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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 (1g/kg) in relation to glucose tolerance in female mice after 1, 3, 8 or 16 weeks of feeding diets containing 11% fat (normal diet-ND), 30% fat (medium-fat diet-MFD) or 58% fat (high-fat diet-HFD; by energy). HFD increased body weight and body fat content, whereas MFD did not. The insulin response (postglucose suprabasal mean 1 and 5 min insulin), was impaired after one week on MFD (481± 33 pM) or HFD (223± 31 pM) compared to the ND (713±46 pM; both $P<0.001$). This was accompanied by impaired glucose elimination compared to ND (both $P<0.001$). Over the 16-week study period, the insulin response adaptively increased in the groups fed 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, suggested 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 high-fat diet in mice, and that MFD-fed mice is a novel non-obese model of glucose intolerance.

Key words: Insulin secretion, dietary fat, beta cell dysfunction, obesity

Glucose intolerance and type 2 diabetes 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, 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 following 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 sixteen-week period with evaluation of the glucose and insulin responses to intravenous glucose after 1, 3, 8 and 16 weeks.

MATERIAL AND METHODS

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-h light-dark cycle, with food and water *ad libitum*. Upon arrival all animals were fed the normal diet (ND), and after one week of acclimatization, the mice were divided into three groups and fed 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 derive 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 (DEXA) using a PIXImus imager (GE Lunar, Madison, WI). The study was approved by the Animal Ethics Committee, Lund, Sweden.

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/mouse fluanison, 0.02 mg/mouse fentanyl (Hypnorm®; Janssen, Beerse, Belgium), and 0.25 mg/mouse midazolam (Dormicum®; Hoffman-LaRoche, Basel, Switzerland) from the retrobulbar, intraorbital, capillary plexus prior to $_{D}$-glucose administration (1 g/kg, volume load 10µl/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 (10µl and 5µl, respectively).

Insulin clearance test

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

Assays

Plasma insulin was analyzed radioimmunochemically using a guinea pig anti-rat insulin antibody, ^{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 anti-human 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) (ABTS) as substrate and the absorbance was measured at 420 nm on a microtiter plate reader (Fluostar/Polarstar Galaxy, BMG Labtechnologies, Offenburg, Germany).

Liver triglyceride content

Liver biopsies (100 mg) were homogenized in ice-cold 20 mM Tris-HCl, 150 mM NaCl, 2 mM 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).

Data analysis and statistics

The insulin response to intravenous glucose was calculated as the mean of suprabasal 1- and 5-min values (AIR = acute insulin response). Glucose elimination was quantified as the glucose elimination constant (K_G); the percentage reduction in circulating glucose between 5 and 20 minutes after intravenous glucose, after logarithmic transformation of the individual glucose values. In the insulin clearance test, the 1-min insulin peak value was set to 100% in each mouse and the elimination of insulin was calculated as % of the peak value. The area under the curve (AUC_{ins}) was calculated by the trapezoid rule. Statistical comparisons were performed using one-way analysis of variance (ANOVA) with Bonferroni correction for mass significance as post hoc test. Statistical significance was considered when $P<0.05$.

RESULTS

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

Body weight gain during the study period was more pronounced in mice fed HFD compared to mice fed MFD ($P<0.001$) or ND ($P<0.001$), whereas no significant difference in body weight was observed between the groups fed ND an MFD (Fig. 1A). Similarly, body fat content was significantly increased in HFD fed mice compared to ND and MFD ($P<0.001$), 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 diets containing increasing amounts of fat (41.1±0.5 (ND), 44.1±0.2 (MFD; $P<0.001$) and 49.9±0.5 (HFD; $P<0.001$) kJ/day and mouse). The metabolic efficiency (consumed energy per gained weight) was significantly lower in mice fed the HFD (441±33 kJ/g) compared to mice fed ND (1032±95 kJ/g, $P<0.001$) and MFD (880±96 kJ/g, $P<0.001$).

Glucose and insulin responses to intravenous glucose

After one week, the insulin response to glucose was reduced in the groups fed MFD or HFD diet compared to 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 ($P<0.001$), 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 to 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=0.006).

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 acute 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.

Insulin clearance

To explore whether the altered insulin response in the high fat diets would be explained by altered insulin clearance, the disappearance of intravenously injected human insulin was measured in plasma from mice fed the ND or the HFD for 8 weeks. After three minutes, 75% of the injected insulin was cleared from the circulation and after 20 min only 2% remained in both ND and in HFD fed mice (Fig 4A). There was no statistical difference in $AUC_{0\text{-}20\ min}$ between the dietary groups (310±11 in ND vs. 335±14 %*20min in HFD), indicating a similar insulin clearance rate between the two dietary groups. Despite injection of insulin, the glucose elimination was severely impaired in the HFD fed group ($K_{G(1\text{-}20min)}$; 6.5±0.4 in ND vs. 3.6±0.2 %/min in HFD, P<0.001), illustrating severe insulin resistance obtained after HF-feeding for 8 weeks.

Liver triglyceride content

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

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 to mice fed a normal 11% fat-containing diet. The impairment was dose-dependent, since compared to 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. Also 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 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 high fat diet 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 non-obese 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 derives from lard and several differences exist between the diets (see Table 1). The present study can not distinguish which factors 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 to a normal diet 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, while polyunsaturated fats improve the insulin sensitivity [18, 19] and, similarly, saturated fat as a risk factor for type 2 diabetes and a protective role of polyunsaturated fat have 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 chronic exposure to fatty acids results in impaired glucose-stimulated insulin secretion (GSIS), whereas acute 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, one 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 [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 is has been observed in type 2 diabetic patient [34,35]. Hyperinsulinemia in HFD-fed mice may therefore be due to compromised hepatic insulin clearance due to liver steatosis rather than 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 to 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 non-obese 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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Legends

Fig. 1. Body weight and body fat content in female C57BL/6J mice. A) Weight curves from animals on normal diet (ND; 11% fat, n=64 at start and 40 at 16 weeks), medium-fat diet (MFD; 30% fat, n=64 at start and 37 at 16 weeks), or high-fat diet (HFD; 58% fat, n=64 at start and 36 at 16 weeks) during 16 weeks of dietary treatment. B) Body fat content was measured using DEXA after 1, 3, 8, 12 and 16 weeks (n=21 in each diet group). Means±SEM are shown. ***P<0.001

Fig. 2. Plasma levels of glucose and insulin and 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 normal diet (ND; 11% fat), medium-fat diet (MFD; 30% fat), or high-fat diet (HFD; 58% fat). Data are presented as means±SEM

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

Fig. 4 Clearance of intravenously injected insulin from the circulation in mice fed normal diet (ND) or high-fat diet (HFD). A) Insulin levels after intravenous injection of 1g/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 min after injection of glucose and insulin. B) Plasma glucose levels after the injection of glucose and insulin.

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
Corn Starch	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 x $1H_2O$	2.9	3.3	4
Vitamin Mix V10001	7.3	8.2	10
Choline Bitartrate	1.5	1.6	2

All three diets were purchased from Research Diets Inc, New Brunswick, NJ. The fat in the diets derive from lard and it contains saturated (SAFA), monounsaturated (MUFA) and polyunsaturated fats (PUFA).

Table 2 Glucose elimination constant (K_G) and the acute insulin response (AIR) following iv glucose administration (1g/kg) in anesthetized female C57BL/6J mice fed the normal diet (ND; 11% fat), medium-fat diet (MFD; 30% fat), or high-fat diet (HFD; 58% fat) for 1, 3, 8 and 16 weeks.

	Diet	**1 week**	**3 weeks**	**8 weeks**	**16 weeks**
$K_{G(\ 5\text{-}20\ min)}$	ND	3.6 ±0.2 (32)	4.0 ±0.3 (29)	4.7 ±0.4 (28)	3.6 ±0.4 (15)
(%/min)	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	ND	713 ±46 (32)	736 ± 66 (29)	776 ± 93 (28)	828 ± 84 (14)
(pmol/l)	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 three different experiments. The number in brackets indicates the number of individuals in each group. ** P<0.01, *** P<0.001 versus ND group.

Figure 1

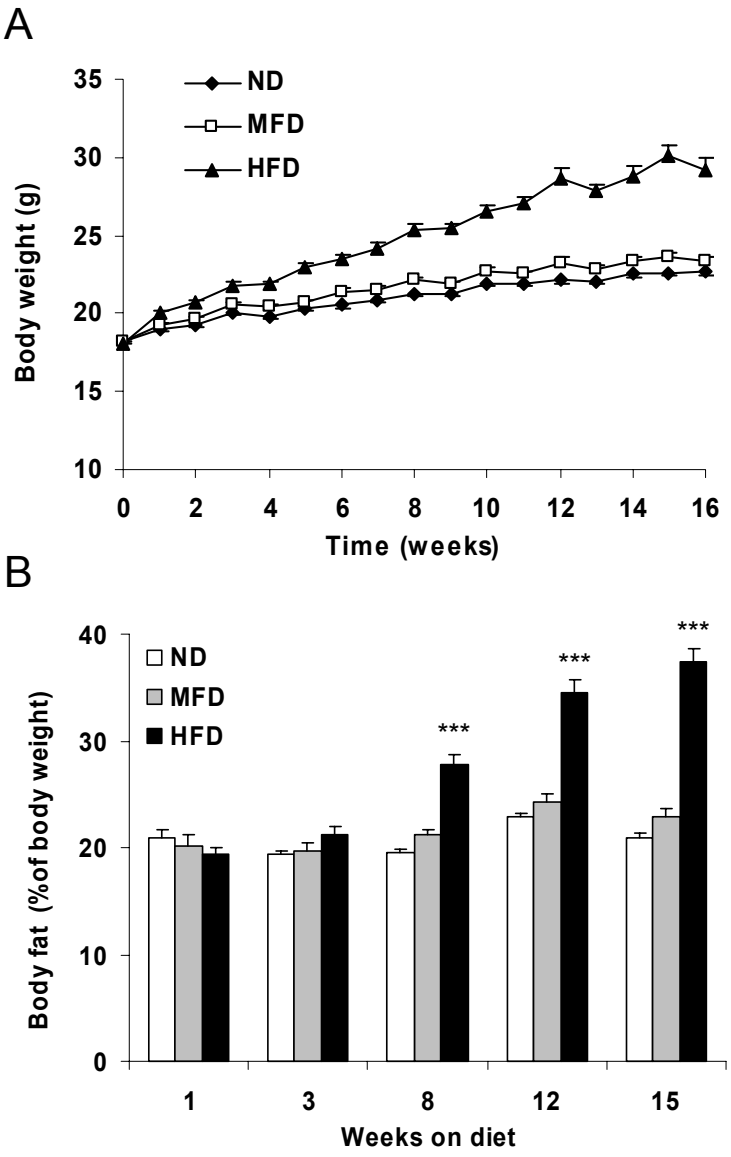
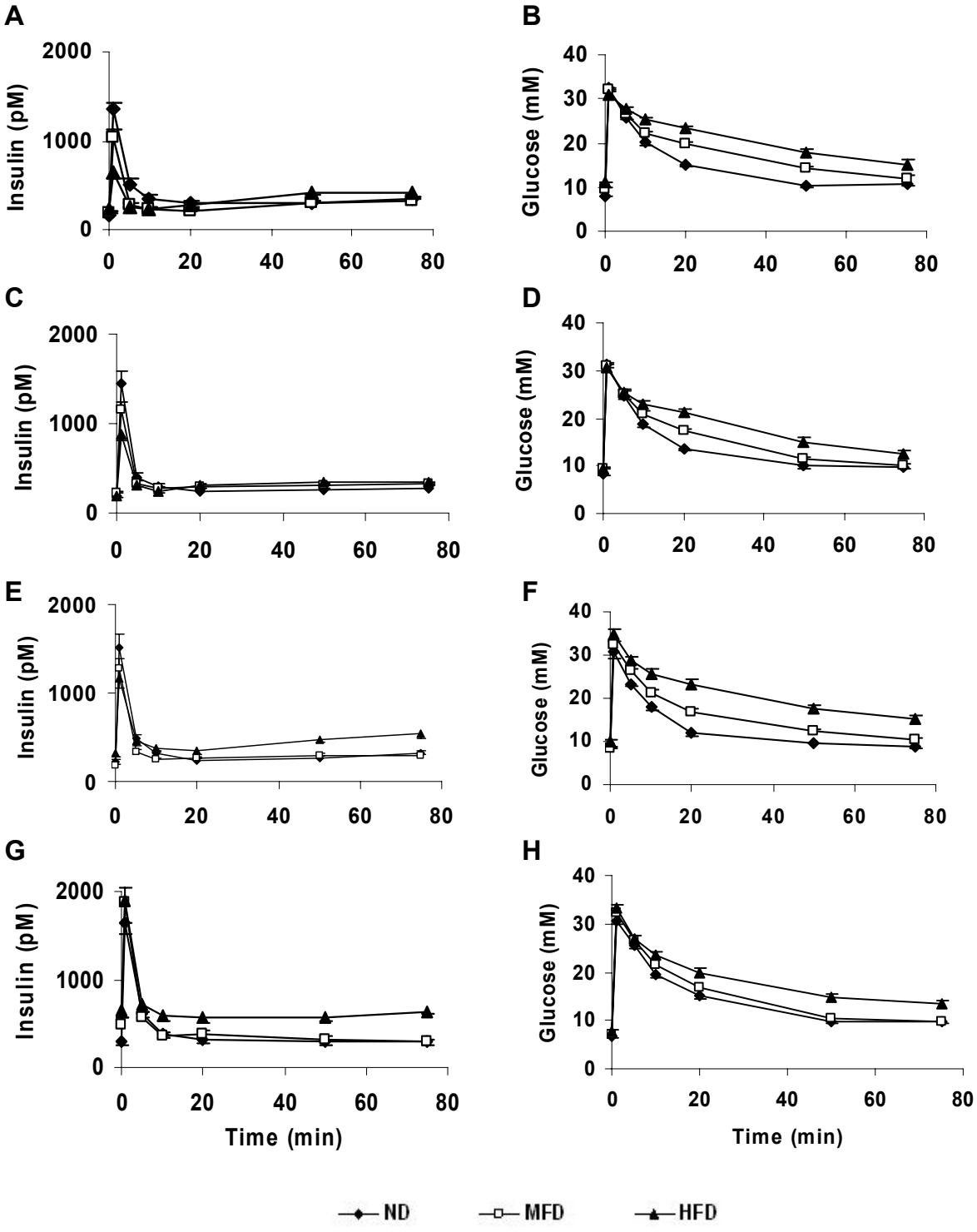

A
ND
MFD
HFD
Body weight (g)
35
30
25
20
15
10
0
2
4
6
8
10
12
14
16
Time (weeks)
B
ND
MFD
HFD
Body fat (%of body weight)
40
30
20
10
0
***
***
***
1
3
8
12
15
Weeks on diet

Figure 2


A
B
C
D
E
F
G
H
Insulin (pM)
Glucose (mM)
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1000
0
40
30
20
10
0
0
20
40
60
80
Time (min)
ND
MFD
HFD

Figure 3

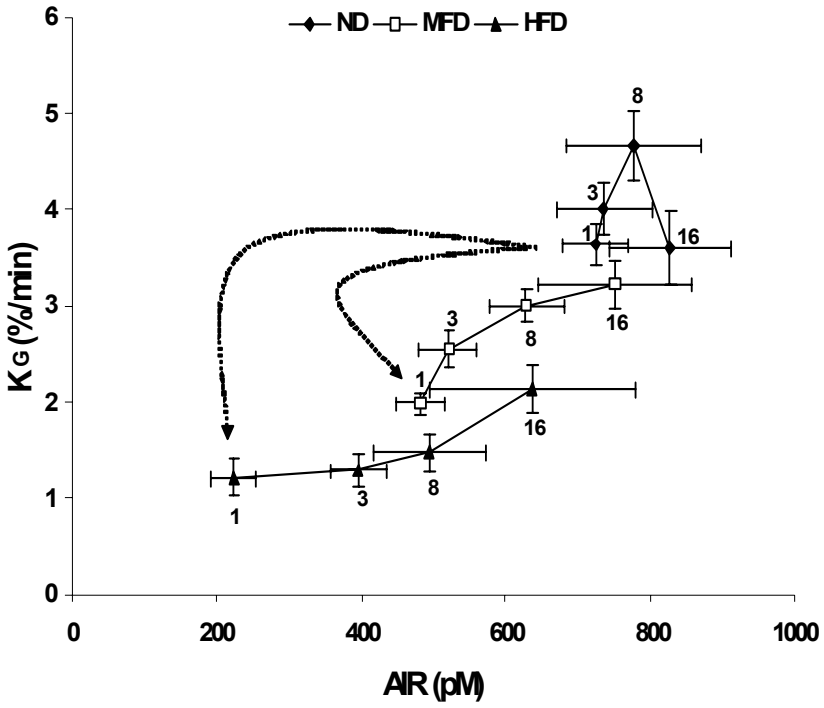
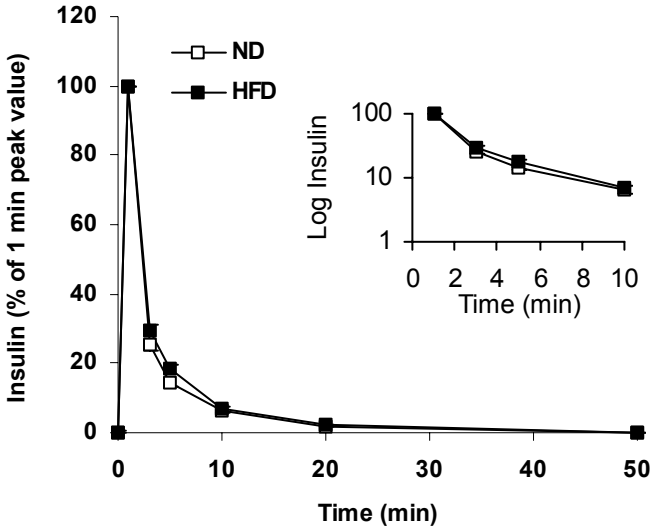
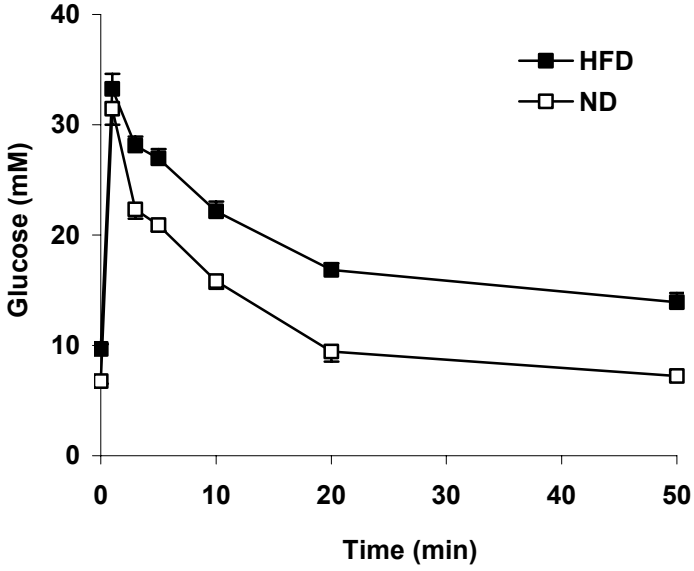


Figure 4

A


120
100
80
60
40
20
0
Insulin (% of 1 min peak value)
ND
HFD
0
10
20
30
40
50
Time (min)
Log Insulin
100
10
1
0
2
4
6
8
10
Time (min)

B


40
30
20
10
0
Glucose (mM)
HFD
ND
0
10
20
30
40
50
Time (min)