Reduction of JNK1 expression with antisense oligonucleotide improves adiposity in obese mice

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Reduction of JNK1 expression with antisense oligonucleotide improves adiposity in obese mice. Am J Physiol Endocrinol Metab 295: E436–E445, 2008. First published June 3, 2008; doi:10.1152/ajpendo.00629.2007.—To investigate the role of JNK1 in metabolism, male ob/ob and diet-induced obese mice were treated with a JNK1-specific antisense oligonucleotide (ASO) or control ASO at 25 mg/kg or saline twice/wk for 6 and 7 wk, respectively. JNK1 ASO reduced JNK1 mRNA and activity by 65–95% in liver and fat tissues in both models. Compared with controls, treatment with JNK1 ASO did not change food intake but lowered body weight, fat pad weight, and whole body fat content. The treatment increased metabolic rate. In addition, the treatment markedly reduced plasma cholesterol levels and improved liver steatosis and insulin sensitivity. These positive observations were accompanied by the following changes: 1) increased mRNA levels of AR-Bt1 and UCP1 by >60% in BAT, 2) reduced mRNA levels of ACC1, ACC2, FAS, SCD1, DGAT1, DGAT2, and RBP4 by 30– 60% in WAT, and 3) reduced mRNA levels of ACC1, FAS, G-6-Pase, and PKCe by 40–70% and increased levels of UCP2 and PPAREx by more than twofold in liver. JNK1 ASO-treated mice demonstrated reduced levels of pIRS-1 Ser302 and pIRS-1 Ser307 and increased levels of pAkt Ser473 in liver and fat in response to insulin. JNK1 ASO-transfected mouse hepatocytes showed decreased rates of de novo sterol and fatty acid synthesis and an increased rate of fatty acid oxidation. These results indicate that inhibition of JNK1 expression in major peripheral tissues can improve adiposity via increasing fuel combustion and decreasing lipogenesis and could therefore provide clinical benefit for the treatment of obesity and related metabolic abnormalities.

insulin sensitivity; metabolic rate; gene expression; antisense

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MATERIALS AND METHODS

ASOs. Two JNK1 ASOs (named as JNK1 ASO and JNK1 ASO no. 2) specifically targeting JNK1 mRNA by binding to distinct regions in the JNK1 mRNA were used for the current study. Their chemical composition and mechanism of action have been described previously (12, 25, 34). A control ASO, which has the same chemical composition as JNK1 ASOs but no complementarity to any known gene sequence in public databases, was also used in the study.

Animal studies. The following investigations were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee at Isis Pharmaceuticals and approved by the committee.

Six-week-old male C57BL6J-Lepob/Lepob (ob/ob) mice were purchased from Jackson Laboratories (Bar Harbor, ME) and housed three animals per cage at 22-25°C with a 12:12-h light-dark cycle and free access to food and water. After 6 days of acclimation with feeding on rodent chow (Lab Diet No. 5015; Purina, St. Louis, MO), BW and body composition were measured by an Echo MRI system (Echo Medical System, Houston, TX), and tail-snip baseline measurement of blood biochemistry was performed (see below for assay methods). The animals were then randomized to three treatment groups (n = 6/group) and injected with JNK1 ASO or control ASO at 25 mg/kg BW or saline twice/wk for 6 wk. ASOs were dissolved in 0.9% saline and administered subcutaneously.

To extend the investigation on the metabolic effects of antisense suppression of JNK1 expression, DIO mice were also treated (injected subcutaneously) with JNK1 ASOs. Six-week-old male C57BL/6J mice were fed a diet containing 58% kcal fat (Research diet D12330; Research Diets, New Brunswick, NJ) for 15 wk to induce obesity and insulin resistance. The animals were then divided into different groups (n = 6/group) and treated with JNK1 ASO or control ASO at a dose of 25 mg/kg BW or saline twice/wk for 7 wk. To confirm an antisense mechanism of action, a second JNK1 ASO (JNK1 ASO no. 2) that targets a different region of JNK1 mRNA was also included in the study to treat a group of mice at the same dose.

During the treatment, weekly food intake and BW were monitored, and body composition and other metabolic measurements were conducted (see below). At the end of the studies, animals were killed. Blood samples were collected by cardiac puncture, and tissues were dissected, weighed, and then saved for further analysis.

For the insulin challenge study, DIO mice were injected subcutaneously with JNK1 ASO or control ASO at a dose of 37.5 mg/kg BW twice/wk for 3 wk. The mice were then fasted overnight and given a bolus intraperitoneal injection of insulin at 2 U/kg BW or vehicle. The animals were then killed, and liver and epididymal white adipose tissue (WAT) were collected and quickly frozen in liquid N2 for further analysis.

Insulin tolerance test and oral glucose tolerance test. Both insulin tolerance test (ITT) and oral glucose tolerance test (OGTT) were conducted in ob/ob mice. ITT was performed 4 h after food withdrawal, and OGTT was performed after overnight food withdrawal. At baseline (0 min), tail blood glucose levels were measured followed by intraperitoneal injection of insulin at 0.8 U/kg BW (R-Insulin; Lilly Research Laboratories, Indianapolis, IN) for ITT or oral administration of glucose at 0.75 g/kg BW for OGTT. Tail blood glucose was then measured at 15- or 30-min intervals for ≤2 h using a Glucometer (Abbott Laboratories, Bedford, MA).

Fig. 1. JNK1 antisense oligonucleotides (ASOs) specifically reduced JNK1 expression in liver and fat. Male ob/ob and diet-induced obese (DIO) mice were treated with JNK1 ASO or control ASO at 25 mg/kg body wt (BW) or with saline twice/wk for 6 and 7 wk. Total RNA was prepared from liver, epididymal white fat [white adipose tissue (WAT)], and interscapular brown fat [brown adipose tissue (BAT)] and used for real-time quantitative RT-PCR analysis to evaluate the expression of JNK1 and JNK2 genes. Tissue homogenates prepared from liver and fat tissues were used for analysis of JNK1 activity with a JNK activity measurement kit (see text for details). JNK1 ASO dramatically reduced JNK1 mRNA in liver, WAT, and BAT (A), with no effect on JNK2 gene expression (B) in ob/ob mice. C: reduction of JNK1 mRNA levels resulted in similar degree of reduction in JNK1 activity. D: dramatic reduction of JNK1 gene expression was also detected in DIO mice treated with either JNK1 ASO or JNK1 ASO no. 2. Data are expressed as means ± SE (n = 5–6). **P < 0.01 vs. saline control; ##P < 0.01 vs. ASO control.
Metabolic rate measurement. Metabolic rate in the mice was measured for a 24-h period using indirect calorimetry (Oxymax System; Columbus Instruments, Columbus, OH).

Biochemical analysis. Plasma insulin was measured with an insulin ELISA kit (ALPCO Diagnostics, Windham, NH). Plasma glucose, total cholesterol and free fatty acid concentrations, and plasma transaminase [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)] activities were measured with a biochemistry analyzer (Olympus AU400; Olympus American, Melville, NY). Liver triglyceride (TG) was measured as previously described (9).

Plasma lipoprotein analysis. Plasma lipoprotein and cholesterol profiling in pooled samples of JNK1 ASO group and control ASO group from the study in ob/ob mice was performed as described (7, 34).

Histological analysis. For hemotoxylin and eosin (H & E) staining, a piece of liver, epididymal WAT, and intrascapular brown adipose tissue (BAT) from ob/ob mice were fixed in 10% buffered formalin and embedded in paraffin wax. For Oil Red O staining, liver samples were collected in embedding medium. Multiple adjacent 4-μm sections were cut and mounted on glass slides. After dehydration, the sections were stained. Images of the histological sections were analyzed.

JNK1 activity assay and Western immunoblotting analysis. Tissue JNK1 activity was analyzed with a JNK activity assay kit (BioVision, Mountain View, CA) according to the manufacturer’s instructions. Briefly, precleared tissue homogenates were immunoprecipitated with a JNK antibody (against both JNK1 and JNK3 but not JNK2). The immunoprecipitates were then used to phosphorylate c-Jun in the presence of ATP. The phosphorylated c-Jun was detected with Western immunoblotting. Because JNK3 is not expressed in liver and fat tissues, the JNK activity in this assay essentially reflects JNK1 activity in these tissues.

For the insulin-signaling assays, equal amounts of total proteins contained in precleared fat or liver homogenates were separated on gradient SDS-PAGE gels (Bio-Rad, Hercules, CA) according to the manufacturer’s instructions. Data are expressed as means ± SE (n = 3–6). *P < 0.05 and **P < 0.01 vs. saline controls; #P < 0.05 and ##P < 0.01 vs. ASO controls.

Results

JNK1 ASO specifically reduced JNK 1 expression. Compared with saline controls, treatment with JNK1 ASO, but not control ASO, reduced JNK1 gene expression in ob/ob mice by >80% in both liver and WAT and by 78% in BAT (Fig. 1A) but did not cause a compensatory change in JNK2 gene expression in any of these tissues (Fig. 1B). Enzymatic assay showed that JNK1 ASO resulted in a decrease in JNK1 activity.
by >95, 80, and 65% in liver, WAT, and BAT, respectively (Fig. 1C). In DIO mice, JNK1 ASO treatment reduced JNK1 mRNA by 78, 66, and 70% in liver, WAT, and BAT, respectively (Fig. 1D). Treatment with the second JNK1 ASO (JNK ASO no. 2) caused similar reduction of JNK1 expression in these tissues (Fig. 1D).

**JNK1 ASO treatment lowered BW and adiposity.** Compared with controls, JNK1 ASO-treated ob/ob mice showed ~20% less BW gain after 6 wk of treatment (Fig. 2A). Significantly lower BW gain was observed in these mice after only 5 wk of treatment. BW in the ASO group began to diverge from that in control groups after 3 wk of treatment (Fig. 2A), which is temporally consistent with the observation that it takes about 2 wk for ASO to reduce the target gene to a substantial degree with the current treatment regimen (8, 25, 34). Although ASO treatment did not change food intake, JNK1 ASO-treated mice showed a >14% lower feed efficiency (Fig. 2B), indicating an increased metabolic rate in these mice. JNK1 ASO-treated mice also showed significantly lower epididymal fat depot weight (Fig. 2C) and percentage of body fat content (Fig. 2D), with no difference in lean body mass (data not shown). Histological examination found a reduction in adipocyte size in both WAT and BAT in the JNK1 ASO treatment group vs. controls (Fig. 2E). Indirect calorimetry measurement showed an increased metabolic rate, as reflected in a higher V\(\dot{O}_2\), in JNK1 ASO-treated mice compared with controls (Fig. 3, A and B), with an unchanged respiratory quotient (data not shown), after only 3 wk of treatment.

In DIO mice, treatment with either of the two JNK1 ASOs did not change food intake (Fig. 4A) compared with controls. However, whereas treatment with either saline or control ASO for 7 wk did not change BW, treatment with JNK1 ASOs lowered BW by >10%, which resulted in a significant difference from the controls (Fig. 4B). Furthermore, both JNK1 ASO-treated groups also showed >35% lower epididymal fat depot weight and >20% lower percentage body fat content (Fig. 4, C and D), with no difference in lean body mass (data not shown). Indirect calorimetry measurement also found that the JNK1 ASO-treated mice had higher V\(\dot{O}_2\) than controls (Fig. 4E), with an unchanged respiratory quotient (Fig. 4F).

**JNK1 ASO treatment improved hepatic steatosis.** Compared with control ASO, JNK1 ASO treatment significantly reduced liver weight in ob/ob mice (Fig. 5A). To examine whether the ASO treatment improved liver steatosis, both liver TG content and histology were analyzed. Liver TG content was found to be >40% lower in the JNK1 ASO-treated group than in controls in ob/ob mice (Fig. 5A). Histological examination with both H & E and Oil Red O staining confirmed a significant improvement in liver steatosis in JNK1 ASO-treated mice (smaller and fewer fat droplets than those in controls; Fig. 5B). In addition, the histological examination did not reveal any sign of ASO-related liver damage. Rather, improved liver steatosis was accompanied by improved liver function, as assessed by plasma ALT and AST measurements (Table 1). JNK1 ASO treatment also lowered liver TG content by >40% in DIO mice (Fig. 5C) without causing liver toxicity, as assessed by plasma ALT and AST levels (Table 1).

**JNK1 ASO treatment lowered plasma cholesterol levels.** Compared with controls, treatment with JNK1 ASO for 3.5 (fasted state) and 6 wk (fed state) lowered plasma total cholesterol levels by 18.5 and 40%, respectively, in ob/ob mice (Table 1). In DIO mice in the fasted state, the ASO treatment for 6 wk lowered plasma total cholesterol by 35% (Table 1). Lipoprotein profile analysis showed that JNK1 ASO treatment lowered the cholesterol content in all three major lipoprotein fractions, namely VLDL, LDL, and HDL cholesterol (Fig. 5D). To investigate whether lowered plasma cholesterol levels with ASO treatment were caused by a reduction in hepatic synthesis and secretion, mouse primary hepatocytes were treated with JNK1 ASO, and then de novo sterol synthesis was determined by measuring the incorporation of \(^{14}C\)acetate into sterols. JNK1 ASO-transfected hepatocytes showed reduced de novo sterol synthesis by 13% compared with controls (Fig. 5E). Furthermore, gene expression analysis found that JNK1 ASO-treated mice had significantly lower hepatic apolipoprotein B-100 (apoB-100) mRNA levels vs. controls (Table 2). Reduction of apoB-100 expression has been well demonstrated to reduce plasma cholesterol levels in rodents and several other species. Therefore, decreased plasma cholesterol levels may be (at least in part) due to decreased hepatic cholesterol secretion.

**JNK1 ASO treatment lowered plasma glucose and insulin levels and improved insulin sensitivity.** Treatment with JNK1 ASO also lowered plasma glucose and insulin levels in both fed and fasted states compared with controls. These effects were very significant, and glucose levels in both models as well as insulin levels in DIO mice were completely normalized, and insulin levels in ob/ob mice were lowered by >50% after 6 wk of treatment (Table 1). To confirm that JNK1 ASO had an insulin-sensitizing effect, both ITT and OGTT were conducted.
in ob/ob mice. Dramatically improved glucose excursion was found during both the ITT (Fig. 6A) and OGTT (Fig. 6B). In addition, a markedly lower level of plasma insulin was observed during OGTT in the JNK1 ASO treatment group vs. controls (Fig. 6B, inset). These data demonstrate that reduction of JNK1 expression with JNK1 ASO significantly improved insulin sensitivity in both models. A trend toward a decrease, although not significant, in plasma free fatty acids and resistin in ob/ob mice treated with JNK1 ASO was observed (Table 1). A trend toward an increase in plasma adiponectin in JNK1 ASO-treated ob/ob mice was also observed (Table 1), indicating that adiponectin secretion per unit of fat tissue was increased considering that total fat content in these mice was decreased.

**JNK1 ASO treatment caused significant changes in the expression of the metabolic genes in liver and fat.** To explore the possible mechanism at the molecular level on how antisense reduction of JNK1 expression caused the above-described positive metabolic effects, quantitative RT-PCR was performed to analyze the expression profile of the metabolic genes in liver and fat. JNK1 ASO treatment was found to increase the mRNA levels of adrenocorticotropin-β3 (AR-β3) and uncoupling protein 1 (UCP1) by 62 and 59%, respectively, in BAT (Fig. 3C). These data are consistent with increased metabolic rate and decreased brown adipocyte size and smaller fat droplets in the fat cells in the ASO-treated ob/ob mice.

In addition, JNK1 ASO treatment caused downregulation of lipogenic gene expression in WAT. The mRNA levels of the lipogenic genes, including acetyl-CoA carboxylase 1 (ACC1), ACC2, fatty acid synthase (FAS), and stearoyl-CoA desaturase-1, were reduced dramatically in the JNK1 ASO-treated ob/ob mice compared with the controls (Table 2). The expression of glycerol kinase, acyl-CoA:diacylglycerol acyltransferase-1 (DGAT1), and DGAT2 was also significantly reduced in these ASO-treated mice (Table 2). However, the treatment did not cause changes in the expression of hormone-sensitive lipase and adipose triglyceride lipase, two key lipolytic genes, in this tissue (Table 2). These data suggest that JNK1 ASO treatment reduced lipogenesis and possibly increased fatty acid oxidation but did not alter lipolysis in WAT. These findings are also consistent with increased whole body metabolic rate and decreased fat pad weight and whole body adiposity.

Fig. 4. JNK1 ASO treatment lowered BW, improved adiposity, and increased metabolic rate in DIO mice. A: compared with controls, treatment with either JNK1 ASO did not change food intake. B: whereas treatment with either saline or control ASO for 7 wk did not change BW, treatment with both JNK1 ASOs lowered BW. Treatment with the JNK1 ASOs also lowered epididymal fat depot weight (C) and %body fat content (D). Indirect calorimetry measurement demonstrated a higher VO2 during dark phase in the ASO-treated mice than controls (E), with an unchanged respiratory quotient (F). Data are expressed as means ± SE (n = 6). #P < 0.05 and ##P < 0.01 vs. saline controls; *P < 0.05 and **P < 0.01 vs. ASO controls.
A marked reduction in the expression of ATP-citrate lyase (ACL), ACC1, and FAS was also found in liver from JNK1 ASO-treated ob/ob mice (Table 2), indicating decreased de novo fatty acid synthesis. However, the treatment did not change the expression of glycerol kinase, DGAT1, and DGAT2 (Table 2), indicating an unchanged hepatic TG synthesis capacity. Although the expression of carnitine palmitoyltransferase I was not changed (data not shown), the expression of both peroxisome proliferator-activated receptor-α (PPARα) and UCP2 in the JNK1 ASO-treated ob/ob mice was greater than twofold higher than that in controls (Table 2), suggesting increased hepatic substrate oxidation. These changes in hepatic gene expression are consistent with decreased hepatic TG content and improved liver steatosis.

Furthermore, JNK1 ASO treatment markedly reduced glucose-6-phosphatase and protein kinase Cε (PKCε) expression in liver and retinol-binding protein 4 (RBP4) expression in WAT and increased hepatic expression of glucokinase and glycogen synthase (Table 2). These data suggest a reduction in hepatic glucose output and may partially account for the improved whole body insulin sensitivity after reduction of JNK1 expression.

The expression of representative genes in DIO mice was also analyzed. In addition to changes similar to what were seen in ob/ob mice, an ~70% increase in the expression of both UCP2 and UCP3 in WAT was found in JNK1 ASO-treated DIO mice vs. controls (Table 3), further suggesting that reduction of JNK1 expression not only inhibits lipogenesis but may also increase metabolic rate. The difference in the expression of some genes such as ACCs in liver and UCPs in WAT between ob/ob mice and DIO mice after JNK1 ASO treatment may be due to their different genetic backgrounds and/or diet feedings.

JNK1 ASO increased fatty acid oxidation and decreased de novo fatty acid synthesis in mouse hepatocytes. To confirm that JNK1 ASO-caused changes in gene expression translated into functional effects, cultured mouse primary hepatocytes had lowered de novo sterol synthesis rate (E) and fatty acid synthesis rate (F) and increased fatty acid oxidation rate (G) vs. controls. Data are expressed as means ± SE (n = 5–6 for in vivo assays; n = 3 for in vitro assays). *P < 0.05 and **P < 0.01 vs. saline controls; #P < 0.05 and ##P < 0.01 vs. ASO controls.

Fig. 5. JNK1 ASO treatment improved liver steatosis and plasma lipoprotein profile. A: treatment with JNK1 ASO for 6 wk not only lowered liver TG content but also lowered liver weight in ob/ob mice. B: improved liver steatosis was confirmed by histological examination showing smaller and fewer fat droplets with both H&E and Oil Red O staining in the JNK1 ASO-treated mice than in controls. C: JNK1 ASO treatment also lowered liver triglyceride (TG) content in DIO mice. D: in addition, JNK1 ASO lowered plasma total cholesterol levels and improved plasma lipoprotein profile. In vitro assays showed that JNK1 ASO-transfected mouse primary hepatocytes had lowered de novo sterol synthesis rate (E) and fatty acid synthesis rate (F) and increased fatty acid oxidation rate (G) vs. controls. Data are expressed as means ± SE (n = 5–6 for in vivo assays; n = 3 for in vitro assays). *P < 0.05 and **P < 0.01 vs. saline controls; #P < 0.05 and ##P < 0.01 vs. ASO controls.
glucokinase; G-6-Pase, glucose-6-phosphatase; GS, glycogen synthase; PKC ε; RBP4, retinol-binding protein 4; apoB-100, apolipoprotein

Table 2. Gene expression in liver, WAT, and BAT of ob/ob mice with different treatments

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Data are expressed as means ± SE (n = 5–6). WAT, white adipose tissue; BAT, brown adipose tissue; ACL, ATP-citrate lyase; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; GyK, glycerol kinase; SCD-1, stearoyl-CoA desaturase-1; DGAT1, acyl-CoA:diacylglycerol acyltransferase; HSL, hormone-sensitive lipase; ATGL, adipose triglyceride lipase; PPARα, peroxisome proliferator-activated receptor-α; UCP, uncoupling protein; AR-β1, adrenocortical β1; GK, glucokinase; G-6-Pase, glucose-6-phosphatase; GS, glycogen synthase; PKCε, protein kinase Cε; RBP4, retinol-binding protein 4; apoB-100, apolipoprotein B-100. The analysis was performed with quantitative RT-PCR. Total RNA was isolated from tissues of ob/ob mice treated with JNK1 ASO or control ASO at 25 mg/kg body wt or with saline twice/wk for 6 wk. *P < 0.05 and †P < 0.01 when compared with either control group.
analyzed. As expected, a much higher level of pAkt Ser\textsuperscript{473} was found in JNK1 ASO-treated mice vs. controls after insulin challenge, although its basal level was lower in the JNK1 ASO-treated mice (Fig. 6D); the latter was probably due to the lower plasma insulin levels in these mice. These data indicate that reduction of JNK1 expression with JNK1 ASO improved insulin signaling activity.

**DISCUSSION**

To investigate the role of JNK1 in metabolism and energy homeostasis, both genetic and dietary mouse models of obesity were chronically treated with JNK1 ASO. JNK1 ASO treatment markedly and specifically reduced the gene expression of JNK1 in both liver and fat tissues, which resulted in a dramatic reduction of JNK1 activity in these tissues. The treatment markedly lowered fed and fasting plasma glucose and insulin levels, improved glucose and insulin tolerance, improved liver steatosis, and lowered plasma cholesterol levels. These data indicate that specific inhibition of JNK1 expression and activity with ASO in the two major metabolic tissues improved adiposity and related metabolic abnormalities in these models.

Whereas a decrease in BW gain was reported in JNK1 knockout mice when fed a normal chow (31) or a high-fat diet (14), those authors did not show any mechanistic data on how deficiency of JNK1 caused lower BW or BW gain, although they reported a slight but insignificant drop in food intake. Studies using small molecules or peptides to directly or indirectly inhibit tissue total JNK activity either did not report the effect of the treatments on BW or adiposity (2, 19, 24) or found other side effects compared with controls. The treatment markedly lowered fed and fasting plasma glucose and insulin levels, improved glucose and insulin tolerance, improved liver steatosis, and lowered plasma cholesterol levels. These data indicate that specific inhibition of JNK1 expression and activity with ASO in the two major metabolic tissues improved adiposity and related metabolic abnormalities in these models.

Table 3. *Gene expression in liver, WAT, and BAT of DIO mice with different treatments*

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<td>100.0±10.5</td>
<td>100.0±5.8</td>
<td>52.0±3.2*</td>
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<td>100.0±6.6</td>
<td>108±6.1</td>
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<td>106.7±11.1</td>
<td>169.2±12.9*</td>
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<td>98.3±8.8</td>
<td>167.8±10.9*</td>
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Data are expressed as means ± SE (n = 6). The analysis was performed with quantitative RT-PCR. Total RNA was isolated from tissues of DIO mice treated with JNK1 ASO or control ASO at 25 mg/kg body wt or with saline twice/wk for 7 wk. *P < 0.01 when compared with either control group.

**Fig. 6.** JNK1 ASO treatment improved glucose tolerance and insulin sensitivity. Insulin tolerance test (ITT) and oral glucose tolerance test (OGTT) were conducted in *ob/ob* mice after 5 and 6 wk of treatment, respectively. JNK1 ASO treatment markedly lowered glucose excursion during both ITT (A) and OGTT (B). A lower plasma insulin level was also found during OGTT in the JNK1 ASO-treated mice (B, inset). Immunoblotting analysis found that the JNK1 ASO-treated mice had lower levels of both phosphorylated insulin receptor substrate-1 (pIRS-1) Ser\textsuperscript{307} (C) and pIRS-1 Ser\textsuperscript{302} (D) and enhanced response of Akt to insulin showing a higher level of pAkt Ser\textsuperscript{473} compared with controls, although their basal level of pAkt Ser\textsuperscript{473} (without insulin challenge) was lower (D) in both WAT and liver, indicating improved insulin signaling in these mice. For the immunoblotting assays, pooled samples with each from 3–4 mice were used. Data are expressed as means ± SE (n = 5–6). **P < 0.01 vs. saline controls; ***P < 0.01 vs. ASO controls.
no change in BW (19). A recent study using Ad-shJNK1 to specifically reduce JNK1 activity in liver did not report the effect of the treatment on BW or adiposity (32). The duration of these studies was only about 1–2 wk, which is too short to see the potential effect (if any) on BW. In the current study, we found that treatment with JNK1 ASO lowered BW or BW gain and adiposity. The treatment did not change food intake but decreased feed efficiency. Indirect calorimetry revealed an increased metabolic rate in the JNK1 ASO-treated mice. Quantitative RT-PCR analysis found increased gene expression in BAT from these mice of AR-β3 and UCP1, two key genes involved in catabolism and fuel combustion in rodents (10, 11, 17). Increased expression of PPARα, UCP2, and UCP3 and decreased expression of ACC2 were also found in either liver or WAT, lending further support to the finding of an increased metabolic rate (6, 15, 22, 23, 27). In addition, a profound decrease in the expression of lipogenic genes and unchanged expression of two key lipolytic genes, hormone-sensitive lipase and adipose triglyceride lipase, were found in WAT, suggesting decreased lipogenesis and unchanged lipolysis after reduction of JNK1 expression in this tissue. A marked decrease in expression of ACC1 and FAS, two key genes involved in de novo fatty acid synthesis, in liver was also detected. Furthermore, increased fatty acid oxidation and decreased de novo fatty acid synthesis were demonstrated directly in JNK1 ASO-transfected hepatocytes. Taken together, these data demonstrate that decreased BW or BW gain and lowered adiposity in the JNK1 ASO-treated mice were attributable to increased fuel combustion/metabolic rate and decreased lipogenesis.

Improved liver steatosis and reduced plasma lipid levels have not been reported either in JNK1-deficient ob/ob mice or in obese mice treated with small molecule JNK inhibitors or JNK-inhibitory peptides (2, 14, 19, 24). A previous study indicated that, compared with wild controls, deficiency of JNK1 prevented liver fatty infiltration but did not change plasma cholesterol levels when the mice were fed a high-fat/high-caloric diet (29). The current study demonstrated that antisense reduction of JNK1 activity not only dramatically lowered liver TG content and improved hepatic steatosis but also markedly improved plasma cholesterol levels in both genetically obese and DIO mice. These changes were accompanied by increased expression of hepatic UCP2 and PPARα genes and decreased expression of the key hepatic lipogenic genes, including ACL, ACC1, and FAS. These changes in gene expression imply an increased shunting of citrate into the TCA cycle and electron transport chain for oxidation and a reduced fatty acid synthesis and increased fatty acid oxidation in JNK1 ASO-treated mice may be due to increased hepatic substrate oxidation and decreased hepatic lipogenesis. This was supported by in vitro studies that showed decreased de novo sterol synthesis and fatty acid synthesis and increased fatty acid oxidation in JNK1 ASO-transfected hepatocytes. In addition, decreased expression of hepatic apoB-100 in JNK1 ASO-treated mice was found. Reduction of hepatic apoB-100 expression lowers plasma cholesterol levels in different models of hyperlipidemia due to reduced hepatic cholesterol synthesis and export (7).

Studies demonstrated that JNKs can interact directly with IRS-1 and cause its phosphorylation at serine positions and inhibit its phosphorylation at tyrosine positions, thereby inhibiting insulin signaling (1, 28). Increased JNK activity was observed in dietary and genetic mouse models of obesity (14). Inhibition of total JNK activity using small molecule JNK inhibitors or peptide inhibitors showed enhanced insulin signaling activity in vitro (24) and improved insulin sensitivity in vivo (2, 19). However, these inhibitors lack selectivity against different JNK isoforms. In this study, we found that specific reduction of JNK1 expression with ASO in liver and fat decreased the levels of both pIRS-1 Ser302 and pIRS-1 Ser307 and increased insulin signaling in both tissues and profoundly improved insulin sensitivity with almost complete normalization of plasma glucose and insulin levels and a dramatic reduction of glucose excursion during ITT and OGTT. The positive change in insulin sensitivity was also found to be accompanied by increased expression of hepatic glucokinase and glycogen synthase, decreased expression of hepatic glucose-6-phosphatase and PKCε, and reduced expression of RBP4 in WAT. Thus, the direct positive effect of JNK1 reduction on insulin signaling and the changes in above gene expression, coupled with the improvement in adiposity, would be expected to result in the observed improvements in insulin sensitivity in the study.

Obesity, which is tightly associated with type 2 diabetes, hyperlipidemia, and fatty liver diseases, has become an epidemic worldwide. However, effective drugs for the treatment of these diseases are scarce. Therefore, an urgent need continues to exist for identifying new therapeutic targets and drug platforms for these disorders. This study demonstrates that JNK1 plays an important role in metabolism and energy homeostasis and that antisense reduction of its expression in liver and fat increases metabolic rate and improves BW and adiposity, which is accompanied by improved liver steatosis, hypercholesterolemia, and insulin sensitivity in both genetically leptin-deficient and DIO mice. Therefore, JNK1 could be a potential therapeutic target for the treatment of obesity and related metabolic disorders.

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REFERENCES