A central role for JNK in obesity and insulin resistance

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Obesity is closely associated with insulin resistance and establishes the leading risk factor for type 2 diabetes mellitus, yet the molecular mechanisms of this association are poorly understood. The c-Jun amino-terminal kinases (JNKs) can interfere with insulin action in cultured cells and are activated by inflammatory cytokines and free fatty acids, molecules that have been implicated in the development of type 2 diabetes. Here we show that JNK activity is abnormally elevated in obesity. Furthermore, an absence of JNK1 results in decreased adiposity, significantly improved insulin sensitivity and enhanced insulin receptor signalling capacity in two different models of mouse obesity. Thus, JNK is a crucial mediator of obesity and insulin resistance and a potential target for therapeutics.

Obesity and type 2 diabetes are the most prevalent and serious metabolic diseases; they affect more than 50% of adults in the USA. These conditions are associated with a chronic inflammatory response characterized by abnormal cytokine production, increased acute-phase reactants and other stress-induced molecules. Many of these alterations seem to be initiated and to reside within adipose tissue, an unusual site for inflammation. Elevated production of tumour necrosis factor (TNF-α) by adipose tissue decreases sensitivity to insulin and has been detected in several experimental obesity models and obese humans. Free fatty acids (FFAs) are also implicated in the aetiology of obesity-induced insulin resistance, although the molecular pathways involved in their action remain unclear. Because both TNF-α and FFAs are potent JNK activators, we asked whether obesity is associated with alterations in stress-activated and inflammatory responses through this signalling pathway and whether JNKs are causally linked to aberrant metabolic control in this state.

We examined JNK activity in liver, muscle and adipose tissues of various models of obesity compared with lean controls to determine whether obesity activates the JNK pathway. In both dietary and genetic (ob/ob) models of obesity, there was a significant increase in total JNK activity in all tissues tested (Fig. 1a). In these tissues there was no apparent difference in the expression of either JNK1 or JNK2 polypeptides, suggesting that the activity of one or both of these kinases is increased in response to obesity.

To test the functional significance of this alteration in the pathogenesis of obesity, insulin resistance and type 2 diabetes, we induced obesity in mice lacking either JNK1 (Jnk1−/−) or JNK2 (Jnk2−/−). Jnk1−/− or Jnk2−/− mice and their control littermates (Jnk1+/+ or Jnk1+/− and Jnk2+/+ or Jnk2+/−) were placed on a high-fat (50% of total calories derived from fat) and high-caloric diet (5286 kcal kg−1; Bioserve, Frenchtown, NJ, USA) along with a control group of each genotype on a standard diet. On the high-fat diet, both controls and Jnk2−/− mice developed marked obesity in comparison with mice kept on standard diet (Fig. 1b and c). Weight gain in Jnk2+/+, Jnk2−/− and Jnk2−/− animals was indistinguishable on either standard or high-fat diet. However, weight gain on both standard and high-fat diets was significantly decreased for the Jnk1−/− group (Fig. 1d and e). Although animals with one targeted allele of Jnk1 (Jnk1+/−) had a body weight intermediate between that of wild-type and Jnk1−/− mice maintained on either diet, these differences did not reach statistical significance (Fig. 1e).

We assessed whether these differences in weight gain were related to alterations in adiposity. Sections of adipose tissue from Jnk1−/− mice exhibited decreased adipocyte size relative to wild-type controls (Fig. 2a). This was not observed in Jnk2−/− mice maintained on either diet, these alterations seem to be initiated and to reside within adipose tissue, an unusual site for inflammation. Elevated production of tumour necrosis factor (TNF)−α by adipose tissue decreases sensitivity to insulin and has been detected in several experimental obesity models and obese humans. Free fatty acids (FFAs) are also implicated in the aetiology of obesity-induced insulin resistance, although the molecular pathways involved in their action remain unclear. Because both TNF-α and FFAs are potent JNK activators, we asked whether obesity is associated with alterations in stress-activated and inflammatory responses through this signalling pathway and whether JNKs are causally linked to aberrant metabolic control in this state.

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of the subcutaneous fat depot was decreased by 33% in Jnk1−/− mice compared with wild-type controls (Fig. 2b). Surprisingly, the weight of the epididymal fat pad was higher in the obese Jnk1−/− group than in the wild-type controls, indicating a redistribution of adipose depots (Fig. 2b). No difference in fat pad weight was evident between Jnk2−/− and wild-type mice in either condition (data not shown). To investigate systemic alterations in adiposity, we next examined total body composition. These studies demonstrated significantly decreased total body adiposity in Jnk1−/− mice compared with controls (Fig. 2c). In contrast, the body composition of the Jnk2−/− group was indistinguishable from wild-type controls (data not shown).

To address alternative causes for decreased body weight in Jnk1−/− mice, we compared lipid metabolism, food intake, intestinal lipid absorption and core body temperature of Jnk1−/− and Jnk1+/+ mice. No significant differences were observed in plasma triglyceride, cholesterol and FFA concentrations between genotypes (data not shown). Examination of faecal lipid content also did not reveal any differences, thus excluding changes in intestinal lipid absorption (Fig. 2d). There was a small and statistically insignificant decrease in daily food intake (0.46 g d−1) and increase (0.32°C) in core body temperature in obese Jnk1−/− mice compared with wild-type mice (Supplementary Fig. 1a and b). Although we cannot rule out the possibility that these small changes might contribute to decreased weight gain, the results strongly suggest that the deficiency in JNK1 is associated primarily with decreased adipocyte size, decreased adiposity and adipose redistribution in the context of dietary obesity.

Adipose tissue can have a substantial impact on systemic glucose homeostasis through production of bioactive molecules. We examined serum concentrations of adipocyte-derived secreted proteins with postulated roles in obesity and insulin action12-15. ACRP30 (30-kDa adipocyte complement-related protein)/adiponectin concentrations in the obese Jnk1−/− mice were significantly higher than in Jnk1+/+ controls (Fig. 2e). In contrast, the concentrations of resistin were lower in Jnk1−/− mice than in Jnk1+/+ animals (Fig. 2f). Because recent studies have indicated a role for adiponectin as a mediator of fatty-acid oxidation and hepatic insulin

Figure 2 Adipose tissue morphology and adiposity in Jnk1−/− mice and wild-type controls. a, b, Histological sections of epididymal fat pads (original magnification x50) (a) and subcutaneous (SC) and epididymal (EP) fat pad weights (b) of 16-week-old male Jnk1−/− and Jnk1+/+ mice (n = 3 in a, n = 9 in b). c-f, Total body composition (c), faecal lipid content (d), serum adiponectin concentration (e) and resistin concentration (f) in Jnk1−/− and Jnk1+/+ mice. Representative immunoblots are shown in insets. Total carcass lipid analysis was performed to determine fat mass of individual mice (n = 6 in each group). Asterisk, statistical significance (P < 0.05) in a two-tailed Student t-test comparing Jnk1+/+ and Jnk1−/− mice.

Figure 3 Metabolic effects of JNK1-deficiency. a-d, Examination of glucose homeostasis by fasting plasma glucose (a) and insulin (b) concentrations and insulin (c) and glucose (d) tolerance tests in lean and obese Jnk1−/− and control male mice at 16 weeks of age. Investigation of the dynamics of the responses to the tolerance tests were done by analysis of variance repeated-measures analysis (Statview 4.01, Abacus Concepts, Berkeley, CA, USA). e-g, Body weight (e) and blood glucose (f) and plasma insulin (g) in ob/ob-Jnk1−/− and ob/ob-Jnk1+/+ mice. Blood weight and blood measurements for ob/ob mice were performed at 4 and 8 weeks of age and after a 6-h daytime food withdrawal. Asterisk, statistical significance (P < 0.001 in c and d; P < 0.01 in e-g). WT, wild type.
sensitivity\(^{12,13}\) and resistin is postulated to have a role in insulin resistance\(^4\), these alterations could affect systemic insulin sensitivity.

To test this possibility, we investigated glucose homeostasis in \textit{Jnk} \(^1\) or \textit{Jnk} \(^2\) mice and in wild-type controls. Obese \textit{Jnk} \(^{1/\circ}\) mice developed mild hyperglycaemia compared with lean wild-type controls (224 ± 20 versus 126 ± 11 mg \text{dL} ^{-1} \text{ (mean ± standard error of the mean; s.e.m.)), \(P < 0.001\). In contrast, obese \textit{Jnk} \(^{1/\circ}\) mice had significantly lower blood glucose concentrations than obese \textit{Jnk} \(^{1/\circ}\) mice (Fig. 3a). At 12 weeks of age, the blood glucose concentration in obese \textit{Jnk} \(^{1/\circ}\) mice was indistinguishable from that of lean \textit{Jnk} \(^{1/\circ}\) or \textit{Jnk} \(^{1/\circ}\) animals (148 ± 15 versus 126 ± 11 and 127 ± 8 mg \text{dL} ^{-1}, \(P = 0.8\)). Obese wild-type mice also developed significant hyperinsulinaemia compared with those on the standard diet (5.5 ± 1.5 versus 0.69 ± 0.1 ng ml \(^{-1}\), \(P < 0.001\)). Blood insulin in obese \textit{Jnk} \(^{1/\circ}\) mice was significantly lower than in obese \textit{Jnk} \(^{1/\circ}\) controls (Fig. 3b) and was indistinguishable from either \textit{Jnk} \(^{1/\circ}\) or \textit{Jnk} \(^{1/\circ}\) lean mice (0.63 ± 0.18 versus 0.69 ± 0.16 and 0.57 ± 0.13 ng ml \(^{-1}\)), \(P = 0.8\). Blood glucose and insulin concentrations in \textit{Jnk} \(^{1/\circ}\) were intermediate between those of \textit{Jnk} \(^{1/\circ}\) and \textit{Jnk} \(^{1/\circ}\) animals, but these differences were statistically insignificant (Fig. 3a and b). Obese \textit{Jnk} \(^{2/\circ}\) mice developed a similar degree of hyperglycaemia and hyperinsulinaemia to that in obese wild-type animals. Blood glucose and insulin concentrations were indistinguishable between the \textit{Jnk} \(^{2/\circ}\), \textit{Jnk} \(^{2/\circ}\) and \textit{Jnk} \(^{2/\circ}\) groups (Supplementary Fig. 2a and b). The increase in blood glucose and insulin in animals on the high-fat diet is a strong indicator of obesity-induced insulin resistance and progression to type 2 diabetes. These results therefore indicate that the \textit{Jnk} \(^1\)- but not \textit{Jnk} \(^2\)-deficient animals are protected from the development of obesity-induced insulin resistance.

To investigate this point further, we performed intraperitoneal insulin and glucose tolerance tests (IITT and IGTT, respectively). The hypoglycaemic response to insulin was lower in obese \textit{Jnk} \(^{1/\circ}\) mice throughout the experiment than in obese \textit{Jnk} \(^{1/\circ}\) animals (Fig. 3c). Again, the glucose disposal curves of obese \textit{Jnk} \(^{1/\circ}\) mice were indistinguishable from those of lean animals. Integration of the area under the glucose disposal curves illustrated an overall difference of 40\% between \textit{Jnk} \(^{1/\circ}\) and \textit{Jnk} \(^{1/\circ}\) mice (Supplementary Fig. 3a and b). IGTT also revealed a higher degree of hyperglycaemia in obese \textit{Jnk} \(^{1/\circ}\) animals throughout the experiment than in obese \textit{Jnk} \(^{1/\circ}\) mice (Fig. 3d). However, in this test the responses recorded in obese \textit{Jnk} \(^{1/\circ}\) mice did not reach those of lean controls, especially in the early phases, indicating residual insulin resistance (Fig. 3d). Interestingly, increased responsiveness in IGTT was even evident in lean \textit{Jnk} \(^{1/\circ}\) mice at the early phase of the experiment. In contrast, obese \textit{Jnk} \(^{2/\circ}\) animals exhibited marked insulin resistance in both IITT and IGTT (Supplementary Fig. 4a and b). The response curves of obese \textit{Jnk} \(^{2/\circ}\) mice were essentially identical to those of obese wild-type animals. In summary, both tests confirm that the \textit{Jnk} \(^1\) substantially decreases the development of insulin resistance associated with dietary obesity.

We next generated genetically obese mice (ob/ob) with targeted mutations in \textit{Jnk} \(^1\) to test the action of \textit{Jnk} \(^1\) in a different and more severe model of obesity. As expected, ob/ob mice developed early-onset and severe obesity (Fig. 3e). However, the extent of weight gain was lower in ob/ob-\textit{Jnk} \(^{1/\circ}\) mice than in ob/ob-\textit{Jnk} \(^{1/\circ}\) animals. Furthermore, at both 4 and 8 weeks of age the blood glucose concentrations were lower in ob/ob-\textit{Jnk} \(^{1/\circ}\) mice than in ob/ob-\textit{Jnk} \(^{1/\circ}\) animals (Fig. 3f). The ob/ob-\textit{Jnk} \(^{1/\circ}\) animals displayed a severe and progressive hyperinsulinaemia (18.4 ± 6.2 and 26.4 ± 7.1 ng ml \(^{-1}\) at 4 and 8 weeks of age, respectively). In contrast, plasma insulin concentrations of ob/ob-\textit{Jnk} \(^{1/\circ}\) were lower (5.7 ± 2.1 and 7.7 ± 2.3 ng ml \(^{-1}\) at 4 and 8 weeks of age, respectively) (Fig. 3g). Thus, \textit{Jnk} \(^1\) deficiency can provide partial resistance against obesity, hyperglycaemia and hyperinsulinaemia even in the most severe form of the disease associated with leptin deficiency in ob/ob mice.

In many but not all functions mediated by JNK, redundancy and molecular compensation were observed\(^{16-20}\). To seek a mechanistic explanation for the involvement of \textit{Jnk} \(^1\) isoforms in obesity-related insulin resistance, we examined total \textit{Jnk} \(^1\) activity in liver, muscle and adipose tissues of lean and obese \textit{Jnk} \(^{1/\circ}\) mice. In \textit{Jnk} \(^1\) immunoblot, \textit{Jnk} \(^1\) and \textit{Jnk} \(^1\) have relative molecular masses of 56,000–54,000 and 46,000–43,000, respectively. Phosphorylation of IRS-1 at Ser 307 in liver cells treated for 1 h with 10 ng ml \(^{-1}\) TNF-\(\alpha\) in the absence (control) or presence of a specific JNK inhibitor SP600125 (Ji) (ref. 26) at 2.5 \(\mu\)M. c-f, Phosphorylation of IRS-1 at Ser 307 (c, d) and insulin receptor (IR) signalling (e, f) in obese \textit{Jnk} \(^{1/\circ}\) and \textit{Jnk} \(^{1/\circ}\) mice. Total and Ser 307-phosphorylated IRS-1 concentrations were determined in liver tissues from lean (L) and obese (O) mice. Representative immunoblots of insulin-stimulated tyrosine phosphorylation (pTyr) of IR and IRS-1 in liver tissues of \textit{Jnk} \(^{1/\circ}\) and \textit{Jnk} \(^{1/\circ}\) mice are shown in e. Each lane represents an individual mouse. Graphs in a, d and f show means ± s.e.m. of the immunoblots. WT, wild type.
the relative abundances of the two isoforms in target tissues.

We next examined potential molecular mechanisms that might underlie the protection from insulin resistance conferred by the loss of Jnk1. Inhibitory serine phosphorylation of insulin receptor substrate (IRS-1)-1 was previously shown to be responsible for both TNF-α-induced and FFA-induced insulin resistance11,12. Direct involvement of JNK in insulin signalling was also suggested, on the basis of experiments in vitro, to be exerted through phosphorylation of IRS-1 at Ser 307 (ref. 2). We examined whether this mechanism is involved in the action of JNK1 on IRS-1 and obesity-induced insulin resistance in vivo. We found increased IRS-1 phosphorylation at Ser 307 in a cellular model of insulin resistance in liver cells treated with TNF-α (Fig. 4b). TNF-α-induced Ser 307 phosphorylation was completely prevented by a JNK inhibitor (Fig. 4b). This TNF-α treatment regimen resulted in a significant decrease in insulin-stimulated tyrosine phosphorylation of IRS-1 (data not shown). We also examined IRS-1 Ser 307 phosphorylation in liver tissue of lean and obese Jnk11−/− and Jnk1+/− mice. The extent of IRS-1 Ser 307 phosphorylation was markedly increased in obese wild-type mice relative to the lean controls (Fig. 4c and d). Most importantly, no such increase could be detected in obese Jnk1−/− mice, demonstrating that Ser 307 of IRS-1 is a target for JNK action in vivo (Fig. 4c and d). Finally, we found that insulin-induced IRS-1 tyrosine phosphorylation was strongly enhanced in livers of obese Jnk1−/− mice in comparison with obese Jnk1+/+ controls (Fig. 4e and f). We also observed improvement in insulin-induced phosphorylation of the 95-kDa β subunit of the insulin receptor (Fig. 4e and f). However, the increase in IRS-1 tyrosine phosphorylation was more marked and was consistent with decreased Ser 307 phosphorylation. These results demonstrate that the absence of Jnk1 enhances the signalling capacity of the insulin receptor, at least in part, through its effects on IRS-1 phosphorylation. It is, of course, possible that additional mechanisms might also be involved in JNK action or the link of serine phosphorylated IRS-1 to insulin resistance. Nevertheless, this study provides evidence that obesity is associated with abnormally elevated JNK activity, predominantly provided by JNK1. Importantly, the absence of JNK1 results in substantial protection from obesity-induced insulin resistance. Abnormal production of inflammatory cytokines such as TNF-α (ref. 3) and increased concentrations of FFAs are crucial players in obesity-induced insulin resistance. Induction of insulin resistance by these mediators involves inhibitory serine phosphorylation of IRS-1 (refs 21, 22). Both TNF-α and FFAs are potent activators of JNK6,8, which in turn phosphorylates IRS-1 at Ser 307 (ref. 2). Our studies provide strong evidence that JNK1 is indeed a crucial component of the biochemical pathway responsible for obesity-induced insulin resistance in vivo. There is also genetic evidence demonstrating that increased JNK activity caused by loss-of-function mutations in the JNK scaffold protein JIP1 is causal to type 2 diabetes in humans23. We therefore suggest that a selective interference with JNK1 activity presents an attractive opportunity for the treatment of human obesity, insulin resistance and type 2 diabetes, the most prevalent metabolic diseases worldwide.

Methods

Generation of mice deficient in JNK1 and JNK2

Generation of Jnk1−/− and Jnk2−/− mice was as described14,15. All experimental mice were backcrossed five generations to C57Bl/6J and generated from intercrosses between these double heterozygotes and groups were derived from littersmates. ob/ob-Jnk1−/− and ob/ob-Jnk1−/− mice were generated by intercrossing Jnk1−/− mice and OB/ob (C57Bl/6J) from our in-house colony at Harvard) animals to generate double heterozygotes and subsequent crosses with OB/ob breeding pairs to create double homozygous mutant mice.

Diet study and metabolic measurements

Male mice of different genotypes were housed in a barrier-free facility and placed on a high-fat/high-carbohydrate diet ad libitum (Diet F3282; Bioserve, Frenchtown, NJ, USA) at 4 weeks of age and were followed for a period of 12 weeks. Biochemical analyses and tolerance tests were performed as described17. The polyclonal rabbit anti-mouse resin antibody was a gift from Affinity Bioreagents Inc.,

Measurement of JNK activity and protein concentrations

Tissue extracts (600 μg of protein) were mixed with 20 μl of glutathione S-transferase (GST)-agarose resin suspension (Sigma) to which 5 μg of GST–c-Jun (1–79) were bound. The mixture was agitated at 4 °C overnight, pelleted by centrifugation and washed twice. JNK activity was measured as described18.

Measurement of insulin receptor and IRS-1 phosphorylation in vivo

After an overnight fast, mice were anaesthetized as described and 25 μl of [125I] insulin (Eli Lilly) or an equal volume of vehicle was administered through the portal vein. Tissues were collected in liquid nitrogen 120 s after injection. Serine phosphorylation of IRS-1 was studied in livers from mice killed without any treatment. Protein extracts (1 mg) from tissue-samples were prepared and analysed as described. Antibodies were purchased from Santa Cruz (anti-insulin-receptor-β and anti-phosphotyrosine) or Upstate Biotechnology (anti-insulin receptor and anti-IRS-1 pser307).

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