HIGHLIGHT

Leptin is a potent anti-diabetic in mice with lipodystrophy and insulin resistance

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Obesity is associated with insulin resistance. However, insulin sensitivity can be even more attenuated in individuals with hardly any fat cells at all. Patients with Berardinelli-Seip congenital lipodystrophy (BSCL) have insulin resistance with secondary hyperinsulinaemia, and insulin-resistant diabetes mellitus (1). They may also have hyperlipidaemia, hepatomegaly, hypertrophic cardiomyopathy, acanthosis nigricans, elevated basal metabolic rate and an excessive food intake. The syndrome is thought to be autosomal recessive, and linkage studies have indicated a locus at chromosome 9q34 (2), whereas 1q21–22 is the locus in a similar syndrome with partial lipodystrophy (3). The region on chromosome 9 harbours the retinoid X receptor α gene (RXRA), which is a potential candidate gene and important for adipocyte differentiation.

Recently, three transgenic mouse models of generalised lipodystrophy have been developed. In all of them the expression of specific genes was directed to white and brown fat cells through use of a vector with a regulatory sequence found in the aP2 gene, which is adipocyte-specific.

A delayed-onset form of lipodystrophy was induced in mice by Burant *et al.* (4), who used the aP2 enhancer/promoter to target the expression of the diphtheria toxin A gene to adipocytes. These mice gradually lost their fat, but did not show any sign of lipodystrophy until 5–6 months of age. At 8–10 months no white adipose tissue was visible, and they developed insulin resistance, diabetes, fatty liver and hypertriglyceridaemia.

A mouse model developed by Moitra *et al.* (5) was lipodystrophic from birth. They directed the expression of an artificial transcription factor designated A-ZIP/F to white and brown fat cells. The transcription factor was constructed so that it made stable promiscuous dimerisation with all members of the Jun and CCAAT/enhancer-binding protein (C/EBP) family and attenuated their activity. Previous studies have shown that these proteins are essential for terminal adipocyte differentiation. The mice had no fat tissue at birth and developed diabetes with increased serum glucose and insulin levels. The liver was filled with lipid and the internal organs were enlarged as in humans with BSCL.

Shimomura *et al.* (6) over-expressed the nuclear form of the sterol regulatory element binding protein-1c (nSREBP-1c) in fat cells in their mouse model of lipodystrophy. SREBP-1c is also known as the adipocyte

determination and differentiation factor (ADD-1). The SREBPs are large proteins attached to the endoplasmic reticulum. They consist of an amino-terminal domain, which is a transcription factor of the basic helix–loop–helix leucine zipper family, a central domain responsible for membrane attachment, and a carboxy-terminal regulatory domain. When cellular sterol levels are low, two proteases are activated and release the domain comprising the transcription factor, which is designated nSREBP. Three SREBPs have been identified. SREBP-1a and SREBP-1c are encoded by the same gene, but nSREBP-1a is a much more potent transcription factor. Both preferentially activate the fatty acid biosynthesis pathway, whereas SREBP-2 favours the activation of cholesterol biosynthesis, but all are capable of activating the same families of genes. When nSREBP-1c was over-expressed in preadipocytes in culture, lipid accumulation was enhanced. In contrast, when Shimomura *et al.* (6) expressed a dominant positive nSREBP-1c in transgenic mice, the animals developed a generalised lipodystrophy as in BSCL.

In all mouse models the physiological features were as in patients with BSCL. The mice had large fatty livers, insulin resistance and hyperglycaemia. Both patients with BSCL and the lipodystrophic mice have very low levels of leptin (4–7). This hormone is secreted by adipocytes and has effects on the hypothalamic control of feeding behaviour and energy expenditure, but also peripherally on fatty acid oxidation (8). Genetic deficiency of leptin has profound consequences both in humans and in the ob/ob mice. Excessive food intake and metabolic disturbances such as obesity and insulin resistance associated with the hypoleptinaemia can be corrected by leptin substitution (9, 10). Shimomura *et al.* (11) investigated whether leptin treatment could correct the insulin resistance observed in their strain of lipodystrophic mice over-expressing nSREBP-1c. The mice were treated with 5 μ g recombinant leptin per day by a continuous infusion through an osmotic minipump. This dose was large enough to normalise plasma leptin levels in both the ob/ob mice and the transgenic nSREBP-1c mice. Leptin substitution normalised the metabolism of ob/ob mice, while the treatment had no metabolic effects in the wild-type animals. Prior to leptin treatment, food intake and body weight were 17 and 10% higher, respectively, in the lipodystrophic mice expressing nSREBP-1c compared with the controls.

Both food intake and body weight were significantly reduced during 12 days of leptin treatment. Plasma insulin and glucose, and liver triglyceride levels were markedly elevated in the transgenic mice, but were normalised by leptin substitution. It was important to exclude the possibility that these effects were secondary to a reduction in food intake and body weight alone. The metabolic effects of a caloric restriction resulting in 30% reduction of food intake and body weight were therefore studied in transgenic and wild-type mice. Hepatic triglyceride content was almost normalised, but no changes in plasma insulin and glucose concentrations were observed during food restriction of the transgenic mice. This was in contrast to the situation in patients with BSCL, which can be controlled by caloric restriction alone (1). Some adipose tissue remains in these mice as in patients with BSCL. Leptin treatment did not change the amount of omental fat. The expression of genes characteristic of adipose tissue such as peroxisome proliferator-activated receptor γ (PPAR γ), adiponin and uncoupling protein 1 remained low. Tumour necrosis factor α (TNF- α) has been implicated in insulin resistance, and the expression of TNF- α was high in omental fat, but was not reduced by leptin treatment.

The report by Shimomura *et al.* (11) indicates that insulin resistance in generalised lipodystrophy is caused by hypoleptinaemia secondary to a failure of adipocyte differentiation, but the mechanism is unknown. One hypothesis is that leptin diverts fatty acids into adipose tissue. An excess of fat storage and metabolism in other tissues than the adipose tissue has been associated with decreased insulin sensitivity. Potent anti-diabetic effects of leptin were recently demonstrated in rats made insulin-deficient and diabetic by streptozotocin (12). Leptin treatment restored euglycaemia both by increasing insulin sensitivity and by mechanisms independent of insulin. An anti-diabetic role of leptin was recently demonstrated in transgenic mice, which over-expressed leptin in their livers (13). These mice had no adipose tissue, but insulin signalling was augmented in both liver and skeletal muscle compared with control mice. Insulin resistance in the lipodystrophic mice seems to be a consequence of hypoleptinaemia and not the absence of adipose tissue.

It is not known from the studies of Shimomura *et al.* (11) whether the anti-diabetic effect of leptin was mediated by the central nervous system (CNS) or by direct effects on peripheral tissues. It has previously been demonstrated that leptin increases hepatic glucose metabolism after both intravenous and intracerebro-ventricular infusion, which suggested that the effects were mediated mainly by signals from the CNS (14). It was recently shown that leptin treatment of mice increases serotonin levels in the diencephalon (15). Insulin sensitivity in patients with BSCL has been normalised by the anorexiatic drug fenfluramine, which also increases serotonin levels in the CNS (1).

However, direct effects of leptin in perfused rat liver on glycogenolysis and gluconeogenesis have also been demonstrated, and indicate that the anti-diabetic effects of leptin probably are a combination of its actions in both the CNS and peripheral tissues (16).

Although insulin resistance in mice with lipodystrophy and insulin resistance can be successfully treated with leptin substitution, insulin sensitivity can also be improved by the thiazolidinedione, troglitazone. Thiazolidinediones are insulin sensitisers that activate PPAR γ , which is most abundant in adipose tissue and important for the differentiation of mature adipocytes. Burant *et al.* (4) showed that troglitazone treatment of mice, which gradually become lipodystrophic by over-expression of the diphtheria toxin in their fat cells, almost normalised plasma levels of glucose, insulin and lipids in the absence of large adipose depots. Leptin levels remained low during the treatment. The effects of leptin and thiazolidinediones in mice with lipodystrophy indicate treatment options for diabetes and hyperlipidaemia in patients with lipodystrophy. These lessons are important not only for the small group of patients with the congenital variant, but also for the growing number of patients with partial lipodystrophy caused by treatment with HIV-1-protease inhibitors (17).

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