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Potentiated β-cell response to non-glucose stimuli in insulin-resistant C57BL/6J mice

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Abstract

Insulin secretion in response to acetylcholine receptor activation by carbachol in insulin resistance induced by 12 weeks of high-fat diet in C57BL/6J mice is exaggerated. To study whether this persists after a longer period of time and also involves other non-glucose stimuli, we fed C57BL/6J mice a high-fat diet for 24 weeks. Both hyperinsulinemia (341 ± 33 vs. 148 ± 15 pmol/l) and slight hyperglycemia (7.8 ± 0.2 vs. 6.1 ± 0.1 mmol/l) were evident at this time point. The insulinotropic response to high dose carbachol (0.53 μmol/kg; 3403 ± 377 vs. 1595 ± 429 pmol/l), to the glucose analogue, 2-deoxyglucose (6 mmol/kg; 2014 ± 315 vs. 1167 ± 200 pmol/l), to cholecystokinin-8 (15.9 nmol/kg; 499 ± 93 vs. 119 ± 40 pmol/l) and to glucagon-like peptide-1 (32 nmol/kg; 307 ± 86 vs. 71 ± 9 pmol/l), were all exaggerated in mice given high-fat diet. In contrast, the insulin response to glucose was impaired. This shows that insulin resistance is accompanied by a general islet supersensitivity to non-glucose stimuli, which persists over a long period of time.

Keywords: Type 2 diabetes; Insulin secretion; 2-Deoxyglucose; Acetylcholine receptor; Cholinergic agonism; GLP-1; Cholecystokinin; Insulin resistance

1. Introduction

Insulin secretion is regulated by insulin sensitivity in that low insulin sensitivity is compensated by increased insulin secretion and hyperinsulinemia (Bergman et al., 1981; Kahn et al., 1993; Larsson and Ahrén, 1996). However, if insulin secretion is insufficient in relation to that required by the concurrent insulin sensitivity, glucose intolerance or type 2 diabetes may develop (Polonsky et al., 1988; Porte, 1991). Consequently, diabetes may, in its early stage, be treated by increasing insulin secretion to adapt to the insulin resistance. To achieve this goal, it is of importance to establish the mechanism of the enhanced insulin secretion during low insulin sensitivity as well as the causes of the failure of this adaptation.

A suitable model to examine these issues is challenging C57BL/6J mice with a high-fat diet, since this model is accompanied by insulin resistance, as evidenced by hyperinsulinemia in combination with severe glucose intolerance (Surwit et al., 1988). We showed recently that after 8 and 12 weeks feeding of C57BL/6J mice with a high-fat diet, insulin secretion induced by the acetylcholine receptor agonist carbachol was significantly exaggerated, whereas that to glucose was impaired when compared to the responses to carbachol and glucose in control diet-fed mice (Ahrén et al., 1997a). This would suggest an enhanced acetylcholine receptor sensitivity in insulin secretion after induction of insulin resistance, which could be an important adaptive β-cell mechanism in insulin resistance for the avoidance of massive hyperglycemia. The suggestion that acetylcholine receptor activity is important in this regard is consistent with several other previous studies in other experimental models in animals (Ahrén et al., 1996; Ahrén and Lundquist, 1982; Chen and Romsos, 1995; Okabayashi et al., 1989; Rohner-Jeanrenaud et al., 1983) and also in humans (DelRio et al., 1997). This would imply that acetylcholine receptor activation of insulin secretion would be a good target for potentiating insulin secretion in insulin resistance, in contrast to agents activating insulin secretion by mechanisms identical to that of glucose, since the response to glucose seems to be impaired.

It is known that insulin secretion is under the influence of a variety of different factors, like nerves and hormones, and several of these factors stimulate insulin secretion by other mechanisms of action than glucose or acetylcholine

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receptor activation (for review see Ahrén, 1996). Whether also these factors might be used for the potentiation of insulin secretion in insulin resistance has not been studied. Specifically, it is not known whether the increased insulinotropic sensitivity in insulin resistance, which we previously observed for carbachol (Ahrén et al., 1997a), is specific for acetylcholine receptor activation. In view of these previous findings, the aim of the present study was two-fold. First, it was considered of importance to examine whether the potentiated insulin response to carbachol in high-fat fed mice persists also after a longer period of time than 8 or 12 weeks. Second, it was considered important to examine if increased insulin response in states of insulin resistance is restricted to acetylcholine receptor activation, or if it is a general phenomenon also involving other non-glucose secretagogues. In the present study, we have therefore fed C57BL/6J mice a high-fat diet for 24 weeks. At the end of the period, we determined the insulin response to four different non-glucose secretagogues: (1) the acetylcholine receptor agonist, carbachol, (2) the glucose analogue, 2-deoxyglucose, (3) the C-terminal octapeptide of cholecystokinin (CCK-8) and (4) the truncated form of glucagon-like peptide-1 (GLP-1). 2-Deoxyglucose induces neuroglycopenia by competing with glucose phosphorylation thereby increasing insulin secretion by stimulating autonomic, mainly parasympathetic, nerve activity (Cramer and Woodward, 1979; Karlsson et al., 1987). CCK-8 is both a gut hormone and a pancreatic neuropeptide with potent insulinotropic activity (Karlsson and Ahrén, 1992), and GLP-1 is a gut hormone of physiological importance for postprandial insulin secretion which has also been suggested to be used in the treatment of diabetes (Ahrén, 1998). Mechanistically, carbachol stimulates insulin secretion by activating phospholipase C (Ahrén et al., 1990), CCK-8 stimulates insulin secretion mainly by activating phospholipases C and A2 (Karlsson and Ahrén, 1992; Malm et al., 1993; Simonsson et al., 1996), whereas, a main signaling mechanism of GLP-1 is activation of adenylate cyclase and formation of cyclic AMP (Ahrén, 1998; Fehmann et al., 1995).

2. Materials and methods

2.1. Animals and diets

Female mice of the C57BL/6J strain (Bomholtgaard Breeding and Research Centre, Ry, Denmark) received either a high-fat diet (Research Diets, N Brunswick, NJ) or a standard rodent chow diet (Lactamin, Stockholm, Sweden) for 24 weeks, starting at the age of 4 weeks. On a caloric base, the high-fat diet consisted of 16.4% protein, 25.6% carbohydrates and 58.0% fat (a total energy content of 23.4 kJ/g), while the control diet consisted of 25.8% protein, 62.8% carbohydrates and 11.4% fat (a total energy content of 12.6 kJ/g). The mice had free access to food and water. Four to five mice were kept per cage in a temperature-controlled (22 ± 1°C) room with a 12-h light-dark cycle with lights on at 0600. The study was approved by the Animal Ethics Committee at Lund University.

2.2. Insulin secretion experiments

After 24 weeks on the respective diets, non-fasted animals were injected intraperitoneally (i.p.) with D-glucose (Fluka Chemie, Buchs, Switzerland) at the dose level of 8.3 or 16.7 mmol/kg, with carbachol (carbamylcholine chloride; BDH, Poole, UK) at 0.16 or 0.53 μmol/kg, with 2-deoxyglucose (Sigma) at 6 mmol/kg, with CCK-8 (Sigma) at 15.9 nmol/kg, with GLP-1 (Saxon, Hannover, Germany) at 32 nmol/kg, or with saline. The doses were selected to yield a half-maximal to maximal insulin secretory response, as inferred from previous in vivo work in mice in this laboratory (Ahrén, 1995; Ahrén and Lundquist, 1981; Ahrén et al., 1997a; Karlsson et al., 1987). The volume load was 10 μl/g body weight. Blood samples were taken at 6 and 10 min. after the respective injection; since initial experiments showed that peak levels of plasma insulin after injection of these secretagogues occurred within this period (data not shown). After centrifugation, plasma was stored at −20°C until assayed.

2.3. Analysis

Plasma insulin was measured radioimmunochemically with a guinea pig anti-rat insulin antibody, ^{125}I-labeled human insulin as tracer and, as standard, rat insulin (Linco Research, St. Charles, MO). The separation of free and bound radioactivity was performed by use of an anti-IgG (goat anti-guinea pig) antibody (Linco). The sensitivity of the assay is 12 pmol/l and the coefficiency of variation is less than 3%. Plasma glucose levels were measured by the glucose oxidase method.

2.4. Calculations and statistics

The results are expressed as the mean ± S.E.M. The mean value of the obtained 6 and 10 min plasma levels for glucose and insulin in each experimental mice were calculated. The mean value for the saline-injected control mice run in the same experiment was then subtracted from the mean value obtained in each experimental mice. To determine the degree of significance, Student's *t*-test for unpaired data was used. A *P*-value less than 0.05 was considered significant.

3. Results

3.1. Body weight and baseline insulin and glucose

Throughout the study, mice given a high-fat diet had higher body weight and they had also increased plasma

Table 1
Body weight and baseline (non-fasted) plasma levels of insulin and glucose at 24 weeks after start of feeding with high-fat diet or control diet in mice of the C57BL/6J strain

	High-fat diet ($n = 67$)	Control diet ($n = 70$)	P-value
Body weight (g)	29.9 ± 0.6	23.7 ± 0.2	$P < 0.001$
Plasma insulin (pmol/l)	341 ± 33	148 ± 15	$P < 0.001$
Plasma glucose (mmol/l)	7.8 ± 0.2	6.1 ± 0.1	$P < 0.001$

Means ± S.E.M. are shown. P-value indicates probability level of random difference between the two groups.

glucose and insulin than control mice, confirming that high-fat diet induces insulin resistance in this strain of mice. The body weight and baseline (non-fasting) values of insulin and glucose after 24 weeks on the respective diets are shown in Table 1.

3.2. Glucose-stimulated insulin secretion

After 24 weeks of high-fat or control diet feeding, glucose was injected i.p. (8.3 or 16.7 mmol/kg). The increase in plasma insulin levels induced by the administration of glucose was not different between the two groups (Fig. 1). However, after administration of glucose at 8.3 mmol/kg, the increase in plasma glucose was higher in the high-fat diet fed animals (13.8 ± 0.7 mmol/l) than in controls (9.4 ± 0.8 mmol/l, $P = 0.002$), in spite of injecting the same amount of glucose in the two groups. This is explained by the glucose intolerance in the animals given high-fat diet. Taking this different degree of glycemia into account, the insulinotropic action of glucose was impaired in animals given high fat diet. In contrast, at the high dose of 16.7 mmol/kg, the increase in plasma glucose was not different between the two groups.

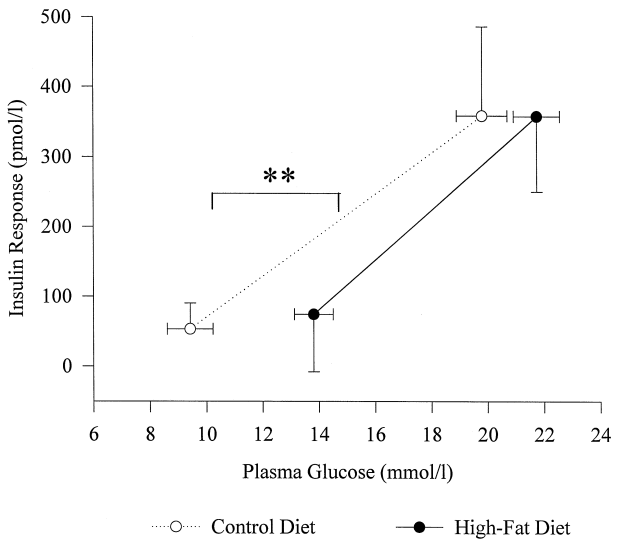

Fig. 1. Glucose-stimulated insulin secretion in vivo at 24 weeks after start of feeding with high-fat diet (●) or control diet (○) in mice of the C57BL/6J strain, as a function of plasma glucose levels. Glucose was injected i.p. at 8.3 or 16.7 mmol/kg and plasma was sampled after 6 and 10 min. Saline-injected control animals were run in the same experiments, and the results are reported as the difference in means ± S.E.M. of the 6 and 10 min values between the experimental and saline groups. Means ± S.E.M. are shown. $n = 5–13$. $** P < 0.01$.

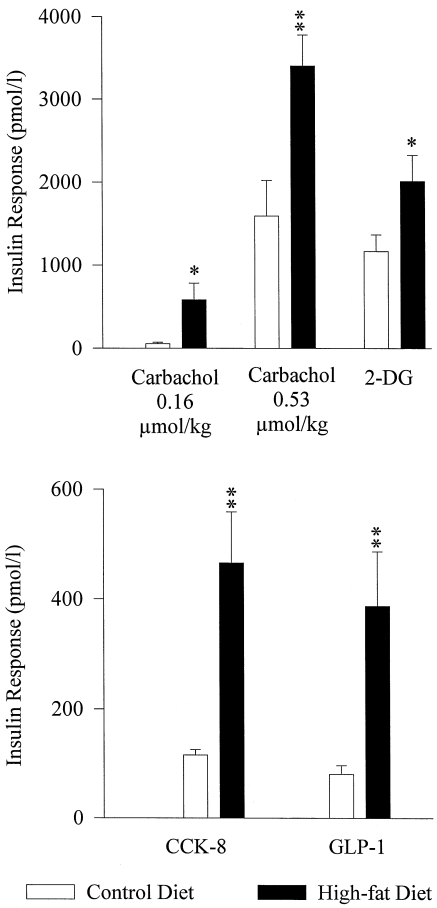

Fig. 2. Upper panel: carbachol- and 2-deoxyglucose-stimulated insulin response in vivo at 24 weeks after start of feeding with high-fat diet (■) or control diet (□) in mice of the C57BL/6J strain. Carbachol was injected i.p. at 0.16 ($n = 5–8$) or 0.53 ($n = 13–20$) µmol/kg and 2-deoxyglucose (2-DG) was injected i.p. at 6 mmol/kg ($n = 17–18$). Lower panel: CCK-8- and GLP-1-stimulated insulin response in vivo at 24 weeks after start of feeding with high-fat diet (■) or control diet (□) in mice of the C57BL/6J strain. CCK-8 was injected i.p. at 15.9 nmol/kg ($n = 15–16$) and GLP-1 was injected i.p. at 32 nmol/kg ($n = 15–16$). Plasma was sampled after 6 and 10 min after injection. Saline-injected control animals were run in the same experiments, and the results are reported as the difference in means ± S.E.M. of the 6 and 10 min values between the experimental and saline groups. $* P < 0.05$, $** P < 0.01$, $*** P < 0.001$.

Table 2
Plasma insulin and glucose at 6 and 10 min after challenging C57BL/6J mice on high-fat or control diet with carbachol, 2-deoxyglucose, CCK-8 or GLP-1 (experimental group) or saline (saline-injected control group run in the same series as the experimental group)

		Plasma insulin (pmol/l)			Plasma glucose (mmol/l)		Number of animals	
		Experimental group	Saline-injected control group	Difference between groups	Experimental group	Saline-injected control group	Experimental group	Saline-injected control group
Carbachol (0.16 μmol/kg)	Control diet	143 ± 22[a]	78 ± 16	62 ± 19	6.7 ± 0.6	7.1 ± 0.5	6	5
	High-fat diet	945 ± 292[a] ($P = 0.028$)	363 ± 151 (ns)	596 ± 234 ($P = 0.040$)	7.3 ± 0.4 (ns)	7.7 ± 0.1 (ns)	8	7
Carbachol (0.53 μmol/kg)	Control diet	1725 ± 619 *	130 ± 16	1595 ± 429	6.5 ± 0.3	6.8 ± 0.2	12	13
	High-fat diet	3658 ± 547[b] ($P = 0.007$)	269 ± 25 ($P < 0.001$)	3403 ± 377 ($P = 0.004$)	7.7 ± 0.4 ($P = 0.034$)	7.3 ± 0.2 (ns)	18	20
2-deoxyglucose (6 mmol/kg)	Control diet	1244 ± 278[b]	163 ± 24	1167 ± 200	15.7 ± 0.5	7.8 ± 0.3	18	17
	High-fat diet	2163 ± 345[b] ($P = 0.046$)	288 ± 32 ($P = 0.011$)	2014 ± 315 ($P = 0.031$)	16.9 ± 0.3 (ns)	8.6 ± 0.2 (ns)	18	18
CCK-8 (15.9 nmol/kg)	Control diet	262 ± 54[a]	143 ± 27	119 ± 40	6.6 ± 0.3	7.0 ± 0.2	16	16
	High-fat diet	675 ± 129[b] ($P = 0.016$)	176 ± 42 (ns)	499 ± 93 ($P = 0.002$)	7.8 ± 0.2 ($P = 0.003$)	8.5 ± 0.3 ($P = 0.002$)	16	15
GLP-1 (32 nmol/kg)	Control diet	228 ± 8[a]	157 ± 31	71 ± 9	8.4 ± 0.2	7.7 ± 0.2	16	15
	High-fat diet	538 ± 139[a] ($P = 0.034$)	231 ± 43 (ns)	307 ± 86 ($P = 0.001$)	8.8 ± 0.4 (ns)	9.5 ± 0.3 ($P < 0.001$)	16	15

Mean plasma insulin and glucose levels at 6 and 10 min after i.p. injection of saline, carbachol, 2-deoxyglucose, CCK-8 and GLP-1, respectively, in C57BL/6J mice fed a high-fat diet or a control diet for 24 weeks. Means + S.E.M. are shown. *n* indicates the number of animals in each group. *P* indicates the probability level of random difference between mice fed the two diets (i.e., vertical comparison). ns = no significant difference. [a] or [b] indicates the probability level of random difference between experimental and saline-injected control group (i.e., horizontal comparison), [a]indicates $P < 0.05$, [b]indicates $P < 0.001$.

3.3. Carbachol- and 2-deoxyglucose-stimulated insulin secretion

The acetylcholine receptor agonist, carbachol, as well as 2-deoxyglucose both stimulated insulin secretion in both high-fat diet fed and control diet fed mice, as evidenced by the significant increase in plasma insulin in the samples taken at 6 and 10 min after the i.p. injection (Fig. 2, Table 2). The potential difference in the insulinotropic response to carbachol and 2-deoxyglucose between the two groups was calculated by subtracting the response in control-fed diet mice from that in high-fat diet fed mice in each individual experiment (there were two or three experiments for each experimental series). It was then seen that the insulinotropic responses to both carbachol (both at 0.16 as well as 0.53 μmol/kg) and to 2-deoxyglucose were exaggerated in mice given high-fat diet compared to those given control diet ($P = 0.040$ for carbachol at 0.16 μmol/kg, $P = 0.004$ for carbachol at 0.53 μmol/kg, and $P = 0.031$ for 2-deoxyglucose). Plasma glucose levels did not change significantly during the studied time period after injection of carbachol. Glucose levels were expectedly elevated by the 2-deoxyglucose administration; the increase was the same in the two groups of animals (Table 2).

3.4. CCK-8- and GLP-1-stimulated insulin secretion

In control diet-fed mice, CCK-8 and GLP-1 slightly increased plasma insulin levels ($P < 0.05$ for both peptides). However, in the high-fat diet fed animals, both CCK-8 and GLP-1 markedly increased plasma insulin levels. When calculating the quantitative difference in insulinotropic action by these two peptide secretagogues in control diet vs. in high-fat diet fed animals, it was revealed that the responses were more than four-fold elevated in the high-fat diet fed animals ($P = 0.002$ for CCK-8 and $P = 0.001$ for GLP-1; Fig. 2, Table 2). Plasma glucose levels were not significantly affected by the administration of CCK-8 or GLP-1 in any of the groups.

4. Discussion

To study the mechanisms behind the islet adaptation to insulin resistance, we have used the C57BL/6J strain of mice fed a high-fat diet. This model is accompanied by insulin resistance in otherwise healthy animals, as evident by hyperinsulinemia in combination with severe glucose intolerance, and only slight obesity (Ahrén et al., 1997a; Surwit et al., 1988). We showed previously that the insulin response to acetylcholine receptor activation by carbachol was potentiated after 8 and 12 weeks feeding of the mice with a high-fat diet, whereas, the insulin response to glucose was impaired (Ahrén et al., 1997a). This suggested that acetylcholine receptor supersensitivity had evolved in conjunction with insulin resistance, which was suggested as a main mechanism for the hyperinsulinemia in these animals (Ahrén et al., 1997a). Also results in other experimental models and in humans have arrived at similar conclusions (Ahrén et al., 1996; Ahrén and Lundquist, 1982; Chen and Romsos, 1995; DelRio et al., 1997; Okabayashi et al., 1989; Rohner-Jeanrenaud et al., 1983). This would suggest that increased acetylcholine receptor activity with ensuing increased insulin secretion supports the organism by providing sufficient amount of insulin during insulin resistance when the insulinotropic action of glucose fails, which would protect against massive hyperglycemia. This could also be a target for future development of new treatment strategies to be used during the early stages of development of diabetes.

In this study, we examined whether the supersensitivity to carbachol persists also after the long-term period of 24 weeks of treatment with high-fat diet, since in our previous study we did not examine the mice after a period of 12 weeks on high-fat diet (Ahrén et al., 1997a). We found that at both low and high doses of carbachol, a potentiated insulin response occurred in high fat-diet fed mice. This exaggerated insulin response to acetylcholine receptor stimulation therefore seems to represent a long-term-compensating mechanism to meet the increased insulin requirement in insulin resistance. Another aim of the present study was to examine whether the increased sensitivity in insulin secretion during insulin resistance is restricted to acetylcholine receptor activation, or also may involve other non-glucose secretagogues. We thereby found that the insulinotropic response to three other non-glucose secretagogues (2-deoxyglucose, CCK-8 and GLP-1) was markedly increased after high-fat diet. These three non-glucose secretagogues are mechanistically different from carbachol and from each other in stimulating insulin secretion. Therefore, our study shows that a potentiating or exaggerating insulinotropic action of non-glucose stimuli in insulin resistance is not restricted to acetylcholine receptor activation and its main signaling pathways, involving phospholipase C activation through mediation by m_3 muscarinic receptors (Ahrén et al., 1996; Karlsson and Ahrén, 1993).

The glucose analogue, 2-deoxyglucose, competes for membrane transport and intracellular glucose phosphorylation and thereby causes neuroglycopenia (Cramer and Woodward, 1979). Through the induction of neuroglycopenia, 2-deoxyglucose stimulates insulin secretion in mice by activating the autonomic nervous system, predominantly the parasympathetic branch (Karlsson et al., 1987). This was previously shown in NMRI mice (Karlsson et al., 1987) and we now show this to be the case also in C57BL/6J mice. The potentiation of insulin secretion by 2-deoxyglucose in animals fed a high-fat diet, when compared with those given control diet, corroborates the potentiated insulinotropic response to acetylcholine receptor activation by carbachol, since a main mechanism of the insulinotropic action of 2-deoxyglucose is activation of the parasympathetic nerves (Karlsson et al., 1987). Hence, also

endogenous activation of the parasympathetic nerves results in a potentiated insulin response in insulin-resistant mice.

CCK-8 is a gut hormone and an islet neuropeptide (Karlsson and Ahrén, 1992), which stimulates insulin secretion by a signaling mechanism involving phospholipases C and A_2 (Karlsson and Ahrén, 1992; Malm et al., 1993; Simonsson et al., 1996). We have previously demonstrated a clearcut insulinotropic action of CCK-8 with a peak at 2 min after its i.v. administration in normal NMRI mice (Ahrén and Lundquist, 1981) and in the present study, we confirm an increase in circulating insulin after the administration of the peptide in C57BL/6J mice fed a control diet. After high-fat diet, a potentiated insulinotropic action of CCK-8 was evident. This result with CCK-8 is in accordance with previous results showing an enhanced responsiveness to CCK-8 in islets from preobese ob/ob mice (Chen and Romsos, 1995) and by findings in Swiss mice, where CCK receptor antagonism exacerbates alloxan-induced diabetes (Parmar et al., 1987). Finally, GLP-1 is an incretin hormone which stimulates insulin secretion through activation of adenylate cyclase and the formation of cyclic AMP (Ahrén, 1998; Fehmann et al., 1995). We have shown previously that GLP-1 increases plasma insulin in NMRI mice (Fridolf et al., 1991; Ahrén, 1995) and here we demonstrate a similar action in C57BL/6J mice. Like for carbachol, 2-deoxyglucose and CCK-8, also the insulinotropic response to GLP-1 was potentiated by high-fat diet in these animals. The results support a previous study in diabetic Zucker rats, showing an increased insulin secretion and a normalized plasma glucose concentration after GLP-1 stimulation (Hargrove et al., 1995). In accordance, studies in Zucker rats (Chan et al., 1984) as well as on islets from preobese ob/ob mice (Chen and Romsos, 1995) have demonstrated an enhanced responsiveness to another adenylate cyclase stimulator, the gastric inhibitory polypeptide (GIP).

Hence, our findings indicate that β-cell compensation during insulin resistance and diabetes development might involve a generalized supersensitivity to non-glucose stimuli. Several explanations might be offered for this phenomenon. One possibility is that the slight hyperglycemia present in the high-fat diet-fed C57BL/6J mice might be triggering a potentiated response to non-glucose secretagogues. For example, the insulin response to GLP-1 in mice was recently shown to be sensitive to small changes in circulating glucose at levels above four mmol/l (Ahrén, 1995). This would imply that a consequence of the slight hyperglycemia would be a potentiated response to non-glucose stimuli restoring the defect direct insulinotropic response to glucose, in analogy with discussions on diabetes pathophysiology in humans (Porte, 1991). Although such a mechanism might contribute, it is not likely that the small increase in circulating glucose in the insulin resistant animals, being only one mmol/l, is the only factor explaining the potentiated non-glucose insulinotropic action. A second possibility is that other circulating agents with increasing levels after high-fat diet, such as lipids and leptin (Ahrén et al., 1997a,b), could augment insulin secretion. This would be possible considering that both free fatty acids (Corkey et al., 1989) and leptin (Tanizawa et al., 1997) under various conditions have been shown to stimulate insulin secretion, although they both also have been shown to inhibit insulin secretion (Sako and Grill, 1990; Emilsson et al., 1997). Hence, their role in the adaptative islet response to insulin resistance remains to be established. A third possibility would be that the potentiated insulin response to non-glucose stimuli might involve increased affinity or enhanced number of muscarinic, GLP-1 and CCK-8 receptors on the β-cell membrane, as was, for example, inferred from a previous study showing that glucose increases the affinity within islet muscarinic receptors (Östenson and Grill, 1987). Finally, it is also possible that alterations in the intracellular transduction systems underlying non-glucose-stimuli-mediated insulin secretion is exaggerated after high-fat diet.

Within the short time period studied after the i.p. challenge with the secretagogues (6 to 10 min), plasma levels of glucose did not change significantly. This might be surprising in view of the large increase in circulating insulin in some of the experimental groups. However, it is known that at least 5 to 10 min are required for insulin to lower glucose in mice (Filipsson et al., 1998) and at least two of the used secretagogues, carbachol and 2-deoxyglucose, stimulate also glucagon secretion in mice (Karlsson and Ahrén, 1987, 1993), which would counteract the glucose lowering action of insulin. Also, catecholamine levels are high during short-term experiments in non-anesthetized mice (Ahrén et al., 1995), which also counteracts a glucose-lowering action of insulin. Hence, marked changes in glucose in these 10 min experiments are not likely to occur.

In this study, we also challenged the C57BL/6J mice with glucose to explore whether the previously observed failing insulin response to glucose persists after 24 weeks on high-fat diet. We found that the high-fat diet-fed mice required a glucose level approximately four mmol/l higher than controls to accomplish an equal insulin response. This suggests an impaired β-cell sensitivity for glucose in mice given a high-fat diet also after 24 weeks of feeding, although the absolute insulin response to the same glucose challenge was the same. This is in accordance with results also from other studies in high-fat diet-fed C57BL/6J mice showing a decreased insulin secretion in vitro (Kaku et al., 1988; Lee et al., 1995).

We conclude that after 24 weeks of high-fat diet exposure in C57BL/6J mice, concomitantly with a failing glucose-stimulated insulin secretion, a general islet supersensitivity to non-glucose stimuli has evoked. We suggest that this phenomenon represents an early islet compensation to low insulin sensitivity. Our results are also of importance for the concept of developing new strategies to

improve failure of insulin secretion during insulin resistance and the development of type 2 diabetes. Since the insulinotropic response to a variety of non-glucose secretagogues is potentiated in the state of insulin resistance, specific activation of these pathways may be targets for new treatments.

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