Review Article

Pathogenesis of Insulin Resistance in Skeletal Muscle

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Insulin resistance in skeletal muscle is manifested by decreased insulin-stimulated glucose uptake and results from impaired insulin signaling and multiple post-receptor intracellular defects including impaired glucose transport, glucose phosphorylation, and reduced glucose oxidation and glycogen synthesis. Insulin resistance is a core defect in type 2 diabetes, it is also associated with obesity and the metabolic syndrome. Dysregulation of fatty acid metabolism plays a pivotal role in the pathogenesis of insulin resistance in skeletal muscle. Recent studies have reported a mitochondrial defect in oxidative phosphorylation in skeletal muscle in variety of insulin resistant states. In this review, we summarize the cellular and molecular defects that contribute to the development of insulin resistance in skeletal muscle.

1. Introduction

Skeletal muscle is the major site for disposal of ingested glucose in lean healthy normal glucose tolerance (NGT) individuals [1–4]. Following a meal, approximately one third of ingested glucose is taken up by the liver and the rest by peripheral tissues, primarily skeletal muscle via an insulin dependent mechanism [1–4]. The postprandial hyperglycemia stimulates insulin secretion from the pancreas and the rise in plasma insulin concentration stimulates glucose uptake in skeletal muscle leading to the disposal of ingested glucose [1–4].

In insulin resistance states, such as T2DM and obesity, insulin-stimulated glucose disposal in skeletal muscle is markedly impaired [1–6]. The decreased insulin-stimulated glucose uptake is due to impaired insulin signaling and multiple postreceptor intracellular defects including impaired glucose transport and glucose phosphorylation, and reduced glucose oxidation and glycogen synthesis [7–10] (Table 1). Although the exact mechanism that leads to the development of insulin resistance in skeletal muscle is not yet fully understood, an increased intramyocellar fat content and fatty acid metabolites have been shown to play a pivotal role in the development of insulin resistance in skeletal muscle [11–15]. The recent studies have reported the existence of a defect in mitochondrial oxidative phosphorylation in skeletal muscle in insulin resistance states [16–20] and suggest that this mitochondrial defect contributes to the increased intramyocellar fat content. In this paper we will summarize the evidence that supports the existence of insulin resistance in skeletal muscle, the cellular mechanism(s) that lead to the development of insulin resistance, and the clinical consequences of insulin resistance in skeletal muscle.

2. Normal Skeletal Muscle Metabolism

Skeletal muscle utilizes both glucose and free fatty acid (FFA) as fuel sources for energy production. During the postabsorptive state, the plasma insulin concentration is low. Since the plasma insulin concentration is the principal factor that restrains lipolysis in adipocytes [21] and stimulates glucose uptake in skeletal muscle [21], during the fasting state, muscle glucose uptake is low and the plasma FFA concentration is elevated. Thus, under fasting conditions, FFA serves as the principal fuel source for energy production in skeletal muscle, while the brain exclusively utilizes glucose.

Following glucose ingestion, the increase in plasma glucose concentration stimulates insulin secretion from the beta cell and the resultant hyperinsulinemia suppresses lipolysis, leading to decline in plasma FFA concentration and subsequent decrease in the rate of lipid oxidation. Simultaneously,

TABLE 1: Defects in Glucose Metabolism in Insulin Resistant Conditions.

Insulin signaling	(1) Reduced insulin receptor tyrosine phosphorylation
	(2) Decreased IRS-1 tyrosine phosphorylation
	(3) Decreased PI3-kinase activation
Glucose transport	(1) Impaired GLUT4 translocation
	(2) Impaired GLUT 12 translocation
Glucose metabolism	(1) Decreased glucose phosphorylation
	(2) Decreased glucose oxidation and glycolytic FLUX
	(3) Impaired glycogen synthase

FIGURE 1: Dose-response curve relating the plasma insulin concentration to the rate of insulin mediated wgole body glucose uptake in controls and type 2 diabetic subjects. $*P < .01$ (from [21]).

insulin stimulates glucose uptake in skeletal muscle, and the increased glucose flux into skeletal muscle, together with the activation of key enzymes in glucose metabolism by insulin, leads a marked increase in muscle glucose oxidation [1]. Thus, under postprandial conditions, for example, mixed meal, muscle energy metabolism switches from predominant oxidizion of fat during the fasting state, to predominant oxidizion of glucose [22]. The ability of skeletal muscle to switch from fat oxidation during the fasting state to glucose oxidation during the postprandial state has been referred to as metabolic flexibility [23].

After glucose is transported into the myocyte via the GLUT4 transporter, it is immediately phosphorylated by hexokinase, and the phosphorylated glucose either is converted to, and stored as glycogen, or enters the glycolytic pathway for oxidation. Approximately 90% of glucose entering the glycolysis is oxidized and the remaining 10% is released as lactate. At low plasma insulin concentration, for example, fasting state, glycogen synthase, and glucose oxidation contribute equally to glucose disposal. However, with increasing plasma insulin concentration, glycogen synthase is activitaed by insulin and glycogen synthesise predominate (~70% of glucose disposal) [24].

3. Insulin Resistance in Skeletal Muscle

The term insulin resistance refers to an impairment in insulin action in insulin-target tissues, such as skeletal muscle, adipocytes, and liver. With respect to skeletal muscle, the primary action of insulin is to stimulate glucose uptake and metabolism [1–4]. In lean healthy individuals insulin stimulates glucose uptake into skeletal muscle in a dose-dependent manner, with a half-maximal effect (EC50) at a plasma insulin concentration ~60 μU/mL [21]. In insulin resistant states, insulin-stimulated glucose uptake is markedly reduced in skeletal muscle (Figure 1).

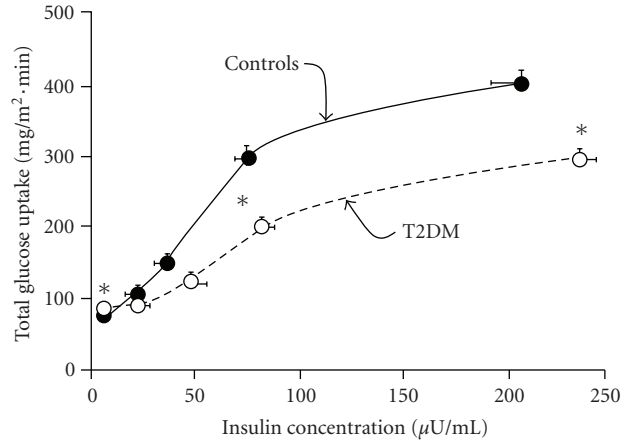Himsworth and kerr [25], using a combined oral glucose and intravenous tolerance test, were the first to demonstrate that tissue sensitivity to insulin is diminished in T2DM patients. In 1975, Ginsberg et al. [26], using the insulin suppression test, provided further evidence that the ability of insulin to promote tissue glucose uptake in T2DM was severely reduced. The most conclusive documentation for increased insulin resistance in skeletal muscle in lean, as well as obese T2DM subjects, has been provided by DeFronzo and colleagues [1–5, 21, 27, 28] and Butterfield and Whichelow [29]. Using the gold standard euglycemic hyperinsulinemic clamp technique to quantify insulin-stimulated glucose uptake, they demonstrated that both lean and obese T2DM subjects have marked decrease (>50%) in whole body glucose disposal during the insulin clamp.

Although glucose disposal during the insulin clamp represents insulin-stimulated glucose uptake by all peripheral tissues, the great majority of this glucose uptake take place in skeletal muscle. Under euglycemic conditions studies, using the insulin clamp in combination with femoral artery and vein catheterization [5] has shown that approximately 80% of total body glucose uptake occurs in skeletal muscle. In response to a physiologic increase in plasma insulin concentration (80–100 μU/mL), leg muscle glucose uptake increases progressively in healthy subjects and reaches a platue value of approximately 10 mg/kg leg wt·min. In contrast, during the last hour of the insulin clamp study, the rate of glucose uptake is reduced by ~50% in lean T2DM subjects (Figure 2). Thus, the dose response curve relating insulin-stimulated glucose uptake and the plasma insulin concentration shifts to the right with an increase in EC50 (to ~120–140 μU/mL) in subjects with T2DM (Figure 1). In addition, the onset of insulin action in skeletal muscle in T2DM subjects is markedly delayed (Figure 2). Even though the insulin infusion is continued for an additional 60 minutes in subjects with T2DM to allow insulin to more fully express its biologic action, glucose uptake remains blunted. These studies indicate that insulin resistance in skeletal muscle in subjects with T2DM is manefisted, not only by a reduction in the magnitude of insulin action, but also by s delayed onset of insulin action to stimulate glucose uptake.
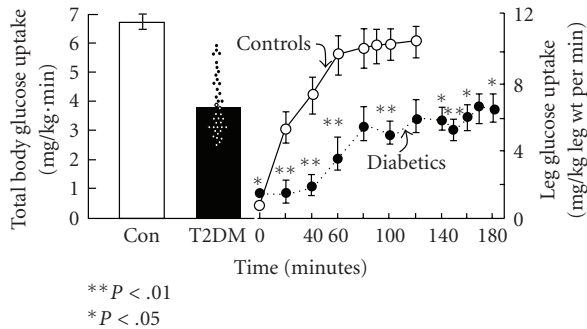
Figure 2: Insulin-stimulated whole body glucose uptake measured with the euglycemic clamp in lean healthy and type 2 diabetic subjects (left) and time course of change in leg glucose uptake in type 2 diabetic and control subjects (right) (from [5]).
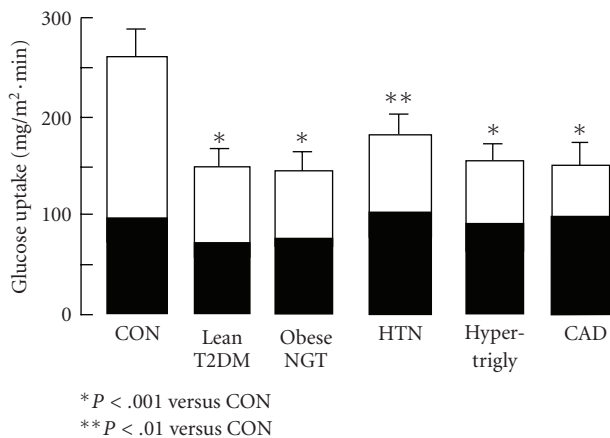

Figure 3: Insulin-stimulated glucose disposal ($40 \, \text{mU/m}^2 \cdot \text{min}$ euglycemic insulin clamp) in lean healthy control (CON) subjects, lean drug naïve type 2 diabetic subjects (T2DM), lean normal-glucose-tolerant (NGT) hypertensive subjects (HTN), NGT hypertriglyceridemic subjects, and nondiabetic subjects with coronary artery disease (CAD). The open portion of the bar represents nonoxidative glucose disposal (glycogen synthesis) and the solid portion represents glucose oxidation. See DeFronzo and Ferrannini [80], DeFronzo [81].

Insulin resistance in skeletal muscle is manifested long before the hyperglycemia becomes evident [2]. Thus, lean healthy offspring of diabetic parents have a significant decrease in insulin-stimulated total body and muscle glucose disposal which is of a similar magnitude to that observed in their diabetic parents [30–32]. Insulin resistance in skeletal muscle also can develop independent of family history of T2DM. Thus, obese NGT subjects without a family history for T2DM [33, 34] and individuals with essential hypertension [35] and ischemic heart disease [36] also have a 35–50% decrease in whole body insulin-mediated glucose disposal (Figure 3). Insulin resistance in skeletal muscle also has been reported in association with the normal ageing process [37], dyslipidemia (increased plasma triglyceride/decreased HDL cholesterol) [4, 26], and in association with many disease states including polycystic ovary syndrome (PCOs) [38], chronic kidney failure [39], heart failure [40], myotonic dystrophy [41], and lipodystrophy [42]. In addition, insulin resistance develops in acute severe illnesses such as injury and sepsis, perhaps secondary to the acute inflammatory state that prevails [43]. Insulin resistance in skeletal muscle also can develop secondary to pharmacological therapy, for example, glucocorticoids [44], anti-HIV therapy [45], and beta blockers [46]. These studies indicate that although insulin resistance in skeletal muscle is a hallmark of T2DM, muscle insulin resistance and the accompanying insulin resistance syndrome are more widely prevalent and the metabolic and clinical consequences of insulin resistance (e.g., increased cardiovascular risk) affect nondiabetic individuals as well. Because the molecular mechanisms that lead to the development of insulin resistance in skeletal muscle have been extensively studied in obese and T2DM subjects, we will focus the discussion on the molecular/biochemical pathways that cause muscle insulin resistance in these two common metabolic disorders. However, similar molecular defects have been reported in other insulin resistant states [39].

Under basal conditions, for example, fasting state, the plasma insulin concentration is low (5–$10 \, \mu\text{U/mL}$). Thus, glucose uptake that takes place in skeletal muscle during the postabsorptive state is insulin independent. The noninsulin dependent glucose uptake in skeletal muscle also is decreased in insulin resistant individuals [47]. The mechanism(s) responsible for the decrease in noninsulin dependent glucose uptake has (have) yet to be defined.

3.1. Relationship between Obesity and Insulin Resistance in Skeletal Muscle.

Obese NGT subjects display marked skeletal muscle insulin resistance compared to lean age and sex matched individuals [33, 34], and the severity of muscle insulin resistance is related to the increase in BMI [34]. Studies in experimental animals and in man have demonstrated that the increase in body weight is accompanied with an increase in skeletal muscle insulin resistance [48–52]. Conversely, weight loss in obese NGT individuals improves/reverses insulin resistance in skeletal muscle [43]. Collectively, these studies demonstrate a causal role of obesity per se in the development of skeletal muscle insulin resistance.

The mechanism via which obesity causes insulin resistance in skeletal muscle is related to the accumulation of fat in the myocytes. Muscle biopsy studies [53, 54] have demonstrated increased triglyceride content in skeletal muscle of obese NGT subjects compared to lean individuals and an inverse relationship between muscle insulin sensitivity and the intramuscular triglyceride content. Although these initial studies documented the importance of increased muscle fat content in the etiology of insulin resistance in obesity, they did not distinguish between the contribution of fat accumulation inside the muscle fiber (intramyocellar, IMCL) versus fat accumulation in the muscle outside the muscle cell (extramyocellar, EMCL). More recent studies [55, 56] using magnetic resonance spectroscopy have demonstrated that although IMCL fat content contributes only a small fraction (~1%) to the total muscle fat content, it plays a key role

in the development of insulin resistance in skeletal muscle compared to EMCL. Muscle insulin resistance strongly correlates with IMCL, independent of total body fat mass and diabetes status [56], while the correlation between EMCL and insulin ressitance is not significant.

Obese NGT individuals are characterized by an increase in plasma free fatty acid (FFA) concentration. The important role of elevated plasma FFA in the pathogenesis of insulin resistance is now well established [12, 14, 57]. Considerable data implicate a causative role for elevated plasma FFA level in insulin resistance in skeletal muscle. The majority of insulin resistant individuals, both diabetic and nondiabetics, are overweight or obese [58], and both lean and obese insulin resistant individuals manifest multiple disturbances in FFA metabolism [59]. Nondiabetic obese and type 2 diabetic individuals are characterized by day-long elevation in the plasma FFA concentration, which fails to suppress normally after ingestion of a mixed meal or an oral glucose load [60]. Elevated plasma FFA levels correlate strongly with reduced insulin-stimulated glucose disposal in skeletal muscle [61]. FFAs are stored within adipocytes in the form of triglycerides and serve as an important source of energy during fasting. Insulin is a potent inhibitor of lipolysis and restrains the release of FFAs from adipocytes. In insulin resistant individuals, for example, nondiabetic obese and lean type 2 diabetic subjects, the ability of insulin to inhibit lipolysis and reduce plasma FFA concentration is markedly impaired [21, 59], leading to an increased rate of lipolysis and chronic elevation in the plasma FFA concentration. It is well established that chronically elevated plasma FFA levels cause insulin resistance in skeletal muscle [1, 12, 14, 62–65]. Both acute (4 hours) and chronic (4 days) physiologic elevation of plasma FFA concentration in insulin sensitive individuals impairs insulin signaling and causes insulin resistance in skeletal muscle [28, 62–68]. The inhibition of insulin signaling and induction of skeletal muscle insulin resistance caused by elevation in the plasma FFA concentration is dose dependent [65]. Conversely, reduction of plasma FFA concentration in insulin resistant individuals, for example, type 2 diabetic subjects, augments insulin-stimulated glucose disposal in skeletal muscle [69–72], and the improvement in insulin sensitivity is closely associated with a reduction in intramyocellular FACoA concentration [69]. Collectively, these results support a causative role of elevated plasma FFA levels in the pathogenesis of insulin resistance in skeletal muscle and have been referred to as "lipotoxicity" [73].

Much evidence supports the hypothesis that the lipotoxic action of FFA on skeletal muscle insulin sensitivity is due to an increase in intramyocellular fat content: (i) in insulin resistant individuals, insulin-stimulated glucose disposal correlates more strongly with intramyocellular fat content than with the extramtocellular fat [55, 56]; (ii) the decrease in insulin resistance brought about by reducing the plasma FFA concentration with acipimox in insulin resistant individuals is associated with a reduction in intracellular FACoA content, and the magnitude of decrease in insulin resistance strongly correlates with the decrease in FACoA content [69]; (iii) the increase in insulin resistance caused by elevating the plasma FFA concentration in insulin sensitive individuals

is associated with an increase in the intramyocellular fat content [74]; (iv) in animals, a high fat diet causes an increase in intramuscular fat content, which correlates closely both with the severity and temporal development of insulin resistance [75, 76]; (v) in rodents, injection of recombinant adiponectin increases fat oxidation, reduces intamyocellular lipid, and improves insulin sensitivity [77]; (vi) muscle-specific over expression of lipoprotein lipase leads to the accumulation of fat/FACoA in muscle and severe muscle insulin resistance, while the fat content and insulin sensitivity in other tissues, that is, liver, remain unchanged [78]; (vii) weight loss, induced by bariatric surgery, results in a complete normalization of insulin sensitivity despite the presence of continued obesity (BMI reduced from 49 to 39 kg/m^2) [79]. The normalization of muscle insulin sensitivity is associated with a reduction of muscle fat content to normal despite a persistent increase in total body fat content [79]. These observations collectively indicate that muscle triglyceride (which is metabolic inert) is not directly involved in determining insulin sensitivity but rather represents a marker of imbalance between lipid supply and lipid oxidation in skeletal muscle, while increases in intramyocellar FACoA and other lipotoxic metabolites (DAG, ceramide) have a more direct role in the development of skeletal muscle insulin resistance.

3.2. Increased Fat Supply or Impaired Mitochondrial Function in Insulin Resistance?

Fat is an important fuel source in skeletal muscle. The accumulation of fat inside the muscle fiber could originate from excess supply of FFA to the muscle, a decreased rate of muscle fat oxidation, or some combination of the two.

3.2.1. Long-Chain Fatty Acid (LCFA) Transport into the Muscle.

Considerable debate exists regarding the mechanism by which LCFAs are transported into skeletal muscle [82–84]. Because LCFAs are highly hydrophobic molecules and easily can cross the plasma membrane of muscle via passive diffusion, the rate of LCFA influx into muscle fiber is, in part, driven by the concentration gradient of LCFA across the plasma membrane. Thus, an increae in plasma FFA concentration is an important regulator of FFA supply to skeletal muscle [82]. Indeed, early studies demonstrated the rate of lipolysis was a key determinant of FFA uptake and oxidation in skeletal muscle and documented a linear relationship between the plasma LCFA concentration and their rate of uptake and oxidation in the muscle [85–87]. More recent studies have reported that under conditions of marked increase in energy demand in the muscle, for example, during exercise, LCFAs uptake initially increases linearly then plateaus [88], suggesting the existence of a facilitated transport mechanism for LCFA into skeletal muscle. Three putative fatty acid binding proteins located at the plasma membrane have been identified: (i) the plasma membrane-bound fatty acid binding protein (FABP); (ii) fatty acid translocase (CD36); (iii) fatty acid transport protein (FATP). There is abundant evidence which indicates that CD36 plays an important role in LCFA uptake into skeletal muscle in rodents and human and its concentration and activity are

increased following exercise [89–96]. However, the transport of LCFA by CD36 into skeletal muscle does not seem to regulate the rate of LCFA oxidation. During conditions of decreased fat oxidation and increased CD36 levels, muscle uptake of LCFA increased, resulting in increased muscle triglyceride synthesis and increased IMCL content [97, 98]. The CD36 content and LCFA transport capacity have been reported to be increased in obese and T2DM individuals and correlate with increased muscle triglyceride content, while fat oxidation is decreased. Further, insulin has been reported to increase CD36 content in skeletal muscle [99].

3.2.2. Fat Oxidation in Insulin Resistance. Several studies have reported decreased fat oxidation in skeletal muscle, independent of the plasma FFA concentration [100–102]. In one study that compared muscle fat oxidation across the leg in obese (BMI = $34 \, kg/m^2$) compared to lean (BMI = $23 \, kg/m^2$) individuals, the rate of fatty acid oxidation was reduced in the obese group despite similar arterial FFA concentrations [100]. These results suggest the presence of impaired muscle fat oxidation independent of fat supply to skeletal muscle. Similar results have been reported in rectus abdominus muscle in morbidly obese individuals [101] and in obese nondiabetic women [102]. Studies in experimental animals also have reported a decreased rate of lipid oxidation in skeletal muscle in obese Zuker rat [103]. Collectively, these studies indicate the existence of an intrinsic defect in mitochondrial capacity to oxidize fat in obesity and T2DM.

3.2.3. Mitochondrial Function in Insulin Resistant Conditions. Since the majority of fat oxidation take place in the mitochondria, impaired fat oxidation in insulin resistant individuals suggests the presence of a mitochondrial defect that contributes to the impaired muscle fat oxidation and increased IMCL fat content. Studies in humans, using molecular, biochemical, and MR spectroscopic techniques, have documented a defect in mitochondrial oxidative phosphorylation in a variety of insulin resistance states. Because of its accessibility, most of these studies have been performed in skeletal muscle. In vivo measurement of oxidative phosphorylation with ^{31}P-NMR has demonstrated impaired ATP synthesis in a variety of insulin resistant states. Lean normal-glucose-tolerant (NGT), insulin-resistant offspring of T2DM parents have a 30–40% decrease in metabolic flux through the tricarboxylic acid cycle [104] and oxidative phosphorylation [31] in skeletal muscle under basal conditions. A similar impairment in resting flux through oxidative phosphorylation also has been reported in subjects with type 2 diabetes [105]. Furthermore, unlike lean insulin-sensitive individuals, both diabetic subjects and NGT insulin-resistant offspring of two diabetic parents fail to increase mitochondrial oxidative phosphorylation flux following insulin stimulation despite a significant increase in glucose disposal in skeletal muscle [105, 106]. Subjects with T2DM also have decreased exercise tolerance and impaired recovery of intracellular phosphocreatinine concentration following exercise [107, 108], indicating that the mitochondrial defect in oxidative phosphorylation may

contribute to the impairment in exercise capacity in insulin-resistant individuals. Insulin resistance also is a characteristic feature of the normal aging process and is associated with a decrease in mitochondrial ATP synthesis rate and an increase in intramyocellar fat content [109]. Lastly, experimental induction of insulin resistance in skeletal muscle, by a physiological increase in plasma FFA concentration in lean healthy insulin-sensitive individuals, is associated with a decrease in oxidative phosphorylation flux in skeletal muscle [110]. Collectively, these results indicate that, regardless of its etiology, insulin resistance in skeletal muscle is associated with decreased mitochondrial oxidative phosphorylation.

3.3. Qualitative or Quantitative Mitochondrial Defect. The mitochondrial defect in ATP synthesis rate documented in vivo with MRS could be due to a reduction in the number of mitochondria in skeletal muscle with normal function of individual mitochondria, an intrinsic defect in a quantitively normal number of mitochondria, or some combination of the two. All three of these scenarios would result in a reduced in ATP synthesis rate measured in vivo with MRS. Other important unanswered questions are whether the mitochondrial defect is inherited or acquired and whether this defect is the cause or the effect of the insulin resistance. An acquired defect potentially could be reversed or prevented, while an inherited defect would be permanent.

Although several lines of evidence support a reduction in mitochondrial number in skeletal muscle in insulin resistant individuals, not all studies have yielded consistent results. Electron microscopic studies have revealed a significant reduction ($\sim$40%) in mitochondrial density in skeletal muscle in lean NGT offspring of type 2 diabetic parents, in obese nondiabetic individuals and in T2DM subjects compared to lean insulin-sensitive, age-matched controls [111, 112]. The decrease in mitochondrial density in skeletal muscle in insulin-resistant individuals is consistent with decreased expression of PGC-1 gene [113], the master regulator for mitochondrial biogenesis. Diabetic subjects have been reported to have a decrease in muscle fiber oxygen consumption measured ex vivo. However, when oxygen consumption was related to mitochondrial copy number, both diabetic and nondiabetic subjects had similar rates of muscle oxygen consumption, implying a decrease in mitochondrial density without an intrinsic mitochondrial defect in T2DM subjects. However, other studies have reported contradictory results. Thus, studies which have assessed mitochondrial copy number in skeletal muscle in insulin-resistant individuals have reported conflicting results [114–116]. Furthermore, insulin resistance, caused by physiological elevation in plasma FFA concentration in lean healthy individuals, was not associated with a significant change in mitochondrial density in skeletal muscle [117].

Several morphological and functional studies support the concept of an intrinsic mitochondrial defect in insulin resistant individuals. EM studies have demonstrated a variety of morphological abnormalities in mitochondria in insulin-resistant individuals [118] and these changes were reversable with weight loss and increased physical activity [119]. Ex vivo measurement of electron transport chain activity (ETC)

[112] and mitochondrial ATP synthesis in isolated mitochondria [120, 121] have demonstrated a decrease in ETC activity and ATP synthesis in obese insulin-resistant individuals compared to lean insulin-sensitive subjects. Since, in these studies [112, 120], ETC activity and mitochondrial ATP synthesis rate were related to the amount of mitochondrial protein and/or citrate synthase activity, these observations indicate the existence of a basic functional defect in mitochondrial activity in skeletal muscle of obese insulin-resistant individuals [115]. Biochemical studies have reported a decrease in the activity of a variety of mitochondrial enzymes [122], also supporting an intrinsic functional mitochondrial defect in type 2 diabetic subjects. An intrinsic mitochondrial defect, with or without a decrease in mitochondrial density, would be expected to result in impaired lipid oxidation, an increase in intramyocellular lipid content, and the development/exacerbation of insulin resistance. Additional studies are needed to resolve some of the inconsistencies reviewed above and to explore the biochemical/molecular basis of the mitochondrial defect measured in vivo in skeletal muscle in insulin resistant individuals.

3.4. Inherited or Acquired Mitochondrial Defect. An inherited mitochondrial oxidative defect in the absence (but especially in the presence) of enhanced FFA influx to skeletal muscle would be expected to lead to intramyocellular fat accumulation. An inherited defect in mitochondrial oxidative capacity should be present at or shortly after birth and, most importantly, not be reversable. With regard to this, several lines of evidence indicate that the mitochondrial defect associated with insulin resistant is reversible, at least in part: (i) modest weight reduction (~10% of body weight), produced by dieting and increased physical activity, reduces insulin resistance and intramyocellular lipid content and these changes are associated with increased fat oxidation in skeletal muscle [123]. Furthermore, the magnitude of increase in fat oxidation following weight loss correlated strongly with the decrease in insulin resistance [123]; (ii) marked weight reduction following bariatric surgery in morbidly obese, severely insulin-resistant subjects completely normalized insulin sensitivity, and correction of the insulin resistance was accompanied by depletion of intramyocellular fat content [124]. Although mitochondrial function was not assessed following weight loss in this latter study, the depletion of intramyocellular fat and normalization of insulin sensitivity suggests a reversible mitochondrial defect in these morbidly obese, insulin-resistant individuals; (iii) the morphological changes which characterize mitochondria in obese insulin-resistant individuals [118] can be reversed following weight loss and increased physical activity [119]; (iv) the improvement in insulin sensitivity following exercise is associated with improved muscle oxidative capacity [125, 126] and increases in both mitochondrial density [127] and activity [128–130]. These observations indicate that the mitochondrial defect which accompanies insulin resistance is, at least in part, reversible. The ability to reverse the defect in mitochondrial oxidative function argues against an inherited defect. Importantly, a mitochondrial defect similar to that observed in insulin-resistant individuals can

be produced in young lean healthy subjects by physiologic elevation of the plasma FFA concentration [110, 131]. In addition, the insulin resistance associated with aging in lean subjects with negative family history of diabetes is associated with a decrease in mitochondrial function [109]. Thus, normal mitochondrial function in young, insulin-sensitive subjects suggests that the mitochondrial defect and insulin-resistance are acquired as part of the normal ageing process.

The evidence reviewed above supports the hypothesis that the mitochondrial defect which accompanies insulin resistance is reversible, at least in part, and is likely to be acquired. Regarding the etiology of the acquired defect in mitochondrial function, in vivo mitochondrial ATP synthesis rate, measured with ^{31}P-NMR strongly and inversely correlates with the fasting plasma FFA concentration [105], suggesting a possible role for elevated plasma FFA/toxic intramyocellar FFA metabolite concentrations in the mitochondrial dysfunction in insulin resistant individuals. With respect to this, we recently have demonstrated that a physiologic increase in FACoA concentration inhibits mitochondrial ATP synthesis in vitro in mitochondria isolated from skeletal muscle of NGT healthy lean subjects [132]. This observation is consistent with the decrease in oxidative phosphorylation observed following elevation in the plasma FFA concentration in lean healthy individuals [110], and it indicates that elevated plasma FFA/intramyocellular metabolite concentration can cause an acquired mitochondrial defect in oxidative phosphorylation.

3.5. Mitochondrial Defect in Insulin Resistance: Cause or Effect? The mitochondrial defect in oxidative phosphorylation described in vivo with MRS and ex vivo in muscle fibers and isolated mitochondria could contribute to the increase in intramyocellar FFA metabolite levels observed in obesity and T2DM and contribute to the insulin resistance. However, one also can imagine another scenario in which the mitochondrial defect results from insulin resistance. If the increase in intramyocellar fat content in insulin resistant individuals was associated with an increase in fat oxidation and excessive production of reactive oxygen species or other toxic metabolites, then the decrease in mitochondrial oxidative phosphprylation could result from the downregulation of mitochondrial function to ameliorate the production of these toxic molecules. Reproduction of the mitochondrial defect in oxidative phosphorylation in normal healthy individuals by elevation of the plasma FFA concentration would argue for a causative role of the mitochondrial defect in insulin resistance. However, since muscle insulin resistance also develops following lipid infusion and since the time course of the development of the mitochondrial defect and insulin resistance were not monitored during lipid infusion [110], one cannot completely exclude the possibility that the mitochondrial defect results secondary to insulin resistance.

Studies in experimental animals and cultured muscle cells have attempted to address the relationship between mitochondrial function and skeletal muscle insulin resistance. Over expression of the PGC-1 alpha gene in skeletal muscle in mice in vivo enhanced mitochondrial activity, augmented the expression of multiple proteins involved in

fat oxidation and glucose transport, and increased by ∼35% insulin-stimulated glucose uptake [133]. Similarly, activation of SIRT1 with resveratrol in mice resulted in increased mitochondrial activity and protected the animals from diet-induced obesity and insulin resistance [134]. Studies in cultured skeletal muscle cells have been inconsistent. Down regulation of mitochondrial function in myotubes with oligomycin, which inhibits mitochondrial ATP synthesis, and with ethidium bromide, which impairs mitochondrial DNA replication, caused an increase in intracellular fat content, impaired insulin signaling, and decreased insulin-stimulated glucose uptake [135]. However, treatment of the muscle cells with azide, an inhibitor of mitochondrial complex IV, increased basal glucose uptake without affecting insulin-stimulated glucose uptake in myotubes [136]. Down regulation of electron transport chain activity in mice by knocking down apoptosis inintiating factor (AIF) resulted in enhanced insulin sensitivity and protection the animal from fat-induced insulin resistance [137].

In summary it can be concluded that both decreased mitochondrial fat oxidation and increased FFA influx into skeletal muscle take place during insulin resistance state. If the rate of fat supply exceeds the demand for fat oxidation, the muscle redirects the fat entering the cell toward triglyceride synthesis leading to increased IMCL content. In contrast if fat oxidation exceeds the rate of fat supply, all fat entering the muscle will be directed to fat oxidation and no fat will accumulate in the muscle. Thus, the IMCL content in skeletal muscle reflects the dynamic balance between the demand for fat oxidation and fat supply to the muscle.

4. Cellular Mechanism of Insulin Resistance

The cellular events via which insulin initiates its stimulatory effect on glucose metabolism start with binding of the hormone to specific receptors that are present on the muscle cell surface [2, 138–140]. The binding of insulin activates the insulin receptor and the activated insulin receptor generates second messengers that activate a cascade of phosphorylation-dephosphorylation reactions that eventually result in the stimulation of intracellular glucose metabolism. The first step in glucose metabolism involves activation of the glucose transport system (GLUT4), leading to glucose influx into muscle cells. The free glucose, which has entered the cell, subsequently is metabolized by a series of enzymatic steps that are under the control of insulin. Of these, the most important are glucose phosphorylation (catalyzed by hexokinase II), glycogen synthase (which controls glycogen synthesis), and phosphofructokinase (PFK) and pyruvate dehydrogenase (PDH) (which regulate glycolysis and glucose oxidation, resp.).

4.1. Insulin Receptor/Insulin Receptor Tyrosine Kinase. The insulin receptor is a glycoprotein comprised of two α-subunits and two β-subunits linked by disulfide bonds [2, 138–140]. The two α-subunits of the insulin receptor are located at the extracellular surface of the muscle plasma membrane and contain the insulin-binding domain. The β-subunits have an extracellular domain, a transmembrane domain, and an intracellular domain that expresses insulin-stimulated kinase activity directed toward its own tyrosine residues. The binding of insulin to the α-subunit causes phosphorylation of the β-subunit on multiple tyrosine residues. The activation of insulin receptor tyrosine kinase activity is essential for the action of insulin on glucose metabolism. Mutagenesis of any of the three major phosphorylation sites (at residues 1158, 1163, and 1162) impairs insulin receptor tyrosine kinase activity, leading to a decrease in the metabolic and growth-promoting effects of insulin [141, 142].

4.2. Insulin Receptor Signal Transduction. Following the activation of insulin receptor tyrosine kinase, specific intracellular proteins, of which at least nine have been identified [138, 143], become phosphorylated. In skeletal muscle insulin-receptor substrate (IRS)-1 serves as the major docking protein and undergoes tyrosine phosphorylation by the activated insulin receptor in regions containing specific amino acid sequence motifs (Figure 4). The phosphorylated motifs serve as recognition sites for proteins containing *src*-homology 2 (SH2) domains. Mutation of these specific tyrosine residues in IRS-1 severely impairs the ability of insulin to stimulate muscle glycogen synthesis, glucose uptake and oxidation, and other acute metabolic- and growth-promoting effects of insulin [142].

The phosphorylated tyrosine residues of IRS-1 mediate an association with the 85-kDa regulatory subunit of phosphatidylinositol 3-kinase (PI3K), leading to activation of the enzyme [138–140, 143, 144]. PI3K is composed of an 85-kDa regulatory subunit and a 110-kDa catalytic subunit. The latter catalyzes the $3'$ phosphorylation of PI 4-phosphate and PI 4,5-diphosphate. Activation of PI3K by phosphorylated IRS-1 leads to activation of protein kinase B/Akt which is a central intermediate for many of the metabolic and growth actions of insulin. It has been identified as one of the kinases responsible for the inactivation of glycogen synthase kinase through its phosphorylation, a process that leads to the activation of glycogen synthase. Akt also phosphorylates the newly identified Akt substrate AS160, leading to its redistribution in the cell and activation of Rab proteins required for the translocation of the vesicles containing GLUT4 to the plasma membrane.

Inhibitors of PI3K impair glucose transport and block the activation of glycogen synthase and hexokinase- (HK-) II expression [138–146] The action of insulin to increase protein synthesis and inhibit protein degradation also is mediated by PI3K.

Other proteins with SH2 domains, including the adapter protein Grb2 and *Shc*, also interact with IRS-1 and become phosphorylated following exposure to insulin [138–140, 143]. Grb2 and *Shc* link IRS-1/IRS-2 to the mitogen-activated protein kinase- (MAPK-) signaling pathway, which plays an important role in the generation of transcription factors and promotes cell growth, proliferation, and differentiation [138, 143]. Inhibition of the MAPK kinase pathway prevents the stimulation of cell growth by insulin but has no effect on the metabolic actions of the hormone [147].
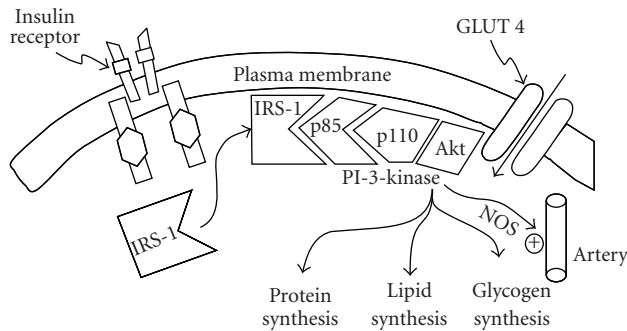
FIGURE 4: Insulin signal transduction system in normal glucose tolerant subjects (see text for a detailed discussion).

Under anabolic conditions, insulin augments glycogen synthesis by simultaneously activating glycogen synthase and inhibiting glycogen phosphorylase [148, 149]. The effect of insulin is mediated through the PI3K pathway, which inactivates kinases such as glycogen synthase kinase-3 and activates phosphatases, particularly PP1. PP1 is believed to be the primary regulator of glycogen metabolism. In skeletal muscle, PP1 associates with a specific glycogen-binding regulatory subunit, causing dephosphorylation (activation) of glycogen synthase. PP1 also phosphorylates (inactivates) glycogen phosphorylase. The precise steps that link insulin receptor tyrosine kinase/PI3K activation to stimulation of PP1 have yet to be defined. Studies [138, 150] have demonstrated convincingly that inhibitors of PI3K inhibit glycogen synthase activity and abolish glycogen synthesis.

4.3. Insulin Receptor Signal Transduction Defects in Insulin Resistance. Early studies have demonstrated a modest 20–30% reduction in insulin binding to monocytes and adipocytes from type 2 diabetic patients, but this has not been a consistent finding [1, 151–154]. The decrease in insulin binding is caused by a reduction in the number of insulin receptors without change in insulin receptor affinity. Some caution, however, should be used in interpreting these studies because muscle and liver, not adipocytes, are the major tissues responsible for the regulation of glucose homeostasis in vivo, and insulin binding to solubilized receptors obtained from skeletal muscle and liver has been shown to be normal in obese and lean diabetic individuals [152, 153, 155]. Moreover, a decrease in insulin receptor number cannot be demonstrated in over half of type 2 diabetic subjects, and it has been difficult to demonstrate a correlation between reduced insulin binding and the severity of insulin resistance [156–158]. A variety of defects in insulin receptor internalization and processing have been described in syndromes of severe insulin resistance and diabetes. The insulin receptor gene, however, has been sequenced in a large number of type 2 diabetic patients from diverse ethnic populations and, with very rare exceptions, physiologically significant mutations in the insulin receptor gene have not been observed [159, 160]. This excludes a structural gene abnormality in the insulin receptor as a common cause of insulin resistance in skeletal muscle.

4.4. Insulin Receptor Tyrosine Kinase Activity. Insulin receptor tyrosine kinase activity has been examined in skeletal muscle from normal-weight and obese diabetic subjects. Most [1, 7, 152, 153, 156, 161] but not all [155] investigators have found a reduction in tyrosine kinase activity that cannot be explained by alterations in insulin receptor number or insulin receptor binding affinity. Restoration of normoglycemia by weight loss, however, has been shown to correct the defect in insulin receptor tyrosine kinase activity [162], suggesting that the defect in tyrosine kinase activity, at least in part, is acquired secondary to some combination of hyperglycemia, distributed intracellular glucose metabolism, hyperinsulinemia, and insulin resistance, all of which improved after weight loss. Exposure of cultured fibroblasts to high glucose concentration also has been shown to inhibit insulin receptor tyrosine kinase activity [163]. Because insulin receptor tyrosine kinase activity assays are performed in vitro, the results of these assays could provide misleading information with regard to insulin receptor function in vivo. To circumvent this problem, investigators have used the euglycemic hyperinsulinemic clamp with muscle biopsies and antiphosphotyrosine immunoblot analysis to provide a "snap shot" of the insulin-stimulated tyrosine phosphorylation state of the receptor in vivo [7]. In insulin-resistant obese nondiabetic subjects, in the NGT insulin resistant offspring of two diabetic parents, and in type 2 diabetic subjects, a substantial decrease in insulin receptor tyrosine phosphorylation has been demonstrated. However, when insulin-stimulated insulin receptor tyrosine phosphorylation was examined in normal-glucose-tolerant insulin-resistant individuals (offspring of two diabetic parents) at high risk for developing type 2 diabetes, a normal increase in tyrosine phosphorylation of the insulin receptor was observed [164]. These findings are consistent with the concept that impaired insulin receptor tyrosine kinase activity in type 2 diabetic patients is acquired secondary to hyperglycemia or some other metabolic disturbance.

4.5. Insulin-Signaling (IRS-1 and PI3K) Defects (Figure 5). The ability of insulin to activate insulin receptor and IRS-1 tyrosine phosphorylation in muscle in obese nondiabetic subjects is modestly reduced; whereas in type 2 diabetics insulin-stimulated insulin receptor and IRS-1 tyrosine phosphorylation are severely impaired [7]. Association of the p85 subunit of PI3K with IRS-1 and activation of PI3K also are greatly attenuated in obese nondiabetic and type 2 diabetic subjects compared with lean healthy controls [7, 165, 166]. The decrease in insulin-stimulated association of the p85 regulatory subunit of PI3K with IRS-1 is closely correlated with the reduction in insulin-stimulated muscle glycogen synthase activity and in vivo insulin-stimulated glucose disposal [7]. Impaired regulation of PI3K gene expression by insulin also is impaired in skeletal muscle, and similar impairment has been demonstrated in adipose tissue of type 2 diabetic subjects [167]. In animal models of diabetes, an 80%–90% decrease in insulin-stimulated IRS-1 phosphorylation and PI3K activity has been reported [168, 169].
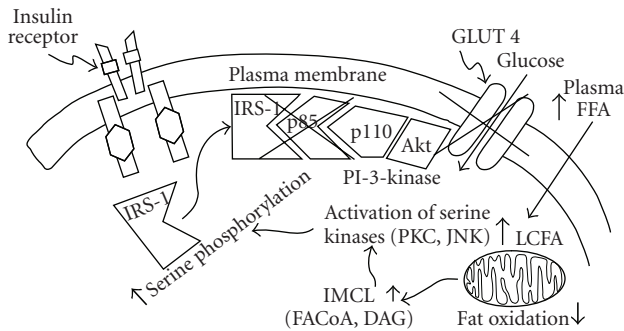
FIGURE 5: In insulin resistant individuals insulin signaling is impaired at the level of IRS-1 leading to decreased glucose transport/phosphorylation/metabolism and impaired nitric oxide synthase activation/endothelial function. Increased intramycellar fat and fatty acid meyabolite content and mitochondrial dysfunction also exist in skeletal muscle in insulin resistant individuals (see text for a detailed discussion).

In the insulin-resistant, normal glucose tolerant offspring of two type 2 diabetic parents, IRS-1 tyrosine phosphorylation and the association of p85 protein/PI3K activity with IRS-1 are markedly decreased despite normal tyrosine phosphorylation of the insulin receptor; these insulin signaling defects are correlated closely with the severity of insulin resistance measured with the euglycemic insulin clamp technique [164]. In summary, impaired association of PI3K with IRS-1 and its subsequent activation are characteristic abnormalities in type 2 diabetics, and these defects are correlated closely with in vivo muscle insulin resistance.

The nature of the defect in IRS-1 that impairs its ability to activate PI3-Kinase does not seem to include a structural defect in IRS-1. A common mutation in the IRS-1 gene (Gly-972-Arg) has been associated with type 2 diabetes, insulin resistance, and obesity, but the physiologic significance of this mutation remains to be established [170]. Rodent and human studies have suggested increased serine phosphorylation of IRS-1 in insulin resistant states [111, 171–173]. In general, serine phosphorylation of IRS-1 negates its ability to undergo tyrosine phosphorylation. Thus, conditions that increase serine phosphorylation of IRS-1 impair tyrosine phosphorylation by the insulin receptor and its activation, thus, leading to an impartment in the insulin signaling cascade. An important, yet an answered, question is the identity of the factor(s) responsible for the activation of serine kinases that phosphorylate IRS-1. Increased activity of protein kinase C (PKC-β and δ has been reported in insulin resistant states and can be induced during lipid infusion [174, 175]. Furthermore, increased intramyocellar levels of fat metabolites, for example, FACoA and DAG, which are strong activators of protein kinase C, have been demonstrated following lipid infusion [176]. Moreover, prevention of the IRS-1 serine phosphorylation by mutating key serine residues in the IRS-1 prevents the development of insulin resistance in skeletal muscle caused by high-fat feeding [169]. Increased stress kinase activity also has been

implicated in contribute to the serine phosphorylation of IRS-1 and subsequent impairment tyrosine phosphorylation [177, 178]. In summary, increased serine phosphorylation of IRS-1 is believed to impair its tyrosine phosphorylation by the insulin receptor and interrupt the insulin signalin cascade, leading to the development of insulin resistance in skeletal muscle.

Because nitric oxide synthase also is activated by the IRS-1/PIS-K pathway, impaired insulin signaling leads to reduced nitric oxide generation and endothelial dysfunction, which have implicated in the development of accelerated atherosclerosis in T2DM individuals.

Insulin resistance in the PI3K signaling pathway contrasts with an intact stimulation of the MAPK pathway by insulin in insulin-resistant type 2 diabetic and obese nondiabetics individuals [7, 165]. Physiologic hyperinsulinemia increases mitogen-activated protein kinase/extracellular signal-regulated kinase 1 activity (MEK-1) and extracellular signal-regulated kinase1/2 phosphorylation activity (ERK) similarly in lean healthy subjects, insulin-resistant obese nondiabetic, and type 2 diabetic patients. Intact stimulation of the MAPK pathway by insulin in the presence of insulin resistance in the PI3K pathway may play an important role in the development of atherosclerosis [7]. If the metabolic (PI3K) pathway is impaired, plasma glucose levels rise, resulting in increased insulin secretion and hyperinsulinemia. Because insulin receptor function is normal or only modestly impaired, especially early in the natural history of type 2 diabetes, this leads to excessive stimulation of the MAPK (mitogenic) pathway in vascular tissues, with resultant proliferation of vascular smooth muscle cells, increased collagen formation, and increased production of growth factors and inflammatory cytokines [179, 180].

4.6. Glucose Transport. Activation of the insulin signal transduction system in skeletal muscle stimulates glucose transport through a mechanism that involves translocation of a large intracellular pool of glucose transporters (associated with low-density microsomes) to the plasma membrane and their subsequent activation after insertion into the cell membrane [181, 182]. There are five major, different facilitative glucose transporters (GLUTs) with distinctive tissue distributions [183, 184]. GLUT4, the insulin regulatable transporter, is found in skeletal muscle, has a K_m of approximately 5 mmol/L, which is close to that of the plasma glucose concentration, and is associated with HK-II [173, 174]. GLUT4 concentration in the plasma membrane increases markedly after exposure to insulin, and this increase is associated with a reciprocal decline in the intracellular GLUT4 pool. A recent study has reported a parallel translocation of GLUT12 from the cytosol to the plasma membrane following insulin stimulation in human skeletal muscle [185].

GLUT1 is the predominant glucose transporter in the insulin-independent tissues (brain and erythrocytes) but also is found in muscle and probably contributes to muscle glucose uptake during the basal state. GLUT1 is located primarily in the plasma membrane where its concentration is

unchanged following exposure to insulin. Thus, it is likely to contribute to muscle glucose uptake during the basal state. It has a low K_m ($\sim$1 mmol/L) and is well suited for its function, which is to mediate basal glucose uptake. It is found in association with HK-I [186].

In insulin resistant individuals with T2DM, glucose transport activity is severely impaired [156, 181, 182, 187–189]. Muscle tissue from lean and obese type 2 diabetic subjects exhibits normal or increased levels of GLUT4 mRNA expression and normal levels of GLUT4 protein, thus demonstrating that transcriptional and translational regulation of GLUT4 is not impaired [190, 191]. Using a novel triple-tracer technique, the in vivo dose-response curve for the action of insulin on glucose transport in forearm skeletal muscle has been examined in type 2 diabetic subjects, and insulin-stimulated inward muscle glucose transport has been shown to be severely impaired [192, 193]. Impaired in vivo muscle glucose transport in type 2 diabetics also has been demonstrated using magnetic resonance imaging [194] and positron emission tomography [195]. Because the number of GLUT4 transporters in the muscle of diabetic subjects is normal, impaired GLUT4 translocation and decreased intrinsic activity of the glucose transporter must be responsible for the defect in muscle glucose transport. Large populations of type 2 diabetics have been screened for mutations in the GLUT4 gene [196]. Such mutations are very uncommon and, when detected, have been of questionable physiologic significance.

4.7. Glucose Phosphorylation. Glucose phosphorylation and glucose transport are tightly coupled processes [197]. Hexokinase isoenzymes (HK-I–IV) catalyze the first committed step of glucose metabolism, the intracellular conversion of free glucose to glucose-6-phosphate (Glu-6-P) [183, 184, 186, 198]. HK-I, HK-II, and HK-III are single-chain peptides that have a very high affinity for glucose and demonstrate product inhibition by Glu-6-P. HK-IV, also called glucokinase, has a lower affinity for glucose and is not inhibited by Glu-6-P.

In human skeletal muscle, HK-II transcription is regulated by insulin, whereas HK-I mRNA and protein levels are not affected by insulin [199, 200]. In response to physiologic euglycemic hyperinsulinemia of 2 to 4 hours' duration, HK-II cytosolic activity, protein content, and mRNA levels increase by 50% to 200% in healthy nondiabetic subjects, and this is associated with the translocation of hexokinase II from the cytosol to the mitochondria. In forearm muscle, insulin-stimulated glucose transport (measured with the triple-tracer technique) is markedly impaired in lean type 2 diabetics [192, 193], but the rate of intracellular glucose phosphorylation is impaired to an even greater extent, resulting in an increase in the free glucose concentration within the intracellular space that is accessible to glucose. These observations indicate that in type 2 diabetic individuals, although both glucose transport and glucose phosphorylation are severely resistant to the action of insulin, impaired glucose phosphorylation (HK-II) appears to be the rate-limiting step for insulin action. Studies using ^{31}P nuclear magnetic resonance in combination with

[1-^{14}C] glucose also have demonstrated that both insulin-stimulated muscle glucose transport and glucose phosphorylation are impaired in type 2 diabetic subjects, but the defect in transport exceeds the defect in phosphorylation [194]. Because of methodologic differences, the results of the triple-tracer technique [192, 193] and magnetic resonance imaging [194] studies cannot be reconciled at present. Nonetheless, these studies are consistent in demonstrating that abnormalities in both muscle glucose phosphorylation and glucose transport are well established early in the natural history of type 2 diabetes and cannot be explained by glucose toxicity.

In healthy nondiabetic subjects, a physiologic increase in the plasma insulin concentration for as little as 2 to 4 hours increases muscle HK-II activity, gene transcription, and translation [201]. In lean type 2 diabetics, the ability of insulin to augment HK-II activity and mRNA levels are markedly reduced compared with controls [199]. Decreased basal muscle HK-II activity and mRNA levels and impaired insulin-stimulated HK-II activity in type 2 diabetic subjects have been reported by other investigators [200, 202]. A decrease in insulin-stimulated muscle HK-II activity also has been described in subjects with IGT [203]. Several groups have looked for point mutations in the HK-II gene in individuals with type 2 diabetes, and, although several nucleotide substitutions have been found, none are close to the glucose and ATP binding sites and none have been associated with insulin resistance [203–205]. Thus, an abnormality in the HK-II gene is unlikely to explain the inherited insulin resistance in common variety type 2 diabetes mellitus.

4.8. Glycogen Synthesis. Impaired insulin-stimulated glycogen synthesis is a characteristic finding in all insulin-resistant states. Obese, IGT, and diabetic subjects have severe impairment in insulin-stimulated glycogen synthase that accounts for the majority of the defect in insulin-mediated whole-body glucose disposal [1, 2, 21, 195, 206–218]. Impaired glycogen synthesis also has been documented in the normal-glucose tolerant offspring of two diabetic parents, in the first-degree relatives of type 2 diabetic individuals, and in the normoglycemic twin of a monozygotic twin pair in which the other twin has type 2 diabetes [206, 210, 211].

Glycogen synthase is the key insulin-regulated enzyme that controls the rate of muscle glycogen synthesis [148, 150, 200, 212–214]. Insulin activates glycogen synthase by stimulating a cascade of phosphorylation-dephosphorylation reactions which ultimately lead to the inhibition of glycogen synthase kinase and activation of PP1 (also called glycogen synthase phosphatase). The regulatory subunit of PP1 has two serine phosphorylation sites, called site 1 and site 2. Phosphorylation of site 2 by cAMP-dependent protein kinase inactivates PP1; whereas phosphorylation of site 1 by insulin activates PP1, leading to the stimulation of glycogen synthase. Phosphorylation of site 1 of PP1 by insulin in muscle is catalyzed by insulin-stimulated protein kinase (ISPK)-1. Because of their central role in muscle glycogen formation, the three enzymes, glycogen synthase, PP1, and

ISPK-1, have been extensively studied in individuals with type 2 diabetes.

Glycogen synthase exists in an active (dephosphorylated) and an inactive (phosphorylated) form [148–150]. Under basal conditions, total glycogen synthase activity in type 2 diabetic subjects is reduced, and the ability of insulin to activate glycogen synthase is severely impaired [7, 215–217]. The ability of insulin to stimulate glycogen synthase also is diminished in the normal glucose-tolerant, insulin-resistant relatives of type 2 diabetic individuals [218]. In insulin-resistant nondiabetic and diabetic Pima Indians, activation of muscle PP1 (glycogen synthase phosphatase) by insulin is severely reduced [219]. Because PP1 dephosphorylates glycogen synthase, leading to its activation, a defect in PP1 appears to play an important role in the muscle insulin resistance of type 2 diabetes mellitus.

The effect of insulin on glycogen synthase gene transcription and translation in vivo has been studied extensively. Most studies have demonstrated that insulin does not increase glycogen synthase mRNA or protein expression in human muscle [201, 220, 221]. Glycogen synthase mRNA and protein levels, however, are decreased in muscle of type 2 diabetic patients, partly explaining the decreased glycogen synthase activity [221, 222]. The major abnormality in glycogen synthase regulation in type 2 diabetes is its lack of dephosphorylation and activation by insulin, as a result of insulin receptor signaling abnormalities.

The glycogen synthase gene has been the subject of intensive investigation, and DNA sequencing has revealed either no mutations or rare nucleotide substitutions that cannot explain the defect in insulin-stimulated glycogen synthase activity [223–225]. The genes encoding the catalytic subunits of PP1 and ISPK-1 have been examined in Pima Indians and Danes with type 2 diabetes [226, 227]. Several silent nucleotide substitutions were found in the PP1 and ISPK-1 genes in the Danish population, but the mRNA levels of both genes were normal in skeletal muscle. No structural gene abnormalities in the catalytic subunit of PP1 were detected in Pima Indians. Thus, neither mutations in the PP1 and ISPK-1 genes nor abnormalities in their translation can explain the impaired enzymatic activities of glycogen synthase and PP1 that have been observed in vivo. Similarly, there is no evidence that an alteration in glycogen phosphorylase plays any role in the abnormality in glycogen formation in type 2 diabetes [228].

In summary, glycogen synthase activity is severely impaired in type 2 diabetic individuals, and the molecular cause of the defect most likely is related to impaired insulin signal transduction.

4.9. Glycolysis. Glucose oxidation accounts for approximately 90% of total glycolytic flux; whereas anaerobic glycolysis accounts for the other 10%. Phosphofructokinase and pyruvate dehydrogenase play pivotal roles in the regulation of glycolysis and glucose oxidation, respectively. In type 2 diabetic individuals, the glycolytic/glucose oxidative pathway has been shown to be impaired [229]. Although one study [230] has suggested that PFK activity is modestly reduced in muscle biopsies from type 2 diabetic subjects, most evidence indicates that the activity of PFK is normal [216, 221]. Insulin has no effect on muscle PFK activity, mRNA levels, or protein content in either nondiabetic or diabetic individuals [221]. PDH is a key insulin-regulated enzyme with activity in muscle that is acutely stimulated by insulin [231]. In type 2 diabetic patients, insulin-stimulated PDH activity has been shown to be decreased in human skeletal muscle [231, 232].

Obesity and type 2 diabetes mellitus are associated with accelerated FFA turnover and oxidation [1, 2, 21, 233], which would be expected, according to the Randle cycle [234], to inhibit PDH activity and consequently glucose oxidation. Therefore, it is likely that the observed defects in glucose oxidation and PDH activity are acquired secondary to increased FFA oxidation and feedback inhibition of PDH by elevated intracellular levels of acetyl-CoA and reduced availability of NAD. Consistent with this scenario, the rates of basal and insulin-stimulated glucose oxidation are not reduced in the normal glucose-tolerant offspring of two diabetic parents and in the first-degree relatives of type 2 diabetic subjects; whereas they are decreased in overtly diabetic subjects.

5. Summary

In summary, a defect in the insulin signaling cascade at the level of IRS-1 is likely the primady defect that leads to insulin resistance in skeletal muscle. Other defects in the insulin signaling pathway, for example, diminished insulin binding, when present, are modest and secondary to downregulation of the insulin receptor by chronic hyperinsulinemia. In insulin resistant individuals with overt hyperglycemia, for example, T2DM, a number of postbinding defects have been demonstrated, including reduced insulin receptor tyrosine kinase activity and altered insulin signal transduction, decreased glucose transport, diminished glucose phosphorylation, and impaired glycogen synthase activity. From the quantitative standpoint, impaired glycogen synthesis represents the major pathway responsible for the insulin resistance and this defect is present long before the onset of overt diabetes, that is, in normal glucose-tolerant, insulin-resistant prediabetic subjects, and in individuals with IGT. The impairment in glycogen synthase activation is likely due to a defect in the ability of insulin to phosphorylate IRS-1, causing a reduced association of the p85 subunit of PI 3-kinase with IRS-1 and decreased activation of the enzyme PI3K.

Increased intramyocellar fat content and fatty acid metabolites, for example, FACoA and DAG, are likely to play a pivotal role in the development of insulin resistance in skeletal muscle. Through activation of serine/threonine kinases and serine phosphorylate the IRS-1, fatty acid metabolites impair IRS-1 phosphorylation by the insulin receptor and lead to the defect in insulin signaling in insulin resistant individuals. The cause for the intramycellar accumulation of fat and fat metabolites has yet to be defined. A mitochondrial defect in oxidative phosphorylation has been reported in insulin resistant individuals. However, the contriution of this mitochondrial defect to the intamyocellar fat accumulation is not yet clear.

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