HHS Public Access

Author manuscript

Diabetes. Author manuscript; available in PMC 2010 December 01.

Published in final edited form as:

Diabetes. 2006 December ; 55(Suppl 2): S9–S15. doi:10.2337/db06-S002.

Molecular Mechanisms of Insulin Resistance in Humans and Their Potential Links With Mitochondrial Dysfunction

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Abstract

Recent studies using magnetic resonance spectroscopy have shown that decreased insulin-stimulated muscle glycogen synthesis due to a defect in insulin-stimulated glucose transport activity is a major factor in the pathogenesis of type 2 diabetes. The molecular mechanism underlying defective insulin-stimulated glucose transport activity can be attributed to increases in intramyocellular lipid metabolites such as fatty acyl CoAs and diacylglycerol, which in turn activate a serine/threonine kinase cascade, thus leading to defects in insulin signaling through Ser/Thr phosphorylation of insulin receptor substrate (IRS)-1. A similar mechanism is also observed in hepatic insulin resistance associated with nonalcoholic fatty liver, which is a common feature of type 2 diabetes, where increases in hepatocellular diacylglycerol content activate protein kinase C-ε, leading to reduced insulin-stimulated tyrosine phosphorylation of IRS-2. More recently, magnetic resonance spectroscopy studies in healthy lean elderly subjects and healthy lean insulin-resistant offspring of parents with type 2 diabetes have demonstrated that reduced mitochondrial function may predispose these individuals to intramyocellular lipid accumulation and insulin resistance. Further analysis has found that the reduction in mitochondrial function in the insulin-resistant offspring can be mostly attributed to reductions in mitochondrial density. By elucidating the cellular and molecular mechanisms responsible for insulin resistance, these studies provide potential new targets for the treatment and prevention of type 2 diabetes. *Diabetes* 55 (Suppl. 2):S9–S15, 2006

Type 2 diabetes is rapidly emerging as one of the greatest global health challenges of the 21st century. The World Health Organization estimates that by the year 2030, ~366 million people will be afflicted with diabetes (1). This looming epidemic is also expected to trigger a steep rise in the complications associated with diabetes, such as ischemic heart disease, stroke, neuropathy, retinopathy, and nephropathy.

Developing better treatments and novel prevention strategies for type 2 diabetes is therefore a matter of great urgency. To accomplish this goal, it is necessary to better understand the pathogenesis of type 2 diabetes. Although the underlying cause remains unknown, recent studies have clearly demonstrated that insulin resistance plays a critical role in the

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This article is based on a presentation at a symposium. The symposium and the publication of this article were made possible by an unrestricted educational grant from Servier.

development of type 2 diabetes (2–5). This brief review will focus on recent magnetic resonance spectroscopy (MRS) studies that have shed new light on the cellular and molecular mechanisms of insulin resistance in humans.

EARLY DEFECTS IN THE PATHOGENESIS OF TYPE 2 DIABETES

Over the past 2 decades, our group has extensively used in vivo MRS to noninvasively probe the cellular and molecular mechanisms of insulin resistance in humans. This technique is ideal for human studies, since it is capable of monitoring particular intracellular metabolite concentrations and kinetics noninvasively and in real time (6–22). Furthermore, in contrast to most other comparable imaging modalities such as positron emission tomography, MRS accomplishes this without the use of ionizing radiation.

Applying ^{13}C MRS to examine rates of insulin-stimulated muscle glycogen synthesis in humans, we were able to demonstrate that skeletal muscle accounts for the majority of insulin-stimulated glucose uptake in both patients with type 2 diabetes as well as age- and weight-matched nondiabetic volunteers (6). Furthermore, we found that the rate of insulin-stimulated muscle glycogen synthesis was decreased by over 50% in patients with type 2 diabetes and that this was the major factor responsible for their insulin resistance under these hyperinsulinemic (~80 μU/ ml)-hyperglycemic (10 mmol/l) clamp conditions (6). To determine the rate-controlling step responsible for this reduced insulin-stimulated rate of muscle glycogen synthesis, we applied ^{13}C and ^{31}P MRS to monitor intracellular glucose, glucose-6-phosphate concentrations, and intramuscular glycogen synthesis under similar conditions (7,8). Using this multinuclear MRS approach, we were able to determine that glucose transport was the rate-controlling step for insulin-stimulated muscle glycogen synthesis in type 2 diabetes, rather than hexokinase. Therefore, glucose transport represents the best target to correct insulin resistance in skeletal muscle in patients with type 2 diabetes. The corollary to these findings is that hexokinase and glycogen synthase activators are probably not very good targets to reverse insulin resistance in skeletal muscle. Indeed, mice with overexpression of hexokinase in skeletal muscle (23) and rats treated with a glycogen synthase kinase-3 inhibitor to activate glycogen synthase (24) were not protected from fat-induced insulin resistance in skeletal muscle.

To gain further insights into the pathogenesis of insulin resistance in skeletal muscle, we examined insulin-stimulated muscle glucose uptake in young lean nonsmoking insulin-resistant offspring of parents with type 2 diabetes. Warram et al. (2) previously demonstrated that these individuals have a high predisposition for developing type 2 diabetes and that insulin resistance is the best predictor for them developing type 2 diabetes. The advantage of studying this cohort of individuals is that they have none of the other confounding factors that may contribute to insulin resistance, such as obesity or hyperglycemia, and the pathogenesis of type 2 diabetes can be examined at its earliest time points. Using similar $^{13}C/^{31}P$ MRS methods, we found that, like their parents with type 2 diabetes, defective insulin-stimulated muscle glycogen synthesis due to defects in insulin-stimulated glucose transport/ phosphorylation was the major factor responsible for their skeletal muscle insulin resistance (9,10). These data suggest that reduced insulin-stimulated glucose

transport/ phosphorylation activity is a very early event in the pathogenesis of type 2 diabetes.

To search for mechanisms responsible for reduced insulin-stimulated glucose transport/ phosphorylation activity, we screened young lean offspring of type 2 diabetic parents and found that fasting plasma fatty acid concentrations were the best predictor for insulin resistance in this otherwise young healthy cohort (25). Subsequently, we and others have found that intramyocellular lipid content assessed by ^{1}H MRS was an even better predictor for insulin resistance in skeletal muscle in both adults (11–13) and children (14). These data are consistent with a previous muscle punch biopsy study in Pima Indians that found a strong relationship between insulin resistance and intramuscular triglyceride content (26).

The mechanism of fat-induced insulin resistance in skeletal muscle has now been mostly elucidated (Fig. 1) (27). Insulin binds to the α subunit of the insulin receptor and activates the tyrosine kinase in the β subunit. The tyrosine kinase phosphorylates the insulin receptor substrate (IRS) proteins, and phosphotyrosine residues on IRS proteins become good targets for the p85 regulatory subunit of phosphatidylinositol (PI) 3-kinase, which in turns catalyzes phosphatidylinositol 4,5 diphosphate into phosphatidylinositol 3,4,5 triphosphate. Downstream molecules such as phosphatidylinositol-dependent kinase (PDK) and protein kinase B (PKB [AKT]) have a pleckstrin homology domain that enables these molecules to migrate toward the plasma membrane (28). In skeletal muscle, this PI 3-kinase–AKT activation is an essential step for insulin-induced GLUT4 translocation, leading to glucose uptake (29). Our group has shown that raising plasma fatty acids in both rodents (15,30) and humans (16) abolishes insulin activation of IRS-1–associated PI 3-kinase activity in skeletal muscle where IRS-1 is most prevalent. Further studies in rodents have pinpointed this defect to a block in insulin receptor tyrosine phosphorylation of IRS-1 (31).

One possibility accounting for this insulin-signaling defect is serine phosphorylation on IRS-1 (Fig. 2). There are over 70 potential serine phosphorylation sites on IRS-1 (30–72), and in general, serine phosphorylation seems to negatively regulate IRS signaling, with a few exceptions (33, 55). Recent studies have demonstrated hyper-serine phosphorylation of IRS-1 on Ser^{302}, Ser^{307}, Ser^{612}, and Ser^{632} in several insulin-resistant rodent models (Fig. 2) (30–34), as well as in lean insulin-resistant offspring of type 2 diabetic parents (17). Furthermore, high-fat diet–induced insulin resistance was abrogated in rodent models where certain Ser/Thr kinases (c-Jun NH_2-terminal kinase [JNK], inhibitor of nuclear factor κB kinase β subunit [IKKβ], S6 kinase 1, and protein kinase C-θ) were either knocked down or pharmacologically inhibited (30,32–35). Further evidence for this hypothesis stems from recent studies in a muscle-specific triple serine to alanine mutant mouse (IRS-1 Ser → Ala^{302}, Ser → Ala^{307}, and Ser → Ala^{612}), which has been shown to be protected from high-fat diet–induced insulin resistance in vivo (36). Based on in vitro studies, serine phosphorylation may lead to dissociation between insulin receptor/IRS-1 and/or IRS-1/PI 3-kinase, preventing PI 3-kinase activation (37–40) or increasing degradation of IRS-1 (41). Taken together, these data suggest that serine phosphorylation on key residues of IRS-1 has an important role in the pathogenesis of muscle insulin resistance. The mechanism of Ser/Thr kinase activation in vivo is still not clear but appears to be secondary to intracellular increases in long-chain fatty acyl CoAs and diacylgycerol (31). Diacylglycerol is an

attractive trigger for fat-induced insulin resistance in skeletal muscle, since it has been shown to increase in muscle during both lipid infusions and fat feeding and it is a known activator of novel protein kinase C (PKC) isoforms. In this regard, PKC-θ and PKC-β and -δ have been shown to be activated during a lipid infusion in rodents (15,31) and humans (42), respectively. Furthermore PKC-θ knockout mice have been shown to be protected from fat-induced insulin resistance in skeletal muscle (30). Intracellular triglyceride (73) and ceramide (74) have also been implicated in fat-induced insulin resistance in muscle; however, recent lipid infusion studies have been able to disassociate fat-induced insulin resistance in skeletal muscle from any increases in ceramide and triglyceride, suggesting that these metabolites may represent more of a marker than the trigger for fat-induced insulin resistance (31).

Recent studies have revealed a similar mechanism for fat-induced insulin resistance in liver (Fig. 1*B*), where accumulation of intracellular lipid metabolites activate a serine kinase cascade involving PKC-ε, leading to decreased insulin receptor kinase activity resulting in *1*) lower insulin-stimulated IRS-2 tyrosine phosphorylation, *2*) lower IRS-2–associated PI 3-kinase activity, and 3) lower AKT2 activity (43). These fat-induced defects in insulin signaling in turn result in reduced insulin stimulation of glycogen synthase activity, resulting in decreased insulin-stimulated hepatic glucose uptake and reduced insulin stimulation of hepatic glucose production. Furthermore, reduced activity of AKT2 results in decreased phosphorylation of forkhead box protein O (FOXO), allowing it to enter the nucleus and activate the transcription of the rate-controlling enzymes of gluconeogenesis (phospho-enolpyruvate carboxykinase, glucose-6-phosphate phos-phatase). Increased gluconeogenesis further exacerbates hepatic insulin resistance and results in fasting hyperglycemia (43,75,76). Mitochondrial glycerol-3-phosphate acyltransferase (mtGPAT) is a key enzyme in de novo fat synthesis in liver, and recent studies in mtGPAT knockout mice have clearly implicated intracellular accumulation of diacylglycerol in triggering fat-induced insulin resistance in liver through activation of PKC-ε (77). These data have important implications for the development of novel therapeutic agents to reverse and prevent hepatic insulin resistance associated with nonalcoholic fatty liver and type 2 diabetes (18).

INSULIN RESISTANCE AND MITOCHONDRIAL DYSFUNCTION

It has been known for many years that severe mitochondrial dysfunction can result in diabetes that is typically associated with severe β-cell dysfunction and neurological abnormalities (78–80). More recent MRS studies, measuring rates of mitochondrial oxidative phosphorylation activity in skeletal muscle, have found that more subtle defects in mitochondrial function may play an important role in the pathogenesis of type 2 diabetes. Using $^{13}C/^{31}P$ MRS, we directly assessed mitochondrial oxidative and phosphorylation activity in the healthy lean elderly volunteers with severe muscle insulin resistance and found that they have an ~40% reduction in rates of oxidative phosphorylation activity associated with increased intramyo-cellular and intrahepatic lipid content compared with BMI activity–matched young control subjects (19). This study suggests that an acquired loss of mitochondrial function associated with aging predisposes elderly subjects to intramyocellular lipid accumulation, which results in insulin resistance through the mechanisms described earlier (Fig. 1).

Using a similar approach, we used ^{31}P MRS to examine mitochondrial function in young lean insulin-resistant offspring of parents with type 2 diabetes (20). Insulin resistance in these individuals could be attributed to severe defects in insulin-stimulated muscle glucose metabolism, which were associated with an ~80% increase in intramyocellular lipid content assessed by ^{1}H MRS. Furthermore, these changes were associated with a 30% reduction in rates of mitochondrial ATP production compared with age-, weight-, and activity-matched insulin-sensitive control subjects (20). To further examine the mechanism responsible for reduced mitochondrial activity in these subjects, we recently assessed mitochondrial density by electron microscopy and found that mitochondrial density was reduced by 38% in the insulin-resistant offspring (17). Taken together, these data suggest that the reduced mitochondrial function observed in the insulin-resistant offspring may be secondary to reduced mitochondrial content and is consistent with previous studies demonstrating lower mitochondrial density in patients with type 2 diabetes (81). Ritov et al. (82) also reported the subsarcolemmal fraction was especially impaired in obese and type 2 diabetic subjects. In agreement with the finding of decreased mitochondrial density using electron microscopy, we also measured the expression of several mitochondrial proteins and found mitochondrial cytochrome-*c* oxidase I to be reduced by ~50% in insulin-resistant offspring and a tendency for succinate dehydrogenase and pyruvate dehydrogenase to be reduced by a similar amount (17). Taken together, these data suggest that insulin-resistant offspring of patients with type 2 diabetes may have an inherited condition that causes a reduction in mitochondrial content in skeletal muscle, which in turn may be responsible for the reduced rates of mitochondrial oxidative phosphorylation predisposing them to intramyocellular lipid accumulation.

MECHANISM OF REDUCED MITOCHONDRIAL BIOGENESIS

An important remaining question is the identification of the factors responsible for the lower mitochondrial density in skeletal muscle of the insulin-resistant offspring. Peroxisome proliferator–activated receptor-γ coactivator (PGC)-1α and PGC-1β are transcriptional factor co-activators that regulate mitochondrial biogenesis and potential candidates in this regard (Fig. 3). In addition AMP kinase, which is activated during exercise and ischemia by a reduction in the ATP/AMP ratio, has been shown to be an important regulator of mitochondrial biogenesis, mediating its effects through MEF2- and CREB-mediated increased PGC-1α expression (83–86). Extracellular stimuli such as cold, thyroid hormone, and exercise stimulate mitochondrial biogenesis through PGC-1 in brown fat and skeletal muscle. Increased PGC-1 protein expression leads to increases in the target genes, including nuclear respiratory factor (NRF)-1. NRF-1 is a transcription factor stimulating many nuclear-encoded mitochondrial genes such as OXPHOS genes and also mitochondrial transcription factor A (mtTFA), a key transcriptional factor for the mitochondrial genome. mtTFA can bind to the D-loop of the mitochondrial genome and increase transcription of mitochondrial genes and replication of mitochondrial DNA (Fig. 3) (87).

Two recent DNA microarray studies found a coordinated reduction in the expression of genes encoded by PGC-1α in the skeletal muscle of type 2 diabetic patients (88,89) and nondiabetic subjects with a family history of diabetes (89). However, in contrast to these studies, we did not observe any difference in the mRNA expression levels for either PGC-1α or PGC-1β in the insulin-resistant offspring. Furthermore, we also examined the mRNA

expression of NRF-1, NRF-2, and mtTFA, which PGC-1α and PGC-1β direct to initiate mitochondrial biogenesis. In contrast to these previous studies, we found no difference in the level of mRNA expression of these factors between the groups (17). These data suggest that there are other unknown factors involved in the regulation of mitochondrial biogenesis responsible for the reduced skeletal muscle mitochondrial content in lean insulin-resistant offspring. The reason for the disparity between our findings and previous studies are not clear but may be related to the fact that our insulin-resistant offspring were young, lean, and healthy, in contrast to other studies where subjects were older, obese, and diabetic (88,89), or in the case of the first-degree relatives, overweight (89). Indeed, a recent study by Ling et al. (90) demonstrated an age-dependent decrease in muscle gene expression of PGC-1α and PGC-1β in young and elderly dizygotic and monozygotic twins without known diabetes. These data suggest that age-related changes in PGC-1 can occur and may account for the disparity between previous reports and our results.

Recent ^{31}P MRS studies have also demonstrated that insulin is an important regulator of mitochondrial ATP synthesis in skeletal muscle of healthy subjects and that insulin-stimulated ATP synthesis is markedly decreased in the muscle of insulin-resistant offspring of parents with type 2 diabetes (21). These changes were associated with parallel reductions in inorganic phosphorus transport into skeletal muscle in the insulin-resistant offspring, suggesting that these two processes may be coupled. However, in contrast to the potentially inherited defects in mitochondrial biogenesis observed in these individuals, it is likely that these defects in insulin-stimulated ATP synthesis and inorganic phosphorus transport may be explained by acquired defects in insulin signaling due to the accumulation of intracellular lipids, as described above. Consistent with this hypothesis are recent studies by Roden and coworkers (22) demonstrating defects in insulin-stimulated ATP synthesis during a lipid infusion in healthy volunteers.

CONCLUSION

In summary, recent MRS studies have revealed important new insights into the pathogenesis of insulin resistance and type 2 diabetes. Specifically, they have identified defects in insulin activation of glucose transport activity as the rate-controlling step responsible for fat-induced insulin resistance in skeletal muscle and have shown that increases in intramyocellular lipid metabolites play a key role in triggering insulin resistance through activation of a serine kinase cascade leading to reduced insulin stimulation of IRS-1 tyrosine phosphorylation. Parallel studies have revealed a similar mechanism for fat-induced hepatic insulin resistance associated with nonalcoholic fatty liver, where increases in hepatocellular diacylglycerol lead to activation of protein kinase C-ε, leading to reduced insulin stimulation of IRS-2 tyrosine phosphorylation resulting in reduced insulin stimulation of glycogen synthase activation and decreased phosphorylation of forkhead box protein O (FOXO), leading to increased hepatic gluconeo-genesis. Finally, recent MRS studies have implicated defects in mitochondrial oxidative phosphorylation activity in causing insulin resistance in both the elderly and young lean insulin-resistant offspring of parents with type 2 diabetes. By elucidating the cellular and molecular mechanisms responsible for insulin resistance, these studies provide potential new targets for the treatment and prevention of type 2 diabetes.

ACKNOWLEDGMENTS

This work was supported by grants from the U.S. Public Health Service (P01 DK-063229, R01 AG-23686, P30 DK-45735, R01 DK-40936, U24 DK-59635, and M01 RR-00125). G.I.S. is the recipient of a Distinguished Clinical Scientist Award from the American Diabetes Association.

Glossary

CREB	cAMP response element binding protein
IRS	insulin receptor substrate
MEF2	myocyte enhancer factor 2
MRS	magnetic resonance spectroscopy
mtTFA	mitochondrial transcription factor A
NRF	nuclear respiratory factor
PGC	peroxisome proliferator–activated receptor-γ coactivator
PI	phosphatidylinositol
PKC	protein kinase C

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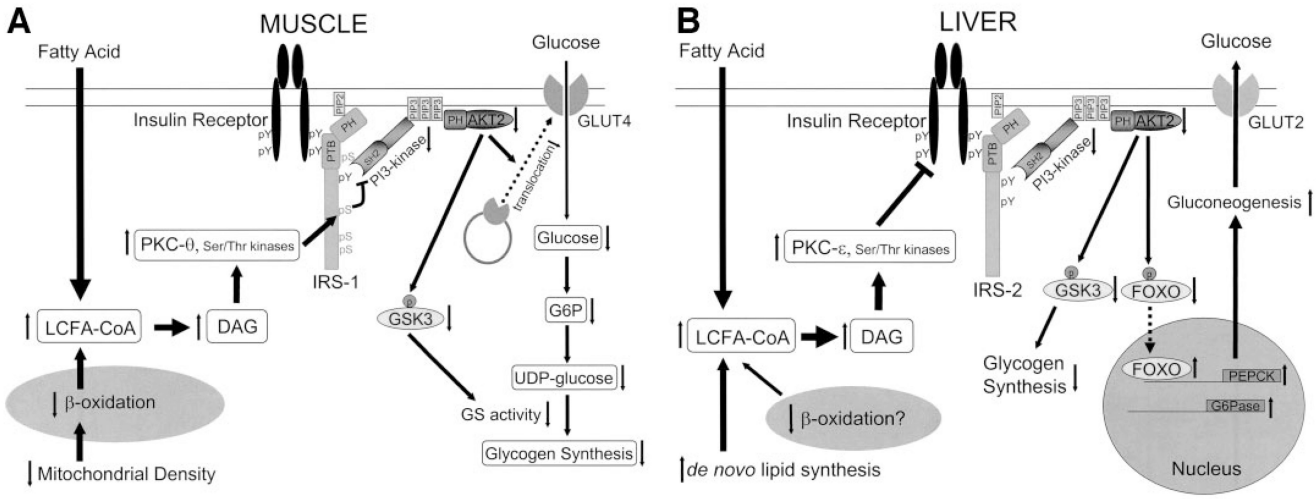

FIG. 1.

The molecular mechanism of fat-induced insulin resistance in skeletal muscle (*A*) and liver (*B*). *A*: Increases in intramyocellular fatty acyl CoAs and diacylglycerol due to increased delivery from plasma and/or reduced β-oxidation due to mitochondrial dysfunction activate serine/threonine kinases such as protein kinase C (PKC-θ rodents, PKC-β and -δ humans) in skeletal muscle. The activated kinases phosphorylate serine residues on IRS-1 and inhibit insulin-induced PI 3-kinase activity, resulting in reduced insulin-stimulated AKT2 activity. Lowered AKT2 activity fails to activate GLUT4 translocation, and other downstream AKT2-dependent events, and consequently insulin-induced glucose uptake is reduced. *B:* Increases in hepatic diacylglycerol content due to increased delivery of fatty acids from the plasma and/or increased de novo lipid synthesis and/or reduced β-oxidation activate protein kinase C-ε (and potentially other serine kinases), leading to reduced insulin receptor kinase activity and reduced IRS-2 tyrosine phosphorylation, resulting in reduced insulin stimulation of glycogen synthase activation and decreased phosphorylation of forkhead box protein O (FOXO), leading to increased hepatic gluconeogenesis. DAG, diacylglycerol; PTB, phosphotyrosine binding domain; PH, pleckstrin homology domain; SH2, src homology domain; GSK3, glycogen synthase kinase-3.

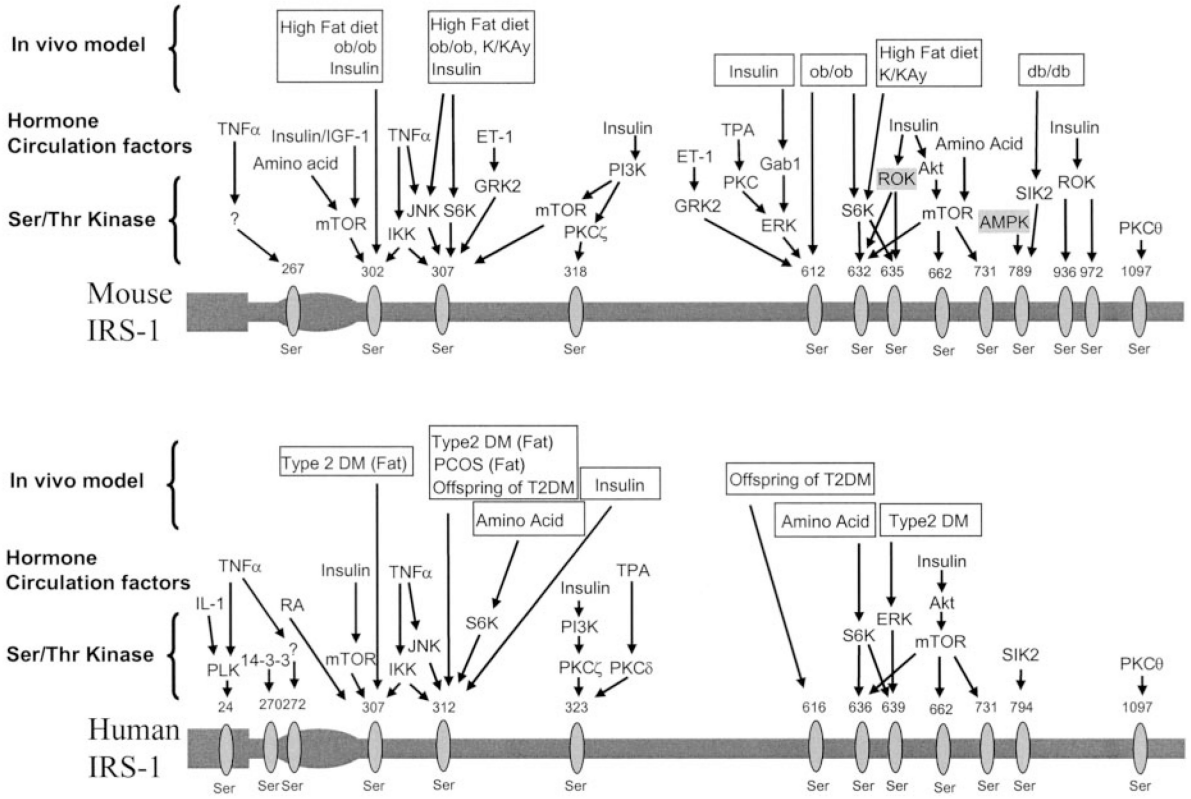

FIG. 2.

Serine/threonine phosphorylation on IRS-1. IRS-1 contains ~70 potential serine/threonine sites. Shown here are the individual residues on mouse IRS-1 and human IRS-1. In addition, the serine/threonine kinases, hormone, and circulating factors that lead to the phosphorylation on IRS-1 at specific sites are shown. The top row illustrates reported animal or human models in which these phosphorylation sites have been confirmed in vivo. Each residue is drawn corresponding to the position based on homology between the humans and mice. PCOS, polycystic ovarian syndrome; T2DM, type 2 diabetes.

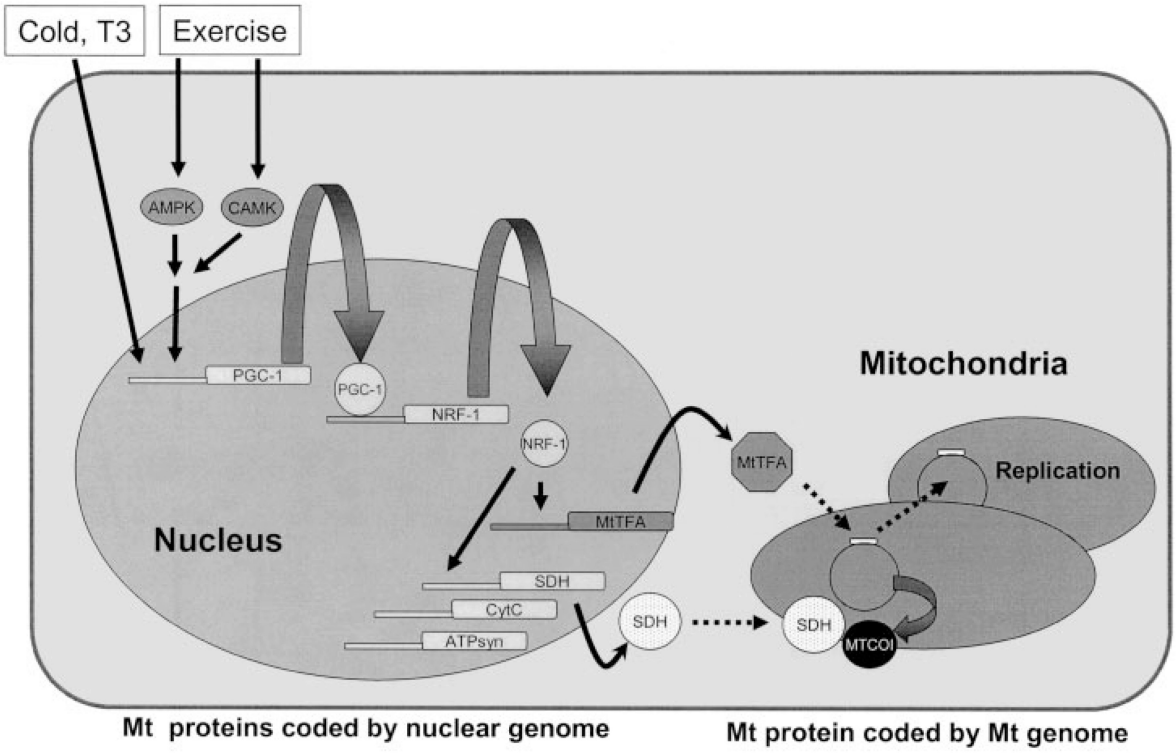

FIG. 3.

The molecular mechanism of mitochondrial (Mt) biogenesis. Mitochondria have their own genome, which encodes 13 proteins, 2 rRNAs, and 22 tRNAs. It is also known that most of the mitochondrial proteins are encoded by the nuclear genome and translated proteins are transported into mitochondria. Extracellular stimuli induce mitochondrial biogenesis through PGC-1 in brown fat and skeletal muscle. Increased PGC-1 protein expression leads to increases in the expression of its target genes, including NRF-1. NRF-1 is a transcription factor stimulating many nuclear-encoded mitochondrial genes such as OXPHOS genes and mtTFA, a key transcriptional factor for the mitochondrial genome. mtTFA can bind to the D-loop of the mitochondrial genome and increase transcription of mitochondrial genes and replication of mitochondrial DNA. ATPsyn, ATP synthase; CytC, cytochrome C; MTCOI, mitochondrial cytochrome c oxidase subunit 1.