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The Obese Göttingen Minipig as a Model of the Metabolic Syndrome: Dietary Effects on Obesity, Insulin Sensitivity, and Growth Hormone Profile

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The objective of the study reported here was to induce obesity in the female Göttingen minipig to establish a model of the human metabolic syndrome. Nine- to ten-month-old female Göttingen minipigs received a high-fat high-energy (HFE) diet or a low-fat, low-energy (LFE) diet. The energy contents derived from fat were 55 and 13 %, respectively. After 5 weeks, animals were subjected to dual energy x-ray absorptiometry (DEXA) scanning, intravenous glucose tolerance testing (IVGTT), and 6-h growth hormone profile recording. After treatment, mean body weight of pigs of the LFE group was 21.0 ± 0.4 kg, and was 26.8 ± 0.2 kg in pigs of the HFE group ($P < 0.0001$). The DEXA scanning indicated that the fat content of the LFE group was 10.0 ± 1.2 % versus 15.2 ± 0.7 % in the HFE group ($P < 0.003$). Triglycerides concentration was significantly ($P < 0.05$) increased in pigs of the HFE group (0.24 ± 0.03 m*M*), compared with that in pigs of the LFE group (0.13 ± 0.04 m*M*). Preprandial plasma glucose and insulin concentrations were not affected, but insulin area under the curve during IVGTT was significantly high in the obese animals. Growth hormone (GH) secretion was low in both groups of pigs.

The obese minipig shares some of the metabolic impairments seen in obese humans, and may thus serve as a model of the metabolic syndrome.

Obesity is a central feature of the metabolic syndrome, formerly called syndrome X. This syndrome includes decreased insulin sensitivity, dyslipidaemia, hypertension, atherosclerosis, and coronary heart disease (1). Several rodent models of obesity are available, in which single gene mutations typically cause obesity (2). Such mutations have also been found to cause obesity in humans, but only account for a small number of cases. Instead, eating patterns and fat content in the diet seem to lead to overweight in people (3, 4). In view of this, animal models of dietary induced obesity have potential interest. The Göttingen minipig might represent an interesting alternative to rodent models. This animal is bred principally for its small size and ease of handling in a laboratory setting, and would thus provide a good model if found metabolically similar to humans. The aim of the study reported here was to compare the effects of a high-fat high-energy diet with those of a high-carbohydrate low-energy diet on body composition and metabolic variables associated with the human metabolic syndrome, such as plasma glucose, insulin, and lipids concentrations, glucose tolerance, and growth hormone (GH) profile.

Materials and Methods

Animals. Twelve female Göttingen minipigs, aged 9 to 10 months and weighing 17.5 ± 0.3 kg, originating from a specific-pathogen-free breeding herd were purchased from Ellegaard (Lille Skensved, Denmark). They were acclimated in a non-barrier stable for three weeks singly in pens measuring 1.5 × 1.5 m. Lights were turned on at 7:00 a.m. and off at 3:30 p.m. Specially trained personnel cared for the animals. The animal facilities and all experimental procedures were approved by The Danish Board of Animal Experimentation (J. No. 1997-101-76).

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Surgery. Two catheters (Cavafix Certo, Braun, Melsungen, Germany) were surgically implanted in the right exterior jugular vein. The tip of the catheter used for infusion was advanced deeper than the tip of the withdrawal catheter to avoid contamination of the latter. The procedure was performed under aseptic conditions. The animals were anesthetized with 0.07 ml/kg of a solution containing tiletamine and zolazepam (both, 25.0 mg/ml; Zoletil, Boehringer Ingelheim Agrovet, Hellerup, Denmark), ketamine (12.5 mg/ml; Ketaminol, Rosco, Tåstrup, Denmark), xylazine (13.0 mg/ml; Rompun Vet, Bayer, Lyngby, Denmark), and methadone (2.5 mg/ml; Nycomed, Roskilde, Denmark). Thereafter, anesthesia was maintained with metomidate hydrochloride (0.03 ml/kg; Hypnodil Vet, Janssen-Cilag A/S, Birkerød, Denmark) as required. Before surgery, all animals were given, benzylpenicillin (0.6 million U; Novocillin, Novo Nordisk A/S, Bagsvaerd, Denmark), intravenously through a catheter in an ear vein. After the procedure, the animals received the analgesic Finadyne (1.0 mg/kg; Scanvet, Fredensborg, Denmark) intramuscularly.

One animal received additional antibiotic treatment with oxytetracycline hydrochloride (100 mg/kg; Terramycin Vet, Pfizer, Ballerup, Denmark), but further complications did not arise during the rest of the study. Due to blockage of catheters, two animals required further surgery. This did not cause detectable changes in weight gain.

Experimental diet. The animals were randomly allocated to two groups matched by weight. One group was fed the low-fat, low-energy (LFE) diet and the other the high-fat, high-energy (HFE) diet purchased at Brogaarden (Gentofte, Denmark). The diets (Table 1; compositions) were prepared to differ in carbohydrate and fat, but not protein content. Thus, when given in the same amounts, the protein source was equal in the two groups. The diet compositions were confirmed by results of chemical analyses (Bioteknologisk Institut, Kolding, Denmark). Differences in body composition and other parameters reflect the higher fat-to-carbo-

Table 1. Composition and calculated energy content of high-fat, high-energy and low-fat, low-energy diets

Ingredients (g/kg)	High fat	Low fat
Corn meal	493	818
Wheat bran	27	27
Casein	148	110
Animal fat	300	13
Vitamins and minerals	32	32
Chemical composition (g/kg)		
Crude protein	170	170
Crude fat	320	50
Metabolizable energy		
Total (MJ/kg)	20.1	13.4
Animal fat (% of total)	51.3	3.3
Vegetable fat (% of total)	3.9	9.8
Fatty acids (% of crude fat)		
Saturated	49	25
Mono-unsaturated	39	30
Polyunsaturated	12	46
Oleic acid	34	28
Total	94	92

MJ = megajoules.

hydrate ratio and/or higher energy content of the HFE diet. The composition of the LFE diet was almost identical to the normal minipig diet (5). Both diets were given in milled form. To adapt animals to the milled diets, they were initially mixed with the standard minipig diet (5) beginning one week after surgery. The experimental diets were fully accepted three weeks after surgery, and were given in portions of 250 g twice daily. Any refusals were included in the next portion to ensure that all individuals received equal amounts of food and protein during the study.

Body weight. A digital scale was used for weighing the conscious animals once a week.

Hormones and metabolites. After 5 weeks, blood was withdrawn and analyzed for GH, insulin-like growth factor I (IGF-I), insulin, C peptide, glucose, glycosylated fructosamine, and lipids concentrations. Blood was collected in tubes containing either EDTA or heparin or no anticoagulant. Serum or plasma samples were then frozen before analyses. After food had been withheld overnight, the animals were placed in familiar cages permitting blood sample collection under non-stressful conditions. Plasma GH was analyzed by use of a previously described enzyme-linked immunosorbent assay (ELISA) (6). Assay of IGF-I, C peptide and insulin was done by use of radioimmunoassay kits (IGF-R20, Mediagnost, Tuebbingen, Germany; kit No. PCP-22K and PI-12K, Linco Research Inc., St, Charles, Mo.). Glucose and lipids were analyzed by use of a Beckman autoanalyzer (CX-5, Beckman, Brea, Calif.). Glycosylated fructosamine was analyzed, using a COBAS MIRA system (Roche Diagnostic Systems, Somerville, N.J.).

Intravenous glucose tolerance testing. In connection with blood sample collection, intravenous glucose tolerance testing (IVGTT) was done. After blood samples had been taken for baseline measurements, an injection of glucose (0.3 mg/kg of body weight, IV) preceded collection of further samples after 5, 10, 30, 60, and 120 min for determination of glucose, insulin, and GH concentrations. There are several reasons for us to perform IVGTT instead of oral GTT (OGTT). The OGTT is considered a tool for epidemiologic studies, with poor reproducibility, and is uncontrolled for differences in absorption of glucose from the gastrointestinal tract (7, 8). Also, the IVGTT can be used to estimate glucose tolerance and insulin resistance during one test (9, 10). Lastly, this test was estimated to the least stressful for the animals as the procedures only required them to be in a familiar cage for the duration of the test (catheters were already implanted for blood sample collection), as opposed to introduction of a esophageal catheter that would be needed for OGTT.

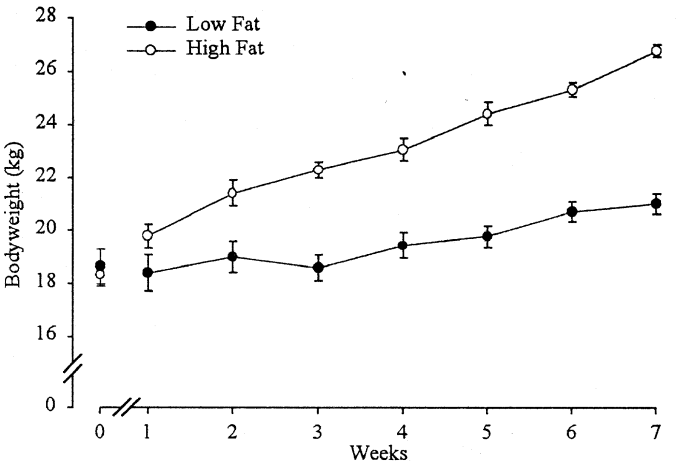

Figure 1. Body weight of female Göttingen minipigs during low-fat, low-energy (Low Fat, n = 5) or high-fat, high-energy (High Fat, n = 6) feeding. Each point represents the mean of the group; error bars indicate SEM. The points at week 0 represent body weight before surgery at the beginning of the study. At that time, body weights were not significantly different on the basis of results of the Student's *t* test. Body weight differed significantly in the two groups during the period from weeks 2 ($P < 0.01$) to 7 ($P < 0.0001$) as analyzed by use of the Student's *t* test. Week 1 represents the first week of full experimental diet, after a period of gradually increasing the amount of experimental diet mixed with normal minipig diet.

Dual energy x-ray absorptiometry scanning. Body composition was determined by use of dual energy x-ray absorptiometry scanning (DEXA)scanning. After 6 weeks of dietary treatment, the animals were anesthetized as described previously, and were placed on the scanner bed (QDR-1000 W, Hologic, Zaventem, Belgium). All bandages were removed before scanning to ensure correct analysis. The procedure was performed, using the Whole Body Analysis protocol in the Hologic software package, which estimates body weight of the scanned individual and calculates the absolute mass of each tissue in relation to this estimate.

Growth hormone profile. After 6 weeks, a GH profile was obtained. Blood was withdrawn every 15 min for 6 h, and feeding was coordinated identically in relation to blood sample collection in all individuals.

Statistics. Data were expressed as mean ± SEM, and were calculated by use of the software program package SAS-STAT univariate procedure (SAS Institute, Cary, N.C.). Comparisons between groups were done by use of the GLM procedure, and a *P*-value < 0.05 was considered significant. Mean values for the GH profiles were calculated after running a smoothing algorithm on the data by use of the software program Pulsar (11).

Results

Growth. Before surgery, the animals were randomly allocated to two weight-matched groups, the LFE and HFE groups, weighing 18.7 ± 0.6 and 18.3 ± 0.4 kg, respectively (Fig. 1). At the end of the study, mean body weight was 21.0 ± 0.4 kg in animals of the LFE group, and was 26.8 ± 0.2 kg in those of the HFE group ($P < 0.0001$). One animal was excluded from the study because of technical problems with blood sample collection.

Body composition. During the study, it was obvious from gross examination that the pigs in the HFE group became rap-

Table 2. Body composition of female Göttingen minipigs after six weeks of low-fat, low-energy (n = 5) or high-fat, high-energy (n =6) diet consumption

	Proportion in relation to body mass (%)			Absolute tissue mass (g)		
Tissue	Low fat	High fat	*P* value	Low fat	High fat	*P* value
Fat	10.0 ± 1.2	15.2 ± 0.7	0.003	1,946 ± 238	3,681 ± 199	0.0003
Lean	87.8 ± 1.1	82.8 ± 0.7	0.003	16,981 ± 390	20,023 ± 256	0.0001
Bone	2.1 ± 0.1	2.0 ± 0.1	0.5	401 ± 13	483 ± 16	0.005

Tissue proportions were calculated as mean ± SEM percentage in relation to total body mass, as estimated by use of dual energy x-ray absorptiometry (DEXA) scanning. Statistical comparisons were made by use of Student's *t* test.

idly obese. This was especially visible in the neck and abdominal regions. The mean body compositions of the two groups were significantly different after 6 weeks of experimental diets. Animals receiving the HFE diet had a larger proportion of total body fat, compared with that for the group receiving the LFE diet ($P < 0.01$). The proportion of lean mass was lower ($P < 0.01$), although absolute lean mass was higher ($P < 0.001$) in animals of the HFE group, compared with those of the LFE group. Further data on body composition are shown in Table 2. When mean body weight was estimated by use of DEXA scanning, it appeared to be 1.5% lower than values obtained by use of a digital scale (data not shown). This difference is well within the normal variation when weighing conscious animals.

Blood lipids concentration. Blood was analyzed for several lipids and metabolites of triglyceride breakdown. When blood samples from animals of the HFE group were centrifuged, lipid droplets were evident in the supernatant of most samples. This was not observed in samples from animals of the LFE group. The HFE diet group had significantly high triglyceride values and high-density lipoprotein (HDL) cholesterol fraction, compared with values in the LFE fed animals. These and other observations on plasma and serum lipids are shown in Table 3.

Preprandial blood glucose, fructosamine, insulin, and C peptide concentrations. Mean preprandial glucose, fructosamine, insulin, and C peptide concentrations were not significantly affected by the HFE diet (Table 4).

Glucose tolerance testing. The area under the curve (AUC) for glucose, insulin, and GH after the glucose injection was determined for each group in Table 4 (for individual data see Fig. 2). The HFE diet caused a significantly ($P < 0.04$) more prominent insulin response, compared with that in animals of the LFE group, whereas glucose values appeared similar in the two groups. Growth hormone peaks were not observed during IVGTT.

Growth hormone profile and IGF-I values. The GH profiles of each animal are shown in Fig. 3, and are based on samples from 10 animals (one animal of the LFE diet group was excluded because of missing samples due to catheter blockage). The smoothed mean values and the mean area over the smoothed basal lines were not significantly different between the two groups (data not shown). The same was true for the IGF-I values, with concentrations of 16.9 ± 3.1 and 21.5 ± 1.0 p*M* in animals of the LFE and HFE groups, respectively. The somewhat lower mean value and larger variation in the LFE group was due to the fact that one pig had low IGF-I values (between 5.0 and 8.6 p*M*) during the study (data not shown). This individual was not identical to the one that had high GH values (Fig. 3).

Discussion

In the study reported here, we examined the metabolism of lean and obese minipigs to identify possible resemblance to that of human obesity and the metabolic syndrome (1). The primary objective was to examine the body compositional and metabolic changes associated with the introduction of a high-fat, high-energy diet. The Göttingen minipig has served as a model for research in the fields of atherosclerosis (12) and hypertension (13), but to our knowledge, reports of metabolic variables of the obese minipig do not exist.

There was a marked response to the high-fat diet in terms of body weight gain. Animals fed the HFE diet became severely obese, and we found a relative content of body fat that was 52 % higher than that in controls. Assuming that animals of the HFE group had initial body composition identical to the composition found in the controls and that the energy cost of depositing fat tissue is 50.2 and 7.44 kJ/g for lean tissue (14, 15), approximately 23% of the excess energy in the HFE diet was deposited as lean tissue and the rest as fat tissue. Thus, the relationship between deposition of fat and lean tissue was found to be a little higher in minipigs than in humans, 3.3 versus 1.6 respectively (15).

Glucose values were not disturbed significantly by the HFE diet. Preprandial glucose concentration, glucose AUC during IVGTT, and glycosylated fructosamine concentration were used as measures of basal, short-term, and long-term (16) glycemic control, respectively. Glycosylation of fructosamine is positively correlated to glucose concentration of the preceding one to three weeks (17); this variable is, therefore, used instead of hemoglobin A_{1C}, which is not applicable to pigs (18). Insulin resistance was evidenced in animals of the obese group, as its plasma concentration increased to significantly higher values in obese than in lean animals in response to identical glucose loads.

Glycemic control in the obese minipig is, thus, similar to what has been observed in obese humans, where euglycemia usually is maintained despite underlying insulin resistance (19, 20). Furthermore, the degree of insulin resistance has been documented to correlate to the severity of central obesity in humans (21). In the study reported here, insulin resistance, represented by insulin AUC during IVGTT, did in fact have significant correlation with total body fat, $R^2 = 0.44$ ($P < 0.03$). Thus, fat depot size in the Göttingen minipig is associated with impairment of insulin sensitivity similarly as in humans.

As expected, we found that consumption of fat of animal origin affected blood lipid concentrations adversely. In the obese pigs, the major changes in blood lipid concentrations consisted of significant increase in triglycerides and HDL cholesterol concentrations. In contrast, a survey of obese humans indicated that the typical obese individual has high triglyceride but decreased HDL cholesterol values (22). However, diet was not controlled for, and in experiments where humans were fed high fat-containing diets, triglyceride and HDL cholesterol values increased (23), or triglycerides concentration increased while HDL cholesterol values did not change (24). The effects seen in our study are, therefore, similar to data presented in comparable human studies. Furthermore, the low fat content of the LFE diet resulted in accordingly low HDL cholesterol values, thus rendering comparison with the human studies difficult.

Table 3. Mean ± SEM preprandial plasma and serum lipid concentrations in female Göttingen minipigs after five weeks of consuming the low-fat, low-energy (n = 5) or high-fat, high-energy (n = 6) diet

Lipid	Low fat	(mmol/L) High fat	*P* value
Triglycerides	0.13 ± 0.04	0.24 ± 0.03	0.05
FFA	0.44 ± 0.09	0.29 ± 0.06	0.2
Glycerol	0.11 ± 0.02	0.09 ± 0.01	0.7
Cholesterol (total)	1.82 ± 0.22	2.03 ± 0.17	0.1
HDL cholesterol	0.77 ± 0.05	1.03 ± 0.07	0.02
-Hydroxybutyrate	0.13 ± 0.02	0.14 ± 0.01	0.6

Statistical comparisons were made by use of Student's *t* test.
FFA = free fatty acids; HDL = high-density lipoprotein.

Table 4. Mean ± SEM preprandial plasma glucose, glycosylated fructosamine, insulin, C peptide, and growth hormone (GH) concentrations and those during intravenous glucose tolerance testing in Göttingen minipigs after five weeks of consumption of low-fat, low energy (n = 5) or high-fat, high-energy (n = 6) diet

Preprandial values	Low fat	High fat	*P* value
Glucose (m*M*)	4.4 ± 0.2	4.5 ± 0.1	0.7
Fructosamine (p*M*)	222 ± 9	207 ± 8	0.2
Insulin (p*M*)	73 ± 9	100 ± 17	0.2
C Peptide	109 ± 9	141 ± 22	0.2
AUC during glucose tolerance testing			
Glucose (m*M* x 120 min)	578 ± 10	636 ± 26	0.1
Insulin (p*M* x 120 min)	13,357 ± 1,113	20,107 ± 2,349	0.04
GH (p*M* x 120 min)	15,764 ± 3,255	10,881 ± 558	0.1
Peak values during glucose tolerance testing			
Glucose (m*M*)	11.7 ± 0.2	12.3 ± 0.3	0.2
Insulin (p*M*)	487 ± 148	520 ± 154	0.7

AUC = area under the curve; statistical comparisons were done by use of Student's *t* test.

The obvious possibility of an abnormal GH/IGF-I axis in the minipig prompted us to examine its GH profile because this hormone may be central to development of the obesity/metabolic syndrome. In obese humans, disturbance of the GH/IGF-I system, with subnormal hormone concentrations, has been observed (25-28), and results of several GH replacement trials have indicated important effects on symptoms of the metabolic syndrome (29-31), such as loss of fat tissue, improvement in hormone concentrations, and insulin resistance. In rats predisposed to obesity, low GH concentration can be detected prior to change in body composition (32), and mice overexpressing GH become obese following inactivation of the transfected gene (33). Thus, in people and animals, GH seems to be important in the control of body fat amount.

There are not, to our knowledge, earlier reports describing GH secretion profiles in minipigs. Surprisingly, basal GH concentration and peak amplitudes were similar in the obese and lean groups. The basal value compares well with what was found previously in Göttingen minipigs (in this instance, only a single GH determination for each individual) (34) and other pig strains (35). The peak amplitudes were low, however, compared with amplitudes found in a large lean pig strain (35) and in lean humans (36). Zenobi and co workers (34) stimulated GH secretion in minipigs by insulin-induced hypoglycemia, which caused a peak response of similar magnitude as that found in our GH profiles. Contrary to expectations (37), we did not find stimulation of GH secretion during IVGTT (data not shown) or difference in GH response between the groups. These data suggest that the GH secretion in the minipig, regardless of body composition, may be similar to what has generally been observed in obese pigs and humans.

Lean and obese animals had IGF-I concentration in the normal range, compared with that in slaughter pigs (38). This was a surprising finding because the low GH values led us to expect similarly low IGF-I values. The IGF-I values usually are slightly reduced in obese humans (39), an association we could not document during this study. A six-week period consuming the HFE diet may be too short for such a change to become apparent.

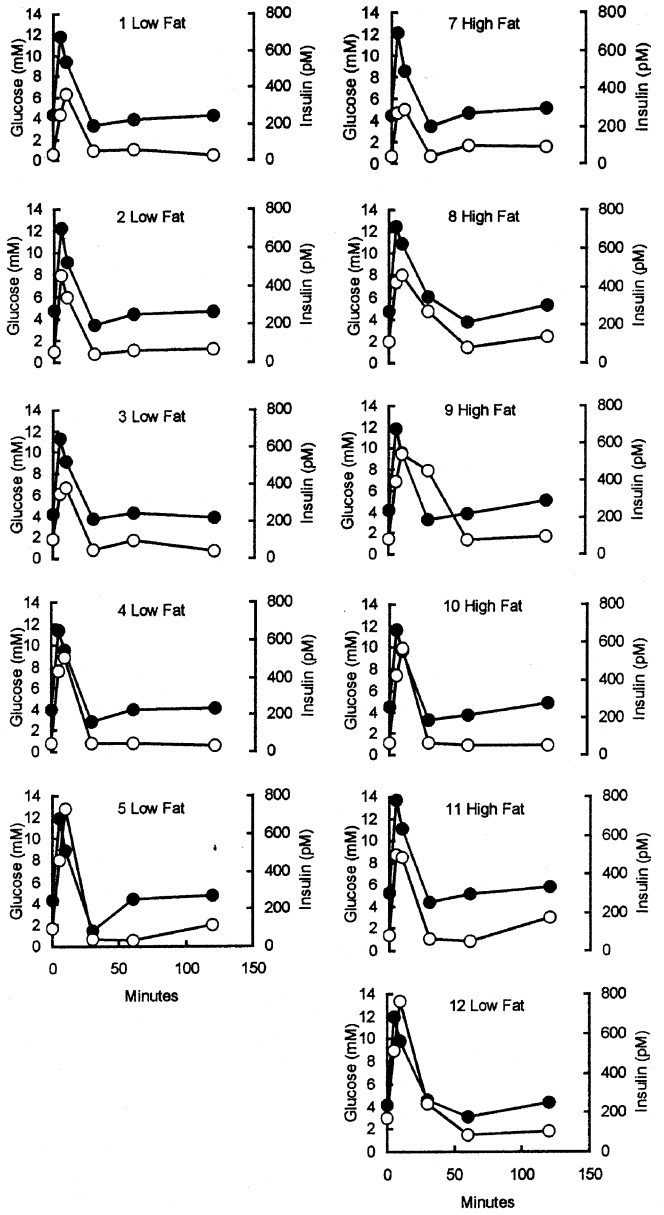

Figure 2. Individual concentrations of plasma glucose (closed circles) and insulin (open circles) during intravenous glucose tolerance testing in female Göttingen minipigs after 6 weeks of low-fat, low-energy (Low Fat, n = 5) or high-fat, high-energy (High Fat, n = 6) feeding. Animals were injected with glucose (0.3 mg/kg) through an indwelling catheter placed in the jugular vein. Blood samples were collected after 5, 10, 30, 60, and 120 min. Statistics of the peak values and the area under the curve are given in Table 4.

Our data and those of the aforementioned studies in humans and animals suggest that low or decreasing GH secretion predisposes a person or animal to obesity, and poses a possible explanation for the tendency of the minipig to readily store body fat if given a dietary source. Postnatal treatment with GH could

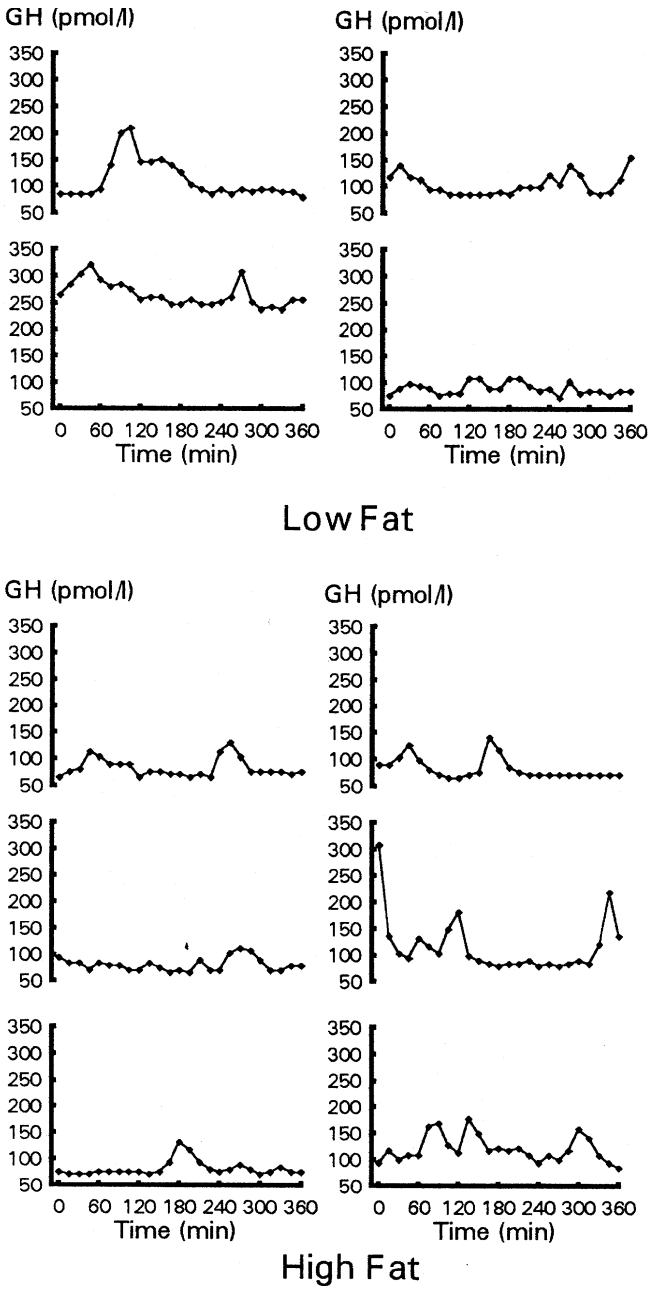

Figure 3. Growth hormone (GH) profiles in female Göttingen minipigs after 6 weeks of low-fat, low-energy (Low Fat, n = 4) or high-fat, high-energy (High Fat, n = 6) feeding. Blood was withdrawn every 15 min for 6 h. Significant differences were not found between the two groups when analyzing smoothed mean values, and area above the smoothed mean by use of Student's *t* test.

shed some light on the role of GH in the control of body composition in the minipig.

In conclusion, the HFE diet induced a major increase in body fat in only six weeks. This was associated with slight insulin resistance, and high blood triglycerides concentration. In this view, the obese Göttingen minipig may provide a good model of some central traits of the human metabolic syndrome. Discrepancies between human and minipig GH secretion remain to be further investigated.

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