Table 2 Changes in the urinary ratio in diseases/treatments.

Diseases/treatments	Change in urinary ratio
Obese subjects	↓
NASH	↓
Mild calorie restriction	→
Pioglitazone	
Responded group (group Y)	↓
Non-responded group (group X)	→

The present study demonstrated an inverse correlation between the urinary ratio and BMI in a large Japanese cohort. Together with that the urinary ratio was also decreased in patients with NASH, decrease in 11β-HSD1 activity in liver of obese individuals may contribute, at least partly, to the fall in the urinary ratio in obesity. Although further studies are warranted, the finding that decrement of the urinary ratio was exaggerated in patients who responded to pioglitazone may be a reflection of 11β-HSD1 inhibition mainly in adipose tissue.

strated that growth hormone supplementation in patients with adult growth hormone deficiency (AGHD) markedly lowered the urinary ratio, reflecting 11β-HSD1 inhibition mainly in adipose tissue [34].

In summary, the present study is the first to provide novel evidence that the urinary ratio in fresh urine reflects a facet of metabolic function in adipose tissue and liver (Table 2), thereby offering a unique method to evaluate the metabolic status and therapeutic effectiveness in human clinics.

Conflicts of interest

None of the authors have any conflict of interest.

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ARTICLE

Beneficial effects of leptin on glycaemic and lipid control in a mouse model of type 2 diabetes with increased adiposity induced by streptozotocin and a high-fat diet

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Abstract
Aims/hypothesis We have previously demonstrated the therapeutic usefulness of leptin in lipoatrophic diabetes and insulin-deficient diabetes in mouse models and could also demonstrate its dramatic effects on lipoatrophic diabetes in humans. The aim of the present study was to explore the therapeutic usefulness of leptin in a mouse model of type 2 diabetes with increased adiposity.
Methods To generate a mouse model mimicking human type 2 diabetes with increased adiposity, we used a combination of low-dose streptozotocin (STZ, 120 μg/g body weight) and high-fat diet (HFD, 45% of energy as fat). Recombinant mouse leptin was infused chronically (20 ng [g body weight]$^{-1}$ h^{-1}) for 14 days using a mini-osmotic pump. The effects of leptin on food intake, body weight, metabolic variables, tissue triacylglycerol content and AMP-activated protein kinase (AMPK) activity were examined.
Results Low-dose STZ injection led to a substantial reduction of plasma insulin levels and hyperglycaemia. Subsequent HFD feeding increased adiposity and induced insulin resistance and further augmentation of hyperglycaemia. In this model mouse mimicking human type 2 diabetes (STZ/HFD), continuous leptin infusion reduced food intake and body weight and improved glucose and lipid metabolism with enhancement of insulin sensitivity. Leptin also decreased liver and skeletal muscle triacylglycerol content accompanied by an increase of α2 AMPK activity in skeletal muscle. Pair-feeding experiments demonstrated that leptin improved glucose and lipid metabolism independently of the food intake reduction.
Conclusions/interpretation This study demonstrates the beneficial effects of leptin on glycaemic and lipid control in a mouse model of type 2 diabetes with increased adiposity, indicating the possible clinical usefulness of leptin as a new glucose-lowering drug in humans.

Keywords High-fat diet · Insulin sensitivity · Leptin · Overweight · Streptozotocin · Tissue triacylglycerol content · Type 2 diabetes

Abbreviations

AMPK	AMP-activated protein kinase
GTT	glucose tolerance test
HFD	high-fat diet
SD	standard diet
STZ	streptozotocin

Introduction

Leptin is an adipocyte-derived hormone that plays a key role in regulating food intake and energy expenditure, and participates in increasing glucose metabolism [1, 2]. Leptin deficiency causes obesity, insulin resistance and diabetes in mice and humans [3–5]. We previously generated transgenic skinny mice (LepTg) overexpressing leptin under the control of the liver-specific human serum amyloid P component promoter [6]. LepTg mice showed elevated

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plasma leptin levels comparable to those of obese human individuals, providing a unique experimental model to investigate various actions of leptin [6–11]. LepTg mice exhibited increased glucose metabolism and insulin sensitivity with augmented liver and skeletal muscle insulin receptor signalling [6]. LepTg mice also exhibited increased lipid metabolism accompanied by increased lipoprotein lipase activity and clearance of triacylglycerol [7]. In addition, LepTg mice had reduced tissue triacylglycerol content along with increased energy expenditure through augmented phosphorylation of AMP-activated protein kinase (AMPK), a key enzyme that mediates the leptin effect on fatty acid β-oxidation in skeletal muscle [8, 9]. Therefore, these findings led us to hypothesise that leptin acts as a glucose-lowering drug with a lipid-lowering effect in vivo.

Given the glucose-lowering action of leptin, we and others have demonstrated that leptin infusion or transgenic overexpression of leptin reverses metabolic abnormalities in different mouse models of lipodystrophy [10, 12]. Recently, we and others confirmed that leptin treatment effectively reduces food intake and improves hyperglycaemia, hypertriacylglycerolaemia and fatty liver in patients with lipoatrophic diabetes [13–16]. In addition, we demonstrated that leptin is useful as a glucose-lowering agent in a mouse model of insulin-deficient diabetes induced by high-dose streptozotocin (STZ) [11]. Leptin infusion reduced the dose of insulin required to improve hyperglycaemia by more than 90%, and prevented insulin-induced body weight gain in STZ-injected mice. However, the therapeutic usefulness of leptin in type 2 diabetes, a more prevalent form of diabetes, remains unclear.

In patients with type 2 diabetes, impaired insulin secretion caused by beta cell dysfunction and insulin resistance in target tissues contributes to increased blood glucose levels [17]. Patients with type 2 diabetes often exhibit dyslipidaemia and an increase of triacylglycerol content in the liver and skeletal muscle [18, 19]. Furthermore, in contrast to patients with lipoatrophic diabetes and insulin-deficient diabetes who are in hypoleptinaemic states [13–16, 20], patients with type 2 diabetes often have increased adiposity and elevated leptin levels.

Previous studies have shown that low-dose STZ injection leads to the partial destruction of pancreatic beta cells and a high-fat diet (HFD) induces insulin resistance in rodents [21–23]. The degree of beta cell destruction and insulin resistance can be adjusted by dosage, duration and condition of STZ injection and HFD feeding [11, 24]. The effects of various glucose-lowering drugs (sulfonylurea, metformin, thiazolidinedione etc) have been examined in mice treated with low-dose STZ and HFD as a model of type 2 diabetes [22, 23]. In the present study, we too generated a mouse model mimicking human type 2 diabetes using low-dose STZ and HFD to examine the effect of leptin infusion. STZ/HFD mice exhibited increased adiposity and disorders in glucose and lipid metabolism accompanied by impaired insulin secretion and insulin resistance. We report here the beneficial effects of leptin infusion on glycaemic and lipid control in this mouse model of type 2 diabetes with increased adiposity.

Methods

Animals Seven-week-old male C57BL/6J mice were purchased from Japan SLC, Shizuoka, Japan. The mice were caged individually and kept under a 12 h light–dark cycle (light on at 09:00 hours) with free access to water and standard diet (SD) (NMF, 14.6 kJ/g, 13% of energy as fat; Oriental Yeast Co., Tokyo, Japan) unless otherwise stated. Animal care and all experiments were conducted in accordance with the Guidelines for Animal Experiments of Kyoto University and were approved by the Animal Research Committee, Graduate School of Medicine, Kyoto University.

Generation of a mouse model of type 2 diabetes One week after purchase, mice were injected i.p. once with vehicle or low-dose STZ (120 μg/g body weight in 10 mmol/l sodium citrate buffer, pH 4.0; Sigma-Aldrich, St Louis, MO, USA) after 4 h of fasting. After 3 weeks, the vehicle-injected mice were randomly divided and placed on SD or HFD (D12451, 19.7 kJ/g, 45% of energy as fat; Research Diets, New Brunswick, NJ, USA) (termed control and HFD mice, respectively), and the STZ-injected mice with similar degrees of hyperglycaemia and body weight were also randomly divided and placed on SD or HFD (termed STZ and STZ/HFD mice, respectively). Each group of mice was fed with either diet for 5 weeks before they were used for the leptin infusion experiment.

Leptin infusion experiments On day 0, a mini-osmotic pump (Alzet model 2002; Alza, Palo Alto, CA, USA) was implanted s.c. in the mid-scapular region of each mouse. The pump delivered saline or recombinant mouse leptin (Amgen, Thousand Oaks, CA, USA) (20 ng [g body weight]$^{-1}$ h^{-1}) s.c. for 14 days. SD or HFD feeding was continued during the leptin infusion experiment.

Food intake, body weight and per cent body fat Food intake was measured before and during the leptin infusion experiment. Body weight was measured on days 0 and 14. Per cent body fat was measured before the leptin infusion experiment under pentobarbital anaesthesia (Nembutal; Dainippon Sumitomo Pharma, Osaka, Japan), using a Latheta LTC-100 (Aloka, Tokyo, Japan).

Metabolic variables Blood was obtained from non-fasted mice between 15:00 and 17:00 hours. Blood glucose levels were determined by the glucose oxidase method using a reflectance glucometer (MS-GR102; Terumo, Tokyo, Japan) on days 0, 4, 7 and 14. Plasma insulin levels were measured by enzyme immunoassay with an Insulin-EIA kit (Morinaga, Tokyo, Japan). Plasma triacylglycerol, NEFA and total cholesterol levels were measured using enzymatic kits (Triglyceride E-test Wako, NEFA C-test Wako and Cholesterol E-test Wako, respectively; Wako Pure Chemicals, Osaka, Japan). Plasma leptin levels were determined using an RIA kit for mouse leptin (Linco Research Immunoassay, St Louis, MO, USA).

Glucose tolerance test (GTT) A GTT was performed on day 10. Mice were injected i.p. with 2.0 mg/g glucose after overnight fasting. Blood glucose and plasma insulin levels were measured at the indicated time points.

Liver and skeletal muscle triacylglycerol content Tissue triacylglycerol content was measured as described previously [7, 8], with modifications. Liver and quadriceps muscle were isolated at the end of the leptin infusion experiment, immediately frozen in liquid nitrogen and lipids extracted with isopropyl alcohol/heptane (1:1 vol./vol.). After evaporating the solvent, the lipids were resuspended in 99.5% (vol./vol.) ethanol, and the triacylglycerol content was measured using the Triglyceride E-test Wako kit.

Isoform-specific AMPK activity AMPK activity was determined as described previously [25, 26], with modifications. To measure α1 and α2 isoform-specific AMPK activity in skeletal muscle, AMPK was immunoprecipitated from muscle lysates (200 μg protein) with specific antibodies against the α1- and α2-subunits (Upstate Cell Signaling Solutions, Lake Placid, NY, USA) bound to Protein A-Sepharose beads, and the kinase activity of the immunoprecipitates was measured using 'SAMS' peptide and [γ-^{32}P]ATP.

Pair-feeding experiments STZ or STZ/HFD mice were fed the same amount of food consumed by the corresponding leptin-infused mice on the previous day, for 14 days. A GTT was performed on day 10 of the experiment. Liver and quadriceps muscle were obtained for triacylglycerol content measurements at the end of the pair-feeding experiment.

Statistical analyses Data are expressed as means±SEM. Comparison between or among groups was by Student's *t* test or ANOVA with Fisher's protected least significant difference test. $p<0.05$ was considered statistically significant.

Results

Generation of a mouse model of type 2 diabetes To generate a mouse model mimicking human type 2 diabetes with impaired insulin secretion and insulin resistance, we used low-dose STZ injection and HFD feeding. As shown in Table 1, HFD feeding effectively increased body weight, per cent body fat and plasma leptin levels in mice. With the development of adiposity, plasma insulin levels substan-

Table 1 Metabolic characteristics of the mouse model of type 2 diabetes

Variable	Mouse group			
	Control	HFD	STZ	STZ/HFD
Food intake (kJ/week)	329.0±9.3	350.7±20.0	365.3±15.1*	422.1±23.1**,†
Body weight (g)	26.8±0.6	34.4±1.3**	26.4±0.4	27.9±0.5†
Body fat (%)	19.8±0.7	40.6±1.1**	18.9±1.0	24.9±1.8*,†
Leptin (ng/ml)	4.7±0.6	26.4±1.0**	4.5±0.5	8.6±0.8**,††
Glucose (mmol/l)	8.3±0.2	9.2±0.4	17.5±2.3**	27.2±1.2**,††
Insulin (pmol/l)	160±28	315±71*	92±12*	160±38
Triacylglycerol (mmol/l)	0.66±0.09	0.86±0.08	1.11±0.14*	1.27±0.28*
NEFA (mEq/l)	0.77±0.06	1.08±0.09*	1.03±0.10*	0.99±0.09*
Total cholesterol (mmol/l)	1.48±0.08	3.61±0.18**	1.49±0.16	3.01±0.19**,††
Liver triacylglycerol content (mg/g tissue)	8.7±1.0	20.0±2.2**	10.2±0.9	27.1±1.7**,††
Skeletal muscle triacylglycerol content (mg/g tissue)	5.6±0.5	8.1±1.2*	5.4±0.5	7.8±0.8*,†

Values are means±SEM for 10–12 mice in each group

C57BL/6J mice were injected with vehicle and fed SD (control) or HFD, or injected with low-dose STZ and fed with SD (STZ) or HFD (STZ/HFD). Food intake for a week, body weight, per cent body fat, blood glucose levels and plasma levels for leptin, insulin, triacylglycerol, NEFA and total cholesterol were measured before the leptin infusion experiment. Blood samples were obtained during ad libitum feeding. Liver and skeletal muscle triacylglycerol contents were measured after the leptin infusion experiment

*$p<0.05$, **$p<0.01$ vs control mice; †$p<0.05$, ††$p<0.01$ vs STZ in STZ/HFD mice

tially increased, although blood glucose levels did not significantly increase, suggesting the development of insulin resistance. HFD feeding also increased plasma NEFA and total cholesterol levels, and liver and skeletal muscle triacylglycerol contents.

Low-dose STZ injection led to a substantial reduction of plasma insulin and hyperglycaemia in mice. Under these conditions, body weight, per cent body fat and plasma leptin levels were unchanged, although food intake was significantly increased. Low plasma insulin levels also led to an increase of plasma triacylglycerol and NEFA levels. Liver and skeletal muscle triacylglycerol contents were unchanged.

On the other hand, subsequent HFD feeding in low-dose STZ injected mice further increased food intake and moderately increased body weight, per cent body fat and plasma leptin levels even with the impairment of insulin secretion. Hyperglycaemia was exacerbated, although plasma insulin levels were mildly elevated, suggesting the development of insulin resistance. Increases of plasma triacylglycerol, NEFA and total cholesterol levels, and liver and skeletal muscle triacylglycerol contents, were also observed in these STZ/HFD mice.

Since STZ/HFD mice manifested increased adiposity and disorders in glucose and lipid metabolism accompanied by impaired insulin secretion and insulin resistance, we used STZ/HFD mice as a model of type 2 diabetes with increased adiposity in the present study.

Effect of leptin on food intake and body weight As shown in Fig. 1a, continuous leptin infusion elevated plasma leptin levels from baseline almost equally in control, HFD, STZ and STZ/HFD mice. Under these conditions, food intake was significantly suppressed in control, STZ and STZ/HFD mice, while that in HFD was unchanged (Fig. 1b). Consistent with food intake, body weight was effectively decreased in control, STZ and STZ/HFD mice, while that in HFD mice was unchanged (Fig. 1c).

Effect of leptin on glucose metabolism In control mice, leptin infusion did not affect blood glucose levels during ad libitum feeding but markedly decreased plasma insulin levels, suggesting the enhancement of insulin sensitivity (Fig. 2a, e). In HFD mice, leptin infusion showed no effect on either blood glucose levels or plasma insulin levels (Fig. 2b, e). On the other hand, both blood glucose levels and plasma insulin levels were effectively decreased after 2 weeks of leptin infusion in STZ and STZ/HFD mice, suggesting the improvement of insulin sensitivity (Fig. 2c–e).

To further evaluate the effect of leptin on glucose metabolism, we performed i.p. GTTs (Fig. 3). In control, STZ and STZ/HFD mice, leptin infusion significantly improved glucose tolerance with reduction of plasma insulin levels not only in the fasting state but also after the glucose load, suggesting an improvement of insulin sensitivity. In contrast, in HFD mice, leptin infusion did not improve glucose tolerance and also did not suppress plasma insulin levels before or after glucose load.

Effect of leptin on plasma lipid profiles Leptin infusion did not affect plasma triacylglycerol, NEFA and total cholesterol levels in control mice (Fig. 4a–c). Leptin infusion also did not change plasma triacylglycerol, NEFA and total cholesterol levels in HFD mice, even though basal plasma NEFA and total cholesterol levels were elevated. In STZ mice, leptin infusion effectively decreased plasma triacylglycerol and NEFA levels, which were elevated at baseline, while leptin infusion did not affect plasma total cholesterol levels, which were not elevated at baseline. In STZ/HFD

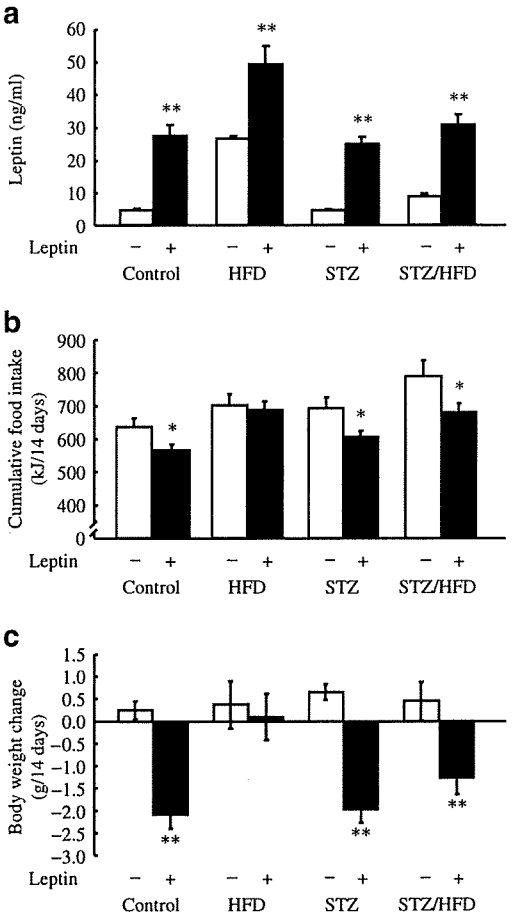

Fig. 1 Effect of leptin on leptin levels, food intake and body weight. Leptin levels on day 14 (**a**), cumulative food intake (**b**) and change in body weight (**c**) after 14 days of leptin infusion in control, HFD, STZ and STZ/HFD mice. Values are means±SEM (n=10–17). *p<0.05, **p<0.01 vs corresponding saline-infused mice

mice, leptin infusion also effectively decreased plasma triacylglycerol, NEFA and total cholesterol levels, which were elevated at baseline.

Effect of leptin on liver and skeletal muscle triacylglycerol contents To assess whether the improvement of glucose metabolism by leptin infusion was associated with the reduction of triacylglycerol content in insulin-target tissues, we examined the effect of leptin infusion on liver and skeletal muscle triacylglycerol contents. As shown in Fig. 5, leptin infusion apparently decreased triacylglycerol contents of both liver and skeletal muscle in control, STZ and STZ/HFD mice, in which glucose metabolism was improved by leptin infusion. In contrast, leptin infusion decreased triacylglycerol content of neither liver nor skeletal muscle in HFD mice, in which glucose metabolism was unchanged by leptin infusion.

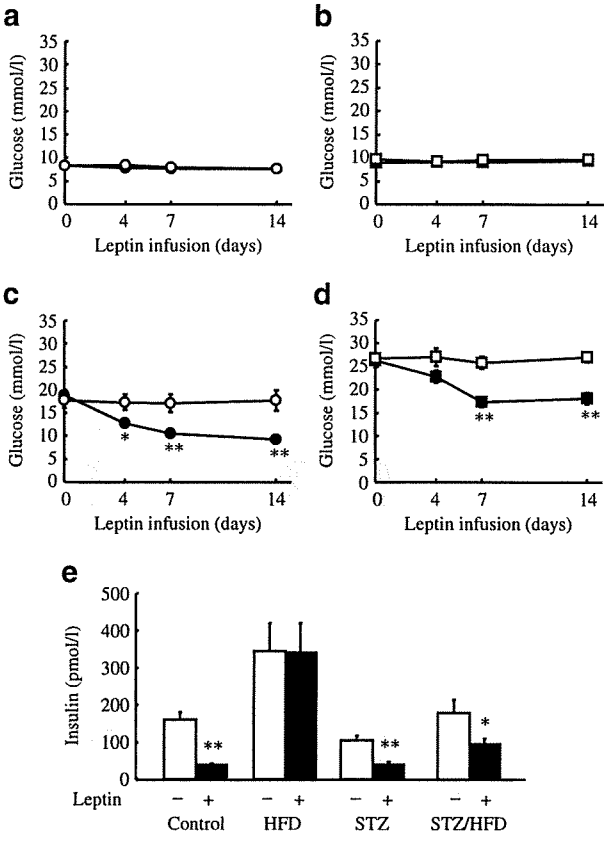

Fig. 2 Effect of leptin infusion for 14 days on blood glucose and plasma insulin levels during ad libitum feeding. Blood glucose levels on days 0, 4, 7 and 14 in control (**a**), HFD (**b**), STZ (**c**) and STZ/HFD mice (**d**). White symbols, saline-infused; black symbols, leptin-infused. **e** Plasma insulin levels during ad libitum feeding on day 14 in control, HFD, STZ and STZ/HFD mice. Values are means±SEM (n=10–17). *p<0.05, **p<0.01 vs corresponding saline-infused mice

Effect of leptin on AMPK activity in skeletal muscle Leptin infusion did not affect α1 isoform-specific AMPK activity in skeletal muscle in any group of mice (Fig. 6a). On the other hand, leptin infusion significantly increased α2 isoform-specific AMPK activity in skeletal muscle in control, STZ and STZ/HFD mice (Fig. 6b). However, no significant increase of α2 AMPK activity in skeletal muscle was observed in HFD mice.

Pair-feeding experiments We investigated whether the reduction of food intake by leptin infusion is the reason

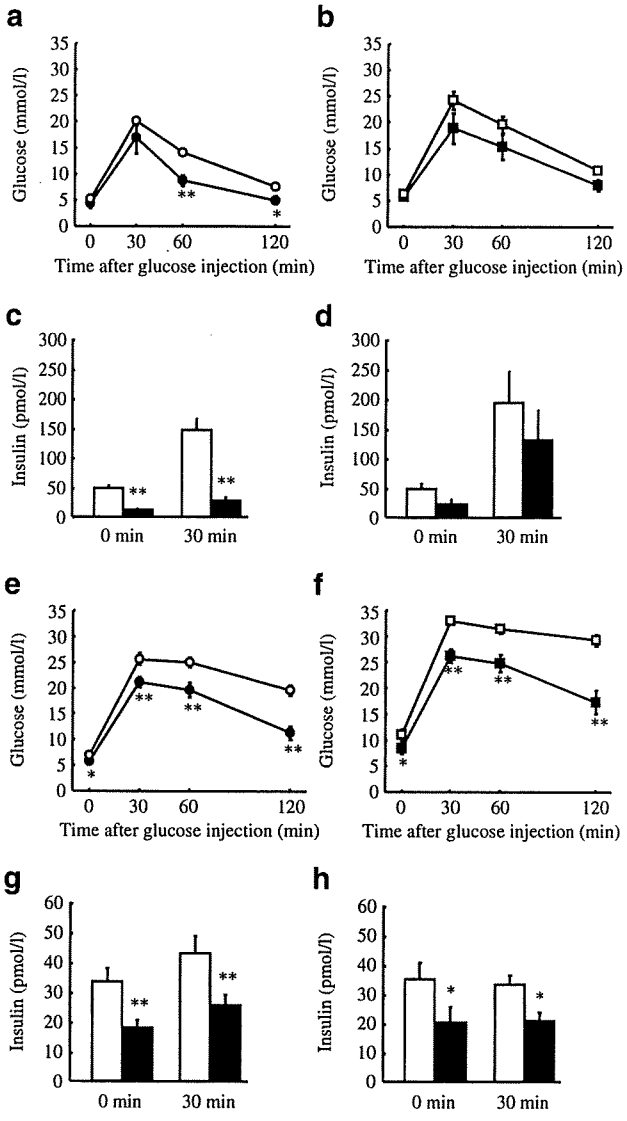

Fig. 3 Effect of leptin on glucose tolerance and insulin secretion during GTTs. Blood glucose and plasma insulin levels were measured at the indicated time points in control (**a, c**), HFD (**b, d**), STZ (**e, g**) and STZ/HFD mice (**f, h**). Values are means±SEM (n=10–17). *p<0.05, **p<0.01 vs corresponding saline-infused mice

for its efficacy in improving glucose metabolism. We pair-fed STZ and STZ/HFD mice the same amount of food consumed by the corresponding leptin-infused mice on the previous day. Pair-feeding did not improve glucose tolerance in GTTs in STZ and STZ/HFD mice (data not shown). Moreover, when compared with basal values (Table 1), no significant decrease of liver and skeletal muscle triacylglycerol contents was observed in pair-fed STZ and STZ/HFD mice (liver triacylglycerol content: 8.3±1.2 and 30.0±5.6 mg/g tissue; skeletal muscle triacylglycerol content: 5.4±0.5 and 6.6±0.5 mg/g tissue, in pair-fed STZ and STZ/HFD mice, respectively, n=5 in each group of mice), in contrast to the corresponding leptin-infused mice (Fig. 5).

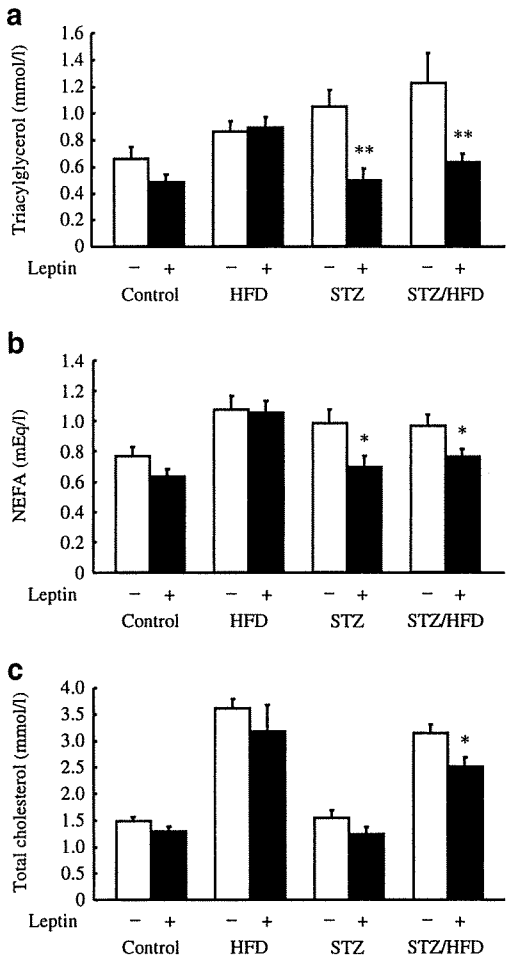

Fig. 4 Effect of leptin on plasma lipid profiles. Plasma triacylglycerol (**a**), NEFA (**b**) and total cholesterol levels (**c**) during ad libitum feeding on day 14 in control, HFD, STZ and STZ/HFD mice. Values are means±SEM (n=10–17). *p<0.05, **p<0.01 vs corresponding saline-infused mice

Discussion

The effectiveness of leptin treatment in diabetes has been reported in patients with leptin deficiency and lipodystrophy and in amenorrhoea in patients with hypothalamic hypogonadism caused by low body weight [5, 13–16, 27]. These patients are in hypoleptinaemic states, and hypoleptinaemia is involved in the pathophysiology of their diseases. However, whether leptin treatment is effective in normo- or hyperleptinaemic states has not been fully examined. The aim of the present study was to explore the therapeutic usefulness of leptin in type 2 diabetes, which is often accompanied by increased adiposity. Type 2 diabetes develops as a result of insulin resistance in target tissues and impaired insulin secretion, accompanied by increased adiposity. To generate a mouse model mimicking human type 2 diabetes, we used a combination of low-dose STZ and HFD. Although high-dose STZ injection generally reduces body weight, with a marked reduction of insulin levels [16], low-dose STZ used in this study did not reduce body weight. In addition, subsequent HFD feeding in low-dose STZ injected mice could increase body weight even

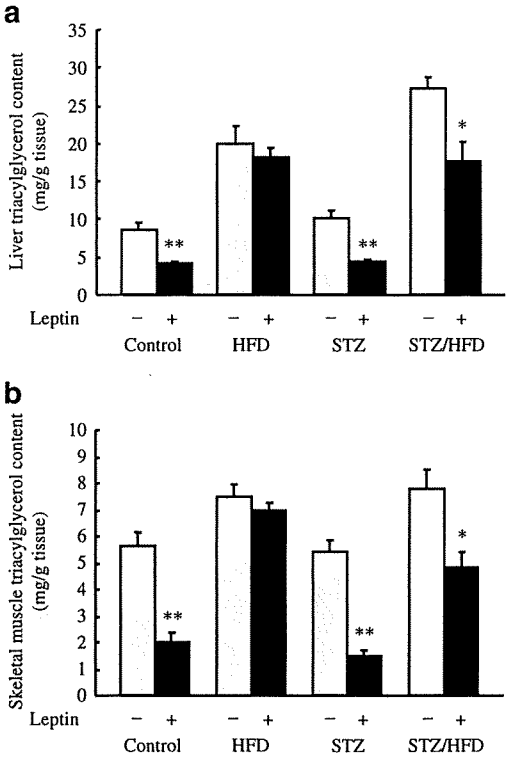

Fig. 5 Effect of leptin on liver and skeletal muscle triacylglycerol contents. Liver (**a**) and skeletal muscle (**b**) triacylglycerol contents on day 14 in STZ and STZ/HFD mice. Values are means±SEM (n=10–13). *p<0.05, **p<0.01 vs corresponding saline-infused mice

with the impairment of insulin secretion in this study. Consistent with the increase in body weight and per cent body fat, STZ/HFD mice showed a nearly twofold increase in plasma leptin levels compared with control mice (Table 1). In humans, plasma leptin levels positively correlated with BMI, and a twofold increase in plasma leptin levels corresponds to a BMI in the range of 25–30 kg/m^2 [28, 29]. According to recent clinical studies, the average BMI in patients with type 2 diabetes is within this overweight range [30–32]. HFD mice showed a larger increase in adiposity and plasma leptin levels than did STZ/HFD mice. However, unlike STZ/HFD mice, HFD mice did not develop hyperglycaemia, because of compensatory hyperinsulinaemia. Therefore, we used STZ/HFD mice as an appropriate model to examine the efficacy of leptin in type 2 diabetes with increased adiposity.

The present study showed that the effect of leptin on food intake and body weight was attenuated in obese HFD mice (Fig. 1b, c). In general, in human obesity and rodent models of diet-induced obesity, even though leptin levels rise proportionally with adiposity, the increased leptin fails to suppress the progression of obesity. Moreover, obese humans and rodents are weakly responsive to exogenously administered leptin in terms of body weight reduction [33, 34]. This leptin ineffectiveness is called leptin resistance. The present study also showed that the effect of leptin on glucose and lipid metabolism was attenuated in obese HFD mice (Figs 2, 3, 4 and 5). In contrast, even under HFD feeding, leptin effectively improved glucose and lipid metabolism in STZ/HFD mice. Impaired insulin secretion caused by STZ injection could reduce the effect of HFD feeding on the development of obesity in STZ/HFD mice. As a result, leptin resistance could be mild, if any, in STZ/HFD mice. The present study demonstrated that leptin could be a glucose-lowering drug for the treatment of type 2 diabetes with impaired insulin secretion.

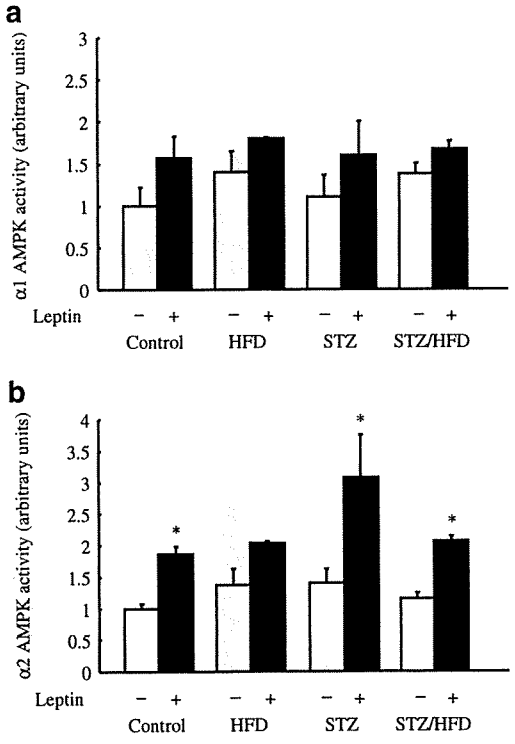

Fig. 6 Effect of leptin on isoform-specific AMPK activity in skeletal muscle. α1 AMPK activity (**a**) and α2 AMPK activity (**b**) on day 14 in soleus muscle of STZ and STZ/HFD mice. Values are means±SEM (n=4–5). *p<0.05 vs corresponding saline-infused mice

Fat accumulation in insulin target tissues is considered to be one of the causes of insulin resistance, and is called lipotoxicity [35, 36]. Indeed, HFD and STZ/HFD mice exhibited insulin resistance and increased liver and skeletal muscle triacylglycerol contents (Table 1). In the present study, we investigated an association between the improvement of glucose metabolism by leptin infusion and the reduction of liver and skeletal muscle triacylglycerol contents. Leptin infusion enhanced insulin sensitivity in control, STZ and STZ/HFD mice, in which it decreased liver and skeletal muscle triacylglycerol contents (Figs 3 and 5). In contrast, leptin infusion did not improve insulin resistance in HFD mice, in which it did not decrease liver and skeletal muscle triacylglycerol contents. Moreover, pair-feeding neither improved glucose tolerance nor decreased the liver and skeletal muscle triacylglycerol contents in STZ and STZ/HFD mice. These results suggest that the improvement of glucose metabolism by leptin infusion is associated with a reduction in liver and skeletal muscle triacylglycerol contents.

Leptin has been shown to selectively stimulate activation of the α2 catalytic subunit of AMPK in skeletal muscle [37]. AMPK is a key enzyme that mediates the leptin effect on fatty acid β-oxidation in skeletal muscle. In the present study, leptin infusion effectively decreased skeletal muscle triacylglycerol content in control, STZ and STZ/HFD mice (Fig. 5b), in which it increased α2 AMPK activity in skeletal muscle (Fig. 6b). In contrast, leptin infusion did not decrease skeletal muscle triacylglycerol content in HFD mice (Fig. 5b), in which it did not increase α2 AMPK activity in skeletal muscle (Fig. 6b). Increased fatty acid β-oxidation through α2 AMPK activation in skeletal muscle is considered to be one of the mechanisms by which leptin decreases skeletal muscle triacylglycerol content [9].

The present study also showed that leptin infusion effectively improved hyperlipidaemia in STZ and STZ/HFD mice (Fig. 4). Increased lipoprotein lipase activity, increased clearance of triacylglycerol [7], reduction of triacylglycerol synthesis by controlling key transcription factors [38] and increased energy expenditure through fatty acid β-oxidation have been reported as mechanisms by

which leptin decreases plasma triacylglycerol levels. The present study demonstrated activation of α2 AMPK activity by leptin infusion in skeletal muscle (Fig. 6b), which might contribute to increased energy expenditure in our leptin-infused STZ and STZ/HFD mice. It is also well known that impaired insulin action induces hyperlipidaemia [39]. It is also possible that leptin improved hyperlipidaemia by enhancement of insulin sensitivity in the present study.

The present study demonstrated that pair-feeding neither improved glucose tolerance nor decreased liver and skeletal muscle triacylglycerol contents in STZ and STZ/HFD mice. Previously, we and others have demonstrated that food intake reduction alone was insufficient for improving glucose and lipid metabolism [6, 10, 12]. It has also been reported that fasting insulin and triacylglycerol levels increased within several days after withdrawal of leptin administration even though the level of food intake remained constant in the patients with lipodystrophy [13]. Furthermore, it has been demonstrated that leptin administration decreases liver and skeletal muscle triacylglycerol contents in patients with lipodystrophy [40]. These results indicate that leptin improves glucose and lipid metabolism independently of the food intake reduction.

With the dose of leptin used in the present study, the plasma leptin levels in STZ/HFD mice increased to the levels of obese HFD mice (mean leptin levels in leptin-infused STZ/HFD mice, 30.8 ng/ml) (Fig. 1a), which can be seen in human obese individuals. In our clinical research on leptin-replacement therapy in patients with generalised lipodystrophy, the peak plasma leptin levels of the 400% dose under the protocol of once-daily injections was 34.5± 2.1 (mean±SE) ng/ml, and the therapy was well tolerated without any adverse effects for about 5 years [15]. In addition, higher leptin levels were obtained in the obese human clinical trial [33]. Therefore, the leptin levels achieved with the dose used in the present study could be clinically applied in humans.

In conclusion, the present study demonstrates that leptin therapy improves glucose and lipid metabolism and enhances insulin sensitivity in a mouse model of type 2 diabetes with an overweight range of adiposity. Our findings indicate that leptin could be a new glucose-lowering drug for the treatment of type 2 diabetes in humans.

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