EXPERIMENTAL STUDY

***In vivo* and *in vitro* suppression by leptin of glucose-stimulated insulin hypersecretion in high glucose-fed rats**

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Abstract

Objectives: Chronic feeding to rats of high glycaemic index (GI) diets results in the hypersecretion of insulin in response to an i.v. glucose load. The first aim of this study was to see if this exaggerated insulin response was accompanied by a hypersensitivity to glucose stimulation in isolated islets *in vitro*. The second aim was to see if the adipocyte factor, leptin, was able to alter insulin secretion in this model both *in vivo* and *in vitro*.

Design and methods: Rats were fed for 6 weeks either a high GI diet in which the carbohydrate component was mostly glucose (GLUC diet) or a low GI diet containing mostly amylose (AMOSE diet). Rats then underwent an i.v. glucose tolerance test (ivGTT) (1 g/kg) with and without a prior infusion of leptin (133 µg/kg per h). Islets were then isolated from these rats and basal and glucose-stimulated insulin secretion (GSIS) measured in both the absence and presence (100 ng/ml) of leptin.

Results and conclusions: Peak insulin response during the ivGTT was 3-fold greater in GLUC rats ($P < 0.001$). Leptin had no effect on AMOSE rat insulin response but lowered the GLUC rat response to AMOSE rat levels. *In vitro*, basal insulin secretion was 4-fold greater in GLUC rats ($P < 0.05$). At 20 mmol/l glucose, there was no further increase in insulin secretion in GLUC rats but a 2-fold increase in AMOSE rats. Leptin had no effect on basal insulin secretion or GSIS in AMOSE rats but reduced basal insulin secretion and GSIS in GLUC rats. These results show insulin hypersecretion in high GI-fed rats may be reduced by leptin.

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Introduction

Dietary carbohydrate consists of many polysaccharides which differ in their rate of digestion and in their effect on postprandial blood glucose levels (1). Long-term feeding to rodents of diets in which the carbohydrate component consists mainly of rapidly absorbed, high glycaemic index (GI) starches causes hypersecretion of insulin in response to an acute glucose challenge (2), suggesting an increased demand for insulin to facilitate peripheral glucose utilisation. However, although tissue-specific dietary-induced changes in insulin sensitivity have been reported in individual tissues in rodent studies (3–5), euglycaemic, hyperinsulinaemic clamp studies have shown no overall whole body change in peripheral glucose uptake in humans (6) or rodents (7) fed these high GI starches. This suggests that the hypersecretion of the β -cell in response to glucose is due to dietary-induced changes in islet metabolism rather than increased peripheral insulin resistance.

Recently it has been shown that receptors for leptin, the adipocyte factor involved in the regulation of food intake, are present in the β -cell (8–11). Leptin at physiological doses has been shown to inhibit both basal

and glucose-stimulated insulin secretion (GSIS) *in vitro* (8, 10–13), although this has not been found in all studies (11, 14, 15). In other studies, both acute and chronic leptin administration have been shown to lower circulating insulin levels *in vivo* (16–18).

The primary aim of this study was to see if the hypersecretion of insulin in response to a glucose challenge *in vivo* in rodents fed a high GI diet was accompanied by a hypersensitivity to glucose stimulation in isolated islets *in vitro*. The second aim was to see if exposure to leptin was able to alter this response both *in vivo* and *in vitro*.

Animals and methods

Animals

Specific pathogen-free male Wistar rats were purchased from the Scientific Research Centre, Perth, WA, Australia, at 6 weeks of age and weighed 180 ± 2 g (S.E.M.) at the commencement of the study. The rats were housed five to a cage in the Biochemistry Animal House, University of Sydney. Animals were maintained

Table 1 Composition of low GI (AMOSE) and high GI (GLUC) diets fed to rats for 6 weeks. Rats were meal fed twice daily (11.5 g/meal).

Component	AMOSE diet (g/kg diet)	GLUC diet (g/kg diet)
Casein	200	200
Gelatine	19	19
D,L-Methionine	2	2
Mineral mix	67	67
Vitamin mix	13	13
Glucose	0	514
Hi Maize flour (60% amylose/40% amylopectin)	514	0
Sucrose	85	85
Unprocessed bran	50	50
Mono-saturated vegetable oil	25	25
Polyunsaturated sunflower oil	25	25
Energy content (kJ/g)	15	15

at 22 °C on a 12 h light:12 h darkness cycle (lights on from 0600 to 1800 h). Approval for the study was obtained from the Sydney University Animal Ethics committee.

Diets

Upon arrival the rats were randomly divided into two groups to be fed either the amylose diet (AMOSE) or the glucose diet (GLUC), the compositions of which are described in Table 1. Both diets contained 65% carbohydrate (55% starch or glucose; 10% sucrose), 22% protein and 11% fat as a percentage of total energy. The diets were prepared weekly and 11.5 g per rat were given to the animals in an unpelleted form, twice daily. Rats were maintained on the diets for 6 weeks and weighed weekly to monitor weight gain.

i.v. glucose tolerance test (ivGTT)

After 6 weeks on the diet, silastic cannulae were inserted into the left and right jugular veins under anaesthesia for infusion of glucose and leptin and for blood sampling. The rats were allowed to recover from surgery for 3–4 days in individual cages and were then fasted overnight before the ivGTT. After a baseline blood sample was taken, leptin-treated rats were given a bolus of recombinant mouse leptin (Amgen, Thousand Oaks, CA, USA) (500 µg/kg) followed by a continuous infusion of leptin (133 µg/kg per h) in 0.9% w/v NaCl for 1 h. This protocol was chosen as it has been used in other rat studies to increase plasma leptin levels to supraphysiological levels and to have an effect on insulin sensitivity during euglycaemic hyperinsulinaemic clamp studies (18). Control rats received vehicle only. A further baseline blood sample was taken and all rats were then given an *i.v.* bolus of 50% glucose (1 g/kg) and blood samples taken at various time points over the next 60 min.

Isolation of the islets of Langerhans and incubation with glucose and leptin

Rats were given 2 days to recover from the ivGTT and were then killed in the fed state under sodium pentobarbitone anaesthesia. Islets were isolated by collagenase digestion (19). For each incubation, 20 islets were handpicked and preincubated in 2 mmol/l glucose at 4 °C for 15 min. The 20 islets were further incubated in 1.0 ml Krebs–Ringer bicarbonate buffer supplemented with 3% fatty acid-free BSA containing either 5 or 20 mmol/l glucose, with or without leptin (100 ng/ml) for 1 h at 37 °C. Incubation medium was stored at –20 °C for subsequent insulin assay. Results are expressed as nanograms insulin per millilitre incubation medium, which represents the insulin secreted by 20 islets in 1 h. Unused islets were frozen for measurement of triglyceride (TG) content.

Serum analyses

Serum leptin was measured using a commercial RIA kit (Linco Research, St Louis, MO, USA). Intraassay variation was 4.2% and interassay variation less than 6.0%. Serum insulin was measured by RIA using rat insulin standards and anti-rat insulin first antibody (Linco). Intraassay variation was 6.5% and interassay variation less than 8.0%. Serum glucose was measured by a glucose oxidase/peroxidase method with 4-amino-antipyrene as the dye, and serum TG and non-esterified fatty acids (NEFA) by commercial *in vitro* enzymatic colorimetric assays (Boehringer Mannheim, Mannheim, Germany and WAKO, Osaka, Japan).

Islet TG assay

TGs were extracted from the islets by chloroform/methanol extraction following sonication of the islets for 60 s. TG content was measured by a colorimetric enzymatic procedure as for serum analyses.

Table 2 Differences in body weight, pancreas weight and serum hormone and metabolite levels between groups of rats fed either a high glucose diet (GLUC) or high amylose diet (AMOSE) for 6 weeks. Results are means $\pm$ S.E.M.. The number in each group is in parentheses.

	GLUC	AMOSE
Final body weight (g)	340 $\pm$ 4 (23)	305 $\pm$ 3 (26) ^a
Weight of pancreas (g)	1.5 $\pm$ 0.1 (10)	1.2 $\pm$ 0.1 (10) ^a
Islet TG content (μ mol/g wet weight)	33.7 $\pm$ 5.0 (12)	8.3 $\pm$ 1.9 (12) ^b
Fed plasma levels (no. per group)	(6)	(12)
Glucose (mmol/l)	9.31 $\pm$ 0.76	10.40 $\pm$ 0.35
Insulin (ng/ml)	5.19 $\pm$ 0.65	5.48 $\pm$ 0.65
Leptin (ng/ml)	5.19 $\pm$ 1.09	3.28 $\pm$ 0.43 ^a
TGs (mmol/l)	1.45 $\pm$ 0.15	1.08 $\pm$ 0.11 ^a
NEFAs (mmol/l)	0.49 $\pm$ 0.10	0.28 $\pm$ 0.04 ^a

^a $P < 0.05$, ^b $P < 0.001$ for differences between GLUC and AMOSE groups.

Statistics

All results are given as means $\pm$ S.E.M. Differences between groups and the effects of leptin were examined using Student's *t*-test or one-way ANOVA using the Statview IV statistical package (Abacus Concepts, Berkeley, CA, USA).

Results

Differences in anthropometric and metabolic parameters after 6 weeks of feeding are summarised in Table 2. GLUC rats gained weight more quickly than AMOSE rats and were significantly heavier by 3 weeks. Both the weight of the pancreas and the TG content of the islets were significantly higher in GLUC rats. There was no difference in plasma glucose and insulin levels but GLUC rats had higher leptin, reflecting the higher body weight, as well as higher TG and NEFA levels.

Glucose responses to the ivGTT are shown in Fig. 1. There was no difference in the basal glucose level, the peak glucose response and the area under the curve (AUC) between GLUC rats and AMOSE rats (Table 3). Leptin infusion for 1 h before the ivGTT had no effect on any of these parameters. Although basal insulin levels were the same, there was a significantly greater insulin response to the glucose load in GLUC rats (Fig. 2) with a higher peak value and an increase in the AUC (Table 3). Prior leptin infusion had no effect on the insulin response in AMOSE rats. However, there was a dramatic decrease in the size of the insulin response in GLUC rats such that there was no difference between AMOSE rats and the leptin-treated GLUC rats.

Islets isolated from GLUC rats had a 4-fold higher insulin secretion rate than those from AMOSE rats after incubation in basal (5 mmol/l) glucose (Fig. 3) ($P < 0.05$). Whereas increasing the glucose concentration to 20 mmol/l caused a 2-fold increase in insulin secretion in the AMOSE group ($P < 0.05$), there was no change in the already-elevated insulin secretion from GLUC rat islets. The addition of leptin to the incubation medium

Figure 1 Plasma glucose response to an i.v. glucose load (1 g/kg) in GLUC rats (upper panel) and AMOSE rats (lower panel) with (○) and without (●) prior leptin infusion. Treated rats were given a bolus of leptin (500 μ g/kg) followed by a continuous infusion of leptin (133 μ g/kg per h) in 0.9% w/v saline for 1 h. Untreated animals received vehicle only. Each point is the mean $\pm$ S.E.M. for at least six rats. Results are summarised in Table 3.

Table 3 Basal plasma hormone and metabolite levels and the glucose and insulin responses to an ivGTT (1 g/kg) in both AMOSE- and GLUC-fed rats with and without prior exposure to leptin. Leptin was given as a bolus of 500 μ g/kg followed by a continuous infusion of leptin (133 μ g/kg) for 1 h. Control animals received vehicle only. Results are means $\pm$ S.E.M.

	GLUC		AMOSE	
	No leptin (n = 8)	Leptin (n = 5)	No leptin (n = 8)	Leptin (n = 6)
Basal levels				
Glucose (mmol/l)	5.4 $\pm$ 0.3	5.9 $\pm$ 0.2	5.7 $\pm$ 0.3	5.7 $\pm$ 0.1
Insulin (ng/ml)	2.0 $\pm$ 0.2	1.7 $\pm$ 0.3 ^a	1.6 $\pm$ 0.3	0.9 $\pm$ 0.2 ^{a,c}
Leptin (ng/ml)	2.8 $\pm$ 0.4	47.0 $\pm$ 2.1 ^b	2.3 $\pm$ 0.4	64.3 $\pm$ 5.7 ^b
TGs (mmol/l)	1.8 $\pm$ 0.2	0.8 $\pm$ 0.3 ^a	1.2 $\pm$ 0.2 ^c	0.9 $\pm$ 0.2
Peak levels				
Glucose (mmol/l)	30.3 $\pm$ 0.8	30.6 $\pm$ 0.6	34.2 $\pm$ 2.7	30.9 $\pm$ 1.3
Insulin (ng/ml)	65.4 $\pm$ 10.5	19.7 $\pm$ 4.0 ^b	14.3 $\pm$ 1.8 ^d	13.6 $\pm$ 1.2
AUC				
Glucose (mmol/l $\times$ h)	590 $\pm$ 26	633 $\pm$ 12	587 $\pm$ 20	653 $\pm$ 11
Insulin (ng/ml $\times$ h)	707 $\pm$ 163	298 $\pm$ 48 ^b	200 $\pm$ 30 ^d	214 $\pm$ 26

^a $P < 0.05$, ^b $P < 0.01$ for effect of leptin treatment. ^c $P < 0.05$, ^d $P < 0.01$ for differences between GLUC and AMOSE.

had no effect on the insulin secretion rate at either glucose concentration in the AMOSE rats. However, in islets from GLUC rats, leptin caused a non-significant decrease in insulin secretion at basal glucose, with greater attenuation of insulin secretion being seen in the presence of 20 mmol/l glucose ($P < 0.05$), such that there was no difference in secretion between islets from GLUC and AMOSE rats.

Figure 2 Plasma insulin response to an i.v. glucose load (1 g/kg) in GLUC rats (upper panel) and AMOSE rats (lower panel) with (○) and without (●) prior leptin infusion. Treated rats were given a bolus of leptin (500 μ g/kg) followed by a continuous infusion of leptin (133 μ g/kg per h) in 0.9% w/v saline for 1 h. Untreated animals received vehicle only. Each point is the mean $\pm$ S.E.M. for at least six rats. Results are summarised in Table 3.

Figure 3 Basal insulin secretion (at 5 mmol/l glucose) and GSIS (20 mmol/l glucose) from islets isolated from GLUC rats (upper panel) and AMOSE rats (lower panel) incubated with (shaded bars) and without (open bars) leptin (100 ng/ml) for 1 h at 37°C. Results are means $\pm$ S.E.M. of values obtained from islets isolated from five rats in each dietary group. Each incubation contained 20 islets in 1.0 ml incubation medium and each point is the mean of five incubations. a: $P < 0.05$ for the effect of leptin treatment. b: $P < 0.05$ for differences between GLUC rats and AMOSE rats. c: $P < 0.05$ for differences between insulin secretion at 5 and 20 mmol/l glucose. All other differences were not significant.

Discussion

Feeding of a diet rich in high GI carbohydrate resulted in an increase in body weight as well as elevated plasma leptin, TG and NEFA levels. This was accompanied by an increased insulin response to glucose *in vivo*, with this hypersensitivity to glucose being maintained in isolated islets. *In vivo*, leptin was able to normalise this response. Leptin also inhibited insulin hypersecretion *in vitro*, showing that the effects of leptin on insulin secretion are direct effects on the islets. There was no effect of leptin on insulin secretion in the AMOSE rats, either *in vivo* or *in vitro*, suggesting that an effect of leptin is only seen when islet conditions predispose the islets to leptin action. In this study the level of hyperleptinaemia achieved *in vivo* was several fold higher than the fed endogenous levels and so it is not possible to say whether this effect would have been seen at more physiological leptin concentrations or whether the elevated fed level in GLUC rats was already attenuating insulin secretion in this model.

Dietary rodent models of insulin resistance, including high fat- and high fructose-fed rats exhibit impaired glucose tolerance as well as reduced glucose uptake during clamp studies (20–22). These animals may exhibit basal hyperinsulinaemia and the impaired glucose tolerance is accompanied by an elevated insulin response. In GLUC rats, there is no difference in glucose tolerance during an ivGTT or in glucose utilisation during clamp studies between low and high GI-fed rats (7). Thus the hypersecretion of insulin during an ivGTT in the GLUC rat does not appear to be due to the development of peripheral insulin resistance, but due to an enhanced sensitivity of the pancreas to glucose as confirmed in the *in vitro* studies. It is interesting that there was no transient hypoglycaemia following the exaggerated insulin response in the GLUC rats, suggesting that the duration of the insulin peak was too short to produce any measurable change in glucose or that the animals are mildly insulin resistant at physiological insulin levels.

GLUC rats exhibit postprandial hyperglycaemia but not fasting hyperglycaemia or elevated plasma glucose following an i.v. glucose load. Thus the hypersensitivity of islets from these rats to glucose appears to be due to exaggerated fluctuations in plasma glucose rather than to any chronic hyperglycaemia. The increased insulin secretion in this study is in contrast to that found with chronic hyperglycaemia where GSIS is suppressed in association with a reduction in GLUT 2 glucose transporters and a decreased activity of glucokinase (23).

Another possible mechanism for the changes in glucose sensitivity in the GLUC rats may be provided by the significantly elevated islet TG content. Both *in vivo* and *in vitro* studies have recognised the importance of fatty acids in the regulation of GSIS. *In vivo*, lowering of plasma NEFA levels in fasted rats prevents insulin secretion in response to glucose (24) and other

secretagogues (25). *In vitro*, chronic fatty acid exposure has been reported to both inhibit and stimulate GSIS (26–28). Hyperinsulinaemia *in vivo* and GSIS *in vitro* have been shown to be highly correlated with the TG content of the pancreas (29). In the prediabetic phase of insulin hypersecretion in Zucker diabetic fatty (ZDF) rats, islet TG content is 5–10 times that of controls (26), similar to the levels found in the GLUC rats in this study. While all this evidence shows that an increase in fatty acid availability enhances insulin secretion, too much TG is toxic with β -cell failure being associated with a pancreatic TG content 50 times that of normal islets (30).

With the exception of those obese rodent models which lack functional leptin receptors in the pancreas (db/db mice and ZDF rats), *in vivo* acute and chronic studies have shown leptin-induced reductions in plasma insulin which are greater in obese animals than in lean animals (16, 17). Thus it appears that an effect of leptin on insulin secretion requires pancreatic TG stores which are both increased and accessible to leptin action. This was confirmed by a recent study in which the over-expression of leptin receptors in ZDF rats was accompanied by a reduction in TG stores (31) and restoration of GSIS (23). Leptin has been shown to alter fatty acid metabolism in the β -cell by depletion of TG stores (32, 33), increasing fatty acid oxidation (32) and increasing the expression of enzymes involved in fatty acid oxidation (34). Thus leptin's effects on GSIS may be due to its effects on islet lipid partitioning with the primary role of leptin in the β -cell being to prevent lipotoxicity. Leptin's effects on insulin secretion involve the activation of ATP-sensitive potassium channels (35). Long-chain acyl-CoA esters bind to and open these channels (36) and so the ability of leptin to alter insulin secretion may involve the formation of these long-chain acyl-CoA esters. Leptin has also been shown to inhibit the transcription of the preproinsulin gene (37) with this effect on insulin production involving different cellular pathways from those controlling insulin secretion.

Leptin production and secretion is regulated by insulin (38) with hyperinsulinaemia preceding or accompanying the development of hyperleptinaemia in different obesity models (39–41). The existence of an adipo-insular axis in which leptin regulates insulin production and secretion and insulin regulates leptin production and secretion has important implications for understanding the development of insulin resistance, obesity and type 2 diabetes in humans (38). The consumption of diets which cause a greater rise in postprandial insulin secretion may contribute to the development of obesity and hyperleptinaemia as seen in this study. High GI diets have also been shown to cause changes in lipid storage enzymes in adipose tissue which favour fat synthesis and deposition (4). Prolonged hyperleptinaemia in turn may lead to suppression of insulin secretion in an attempt to limit the size of these fat stores and

subsequently the development of insulin resistance and diabetes. In a recent paper, Ferrannini *et al.* (42) established that more obese subjects are hyperinsulinaemic than are insulin-resistant suggesting that hypersecretion of insulin is not just a compensatory mechanism in response to peripheral insulin resistance but is a primary event in the development of obesity. Thus dietary strategies designed to reduce postprandial hyperinsulinaemia, i.e. diets in which the carbohydrate component consists mainly of low GI starches, could be important in the prevention of weight gain in humans.

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