Insufficient islet compensation to insulin resistance vs. reduced glucose effectiveness in glucose-intolerant mice

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Ahrén, Bo, and Giovanni Pacini. Insufficient islet compensation to insulin resistance vs. reduced glucose effectiveness in glucose-intolerant mice. *Am J Physiol Endocrinol Metab* 283: E738–E744, 2002. First published June 11, 2002; 10.1152/ajpendo.00199.2002.—This study evaluated the relative contribution of insulin-dependent mechanisms vs. mechanisms independent on dynamic insulin for glucose intolerance induced by high-fat diet. C57BL/6J mice underwent a frequently sampled intravenous glucose tolerance test (1 g/kg glucose) at 1 wk and 1, 3, and 10 mo after initiation of a high-fat diet (58% fat; control diet 11% fat) to measure glucose effectiveness (S_G) and disposition index (DI), i.e., insulin sensitivity (S_I) times early or total insulin secretion. Glucose disappearance (K_G) and S_I were reduced in high-fat-fed mice at all time points. Total (50 min) insulin secretion was sufficiently increased at all time points to compensate for the reduced S_I, as judged by normal $DI_{50\ min}$. In contrast, early (10 min) insulin secretion was not sufficiently increased; $DI_{10\ min}$ was reduced after 1, 3, and 10 mo. S_G was reduced after 1 wk; the reduction persisted throughout the study period. Thus glucose intolerance induced by high-fat diet is, in early phases, solely explained by reduced glucose effectiveness, whereas insufficient early insulin secretion is of importance after long-term feeding.

glucose tolerance; glucose intolerance; insulin secretion; high-fat diet

TYPE 2 DIABETES OCCURS when the β-cells fail to adequately compensate a reduced insulin sensitivity with a sufficient insulin secretion (6, 7, 16, 19, 28). Also, glucose uptake mediated by mechanisms independent of dynamic insulin, the so-called glucose effectiveness (S_G), importantly contributes to glucose tolerance and is an independent risk factor for type 2 diabetes (1, 2, 8, 17). The relative impact of reduced β-cell compensation vs. S_G for development of glucose intolerance is, however, not known. Therefore, we have explored in this study the temporal relationship between insulin-dependent mechanisms and processes being independent of dynamic insulin in the standardized experimental model of mice fed a high-fat diet by use of the frequently sampled intravenous glucose tolerance test (FSIVTT) recently developed for use in mice (26). High-fat diet is known to be linked to both islet dysfunction and poor glucose effectiveness (9, 13, 18, 21, 22, 24, 32). The high-fat diet mouse model has previously been shown to be associated with insulin resistance together with glucose intolerance (4, 5, 11, 15). This model is therefore suited for studies on the temporal relationship between insulin-dependent mechanisms and mechanisms independent of dynamic insulin for the glucose intolerance accompanying insulin resistance caused by high-fat diet.

The study also evaluated the relative contribution of early vs. total insulin secretion for development of glucose intolerance in insulin resistance. This is of interest because recent studies have suggested that the initial insulin response more importantly contributes to glucose tolerance than does total insulin secretion. Thus a negative correlation has been reported between the 30-min insulin response to oral glucose as a marker for early insulin secretion and the 120-min glucose value as a marker of glucose tolerance (23). Furthermore, prevention of the early insulin response results in glucose intolerance (3, 33), and a brief administration of a minute amount of insulin during the first 15 min after food intake markedly improves glucose tolerance in subjects with obesity (33) and type 2 diabetes (10).

MATERIALS AND METHODS

Animals. A total of 119 experiments were performed in nonfasted female C57BL/6J mice (Bomholdtgaard Breeding and Research Centre, Ry, Denmark) weighing 20–25 g at the start of the study and kept five per cage on a 12:12-h light-dark schedule (lights on at 0600). Experiments were performed in late morning without food being removed from the cages. Previous experiments in the laboratory have shown that results with this approach are the same as when food is removed from the cage 4 h before experiments. The animals were obtained at 4 wk of age. On *day 4* after arrival, mice received a high-fat diet consisting of 58% fat, 25.6% carbohydrate, and 6.4% protein (total energy 23.4 kJ/g; Research Diets, New Brunswick, NJ). Controls were fed with a standard pellet diet (fat 11.4%, carbohydrate 62.8%, protein 25.8%, total energy 12.6 kJ/g; Research Diets). Tap water was given ad libitum. The experimental procedures were approved by the Ethics Committee of Lund University.

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Experiments. Intravenous glucose tolerance tests (IVGTT) were performed at 1 wk and 1, 3, and 10 mo after the high-fat diet was started. The animals were anesthetized with intraperitoneal midazolam (Dormicum, 0.14 mg/mouse; Hoffman-La Roche, Basel, Switzerland) and a combination of fluanison (0.9 mg/mouse) and fentanyl (Hypnorm, 0.02 mg/mouse; Janssen, Beerse, Belgium). After 30 min, a blood sample (75 μl) was taken from the retrobulbar, intraorbital, capillary plexus in a 100-μl pipette, which had been pre-rinsed in heparin solution (100 U/ml in 0.9% NaCl; Lövens, Ballerud, Denmark). Thereafter, D-glucose (British Drug Houses, Poole, UK) was injected intravenously over 3 s at the dose of 1 g/kg in a tail vein without flushing of the 27-gauge needle after injection. The volume load was 10 μl/g body wt. New blood samples (75 μl each) were taken at 1, 5, 10, 20, 50, and 75 min. In another series of experiments, an insulin-modified IVGTT was undertaken. Animals then underwent the IVGTT as the regular test, with the difference that human insulin (Actrapid, 1 U/kg; Novo Nordisk, Bagsvaerd, Denmark) was administered intravenously at 10 min after glucose, immediately after taking the 10-min sample.

Data analysis. Insulin and glucose data from the IVGTT were analyzed with the minimal model technique, as already reported in detail (26). The model assumes a first-order, nonlinear insulin- and glucose-controlled glucose kinetics following exogenous glucose injection. This analysis provides parameter S_I (insulin sensitivity index), which is defined as the ability of insulin to enhance net glucose disappearance and to inhibit glucose production, and the parameter S_G (glucose effectiveness), representing net glucose disappearance per se from plasma without any change in dynamic insulin. Both the early and total insulin responses to intravenous glucose were calculated by the trapezoid rule as the suprabasal area under the insulin curve ($AUC_{insulin}$) for the first 10 min (ΔAUC_{0-10}) or the $AUC_{insulin}$ for the total 50 min (AUC_{0-50}) after the glucose challenge. We also determined the unitless index DI (global disposition index) by multiplying S_I times ΔAUC_{0-10} or times AUC_{0-50} ($DI_{10\ min}$ and $DI_{50\ min}$, respectively). These indexes are an extension of the concept proposed in dogs by Bergman et al. (7) and in humans by Kahn et al. (16) and describe the insulin effect by

including both insulin action and insulin secretion. The net glucose elimination rate after the glucose injection [the glucose tolerance index (K_G)] was calculated as the slope for the interval 1–20 min after glucose injection of the logarithmic transformation of the individual plasma glucose values (26). The relative contribution of the insulin-mediated processes and the processes independent of dynamic insulin for glucose intolerance was assessed as previously described in detail (26), considering, as has been demonstrated (34), that intravenous glucose tolerance is a linear combination of these two processes. To evaluate the relative contribution of S_G and DI to the changes of K_G, we applied the definition and the calculations of sensitivity, i.e., the quantification of changes of K_G for a unit change of S_I or DI (26), that yield the value (as percentage) of the two contributions. The detailed description of the method to calculate these sensitivities and the relative contributions has been reported in Ref. 26.

Statistics. Data and results are reported as means ± SE unless otherwise designated. Statistical comparisons between any group or subgroup with the relative controls were performed with an unpaired *t*-test. ANOVA was exploited for multiple comparisons.

RESULTS

Regular IVGTT after high-fat feeding. The two groups of mice being fed a high-fat diet or a control diet for 10 mo underwent a regular FSIVGTT at 1 wk and 1, 3, and 10 mo after introduction of diet. Figure 1 shows the body weight, baseline glucose and insulin levels, and glucose tolerance as calculated by the glucose elimination rate during *minutes 1–20* after glucose injection (K_G). It was seen that body weight was already significantly higher in high-fat-fed mice after 1 wk, whereas glucose and insulin levels were elevated after 1 mo on the high-fat diet. The mice on the high-fat diet were glucose intolerant throughout the study, as K_G was significantly lower than in control diet-fed mice at all studied time points. Figure 2 shows the time courses of the glucose and insulin levels during the regular IVGTT, and Fig. 3
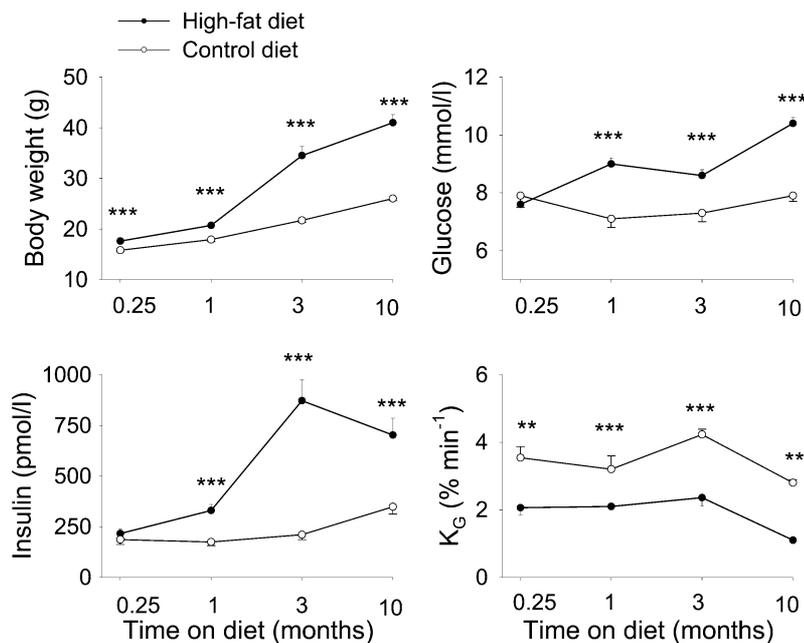

Fig. 1. Body weight, baseline levels of glucose and insulin, and glucose disposal (K_G) after iv glucose challenge in anesthetized nonfasted mice given a control diet or a high-fat diet for 1 wk or 1, 3, or 10 mo. After 1 wk, 1 mo, and 3 mo, there were 8 mice in each group, whereas after 10 mo the number of animals was 24 (high-fat diet) or 23 (control diet). Means ± SE are shown. Asterisks indicate the probability level of random difference (**$P < 0.01$, ***$P < 0.001$).
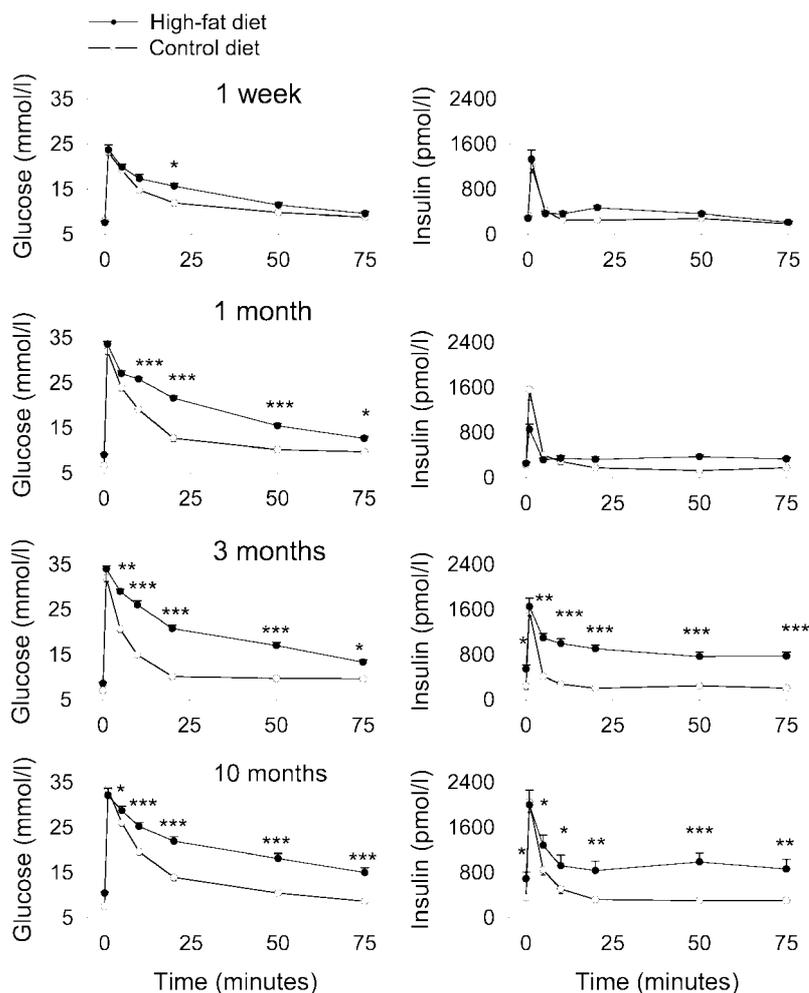
Fig. 2. Glucose and insulin levels before and after the iv injection of glucose (1 g/kg) to anesthetized mice given a control diet or a high-fat diet for 1 wk or 1, 3, or 10 mo. After 1 wk, 1 mo, and 3 mo, there were 8 mice in each group, whereas after 10 mo the number of animals was 24 (high-fat diet) or 23 (control diet). Means ± SE are shown. Asterisks indicate the probability level of random difference between the groups (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

shows the calculated parameters from the minimal-model analyses of glucose and insulin data obtained from the FSIVGTT. S_I increased slightly by time in control mice over the first 3 mo, being higher at 3 mo than at 1 wk ($P = 0.015$), whereafter it fell. Throughout the study period, however, S_I was significantly lower in high-fat-fed mice than in mice fed a control diet, i.e., already after 1 wk. The lowered S_I was paralleled by an increased insulin secretion in high-fat-fed mice, when calculated as the total 50-min $AUC_{insulin}$, which was significantly higher in high-fat-fed mice than in mice fed a control diet at all time points (Fig. 3). AUC_{0-50} and S_I displayed a hyperbolic relationship to each other (Fig. 4). The increase in AUC_{0-50} induced by high-fat feeding matched the reduction in S_I; hence, the DI_{50}, i.e., the product of insulin secretion times insulin sensitivity, was not significantly different between the two groups of animals. In contrast, the first 10-min insulin response to glucose (ΔAUC_{0-10}) was significantly increased only after 3 and 10 mo on a high-fat diet and was therefore not elevated in high-fat-fed mice compared with mice fed a control diet at 1 wk and 1 mo. Hence, the 10-min insulin response was not compensatorily increased in relation to the low S_I after 1 wk or 1 mo on high-fat diet. When calculated for this early 10-min insulin response (DI_{10}), the DI was there-

fore lower in high-fat-fed mice than in mice fed a control diet at all time points, being significant at 1 mo ($P = 0.012$), 3 mo ($P = 0.013$), and 10 mo ($P = 0.017$). S_G did not change significantly over time in control mice and was significantly lower in high-fat-fed mice than in mice fed a control diet at all time points. In Table 1, a summary of the parameters calculated after 10 mo of high-fat diet is reported.

Contribution of metabolic parameters to glucose tolerance. Across all animals, K_G correlated to both DI (DI_{10} $r = 0.51$, $P < 0.001$; DI_{50} $r = 0.42$, $P < 0.001$) and S_G ($r = 0.56$, $P < 0.001$; Fig. 5). To calculate the relative contribution of the two DIs vs. S_G for the development of glucose intolerance, multiple regression analysis was performed, with K_G as the independent variable and these parameters as dependent variables. It was found that, after 1 wk on high-fat diet, S_G ($r = 0.85$, $P < 0.001$) but not DI (either one) correlated independently with K_G. In contrast, after 1 mo on high-fat diet, only DI_{10} ($r = 0.75$; $P = 0.001$), but not S_G or DI_{50}, correlated with K_G, and after 3 mo, both DIs ($r = 0.87$, $P < 0.001$), but not S_G, correlated with K_G. Finally, after 10 mo, all three parameters correlated independently with K_G ($r = 0.78$; $P < 0.001$). When the relative contribution of DI_{50} vs. S_G to K_G was calculated, all control and high-fat-fed mice, re-
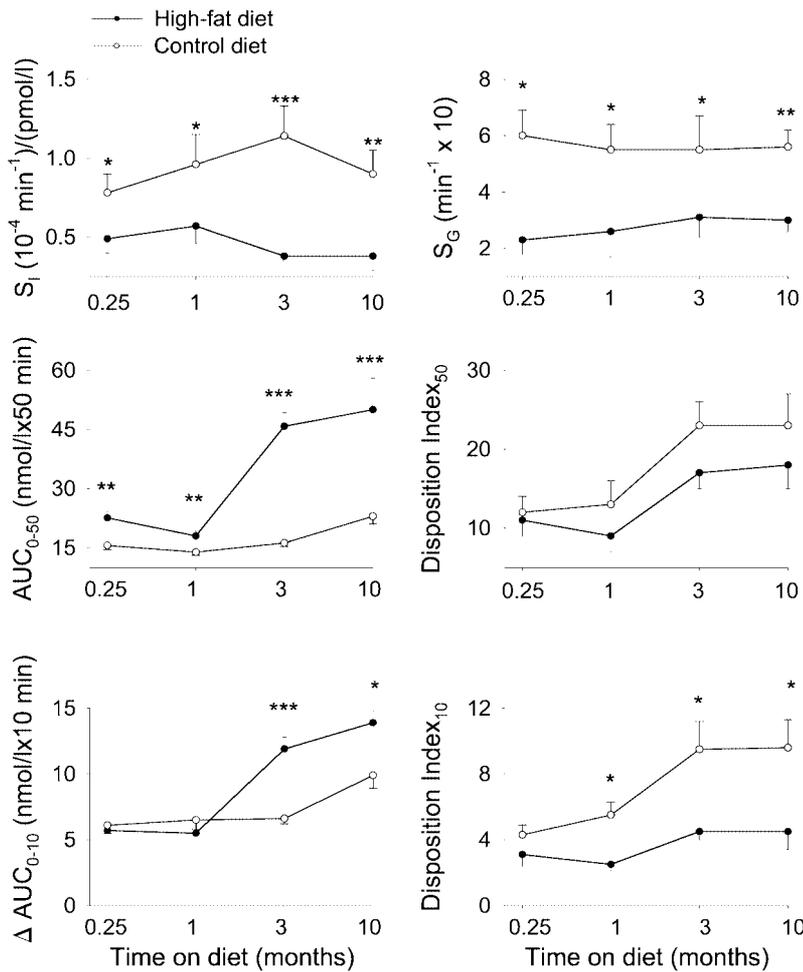
Fig. 3. Insulin sensitivity index (S_I), glucose effectiveness (S_G), area under the 50-min insulin curve (AUC_{0-50}), 50-min disposition index (DI_{50}), suprabasal 10-min insulin curve (ΔAUC_{0-10}), and 10-min DI (DI_{10}) in mice given a control diet or a high-fat diet for 1 wk or 1, 3, or 10 mo as achieved by the minimal-model data analyses of glucose and insulin levels after an iv glucose tolerance test (IVGTT). After 1 wk, 1 mo, and 3 mo, there were 8 mice in each group, whereas after 10 mo the number of animals was 24 (high-fat diet) or 23 (control diet). Means ± SE are shown. Asterisks indicate the probability level of random difference (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

spectively, were included in the analysis. It was found that, in controls, S_G contributed by 72%, whereas DI_{50} contributed by 28% to K_G. In contrast, in high-fat-fed mice, the contribution by DI_{50} was considerably increased, being 54.3%, vs. only 45.7% for S_G.

Insulin-modified IVGTT after high-fat feeding. Also at 10 mo, a modified IVGTT was performed in which insulin was injected 10 min after glucose, which re-

Fig. 4. S_I vs. the area under the 50-min insulin curve (AUC_{0-50}) in mice given a control diet ($n = 47$) or a high-fat diet ($n = 48$) for 1 wk or 1, 3, or 10 mo.

sulted in very high insulin levels at *minute 20* (Fig. 6). The aim of this set of experiments was to evaluate whether reduced glucose tolerance in animals under high-fat diet was reversed with very high levels of ambient insulin. The total insulin levels (AUC_{0-50}) were very high in this test, whereas the early insulin response (ΔAUC_{0-10}) was not significantly different from the values obtained in the regular IVGTT. We confirmed the reduction of S_I, DI (both DIs), and S_G in mice fed a high-fat diet (Table 1). Also with the insulin-modified IVGTT, despite a very high ambient insulin, the high-fat-fed animals displayed a lower K_G ($P = 0.002$). It is worth noting that K_G in the insulin-modified IVGTT in high-fat-fed mice was higher than in control mice during the regular IVGTT, being calculated at two different ambient insulin levels. Finally, it is also interesting to observe that the value of S_I in both tests in the control animals was virtually the same (Table 1), supporting the robustness of this parameter in situations characterized by different insulin concentrations.

DISCUSSION

This study examined the relative contribution of insulin-dependent mechanisms vs. processes independent of dynamic insulin for impaired glucose tolerance
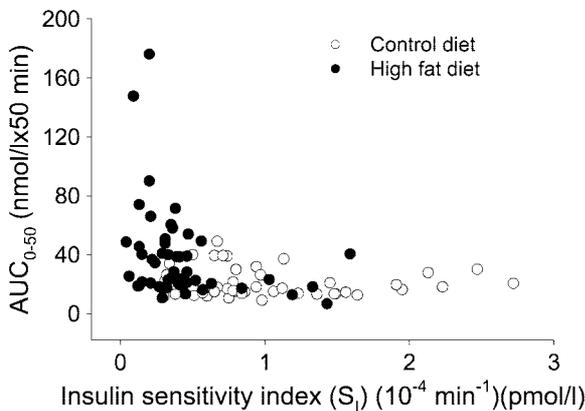
Table 1. *Metabolic parameters in mice receiving high-fat diet for 10 mo vs. controls during regular or insulin-modified IVGTT*

Parameter (Unit)	Regular IVGTT			Insulin-modified IVGTT		
	High-fat diet ($n = 24$)	Control diet ($n = 23$)	P value	High-fat diet ($n = 12$)	Control diet ($n = 12$)	P value
ΔAUC_{0-10}, $nmol \cdot 1^{-1} \cdot 10\ min$	13.9 ± 0.9	9.9 ± 1.0	0.046	10.3 ± 2.2	7.3 ± 1.1	0.042
AUC_{0-50}, $nmol \cdot 1^{-1} \cdot 50\ min$	50 ± 8	23 ± 2	0.002	222 ± 24	325 ± 60	NS
K_G, $\%\ min^{-1}$	1.9 ± 0.1	4.6 ± 0.3	<0.001	3.6 ± 0.2	5.0 ± 0.2	0.002
S_I, $pmol \cdot 1^{-1} \cdot 10^{-4}\ min^{-1}$	0.38 ± 0.09	0.90 ± 0.15	0.002	0.13 ± 0.02	1.02 ± 0.25	0.002
$DI_{10\ min}$	4.5 ± 1.1	9.6 ± 1.7	0.017	2.3 ± 0.9	8.4 ± 0.9	<0.001
$DI_{50\ min}$	18 ± 3	23 ± 4	NS	22 ± 5	262 ± 62	<0.001
S_G, min^{-1}	0.030 ± 0.004	0.056 ± 0.006	<0.001	0.043 ± 0.006	0.097 ± 0.015	<0.001

Values are means $\pm$ SE. IVGTT, intravenous glucose tolerance test; P value, probability level of random difference between the two groups; ΔAUC_{0-10} and AUC_{0-50}, suprabasal 10- and and total 50-min insulin concentrations, respectively, after iv glucose challenge; K_G, 1- to 20-min glucose disappearance rate; S_I, insulin sensitivity index; DI_{10} and DI_{50}, disposition indexes (i.e., S_I times ΔAUC_{0-10} or AUC_{0-50}, respectively); S_G, glucose effectiveness (insulin-independent glucose uptake); NS, not significant.

after high-fat feeding in mice. Furthermore, with regard to insulin-dependent mechanisms, the study also evaluated the relative importance of defective early insulin response to the glucose challenge vs. the total insulin secretion over 50 min. A main novel finding is

the demonstration that glucose intolerance in the early stages after initiation of insulin resistance is dependent on a reduction of glucose-controlled glucose clearance (S_G), whereas after a longer period of time, when obesity has progressed, insufficient islet compensation to insulin resistance contributes to a higher extent. A second main novel finding is the demonstration that defective compensation of the early insulin response to glucose more importantly than total insulin secretion contributes to defective insulin-dependent mechanisms responsible for glucose intolerance. These results are of importance for our understanding of basic mechanisms for glucose intolerance as well as for identifying targets for treatment.

High-fat feeding is known to induce insulin resistance, as reported from studies in mice (4, 5, 11, 15), dogs (18, 24, 29), rats (31), and humans (9, 21, 22, 32). It has been shown that this is associated with impaired insulin signaling and translocation of GLUT4 in skeletal muscle (15). We show here a marked reduction of insulin sensitivity, as is evident by the low S_I, in high-fat-fed mice. A lower S_I in the high-fat-fed mice compared with the control mice was already evident at 1 wk after introduction of a high-fat diet and persisted throughout the entire study period. This may be caused by the fat loading per se in combination with the increased degree of obesity.
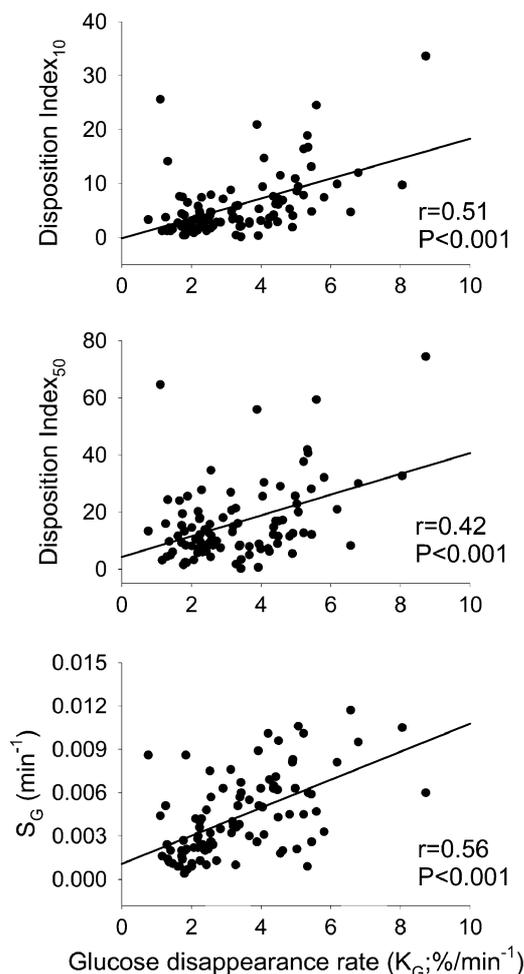
Fig. 5. Linear regression between K_G and DI_{10} (*top*), DI_{50} (*middle*) and S_G (*bottom*) in mice given a control or a high-fat diet (total no. of animals = 95) for 1 wk or 1, 3, or 10 mo. *r*, Linear regression coefficient; *P*, the probability level of random regression.
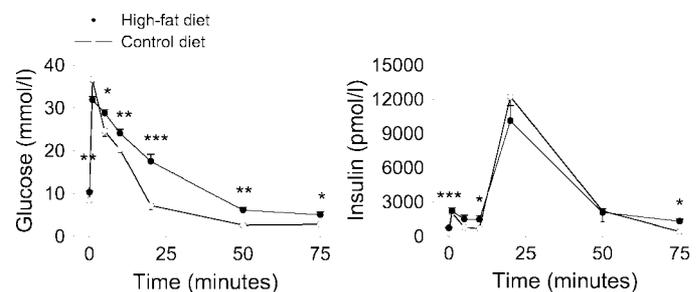
Fig. 6. Glucose and insulin levels before and after the iv injection of glucose (1 g/kg), when insulin was injected at 10 min in anesthetized mice given a control diet or a high-fat diet for 10 mo. There were 12 animals in each group. Means $\pm$ SE are shown. Asterisks indicate the probability level of random difference (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

The insulin response to the intravenous glucose was augmented in mice fed a high-fat diet compared with control mice. This is a consequence of the reduced insulin sensitivity and illustrates the islet compensation to insulin resistance as initially shown by Bergman et al. (7) and later explored in detail by Kahn et al. (16). We found that total insulin secretion, as judged by the 50-min $AUC_{insulin}$ during the regular IVGTT, was increased by $\sim40\%$ in mice on a high-fat diet. This increase perfectly matched the 40% reduction in S_I. In contrast, the compensatory augmentation of the early (10 min) insulin response to glucose was slow and delayed compared with the augmented total insulin secretion and took, in fact, 3 mo to evolve. Thus there was no significant compensatory increase in the early insulin response to glucose in the high-fat diet after 1 wk or 1 mo despite the reduced S_I. Furthermore, when the compensation in this parameter appeared, i.e., after 3 mo and 10 mo, it was lower than what was required for the reduced S_I. Because at these time points glucose tolerance was markedly impaired, the results suggest that the defective early insulin response to glucose is of major importance for the development of glucose intolerance. It should be emphasized that the defective early insulin response was evident despite a marked increase in insulin secretion, because ΔAUC_{0-10} was 156% higher in high-fat diet-fed mice than in controls, yet DI_{10} was reduced by almost 40%. To be sufficient, an $\sim50\%$ higher insulin secretion would be required in the mice with low S_I to result in a normal DI. At present, the mechanism of this failure to adequately compensate for the reduced insulin secretion is not known. It should be emphasized that the study explored the insulin response to glucose in vivo. Thus the insulin response to glucose depends both on the function of the β-cells and on in vivo factors influencing the β-cells, such as the autonomic nerves, circulating nutrients, and hormones. It has previously been shown that high-fat feeding in mice is associated with an $\sim50\%$ increase in β-cell mass (25). This would suggest that it is not a defective increase in β-cell mass that explains the poor compensation. However, further studies have to analyze the exact mechanism behind the defective adaptation.

The compensatory increase in insulin secretion in insulin resistance makes it impossible to quantify insulin secretion independent of the knowledge of insulin sensitivity. Hence, the demonstration of the defective insulin secretion in high-fat-fed mice would not be possible without the knowledge that S_I is markedly reduced, which requires an even higher insulin secretion than was evident. This failure to determine both processes for accurate determination of their contributions may underlie previous conclusions that reduced insulin action is a primary defect for the development of glucose intolerance and type 2 diabetes (20, 30). In fact, it seems to be the defect of insufficient β-cell compensation to the reduced insulin action that is the main event underlying progression to glucose intolerance and type 2 diabetes (14, 19). This is supported by the present standardized model, which expresses insulin resistance as the temporally initial defect, and glucose tolerance is worsened by inappropriate β-cell compensation in the early insulin secretion.

To further evaluate in detail the limits of insulin compensation for reduced insulin sensitivity and to assess whether very high insulin levels could ameliorate glucose tolerance during a high-fat diet, we also exploited the insulin-modified IVGTT, which has never been performed previously in mice. Also, this test showed that S_I was markedly lower in high-fat-fed vs. control mice, causing again a sustained reduction of DI. Glucose disposal as judged by the K_G was also lower in high-fat diet-fed mice, although glucose elimination was higher than that of the regular IVGTT due to the very high insulin levels. This shows that insulin administration improves glucose elimination in high-fat-fed mice, but the glucose disposal remains lower than in control mice to which the same amount of insulin is administered.

In the present study, we exploited the minimal model of glucose disappearance to calculate insulin sensitivity in mice. This method was recently validated against the gold standard hyperinsulinemic euglycemic glucose clamp technique both in control animals and in mice on a high-fat diet (26), thus yielding confidence in the results obtained here. An additional interesting result of the present study was that S_I was not different in control animals when obtained with the insulin-modified test or with the regular test, where much lower levels of insulin are present. This further demonstrates that S_I from the minimal model is a robust and consistent parameter in rodents also, this having already been shown in humans (27).

The minimal-model analysis of the data during the IVGTT allowed calculation not only of insulin sensitivity and insulin secretion but also of S_G and confirmed that S_G largely contributes to glucose disposal in normal mice, because K_G was dependent on S_G by 72% (26). In high-fat-fed mice, S_G contributed instead only by 45% to K_G, and this was explained by the significantly lower S_G that was already evident after only 1 wk of feeding. This shows that the metabolic derangement due to the high-fat diet involves both mechanisms responsible for glucose disappearance from the beginning. The reduction in S_G was largest in the early time points. Thus, after 1 wk on a high-fat diet, S_G was reduced by >60%, whereas S_G thereafter gradually improved and was reduced by only 25% after 3 mo. Therefore, S_G shows a different time pattern after initiation of high-fat feeding from S_I, which deteriorated progressively throughout the study period. Although there is no evidence of a synergistic process between S_I and S_G (8), it can be speculated that this mechanism of increasing S_G is in the long run an attempt to counterbalance the elevated insulin resistance. The molecular basis for the reduced S_G in high-fat-fed mice remains to be studied, however.

In summary, glucose intolerance in insulin resistance in mice is caused by a combination of reduced glucose effectiveness and insufficient islet compensation to insulin resistance, the latter involving the early 10-min insulin response. Reduced glucose effectiveness and defective early 10-min insulin response are, therefore, in addition to insulin resistance, targets for treatment of high-fat diet-associated glucose intolerance.

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