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Statin modulates insulin signaling and insulin resistance in liver and muscle of rats fed a high-fat diet

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Abstract

Recent studies have shown that statins might have relevant effects on insulin resistance in animal models and in humans. However, the molecular mechanisms that account for this improvement in insulin sensitivity are not well established. The aim of the present study was to investigate the effect of a statin on insulin sensitivity and insulin signaling in liver and muscle of rats fed on a high-fat diet (HFD) for 4 weeks, treated or not with lovastatin during the last week. Our data show that treatment with lovastatin results in a marked improvement in insulin sensitivity characterized by an increase in glucose disappearance rate during the insulin tolerance test. This increase in insulin sensitivity was associated with an increase in insulin-induced insulin receptor (IR) tyrosine phosphorylation and, in parallel, a decrease in IR serine phosphorylation and association with PTP1B. Our data also show that lovastatin treatment was associated with an increase in insulin-stimulated insulin receptor substrate (IRS) 1/phosphatidylinositol 3-kinase/Akt pathway in the liver and muscle of HFD-fed rats in parallel with a decrease in the inflammatory pathway (c-jun N-terminal kinase and I kappa β kinase (IKKβ)/inhibitor of κB/nuclear factor κB) related to insulin resistance. In summary, statin treatment improves insulin sensitivity in HFD-fed rats by reversing the decrease in the insulin-stimulated IRS-1/phosphatidylinositol 3-kinase/Akt pathway in liver and muscle. The effect of statins on insulin action is further supported by our findings that HFD rats treated with statin show a reduction in IRS-1 serine phosphorylation, I kappa kinase (IKK)/inhibitor of κB/nuclear factor κB pathway, and c-jun N-terminal kinase activity, associated with an improvement in insulin action. Overall, these results provide important new insight into the mechanism of statin action in insulin sensitivity.

1. Introduction

Insulin uses 2 main signaling pathways, the phosphatidylinositol 3-kinase (PI3K)-AKT/protein kinase B (PKB) pathway, which is responsible for most of the metabolic actions of the hormone, and the Ras–mitogen-activated protein kinase pathway, which regulates expression of some genes and cooperates with the PI3K pathway to control cell growth and differentiation [1]. The (PI3K)-AKT pathway initiates when the insulin receptor (IR) undergoes autophosphorylation after insulin binding and phosphorylates a number of intracellular proteins including IR substrate proteins (IRSs; IRS-1 and IRS-2 are the most important) [2]. After tyrosine phosphorylation, IRS-1 and IRS-2 bind and activate the PI3K [2,3]. Downstream to PI3K, activation of a serine/threonine kinase (Akt) occurs, which in turn stimulates glucose transport in the muscle and adipose tissue, stimulates glycogen synthesis in the liver and muscle, and stimulates lipogenesis in the adipose tissue [4]. Therefore, the PI3K/Akt pathway has an important role in the metabolic effects of insulin.

An important mechanism that may contribute to the dysregulation of the insulin-signaling pathway includes serine 307 (Ser^{307}) phosphorylation of IRS proteins by c-jun N-terminal kinase (JNK) [5]. The JNK is a member of the mitogen-activated protein kinase family [6,7] and can be activated by tumor necrosis factor α [8] and interleukin 1β [9,10], both proinflammatory cytokines. Serine 307 is located next to the phosphotyrosine (pY)-binding domain in IRS-1, and its phosphorylation inhibits the interaction of the pY-binding domain with the phosphorylated NPEY motif in the activated IR, causing insulin resistance [11].

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Furthermore, JNK might serve as a feedback inhibitor during insulin stimulation [5,11].

In rodents and humans, the dietary intake of high amounts of fat has been shown to be associated with increased adiposity and insulin resistance [12]. A recent study has shown that a high-fat diet (HFD) induces a tissue-specific regulation of glucose transport with reduced glucose uptake and insulin signaling in muscle that is accompanied by an increased insulin-stimulated glucose uptake in adipose tissue [13]. In this context, insulin resistance could be tissue specific to the muscle, liver, and hypothalamus, whereas adipose tissue remains sensitive to insulin.

Another mechanism involved in HFD-dependent insulin resistance is the activation of the proinflammatory I kappa β kinase (IKKβ)/inhibitor of κB (IκB)/nuclear factor κB (NFκB) pathway [14-16]. I kappa kinase (IKK) is a serine kinase; and its activation phosphorylates the IκB, a cytoplasmic protein that inhibits nuclear translocation of NFκB, a family of transcription factors that function as homo- or heterodimers in the regulation of the expression of proinflammatory, immunomodulatory, and antiapoptotic genes [17]. After its phosphorylation, IκB is ubiquitinated and degraded in the proteosome, releasing NFκB for translocation to the nucleus and activation of gene expression. It has been proposed that NFκB-increased activation may play an important role in the pathogenesis of insulin resistance [14-17].

Since their discovery in the early 1970s, statins have been used to lower cholesterol, acting as 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors [18,19]. However, recent studies have shown that statins might have additional relevant effects on insulin resistance in animal models [20,21] and in humans [22,23]. However, the molecular mechanisms that account for this improvement in insulin sensitivity are not yet well established. The aim of the present study is to investigate the effect of a statin on insulin sensitivity and insulin signaling in the liver and muscle of HFD-fed rats.

2. Materials and methods

2.1. Materials

Male Wistar rats were provided by the State University of Campinas-Central Breeding Center (Campinas, SP, Brazil). Antiphosphotyrosine and anti-IR (αIR) antibodies anti–IRS-1, anti-Akt1/2, and anti-pJNK were from Santa Cruz Technology (Santa Cruz, CA). Anti-pAkt was from Cell Signaling Technology (Beverly, MA). Anti-PI3K and anti–phospho-IRS-1^{ser307} were obtained from Upstate Biotechnology (Lake Placid, NY). Human recombinant insulin (Humulin R) was purchased from Eli Lilly (Indianapolis, IN). Routine reagents were purchased from Sigma Chemical (St Louis, MO) unless otherwise specified. [^{125}I]-Protein A was from Amersham Biosciences Group (Little Chalfont, United Kingdom).

2.2. Animals

Four-week-old male Wistar rats were randomly divided into 3 groups with similar body weights and fed for 4 weeks: control group (C), fed standard rodent chow and water ad libitum (protein, 20 kcal%; carbohydrate, 70 kcal%; lipid, 10 kcal%); HFD group, fed a fat-rich chow and water ad libitum (protein, 20 kcal%; carbohydrate, 35 kcal%; lipid, 45 kcal%, predominantly in the form of lard); and lovastatin group (L), fed a fat-rich chow and water ad libitum and treated daily with lovastatin (6 mg/kg) by oral gavage at night during the last week of the experiment. All experiments were approved by the Ethics Committee of the State University of Campinas.

2.3. Methods

2.3.1. Tissue extraction and immunoprecipitation

Food was withdrawn 12 to 14 hours before the experiments. One hour before the procedure, the animal received the last dose of drug or vehicle. Rats were anesthetized with sodium thiopental (25 mg/kg body weight, intraperitoneally) and were used 10 to 15 minutes later, that is, as soon as anesthesia was ensured by the loss of pedal and corneal reflexes. The abdominal cavity was opened, the cava vein was exposed, and 0.5 mL of isotonic sodium chloride solution or insulin (6 μg) was injected. Fragments of liver and soleus muscle were then collected at 30 and 90 seconds, respectively. The fragments were minced coarsely and homogenized immediately in extraction buffer (1% Triton-X 100, 100 mmol/L Tris [pH 7.4] containing 100 mmol/L sodium pyrophosphate, 100 mmol/L sodium fluoride, 10 mmol/L EDTA, 10 mmol/L sodium vanadate, 2 mmol/L phenylmethylsulfonyl fluoride, and 0.1 mg of aprotinin per milliliter) at 4°C with a Polytron PTA 20S generator (model PT 10/35; Brinkmann Instruments, Littau-Luzern, Switzerland) operated at maximum speed for 30 seconds. The extracts were centrifuged at 15 000 rpm and 4°C in a Beckman 70.1 Ti rotor (Palo Alto, CA) for 45 minutes to remove insoluble material, and aliquots of the resulting supernatants containing 2.0 mg of total protein of these tissues were used for immunoprecipitation with 10 μL of polyclonal antibodies: anti-αIR, αIRS-1, anti-Akt (Cell Signaling Technology), anti–phospho-[Thr183]-JNK, anti–phospho-IRS-1^{ser307}, and anti-IκBα and Protein A Sepharose 6MB or Protein A/G Plus (Pharmacia, Uppsala, Sweden). Tissue extraction and immunoprecipitation overnight were followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), transfer to nitrocellulose membranes, and blotting with anti-pY, αIR, anti–IRS-1, or anti-p85/PI3 kinase antibodies.

2.3.2. Protein analysis by immunoblotting

The precipitated proteins and/or whole-tissue extracts were treated with Laemmli sample buffer [24] containing 100 mmol/L dithiothreitol and heated in a boiling water bath for 5 minutes, after which they were subjected to SDS-PAGE in a Bio-Rad (Hercules, CA) miniature slab gel apparatus

Table 1
Serum insulin and plasma fasting glucose levels

	C	HFD	L
Plasma glucose (mg/dL)	95 ± 11	118 ± 6	112 ± 11
Serum insulin (ng/mL)	2.22 ± 0.12 *	3.76 ± 0.24 **	2.68 ± 0.08

Serum insulin and plasma glucose levels. Data are expressed as mean ± SEM. Each group was composed of 6 animals.
* $P < .001$: C vs HFD.
** $P < .05$: HFD vs L.

(Mini-Protean). For total extracts, 250 μg of proteins were subjected to SDS-PAGE. Electrotransfer of proteins from the gel to nitrocellulose was performed for 120 minutes at 120 V in a Bio-Rad Mini-Protean transfer apparatus [25]. Non-specific protein binding to the nitrocellulose was reduced by preincubating the filter for 2 hours in blocking buffer (5% nonfat dry milk, 10 mmol/L Tris, 150 mmol/L NaCl, 0.02% Tween 20). The nitrocellulose blot was incubated with specific antibodies overnight at 4°C and then incubated with 2 mCi FS[^{125}I]-labeled protein A (30μCi/μg). The results were visualized by autoradiography with preflashed Kodak XAR film (Manaus, Amazonas-Brazil). Band intensities were quantified by optical densitometry (model GS300; Hoefer Scientific Instruments, San Francisco, CA).

2.3.3. Insulin tolerance test

All the 3 groups were submitted to an intravenous insulin tolerance test (1 U/kg body weight of insulin, intravenously); and samples for blood glucose measurements were collected at 0 (basal), 4, 8, 12, and 16 minutes after injection. Rats were anesthetized with sodium thiopental (25 mg/kg body weight, intraperitoneally) as described above, 40μL of blood was collected from their tails, and blood glucose concentration was measured by the glucose-oxidase method. Thereafter, the rate constant for plasma glucose disappearance (K_{itt}) was calculated using the formula $0.693/(t_{1/2})$. The plasma glucose $t_{1/2}$ was calculated from the slope of the least squares analysis of the plasma glucose concentrations during the linear phase of decline [26].

2.3.4. Serum insulin analysis

Serum insulin levels were analyzed by radioimmunoassay, as previously described [27].

2.3.5. Statistical analysis

All groups of animals were studied in parallel. Comparisons between different groups were performed by using Student *t* test for unpaired samples and analysis of variance as appropriate. The level of significance adopted was $P < .05$.

3. Results

3.1. Animal characteristics

Body weights were higher in HFD-fed rats than in the control group (HFD: 412 ± 5 g vs C: 347 ± 12 g, $P < .05$) and in the lovastatin group compared with control rats (L: 426 ± 20 g vs C: 347 ± 12 g, $P < .05$). The body weights of HFD-fed rats and the rats of the lovastatin group were not statistically different. The insulin levels in HFD-fed animals were higher than those in the control group (C: 2.22 ± 0.12 ng/mL vs HFD: 3.76 ± 0.24 ng/mL, $P < .001$). The group fed on the diet and treated with lovastatin presented insulin levels that were similar to those of the controls and statistically different from the animals fed the HFD (HFD: 3.76 ± 0.24 ng/mL vs L: 2.68 ± 0.08 ng/mL, $P < .05$). We did not find any difference in fasting plasma glucose concentrations between the 3 groups. Table 1 shows the results of serum insulin and fasting plasma glucose.

The HFD animals were more insulin resistant than the control rats, as expressed by their lower plasma glucose disappearance rates measured by the insulin tolerance test (K_{itt}) (C: 4.3%/min ± 0.6%/min vs HFD: 1.7%/min ± 0.4%/min, $P < .05$). The use of lovastatin increased the insulin sensitivity, as shown by the K_{itt} (L: 3.3%/min ± 0.5%/min), which was different from the HFD group ($P < .05$) (Fig. 1).

3.2. Insulin signaling in liver of controls, HFD-fed rats, and lovastatin-treated rats

There were no differences in IR protein levels between the 3 groups. The insulin-induced IR tyrosine phosphorylation in the liver of HFD rats was decreased when compared with control animals. The animals treated with lovastatin presented insulin-induced IR tyrosine phosphorylation levels that were similar to those of controls (C: 100% ± 4% vs HFD: 48% ± 5%, $P < .001$; HFD vs L: 101% ± 9%, $P < .001$; Fig. 2A and B). The IR serine phosphorylation in the liver of HFD rats was increased when compared with control animals; and after the treatment with lovastatin, the IR serine phosphorylation was similar to that of the control group (C: 100% ± 10% vs HFD: 157% ± 8%, $P < .05$; HFD:

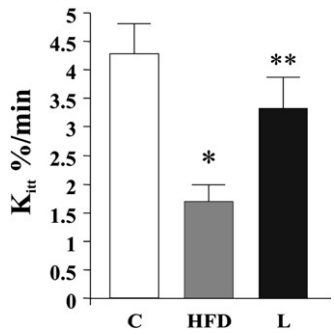

Fig. 1. All 3 groups were submitted to an intravenous insulin tolerance, as described in Materials and methods. Blood samples were collected from the tails of anesthetized rats at 0 (basal), 4, 8, 12, and 16 minutes after injection of 1 U/kg body weight of insulin intravenously; glucose concentration was measured by the glucose-oxidase method; and the rate constant for plasma glucose disappearance (K_{itt}) was calculated. The HFD-fed rats were more resistant than the control rats as shown by K_{itt} (C: 4.3%/min ± 0.6%/min vs HFD: 1.7%/min ± 0.4%/min, $P < .05$), and the use of lovastatin increased the insulin sensitivity (K_{itt} L: 3.3%/min ± 0.5%/min vs HFD: 1.7%/min ± 0.4%/min, $P < .05$).

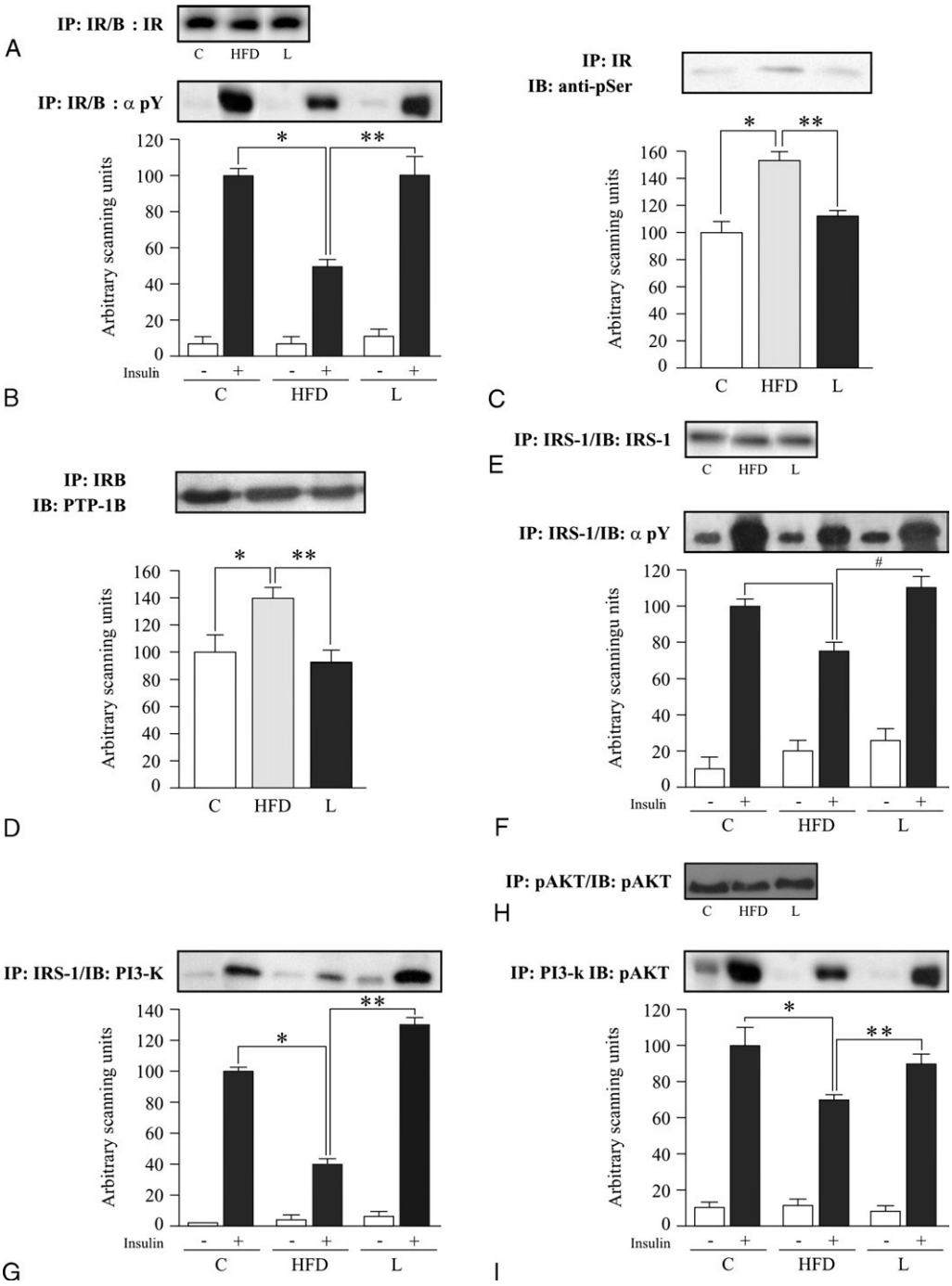

Fig. 2. Insulin signaling in the liver of control animals, animals fed on HFD for 4 weeks, and animals fed on HFD for 4 weeks and treated with lovastatin during the last week. In animals infused with insulin, the samples were extracted at 30 seconds after insulin injection. A, Immunoprecipitation (IP) with αIR and immunoblotting (IB) with α-IR antibodies. B, IP with α-IR and IB with α-PY. C, IP with αIR and IB with antiphosphoserine antibodies. D, IP with αIR and IB with anti-PTP1B antibodies. E, IP with α-IRS-1 and IB with α-IRS-1. F, IP with α-IRS-1 and IB with α-PY. G, IP with α-IRS-1 and IB with PI3K. H, IB with α-AKT1/2 antibodies. I, IB with α-PAKT. Data are mean ± SEM of 10 independent experiments; that is, 10 different cohorts of animals were fed on a control diet, HFD for 4 weeks, or HFD for 4 weeks plus lovastatin treatment during the last week. *HFD vs C, $P < .001$; **HFD vs L, $P < .001$; #L vs HFD, $P < .05$.

157% ± 8% vs L: 112% ± 4%, $P < .05$; Fig. 2C). A similar behavior was seen for the association of IR and PTP1B (C: 100% ± 12% vs HFD: 140% ± 8%, $P < .05$; HFD: 140% ± 8% vs L: 95% ± 10%, $P < 0.05$; Fig. 2D). Despite similar levels of IRS-1 in the liver of the 3 groups, there was a decrease in insulin-stimulated IRS-1 tyrosine phosphorylation in the HFD group with an increase after the use of the drug (C: 100% ± 3%, HFD: 76% ± 7%, and L: 112% ± 14%,

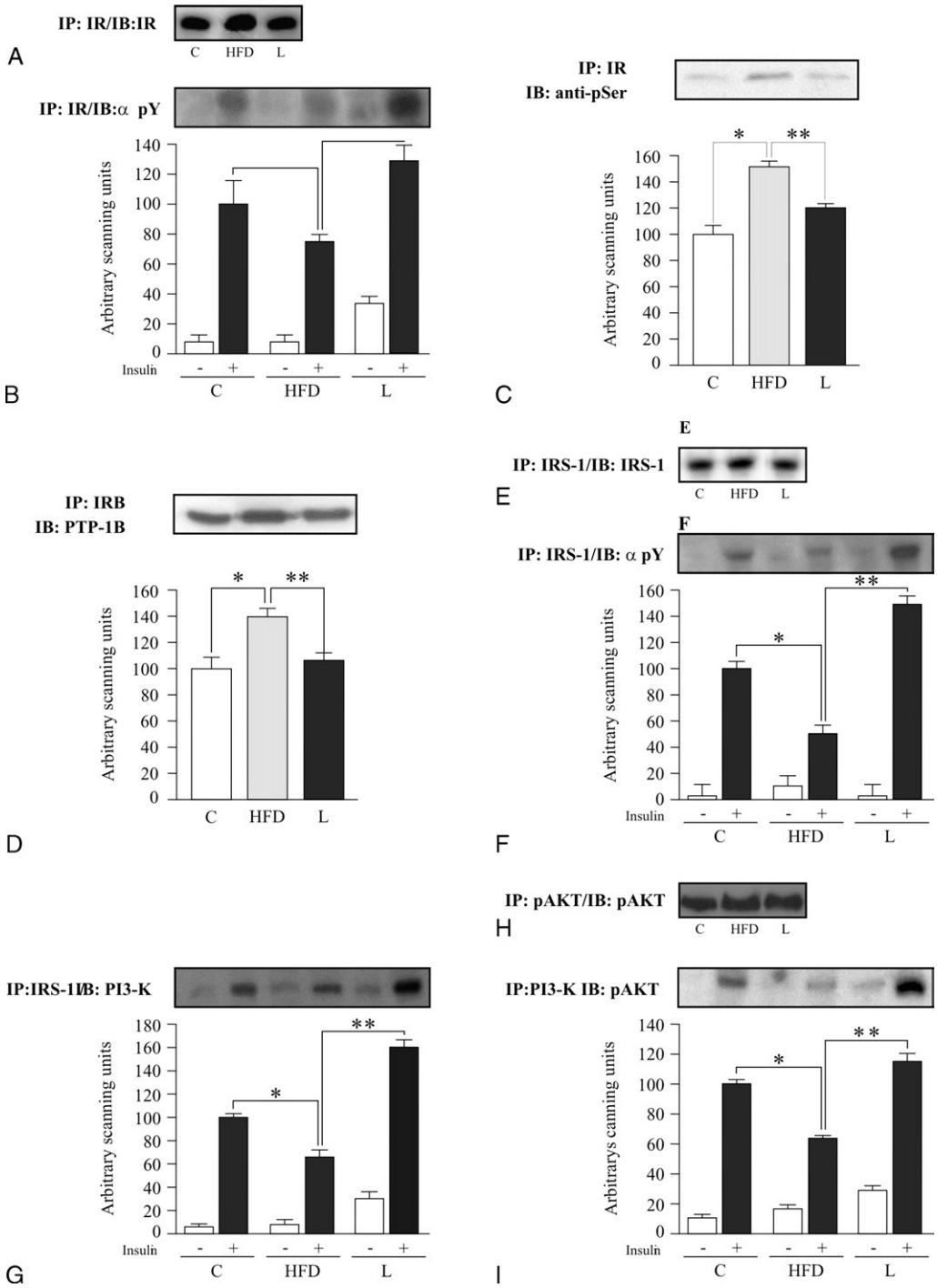

Fig. 3. Insulin signaling in the skeletal muscle of control animals, animals fed on HFD for 4 weeks, and animals fed on HFD for 4 weeks and treated with lovastatin during the last week. In animals infused with insulin, the samples were extracted at 90 seconds after insulin injection. A, IP with α-IR and IB with α-IR antibodies. B, IP with α-IR and IB with α-PY. C, IP with α-IR and IB with antiphosphoserine antibodies. D, IP with αIR and IB with anti-PTP1B antibodies. E, IP with α-IRS-1 and IB with α-IRS-1. F, IP with α-IRS-1 and IB with α-PY. G, IP with α-IRS-1 and IB with PI3K. H, IB with α-AKT1/2 antibodies. I, IB with α-PAKT. Data are mean ± SEM of 10 independent experiments. *HFD vs C, $P < .001$; **HFD vs L, $P < .001$.

$P < .05$ between HFD animals and lovastatin group; Fig. 2E and F). The IRS-1/PI3K association presented a similar behavior (C: 100% ± 0.5% vs HFD: 40% ± 4%, $P < .001$; HFD vs L: 121% ± 4%, $P < .001$; Fig. 2G). There were no differences in Akt protein levels between the groups (Fig. 2H). However, animals fed on the HFD presented a decrease in insulin-induced Akt serine phosphorylation, which was reversed after lovastatin treatment (C: 100% ± 5% vs HFD: 70% ± 2%, $P < .001$; HFD vs L: 95% ± 5%, $P < .001$; C vs L, $P < .05$; Fig. 2I).

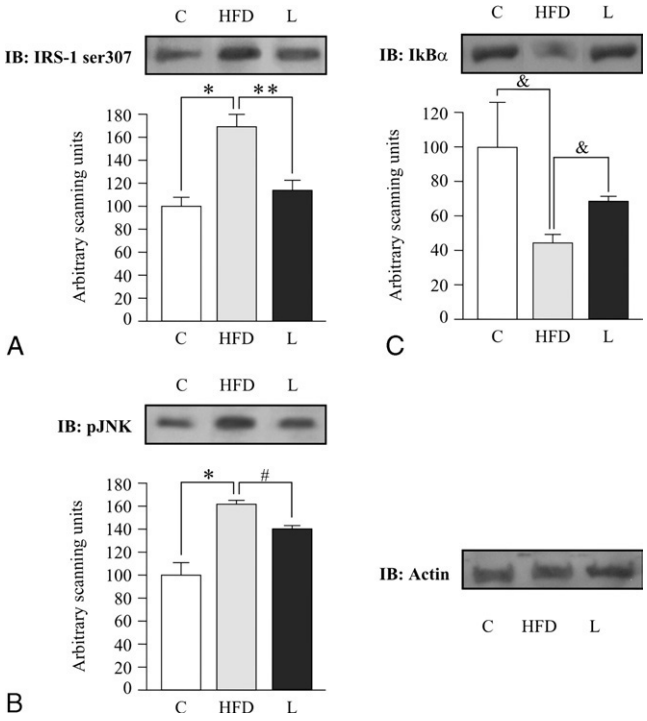

Fig. 4. Insulin signaling in the liver of control animals, animals fed on HFD for 4 weeks, and animals fed on HFD for 4 weeks and treated with lovastatin during the last week. A, IB with α-IRS-1^{ser307} antibodies. B, IB with α-JNK1. C, IB with IκBα. D, IB with antiactin antibodies. Data are mean ± SEM of 10 independent experiments. *HFD vs C, $P < .001$; **HFD vs L, $P < .001$; #HFD vs L, $P < .01$; &C vs HFD and HFD vs L, $P < .05$.

3.3. Insulin signaling in the skeletal muscle of controls, HFD-fed rats, and lovastatin-treated rats

There were no differences in IR protein levels in the skeletal muscle of controls, HFD-fed animals, and lovastatin-treated animals (Fig. 3A). Despite an apparent decrease in insulin-stimulated IR tyrosine phosphorylation in the HFD-fed animals and increase after use of lovastatin, there were no statistical differences between the groups (C: 100% ± 17%, HFD: 76% ± 4%, and L: 129% ± 23%; Fig. 3B). The IR serine phosphorylation in the muscle of HFD rats was increased when compared with control animals (C: 100% ± 8% vs HFD: 155% ± 8%, $P < .05$; Fig. 3C). In the group fed on the HFD and treated with lovastatin, the IR serine phosphorylation was similar to that of the control group (HFD: 155% ± 8% vs L: 116% ± 5%, $P < .05$; Fig. 3C). A similar behavior was seen for the association of IR and PTP1B (C: 100% ± 10% vs HFD: 140% ± 8%, $P < .05$; HFD: 140% ± 8% vs L: 105% ± 8%, $P < .05$; Fig. 3D). However, although no differences were observed in IRS-1 protein levels, the HFD-fed animals presented a significantly reduced insulin-stimulated IRS-1 tyrosine phosphorylation compared with control rats and an increased phosphorylation after the use of the drug (C: 100% ± 5% vs HFD: 51% ± 7%, $P < .001$; HFD vs L: 151% ± 3%, $P < .001$; Fig. 3E and F). The IRS-1/PI3K association presented similar results (C: 100% ± 1% vs HFD: 54% ± 20%, $P < .001$; HFD vs L: 179% ± 11%, $P < .001$; Fig. 3G). There were no differences in Akt protein levels between the groups (Fig. 3H); however, the HFD-fed rats presented a decrease in insulin-induced Akt serine phosphorylation that was reversed by the use of lovastatin (C: 100% ± 2% vs HFD: 64% ± 2%, $P < .001$; HFD vs L: 116% ± 7%, $P < .001$, Fig. 3I).

3.4. Phospho-IRS-1^{ser307}, phospho-JNK, and IκBα in the liver of controls, HFD-fed rats, and lovastatin-treated rats

The consumption of the HFD significantly increased the levels of phospho-IRS-1^{ser307} of the rats in the HFD group compared with those of the control rats, and the treatment with lovastatin decreased it to values similar to those of controls (C: 100% ± 5% vs HFD: 171% ± 20%, $P < .001$; HFD vs L: 113% ± 12%, $P < .001$; Fig. 4A). Similarly, the HFD-fed animals presented a significant increase in the levels of phospho-[Thr183]-JNK compared with controls; and this increase was reversed in the group of animals treated with lovastatin (C: 100% ± 7% vs HFD: 162% ± 3%, $P < .001$; HFD vs L: 141% ± 3%, $P < .01$; Fig. 4B). There was a decrease in IκBα in HFD-fed rats, suggesting an activation of IKKβ, which was reversed in the lovastatin group (C: 100% ± 6%, HFD: 44% ± 4%, and L: 67% ± 2%; Fig. 4C).

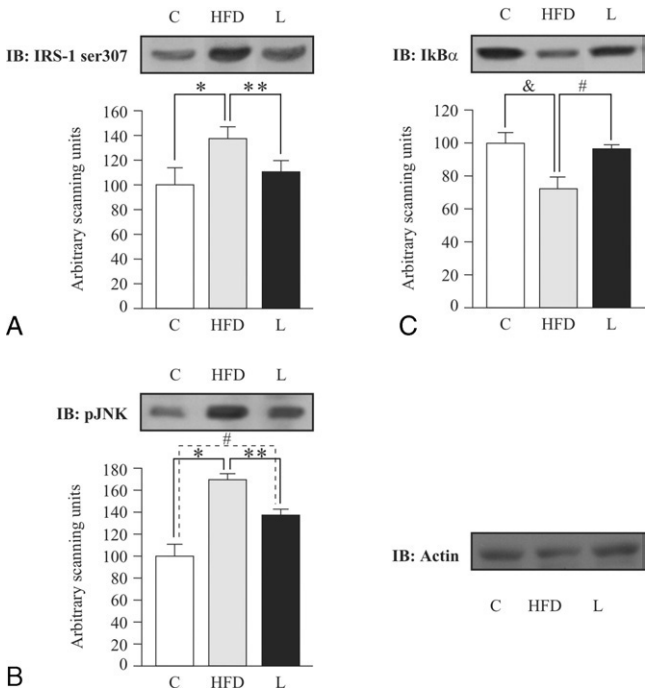

Fig. 5. Insulin signaling in the skeletal muscle of control animals, animals fed on HFD for 4 weeks, and animals fed on HFD for 4 weeks and treated with lovastatin during the last week. A, IB with α-IRS-1^{ser307} antibodies. B, IB with α-JNK1. C, IB with IκBα. D, IB with antiactin antibodies. Data are mean ± SEM of 10 independent experiments. *HFD vs C, $P < .001$; **HFD vs L, $P < .001$; #C vs L, $P < .01$; &C vs HFD and HFD vs L, $P < .05$.

3.4.1. Phospho-IRS-1^{ser307}, phospho-JNK, and IκBα in the skeletal muscle of controls, HFD-fed rats, and lovastatin-treated rats

The levels of phospho-IRS-1^{ser307} increased in the group of animals treated with the HFD compared with the control rats, and this increase was reversed in the lovastatin-treated rats compared with the controls (C: 100% ± 14% vs HFD: 137% ± 7%, $P < .001$; HFD vs L: 110% ± 12%, $P < .05$; Fig. 5A). Increased phospho-[Thr183]-JNK was observed in the HFD-fed rats compared with controls, and a reversal of this increase was also seen in the lovastatin-treated animals (C: 100% ± 6% vs HFD: 172% ± 2%, $P < .001$; HFD vs L: 138% ± 2%, $P < .001$; C vs L, $P < .01$; Fig. 5B). The use of the HFD decreased the IκBα in the HFD group compared with control animals, whereas treatment with lovastatin reversed this decrease to values similar to those of controls rats (C: 100% ± 7% vs HFD: 72% ± 6%, $P < .05$; HFD vs L: 97% ± 3%, $P < .01$; Fig. 5C).

4. Discussion

In the present study, we demonstrated that lovastatin, an 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, has insulin-sensitizing properties in HFD-fed rats. Treatment with lovastatin resulted in a marked improvement in insulin sensitivity characterized by an increase in the glucose disappearance rate during the insulin tolerance test. This increase in insulin sensitivity was associated with an increased insulin-stimulated IRS-1/PI3K/Akt pathway in the liver and muscle of HFD-fed rats in parallel with a decrease in the inflammatory pathway (JNK and IKK/IκB/NFκB) related to insulin resistance.

Our data showing an improvement in insulin sensitivity in statin-treated HFD-fed rats are in agreement with the results of 3 separate studies in humans [28-30]. In these studies, the authors used both simvastatin and cerivastatin and measured insulin action by euglycemic hyperinsulinemic glucose clamp and by homeostatic model assessment. In other studies, the improvement in insulin action by statin was not demonstrated, probably because of the lack of direct assessment of insulin action or inclusion of patients using other drugs that interfered in insulin action [31,32]. A retrospective cohort study using Saskatchewan health databases found that the use of statin was associated with a delay of 10 months in starting insulin treatment in patients with type 2 diabetes mellitus [33]. In Zucker rats, atorvastatin resulted in a dose-dependent increase in insulin sensitivity [21].

In the present study, the increase in insulin sensitivity induced by lovastatin was associated with an increase in insulin-stimulated IR tyrosine phosphorylation in parallel with a reduction in IR serine phosphorylation and also in the IR/PTP1B association in liver and muscle. Previous data showed that an increase in IR serine phosphorylation is associated with impairment in insulin-induced activation of its receptor [34-36]. Another mechanism that controls IR function is the activity of PTP1B, and previous studies have demonstrated an increase in IR/PTP1B association in situations of insulin resistance [36,37]. In this regard, the reversal of increased IR serine phosphorylation and also of IR/PTP1B association induced by lovastatin may have a role in the improved insulin sensitivity induced by this drug. Our data also show that lovastatin treatment was associated with an increase in the insulin-stimulated IRS-1/PI3K/Akt pathway. This finding is consistent with studies demonstrating statin-induced activation of Akt/PKB [38,39]. A previous study suggested that Akt/PKB activation by statin is PI3K dependent [39]. Our data showing an increase in the insulin-induced activation of upstream regulators of Akt/PKB, such as IRS-1 and PI3K, in the liver and muscle of HFD-fed rats reinforce this previous study. This effect of statin may have an important role in the improvement of insulin sensitivity in HFD-fed animals because this pathway has been implicated in glucose transport in muscle and in glycogen synthesis in liver and muscle [40,41].

Serine phosphorylation of IRS proteins is believed to be a major mechanism of suppression of IRS-1 activity that contributes to insulin resistance [40]. One possible kinase that might increase IRS-1 serine phosphorylation in HFD-fed rats is JNK [13]. Our data showing that statin reversed Ser^{307} IRS-1 phosphorylation and blunted JNK activation in liver and muscle provide new insights into the mechanism of statin improvement in insulin action.

Another mechanism involved in HFD-dependent insulin resistance is the activation of the proinflammatory IKK/IκB/NFκB pathway [14,16]. We have also demonstrated a decrease in the level of IκB in the liver and muscle of rats fed on a HFD compared with control animals, suggesting an activation of this pathway. Interestingly, the statin treatment also reversed this activation. Hence, in our study, the use of lovastatin in rats on HFD reversed the deleterious effects of the insulin signaling pathway with regard to JNK activation and IKK/IκB/NFκB pathway.

Clinical and experimental studies strongly support an anti-inflammatory role for statins. Liver is a major site of action of statins, particularly in the inhibition of cholesterol synthesis. However, the hepatocyte is also a source of proinflammatory mediators; and a decrease in the expression of these factors could be an important mechanism of the anti-inflammatory action of these drugs [42]. The cholesterol synthesis is a complex process that generates isoprenoids, such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate, that serve as lipid attachments for a variety of signaling molecules, such as guanosine-3-phosphate (GTP)-binding protein Ras and its related Ras-like proteins, such as Rho, Rac, Rab, Rap, and Ral [43,44]. The translocation of Ras to the membrane is necessary for activity and is dependent on farnesylation. Similarly, attachment of Rho and Rac to the membrane is required for activity; but in contrast to Ras, these factors undergo geranylgeranylation. Ras has been associated with cellular proliferation, Rac with

generation of reactive oxygen species, and Rho with activation of proinflammatory pathways [41,44]. Interestingly, Rho and Rac proteins can induce NFκB activity by a mechanism that induces phosphorylation of IκB and nuclear accumulation of NFκB [45,46]. In the case of NFκB, statins have also been shown to limit NFκB nuclear accumulation and DNA binding, perhaps via an increase in the expression of IκB [47,48]. In addition, statins have been shown to reduce the expression of c-jun [48], indicating a possible effect on JNK activity. Our data showing that statin blunted the activation of IKK/IκB/NFκB and JNK in liver and muscle of HFD-fed rats indicate a possible mechanism for their anti-inflammatory effect on insulin action. In addition to alterations described in this study induced by the HFD, other mechanisms can also induce insulin resistance [49-51]. We cannot exclude the possibility that lovastatin could also be acting through other mechanisms, contributing to its effect of improving insulin action.

In summary, statin treatment improves insulin sensitivity in HFD-fed rats by reversing the decrease in the insulin-stimulated IRS-1/PI3K/Akt pathway in the liver and muscle. The effect of statins on insulin action is further supported by our findings that HFD rats treated with statin demonstrate a reduction in IRS-1 serine phosphorylation, IKK/IκB/NFκB pathway, and JNK activity associated with an improvement in insulin action. Overall, these results provide important new insight into the mechanism of statin action in insulin sensitivity.

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