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Temporal and dietary fat content–dependent islet adaptation to high-fat feeding–induced glucose intolerance in mice

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Abstract

The high fat–fed mouse is an experimental model for studies of islet dysfunction as a mechanism for glucose intolerance and for evaluation of therapeutic targets. This model is, however, dynamic with a temporal and dietary fat content–dependent impact on islet function and glucose tolerance, the details of which are unknown. This study therefore examined the time course of changes in the insulin response to intravenous glucose (1 g/kg) in relation to glucose tolerance in female mice after 1, 3, 8, or 16 weeks of feeding with diets containing 11% fat (normal diet [ND]), 30% fat (medium-fat diet [MFD]), or 58% fat (high-fat diet [HFD]; by energy). High-fat diet increased body weight and body fat content, whereas MFD did not. The insulin response (postglucose suprabasal mean 1- and 5-minute insulin) was impaired after 1 week on MFD (481± 33 pmol/L) or HFD (223 ± 31 pmol/L) compared with ND (713 ± 46 pmol/L, both $P < .001$). This was accompanied by impaired glucose elimination compared with ND (both $P < .001$). Over the 16-week study period, the insulin response adaptively increased in the groups fed with HFD and MFD, to be not significantly different from ND after 16 weeks. This compensation normalized glucose tolerance in MFD, whereas the glucose tolerance was still below normal in HFD. Insulin clearance, as judged by elimination of intravenous human insulin, was not altered in HFD, suggesting that the observed changes in insulin responses to glucose are due to changes in insulin secretion rather than to changes in insulin clearance. We conclude that time- and dietary fat–dependent dynamic adaptive islet compensation evolves after introducing HFD in mice and that MFD-fed mice is a novel nonobese model of glucose intolerance.

1. Introduction

Glucose intolerance and type 2 diabetes mellitus are characterized by insufficient islet compensation to insulin resistance [1-5]. These conditions are associated with high dietary fat intake [6-9] and may be due to insulin resistance and beta-cell dysfunction instituted by fatty acid species [10-12]. The model of high fat–fed C57BL/6J mouse has been developed to study the impact of dietary fat for islet dysfunction in the pathogenesis of type 2 diabetes mellitus and to explore novel therapeutic targets [13,14]. This model is associated with insulin resistance as demonstrated by the euglycemic clamp technique and estimation of the insulin sensitivity index from data obtained from the intravenous glucose tolerance test [15]. The model is also associated with glucose intolerance, as evident by impaired glucose disposal after glucose challenge [13-15]. As has previously been observed during long-term studies, the high fat–fed mouse model is a dynamic model in which compensatory adaptations may change by time. Hence, one study demonstrated failure of insulin secretion to compensate for insulin resistance during the first 4 weeks of dieting with a diet containing a very high (58%) amount of fat, followed by a clear compensatory increase at later time points, which nevertheless was insufficient for the insulin resistance [16]. Similar studies with more moderate increases in dietary fat content are not available yet. Therefore, to explore the impact of dietary fat to the dynamic temporal development of islet adaptation to insulin resistance, we have in this study fed mice with diets containing different amounts of fats (11%, 30%, or 58% fat from lard) over a 16-week period with evaluation of the glucose and insulin responses to intravenous glucose after 1, 3, 8, and 16 weeks.

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2. Materials and methods

2.1. Animals

Eight-week-old female C57BL/6JBomTac mice were obtained from Taconic, Skensved, Denmark, and kept in a temperature-controlled room (22°C) on a 12-hour light-dark cycle, with food and water ad libitum. On arrival, all animals were fed with the normal diet (ND), and after 1 week of acclimatization, the mice were divided into 3 groups and fed with ND, medium-fat diet (MFD), or high-fat diet (HFD; all diets were from Research Diets, New Brunswick, NJ; for compositions, see Table 1). Sixty-four mice were started on each diet. The fat in the diets was from lard, which consists of 40% saturated fats, 48% monounsaturated fats, and 12% polyunsaturated fats. The amounts of fat in the different diets are given in Table 1. Body weight and food consumption were measured weekly. After 1, 3, 8, 12, and 16 weeks on the different diets, body fat content was determined by dual x-ray absorptiometry using a PIXImus imager (GE Lunar, Madison, WI). The study was approved by the Animal Ethics Committee, Lund, Sweden.

2.2. Intravenous glucose tolerance test

Intravenous glucose tolerance test (IVGTT) was performed on 4-hour fasted animals after 1, 3, 8, and 16 weeks of dietary treatment. Blood samples (50 μL) were collected from mice anesthetized with 0.5 mg fluanisone per mouse, 0.02 mg fentanyl per mouse (Hypnorm; Janssen, Beerse, Belgium), and 0.25 mg midazolam per mouse (Dormicum; Hoffman-LaRoche, Basel, Switzerland) from the retrobulbar, intraorbital, capillary plexus before D-glucose administration (1 g/kg, volume load 10 μL/g). Additional blood samples were drawn after 1, 5, 10, 20, 50, and 75 minutes from each mouse. Plasma was separated and stored at −20°C until analyzed for insulin and glucose (10 and 5 μL, respectively).

Table 1
Diet compositions

	ND	MFD	HFD
Protein (%)	16	16	16
Carbohydrate (%)	73	54	26
Fat (%)	11	30	58
SAFA (%)	4.4	12	23.2
MUFA (%)	5.3	14.4	27.8
PUFA (%)	1.3	3.6	7
kJ/g	17.05	19.15	23.30
Ingredients (g/kg)			
Casein, 80 Mesh	166.8	187.4	228
DL-Methionine	1.5	1.6	2
Maltodextrin 10	124.4	139.7	170
Sucrose	128.0	143.8	175
Cornstarch	482.9	320.5	0
Lard	47.6	152.1	358.5
Mineral mix S10001	29.3	32.9	40
Sodium bicarbonate	7.7	8.6	10.5
Potassium citrate × 1 H_2O	2.9	3.3	4
Vitamin mix V10001	7.3	8.2	10
Choline bitartrate	1.5	1.6	2

All 3 diets were purchased from Research Diets. The fat in the diets were from lard and it contains saturated (SAFA), monounsaturated (MUFA), and polyunsaturated fats (PUFA).

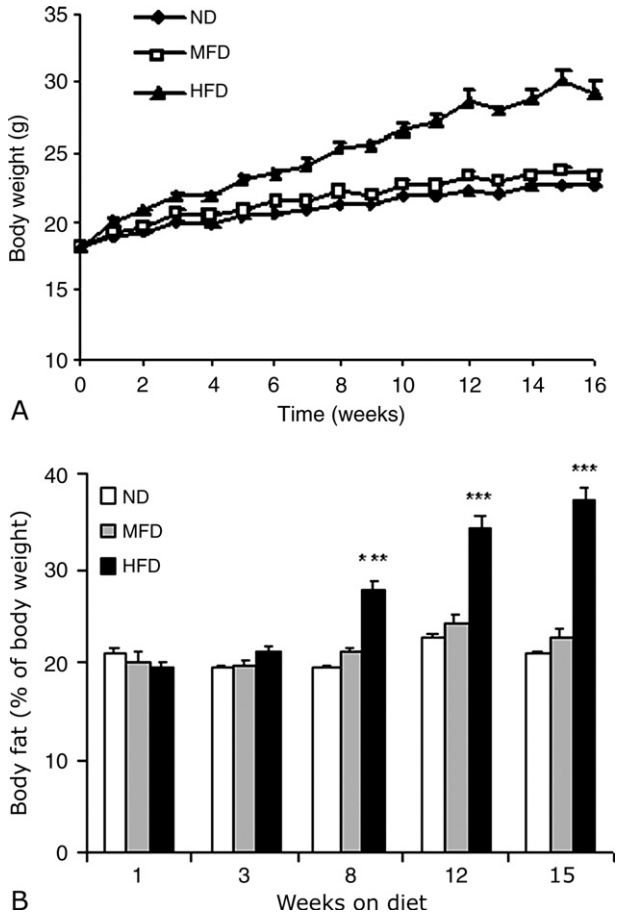

Fig. 1. Body weight and body fat content in female C57BL/6J mice. A, Weight curves from animals on ND (11% fat, n = 64 at start and n = 40 at 16 weeks), MFD (30% fat, n = 64 at start and n = 37 at 16 weeks), or HFD (58% fat, n = 64 at start and n = 36 at 16 weeks) during 16 weeks of dietary treatment. B, Body fat content was measured using dual x-ray absorptiometry after 1, 3, 8, 12, and 16 weeks (n = 21 in each diet group). Means ± SEM are shown. ***$P < .001$.

2.3. Insulin clearance test

Mice fed with the ND or HFD were given a human insulin analogue (Actrapid, Novo Nordisk, Bagvaerd, Denmark) intravenously together with D-glucose to estimate the insulin clearance rate, and the elimination of insulin from the circulation was measured. The mice had been fed with the different diets for 8 weeks before the insulin clearance test. A basal blood sample was drawn immediately before intravenous injection of insulin (0.1 U/kg Actrapid) and glucose (1 g/kg, volume load 10 μL/g) as described above for IVGTT. Additional blood samples were drawn 1, 3, 5, 10, 20, and 50 minutes after the injection. Plasma was separated and stored at −20°C until analysis of insulin and glucose (10 and 5 μL, respectively).

2.4. Assays

Plasma insulin was analyzed radioimmunochemically using a guinea pig antirat insulin antibody, iodine 125 (^{125}I)–labeled human insulin as tracer, and rat insulin as standard (Linco Research, St Charles, MO). The clearance of injected human insulin from plasma was measured using a guinea pig antihuman insulin antibody, ^{125}I-labeled human insulin as tracer, and human insulin as standard (Linco). Glucose was measured with the glucose oxidase method using 2,2′ -azino-bis(3-ethyl-benzothialozine-6-sulfonate) as substrate, and the absorbance was measured at 420 nm on a microtiter plate reader (Fluostar/Polarstar Galaxy, BMG Labtechnologies, Offenburg, Germany).

2.5. Liver triglyceride content

Liver biopsies (100 mg) were homogenized in ice-cold 20 mmol/L Tris-HCl, 150 mmol/L NaCl, 2 mmol/L EDTA, and 1% Triton X-100, pH 7.5. Triglycerides were extracted from the tissue homogenates with chloroform/methanol (2:1). The amount of extracted triglycerides was measured using a commercially available kit (Infinity Triglycerides Liquid Stable Reagent, Thermo Electron, Melbourne, Australia), using triolein (Sigma) as standard. The triglyceride content was correlated to the total protein content in the liver homogenates, determined with the BCA Protein Assay kit (Pierce, Rockford, IL).

2.6. Data analysis and statistics

The insulin response to intravenous glucose was calculated as the mean of suprabasal 1- and 5-minute values (acute insulin response [AIR]). Glucose elimination was quantified as the glucose elimination constant (K_G), that is, the percentage of reduction in circulating glucose between 5 and 20 minutes after intravenous glucose, after logarithmic transformation of the individual glucose values. In the

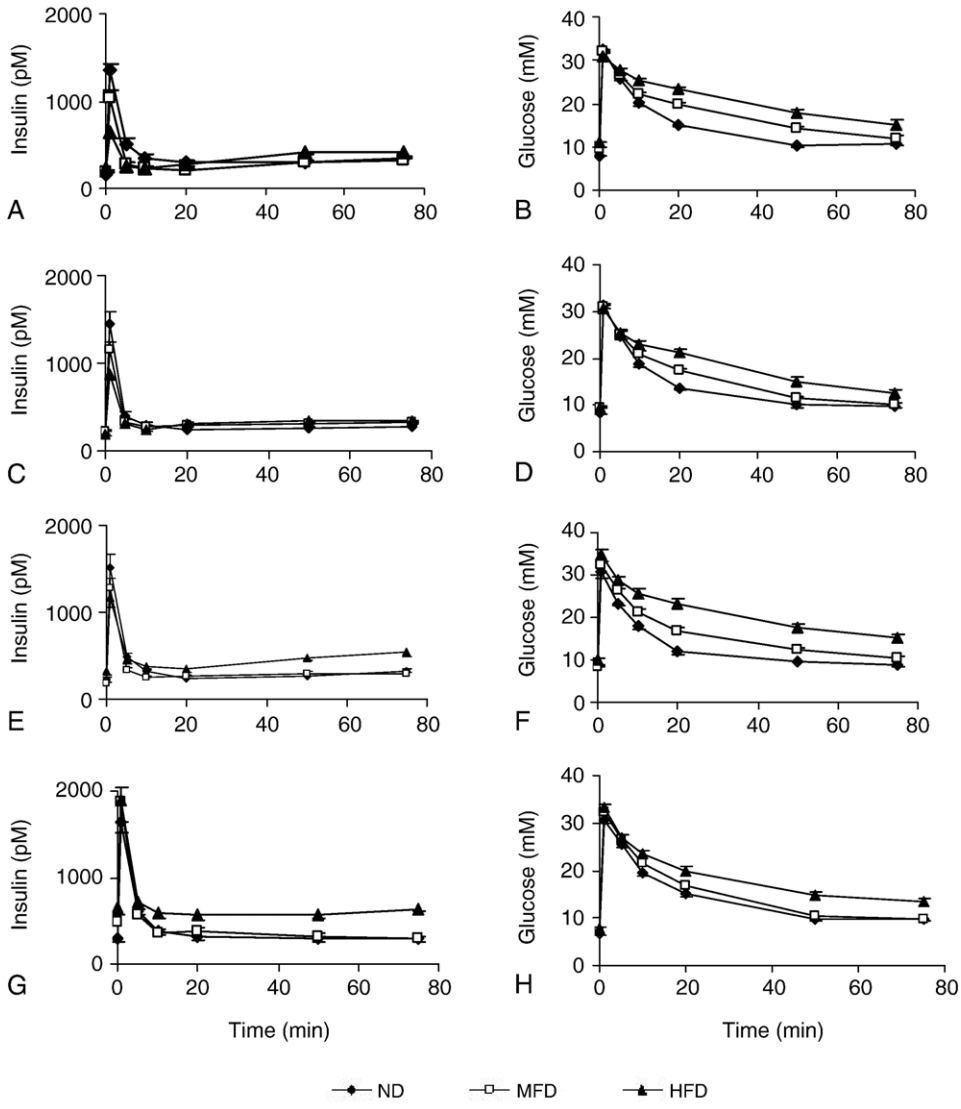

Fig. 2. Plasma levels of glucose and insulin in female C57BL/6J mice during IVGTT (1 g/kg glucose) performed after 1 (A, B), 3 (C, D), 8 (E, F), or 16 (G, H) weeks on ND (11% fat), MFD (30% fat), or HFD (58% fat). Data are presented as means ± SEM.

Table 2
Glucose elimination constant (K_G) and the AIR after intravenous glucose administration (1 g/kg) in anesthetized female C57BL/6J mice fed with the ND (11% fat), MFD (30% fat), or HFD (58% fat) for 1, 3, 8, and 16 weeks

	Diet	1 wk	3 wk	8 wk	16 wk
$K_{G(5\text{-}20\ min)}$ (%/min)	ND	3.6 ± 0.2 (32)	4.0 ± 0.3 (29)	4.7 ± 0.4 (28)	3.6 ± 0.4 (15)
	MFD	2.0 ± 0.1** (28)	2.5 ± 0.2** (32)	3.0 ± 0.2** (29)	3.2 ± 0.2 (17)
	HFD	1.2 ± 0.2** (20)	1.3 ± 0.2** (27)	1.5 ± 0.2** (25)	2.1 ± 0.2* (20)
AIR (pmol/L)	ND	713 ± 46 (32)	736 ± 66 (29)	776 ± 93 (28)	828 ± 84 (14)
	MFD	481 ± 33** (32)	520 ± 41* (32)	629 ± 51 (29)	751 ± 106 (17)
	HFD	223 ± 31** (32)	396 ± 40** (27)	495 ± 76 (25)	636 ± 142 (20)

The values are means ± SEM of data obtained from 3 different experiments. The number in parentheses indicates the number of individuals in each group.
* $P < .01$ vs ND group.
** $P < .001$ vs ND group.

insulin clearance test, the 1-minute insulin peak value was set to 100% in each mouse and the elimination of insulin was calculated as the percentage of the peak value. The area under the curve (AUC_{ins}) was calculated by the trapezoid rule. Statistical comparisons were performed using 1-way analysis of variance with Bonferroni correction for mass significance as post hoc test. Statistical significance was considered when $P < .05$.

3. Results

3.1. Body weight, body fat content, energy intake, and metabolic efficiency

Body weight gain during the study period was more pronounced in mice fed with HFD compared with mice fed with MFD ($P < .001$) or ND ($P < .001$), whereas no significant difference in body weight was observed between the groups fed with ND and MFD (Fig. 1A). Similarly, body fat content was significantly increased in HFD-fed mice compared with those fed with ND and MFD ($P < .001$), whereas there was no significant difference in body fat content between ND- and MFD-fed mice during the 16-week study (Fig. 1B). Energy intake was significantly increased in mice fed with diets containing increasing amounts of fat (41.1 ± 0.5 kJ [ND], 44.1 ± 0.2 kJ [MFD; $P < .001$], and 49.9 ± 0.5 kJ [HFD; $P < .001$] per day and per mouse). The metabolic efficiency (consumed energy per gained weight) was significantly lower in mice fed with the HFD (441 ± 33 kJ/g) compared with mice fed with the ND (1032 ± 95 kJ/g, $P < .001$) and MFD (880 ± 96 kJ/g, $P < .001$).

3.2. Glucose and insulin responses to intravenous glucose

After 1 week, the insulin response to glucose was reduced in the groups fed with MFD or HFD compared with those fed with ND (Fig. 2A, Table 2). This was accompanied by impaired glucose elimination (Fig. 2B, Table 2). During the subsequent study period, the insulin response to glucose was compensatory increased in both MFD and HFD (Fig. 2C, E, and G); after 8 weeks, the insulin response to glucose in MFD was not significantly different from that in ND, whereas in HFD AIR was still ~40% of the AIR in the controls (Table 2). Concomitantly, glucose elimination was gradually improved in MFD, still being significantly lower than in the ND group after 8 weeks ($P < .001$), but restored and not different from ND (Fig. 2H, Table 2). In contrast, in HFD-fed mice, glucose elimination was not improved after 8 weeks, compared with after 1 week, in spite of the marked increase in AIR, and after 16 weeks, glucose elimination was improved, but still significantly lower than in ND- and MFD-fed mice ($P = .006$).

The time course of the effects of the different diets on glucose tolerance and insulin secretion is illustrated in Fig. 3. The dotted line shows the immediate effects seen after 1 week in MFD and HFD, respectively. In both these diets, AIR was markedly suppressed after 1 week along with reduced K_G, and during subsequent weeks, the adaptive compensation was evident in both MFD and HFD.

3.3. Insulin clearance

To explore whether the altered insulin response in the high-fat diets would be explained by altered insulin clearance, we measured the disappearance of intravenously injected human insulin in plasma from mice fed with the ND or the HFD for 8 weeks. After 3 minutes, 75% of the

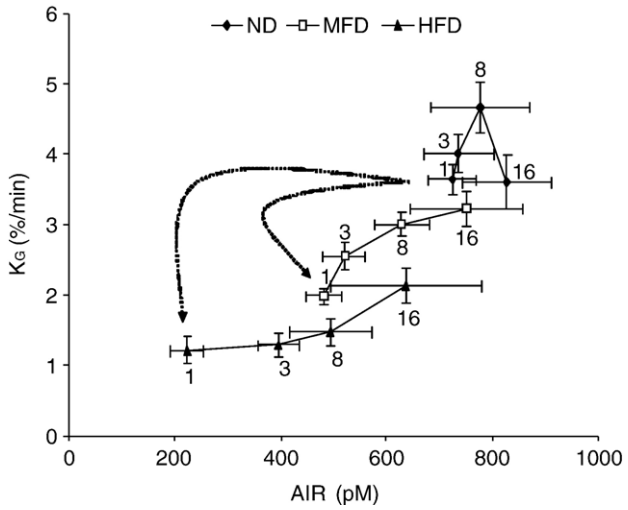

Fig. 3. Glucose elimination [$K_{G(5\text{-}20\ min)}$] as a function of the AIR (suprabasal 1-5 minutes) after intravenous administration of glucose (1 g/kg) in anesthetized female C57/Bl/6J mice fed with the ND (11% fat), MFD (30% fat), or HFD (58% fat) for 1, 3, 8, and 16 weeks (indicated as 1, 3, 8, and 16 in the figure). Data as means ± SEM are shown.

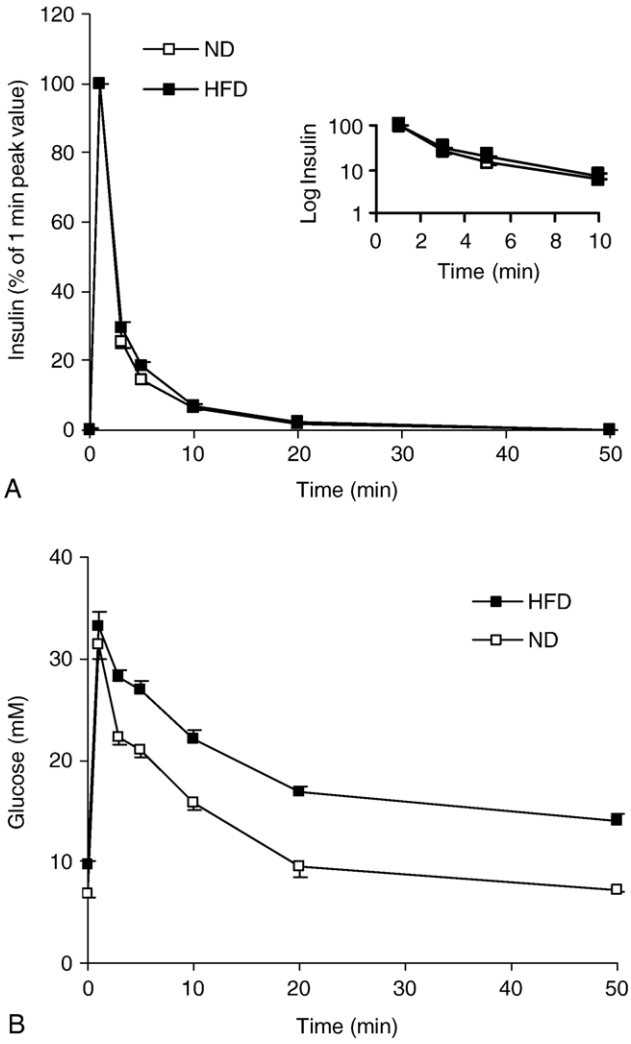

Fig. 4. Clearance of intravenously injected insulin from the circulation in mice fed with ND or HFD. A, Insulin levels after intravenous injection of 1 g/kg glucose together with 0.1 U/kg insulin (Actrapid) in anesthetized mice. The inset figure represents the logarithmic transformation of the insulin values between 1 and 10 minutes after injection of glucose and insulin. B, Plasma glucose levels after the injection of glucose and insulin.

injected insulin was cleared from the circulation and after 20 minutes only 2% remained in both ND- and HFD-fed mice (Fig. 4A). There was no statistical difference in $AUC_{0\text{-}20\ min}$ between the dietary groups (310% ± 11% × minute in ND vs 335% ± 14% × minute in HFD), indicating a similar insulin clearance rate between the 2 dietary groups. Despite injection of insulin, the glucose elimination was severely impaired in the HFD-fed group [$K_{G(1\text{-}20\ min)}$; 6.5% ± 0.4% per minute in ND vs 3.6% ± 0.2% per minute in HFD, $P < .001$], illustrating severe insulin resistance obtained after HF feeding for 8 weeks.

3.4. Liver triglyceride content

Liver triglyceride content was measured in mice fed with ND or HFD for 12 weeks. There was no significant difference in triglyceride content between the 2 dietary groups (128 ± 18 μg/mg protein in ND vs 141 ± 13 μg/mg protein in HFD, n = 20 in each group).

4. Discussion

By feeding mice diets with 30% or 58% fat on energy basis, marked impairment of the insulin response to glucose developed already after 1 week compared with mice fed a normal 11% fat-containing diet. The impairment was dose-dependent, because compared with the ND group, AIR was reduced by ~30% in the MFD group, whereas the corresponding reduction in the HFD group was ~70%. This rapid impairment is in agreement with previous studies in HFD-fed mice [14-17]. After the initial failure, there was a compensatory increase in the insulin response. In MFD, the insulin response to glucose was normalized over the 16-week study period. In addition, in the HFD group, an improvement in the insulin response to glucose was observed over the 16-week study period, although still a reduction of ~25% was observed at that time point in spite of an approximately 3-fold increase in insulin response over the period. Glucose tolerance also improved and was normalized after the 16-week study period in the group fed with MFD. In contrast, in the HFD group, there was a slight improvement in glucose tolerance after 16 weeks on HFD, but it was significantly lower than in MFD- and ND-fed mice. These data thus show the temporal development of islet compensation in mice fed with HFD over the 16-week study period, which compensates the reduction in insulin sensitivity in MFD-fed mice, but does not compensate the insulin resistance in HFD-fed mice. This in turn emphasizes and underlines that the MFD-fed mouse is a novel nonobese model, illustrated by normal body weight and body fat content, developing mild glucose intolerance and a preserved adaptive islet response.

The dietary fat used in this study was from lard, and several differences exist between the diets (see Table 1). The present study cannot distinguish the factors that are of importance for the observed differences between the diet, although the large difference in fat content is the most likely explanation. The diets consist of both saturated fat (40%) and unsaturated fat (60%), giving 12% of total energy intake from saturated fats in the MFD and 23.2% in the HFD. Of importance is that the MFD represents a diet with similar composition of fat compared with a ND in humans, which, again, underlies the potential of this diet for further studies. In contrast, the HFD contains a high level of saturated fat, which is not commonly seen in humans. Saturated fats induce insulin resistance, whereas polyunsaturated fats improve the insulin sensitivity [18,19] and, similarly, saturated fat is a risk factor for type 2 diabetes mellitus and a protective role of polyunsaturated fat has been established also in humans [20]. This difference may be explained by altered cell membrane structure, particularly in skeletal muscle cells, resulting in insulin resistance [21,22], although this process may take several months [23]. High

dietary fat intake may also affect glucose metabolism through changes in plasma free fatty acids because long-term exposure to fatty acids results in impaired glucose-stimulated insulin secretion (GSIS), whereas short-term exposure stimulates GSIS [12]. In fact, the potentiating effect of fatty acids on GSIS has been shown to be crucial for maintaining normoglycemia in the face of insulin resistance [12]. In our study, 1 week on MFD or HFD resulted in impaired insulin response to glucose, and, although insulin secretion was not measured directly, it is possible that after this short period of elevated dietary fatty acids, GSIS is impaired due to a blunted response to the circulating lipids. Over time, the islet sensitivity to fatty acids improved, possibly through adaptively altered fatty acid metabolism [24]. This suggests that increasing the amount of dietary fat dose-dependently compromises the beta cells as a sign of lipotoxicity [25,26]. A recent study, which showed improved glucose intolerance in HFD-fed mice by the fat-reducing compound acipimox [27], supports that it is the high lipidemia after HFD that contributes to the impaired insulin response.

The liver plays an important role in regulating whole-body glucose production and hepatic insulin resistance results in excess glucose production contributing to hyperglycemia in type 2 diabetes mellitus [28,29]. The liver also plays an important role in regulating the clearance of insulin from the circulation [30,31]; liver steatosis compromises the clearance of insulin [32]. Fat-rich food has been suggested to cause peripheral insulin resistance through accumulation of triglycerides in non-adipocytes [33], and accumulation of liver triglycerides has been observed in type 2 diabetic patients [34,35]. Hyperinsulinemia in HFD-fed mice may therefore be due to compromised hepatic insulin clearance due to liver steatosis rather than due to augmented insulin secretion as compensation to insulin resistance. In this study, we therefore estimated insulin clearance by a bolus injection of human insulin analogue in ND- and HFD-fed mice. Although the conclusion of this result is limited by the use of human rather than murine insulin, we found no significant effect on the clearance of insulin from the circulation in HFD-fed mice compared with ND-fed mice, suggesting that the HFD-induced hyperinsulinemia is not caused by impaired insulin clearance. This was further supported by the finding that there was no significant accumulation of triglycerides in the liver after 12 weeks on the diets, indicating maintained liver lipid metabolism also in HFD-fed mice.

This study thus present findings that by modulating the fat content of the diet, temporal and dose-related standardized differential glucose intolerance is seen with a dynamic islet response, which is dependent on both time and dietary fat content. The study also shows that a moderate increase in dietary fat (30%, MFD) results in a nonobese model of glucose intolerance with temporal islet compensation, in contradiction to the extreme HFD (58%), which initiates a more severe compromise of the islet compensation to insulin resistance.

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