The augmenting effect on insulin secretion by oral versus intravenous glucose is exaggerated by high-fat diet in mice

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Abstract

To study whether the incretin effect is involved in adaptively increased insulin secretion in insulin resistance, glucose was infused at a variable rate to match glucose levels after oral glucose (25 mg) in normal anesthetized C57BL/6J female mice or in mice rendered insulin resistant by 8 weeks of high-fat feeding. Insulin response was markedly higher after oral than i.v. glucose in both groups, and this augmentation was even higher in high-fat fed than normal mice. In normal mice, the area under the curve ($AUC_{insulin}$) was augmented from $4 \cdot 0 \pm 0 \cdot 8$ to $8 \cdot 0 \pm 1 \cdot 8$ nmol/l$\times$60 min by the oral glucose, i.e. by a factor of 2 ($P=0 \cdot 023$), whereas in the high-fat fed mice, $AUC_{insulin}$ was augmented from $0 \cdot 70 \pm 0 \cdot 4$ to $12 \cdot 4 \pm 2 \cdot 5$ nmol/l$\times$60 min, i.e. by a factor of 17 ($P<0 \cdot 001$).

To examine whether the incretin hormone glucagon-like peptide-1 (GLP-1) is responsible for this difference, the effect of i.v. GLP-1 was compared in normal and high-fat fed mice. The sensitivity to i.v. GLP-1 in stimulating insulin secretion was increased in the high-fat diet fed mice: the lowest effective dose of GLP-1 was 650 pmol/kg in normal mice and 13 pmol/kg in the high-fat diet fed mice. We conclude that 1) the incretin effect contributes by $\sim$50% to insulin secretion by the oral glucose in normal mice, 2) this effect is markedly exaggerated in insulin-resistant mice fed a high-fat diet, and 3) this augmented incretin contribution in the high-fat fed mice may partially be explained by GLP-1.

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Introduction

The entero–insular axis represents the physiological regulation of islet function by the gut (Unger & Eisentraut 1969). A key component of this axis is the incretin effect, which augments increase in insulin secretion following oral versus i.v. glucose (Elrick *et al*. 1964, Perley & Kipnis 1967). This effect is partially achieved by gastrointestinal hormones that are released during meal ingestion and augment glucose-stimulated insulin secretion; the two most important being glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP; Vilsboll & Holst 2004, Drucker 2006). The effect is, however, also partially achieved by autonomic nerves activated by oral glucose. Thus, cholinergic nerves activated during meal ingestion may stimulate insulin secretion (Ahrén 2000). In humans, a study with careful matching of glucose levels after oral versus i.v. administration has shown that the incretin effect contributes to 70–90% of the insulin response to oral glucose (Nauck *et al*. 1986). Similarly, also in pigs, the incretin effect contributes to a large degree of the insulin response to oral glucose (Lindkaer- Jensen *et al*. 1975).

Insulin resistance upregulates insulin secretion, and if this adaptation fails, impaired glucose tolerance or type 2 diabetes develops (Ahrén & Pacini 2005). The mechanisms of upregulated insulin secretion in insulin resistance may include slight elevation of glucose or free fatty acids (Ahrén & Pacini 2003). Whether the upregulation of insulin secretion also involves the incretin effect is, however, not known. To study this possibility, incretin contribution to the insulin response to oral glucose was examined in the animal model of high-fat feeding of mice. In this model, insulin resistance develops, and on a long-term basis, this is compensated by increased insulin secretion (Pacini *et al*. 2001, Ahrén & Pacini 2002, Winzell & Ahrén 2004). Previous studies have inferred that there is an upregulation of the insulin response also to non-glucose stimuli such as carbachol (cholinergic agonist), 2-deoxy-glucose (activating autonomic nerves), and GLP-1 in this model (Ahrén *et al*. 1997, Simonsson & Ahrén 1998). However, these studies have used exogenous administration of secretory agents and, therefore, whether the endogenous incretin effect *per se* is altered in insulin resistance is still not known.

In this study, we have therefore examined the incretin contribution to insulin secretion during oral glucose in normal and high-fat fed insulin-resistant mice. We estimated insulin secretion after oral and i.v. glucose administration when the glucose levels were matched in these two conditions. We also examined the GLP-1 response to the oral glucose load and the insulin response to low-dose GLP-1 in the model for examining

the possibility that GLP-1 may contribute to the augmented incretin effect in insulin resistance.

Materials and Methods

Animals

Eight-week-old female C57BL/6J mice were obtained from Taconic, Skensved, Denmark, and kept in a temperature-controlled room (22 °C) on a 12 h light:12 h darkness cycle. The animals were divided into two groups and given a standard pellet diet (fat 10%, carbohydrate 70%, protein 20% on an energy basis, total energy 16·1 kJ/g) or a high-fat diet (fat 60%, carbohydrate 20%, protein 20% on an energy basis, total energy 22·0 kJ/g) for 8 weeks. The main difference between the two diets is that the high-fat diet has a high content of lard (317 g/kg) and 32 g/kg soybean oil compared with the normal diet (19 g/kg lard and 24 g/kg soybean oil), and that the bulk of the normal diet is cornstarch (299 g/kg) that is not included in the high-fat diet. Tap water was made available *ad libitum*. This study was approved by the Ethics Committee in Lund/Malmö.

Oral glucose administration

The oral glucose test was performed late in the morning after the removal of food from the cages 16 h earlier. The animals were anesthetized with an i.p. injection of midazolam (0·1 mg/mouse, Dormicum; Hoffman-La-Roche, Basel, Switzerland) and a combination of fluanison (0·4 mg/mouse) and fentanyl (0·013 mg/mouse; Hypnorm; Janssen, Beerse, Belgium). Thirty minutes later, a blood sample was taken from the retrobulbar, intraorbital, capillary plexus into heparinized tubes. Then, D-glucose (Sigma–Aldrich) was dissolved in saline and administered orally through a gastric tube (outer diameter 1·2 mm, 25 mg/mouse). The load volume was 10 µl/g body weight. At specific time points after glucose administrations (0, 15, 30, 60, and 120 min), blood samples, 50 µl each, were collected. Anesthesia was maintained throughout the 120-min study period. Blood was collected in heparinized tubes and immediately centrifuged, whereupon plasma was separated and stored at −20 °C until analysis for insulin, glucose and active GLP-1.

i.v. glucose infusion

The i.v. glucose test was performed late in the morning after the removal of food from the cages 16 h earlier. The animals were anesthetized as described above. Ten minutes later, the right jugular vein and the left carotid artery were catheterized. The venous catheter was used for infusion of glucose and the arterial catheter for sample collection. After another 30 min, a variable rate glucose infusion was started. The glucose solution was 20 g/dl and plasma glucose was determined every 5 min by the glucose dehydrogenase technique with the use of a Hemocue (Ängelholm, Sweden). The glucose infusion rate was adjusted to achieve the same glucose level as obtained during the oral glucose test when glucose was administered at a dose of 25 mg. Blood samples for the determination of insulin and C-peptide were taken at 0, 15, 30, 60 and 120 min, i.e. at the same time points as in the oral test.

i.v. GLP-1 test

Animals were anaesthetized as above. Glucose alone (0·35 g/kg) or glucose together with GLP-1 (13, 130, or 650 pmol/kg; Sigma) was injected intravenously; blood samples for the analysis of insulin were taken before the i.v. injection and 1 and 5 min after injection.

Analyses

Plasma insulin was analyzed radioimmunochemically with a double antibody assay using a guinea pig anti-rat insulin antibody, ^{125}I-labeled human insulin as the tracer, and rat insulin as the standard (Linco Research, St Charles, MO, USA). Plasma C-peptide was analyzed radioimmunochemically with a double antibody assay using guinea pig anti-rat C-peptide antibody (which cross-reacts to 100% with mouse C-peptide), rat C-peptide standard, and ^{125}I-rat C-peptide as the tracer (Linco). Plasma levels of active GLP-1 were determined by the ELISA technique using monoclonal guinea pig antibodies specific for the active (intact) form of GLP-1 (Linco; Persson *et al.* 2000). Plasma glucose was measured with the glucose oxidase method using 2,2′-azino-bis(3-ethyl-benzothialozine-6-sulfonate) as the substrate, and the absorbance was measured at 420 nm on a microtiter plate reader (Fluostar/Polarstar Galaxy; BMG Labtechnologies, Offenburg, Germany).

Calculations and statistical analysis

The values are reported as means±S.E.M. area under the curve (AUC) was determined by the trapezoidal rule for glucose, insulin, and C-peptide data during the time period of 0–60 min after the oral or the matching i.v. glucose infusion. After the i.v. injection of glucose alone or in combination with GLP-1, the acute insulin response (AIR) was estimated as the suprabasal mean 1 and 5 min insulin, i.e., the mean of insulin at 1 and 5 min subtracted by the baseline insulin. Statistical comparisons were performed using one-way ANOVA with Bonferroni correction for mass significance as *post hoc* test. Statistical significance was considered when $P < 0·05$.

Results

Baseline levels

High-fat diet was given for 8 weeks. Table 1 shows that body weight was higher in the high-fat fed mice than in animals

Table 1 Body weight and baseline levels of glucose, insulin, and C-peptide in anesthetized C57BL/6J female mice fed normal or high-fat diet. Means±S.E.M. are shown

Variable	Normal diet-fed mice ($n=41$)	High-fat diet fed mice ($n=33$)	*P*
Body weight (g)	20·7±0·2	34·7±0·6	<0·001
Glucose (mmol/l)	5·6±0·2	6·6±0·2	<0·001
Insulin (pmol/l)	147±12	458±44	<0·001
C-peptide (pmol/l)	497±30	1310±76	<0·001

n indicates number of animals. *P* indicates the probability level of random difference between the two groups.

given the normal diet. Furthermore, plasma glucose levels were slightly elevated as were the levels of insulin and C-peptide. The hyperinsulinemia in spite of the slight hyperglycemia shows the insulin resistance in the high–fat diet fed mice.

Glucose levels during oral and i.v. glucose administration with matched glucose levels

To estimate the incretin effect, each mouse was given 25 mg glucose orally. This increased the plasma glucose levels to a peak at 15 min in both the normal diet-fed mice (15·7±0·4 mmol/l) and the mice fed the high-fat diet (17·7±0·4 mmol/l). Thereafter, the glucose levels fell to reach baseline levels after 120 min (Fig. 1). The AUC$_{glucose}$ during the oral glucose was significantly higher in the high-fat fed mice (605±31 mmol/l×120 min) than that in normal mice (396±29 mmol/l× 120 min) ($P<0·001$). For the estimation of the incretin effect, a variable rate glucose infusion was given intravenously to match the glucose levels after the oral glucose. During the i.v. test, glucose was determined 'bedside' to achieve matching. Figure 1 shows that the matching between the two conditions with regard to glucose, which was excellent in both groups during the initial 60 min with no significant difference between the two tests. There was a high correlation between the four mean glucose levels during the two tests, in both the normal diet-fed mice ($r=0·98$, $P=0·022$) and the high-fat fed mice ($r=0·99$; $P=0·008$). By contrast, during the second hour of experiment, the glucose levels obtained during the i.v. test were consistently higher than those during the oral test, despite the fact that the glucose infusion was terminated at 20 min. Figure 1 also shows the glucose infusion rates (stopped at 20 min) to achieve the glucose levels matching those of the oral administration and the glucose concentration obtained during the infusion in relation to the desired levels achieved during the oral glucose administration. The total amount of glucose infused was approximately the same in the two groups of mice during the first 15 min but higher in the mice given the high–fat diet during the interval between 15 and 20 min
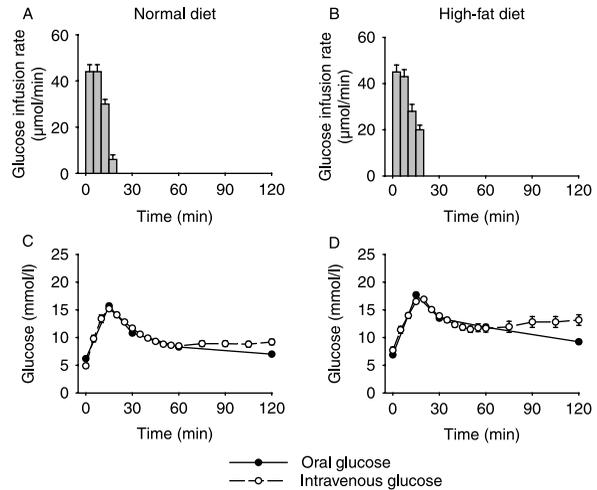

Figure 1 Upper panels show the glucose infusion rates during i.v. administration of glucose to match the glucose levels achieved during oral glucose administration at 25 mg in (A) normal mice or (B) high-fat fed mice. Lower panels show the achieved plasma glucose levels during oral or i.v. glucose administration in (C) normal mice or (D) high-fat fed mice. There were 11–30 animals in each group. Means±S.E.M. are shown.

(20·2±2·1 μmol/min versus 6·1±1·9 μmol/min, $P<0·001$). When correcting for the difference in body weight, the amount of glucose infused was slightly lower in mice fed the high–fat diet (27·8±0·7 μmol/g body weight) when compared with normal mice (33·0±1·3 μmol/g body weight, $P=0·018$).

Insulin secretion

Figure 2 shows insulin and C-peptide levels during the oral and i.v. glucose challenges in the two groups of animals. Insulin and C-peptide levels were significantly higher during the oral tests at 15 min in both the normal and high-fat fed animals. Table 2 shows AUC of the 0- to 60-min time interval, which is the time interval when the glucose levels were perfectly matched. AUC$_{insulin}$ and AUC$_{C-peptide}$ were significantly higher after the oral glucose challenge than during the i.v. glucose challenge in both the groups. The augmentation of the insulin and C-peptide responses by oral versus i.v. glucose was, however, higher in the high-fat fed mice. Thus, in normal mice, AUC$_{insulin}$ from the oral glucose was twice as much as that from i.v. glucose (8·0±1·8 vs 4·0±0·8 nmol/l×60 min; $P=0·023$), whereas in the high-fat fed mice, AUC$_{insulin}$ was augmented with the oral glucose by a factor of 17 (12·4±2·5 vs 0·7±0·47 nmol/l×60 min; $P<0·001$).

GLP-1 response to oral glucose

To study the possible contribution by GLP-1 to the augmented insulin response to oral versus i.v. glucose in the
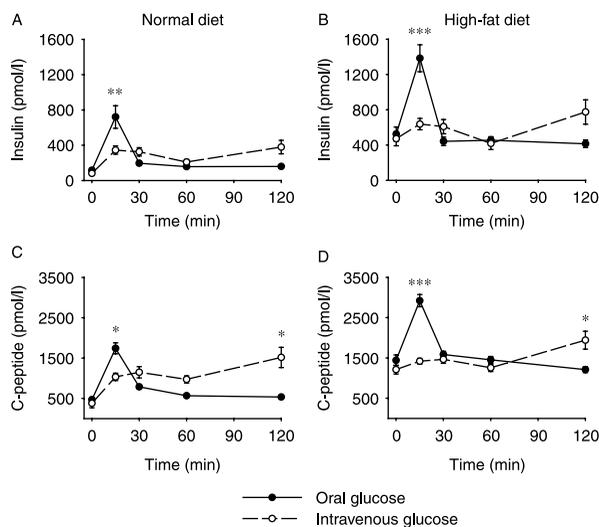
Figure 2 Plasma levels of insulin and C-peptide before and after oral administration of glucose at 25 mg and the corresponding results from the experimental series with i.v. glucose infusion to match the glucose levels achieved during the oral test in anesthetized C57BL/6J female mice fed (A and C) normal diet or (B and D) high-fat diet. There were 11–30 animals in each group. Means±S.E.M. are shown. Asterisks indicate the probability level of random difference between the tests (*$P<0.05$, **$P<0.01$, ***$P<0.001$).

Table 2 Suprabasal area under the curves for glucose, insulin, and C-peptide during time period 0–60 min following administration of oral glucose (25 mg) or i.v. glucose to match the glucose levels during the oral glucose test in anesthetized C57BL/6J mice fed normal or high-fat diet. Means±S.E.M. are shown

	Oral glucose	Intravenous glucose	P
Normal diet			
n	30	13	
Glucose (mmol/l× 60 min)	151±7.4	155±7.4	NS
Insulin (nmol/l× 60 min)	8.0±1.8	4.0±0.8	0.023
C-peptide (nmol/l× 60 min)	17.9±1.9	11.9±1.2	0.036
High-fat diet			
n	28	11	
Glucose (mmol/l× 60 min)	187±8.5	184±8.4	NS
Insulin (nmol/l× 60 min)	12.4±2.5	0.70±0.47	<0.001
C-peptide (nmol/l× 60 min)	21.2±2.7	2.2±1.2	<0.001

n indicates number of animals. *P* indicates the probability level of random difference between the two groups.

high-fat fed mice, the plasma levels of active GLP-1 were determined before and at 15 and 30 min after the oral glucose load (25 mg). It was found that the baseline GLP-1 levels were 2.7±0.3 pmol/l in mice fed a normal diet ($n=32$) and 2.3± 0.2 pmol/l in mice fed a high-fat diet ($n=33$, $P=$NS). After the oral glucose load, no significant increase in GLP-1 levels was observed in any of the groups; the 15-min GLP-1 levels were 3.1±0.8 pmol/l in normal diet fed mice ($n=11$) and 2.2±0.2 pmol/l in the high-fat fed mice ($n=11$, $P=$NS).

Insulin response to i.v. GLP-1

To study the effect of i.v. administration of low-dose GLP-1 on insulin secretion, the peptide was injected at 13, 130, or 650 pmol/kg together with glucose (0.35 g/kg) in the normal diet fed and high-fat diet fed mice. The glucose levels at the 1-min time point were 13.8±0.3 mmol/l in the normal diet-fed mice ($n=29$) and 16.1±0.5 mmol/l in the high-fat diet fed mice ($n=25$) in all groups. It was found that the insulin response to i.v. glucose was markedly suppressed in the high-fat diet fed mice compared with normal mice, which is similar to that in the experiments with matching glucose infusion. Thus, as is seen in the upper panel of Fig. 3, the AIR after glucose alone was 938±101 pmol/l in the normal mice ($n=9$) and 44±7 pmol/l in the high-fat diet fed mice ($n=7$; $P<0.001$). The i.v. administration of GLP-1 together with glucose increased the insulin response. However, the lowest effective dose of GLP-1 in this respect was different between the groups. Thus, the lowest GLP-1 to achieve an augmentation of the insulin response to i.v. glucose was 650 pmol/kg in the normal mice but only 13 pmol/kg in the high-fat diet fed mice, indicating an increased sensitivity to GLP-1 after high-fat feeding. Furthermore, as is seen in the middle panel of Fig. 3, while the fold increase in the AIR was markedly higher in normal mice after glucose alone (4.7±0.8) than in the high-fat diet fed mice (0.2±0.2; $P<0.001$), the fold increase by GLP-1 at 650 pmol/kg together with glucose was not different between the groups (being 6.3±0.9 in normal mice versus 5.1±0.9 in high-fat diet fed mice, $P=$NS). In fact, the markedly suppressed insulin response to glucose in the high-fat fed mice was normalized by GLP-1 at 650 pmol/kg. The increased efficacy of GLP-1 in the high-fat fed mice is also illustrated by calculating the fold increase in AIR by GLP-1 over glucose alone in the two groups (lower panel, Fig. 3). Thus, at 650 pmol/kg, this increase was 25.1±9.1 in the high-fat diet versus 1.5±0.2 in normal mice ($P=0.001$).

Discussion

The main novelty of this study is the demonstration of a large incretin contribution to the insulin response to oral glucose in normal mice, and that this incretin effect is augmented in the insulin-resistant high-fat fed mice. Furthermore, the study also demonstrates an increased sensitivity to exogenous GLP-1 in augmenting glucose-stimulated insulin secretion
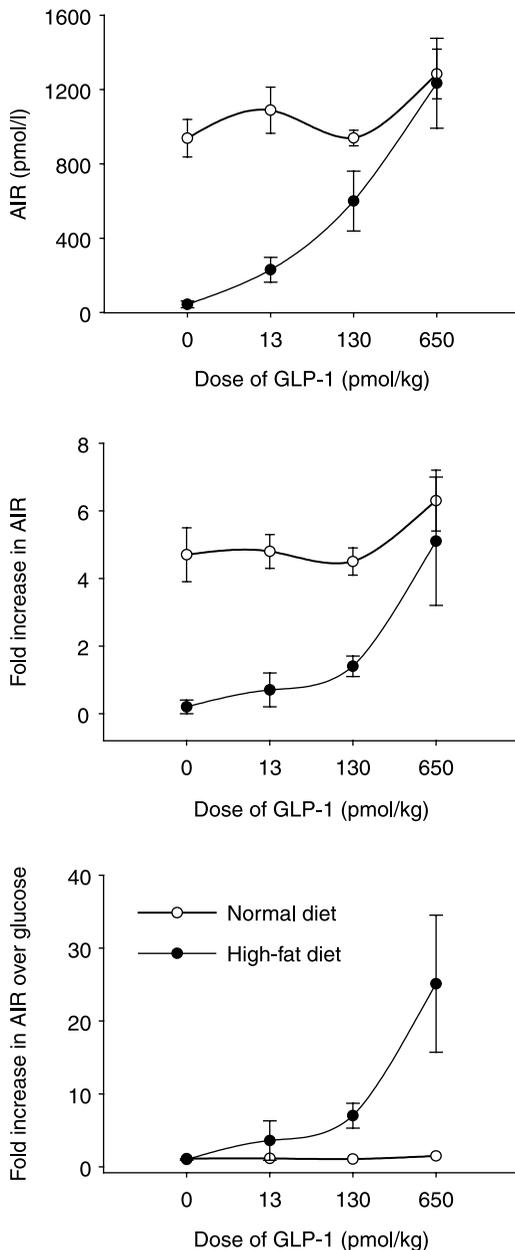
Figure 3 Insulin response to i.v. administration to glucose alone (0·35 g/kg) or to glucose in combination with GLP-1 at 13, 130, or 650 pmol/kg in high-fat fed mice and in normal mice. Upper panel shows the AIR (acute insulin response, i.e., the mean suprabasal 1 and 5 min insulin after i.v. administration); middle panel shows the fold increase in AIR over baseline insulin; lower panel shows the fold increase in AIR when related to fold increase by glucose alone (the mean of which was set to 1). There were 3–9 mice in each group. Means±s.e.m. are shown.

in the high-fat fed mice, in spite of a suppressed response to glucose alone. This suggests that GLP-1 may contribute to the augmented insulin response to oral glucose in the high-fat fed mice, although other mechanisms may be important as well.

The incretin contribution to the insulin response to oral glucose was evaluated by a variable rate i.v. glucose infusion with the measurement of glucose every 5 min to match the glucose levels seen during the oral test. The matching between the two conditions was good during the first 60 min. At the end of the second hour, however, the glucose levels were consistently higher during the i.v. test than during the oral test, in spite of the termination of glucose infusion at 20 min. The explanation for this could be the markedly lower insulin levels during the initial 15 min of the i.v. experiments, which results in a lower glucose clearance and a possible less inhibition of the action of the counterregulatory hormones during the i.v. test. The glucose infusion rate was similar in the two groups to achieve the desired glucose levels, although the total amount of glucose infused per gram body weight was lower in the high-fat fed mice. This was seen in the presence of hyperinsulinemia in the high-fat fed mice. This is a sign of insulin resistance, although it has to be emphasized that the variable rate glucose infusion in the presence of varying insulin levels is not a steady state condition, under which a true insulin sensitivity is possible to determine. Previous studies using the conventional euglycemic–hyperinsulinemic clamp technique have shown that the high-fat fed mice are clearly insulin resistant (Pacini *et al*. 2001).

The main aim of our study was to compare the contribution by incretin factors to insulin secretion during the oral glucose in normal versus insulin-resistant animals. To that end, we compared the incretin contribution to the insulin response to oral glucose in the normal mice with that in the high-fat fed mice. This model has previously been shown to induce insulin resistance (Pacini *et al*. 2001, Winzell & Ahrén 2004). On a short-term basis, such as the 8-week period used in this study, the high-fat food also results in impaired glucose-stimulated insulin secretion (Ahrén *et al*. 1997, Simonsson & Ahrén 1998), whereas on a long-term basis, upregulated insulin secretion develops (Winzell *et al*. 2007). In the present study, impaired glucose-stimulated insulin secretion was evident in the high-fat fed mice by estimating the insulin response to i.v. glucose. By contrast, we found an increased sensitivity to the incretin effect in the high-fat diet fed mice. Thus, whereas the insulin response in the normal mice was approximately double during the matching 60-min study period after oral glucose compared with i.v. glucose, in the high-fat fed mice, the insulin response to oral glucose was augmented 17-fold when compared with i.v. glucose. This study therefore clearly shows the existence of the incretin effect in mice, and that the augmentation of insulin secretion by oral versus i.v. glucose is more pronounced in the high-fat fed than normal mice. This latter conclusion supports an earlier report that the oral glucose elicits a more pronounced augmentation of glucose-stimulated insulin secretion in the high-fat fed versus control rats, although in that study the glucose levels were not matched between the two conditions (Collier *et al*. 1985).

Previous studies have used other approaches than this gold standard technique to quantify the incretin concept in rodents. One approach has been to inhibit incretin hormone action by receptor antagonists or peptide immunoneutralization; these interventions have resulted in glucose intolerance and an insulin response after the oral glucose, which is reduced by ∼50% (Tseng *et al.* 1999, Filipsson *et al.* 2000, Lewis *et al.* 2000). Another approach used mice with genetic deletion of the incretin hormone receptors; such studies have shown that mice with double knockout of the GLP-1 and GIP receptors have reduced the plasma insulin levels (∼50%) after oral glucose but not after i.v. glucose (Hansotia *et al.* 2004). A third approach has been to use mice with genetic deletion of neuropeptide receptors, thereby interrupting the neural responses; such studies have shown that mice with genetic deletion of the pituitary adenylate cyclase (PAC1) receptor (the receptor for PAC-activating polypeptide, PACAP) have impaired insulin response to oral administration of glucose (Ahrén 2006). Although these studies present evidence for the existence of the contribution by gut hormones and neuropeptides to the insulin response to oral glucose in rodents, they do not allow quantification of this contribution. Instead, quantification of the contribution by hormones and nerves to prandial insulin secretion requires studies comparable with the human study (Nauck *et al.* 1986), i.e. estimation of the differences in insulin secretion after careful matching of the glucose levels after oral versus i.v. glucose administration, as performed in this study.

One implication of the results in this study is that other stimulants in concert with glucose elicit a higher insulin secretory response in the high-fat fed mice, in comparison with the blunted insulin response that is seen after stimulation with glucose alone. One possibility would be that the increase in plasma GLP-1 after the oral glucose would be higher in the high-fat diet fed than normal mice. We have previously shown that the oral glucose elicits a GLP-1 response in mice at a glucose load of 50 or 75 mg but not at 17 mg (Persson *et al.* 2000). We show here that there was no significant increase in GLP-1 levels after oral administration of 25 mg in the mice, and therefore confirm the previous report that in mice an oral dose of 50 mg is required for eliciting a plasma GLP-1 response, when determined by the available assay techniques. This suggests that increased circulating GLP-1 is less likely to contribute to the augmented insulin response to oral glucose in mice. Another option, however, could be an increased response to GLP-1 in the high-fat diet fed mice in spite of the impaired response to glucose alone. Previous studies have suggested that exogenous administration of GLP-1 at a very high, supraphysiological dose (32 nmol/kg) resulted in a more pronounced insulin response in the high-fat diet fed than normal mice (Simonsson & Ahrén 1998). In the present study, we examined the insulin response to GLP-1 in the high-fat diet fed and normal mice by giving much lower doses that are more related to physiological conditions. We show that the sensitivity to GLP-1 in stimulating insulin secretion was augmented in the high-fat diet fed mice and in fact that GLP-1 could normalize the suppressed insulin response to glucose in this group. This would suggest that the augmented incretin effect in the high-fat diet fed mice is dependent on increased sensitivity of the β-cells to the incretin rather than to increased release, although this needs to be explored in mice detail.

In conclusion, in this study, we have demonstrated that the incretin effect (mediated by neural or hormonal mechanisms) contributes substantially to insulin secretion to the oral glucose load in mice, and that this effect is augmented in the insulin-resistant high-fat fed mice. This suggests that the adaptive islet response to insulin secretion may involve increased contribution by the incretin effect. The mechanism of this remains to be established, and this study suggests that GLP-1 may contribute.

Acknowledgements

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