Leptin Impairs Insulin Signaling in Rat Adipocytes

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Leptin modulates glucose homeostasis by acting as an insulin-sensitizing factor in most insulin target tissues. Nevertheless, insulin-dependent glucose uptake in white adipose tissue decreases after in vivo treatment with leptin. Moreover, elevated leptin concentrations inhibit insulin metabolic effects in adipocytes. Here we studied both, direct and centrally mediated effects of leptin on insulin signaling in rat adipocytes. Adipocyte incubation with low leptin concentrations did not modify the insulin stimulation of mitogen-activated protein kinase (MAPK). However, at elevated concentrations, leptin impaired insulin-stimulated MAPK activity, glycogen synthase kinase (GSK)3β phosphorylation, and insulin receptor tyrosine phosphorylation without altering vanadate stimulation. An increase of suppressor of cytokine signaling-3 protein was also observed. Central administration of leptin decreased insulin effects on adipocyte MAPK and GSK3β phosphorylation. In insulin-resistant aged rats with hyperleptinemia and central leptin resistance, insulin poorly stimulated adipocyte insulin responsiveness. Food restriction increased MAPK stimulation by insulin and restored the ability of centrally infused leptin to attenuate adipocyte insulin signaling in aged rats. We conclude that leptin can modulate, in an inhibitory manner, adipocyte insulin signaling by two different ways: as an autocrine signal and, indirectly, through neuroendocrine pathways. These mechanisms may be of relevance in situations of hyperleptinemia, such as aging and/or obesity. Diabetes 53:347–353, 2004

Leptin is a hormone, mainly produced by the adipose tissue, involved in the regulation of energy balance (1–3). Compelling evidence indicates that leptin serves as a mediator in the cross-talk between the peripheral and central nervous system to adapt metabolic and neuroendocrine function to changes in the nutritional state of the organism (4).

Considerable experimental data indicate that leptin modulates glucose homeostasis (5). In most cases (6–8), leptin appears to act as an insulin-sensitizing factor at the whole body level in rats. Nevertheless, studies on glucose uptake by different tissues after leptin treatment suggest that the hormone exerts tissue-specific effects. Thus, microinjection of leptin into ventromedial hypothalamus increases glucose uptake in brown adipose tissue and heart and skeletal muscles but not in white adipose tissue (9). A subcutaneous infusion of leptin for 7 days combined with a euglycemic-hyperinsulinemic clamp induces an increase of glucose uptake in brown adipose tissue and skeletal muscles but a decrease in white adipose tissue (6,10).

Further evidence for the role of leptin on glucose homeostasis was obtained in ob/ob and in lipodystrophic mice, which lack detectable amounts of circulating leptin. In both cases, peripheral treatment with leptin alone (11–13) or in combination with the adipocyte-derived hormone adiponectin (14) reversed the characteristic diabetic phenotype and insulin resistance of these animals, and the same has been observed in lipodystrophic patients under chronic leptin treatment (15). In contrast, in hyperleptinemic db/db mice and fa/fa rats, which have mutations in the leptin receptor, and in obese rats and humans with hyperleptinemia, leptin administration does not improve glucose tolerance and insulin sensitivity, probably due to the existence of leptin resistance (5).

The molecular bases for the metabolic effects of leptin are not completely understood. Experimental evidence indicates that leptin acts predominantly in the central nervous system, mainly in the hypothalamus, bringing about effects on appetite and in neuroendocrine pathways, as well as on autonomic nerves, which are transmitted to the periphery (4). On the other hand, expression of the leptin receptor has been observed in peripheral tissues, including pancreatic β-cells, liver, fat, and muscle (5), suggesting direct effects of leptin that are independent of central pathways. Although the relative importance of peripheral versus central actions of leptin on metabolic effects of the hormone remains unknown, direct effects of leptin on liver triglyceride content (16) and on skeletal and cardiac muscle fatty acid oxidation (17,18) have been recently described. Additionally, a direct fat-depleting effect at supraphysiological concentrations of leptin has been reported (19).

In nonadipose insulin-target tissues, leptin prevents triglyceride accumulation, thus contributing to the maintenance of insulin sensitivity (20). However, in white adipose tissue, the effect of leptin on insulin sensitivity

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GSK, glycogen synthase kinase; IRS, insulin receptor substrate; MBP, myelin basic protein; MAPK, mitogen-activated protein kinase; SOCS, suppressor of cytokine signaling.

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remains controversial. As mentioned above, while in vivo treatment with leptin reduces insulin-dependent glucose uptake of white adipose tissue (6,9,10), leptin incubation of isolated adipocytes has been reported to impair insulin stimulation of glucose transport and most of the metabolic effects of insulin (21), to impair only the lipogenic action of insulin (22,23), and to not influence insulin-mediated effects (24,25). On the other hand, the effect of leptin on specific insulin-signaling events has not been studied, and the data from studies in cell lines have been controversial (5).

Several studies have demonstrated that decreasing visceral fat by caloric restriction (26) or by its surgical removal (27) prevent the onset of overall insulin resistance. Additionally, fat-specific GLUT4 gene inactivation causes glucose intolerance and hyperinsulinemia (28), whereas GLUT4 overexpression in fat tissue enhances whole-body insulin sensitivity and glucose tolerance in diabetic mice (29), suggesting that the insulin sensitivity of adipose tissue might play a pivotal role in the development of whole-body insulin resistance and glucose intolerance.

Hyperleptinemia develops in most cases of obesity and during aging in rodents and humans (1,30,31). Thus, it could be possible that the elevated levels of circulating leptin in these cases might play a key role in the development of insulin resistance in adipose tissue, leading to overall insulin insensitivity.

In the present work, we studied the effect of leptin administration, in vivo and in vitro, on insulin signaling in isolated adipocytes from mature Wistar rats. Additionally, in aged rats that presented hyperleptinemia, we analyzed whether a decrease in the circulating leptin concentration after caloric restriction improved insulin signaling in adipocytes and restored the ability of centrally administered leptin to modulate adipocyte insulin responsiveness.

RESEARCH DESIGN AND METHODS

Male Wistar rats (3 and 24 months old) from our in-house colony (Centre of Molecular Biology, Madrid, Spain) were used throughout this study. Rats were housed in climate-controlled quarters with a 12-h light cycle and ad libitum standard laboratory diet and water. For studies with food-restricted animals, 21-month-old male Wistar rats were placed in individual cages and fed daily with an amount of diet equal to 75–85% of the normal food intake until they reached a reduction of ~25% of their body weight. The amount of diet provided was adjusted weekly to maintain this body weight up to age 24 months (32). The animals were handled according to the laws of the European Union and the guidelines of the National Institutes of Health, and the experimental protocols were approved by the institutional committee of bioethics.

Isolation and fractionation of adipocytes for in vitro experiments.

Adipocytes were prepared by the collagenase method using Dulbecco’s modified Eagle’s medium containing 25 mM HEPES, pH 7.4, and 4% BSA. Cells were washed in HEPES-buffered balanced salt solution (pH 7.4) containing 20 mM HEPES, 120 mM NaCl, 1.2 mM MgSO4, 2 mM CaCl2, 2.5 mM KCl, 1.0 mM Na2HPO4, 1% BSA, and 1 mM sodium pyruvate (33) and incubated in the same buffer (1 ml cells per 3 ml medium) at 37°C for 6 h in the presence or absence of the indicated concentration of rat recombinant leptin (Sigma, St. Louis, MO), following the treatment, cells were incubated in the absence or presence of 16 mM porcine insulin or 1 mM Na1VO4 (Sigma). After 5 min, the medium was aspirated, cells were washed in PBS, lysed in homogenization buffer, and centrifuged at 8,000g for 5 min and the infranatants solubilized at 4°C for 30 min as previously described (34,35). Infranatants (cytosolic extracts) were used for determination of mitogen-activated protein kinase (MAPK) activity and glycogen synthase kinase (GSK3) phosphorylation. To evaluate insulin receptor phosphorylation and the amount of suppressor of cytokine signaling (SOCS)-3 protein, cells lysed in homogenization buffer were centrifuged at 5,000g for 5 min and the infranatants solubilized at 4°C for 30 min in the presence of 1% Triton-X-100 and 1 mg/ml each of leupeptin, pepstatin, and aprotinin.

Assay of MAPK activity. MAPK activity was determined in anti-MAPK immunoprecipitates using myelin basic protein (MBP) as substrate. Cytosolic extracts (~500 µg protein) were immunoprecipitated with anti-MAPK (1/200 dilution, UBI, Lake Placid, NY) following the manufacturer’s protocol. Immunoprecipitates were incubated for 20 min with 0.5 mg/ml MBP and 50 µmol/l [γ-32P]ATP, and phospho-MBP was resolved on 12% SDS-PAGE, detected by autoradiography, and quantitated by scanning densitometry as previously described (35). Alternatively, bands were cut off the gel and phospho-MBP was quantitated by scintillation counting. The amount of MAPK phosphorylated in cytosolic extracts was determined by Western blot analysis with the same antibody and was used to normalize the values of MBP phosphorylation.

Determination of phosphorylated GSK3. Cytosolic proteins (~20 µg) were resolved by 10% SDS-PAGE and then transferred to nitrocellulose membranes. Membranes were blocked in Tris-buffered saline containing 5% fat, skimmed, dry milk and incubated overnight at 4°C with anti-phospho-GSK3b (Ser 9) polyclonal antibodies (Cell Signaling, Beverly, MA) at a 1:500 dilution. Phosphorylated GSK3 was visualized after incubation with goat anti-rabbit peroxidase-conjugated IgG (Nordic Immunologic, Tiburg, the Netherlands) using the enhanced chemiluminescence method and quantitated by densitometric scanning. Membranes were reblotted with anti-GSK3b polyclonal antibodies (dilution 1:500; Biosource, Camarillo, CA) followed by incubation with horse anti-mouse peroxidase-conjugated IgG (dilution 1:1,000; Vector, Burlingame, CA) to determine the relative amount of GSK3 proteins in the cytosolic extracts.

Measurement of SOCS-3 and insulin receptor tyrosine phosphorylation. Cell lysates (~500 µg protein) were immunoprecipitated with 5 µg anti-human SOCS-3 (H-103) or 5 µg insulin Rδ (C-19) polyclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA). Precipitated proteins were resolved by SDS-PAGE, transferred to nitrocellulose, and visualized by incubation with SOCS-3 (H-103) or anti-phosphotyrosine (UBI) monoclonal antibodies (dilution 1:500; Biosource, Camarillo, CA) followed by incubation with horse anti-mouse peroxidase-conjugated IgG (dilution 1:1,000; Santa Cruz Biotechnology).

Leptin administration. Rats were anesthetized by inhalation of a mixture of O2, NO, and isoflurane (Pharmacia-Upjohn, Barcelona, Spain) and placed in a stereotaxic frame (David Kopf, Tujunga, CA). An opening of the skull was made with a dental drill at ~1.6 mm lateral to the midline and 0.8 mm anterior to the bregma (32). A cannula connected to an osmotic minipump (Alzet, Palo Alto, CA) was implanted in the right lateral cerebral ventricle. Osmotic minipumps, with a releasing rate of 1 µl/h, were filled with rat leptin (Sigma), which was diluted as indicated by the manufacturer. Leptin concentrations were adjusted to 0.41 µg/g (10 µg/day) and 0.0082 µg/g (0.2 µg/day) with PBS. Control rats were implanted with a minipump containing vehicle of the same osmolarity of the leptin dilution. After 7 days, the rats were killed and isolated adipocytes were prepared from fat tissue as described by Molero et al. (35).

RNA extraction and RT-PCR. Total RNA was isolated from white adipose tissue using the RN-Easy minikit (Qiagen, Hilden, Germany). Primers used for SOCS-3 and actin cDNA synthesis and for PCR were essentially the same as those described previously (36). The template was denatured for 5 min at 94°C, followed by 30 cycles (25 cycles for actin amplification) with the following temperatures: denaturation, 1 min at 94°C; annealing, 2 min at 60°C; and elongation, 1 min at 72°C. Reactions were finished with a final extension of 10 min at 72°C. The samples were electrophoresed and analyzed as described (36).

Statistical analysis. Statistical analysis was performed using the Probit software (BBN Systems and Technologies, Cambridge, MA). Significant differences between groups were determined by one-way ANOVA followed by the Duncan test for multiple comparisons or Student’s t test.

RESULTS

Effect of leptin on insulin stimulation of MAPK in isolated adipocytes. We studied the effect of incubation with different leptin concentrations over 6 h on insulin-stimulated MAPK activity in adipocytes isolated from 3-month-old rats. Insulin stimulates MBP phosphorylation by anti-MAPK immunoprecipitates ~2.8-fold in cells incubated in the absence of leptin. Preincubation of adipocytes up to a level of 0.5 mM/1 leptin (which is slightly higher than the physiological concentration of leptin in mature rats) does not significantly modify the insulin effect on MBP phosphorylation. In contrast, preincubation of adipo-
cytes with higher leptin concentrations (5–50 nmol/l) brings about a significant decrease in the insulin stimulation of MBP phosphorylation (Fig. 1A and B). No significant differences were found in basal activities determined after scintillation counting of the bands, which were 3.9 ± 0.6, 4.0 ± 0.9, 4.3 ± 0.9, and 4.1 ± 0.8 fmol·20 min⁻¹·µg⁻¹ of immunoprecipitated protein, in adipocytes preincubated without leptin or with 0.5, 5, and 50 nmol/l leptin, respectively. To investigate the molecular mechanisms involved in leptin action on insulin signaling to MAPK, adipocytes preincubated or not with 50 nmol/l leptin were stimulated with 1 mmol/l vanadate. In contrast to insulin, vanadate similarly stimulates the MAPK activity of adipocytes treated with or without leptin (Fig. 1C and D).

**Effect of leptin on insulin-dependent GSK3 phosphorylation.** As is shown in Fig. 2, insulin stimulates the phosphorylation of GSK3β in adipocytes incubated in the absence of leptin by approximately threefold. When adipocytes are preincubated with 50 nmol/l leptin for 6 h, the stimulatory effect of insulin is significantly lower (~2.1-fold). On the other hand, vanadate stimulates GSK3β phosphorylation ~2.5-fold, and preincubation of adipocytes with leptin does not decreases its stimulatory effect (Fig. 2A and B). Preincubation with leptin did not modify the basal phosphorylation of GSK3β (0.58 ± 0.18 and 0.46 ± 0.07 arbitrary units for cells incubated without or with leptin, respectively; n = 5, P = 0.43).

**Effect of leptin on SOCS-3 expression and insulin receptor tyrosine phosphorylation.** As shown in Fig. 3A and B, incubation of adipocytes with 50 nmol/l leptin for 6 h significantly increases the cellular amount of SOCS-3 (~50%). Moreover, in cells preincubated with leptin, insulin-dependent insulin receptor β-subunit phosphorylation is markedly lower than in adipocytes incubated without leptin (Fig. 3C and D). Basal insulin receptor phosphorylation was not significantly different between cells preincubated without or with leptin (0.67 ± 0.05 and 0.63 ± 0.10 arbitrary units, respectively; n = 4, P = 0.28).

**Effect of central administration of leptin on adipocyte insulin responsiveness.** We next examined the effect of prolonged central administration of leptin on the stimulation of MAPK and GSK3β phosphorylation by insulin in isolated adipocytes from 3-month-old rats. We have previously shown that intracerebroventricular administration of a daily amount of 0.2–10 µg leptin during 7 days results in a dose-dependent decrease in food intake and body weight, without changes in circulating levels of leptin (32). Serum leptin concentrations at the end of the exper-
ment were 1.4 ± 0.1, 1.4 ± 0.4, and 1.1 ± 0.3 ng/ml for rats infused with saline or with 0.2 and 10 μg/day leptin, respectively. As shown in Fig. 4, in adipocytes isolated from rats infused with saline, insulin stimulates MAPK activity >2.2-fold. In contrast, in fat cells from rats infused with two different doses of leptin, the insulin effect on MAPK is significantly lower (<1.4-fold). Leptin infusion for 1 week did not modify the basal MAPK activity of adipocytes (1.3 ± 0.2, 1.03 ± 0.2, and 1.13 ± 0.28 arbitrary units for rats treated with saline or 0.2 and 10 μg/day leptin, respectively; P = 0.73). Figure 4 also shows that the stimulation of adipocyte GSK3β phosphorylation by insulin is decreased after the infusion of a daily dose of 0.2 μg leptin, without significant changes in the basal phosphorylation (0.25 ± 0.07 and 0.44 ± 0.06 arbitrary units, for saline-infused and leptin-treated animals, respectively; n = 4, P = 0.11).

Central effect of leptin on adipocyte insulin signaling in leptin-resistant aged rats: effect of food restriction. We next focused our attention on the stimulation of MAPK by insulin in adipocytes from 24-month-old rats, which show hyperleptinemia and central leptin resistance (31,32), in addition to overall (37) and adipocyte (34,35,37) insulin resistance. As shown in Fig. 5, in adipocytes from aged rats fed ad libitum and infused intracerebroventricularly with saline over 7 days, insulin elicits poor stimulation of MAPK activity (1.4-fold), which is similar to that observed previously in adipocytes from 24-month-old animals without pump implantation (35). Infusion of a daily dose of 0.2 μg leptin for 7 days did not modify the circulating leptin concentration (8.8 ± 2.7 in saline vs. 9.0 ± 3.2 ng/ml in leptin-infused rats) and did not cause further inhibition of insulin stimulation of adipocyte MAPK (Fig. 5). Basal MAPK activities were similar in leptin-treated and saline-infused animals (0.75 ± 0.21 and 0.40 ± 0.12 arbitrary units, respectively; n = 5, P = 0.4). The food restriction used in these experiments lowers serum leptin levels to 2.7 ± 0.4 ng/ml, a value close to that of young rats, and improves adipocyte insulin sensitivity as demonstrated by the fact that insulin stimulates MAPK activity >1.9-fold (Fig. 5). Interestingly, it also restores the ability of intracerebroventricularly infused leptin to modulate adipocyte insulin responsiveness as demonstrated by the low stimulation of MAPK activity by insulin (1.2-fold) in adipocytes from food-restricted old rats infused daily with 0.2 μg leptin. As in the case of old animals fed ad libitum, this treatment did not increase serum leptin levels (1.4 ± 0.2 ng/ml), and basal MAPK activities for saline-

![FIG. 3. Effect of leptin on adipocyte SOCS-3 protein expression and IR tyrosine phosphorylation. A: A representative blot illustrating the presence of SOCS-3 and insulin receptor α in cell lysates. B: Amount of SOCS-3 relative to the amount of insulin receptor-α. Data are means ± SE (n = 5) and refer to the amount of cells treated without leptin. *P < 0.05 vs. cells without leptin. C: Representative autoradiogram illustrating the tyrosine phosphorylation of the insulin receptor-β subunit. D: Insulin receptor β phosphorylation. Data show the fold stimulation by insulin and are the means ± SE (n = 4). *P < 0.01 vs. cells treated without leptin. IR, insulin receptor.](image)

![FIG. 4. Effect of centrally infused leptin on insulin stimulation of adipocyte MAPK and GSK3β phosphorylation. A: Representative autoradiograms illustrating the insulin-dependent phosphorylation of MBP and GSK3β in adipocytes from rats treated or not intracerebroventricularly with leptin. B: Fold stimulation by insulin. Data are means ± SE of four to six animals per group. For MBP phosphorylation, one-way ANOVA indicates a significant effect of leptin (P = 0.01). *P < 0.05; **P < 0.01 vs. saline-infused animals. i.c.v., intracerebroventricular.](image)
infused and leptin-treated food-restricted rats were similar (0.85 ± 0.12 and 0.93 ± 0.1 arbitrary units, respectively; n = 5, P = 0.65). Low insulin sensitivity in adipocytes from older rats could be due to the increased expression of SOCS-3 mRNA observed in adipose tissue of 24-month-old animals if compared with 3-month-old rats. Interestingly, the restoration of insulin responsiveness after food restriction is paralleled by a decrease in SOCS-3 mRNA in adipose tissue (Fig. 6).

FIG. 5. Stimulation of MAPK by insulin in old rat adipocytes: the effect of centrally infused leptin and food restriction. Leptin (0.2 μg/day) or saline was infused intracerebroventricularly over 7 days in 24-month-old rats, which were either fed ad libitum (AL) or under food restriction (FR). A: Representative autoradiogram illustrating the insulin-dependent phosphorylation of MBP. B: Quantitation of phospho-MBP. The figure shows the fold stimulation by insulin, and data are means ± SE of six to eight animals. *P < 0.05 vs. all other groups.

DISCUSSION
Since its identification as an adipocyte-derived hormone, leptin has been demonstrated to play a key role in the regulation of energy balance, modulating appetite, energy expenditure, and partitioning of fuels in the organism (4). Compelling evidence suggests that fat tissue is not only the main source of leptin but also a target of it. The physiological effects of leptin on adipose tissue can be mediated by neural circuits and/or elicited in an autocrine manner (4). Most of the effects of leptin are likely mediated by its interaction with hypothalamic leptin receptors. Nevertheless, under conditions of pathological or experimentally induced hyperleptinemia, direct metabolic actions on several tissues, including fat tissue, have been reported (16–19). In this work, we studied the effect of leptin on adipocyte insulin signaling. Direct action was analyzed using isolated fat cells preincubated over 6 h with 50 nmol/l leptin, conditions previously shown to inhibit insulin metabolic action in adipocytes (21). On the other hand, neural-mediated action was studied in adipocytes isolated from intracerebroventricularly leptin-infused rats over 7 days, under conditions that do not increment the level of circulating leptin, precluding a direct effect of the hormone (32).

The data reported herein indicate that prolonged incubation of adipocytes with leptin impairs the two main branches of insulin signal transduction (38), as is shown by the lower stimulation of both MAPK activity and phosphorylation of GSK3. Our data also indicate that leptin treatment does not impair the activation of insulin signal pathways by vanadate, which stimulates insulin receptor substrate (IRS)-1–associated phosphatidylinositol 3-kinase in adipocytes (34) without activating insulin receptor autophosphorylation. As stimulation of phosphatidylinositol 3-kinase plays a key role in the phosphorylation and subsequent inhibition of GSK3 in response to insulin (38) as well as in the stimulation of MAPK by insulin and vanadate in adipocytes (35), our data suggest that leptin impairs insulin signaling at an early step of the signal cascade.

Leptin is known to induce the expression of SOCS-3 in hypothalamic nuclei (39) and in cell lines (40,41), which is involved in a negative feedback loop to terminate leptin signaling (20). Additionally, SOCS-3 has been shown to inhibit insulin signaling in several cell lines (42–44). The data in this work demonstrate that treatment of adipocytes with 50 nmol/l leptin increases the cellular amount of SOCS-3. Different mechanisms of insulin-signaling inhibition by SOCS-3 have been proposed for different cell lines. In COS-7 fibroblasts, SOCS-3 inhibits the insulin-dependent phosphorylation of IRS-1 but not the autophosphorylation of the insulin receptor (42), suggesting that it competes with IRS-1 for binding to the insulin receptor. In contrast, in HepG2 cells, SOCS-3 associates with the insulin receptor and suppress its insulin-dependent autophosphorylation (44). Although we were unsuccessful in observing a direct association between the insulin receptor and SOCS-3, our data indicate that in leptin-treated adipocytes, insulin-dependent autophosphorylation is markedly decreased. Therefore, it can be speculated that the increased amount of SOCS-3 could play a role in this inhibition, such as that which occurs in HepG2 cells. The
decreased insulin receptor autophosphorylation observed in leptin-treated adipocytes would explain the lower stimulation by insulin of GSK3 phosphorylation and MAPK activity, as well as its normal stimulation by vanadate.

From the former data it can be concluded that leptin, at elevated concentrations, is able to downmodulate insulin signaling in adipose cells, whereas lower concentrations have no evident effect on insulin signaling. The concentration of leptin that is effective in inhibiting insulin signaling is similar to that previously shown (21) to cause the inhibition of insulin stimulation of glucose transport and other metabolic effects in adipocytes. Although physiological serum leptin concentrations are markedly lower, in situations of obesity and/or aging, circulating leptin levels can be >3 nmol/l and the interstitial concentration of leptin surrounding adipocytes might be extremely high (45). Therefore, our data suggest that in these circumstances that are characterized by hyperleptinemia and central leptin resistance, leptin might act as an autocrine factor that induces insulin resistance in adipose tissue.

The data in this work also show that leptin, acting through the central nervous system, modulates adipocyte insulin sensitivity in 3-month-old rats, as demonstrated by the inhibition of the insulin effect on MAPK activity and GSK3β phosphorylation. This effect must be mediated by the autonomic nervous system because the central infusion of leptin does not increase the leptin concentration in plasma. Nevertheless, because the intracerebroventricular concentration reached after leptin infusion is unknown, it is difficult to evaluate whether physiopathological increases in serum leptin concentration would be able to induce intracerebroventricular leptin levels sufficient to exert this inhibitory effect on adipocyte insulin signaling. Although intracerebroventricular leptin infusion causes a decrease in food intake and body weight (32), it is unlikely that those changes account for the lower insulin sensitivity of adipocytes because lowering body weight by caloric restriction is associated with an improvement of insulin signaling. These data agree with previous results (6,9,10), indicating that leptin has insulin-desensitizing effects, specifically in white adipose tissue. As the main role of insulin in adipose tissue is to promote the incorporation of glucose into triglycerides, this central effect of leptin on insulin signaling might contribute to the control of fat storage in addition to the downregulation of the lipogenic enzymes that it induces (46). Accretion of fat tissue seems to be a cause of whole-body insulin resistance. In fact, surgical removal of visceral adipose tissue has been reported to prevent insulin resistance in aging rats (27). Thus, the central action of leptin is likely playing a role in preserving the overall insulin sensitivity of the organism.

Aging rats develop central leptin resistance (30–32,36), fat tissue expansion, and compensatory hyperleptinemia. Our data show that this brings about an increase in SOCS-3 expression in adipose tissue. These data agree well with the observed increment in SOCS-3 in leptin-treated adipocytes that is reported herein. This increase in SOCS-3 is likely a mechanism to block autosuppression of adipose tissue by leptin, allowing adipocytes to meet the elevated physiological demand of this factor, as has been previously reported (45) for other models of nongenetic obesity. Additionally, elevated levels of SOCS-3 might induce a persistent impairment of insulin signaling, which agrees with the poor effect of insulin on MAPK activity observed in aged rats. Central infusion of leptin has no additional inhibitory effect on insulin sensitivity. This is in agreement with the presence of central leptin resistance in these rats (32). Prolonged moderate caloric restriction lowers adiposity index and hyperleptinemia in aged rats if compared with aged-matched animals fed ad libitum (32). As shown here, this improves insulin sensitivity in adipose tissue in parallel with a decrease in SOCS-3 expression, indicating that the impairment of insulin signaling mediated by leptin in the adipose tissue of aged rats can be reversed. As reported previously (32,36), caloric restriction also improves central leptin sensitivity. This agrees well with the restoration of the central leptin effect on adipocyte insulin sensitivity, as manifested by the marked inhibition of the effect of insulin on MAPK in adipocytes from leptin-infused, food-restricted aged rats.

In conclusion, the data in this work demonstrate that leptin can downmodulate insulin signaling in rat adipocytes in two different ways. In animals with normal physiological circulating leptin concentrations, leptin action takes place by stimulation of hypothalamic neural circuits and the autonomic nervous system, likely preventing excessive fat storage. On the other hand, at elevated leptin concentrations, leptin also inhibits insulin signaling, impairing insulin receptor autophosphorylation. This could be related to the increased expression of SOCS-3 protein reported herein. Hypothalamic-independent modulation of adipocyte insulin signaling could be relevant under physiological situations of hyperleptinemia and central leptin resistance, such as aging and obesity. The data presented herein also suggest that as central leptin resistance develops with aging and/or obesity, the increase in fat mass leads to hyperleptinemia. This circumstance induces the expression of SOCS-3, which would block the lipopenic effect of leptin (45) and likely inhibit insulin signaling in fat tissue. As suggested by others (47), this could represent an early step leading to the overall insulin resistance characteristic of aged and/or obese rats. Interestingly, caloric restriction restores adipocyte insulin sensitivity as well as the hypothalamic-dependent leptin modulation of adipocyte insulin signaling.

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