Increased basal level of Akt-dependent insulin signaling may be responsible for the development of insulin resistance

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1Translational Biology, The Hamner Institutes for Health Sciences, Research Triangle Park; 2Department of Internal Medicine (Endocrinology), Duke University Health System, Durham, North Carolina; 3The First Affiliated Hospital of the University of South China, Hengyang, Hunan, People’s Republic of China; and 4Department of Medicine (Endocrinology), University of Virginia, Charlottesville, Virginia

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Liu HY, Hong T, Wen GB, Han J, Zuo D, Liu Z, Cao W. Increased basal level of Akt-dependent insulin signaling may be responsible for the development of insulin resistance. Am J Physiol Endocrinol Metab 297: E898–E906, 2009. First published July 28, 2009; doi:10.1152/ajpendo.00374.2009.—A majority of subjects with insulin resistance and hyperinsulinemia can maintain their blood glucose levels normal for the whole life presumably through protein kinase B (Akt)-dependent insulin signaling. In this study, we found that the basal Akt phosphorylation level was increased in liver and gastrocnemius under the high-fat diet (HFD). Levels of mitochondrial DNA and expression of some mitochondrial-associated genes were decreased by the HFD primarily in liver. Triglyceride content was increased in both liver and gastrocnemius. Insulin sensitivity was decreased by the HFD. All of these changes were largely or completely reversed by treatment of animals with the phosphatidylinositol 3-kinase inhibitor LY-294002. Together, our results indicate that increased basal Akt-dependent insulin signaling suppresses mitochondrial function, increases ectopic fat accumulation, induces increased basal Akt-dependent insulin signaling suppresses mitochondrial function, increases ectopic fat accumulation, induces oxidative stress, and desensitizes insulin signaling in subjects with insulin resistance and hyperinsulinemia.

protein kinase B

INSULIN RESISTANCE IS CLEARLY ASSOCIATED with the positive energy imbalance due to overeating and/or lack of physical activity. The primary player that converts the positive energy imbalance into insulin resistance has been intensively investigated, but remains to be established. The excess calories are stored as fat in the adipose tissue and/or ectopically in nonadipose tissues. Ectopic fat accumulation in liver and skeletal muscles plays a critical role in the development of insulin resistance, which may not occur in the absence of ectopic fat accumulation no matter how severe obesity is (20). Insulin resistance may develop whenever ectopic fat accumulation occurs no matter how small animals are (48). Nutrients cannot be converted into fat effectively, even in the presence of excessive food intake when the insulin receptor is fat-specifically knocked out (19). In the absence of insulin, the accumulated fat is “melted” away through increased lipolysis and fat oxidation and decreased lipogenesis. Thus insulin is required for converting the excess calories into fat and keeping fat (including ectopic fat accumulation).

Fat and its metabolites [free fatty acid (FFA), diacylglycerol, and ceramide] can induce insulin resistance through various signaling pathways such as mitogen-activated protein kinase (c-Jun NH2-terminal kinase, extracellular signal-regulated kinase 1/2, and p38), protein kinase C, nuclear factor-κB, and endoplasmic reticulum stress (13, 18, 29, 50, 53). However, induction of insulin resistance in mice by obesity or the high-fat diet (HFD) is prevented when production of mitochondrial (mt)-derived reactive oxygen species (ROS) is blocked (16, 22, 37). Induction of insulin resistance in cultured cells is also blocked when mtROS is scavenged (16). Oxidative stress has been shown to be a precursor of insulin resistance (31). Without oxidative stress, insulin resistance may not occur, even in the presence of ectopic fat accumulation (33, 39). It is noteworthy that ROS is capable of activating all of the above-described signaling pathways that are known to be involved in insulin resistance (10). Therefore, mtROS plays a critical/necessary role in the development of insulin resistance.

It is known that availability of ADP, a necessary substrate for ATP synthesis, is a rate-limiting factor of mtROS production. For example, addition of ADP to isolated mitochondria can basically eliminate mtROS production (28). ADP is transported into mitochondrial matrix through the adenine nucleotide translocator (Ant), which is located at the mitochondrial inner membrane (47). Fat accumulation is capable of affecting ADP transport through Ant. First, the overexpanded adipose tissue secretes inflammatory cytokines such as tumor necrosis factor-α, which is a known inhibitor of Ant (52). Second, accumulated lipids such as FFA are activated as long-chain acyl-CoA, which is another known inhibitor of the Ant-dependent transport of ADP (25, 35, 40, 41, 49).

mtROS production is also influenced by mitochondrial capacity. Mitochondrial membrane potential and mtROS production is bound to increase when the even perfectly normal or increased mitochondrial mass is overloaded by NADH from excess nutrients because ADP availability for converting proton gradient into ATP is not unlimited, in particular, when ATP is not even consumed and turned into ADP due to lack of physical activity (5). We have recently shown that prolonged exposure of hepatocytes to insulin reduces mitochondrial mass through the protein kinase B (Akt)-dependent insulin signaling (27). In this study, we tested the hypothesis that excess exposure to the Akt-dependent insulin signaling promotes ectopic...
fat accumulation while inhibiting mitochondrial production, leading to oxidative stress and insulin resistance.

MATERIALS AND METHODS

Reagents. Antibodies against total/phospho-Akt were from Cell Signaling Technology (Danvers, MA). The antibodies against peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α (H-300), nuclear respiratory factor 1 (NRF-1; catalog no. sc-33771), and TFAM (catalog no. sc-23588) were all from Santa Cruz Biotechnology (Santa Cruz, CA). The anti-PGC-1β antibody (914-1023) was purchased from Abnova (Walnut, CA). LY-294002 was from Calbiochem (San Diego, CA). The GSH/GSSG-412 assay kit was from Bioxytech (Foster City, CA). Blood glucose concentrations were measured by using the Breese 2 glucose meter (Bayer HealthCare). Plasma insulin levels were measured with a Linco insulin enzyme-linked immunosorbent assay kit. Protein assay kits were from Bio-Rad (Hercules, CA). Triglyceride (TG) content was measured by using a kit from Sigma. Other materials were all obtained commercially and are of analytical quality.

Animal experiments. Animals were housed under the usual day (12 h daylight) and night (12 h darkness) circadian and fed ad libitum. 1) To examine the effect of the HFD on mitochondrial biogenesis, ectopic fat accumulation, and oxidative stress, C57BL/6 (B6) mice were fed with either normal rodent chow diet (ND) or HFD (Research Diets no. D12330: 58.0 kcal/100 kcal fat, 16.0 kcal/100 kcal protein, and 26 kcal/100 kcal carbohydrate) for 8 wk (7, 44, 51). 2) To study the effect of insulin signaling blockade, B6 mice were similarly fed with either ND or HFD as described above for 4 wk. During the last week of the ND or HFD, some mice were administered the phosphoinositide (PI) 3-kinase inhibitor LY-294002 (10 mg/kg ip, once a day at 9:00 A.M. × 7 days). All animal studies were approved by the Institutional Animal Care and Use Committee of The Hamner Institutes for Health Sciences and fully complied with the guidelines from the National Institutes for Health.

Calculation of insulin sensitivity. Insulin sensitivity was evaluated by measuring levels of blood glucose and plasma insulin and calculated as previously described (24): quantitative insulin sensitivity check index \( \text{QUICKI} = \frac{1}{\log(I_0) + \log(G_0)} \) and homeostasis model assessment \( \text{HOMA} = \frac{(G_0 \times I_0)}{22.5} \), where \( I_0 \) is fasting insulin (μU/ml) and \( G_0 \) is fasting glucose (mg/dl) for QUICKI. For HOMA \( G_0 = \text{mmol/l} \).

Measurement of mitochondrial DNA. Total DNA was extracted from tissues or cultured cells using the Qiagen DNA extraction kit. DNA concentrations were determined by the NanoDrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE). Total DNA (1 ng) was used to determine the ratio of mitochondrial cyclooxygenase...
Numerous studies have demonstrated that mitochondrial dysfunction, ectopic lipid accumulation, and oxidative stress have been implicated in insulin resistance (2, 10, 36). Here, we addressed the role of basal Akt-dependent insulin signaling in mitochondria in HFD-induced insulin resistance and hyperinsulinemia. To this end, we fed B6 mice with the HFD for 8 wk, followed by evaluation of insulin sensitivity, mitochondrial production/biogenesis, TG content, and oxidative stress in liver and skeletal muscle. As shown in Fig. 1A, fasting blood glucose levels were not changed while plasma insulin level was significantly increased by the HFD, indicating insulin sensitivity was decreased (Fig. 1A). Importantly, basal Akt phosphorylation was significantly elevated in both liver and skeletal muscle gastrocnemius (Fig. 1C), suggesting that the basal level of Akt-dependent insulin signaling is increased in the presence of insulin resistance and hyperinsulinemia.

Next, mitochondrial production was evaluated by measuring mitochondrial DNA (mtDNA) and expression of genes involved in mitochondrial production. As shown in Fig. 2A, levels of mtDNA were decreased in both liver and gastrocnemius. 

### RESULTS

Mitochondrial production/biogenesis is decreased while ectopic fat accumulation and oxidative stress are increased in the presence of insulin resistance. Basal Akt phosphorylation is increased in mice under the HFD, and is associated with decreased mitochondrial production and increased ectopic fat accumulation and oxidative stress. HFD is known to induce obesity and insulin resistance (11, 43). What remains unestablished is the primary in vivo mediator of insulin resistance. Because roles for mitochondrial dysfunction, ectopic lipid accumulation, and oxidative stress have been implicated in insulin resistance (2, 10, 36), we fed B6 mice with the HFD for 8 wk, followed by evaluation of insulin sensitivity, mitochondrial production/biogenesis, TG content, and oxidative stress in liver and skeletal muscle. As shown in Fig. 1A, fasting blood glucose levels were not changed while plasma insulin level was significantly increased by the HFD, indicating insulin sensitivity was decreased (Fig. 1B). Importantly, basal Akt phosphorylation was significantly elevated in both liver and skeletal muscle gastrocnemius (Fig. 1C), suggesting that the basal level of Akt-dependent insulin signaling is increased in the presence of insulin resistance and hyperinsulinemia.

### Table 1. Transcript levels of mitochondrial-related genes in liver and gastrocnemius of mice described in Fig. 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>HFD vs. ND (fold)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATP5a1</td>
<td>0.868±0.0813</td>
<td>0.1918</td>
</tr>
<tr>
<td>COXIV</td>
<td>0.688±0.0928</td>
<td>0.0281*</td>
</tr>
<tr>
<td>Cyc</td>
<td>0.662±0.1075</td>
<td>0.0333*</td>
</tr>
<tr>
<td>ERRα</td>
<td>0.530±0.0433</td>
<td>0.0606</td>
</tr>
<tr>
<td>Nduv1</td>
<td>0.613±0.0763</td>
<td>0.0011†</td>
</tr>
<tr>
<td>NRF1</td>
<td>0.822±0.0552</td>
<td>0.0963</td>
</tr>
<tr>
<td>PGC-1α</td>
<td>0.980±0.1124</td>
<td>0.8932</td>
</tr>
<tr>
<td>PGC-1β</td>
<td>1.350±0.1491</td>
<td>0.0458*</td>
</tr>
<tr>
<td>COXI</td>
<td>0.747±0.0667</td>
<td>0.2668</td>
</tr>
<tr>
<td>Sdhc</td>
<td>0.663±0.0747</td>
<td>0.0233*</td>
</tr>
<tr>
<td>Tfam</td>
<td>0.580±0.0463</td>
<td>0.0114*</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATP5a1</td>
<td>1.121±0.1112</td>
<td>0.4820</td>
</tr>
<tr>
<td>COXIV</td>
<td>0.947±0.1579</td>
<td>0.7860</td>
</tr>
<tr>
<td>Cyc</td>
<td>0.792±0.0811</td>
<td>0.3820</td>
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<tr>
<td>ERRα</td>
<td>1.774±0.2453</td>
<td>0.2800</td>
</tr>
<tr>
<td>Nduv1</td>
<td>1.013±0.2046</td>
<td>0.9692</td>
</tr>
<tr>
<td>NRF1</td>
<td>1.100±0.0965</td>
<td>0.4352</td>
</tr>
<tr>
<td>PGC-1α</td>
<td>0.264±0.1007</td>
<td>0.1388</td>
</tr>
<tr>
<td>PGC-1β</td>
<td>1.619±0.3582</td>
<td>0.1364</td>
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<tr>
<td>COXI</td>
<td>0.903±0.1679</td>
<td>0.7101</td>
</tr>
<tr>
<td>Sdhc</td>
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<td>0.6686</td>
</tr>
<tr>
<td>Tfam</td>
<td>0.974±0.2193</td>
<td>0.9235</td>
</tr>
</tbody>
</table>

Values are means ± SE. HFD, high-fat diet; ND, normal diet. See Supplemental Table 2 for abbreviations. *P < 0.05 and †P < 0.01.
mius of mice under the HFD. Transcript levels of some key genes involved in mitochondrial production such as COXIV, NDAH dehydrogenase, and succinate dehydrogenase were significantly decreased in liver, although not in gastrocnemius (Table 1). Changes in levels of PGC-1α and β seemed complicated. Levels of PGC-1α transcripts tended to decrease in gastrocnemius, but not altered in liver by the HFD, with neither of them reaching statistical significance (Table 1). However, PGC-1α protein level was decreased in both liver and gastrocnemius by the HFD (Fig. 3A). In contrast, levels of PGC-1β transcripts and protein were increased in liver and gastrocnemius (Table 1 and Fig. 3B). No significant change in levels of NRF-1 transcripts was observed. However, NRF-1 protein was decreased in liver (Fig. 3C). Levels of both transcripts and protein of mitochondrial transcription factor A (Tfam) were decreased in liver (Table 1 and Fig. 3C). Because Tfam is essential for mitochondrial production (23), these results show that mitochondrial production is reduced by the HFD.

Because insulin is the primary regulator of lipogenesis and fat oxidation, lipid content in liver and gastrocnemius and transcript levels of some key genes, which are involved in fatty acid β-oxidation and lipogenesis, were measured. As shown in Fig. 2B, TG content was increased in both liver and gastrocnemius by the HFD. Transcript levels of some key genes involved in fat oxidation such as medium-chain acyl-CoA dehydrogenase and long-chain acyl-CoA dehydrogenase were significantly decreased in liver (P < 0.05), but were not altered significantly in gastrocnemius (Table 2). Transcript levels of some key genes involved in lipogenesis, including fatty acid synthase, sterol regulatory element-binding protein-2, and HMG-CoA reductase were increased significantly in liver, but the increase did not reach statistical significance in gastrocnemius (Table 3). These results indicate that fat accumulation is increased in both liver and skeletal muscles through inhibition of fat oxidation and stimulation of lipogenesis under the HFD.

To evaluate oxidative stress, we quantified the ratio between the reduced and oxidized glutathione (GSH/GSSG) and transcript levels of some oxidation-responsive genes. As shown in Fig. 2C, the GSH-to-GSSG ratio was decreased in both liver and gastrocnemius by the HFD. Transcript levels of some oxidation-responsive genes, including glutathione peroxidase, catalase, and heme oxygenase 1, were increased in liver (P < 0.05) or tended to increase in gastrocnemius (P > 0.05) (Table 4).

![Fig. 3. Peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α protein level is decreased while PGC-1β protein level is increased in the presence of insulin resistance and hyperinsulinemia. PGC-1α (A) and PGC-1β (B) protein were detected by immunoblotting and quantified in liver and gastrocnemius of mice described in Fig. 1. C: protein levels of nuclear respiratory factor (NRF)-1 and transcription factor A (TFAM) in liver were measured by immunoblotting. β-Actin protein levels were also evaluated by immunoblotting and quantified. Results represent means ± SE of mice in each group. *P < 0.05 and ***P < 0.001 vs. ND.](http://ajpendo.physiology.org/)

**Fig. 3.** Peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α protein level is decreased while PGC-1β protein level is increased in the presence of insulin resistance and hyperinsulinemia. PGC-1α (A) and PGC-1β (B) protein were detected by immunoblotting and quantified in liver and gastrocnemius of mice described in Fig. 1. C: protein levels of nuclear respiratory factor (NRF)-1 and transcription factor A (TFAM) in liver were measured by immunoblotting. β-Actin protein levels were also evaluated by immunoblotting and quantified. Results represent means ± SE of mice in each group. *P < 0.05 and ***P < 0.001 vs. ND.
Again, as shown in Fig. 4, this decrease in oxidative stress is likely caused by fats directly. Because basal Akt phosphorylation was increased by the HFD, but blunted by the PI 3-kinase inhibitor LY-294002, which was administered during the nonfeeding phase (daytime). Levels of fasting blood glucose were not altered by either HFD or LY-294002 (Fig. 4C). However, fasting plasma insulin level was significantly increased by the HFD, and the increase was prevented by LY-294002 (Fig. 4D). As a result, the overall insulin sensitivity evaluated by HOMA and QUICKI was increased by treatment with LY-294002 during the time when insulin was not highly demanded (Fig. 4, E and F).

In the meantime, levels of mtDNA were decreased by the HFD, but the decrease was reversed by treatment with LY-294002 (Fig. 5A). Again, TG content was increased in both liver and gastrocnemius by the HFD, and the increase was largely prevented by LY-294002 (Fig. 5B). Similarly, the GSH-to-GSSG ratio was decreased by the HFD, and the decrease was significantly reversed by LY-294002 in liver (P < 0.05) and gastrocnemius (P = 0.09) (Fig. 5C). Application of LY-294002 did not alter mtDNA, TG content, or the GSH-to-GSSG ratio in mice under the ND. Together, these results show that reduction of the basal insulin signaling activity in mice under the HFD during the time when insulin is not highly demanded protects mitochondrial production, prevents ectopic fat accumulation, reduces oxidative stress, and improves insulin sensitivity.

**DISCUSSION**

Mitochondrial dysfunction and insulin resistance. Although mitochondrial dysfunction is a cardinal feature of insulin resistance (1, 47), the definition and reports about its role in the development of insulin resistance remain controversial. The basic function of mitochondria is to produce ATP. Another permanent product of mitochondrial activity is ROS. Some studies have recently shown that it may be beneficial when mitochondrial ATP production is decreased to a certain degree. For example, decreased mitochondrial ATP production by knocking out apoptosis-inducing factor (AIF) activates AMP-activated protein kinase and consequently stimulates fat oxidation, and enhances glycolysis. As a result, mice are more resistant to obesity and development of insulin resistance than normal mice (37). In contrast, decreased mitochondrial ATP production by knocking out the Frataxin gene is associated with insulin resistance and type 2 diabetes mellitus in mice.
Mutations of the Frataxin gene are associated with diabetes in humans (38). The exact reason for these opposite results remains unclear. However, it is clear that Frataxin mice have increased oxidative stress while AIF mice do not have increased oxidative stress. Therefore, the consequence of the so-called mitochondrial dysfunction is not determined by the level of ATP production, but is determined by the level of mtROS production.

Production of mtROS is influenced by the integrity of individual mitochondria. For example, mutations or deletions of certain mitochondrial proteins such as Frataxin and AIF as described above alter mtROS production and insulin sensitivity (45, 47). More importantly, mtROS production is also determined by the status of mitochondrial function. The status of mitochondria function is heavily influenced by the NADH load, which in turn is determined by the nutrient load (5, 47). When the NDAH load exceeds mitochondrial capacity, proton gradient over the mitochondrial inner membrane and mitochondrial membrane potential are increased and the electron flux in the mitochondrial respiration chain is slowed, resulting in increased ROS production and decreased insulin sensitivity.

That is why animals on the HFD may still develop insulin resistance, even in the presence of increased mitochondrial production (14). In this study, our results show that mitochondrial production is decreased by hyperinsulinemia induced by the HFD. The decreased mitochondrial production may lead to absolute or relative overloading of mitochondria by nutrients, and contribute to increased oxidative stress and insulin resistance. Besides, ectopic lipid accumulation caused by hyperinsulinemia can directly cause/aggravate mitochondrial dysfunction, oxidative stress, and insulin resistance through inhibition of the ANT-dependent ADP transport as described in the Introduction.

Role of insulin in mitochondrial production/biogenesis. The effect of insulin on mitochondrial production is complex, and reports about it are conflicting. Theoretically, insulin can both promote and inhibit mitochondrial production. Insulin is a potent stimulator of protein synthesis, i.e., insulin can promote mitochondrial protein synthesis through stimulation of protein translation. In contrast, insulin may inhibit transcription of many mitochondrion-related genes as described in this study. It is known that PGC-1α is a potent coactivator of the central...
transcription factor NRF-1 in regulating transcription of the Tfam gene, which controls the sole mitochondrial DNA promoter and replication of mitochondrial DNA (23). Insulin can inhibit the function of PGC-1α through at least two different ways. First, insulin has been shown to inhibit expression of the PGC-1α gene in adipocytes and skeletal muscle (34, 42). In this study, we show that levels of PGC-1α protein are decreased in both liver and gastrocnemius of mice with hyperinsulinemia although mRNA level was not altered significantly. In contrast, both mRNA and protein of PGC-1β were increased by the HFD in both liver and gastrocnemius. Second, it has been shown that insulin can inhibit the function of PGC-1α through protein phosphorylation (26). Importantly, our results show that levels of both mRNA and protein of the Tfam gene were decreased in the liver in the presence of hyperinsulinemia. Because Tfam is downstream of NRF-1 and PGC-1 and is necessary for mitochondrial DNA replication and expression of the genes encoded by the mitochondrial DNA (23), insulin appears to suppress mitochondrial production by inhibiting functions of PGC-1α and Tfam. It is noteworthy that the expression level of PGC-1β is increased in the presence of insulin resistance/hyperinsulinemia induced by the HFD. This suggests at least two possibilities. First, PGC-1β cannot replace PGC-1α in terms of promoting mitochondrial production in liver. Second, when Tfam is insufficient, mitochondrial production is reduced no matter how much PGC-1 (α and β) is available. Thus insulin suppression of mitochondrial production is complicated and demands further studies.

Role of excessive exposure to insulin in the development of insulin resistance and its associated health problems. Plasma insulin level is increased after each food intake and returns to a low basal level within hours when all nutrients leave the circulation. The time with increased plasma insulin level varies with the amount and components of ingested food. The more food is ingested, the longer it takes to digest and absorb the food, i.e., nutrients stay in the blood for a longer period when
overeating occurs. As a result, it takes a longer time for insulin to drive nutrients away from the blood into tissues to be stored primarily as fat. During the early stage of overeating, increased levels of nutrients and plasma insulin after each meal may not last long enough to cause insulin resistance. Therefore, insulin efficiently converts excessive calories into fat storage initially in adipose tissue and then in nonadipose tissues such as liver and skeletal muscles. It is noteworthy that, in the absence of insulin receptor, nutrients cannot be converted into fat storage efficiently (6, 19.) When fat accumulation in adipose tissue and nonadipose tissues reaches a certain level, lipids and adipokines may induce insulin resistance and hyperinsulinemia through mtROS, leading to increased basal Akt-dependent insulin signaling as shown in this study. When the increased basal Akt-dependent signaling works hard to maintain the blood glucose level normal, it may aggravate ubiquitous fat accumulation, suppress mitochondrial production, and enhance oxidative stress and insulin resistance. S6 kinase (S6K) is an established substrate of mammalian target of rapamycin, which in turn is activated by Akt, and has been shown to induce insulin resistance through serine phosphorylation of insulin receptor substrate (IRS)-1 (46). When the basal Akt-dependent insulin signaling is increased, the S6K-dependent mechanism may also contribute to the development or aggravation of insulin resistance through IRS-1 serine phosphorylation. The basic function of S6K is to promote protein synthesis. Our results show that protein levels of PGC-1α and Tfam, key mitochondrial biogenic genes, are actually decreased in the presence of insulin resistance/hyperinsulinemia induced by the HFD. Thus it does not seem that S6K is involved in insulin suppression of mitochondrial production.

In contrast, reduced exposure to insulin is associated with the exact opposite outcomes. Animals with low basal levels of Akt-dependent insulin signaling due to caloric restriction, growth hormone deficiency, or growth hormone receptor knockout have elevated mitochondrial biogenesis, decreased fat mass and oxidative stress, and better insulin sensitivity (3, 8, 15). Interestingly, even intermittently reduced exposure to insulin appears to be equally beneficial. Similar to the animals under the continuous caloric restriction, animals under the intermittent caloric restriction have decreased basal plasma insulin level, elevated mitochondrial biogenesis, reduced oxidative stress, and increased insulin sensitivity (4, 17, 30, 32).

In this study, mice under the HFD had increased basal Akt-dependent insulin signaling together with increased ectopic fat accumulation, elevated oxidative stress, decreased mitochondrial production, and reduced insulin sensitivity. However, all of these changes were reversed when the basal Akt-dependent insulin signaling was reduced by application of the PI 3-kinase inhibitor LY-294002 during the daytime when mice were usually sleeping and insulin was not highly demanded. Reduction of the basal Akt-dependent insulin signaling may increase insulin sensitivity through several possibilities. When the Akt-dependent insulin signaling is blunted or blocked, the following events may occur: 1) decreased lipogenesis, minimizing fat accumulation (including ectopic fat); 2) increased lipolysis, also leading to decreased (ectopic) fat accumulation; 3) increased fatty acid oxidation, presumably leading to decreased accumulation of long-chain acyl-CoA at mitochondrial inner membrane and increased ADP availability in mitochondrial matrix; 4) increased production of new mitochondria; and 5) increased autophagy-dependent removal of aged/dysfunctional mitochondria (unpublished observation). All of these events can lead to better mitochondrial and cellular functions, decreased oxidative stress, and improved insulin sensitivity. Another likely consequence of the blunted/blocked PI 3-kinase-dependent insulin signaling as described in this study is the increased blood glucose level (diabetes). However, this increase should be temporary, as evidenced by the normal fasting blood glucose level in this study. Importantly, our separate study has shown that high levels of glucose in either animals or cultured hepatocytes do not cause insulin resistance in the absence of insulin (data not shown). In summary, our results suggest that: 1) the basal level of Akt-dependent insulin signaling is increased in the presence of insulin resistance/hyperinsulinemia induced by the HFD; and 2) appropriate blockade of the continuously elevated Akt-dependent insulin signaling may be an effective way to reverse the suppressed mitochondrial production, increased ectopic fat accumulation, elevated oxidative stress, and insulin resistance in subjects with insulin resistance and hyperinsulinemia.

GRANTS

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