glucose uptake (181). Generally, n-3 PUFAs seem to prevent the decrease of PI3K activity and minimize the GLUT4 depletion in skeletal muscle that would normally occur in lipid-induced insulin desensitization. Finally, it is interesting that n-3 PUFAs are preferentially oxidized over saturated fatty acids (167). These fatty acid types can modify fuel partitioning within the cell and upregulate genes involved in lipid oxidation such as PPARs and therefore possibly discourage IMTG accumulation (187).

With mounting evidence supporting the benefits of increased n-3 PUFA dietary intake, the American Diabetes Association has recommended the public consume 2-3 servings of fish rich in n-3 PUFAs per week (188). Unfortunately, despite the benefits that n-3 PUFA intake may have, there is still no definitive proof that these fats can actually reverse insulin resistance. Most intervention trials have not shown any benefit from consuming these fatty acid types in T2DM patients (189-191). Rather, their use should be a part of a healthier diet geared toward prevention of T2DM. Regardless, fish oil seems to have many beneficial effects in healthy subjects such as decreasing plasma triacylglycerols; therefore, increasing one's dietary intake should prove helpful.

The beneficial effects seen with n-3 PUFAs have not been observed consistently with n-6 PUFAs; their effect on lipid-mediated insulin resistance in skeletal muscle has been mixed. For example, the n-6 PUFA arachidonic acid was seen as fairly effective in preventing alloxan-induced diabetes in male Wistar rats (192), and treatment of genetically diabetic GotoKakizaki rats with arachidonic acid significantly improved insulin sensitivity (193). Results seen with lipid-induced insulin resistance have been less impressive, however. Marotta et al (194) showed a significant increase in intramyocellular triacylglycerol concentrations in rats given a hypercaloric diet rich in n-6 PUFAs (sunflower oil). In contrast, little effect in these muscle lipid concentrations was seen when the n-6 PUFAs were substituted with either saturated or monounsaturated fat (194). Another study showed that a diet rich in n-6 PUFAs led to blunted signaling of IR and IRS-1 tyrosine phosphorylation and seemed to inhibit PI3K activity as well as reducing GLUT4 protein content (183). In comparison, this same study showed that a diet rich in n-3 PUFAs in addition to the n-6 PUFAs completely maintains insulin sensitivity, offsetting these effects. Lipid-induced stimuli that lead to c-JUN NH₂-terminal kinase (JNK) activation, such as the n-6 fatty acid linoleate (195), seem to inhibit IRS-1 function through serine³⁰⁷ phosphorylation, thereby interrupting the IRS-insulin receptor interaction (196) or promoting IRS protein degradation (197). Gao et al (195) found that activation of PKC θ contributes to JNK activation and that JNK mediates PKC θ signals for serine phosphorylation and degradation of IRS-1. Conversely, skeletal muscle PKC θ translocation to the membrane induced by high-fat feeding in rats was reversed by



acute dietary manipulation, specifically feeding of a high-glucose and low-fat meal (198).

On a final note, high dietary intake of *trans*-fatty acids seems to be associated with various deleterious effects, similar to intake of saturated fatty acids. Although small amounts of *trans*-fatty acids are present in nature, the artificial processing of unsaturated fats via the addition of hydrogen creates a chemically stable lipid that is currently used in a wide variety of processed foods. These hydrogenated lipids differ from their *cis*-unsaturated counterparts by spatial configuration, possessing the straight unknicked structure similar to saturated fatty acids yet still display a degree of unsaturation. Little has been done to examine the effect that these fats have on lipid-mediated insulin resistance seen in skeletal muscle. One study that examined whether *cis* and *trans*-fatty acids of the same length acutely influence insulin release and glucose oxidation in isolated mouse pancreatic islet cells found that the *trans*-fatty acids elicited a greater insulin output than did their *cis* counterparts and additionally the *cis* isomers significantly inhibited glucose oxidation compared with the *trans*-fatty acids (199). Another study that compared both saturated and *trans*-fatty acids with monounsaturated fatty acids in healthy subjects showed no difference in insulin sensitivity and glucose oxidation (200). The literature overall seems to suggest that *trans*-fatty acids have no significant effect on insulin sensitivity in lean, healthy persons (200, 201), yet an elevated insulin response may occur in persons with T2DM (202). Large randomized controlled trials need to be done, and any possible link between these fatty acid types and specific lipid metabolites is unknown at this time.

EXERCISE MODULATION OF SKELETAL MUSCLE INSULIN SENSITIVITY AND LIPID METABOLISM

Endurance exercise improves skeletal muscle insulin sensitivity, and the mechanism of action is fairly well described. Notable points in skeletal muscle insulin signal modulation via this type of exercise include increases in GLUT4 protein concentrations and increased activities of both glycogen synthase and hexokinase, the enzyme that phosphorylates glucose (203, 204). As previously mentioned, endurance athletes are quite insulin-sensitive yet have high IMTG concentrations (17). Some studies have shown that placing sedentary adults on an endurance exercise program improves insulin sensitivity while increasing IMTG concentrations (16, 205). The effect of exercise is, of course, whole-body mediated, but in these studies, the improved insulin sensitivity in the presence of increased IMTG concentrations is most likely the result of more efficient lipid turnover in that the muscle is becoming more adept at lipid uptake, transport, utilization, and oxidation. Indeed, Menshikova et al (206) showed improvements in mitochondrial biogenesis and electron transport chain activity in older persons after 12 wk of endurance training. Bruce et al (14) obtained similar results in obese persons, although their IMTG concentrations remained relatively unchanged. Therefore, the capacity for lipid oxidation is increased, yet given the IMTG increase noted in some of these studies, greater FFA delivery and uptake must also be occurring (207, 208). The increase in lipid uptake most likely represents, again, an adaptation by the muscle to the increased metabolic demands that arise from strenuous physical exertion. This, coupled with increased FFA delivery to the exercising muscles, an expected physiologic response, would help to explain increased


IMTG concentrations. The improvements in insulin sensitivity despite the increase in IMTG are likely related to reductions in deleterious lipid metabolites from a greater lipid flux. In the study by Bruce et al (14), obese subjects were exposed to endurance training, which yielded reductions in both intramyocellular DAG and ceramide content. Reductions in lipid metabolite concentrations may partly explain the improvements in GLUT4 translocation and activities of hexokinase and glycogen synthase. There is also some evidence suggesting that endurance training reduces susceptibility of skeletal muscle to lipid peroxidation (209). This may lead to further improvements in mitochondrial function. Lastly, the antiinflammatory effects of exercise are well known [reviewed by Petersen and Pedersen (210)], and studies have shown that exercise reduces TNF- α concentrations, which may in part explain the increases in GLUT4 expression.

In addition to endurance exercise, resistance training should also be regarded as an essential component in an individual's daily lifestyle. From a physiologic point of view, it is well recognized that endurance exercise increases capillary density, improves blood flow to the muscles and skeletal muscle mitochondrial biogenesis, and enhances translational stability of key proteins involved in insulin signal transduction (203). However, endurance exercise does not substantially affect skeletal muscle hypertrophy and strength compared with resistance training. Because resistance training increases skeletal muscle mass (211), it can augment whole-body glucose disposal capacity (212–214). Furthermore, studies have shown that even a single resistance exercise training session can improve insulin sensitivity for up to 24 h after cessation of exercise (214–216) and that these benefits are possibly attributed in part to reductions in IMTG stores (217). At first, this may seem contradictory to studies that have shown increases in IMTG from endurance exercise, which imply a discrepancy dependent on exercise type. However, it is important to distinguish between a single training session and multiple training sessions. Many studies examining a single endurance bout have also shown reductions in IMTG concentrations (218–220). It is widely agreed that to really achieve any substantial long-lasting benefit from physical exercise, the activity must be consistently repeated throughout one's life. A single training session of either endurance or resistance exercise will undoubtedly lead to reduced IMTG concentrations, because these lipids have been shown to be a major fuel source in both exercise types, depending on the intensity of the exercise; though, admittedly, this is still rather controversial [reviewed by van Loon (221)]. The enhanced lipid turnover seen with endurance exercise (14, 222) is a consequential adaptation to the metabolic demands of the body. Unfortunately, studies on the metabolic demands of resistance training are few. This is likely due to the methodologic difficulties associated with the non-steady state conditions of this type of exercise. Regardless, studies that use exercise, be it endurance or resistance training, have consistently shown improvements in skeletal muscle insulin sensitivity, and any so called "paradox" with regard to IMTG concentrations is explained when examining lipid turnover.

CONCLUSIONS

Insulin resistance is a highly complex condition, and the molecular details of its pathology have yet to be completely deciphered, particularly in relation to IMTG accumulation. Given the



overall mass of skeletal muscle coupled with its role in whole-body glucose homeostasis, understanding the etiology of insulin resistance in this particular tissue is important. IMTG deposition is deleterious when accompanied with reduced lipid turnover, as evident by the fact that endurance-trained persons have high IMTG concentrations yet are also quite insulin sensitive. Exercise improves insulin sensitivity by increasing the expression and activity of notable enzymes that are important to glucose uptake. Additionally, exercise improves lipid flux, and, therefore, high IMTG concentrations may represent an adaptive physiologic response to training. In contrast, obesity is associated with a reduced capacity for lipid oxidation, which by itself leads to IMTG deposition. However, it is unclear whether the reduced mitochondrial efficiency in obese skeletal muscle is a cause or consequence of IMTG deposition, because lipid peroxidation is known to induce mitochondrial damage. The quantity of IMTG in the obese state serves as a marker for the potential build up of specific lipid metabolites, the identity of which may be influenced by dietary fat composition. These metabolites have been shown to interfere with PI3K activation through activation of nPKCs that in turn lead to excessive serine phosphorylation of IRS. Additionally, the fatty acid composition of the sarcolemma can also be influenced by diet, thus representing another means of affecting insulin sensitivity. However, human evidence that conclusively links dietary fat composition with both IMTG accumulation and insulin resistance is lacking, though both observational and animal studies are suggestive of an effect. n-3 Fatty acids seem to improve skeletal muscle insulin sensitivity, whereas saturated fats and possibly *trans*-unsaturated fats seem to do the opposite. Mixed results are often seen with n-6 fatty acids. Overall, it seems clear that a long-term exercise program, composed of both endurance and strength training, along with reductions in saturated fat intake, will prevent the occurrence of insulin resistance in the general population and improve insulin sensitivity in the obese population. 

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