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Genetic disruption of protein phosphatase 5 in mice prevents high-fat diet feeding-induced weight gain

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ABSTRACT

The role of serine/threonine protein phosphatase 5 (PP5) in the development of obesity and insulin resistance associated with high-fat diet-feeding (HFD) was examined using PP5-deficient mice (*Ppp5c*$^{-/-}$). Despite similar caloric intake, *Ppp5c*$^{-/-}$ mice on HFD gained markedly less weight and did not accumulate visceral fat compared to wild-type littermates (*Ppp5c*$^{+/+}$). On a control diet, *Ppp5c*$^{-/-}$ mice had markedly improved glucose control compared to *Ppp5c*$^{+/+}$ mice, an effect diminished by HFD. However, even after 10 weeks of HFD glucose control in *Ppp5c*$^{-/-}$ mice was similar to that observed in *Ppp5c*$^{+/+}$ mice on the control diet. Thus, PP5 deficiency confers protection against HFD-induced weight gain in mice.

1. Introduction

In modern societies, many humans have adopted a life style that is sedentary and combined with excessive caloric intake. This life style can shift normal metabolism in favor of pathways that promote the storage of energy as fat. The resulting obesity has become an important health problem [1], because excess adiposity greatly increases the risk of developing a variety of pathological conditions, including insulin resistance [2], dyslipidemia, type 2 diabetes mellitus (T2DM) [3] and cardiovascular disease [4].

In conjunction with an expansion of adipose tissue mass, there is often a development of chronic low-grade inflammation, recognized by elevated levels of proinflammatory cytokines released from macrophages that invade adipose tissue [5]. Release of these so-called adipokines, when combined with an excessive amount of fatty acids, further contributes to the development of insulin resistance and impaired glucose metabolism in muscle, liver, adipose tissue and pancreatic β-cells [6]. As insulin resistance worsens, elevated levels of glucose and fatty acids combine to produce a so-called glucolipotoxic effect, in which reactive oxygen species (ROS) are produced at increased levels [7–9]. High levels of ROS cause oxidative damage, and as pancreatic β-cells express low levels of ROS-scavenging enzymes [10,11], these cells are particularly susceptible to ROS. Notably, when present at high levels, ROS can initiate apoptosis in isolated human pancreatic islets [12].

PP5 (*PPP5C*, *Ppp5c*) is a PPP-family serine/threonine protein phosphatase that may act as a double-edge sword in the development of T2DM. PP5 is known to interact with several stress proteins, including apoptosis signal-regulating kinase 1 (ASK1), heat shock protein-90 (HSP-90), and HSP-90 chaperone complex proteins (glucocorticoid receptor [GR], STIP1, CDC37) [13–15]. The expression and activation of PP5 is responsive to ROS [16]. Following ROS exposure PP5 acts to suppress ASK1 signaling; thus, slowing the activation of JNK and progression into apoptosis [13,16]. In both insulin-secreting MIN6 cells and pancreatic islets isolated from mice, the loss of PP5 is associated with increased JNK phosphorylation and apoptosis following treatment with saturated fatty acids and H_2O_2 [17]. These studies indicate that PP5 protects pancreatic β-cells from ROS-induced apoptosis, suggesting that PP5 may help guard against the development of T2DM. Nonetheless, PP5 is also known to interact with HSP-90/GR complexes. In GR-signaling, the suppression of PP5 with siRNA or antisense oligonucleotides is associated with enhanced dexamethasone-induced gene expression [18] increased accumulation of nuclear GRs [19], and altered patterns of GR phosphorylation [20,21]. Glucocorticoids produce an immunosuppressive response that may be

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desirable to counter the production of proinflammatory cytokines. However, glucocorticoids also produce a "pro-diabetic" effect, acting to enhance glucose production in the liver, impair insulin-dependent glucose uptake in peripheral tissues, and inhibit insulin secretion from pancreatic β-cells [22]. Therefore, it is not yet clear if the actions of PP5 during the development of insulin resistance and T2DM in animals will be harmful or beneficial.

The high conservation of PP5 among species (at the level of amino acids, mouse, rabbit, bovine and human PP5 protein share ~98% identity) suggests that the actions of PP5 are conserved in mammals. To help better understand the roles of PP5 in biology, we generated congenic strains of mice in which exon 1 of the *PPP5C* gene was removed genetically (PP5 knockout mice, *Ppp5c*$^{-/-}$) in the C57Bl/6J background [23]. Studies conducted with embryonic fibroblasts and pancreatic islets isolated from the *Ppp5c*$^{-/-}$ mice confirmed the role of PP5 in the suppression of signaling cascades induced by several forms of genomic stress, including ROS- and fatty acid-induced apoptosis [17,23]. However, when compared to their wild-type littermates, the mice lacking PP5 weighed less, and no loss of β-cell mass was observed in the *Ppp5c*$^{-/-}$ mice. Further analysis revealed that *Ppp5c*$^{-/-}$ mice had lower fasting glycemia and eliminated glucose faster during an glucose tolerance test, but they retained normal insulin sensitivity and normal fasting insulin levels [17]. Working with cells derived from an independently generated line of PP5 knockout mice, Hinds et al. reported that in adipogenically differentiated mouse embryonic fibroblasts (MEFs) PP5 may act as a permissive enzyme for adipogenesis, by simultaneously suppressing the phosphorylation of GR-α and peroxisome proliferator activated receptor γ (PPARγ) at sites which facilitate lipolysis or lipogenesis, respectively [24]. Here, we report on a high-fat diet-feeding study which revealed that even on a diet in which ~60% of the calories are derived from fat, a genetic deletion of PP5 prevents weight gain in mice. These observations suggest that inhibitors of PP5 may indeed be useful to combat the development of obesity, and in doing so, prevent obesity-induced insulin resistance.

2. Methods

2.1. Animals and treatment

The experiments were performed on male PP5 knockout animals (*Ppp5c*$^{-/-}$) that were developed previously [23], using wild-type (*Ppp5c*$^{+/+}$) littermates as controls. Mice of both genotypes (8 weeks of age) were placed on either a high-fat diet (HFD: 20% protein, 20% carbohydrates, 60% fat), or on a control diet (CD: 20% protein, 70% carbohydrates, 10% fat) for ten weeks. Body weight and the intake of water and food were monitored weekly. Fasting blood glucose (FBG) was measured before the study and then after five and nine weeks on the indicated diets. At the same time points, blood samples were collected. The penultimate week, mice were allocated to either an intraperitoneal (i.p.) glucose- or insulin tolerance test (IPGTT, IPInsTT) to evaluate glucose tolerance and insulin sensitivity, respectively, as previous described [17].

Briefly, prior to the IPGTT mice were fasted 4 h before they received i.p. injections containing a 30% glucose solution (2 g/kg body weight) (Fresenius Kabi, Uppsala, Sweden). For the IPInsTT, random-fed mice were injected i.p. with human recombinant insulin (Humalog®, Eli Lilly, Indianapolis, IA) equivalent to 1 unit/kg body weight blood was drawn from the tail vein and glycaemia was measured using a glucometer (OneTouch® Ultra® 2, LifeScan Inc., Milpitas, CA), immediately before (time 0), 5, 15, 30 and 60 min after the injection. Area under the curve (AUC) for the IPInsTT was calculated between 0 and 60 min and the rate for glucose disappearance (K_{itt}) was calculated from the slope of the regression line obtained between 0 and 30 min. The study was performed according to the guidelines of Karolinska Institutet and approved by the local animal ethics committee. Additional details on the diet treatment are provided as Supplementary material.

2.2. Insulin determination

Serum levels of insulin were determined using mouse insulin ELISA according to the manufacturer's instructions (Mercodia, Uppsala, Sweden).

2.3. Statistical analysis

Data are presented as mean values ± standard error of the mean (mean ± S.E.M.). Student's *t*-test was used when comparing the difference between two groups. For multiple comparisons, differences were determined by one- or two-way ANOVA followed by Bonferroni's *post hoc* test (GraphPad Prism version 5). A value of $P < 0.05$ was considered statistically significant.

3. Results

Despite similar feeding behavior, *Ppp5c*$^{-/-}$ mice are less prone to diet-induced weight gain when compared with their *Ppp5c*$^{+/+}$ littermates *Ppp5c*$^{-/-}$ and littermate *Ppp5c*$^{+/+}$ mice were divided into four cohorts. Each cohort was then placed on either a control or a high-fat diet (HFD) for 10 weeks. Weight gain and food/water consumption were measured weekly. Comparison of food intake between cohorts revealed that both *Ppp5c*$^{+/+}$ and *Ppp5c*$^{-/-}$ mice on the HFD had a higher caloric intake than mice on the control diet. However, no difference in energy intake between the *Ppp5c*$^{+/+}$or *Ppp5c*$^{-/-}$ mice on either diet was observed (Fig. 1a). Water consumption was also similar among cohorts (Fig. 1b). As known for the C57Bl/6J mouse strain [25], comparison of *Ppp5c*$^{+/+}$ mice on HFD or control diet revealed that mice on the HFD gained more weight than the mice fed the control diet. In contrast, the weight gained by the *Ppp5c*$^{-/-}$ cohort on the HFD was not statistically different from the *Ppp5c*$^{-/-}$ or the *Ppp5c*$^{+/+}$ cohorts fed the control diet (Fig. 2a). Comparison of cohorts on the HFD revealed that, starting at week four, there was a significant difference in body weight between *Ppp5c*$^{-/-}$ and *Ppp5c*$^{+/+}$ animals. Interestingly, even after ten weeks the *Ppp5c*$^{-/-}$ mice on the HFD were similar in weight to their wild-type littermates receiving the control diet.

Analysis of tissue mass after sacrifice revealed that the weight of perirenal, epididymal and subcutaneous fat deposits all increased in the *Ppp5c*$^{+/+}$ mice after HFD feeding as compared to the *Ppp5c*$^{+/+}$ cohort on the control diet. This increase in fat deposits was not observed in the *Ppp5c*$^{-/-}$ mice (Fig. 2b). Notably, the perirenal, epididymal and subcutaneous fat tissues were significantly smaller when the *Ppp5c*$^{-/-}$ mice were compared with their *Ppp5c*$^{+/+}$ littermates. In contrast, a decrease in the weights of other tissues (e.g., spleen, heart, kidney, muscle, pancreas, brain) was not observed in the *Ppp5c*$^{-/-}$ mice (data not shown).

3.1. Fasting serum insulin levels in *Ppp5c*$^{-/-}$ mice are not elevated in response to the HFD

Fasting serum insulin and blood glucose (FBG) levels were measured to detect diet-induced differences in insulin levels or glycemia between the cohorts (Fig. 3). Similar to our previous report [17], at the start of the trial, fasting insulin levels of the 8-week-old mice were similar in the *Ppp5c*$^{-/-}$ and *Ppp5c*$^{+/+}$ animals. After nine weeks on the diets, a statistically significant increase in fasting insulin levels was observed in the *Ppp5c*$^{+/+}$ cohort on HFD, as compared to all other cohorts. In contrast, *Ppp5c*$^{-/-}$ mice