Increased insulin secretion and normalization of glucose tolerance by cholinergic agonism in high fat-fed mice

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Ahren, Bo, Per Sauerberg, and Christian Thomsen. Increased insulin secretion and normalization of glucose tolerance by cholinergic agonism in high fat-fed mice. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E93–E102, 1999.— Increased insulinotropic activity by the cholinergic agonist carbachol exists in insulin-resistant high-fat-fed C57BL/6j mice. We examined the efficiency and potency of carbachol to potentiate glucose-stimulated insulin secretion and to improve glucose tolerance in these animals. Intravenous administration of carbachol (at 15 and 50 nmol/kg) markedly potentiated glucose (1 g/kg)-stimulated insulin secretion in mice fed both a control and a high-fat diet (for 12 wk), with a higher relative potentiation in high-fat-fed mice measured as increased (1–5 min) acute insulin response and area under the 50-min insulin curve. Concomitantly, glucose tolerance was improved by carbachol. In fact, carbachol normalized glucose-stimulated insulin secretion and glucose tolerance in mice subjected to a high-fat diet. Carbachol (>100 nmol/l) also potentiated glucose-stimulated insulin secretion from isolated islets with higher efficiency in high-fat-fed mice. In contrast, binding of the muscarinic receptor antagonist [N-methyl-3H]scopolamine to islet muscarinic receptors and the contractile action of carbachol on ileum muscle strips were not different between the two groups. We conclude that carbachol normalizes glucose tolerance in insulin resistance.

acetylcholine receptors; carbachol; glucose intolerance; C57BL/6j mice

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lated islets in vitro. Furthermore, we have in the present study also examined the binding of the muscar- 

inotropic action of carbachol is a result of increased 

secretion, is restricted to islets or is a more general 

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METHODS

Animals and diets. Female mice of the C57BL/6J strain (Bomholagaard Breeding and Research Centre, Ry, Denmark) received either a high-fat diet or a standard rodent chow diet (Research Diets, New Brunswick, NJ) for 12 wk, starting at the age of 4 wk. On a caloric basis, 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), whereas 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 12:12-h light-dark cycle with lights on at 6 AM. The study was approved by the Animal Ethics Committee at Lund University.

Insulin secretion experiments in vivo. After 12 wk on the respective diets, nonfasted animals (n = 86) were anesthe-

ized with an intraperitoneal injection of midazolam (Dormi-
cum, Hoffmann-La Roche, Basel, Switzerland; 0.4 mg/mouse) and a combination of fluanisone (0.9 mg/mouse) and fentanyl (Hynorm, J anssen, Beerse, Belgium; 0.02 mg/mouse). There-

after, a blood sample was taken from the retrobulbar, intraro-

bital, capillary plexus in heparinized tubes, and then D-glucose (British Drug Houses (BDH), Poole, UK; 1 g/kg) was injected 

rapidly intravenously either alone or together with carbachol (carbamylcholine chloride; BDH) at different dose levels. The 

volume load was 10 µl/g body weight. Blood samples were taken 

immediately before the intravenous injection and 1, 5, 20, and 

50 min after. Repeated experiments in our laboratory have shown that the insulin response to carbachol in anesthetized 

mice peaks at 1 min after the intravenous injection (unpublished observations). After centrifugation, plasma was stored 

at −20°C until assayed.

Insulin secretion experiments in vitro. At 2–3 wk after the 
in vivo studies, pancreatic islets were isolated with the collagenase isolation technique. In brief, the pancreas was 

regradedly filled with 3 ml of Hank’s balanced salt solution (Sigma), supplemented with 0.4 mg/ml of collagenase P (activity 1.86 U/mg; Boehringer-Mannheim, Mannheim, Germany). The pancreas was subsequently removed and incubated for 20 min at 37°C. After being rinsed, the islets were handpicked under a stereomicroscope and incubated at 37°C in 1 ml of incubation medium (pH 7.36) supplemented with 0.1% human serum albumin (Sigma) and 3.3 mmol/l glucose. The medium con- 

sisted of (in mmol/l): 125 NaCl, 5.9 KCl, 1.2 MgCl2, 1.28 CaCl2 (all Sigma), and 25 HEPES (Boehringer-Mannheim). After the preincubation, groups of three islets were transferred into separate chambers containing 200 µl of the medium supplemented with glucose at various concentrations. After incubation at 37°C for 60 min, 25 µl of the medium were collected from each chamber and stored at −20°C until analysis.

Islet binding studies. Islets were isolated as described in insulin secretion experiments in vitro by the collagenase isolation technique and handpicked under a stereomicroscope. Islets were then homogenized for 30 s (Ultra-Turrax homogenizer) in 10 ml of medium consisting of 50 mmol/l NaH2PO4/NaH2PO4 and 2 mmol/l MgCl2 with the addition of bovine serum albumin (1 mg/ml), bacitracin (0.2 mg/ml), aprotinin (500 kallikrein-inhibitor units/ml; all Sigma), and Pefabloc (Boehringer-Mannheim, 0.1 mmol/l), pH 7.4. The homogenate was then centrifuged (50,000 g) for 20 min at 2°C. The pellet was resuspended in 20 ml of the medium with the additions as above; the membranes suspended were used for binding assay. The membranes (from 30 islets/tube) were incubated in the medium for 2 h at 25°C in the presence of the muscarinic receptor antagonist [3H]NMS (specific activity, 80 Ci/mmol; New England Nuclear, Boston, MA) in a final volume of 1 ml. After the 2-h incubation, free and bound radioactivity was separated by filtration over Whatman GF/B filters that were washed with 3 × 5 ml of ice cold phosphate assay buffer.

Ileum contractions. The C57BL/6J mice were killed by means of cervical dislocation, the terminal 15 cm of ileum were removed, and 1.5- to 2.0-cm lengths were prepared and mounted in 10-ml organ baths containing calcium-deficient medium of the following composition (in mM; all Sigma): 137 NaCl, 2.68 KCl, 0.9 CaCl2, 1.05 MgCl2, 11.9 NaHCO3, 0.42 NaH2PO4, and 5.5 glucose. The organ bath was maintained at 37°C and gassed with 95% O2-5% CO2. The mechanical activity of the muscle was measured by a HSE 351 isometric transducer connected via a HSE bridge amplifier to a poten-
tiometric pen recorder (Hugo Sachs Elektronik). Resting tension was 1 g, and the tissue was left to equilibrate for 1 h. Dose-response curves to added carbachol were constructed sequentially with 30-s contact time with a drug concentration being added every 3 min. Contractions were calculated as millimeter responses.

Calculations and statistics. The results are expressed as means ± SE. The acute insulin response (AIR) to glucose with or without carbachol was calculated as the mean of suprabasal 1- and 5-min postchallenge plasma insulin levels. Area under the curve (AUC) was calculated for suprabasal plasma insulin (AUCinsulin) and plasma glucose (AUCglucose) levels, respectively, for the entire 50-min study period with the trapezoidal rule. To determine the degree of significance, Student’s t-test for unpaired data was used. When comparing the dose-response relationships, ANOVA with Bonferroni post hoc analysis for multiple comparisons was undertaken. Pearson’s product-moment correlation was used to estimate linear relationships between variables. A P value < 0.05 was considered significant.
RESULTS

Body weight and baseline insulin and glucose. Throughout the study, mice given a high-fat diet had higher body weight than control diet-fed mice and they also had increased plasma glucose and insulin levels, confirming that high-fat diet induces insulin resistance in this strain of mice. The body weight and baseline (nonfasting) values of insulin and glucose at 4, 8, and 12 wk on the respective diets are shown in Fig. 1. The results in all time points after the start of the high-fat diet are statistically significant between the two groups ($P < 0.05$ or less).

Insulin secretion and glucose tolerance after intravenous glucose with or without carbachol. After a period of 12 wk on the respective diets (age 16 wk), the animals underwent an in vivo experiment with the intravenous challenge of glucose (1 g/kg) alone or together with different concentrations of carbachol (ranging from 1.5 to 50 nmol/kg). Figure 2 shows the plasma insulin levels in the two groups of animals in this experimental series. The intravenous injection of glucose elicited a rapid insulin response in both groups of mice with a maximal increase in plasma insulin already at 1 min after injection. The peak insulin level after glucose administration was significantly potentiated by carbachol at the two highest doses (15 and 50 nmol/kg) in both normal fed ($P = 0.017$ for 15 nmol/kg; $P < 0.001$ for 50 nmol/kg) and high fat-fed animals ($P < 0.001$ for both doses). In contrast, at 1.5 and 5 nmol/kg, carbachol did not significantly affect the peak plasma insulin levels. Figure 3 shows the corresponding plasma glucose levels. Plasma glucose levels peaked at 1 min after the injection of glucose and carbachol.
after injection. In control diet-fed mice, plasma glucose levels thereafter declined to reach baseline values within the 50-min study period. However, in high fat diet-fed animals, plasma glucose levels were still elevated above the preinjection levels at 50 min after glucose administration ($P < 0.001$). At this time point, high fat diet-fed animals injected with glucose and carbachol at 50 nmol/kg had lower plasma glucose than animals injected with glucose alone ($P = 0.029$), whereas high fat diet-fed mice injected with carbachol at lower dose levels had a 50-min plasma glucose level not significantly different from that in glucose-injected controls.

Figure 4 shows the calculated AIR, $AUC_{insulin}$, and $AUC_{glucose}$ as a function of dose of carbachol in mice fed a high-fat and a normal diet. In controls injected with glucose alone, i.e., without carbachol, AIR ($P = 0.018$) and $AUC_{insulin}$ ($P = 0.042$) were both 36% lower in mice fed a high-fat diet vs. those fed a control diet, whereas $AUC_{glucose}$ was 43% higher ($P < 0.001$) as a sign of the impairment of glucose-stimulated insulin secretion and glucose tolerance that accompanies insulin resistance. AIR and $AUC_{insulin}$ were potentiated by carbachol at dose levels of 15 and 50 nmol/kg in both groups of animals ($P < 0.05$), whereas at the two lower dose levels (1.5 and 5 nmol/kg), carbachol had no significant effect. Furthermore, $AUC_{glucose}$ was reduced by carbachol at 15 and 50 nmol/kg in both groups ($P < 0.05$). After administration of carbachol at 15 and 50 nmol/kg to mice fed a high-fat diet, $AUC_{glucose}$ did not differ significantly from the $AUC_{glucose}$ in control diet-fed

**Fig. 3.** Plasma glucose concentrations immediately before and at 1, 5, 20, and 50 min after iv injection of glucose (1 g/kg) alone (control, $n = 14$) or together with carbachol at 1.5, 5, 15, or 50 nmol/kg in C57BL/6J mice given a control (A) or high-fat (B) diet ($n = 5–9$ in each group). Data are means ± SE. For explanation of significant differences, see text.

**Fig. 4.** Calculated acute insulin response (AIR, i.e., the mean of suprabasal 1- and 5-min plasma insulin after iv administration), total suprabasal 50-min area under insulin curve ($AUC_{insulin}$), and total suprabasal 50-min area under the glucose curve ($AUC_{glucose}$) after iv injection of glucose (1 g/kg) alone or together with carbachol at 1.5, 5, 15, or 50 nmol/kg in C57BL/6J mice given a control or high-fat diet ($n = 5–14$ in each group). Data are means ± SE. Asterisks indicate probability level of random difference between 2 groups. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. 

Asterisks indicate probability level of random difference between 2 groups. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. 

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animals given glucose alone, i.e., carbachol normalized glucose tolerance.

Figure 5 shows the AIR and $AUC_{glucose}$ in relative response to glucose alone after administration of carbachol, expressed as percentage of the response to glucose alone in the two groups of mice. At 50 nmol/kg, carbachol increased AIR in high fat diet-fed mice by $479 \pm 86\%$ vs. by only $237 \pm 36\%$ in normal diet-fed mice ($P = 0.019$), and the corresponding values at 15 nmol/kg carbachol were $392 \pm 64\%$ in high fat-fed animals vs. $127 \pm 39\%$ in mice fed a normal diet ($P = 0.002$). Moreover, the $AUC_{glucose}$ was reduced by carbachol at 50 nmol/kg by $38 \pm 8\%$ in high fat diet-treated animals vs. $18 \pm 6\%$ in normal animals ($P = 0.026$). Also, when given at 15 nmol/kg, carbachol reduced $AUC_{glucose}$ by $36 \pm 6\%$ in high fat diet-fed animals and by $16 \pm 5\%$ in control diet-fed animals ($P = 0.012$).

Relation between plasma insulin and plasma glucose. Across all animals and when results were included from all time points ($n = 430$), plasma glucose correlated significantly with plasma insulin ($r = 0.58, P < 0.001$) and the correlation was stronger when plasma insulin levels were transformed logarithmically ($r = 0.71, P < 0.001$). This correlation was evident in both control diet-fed and high fat-fed mice and in mice injected both with glucose alone and with carbachol at the different dose levels (Table 1). The slope of the regression differed between high fat-fed and control diet-fed mice, however. Thus, in animals injected with glucose alone, the slope of the regression was lower in high fat-fed than in control diet-fed mice ($P < 0.001$) as a sign of impaired glucose sensitivity to increase plasma insulin in mice fed a high-fat diet. Furthermore, the interception of the regression with the $y$-axis was higher in high fat-fed than in control diet-fed mice ($P < 0.001$). Carbachol administration at 15 and 50 nmol/kg increased the slope of the regression in both high fat-fed and control diet-fed animals ($P < 0.001$) without significantly affecting the interception of the regression with the $y$-axis. In fact, the slope of the regression after carbachol administration in high fat diet-fed mice did not differ significantly from that in control mice given glucose alone, indicating that carbachol normalized the relation between glucose and plasma insulin in insulin-resistant C57BL/6J mice.

Relation between insulin secretion and glucose tolerance. Figure 6 shows the ratio between AIR and $AUC_{glucose}$, which is a measure of the relation between insulin secretion and glucose tolerance, in the two groups after administration of the various doses of carbachol. It is seen that after administration of glucose alone or glucose with the low doses of carbachol, animals fed a control diet had a higher AIR-to-$AUC_{glucose}$ ratio than animals fed a high-fat diet ($P = 0.020$), indicating insufficient insulin secretion in relation to the ambient glucose tolerance in high fat diet-fed animals. However, at the two highest doses of carbachol (15 and 50 nmol/kg), the AIR-to-$AUC_{glucose}$ ratio did not differ significantly between animals given control diet and animals given high-fat diet. This indicates a normalization of the relation between insu-

| Table 1. Slope and interception with $y$-axis of regression with plasma glucose and logarithmically transformed plasma insulin in high fat-fed and normally fed C57BL/6J mice injected iv with glucose alone or with carbachol at 4 different dose levels |
|---|---|---|---|
| Carbachol Dose, nmol/kg | Coefficient of Regression ($r^2$) | Slope, log pmol/l mmol/l $\times 1000$ | Interception with $y$-Axis, log pmol/l |
| **High-fat diet** | | | |
| 0 (14) | 0.59 | 24 $\pm$ 6 | 2.03 $\pm$ 0.09 |
| 1.5 (5) | 0.50 | 21 $\pm$ 8 | 2.28 $\pm$ 0.18 |
| 5.0 (5) | 0.55 | 21 $\pm$ 7 | 2.28 $\pm$ 0.14 |
| 15 (10) | 0.78 | 38 $\pm$ 4 | 2.22 $\pm$ 0.091 |
| 50 (9) | 0.79 | 42 $\pm$ 5 | 2.16 $\pm$ 0.11 |
| **Control diet** | | | |
| 0 (14) | 0.82 | 38 $\pm$ 3 | 1.90 $\pm$ 0.063 |
| 1.5 (5) | 0.81 | 44 $\pm$ 7 | 1.87 $\pm$ 0.13 |
| 5.0 (5) | 0.83 | 38 $\pm$ 5 | 1.76 $\pm$ 0.11 |
| 15 (10) | 0.92 | 51 $\pm$ 3 | 1.86 $\pm$ 0.06 |
| 50 (9) | 0.90 | 52 $\pm$ 4 | 1.97 $\pm$ 0.07 |

Values are means $\pm$ SE; no. of animals in parentheses. Glucose alone injection, 1 g/kg. *All regressions are significant ($P < 0.001$).

Fig. 5. Calculated AIR (i.e., mean of suprabasal 1- and 5-min plasma insulin after iv administration) and total suprabasal 50-min $AUC_{glucose}$ after iv injection of glucose (1 g/kg) together with carbachol at 1.5, 5, 15, or 50 nmol/kg in C57BL/6J mice given a control or high-fat diet ($n = 5-14$) as expressed in percentage of respective control (injection of glucose alone). Data are means $\pm$ SE. Asterisks indicate probability level of random difference between 2 groups. *$P < 0.05$; **$P < 0.01$. 

Figure 6 shows the ratio between AIR and $AUC_{glucose}$, which is a measure of the relation between insulin secretion and glucose tolerance, in the two groups after administration of the various doses of carbachol. It is seen that after administration of glucose alone or glucose with the low doses of carbachol, animals fed a control diet had a higher AIR-to-$AUC_{glucose}$ ratio than animals fed a high-fat diet ($P = 0.020$), indicating insufficient insulin secretion in relation to the ambient glucose tolerance in high fat diet-fed animals. However, at the two highest doses of carbachol (15 and 50 nmol/kg), the AIR-to-$AUC_{glucose}$ ratio did not differ significantly between animals given control diet and animals given high-fat diet. This indicates a normalization of the relation between insu-

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lin secretion and glucose tolerance by carbachol in high fat diet-fed animals.

Insulin secretion in isolated islets. Figure 7 shows that carbachol-stimulated insulin secretion from islets isolated both from high fat diet- and control diet-fed mice. The effect of carbachol was dose dependent with a maximal effect obtained at 1 µmol/l (control diet-fed mice) and 10 µmol/l (high fat-fed mice). Furthermore, the insulinotropic action of carbachol was glucose dependent because the effect was only minimal at 5.6 mmol/l glucose but marked at 11.1 mmol/l glucose. At dose levels >1 µmol/l, carbachol significantly stimulated insulin secretion more markedly in islets from high fat diet-fed mice than in islets from control diet-fed mice (P < 0.05 or less). At very high-dose levels (>100 µmol/l), the insulinotropic action of carbachol was lower than at lower dose levels, but still with a higher efficiency in islets isolated from high fat-fed mice.

Carbachol binding to isolated islets. Figure 8 shows the Scatchard plot of the binding of [3H]NMS to islets from mice of the two groups, each point consisting of 30 islets. It is seen that the binding kinetics were not different for the two groups of animals (n = 3 for each group). The dissociation constant (Kd) for binding was 0.17 ± 0.02 nmol/l in mice fed a control diet vs. 0.22 ± 0.04 nmol/l in mice fed a high-fat diet, and the corresponding values for maximum binding capacity (Bmax) were 0.46 ± 0.04 and 0.47 ± 0.07 fmol/30 islets, respectively.

Ileum contractions. When carbachol was added to the ileum from the mice, a contraction was induced. There was no difference, however, in the contractile response to carbachol between control diet- and high fat diet-fed mice (Fig. 9).

DISCUSSION

In this study, we confirm that mice of the C57BL/6J strain fed a high-fat diet develop slight hyperglycemia and hyperinsulinemia in association with the increased body weight as a sign of insulin resistance (7, 35, 41). It is known that these mice exhibit impaired insulin secretion after glucose administration as a sign of islet dysfunction (7, 27, 41). We previously showed that the administration of the muscarinic agonist carbachol induces an exaggerated insulin secretory response under baseline conditions, i.e., without any concomitant administration of another insulin secretagogue, in these insulin-resistant mice (7, 35). This suggested that although the insulin secretory sensitivity to glucose is impaired, the response to cholinergic agonism is potentiated. In this study, we have examined the influence of
carbachol on glucose-stimulated insulin secretion and glucose tolerance in mice fed a high-fat diet. We also characterized the dose-response relationships and examined the direct in vitro influence of carbachol on isolated islets. We showed that carbachol also potentiates glucose-induced insulin secretion in the mice fed a high-fat diet and a control diet. Because the glucose-induced insulin secretion per se was lower in mice fed a high-fat vs. a control diet and the resulting insulin secretory response to glucose plus carbachol was the same in the two groups of animals, the net effect of carbachol to augment glucose-stimulated insulin secretion was more marked in the mice fed a high-fat diet. This was particularly evident when calculating AIR, i.e., the insulin response during the first 5 min, which represents the first phase insulin secretion. Hence, also with respect to glucose-stimulated insulin secretion, an exaggerated insulino-tropic response to carbachol is evident after high-fat diet in the C57BL/6J mice. This further supports the notion that insulin resistance is accompanied by an adaptively increased cholinergic sensitivity in the islets, which previously has been observed in obese and preobese ob/ob mice (4, 12). Also in obese, insulin-resistant humans, an exaggerated insulin response to cholinergic activity has been documented (13). The hyperinsulinemia in insulin resistance might therefore be dependent on an increased cholinergic sensitivity in the islets.

It is necessary to comment on a few aspects of the design of the present study. First, we used the increase in plasma insulin levels as a determinant for insulin secretion. Although this is a valid parameter used in a number of studies, a drawback is that also possible differences in insulin clearance between the two groups of animals might contribute to the parameter. This might be important because it is known that insulin resistance per se might be accompanied by reduced insulin clearance (19, 43). However, we also showed that carbachol induced a more marked insulin secretion in islets isolated from high fat-fed mice vs. from control mice. Therefore, although reduced insulin clearance might contribute to the raised plasma insulin levels after carbachol in high fat-fed mice, the exaggerated insulin response to carbachol also represents an exaggerated insulin secretion. Second, we used the cholinergic agonist carbachol to activate the cholinergic receptors, and when given centrally (in the cerebroventricular system), it is known that carbachol induces a “stress” response involving hyperglycemia and hyperglucagonemia with unchanged insulin levels (45). However, we do not consider such an action to contribute to the observed effects on plasma insulin and glucose in our experiments, because carbachol, due to its polar structure, does not pass the blood-brain barrier when given peripherally (37). Furthermore, we have previously shown that methylxanthine, which also does not pass the blood-brain barrier, inhibits carbachol-stimulated insulin secretion in mice (3). This suggests that the effects of carbachol in our present study are exerted peripherally. Third, even though the effects of carbachol are peripherally mediated, it is possible that the drug also exerts indirect, islet-independent, peripheral actions. For example, carbachol has been shown to stimulate epinephrine release from the adrenals (24) and secretion of glucagon-like peptide-1 (GLP-1) from the gut enteroglucagon cells (11), which might contribute to the insulino-tropic action of the drug. The possibility that the stimulatory action of epinephrine (the β-adrenergic receptor activation) contributes to the insulino-tropic action of carbachol is, however, unlikely, because we have shown that propranolol, inhibiting β-adrenergic receptor-activated insulin secretion, does not inhibit carbachol-stimulated insulin secretion in mice (3). Whether GLP-1, and possibly also gastric inhibitory polypeptide (GIP), contribute to the insulino-tropic action of carbachol is, however, likely, because we have shown that propranolol, inhibiting β-adrenergic receptor-activated insulin secretion, does not inhibit carbachol-stimulated insulin secretion in mice (3). Whether GLP-1, and possibly also gastric inhibitory polypeptide (GIP), contribute to the insulino-tropic action of the drug, however, remains to be studied in more detail. Nevertheless, the in vitro results, showing that carbachol potently stimulates insulin secretion from isolated islets, suggest that the insulino-tropic action of the drug as observed in the present study is most likely caused by a direct islet action. Finally, although induction of anesthesia and the experimental procedure might have induced a stress response in the animals, the stress level is remarkable low in this type of experiments. For example, we have previously shown that anesthesia reduces the circulating levels of catecholamines by ~80% when compared with studies in nonanesthetized animals (2, 15); hence, our experimental model provides a good technique associated with only a low degree of stress. The potential influence of the unavoidable stress on the metabolic status of the animals has not been established in detail. However, we have shown that circulating glucose does not increase after saline injection in anesthetized mice, which suggests that the model is not associated with significant metabolic responses associated with stress (15).

In this study, we characterized the relationship between dose of carbachol and effects on glucose-stimulated insulin secretion and glucose tolerance in the two groups of mice. We showed that a threshold for a
potentiated glucose-stimulated insulin secretion by carbachol in mice exists at a dose between 5 and 15 nmol/kg and that this threshold was similar in mice fed a high-fat diet and in those fed a control diet. Therefore, the increased insulinotropic response to carbachol in high fat-fed animals is reflected as increased efficiency rather than increased potency. To examine whether this increased efficiency is also evident in islets, we performed an in vitro study. The results show that carbachol dose and glucose dependently stimulated insulin secretion from islets isolated both from control diet- and high fat diet-fed mice. Furthermore, the pattern of the effects of carbachol also shows that in vitro it is the efficiency rather than the potency that is increased. In both groups, a “bell-shaped” dose-response relationship existed between dose of carbachol and insulin secretion, with diminished action at high-dose levels. This is a well-known phenomenon for cholinergic agonism in different in vitro systems (21, 25, 30, 33, 34, 38). We also show that the binding of carbachol to islets isolated from the control diet-fed and high fat diet-fed animals was the same. These results together indicate that the increased activity of carbachol to stimulate insulin secretion in insulin-resistant mice is not dependent on increased receptor sensitivity but rather on increased intrinsic activity subsequent to acetylcholine-receptor activation in islets. This may be due to increased efficiency in the signaling pathways activated by these receptors.

Our study on the contractile response to carbachol in the ileum preparations from the two groups of mice showed no difference between control diet fed- and high fat-fed mice. This shows that the increased responsiveness to carbachol in insulin secretion is not reflective of a generalized increased responsiveness to muscarinic agonism after high-fat feeding. It is also important to acknowledge that the high fat-fed mice had a reduced insulin secretory response to glucose per se, which was evident both by the lower AIR and AUC\textsubscript{insulin}, as well as by the lower slope between plasma levels of glucose and insulin. Hence, an exaggerated signaling is probably not a general phenomenon in the islets, but specific to certain secretagogues. In this context, it should be mentioned that we have previously shown that the insulinotropic response also to cholecystokinin and to the glucose-incretin hormone GLP-1 are exaggerated in C57BL/6J mice (35), suggesting a generalized increased responsiveness to nonglucose insulin secretagogues.

The relation between circulating glucose and insulin was linearly correlated both in control diet-fed and in high fat-fed mice, and the relation was improved by logarithmic transformation of the insulin data. However, as is shown in detail in Table 1, the slope of the relation was lower in high fat-fed animals than in control diet-fed animals, illustrating the insensitivity for glucose for the insulin secretion in insulin resistance. By challenging the mice with intravenous carbachol, we increased the slope of the relation between glucose and insulin. Of importance is that the slope was the same in control diet-fed animals given glucose as in high fat-fed mice given glucose + carbachol, suggesting that carbachol normalizes the insulin-glucose relation in insulin-resistant mice.

The mice fed the high-fat diet had a markedly impaired glucose tolerance, as judged by the marked increase in AUC\textsubscript{glucose} after the glucose administration and the finding that plasma glucose levels after glucose administration were still elevated after 50 min. The potentiated glucose-stimulated insulin secretion resulted in reduction in AUC\textsubscript{glucose} as a sign of improvement of glucose tolerance. In fact, the AUC\textsubscript{glucose} in high fat-fed animals given glucose plus carbachol (15 or 50 nmol/kg) was the same as in control diet-fed animals given glucose alone, and, furthermore, the ratio between AIR to AUC\textsubscript{glucose} was not different between the two groups of animals given carbachol at the higher dose levels. This further suggests that carbachol has the ability through its potentiated action on insulin secretion to normalize glucose tolerance in high fat-fed mice. Still, however, the 50-min glucose levels were slightly higher than baseline in high fat diet-fed mice given carbachol at the highest dose level, suggesting that an even more efficient insulinotropic signal is required for complete normalization of the glucose intolerance. This is, however, not possible with carbachol due to its muscarinic receptor subtype nonspecific action. Although not studied directly in the present study, the improved glucose tolerance after carbachol administration is probably mediated by the increased plasma insulin levels rather than by any direct action of carbachol on peripheral insulin sensitivity, because a recent study in rats showed that administration of acetylcholine did not affect insulin sensitivity during a euglycemic clamp study (46).

In conclusion, the study has presented evidence that exogenous administration of the muscarinic agonist carbachol elicits a more marked insulin secretory response in high fat-fed insulin-resistant mice than in control mice, with improved glucose tolerance as a result. The exaggerated insulin response is exerted mainly through increased efficiency rather than increased potency. Furthermore, the study also shows that islet binding of muscarinic antagonist is not altered in mice fed a high-fat diet and that the ileum contraction activity of carbachol is not different between the groups. The study thus suggests that insulin resistance is associated with increased islet, but not general, muscarinic sensitivity. This may be a mechanism underlying the hyperinsulinemia in insulin resistance. It may also be suggested that development of islet-specific muscarinic agonists, with lesser general muscarinic activity, might be a feasible target to improve failure of insulin secretion in insulin resistance during the development of type 2 diabetes. The rapid potentiation of the immediate (first 5 min) glucose-stimulated insulin secretion is in this context of great interest, because impairment of first phase insulin secretion is an important islet defect in glucose intolerance and type 2 diabetes (6, 16).

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