Early changes in insulin secretion and action induced by high-fat diet are related to a decreased sympathetic tone


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Cruciani-Guglielmacci, C., M. Vincent-Lamon, C. Rouch, M. Orosco, A. Ktorza, and C. Magnan. Early changes in insulin secretion and action induced by high-fat diet are related to a decreased sympathetic tone. *Am J Physiol Endocrinol Metab* 288: E148–E154, 2005. First published September 7, 2004; doi:10.1152/ajpendo.00225.2004.—To evaluate the relationship between the development of obesity, nervous system activity, and insulin secretion and action, we tested the effect of a 2-mo high-fat diet in rats (HF rats) on glucose tolerance, glucose-induced insulin secretion (GHS), and glucose turnover rate compared with chow-fed rats (C rats). Moreover, we measured pancreatic and hepatic norepinephrine (NE) turnover, as assessment of sympathetic tone, and performed hypothalamic microdialysis to quantify extracellular NE turnover. Baseline plasma triglyceride, free fatty acid, insulin, and glucose concentrations were similar in both groups. After 2 days of diet, GHS was elevated more in HF than in C rats, whereas plasma glucose time course was similar. There was a significant increase in basal pancreatic NE level of HF rats, and a twofold decrease in the fractional turnover constant was observed, indicating a change in sympathetic tone. In ventromedial hypothalamus of HF rats, the decrease in NE extracellular concentration after a glucose challenge was lower compared with C rats, suggesting changes in overall activity. After 7 days, insulin hypersecretion persisted, and glucose intolerance appeared. Later (2 mo), there was no longer insulin hypersecretion, whereas glucose intolerance worsened. At all times, HF rats also displayed hepatic insulin resistance. On day 2 of HF diet, GHS returned to normal after treatment with oxymetazoline, an α2-adrenoceptor agonist, thus suggesting the involvement of a low sympathetic tone in insulin hypersecretion in response to glucose in HF rats. In conclusion, the HF diet rapidly results in an increased GHS, at least in part related to a decreased sympathetic tone, which can be the first step of a cascade of events leading to impaired glucose homeostasis.

Overweight and obesity are known to be among the main environmental factors responsible for the metabolic syndrome that could lead to type 2 diabetes in predisposed subjects (7, 33, 34). Among early events characterizing premetabolic syndrome states, glucose-induced insulin oversecretion associated with insulin resistance is frequently observed (15, 33). This period of insulin hypersecretion may be followed by a further deterioration of insulin secretion in response to glucose (a pancreatic failure and the setting of type 2 diabetes; see Refs. 14 and 15). These abnormalities could be related to impaired free fatty acid (FFA) metabolism (33), which is known to contribute to the deterioration of both insulin secretion (47) and action (13, 46).

From studies performed on different animal models, it appeared that high-fat (HF) diet-related overweight induces an increase in plasma triglyceride (TG) concentration (6, 10, 11, 30) and/or TG ectopic storage (5, 10), a situation that may produce lipotoxicity at both pancreatic (3) and peripheral levels (10). On the other hand, it has been proposed that diet-induced dysfunction of the autonomic nervous system could also be an important component of premetabolic syndrome states (re-viewed in Refs. 23 and 24). For example, Levin (28) showed that there was a reduced norepinephrine (NE) turnover in organs, including the brain, in obesity-prone rats before any increase in body weight. Furthermore, the high prevalence of obesity and type 2 diabetes in the Pima Indian community might be the consequence of a “thrifty genotype,” including a low sympathetic activity (40).

In the present study, normal Wistar rats were submitted to a HF diet to study its short- and long-term effects on insulin secretion and action. We found that HF rats rapidly displayed insulin hypersecretion in response to glucose and hepatic insulin resistance (after 2 days of diet), followed by glucose intolerance (7 days) and the loss of pancreatic hypersecretion of insulin (2 mo). Furthermore, we aimed to test whether the HF diet could induce alterations in central nervous system (CNS) activity, focusing only on the very beginning of the diet (2 days) when the alteration of insulin secretion and action appeared. Indeed, dyslipidemia is often associated with autonomic nervous system dysfunction (29, 47), and it has been previously shown that a decreased sympathetic tone could be partially responsible in insulin hypersecretion in response to glucose (31).

Materials and Methods

Animals. The experimental protocol was approved by the institutional animal care and use committee of University Paris 7. Male Wistar rats were randomized at 6 wk of age into the following two groups: one group received standard laboratory chow (113; UAR, Epinay sur Orge, France) ad libitum and the other a lard-based HF diet (231H, UAR) ad libitum (HF rats). The HF diet contained 40% fat (32.5% as lard and 7.5% as maize oil) vs. 4.5% for the chow diet, and the total energetic values were, respectively, 5,100 and 3,000 kcal/kg. Rats were housed individually in stainless steel cages in a room maintained at 24 ± 3°C with lights on from 7:00 AM to 7:00 PM; they had free access to water.

Food intake. Daily food intake was measured by weighing the pellets and the HF diet between 9:00 and 10:00 AM.

Glucose-induced insulin secretion. Insulin secretion in response to glucose was investigated every day after the beginning of the diet.
Briefly, a single dose of glucose was injected intraperitoneally (1 g/kg body wt) in rats deprived of food for 4 h. Blood samples were drawn from caudal vessels at time 0, 5, 10, 15, 20, 30, and 60 min after glucose injection. Glycemia was immediately measured using a glucose analyzer (Roche Diagnostics, Meylan, France). Plasma was then removed and stored at −20°C until RIA of insulin.

To appreciate the possible involvement of changes in sympathetic activity in the control of insulin secretion after 2 days of diet, glucose-induced insulin secretion (GHS) was also studied in the presence of an α2A-adrenergoreceptor agonist, oxymetazoline (Sigma Chemical, St. Louis, MO). The insulin response was tested in the presence of 0.1, 1, 10, and 1,000 pmol oxymetazoline/kg body wt, injected intraperitoneally 5 min before glucose loading. We measured the insulinogenic index from time 0 (glucose load) to time 60 min (end of the test). The time points were 0, 5, 10, 15, 20, 30, and 60 min. Each concentration corresponded to a different group of rats.

Glucose turnover rate. Measurements were performed in the basal state and during euglycemic-hyperinsulinemic clamps. Rats deprived of food for 4 h were anesthetized with pentobarbital sodium (50 mg/kg ip). A catheter was inserted in the right jugular vein for blood sampling. Infusions (insulin, labeled and unlabeled glucose) were carried out using butterfly needles that were inserted in the saphenous vein.

A priming dose of [3-3H]glucose (5 μCi) was injected through the saphenous vein at −50 min, followed by a continuous infusion at a rate of 0.2 μCi/min throughout the study. Blood samples were drawn at −15, −10, and 0 min during the basal period and at time 70, 80, and 90 min during the steady-state euglycemic clamp for glucose disposal rate evaluation.

Euglycemic-hyperinsulinemic clamps. Before insulin infusion, two sets of blood samples were drawn for determinations of basal blood glucose, plasma insulin, and nonesterified fatty acid (NEFA) levels. A priming dose of insulin (20 mU Actrapid; Novo, Copenhagen, Denmark) was dissolved in isotonic saline and injected through a saphenous vein followed by a continuous infusion of insulin (0.4 U·kg⁻¹·h⁻¹) at a constant rate of 20 μl/min.

During clamps, blood was sampled by caudal vessels every 5 min to determine glycemia and to adjust the rate of unlabeled glucose infusion to maintain euglycemia (glycemia between 4.5 and 6 mM). The euglycemic clamp was attained within 30–40 min and maintained for 40 min thereafter. Steady-state specific glucose radioactivity and plasma glucose and insulin concentrations were determined during the last 20 min of the clamp.

Analytic methods. Blood glucose was determined using a glucose analyzer (Roche Diagnostics). For the assay of [3-3H]glucose radioactivity, blood samples were deproteinized with Ba(OH)₂ and ZnSO₄, deproteinized according to the formula: 

\[ \text{Rd} \] 

rate of disappearance, reflecting glucose utilization. \( \text{Rd} \) was calculated according to the formula: 

\[ \text{Rd} = \frac{\text{Ri} - \text{Ro}}{t} \] 

[disintegrations·min⁻¹ (dpm)·min⁻¹] divided by blood glucose specific activity (dpm/mg), during the last 20 min of the glucose clamp (50–70 min after the onset of insulin infusion). Results are given as means ± SE.

Pancreas, liver, and brain NE turnover. We used the synthesis inhibition method by Brodie et al. (8). At 2 h after lights on, rats were injected with α-methyl-DL-p-tyrosine, a tyrosine hydroxylase inhibitor (250 mg/kg; Sigma) that prevents the conversion of tyrosine to dihydroxyphenylalanine (DOPA), the rate-limiting step of catecholamine synthesis. Next, 3 h after this injection, one group of rats (time 3 h) was killed, and pancreas, liver, and whole brain were rapidly removed and frozen in liquid nitrogen. The other group (time 6 h) received an additional 125 mg/kg ip α-methyl-DL-p-tyrosine. These rats were killed 3 h later, and the organs were also removed and frozen. Another group (time 0 h) of rats was not injected with the inhibitor; these rats were killed at the beginning of the experiment to determine the basal level of NE concentration, NE₀, expressed as nanograms per gram of organ. Because the concentration of NE ([NE]) in tissues declined exponentially after blockade of NE synthesis, we could obtain from the slope of the line of log[NE] vs. time, k, the rate constant of NE efflux (expressed in h⁻¹). The product of k and NE₀ will give the rate of NE efflux, which is equal to the rate of NE synthesis and will be called turnover rate (TR, expressed in ng·g⁻¹·h⁻¹, for details, see Ref. 8). Organ NE levels were determined using RPLC with a C18 reverse-phase column (150 × 2 mm Beckman column packed with Ultrasphere ODS C18, 5 μm average particle size) and electrochemical detection (Kontron 402) after alumina extraction. Calibration curves were made with spiked plasma or homogenate. Recoveries were calculated on the basis of peak heights measured by an integrator (Shimazu, Kyoto, Japan).

Liver analysis: glycogen and TG contents. Liver (~0.2 g) was ground in distilled water with Ultraturrax (ice-cold procedure), and then TG were assayed in the homogenate using the commercial kit “GPO triinder” (Sigma Diagnostic). Glycogen was also measured in the homogenate using the method of Roehrig and Alfre (42); it was hydrolyzed by amyloglucosidase treatment without preliminary extraction, and glucose was dosed by a colorimetric method using glucose oxidase.

Microdialysis procedure. A detailed description of surgical and technical procedures has been given previously (43). Briefly, at least 5 days before the experiment, the animal was placed in a stereotaxic frame (Kopf Instruments). A guide cannula was implanted to insert the microdialysis probe with its upper part in the paraventricular nucleus (PVN) of the hypothalamus and the lower part in the ventromedial hypothalamus (VMH). The coordinates of the guide tip were (according to the atlas of Paxinos and Watson; Ref. 37) −1.9 mm posterior to bregma, 0.5 mm lateral, and 7 mm ventral to the dura. The dialysis probe protruded 2 mm beyond the guide tube, and its tip reached a point 9 mm ventral to bregma. The guide cannula and the venous catheter were fixed to the skull with stainless steel screws and dental cement.

On the day of the experiment, a system of tubing connected to a two-channel swivel placed on the beam allowed infusion of fluid in the probe and sampling. The flow rate (2 μl/min) allowed the collection of 30–50 μl samples every 15 min. The dialysis probe was inserted through the guide at 9:00 AM, and the infusion was performed with a Ringer-type solution containing 147 mM Na⁺, 2.3 mM Ca²⁺, 4 mM K⁺, and 155.6 mM Cl⁻. The first microdialysis samples were taken 4 h after insertion of the probe; this delay is necessary to achieve stable catecholamine levels. After four 15-min baseline samples were collected, the rats were subjected to an intraperitoneal glucose injection (1 g/kg body wt). Sampling was carried on every 15 min after the injection during at least 3 h.

Measurement of hypothalamic NE. The dialyses were analyzed by means of reverse-phase liquid chromatography with electrochemical detection (Decade; Antec) at a potential of 750 mV. The chromatographic system consisted of a 20-μl sample loop leading to a 10-cm column (Colochrom) with a 3.2-mm internal diameter and 3 μm C18 packing. The mobile phase consisted of an acetate buffer containing 100 μM EDTA, 1 mM octanesulfonic acid, and 4% vol/vol acetonitrile at pH 3.1. This system allowed detection of NE at a sensitivity of 0.1 nA and separation of it from the other monoaminergic compounds.

The mean NE levels of the four baseline samples (before the glucose injection) were calculated for each animal. The percentage of variation relative to the basal levels was calculated for each animal at...
each time point. The results were then expressed as means of percentage of baseline ± SE.

Statistical analysis. Statistical analyses were performed using a two-factor repeated-measures ANOVA. Comparison between control and HF rats was assessed by a nonpaired Student’s t-test. P < 0.05 was chosen as statistical significance.

RESULTS

Body weight, food intake, and basal plasmatic parameters. Rats submitted to the HF diet showed significant overweight after 5 wk of diet (data not shown), and overweight persisted throughout after the diet (Table 1). The energetic intake during

Table 1. Body weight, food intake, and basal plasma parameters 2 days, 1 wk, and 8 wk after the beginning of the diet

<table>
<thead>
<tr>
<th></th>
<th>2 Days</th>
<th>1 Week</th>
<th>8 Weeks</th>
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<tbody>
<tr>
<td></td>
<td>Chow</td>
<td>HF</td>
<td>Chow</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>163 ± 1.8</td>
<td>162 ± 1.3</td>
<td>195 ± 4.4</td>
</tr>
<tr>
<td>24-h Food intake, kcal/kg body wt</td>
<td>299 ± 5.8</td>
<td>680 ± 67*</td>
<td>333 ± 23</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>5.51 ± 0.1</td>
<td>5.44 ± 0.1</td>
<td>5.33 ± 0.2</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>351 ± 72</td>
<td>330 ± 72</td>
<td>284 ± 35</td>
</tr>
<tr>
<td>Ghrelin, pg/ml</td>
<td>640 ± 73</td>
<td>353 ± 55*</td>
<td>781 ± 69</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>1.56 ± 0.14</td>
<td>1.77 ± 0.23</td>
<td>1.93 ± 0.16</td>
</tr>
<tr>
<td>FFA, μmol/l</td>
<td>425 ± 84</td>
<td>460 ± 18</td>
<td>462 ± 51</td>
</tr>
<tr>
<td>TG, μmol/l</td>
<td>630 ± 180</td>
<td>554 ± 62</td>
<td>672 ± 101</td>
</tr>
</tbody>
</table>

Values are means ± SE. HF, high-fat diet; FFA, free fatty acid; TG, triglyceride. *P < 0.01 vs. chow group.

Fig. 1. Time course of plasma glucose and insulin concentrations in response to glucose load in chow-fed (dotted line) and high-fat (solid line) rats after 2 days of diet (A and B), 1 wk of diet (C and D), and 8 wk of diet (E and F). Values are means ± SE; n = 9 rats/group. *P < 0.05, significantly different from controls.
24 h was doubled in HF rats vs. chow-fed rats whatever the time of the measurement (Table 1), whereas daily food intake, as expressed in grams per gram body weight, was similar between groups (data not shown). Basal plasma glycemia, insulinemia, NEFA, and TG concentrations did not change throughout the study, and the HF diet had no effect on any of these parameters. We observed a decrease in plasma ghrelin concentration after 2 and 7 days of diet and an increase in leptinemia that reached significance after 7 days of diet (Table 1).

**GIIS.** As shown in Fig. 1, the time course of glycemia after glucose loading was similar in both groups after 2 days of diet (Fig. 1A). In contrast, the increase in plasma insulin concentration was much higher in HF rats than in control rats (Fig. 1B). After 1 wk of diet, insulin hypersecretion persisted (Fig. 1D), and the increase in plasma glucose concentration was significantly higher in HF rats than in control rats (Fig. 1C), indicating glucose intolerance. After 8 wk of diet, GIIS was not significantly different from controls (Fig. 1F), whereas glucose intolerance worsened (Fig. 1E). Indeed, the area under the curve (AUC) of glycemia in HF rats after 8 wk of diet is significantly higher than the AUC after 1 wk of diet (342 ± 11006 22 vs. 185 ± 11006 7, \( P < 0.001 \)).

**GIIS in control and HF rats after 2 days of diet in the presence of oxymetazoline.** In the absence of oxymetazoline (Fig. 2), the insulinogenic index \((\Delta I/\Delta G)\) was 2.5-fold higher in HF rats vs. control rats after 2 days of diet, which expresses insulin hypersecretion in response to glucose (also shown in Fig. 1, A and B). In HF rats, the injection of oxymetazoline significantly reduced insulin secretion in response to glucose in a dose-dependent manner: \(\Delta I/\Delta G\) was reduced by 24% vs. the basal value in the presence of 0.1 pmol/kg oxymetazoline and significantly reduced by 65, 74, and 93% in the presence of 1, 10 and 1,000 pmol/kg, respectively. In contrast, in control rats, \(\Delta I/\Delta G\) remained unchanged when animals were given 0.1, 1, and 10 pmol/kg oxymetazoline, whereas it was reduced by 74% in the presence of 1,000 pmol/kg.

**Glucose TR under basal and euglycemic-hyperinsulinemic clamp conditions.** Figure 3 shows the glucose TR under both basal and hyperinsulinemic-euglycemic clamp conditions in HF rats and chow-fed rats after 2 days of diet. Basal turnover was similar in both groups. During hyperinsulinemic clamps, plasma insulin concentration was increased by about six times above the basal value in both groups with insulin infusions of 0.4 U·kg\(^{-1}\)·h\(^{-1}\). Under this condition, hyperinsulinemia was similar in both groups and reached \( \sim 1,500 \) pM (basal plasma insulin concentration in both groups was \( \sim 250 \) pM). The insulin-induced increase in glucose utilization (i.e., \( R_d \)) was similar in chow-fed and HF rats (Fig. 3A); in contrast, the decrease in hepatic glucose production (i.e., \( R_a \)) was attenuated in HF rats compared with controls (Fig. 3B). Therefore, hepatic insulin resistance was present as early as after 2 days of HF diet and persisted throughout the study (data not shown).

**Hepatic aspects.** After 2 days of HF diet, there was a significant increase in hepatic TG content (50.3 ± 3 mg/g liver vs. 31.7 ± 2.4 mg/g in chow-fed rats, \( P < 0.01 \)) and a decrease in glycogen content (17.8 ± 1.4 vs. 28.5 ± 3.8 mg/g, \( P < 0.01 \)).

**Tissue NE turnover.** In pancreas and brain, there was a significant increase in NE\(_0\) in HF rats and a slight increase (not significant) in liver (Table 2). The fractional turnover constant.
(k) was twofold decreased in pancreas and liver of HF rats, which indicated a twofold increase in NE half-life in these organs, whereas it remained constant in the whole brain. The TR was calculated by multiplying NE0 and k. It reached significance only in brain, since in liver and pancreas these parameters were varying in opposite ways (Table 2).

Hypothalamic NE. Basal hypothalamic extracellular NE levels were similar in both groups (−20 pg/20 μl). During the 30 min after the intraperitoneal glucose injection (1 g/kg body wt), we observed a drastic decrease (−78 ± 3%) in extracellular NE concentration in the chow-fed group (Fig. 4), whereas the decrease was much smaller in HF rats (−26 ± 5%). This discrepancy between groups could indicate changes in nervous activity of neurons in the VMH/PVN area.

### DISCUSSION

This study demonstrates that HF diet rapidly results in an increased GSIS (2 days) and a hepatic insulin resistance, followed by a glucose intolerance (1 wk). After 8 wk, insulin hypersecretion in response to glucose is not observed anymore, which can be explained by the inability of the pancreas to maintain hypersecretion for facing long-standing insulin resistance. Rats submitted to the HF diet became massively glucose intolerant, but they did not display basal hyperglycemia or hyperinsulinemia. Moreover, 60 min after glucose injection, their glycemia came back to the level of control rats (Fig. 1), indicating that insulin was still efficient enough to maintain basal normoglycemia.

Because some studies showed that exaggerated insulin secretion can be the first step of a cascade of events leading to an impaired insulin secretion and glucose homeostasis (3, 16), and finally to type 2 diabetes (15), we then decided to focus on day 2 of the HF diet, when insulin hypersecretion in response to glucose and hepatic insulin resistance appeared, i.e., the key period for the further deterioration of glucose homeostasis.

Increased TG storage in the liver was probably the main change responsible for this feature, since accumulation of lipid esterification products has been shown to play an important role in the setting of hepatic insulin resistance (25, 27, 48), and several studies showed that hepatic insulin resistance could be reversed by a decrease in TG content (27). We observed a lower glycogen storage in our model, which is consistent with studies indicating that glycogen storage is inversely correlated with TG storage in liver of insulin-resistant HF rats (25).

Because there was no increase in circulating FFA in HF rats throughout the diet, insulin hypersecretion observed after 2 days could be interpreted as a response to an increased insulin demand, caused by hepatic insulin resistance (49), rather than a direct effect of fatty acid on the β-cell. Modifications of basal NE content in pancreas and k in both liver and pancreas could also account for alterations in insulin secretion and efficiency on the liver (2), as a reflection of sympathetic nervous changes. Moreover, an increase in basal NE content is often associated with diabetes (1). Another interesting finding from our study was a change in central noradrenergic activity in HF rats, put in evidence by a lower decrease in NE extracellular concentration in response to glucose stimulus (microdialysis study) and an increase in brain NE turnover, after 2 days of diet. Because both the pancreas (2) and target organs of insulin are richly innervated and controlled by the autonomic nervous system (reviewed in Refs. 36 and 37), whose fibers are emerging from the VMH area (26), we postulated that the modifications of PVN/VMH noradrenergic activity could result in a decreased sympathetic tone involved in β-cell hyperresponsiveness. Consistent with this hypothesis, it has already been shown that a decrease in the sympathetic tone was responsible, at least in part, for insulin hypersecretion in rats infused with lipids (31). Because it is well established that the NE released by the postsynaptic nerve exerts its inhibitory effect on insulin secretion through α2A- and adrenoreceptors (2, 4), we used oxymetazoline, an α2A- adrenoreceptor agonist, to overcome the postulated decrease in sympathetic tone. With the use of increasing amounts of oxymetazoline, β-cell hyperresponsiveness was progressively blunted in HF rats; the dose of 1 pmol/kg oxymetazoline completely normalized their GSIS compared with controls, clearly indicating the involvement of a low sympathetic activity in the high insulin response in HF rats. Because α2A-adrenoreceptors are present not only on the β-cell membrane but also in some hypothalamic areas (12), it can be hypothesized that this low activity may be of peripheral and/or central origin. The normalization of insulin secretion with oxymetazoline was associated with a higher glycemia profile, thus attesting that the higher GSIS in HF rats was necessary to compensate liver insulin resistance. In control rats, the lower doses of oxymetazoline had no detectable effect.
REFERENCES


