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► **To cite this version:**

Natalia Sinitskaya, Sylviane Gourmelen, Carole Schuster-Klein, Béatrice Guardiola-Lemaitre, Paul Pevet, et al. Increasing the fat-to-carbohydrate ratio in a high-fat diet prevents the development of obesity but not a prediabetic state in rats. *Clinical Science*, 2007, 113 (10), pp.417-425. 10.1042/CS20070182 . hal-00479385

HAL Id: hal-00479385

<https://hal.science/hal-00479385>

Submitted on 30 Apr 2010

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Paper No CS2007/0182

Increasing fat-to-carbohydrate ratio in a high-fat diet prevents the development of obesity but not prediabetic state in rats

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Short title: Obesogenic and diabetogenic effects of high-fat diets

Key words:

Obesity, insulin resistance, metabolic syndrome, leptin, adiponectin

Abbreviations:

BMI, body mass index; CHO, carbohydrate; HCD, high-carbohydrate diet; HFD, high-fat diet
HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol

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ABSTRACT

Metabolic disorders induced by high-fat feeding in rodents evoke some, if not all the features of the human metabolic syndrome. The occurrence and severity of metabolic disorders, however, varies according to rodent species, and even strain, as well as the diet. Therefore we investigated obesogenic and diabetogenic long-term effects of 3 high-fat diets differing by their fat/carbohydrate (CHO) ratios. Sprague-Dawley rats were fed for 10 weeks with either a control, high-carbohydrate, low-fat diet (HCD; 12/64/24 ratio of fat/CHO/protein, 3.7 kcal/g), a high-fat, medium-carbohydrate diet (HFD1; 53/30/17 ratio of fat/CHO/protein, 4.7 kcal/g), a very high-fat, low-carbohydrate diet (HFD2; 67/9/24 ratio of fat/CHO/protein, 5.2 kcal/g), or a very high-fat, carbohydrate-free diet (HFD3; 75/0/25 ratio of fat/CHO/protein, 5.9 kcal/g). Compared to the control diet (HCD), rats fed with high fat combined with more (HFD1) or less (HFD2) of CHO exhibited higher body mass index (+13 and +10% BMI, respectively; $P < 0.05$) and abdominal fat (+70% in both HFD1 and HFD2; $P < 0.05$), higher plasma leptin (+130 and +135%, respectively; $P < 0.05$), lower plasma adiponectin levels (-23 and -30%, respectively; $P < 0.05$), and impaired glucose tolerance. Only the HFD1 group showed insulin resistance. By contrast, a very high-fat diet devoid of CHO (HFD3) led to impaired glucose tolerance, insulin resistance and hypoadiponectinemia (-50%, $P < 0.05$) while BMI, adiposity and plasma leptin did not differ from respective values in animals fed with the control diet. We conclude that increasing fat-to-carbohydrate ratio to the uppermost (i.e., carbohydrate-free) in a high-fat diet prevents the development of obesity, but not the prediabetic state (i.e., altered glucose tolerance and insulin sensitivity).

INTRODUCTION

Spontaneous mutations and gene targeting in rodents have triggered remarkable progress for identifying key genes involved in the regulation of adiposity. In humans, however, monogenic causes of obesity are rare. Rather, the risk of developing obesity appears largely polygenic in combination with a large spectrum of environmental obesogenic influences.

Food and beverages rich in energy, fat and/or sugar are now commonly found in modern societies. Besides genetic predisposition [1,2], physical inactivity [3,4] and perinatal environment [5,6], such diets have long been recognized as major causes to the obesogenic environment in humans [7,8]. The interactions of these diets with the physiological systems that regulate energy metabolism and body composition are thus an area of considerable importance. Major complications of obesity include type 2 diabetes, insulin resistance and increased risk of cardiovascular disease [3,9]. The simultaneous occurrence of obesity, and particularly of visceral origin, dyslipidemia, insulin resistance and hypertension is classically referred to as the so-called “metabolic syndrome” [9-11].

A number of different types of high-fat and/or high-energy diets have already been used to induce obesity and/or mimic the human metabolic syndrome in rodents [12-17]. Most studies have used only one high-fat formula compared with the standard chow diet. To take into account the relative amounts of macronutrients, we aimed at testing specifically the possible importance of the fat/carbohydrate ratio in a high-fat diet.

Therefore, the current study was undertaken to characterize the occurrence of various aspects of the metabolic syndrome (namely, prevalence of obesity, dyslipidemia, glucose tolerance and insulin resistance) in rats placed on a high-carbohydrate, low-energy diet (chow) compared to three high-fat, high-energy diets differing in their respective amounts of saturated fat and carbohydrate.

MATERIALS AND METHODS

Ethics

All experiments were performed in accordance with the rules of the European Committee Council Directive of November 24, 1986 (86/609/EEC) and the French Department of Agriculture (license n°67-88).

Animals

48 male Sprague-Dawley rats (Charles River Laboratories, L'arbresle, France) weighing around 120 g upon their arrival, were housed individually, kept at $21 \pm 1^\circ\text{C}$ under a 12:12 h light-dark cycle (lights on at 07:00 AM) and had ad libitum access to food (standard low-fat diet, 105, SAFE, 89290 Augy, France) and tap water for 2 weeks.

Experimental design

Rats were then divided into 4 groups: the first group of 12 control rats was fed a standard high-carbohydrate, low-fat pelleted diet (HCD; 3.7 kcal/g; 229H, SAFE; [Table 1](#)). The second group of 12 rats received a high-fat, medium-carbohydrate pelleted diet (HFD1; 4.7 kcal/g, SAFE; [Table 1](#)). The third group of 12 rats were fed with a very high-fat, low-carbohydrate diet (HFD2; 5.2 kcal/g; 231H, SAFE; [Table 1](#)). The fourth group of 12 rats received a very high-fat, carbohydrate-free diet (HFD3; 5.9 kcal/g, SAFE; [Table 1](#)). Diets were vacuum-packed and stored in a fresh (8°C) room before use. Animals were given fresh food once a week. Body mass was measured every week. Due to considerable wasting (especially for HFD2 and 3), we were not able to quantify with precision daily food intake according to the diet. One ill animal from the HCD group had to be taken out of the experiment, leading to $n=11$ control animals.

After 8 weeks of experimental diet, rats were fasted overnight and tested for glucose tolerance 2 h after light onset. After 10 weeks of experimental diet, rats were fasted overnight and tested for insulin sensitivity 2 h after light onset. In the morning of the following day, all rats were deeply anesthetized with isoflurane and their body length and mass were determined before decapitation. Thereafter, blood was immediately collected and centrifugated. Fat tissues were dissected and weighed. The sum of the mass of epididymal fat pads and retroperitoneal fat expressed as percentage of body mass was used as an index of adiposity.

Methods of analysis

To test oral glucose tolerance, blood glucose was assessed in overnight fasted rats just before, 30 min, 1 and 2 h following an oral administration of glucose (2 g/kg, Sigma, St Louis, MO, USA) or water. Blood samples were collected via tail veins and blood glucose was immediately determined (Glucotrend premium kit, Roche Diagnostics, Meylan, France). To optimize timing of blood sampling and avoid any time-of-day effect, 24 rats were sampled, half of them receiving an either glucose or water. For each condition, 3 rats per diet were tested.

To assess insulin-induced hypoglycaemia, overnight fasted rats received an s.c. injection of 1 IU/ kg human insulin (Umuline NPH, Lilly France, Suresnes, France) 2 h after light onset. Blood glucose collected as above was assessed just before, 30 min, 1 and 2 h after insulin treatment. During the sampling period, rats had no access to food. Again, to optimize timing of blood sampling and avoid any time-of-day effect, 24 rats (n=6 per diet) were tested with insulin only, because no major effect of manipulation and/or water injection was detected in the glucose tolerance test.

Commercial enzymatic assays were used to determine plasma free fatty acids with RxL Dade Boehringer analyzer (Diamond Diagnostics, Holliston, MA, USA) and plasma triglycerides, total cholesterol and high high-density lipoprotein-cholesterol (HDL-C) using Advia 1650 analyzer (Bayer Diagnostics, Tarrytown, NY). Low-density lipoprotein-cholesterol (LDL-C) was calculated as follows:

$$\text{LDL-C} = \text{Total Cholesterol} - (\text{HDL-C}) - (\text{Triglycerides}/2.2).$$

Plasma insulin was determined by an ELISA kit for rats (EZRFI-13K, Linco Research, Inc., St Charles, MO, USA) with intra- and inter-assay coefficients of variation (%CV) of 1.9 ± 0.6 and 7.6 ± 0.8 %, respectively. The limit of sensitivity of insulin assay was 0.2 ng/ml. Plasma leptin was determined by an ELISA kit for rats (EZRL-83K, Linco Research) with 2.2 ± 0.2 intra- and 3.4 ± 0.3 inter-assay %CV. The limit of sensitivity of leptin assay was 0.04 ng/ml. Plasma adiponectin was determined by an ELISA kit for rats (EZRADP-62K, Linco Research) with 1.3 ± 0.2 intra- and 7.0 ± 0.4 inter-assay %CV. The limit of sensitivity of adiponectin assay was 0.155 ng/ml.

Homeostatic model assessment (HOMA) used to assess B-cell function and insulin resistance (IR) was calculated as follows:

$\text{HOMA-IR} = (\text{Fasting plasma glucose} \times \text{Fasting plasma insulin}) / 22.5$ as described by Matthews et al. [18].

Statistical analysis

To test the effects of diet (HCD, HFD1, HFD2 or HFD3) and time (weeks), data were processed with analyses of variance (ANOVA) with repeated measures or not, depending on the parameter considered. If significant main effects or significant interactions were detected ($P < 0.05$), *post-hoc* comparisons were performed with Tukey HSD test. Values are means \pm SEM. For assessment of correlations, data were fitted to the following equation (SigmaPlot software, Jandel Scientific, Chicago, USA): $y = y_0 + a \cdot x$ where a was the slope and y_0 the intercept.

RESULTS

Body mass and adiposity

Both HFD1 and HFD2 led to increased body mass compared to HCD control animals ($+11 \pm 2$ and $+13 \pm 4$ %, respectively; $P < 0.05$) 10 weeks after the start of the experiment. Note that such a difference was significant after 6 weeks of high-fat diets up to the end of the experiment. In contrast, HFD3 without carbohydrate did not significantly modify gain in body mass over the experiment compared to rats fed with HCD ($+3 \pm 2$ %; NS; Fig. 1A). This lack of body mass difference cannot be attributed to a slower growth in the HFD3 group (Fig. 1B). Accordingly, the body mass index was larger in rats fed with HFD1 and HFD2, but not with HFD3, compared to control animals fed with HCD (Fig. 1C).

Interestingly, adiposity index (epididymal and retroperitoneal white fat as percentage of body mass) was larger in animals with diets containing both high-fat and a certain amount of carbohydrate (i.e., HFD1 and HFD2) compared to rats fed with either low-fat, high carbohydrate diet (HCD) or high-fat, carbohydrate-free diet (HFD3; Fig. 2).

Glucose tolerance

Fasting blood glucose, determined in the morning (i.e., 2 h after the onset of light), did not differ significantly between the feeding conditions ($P > 0.05$; Fig. 3A). Whatever the diet considered, handling and oral administration of water did not modify significantly basal blood glucose during the 2-h period of blood sampling ($P > 0.05$; Fig. 3B). In control rats, an oral bolus of glucose led to a significant increase in blood glucose, which returned to basal levels 2 h later (Fig. 3A). Blood glucose was increased by almost two-fold in rats with either HFD2 or HFD3 (Fig. 3A), while only a trend for increase was visible for the HFD1 group (Fig. 3A). In addition, 2 h after glucose administration, in contrast to the control group, blood glucose in

the three groups of animals fed with high-fat diet (HFD1-3) was still larger than the basal values ($P < 0.05$; Fig. 3A), indicating a reduction in glucose tolerance.

Insulin resistance

In response to an acute administration of insulin after an overnight fast, control rats fed with HCD displayed the expected lowering in blood glucose that levelled back to normal values 2 h after the treatment (Fig. 3C). Albeit reduced, insulin-induced hypoglycaemia was not significantly impaired in rats fed with HFD2 compared to control rats, whatever the time point considered ($P > 0.05$; Fig. 3C). Unlike these limited alterations in the HFD2 group, blood glucose in HFD1 and HFD3 rats did not change at all during the testing period, leading to higher glucose levels 30 min and 1 h after insulin treatment in comparison with those in control rats ($P < 0.05$; Fig. 3C), suggesting a reduced whole-body sensitivity to insulin.

Hormonal changes

Morning levels of plasma insulin in overnight fasted rats tended to be higher in the three HFD1-3 groups compared to the control HCD, but these differences did not reach significance (Fig. 4A). In keeping with adiposity data (Fig. 2), plasma leptin in HFD1 and HFD2 rats was significantly higher than that in HCD rats ($P < 0.05$), while intermediate (but not significant) values of plasma leptin were found in HFD3 rats (Fig. 4B). Finally, plasma adiponectin was significantly reduced in the three groups of animals fed with high-fat diet (HFD1-3) compared than the control group ($P < 0.05$). Surprisingly, the lowest level of plasma adiponectin was detected in rats fed with HFD3 (Fig. 4C).

Homeostatic model assessment and insulin resistance (HOMA-IR) and correlations with adipokins

HOMA-IR tended to higher in HFD1 (8.6 ± 1.8 a.u.), HFD2 (9.1 ± 2.5 a.u.) and HFD3 groups (8.9 ± 2.5 a.u.) compared to control group (HCD: 3.5 ± 0.9 a.u.), but these differences did not reach significance ($P > 0.05$). Plasma adiponectin correlated neither with fat mass (Fig. 5A), nor with HOMA-IR (Fig. 5B). By contrast, plasma leptin was significantly correlated with both fat mass (Fig. 5C) and HOMA-IR (Fig. 5D)

Circulating lipids

Concerning total cholesterol, there was an apparent increase in all rats fed with high-fat diet, but the differences with control values were only significant in rats with HFD2 and HFD3

($P < 0.05$; Fig. 6A). Furthermore, LDL-cholesterol was clearly altered in HFD3- fed rats, but not with the HFD1 diet (Fig. 6B). Although not significant, LDL-cholesterol level tended to be increased in HFD2-fed rats.

Plasma triglycerides were not markedly modified by the diet, except a higher level in rats fed with HFD1 compared to that in the HFD3 group, Fig. 6C). Moreover, free fatty acids were increased by high-fat diet in HFD2 rats ($P < 0.05$), but this higher level did not reach the threshold of statistical significance in HFD1 and HFD3 animals (Fig. 6D).

DISCUSSION

The present study demonstrates that depending on respective amounts of saturated fat and carbohydrate, high-fat/high-energy diets lead to differential metabolic abnormalities. The two high-fat diets combined with moderate- to low-carbohydrate content (HFD1 and HFD2) were both obesogenic (i.e., causing high adiposity and plasma leptin as well as low plasma adiponectin) and diabetogenic (i.e., inducing insulin resistance), while the high-fat, carbohydrate-free diet did not produce abdominal obesity but led to insulin resistance and, by increasing LDL-C, also led to higher risk of cardiovascular disease.

High-fat diets have been widely used in rodents to provide experimental models of the human metabolic syndrome that simultaneously associate obesity, dyslipidemia, diabetes-related alterations, and eventually atherosclerotic cardiovascular disease. The present study confirms the usefulness of diets enriched in saturated fat to produce metabolic abnormalities mimicking the metabolic syndrome. A large number of experiments studying the metabolic consequences of high-fat diets have been already performed in various strains of rats such as Sprague-Dawley (e.g., [12,13,16], [this study](#)), Wistar (e.g., [15]) or Long-Evans (e.g., [14,17]). The increase in body mass we obtained with both HFD1 (i.e., +11%) and HFD2 (i.e., +13%) diets is roughly consistent with previous experiments considering all the experimental animals (i.e., with no post-hoc selection according to body mass gain) and having the same duration as ours (e.g., +10% after 10 wks, [17]; +10 % after 12 wks, [15]). The accretion of body fat in HFD1 and HFD2 rats was even clearer when considering BMI and adiposity values.

Moreover, we aimed at investigating the possible importance of the saturated fat/carbohydrate ratio in a high-fat diet. This is why we compared the effects of three diets enriched in saturated fat differing mainly by their fat/carbohydrate ratio, while their protein content remained largely unchanged (range 17-25% in metabolizable energy). The most pronounced manifestations of abdominal obesity and insulin intolerance were observed after feeding with HFD1, that is, a diet containing high-fat, medium-carbohydrate proportions (in energy). Rats with this diet gained more body mass, had larger body mass index, were fatter with high leptinemia and low adiponectinemia, and displayed physiological signs not only of impaired glucose tolerance, but also of whole-body insulin resistance, compared to animals fed a standard, high-carbohydrate diet (HCD). HFD2, the second diet which the metabolic consequences were evaluated, contained a very high-fat (>50%), low-carbohydrate diet. HFD2 led roughly to metabolic abnormalities comparable with HFD1, except that the glycemic responses to insulin treatment were less altered, being close to values in the control

group (HCD). Dyslipidemia has been difficult to obtain in rats, even after modifications of the fat/carbohydrate ratio. Here only HFD2 and HFD3 rats showed altered profiles of circulating lipids. The increase in plasma free fatty acids was only significant with this HFD2 diet. Nevertheless, except for dyslipidemia, the present results suggest that HFD1 is a better experimental model of the human metabolic syndrome than the two other high-fat diets tested here.

Diets with different ratios of fat/carbohydrate ratios have been already studied (e.g., in mice, [19]; in rats, [20-23]). The overall conclusion of these studies is as follows: the more the caloric content derives from dietary saturated fat, the fatter and the less glucose-tolerant become the animals. To our knowledge, however, the previous studies did not investigate directly a carbohydrate-free, high-fat diet compared to high-fat diet with variable amounts of carbohydrate, while keeping protein content comparable between the diets to avoid any deficit in body growth.

A question that arises from the present study in which diets were not isoenergetic is whether the increase in body fat is due to the larger caloric content of the high-fat diets or to the larger percentage of energy as fat. Overall, the degree of obesity was not correlated with the caloric diet because the highest body mass index and adiposity were found in rats fed with the lowest energy content (i.e., HFD1 with 4.7 kcal/g) among the three high-fat diets studied. Conversely, the highest caloric diet (i.e., HFD3 with 5.9 kcal/g) did not lead to a significant increase of body mass index or adiposity in comparison with control animals. Therefore, these opposite responses clearly indicate that the different caloric contents in the diets proposed here are not the main factor causing fat accretion. Alternatively, even if we could not measure caloric intake in the present experiment, changes in palatability and/or texture may have altered food intake to some extent.

In addition to the fat content in the diet, its composition in fatty acids has been shown to trigger differentially obesogenic factors. In particular, a higher proportion of saturated fatty acids is closely associated with resulting increased adiposity and insulin resistance [24]. By contrast, dietary unsaturated fats, particularly n-3 fatty acids from fish, have been demonstrated to limit fat accretion and hyperleptinemia [24,25] and to increase the thermogenic activity of brown adipose tissue [26]. In addition, unsaturated fatty acids, again especially n-3 [27,28] but also n-6 polyunsaturated fatty acids provided in a semisynthetic diet [21], have beneficial consequences on plasma cholesterol and insulin resistance. Therefore, a potential bias in the effects of the HFD3 diet is that it contains a larger proportion of corn oil (21%) than the other (including control) groups (6-12%). Because the rats fed with the HFD3

regimen were insulin-resistant, displayed impaired glucose tolerance, had the highest plasma total cholesterol and cholesterol with LDL, these effects are actually opposite to those expected from known benefits of (poly-)unsaturated fat consumption. First, it should be reminded that that the larger proportion of mono- and poly-unsaturated fats in the HFD3 comes from vegetal (i.e., corn), and not fish source. Second, corn oil contains mainly n-6 fatty acids (linoleic acid) and this oil is not recognized as a main source for n-3 fatty acids. Taken together, the most parcimonious hypothesis to explain the lack of obesogenic effects and the clear diabetogenic properties of HFD3 is that these differential changes are due to the largest proportion of energy derived from fat (both saturated and unsaturated) and/or to the absence of carbohydrate in this diet. Because the adiposity in HFD3-fed rats did not match that expected from the two other high-fat regimens with variable content in carbohydrate (HFD1 and HFD2), we rather favour the second hypothesis (i.e., carbohydrate-free diet) to be the main cause of the observed metabolic changes in HFD3-fed rats. In accordance with this interpretation, a recent study showed that a carbohydrate-free, high-fat diet also leads to a reduction in the body mass gain and development of body fat [23], even if in this study the lipid/protein ratio was close to 0.8 compared to 3 in the present work. Rather than a lack of dietary carbohydrate-induced thermogenesis, enzymatic activities measured by Pichon and colleagues in the white adipose tissue and the liver suggest that such a reduced gain of body fat in carbohydrate-free diets can be attributed to reduced hepatic lipogenesis [23].

When rats were challenged with exogenous insulin, there was a whole-body insulin resistance, indicative of compromised β -cell function, in both HFD1- and HFD3-fed rats, while only a trend for alteration was noted in HFD2-fed rats. Probably as a consequence of insulin resistance, all the high-fat diets tended to produce a hyperinsulinemia that might have been significant if the experiment was continued further.

The increase in body fat in HFD1- and HFD2-fed rats was associated with higher levels of plasma leptin, a hormone secreted by the adipocytes and thought to signal metabolic status from the adipocytes to peripheral tissues and the brain. Accordingly, in the HFD3 group that did not display increased adiposity, leptinemia was not significantly different from animals fed with the control diet. As expected, there was a strong correlation between plasma leptin and abdominal fat mass irrespective of the diet.

Adiponectin is another adipokine secreted by the adipocytes. Its role and regulation differ largely from leptin, because the development of the metabolic syndrome and diabetes is associated with a downregulation of adiponectinemia. Furthermore, adiponectin is now known to have potent insulin-sensitizing effects [29]. It is noteworthy that low levels of

plasma adiponectin was observed not only in HFD1 and HFD2 animals (i.e., those with increased body fat), but also in HFD3 rats (i.e., those with an adiposity close to normal values). This observation raised doubts of whether low plasma adiponectin can be used as a reliable marker of the metabolic syndrome [30]. In humans it has been proposed hypoadiponectinemia is more closely related to insulin resistance than adiposity *per se* [31,32]. The relationship was not clearly confirmed by our data in rats.

In conclusion, we propose that the carbohydrate/fat ratio of the diet may be important in the etiology of the metabolic syndrome linked to high fat diets. Still, we do not mean that certain high-carbohydrate, low-fat diets may not also predispose and/or generate a metabolic syndrome, but the present study was specifically focused on the carbohydrate/fat ratio during high-fat diet. The two diets combining high-fat and significant amount of carbohydrate led to a pre-diabetic state associated with an increase in both adiposity and leptinemia. Of interest, the high-fat, carbohydrate-free diet had only diabetogenic properties, but no obvious obesogenic features. Further experiments will be needed to understand the mechanisms underlying such a limited fat accretion (reduced hepatic lipogenesis?).

Even if it is clearly premature to consider the present data in a clinical perspective, defining appropriate diets on a long-term scale for preventing and/or treating the metabolic syndrome is still a crucial and complex issue. New dietary strategies using low-carbohydrate diets are emerging [33,34].

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FIGURES LEGENDS

Figure 1: Changes in body mass (panel A) and length (panel B), and body mass index (panel C) in rats fed either with low-fat, high carbohydrate diet (HCD, white circles or bars), high-fat, medium-carbohydrate diet (HFD1, light grey squares or bars), very high-fat, low-carbohydrate diet (HFD2, dark grey triangles or bars) or very high-fat, carbohydrate-free diet (HFD3, black circles or bars). * $P < 0.05$ compared to the control HCD group.

Figure 2: Adiposity, as determined by the ratio of abdominal (i.e., epididymal and retroperitoneal) white fat, in rats fed either with low-fat, high carbohydrate diet (HCD, white bar), high-fat, medium-carbohydrate diet (HFD1, light grey bar), very high-fat, low-carbohydrate diet (HFD2, dark grey bar) or very high-fat, carbohydrate-free diet (HFD3, black bar). * $P < 0.05$ compared to the control HCD group.

Figure 3A: Time-course of glucose tolerance following an oral administration of glucose (2 g/kg) in rats fed either with low-fat, high carbohydrate diet (HCD, white circles), high-fat, medium-carbohydrate diet (HFD1, light grey squares), very high-fat, low-carbohydrate diet (HFD2, dark grey triangles) or very high-fat, carbohydrate-free diet (HFD3, black circles). a, $P < 0.05$ for all groups compared to T0 min; b, $P < 0.05$ for all HF groups compared to T0; n.s., non-significant (HCD group at T120 min compared to T0 min).

3B: Time-course of changes in plasma glucose after an oral administration of water in rats fed either with low-fat, high carbohydrate diet (HCD, white circles), high-fat, medium-carbohydrate diet (HFD1, light grey squares), very high-fat, low-carbohydrate diet (HFD2, dark grey triangles) or very high-fat, carbohydrate-free diet (HFD3, black circles). No significant effect was detected.

3C: Time-course of changes in plasma glucose after insulin (1 IU/kg) injection in rats fed either with low-fat, high carbohydrate diet (HCD, white circles), high-fat, medium-carbohydrate diet (HFD1, light grey squares), very high-fat, low-carbohydrate diet (HFD2, dark grey triangles) or very high-fat, carbohydrate-free diet (HFD3, black circles). * $P < 0.05$ in HFD1 and HFD3 groups compared to the control HCD group at the same time-point.

Figure 4: Plasma insulin (A), leptin (B) and adiponectin (C) in rats fed either with low-fat, high carbohydrate diet (HCD, white bar), high-fat, medium-carbohydrate diet (HFD1, light

grey bar), very high-fat, low-carbohydrate diet (HFD2, dark grey bar) or very high-fat, carbohydrate-free diet (HFD3, black bar). * $P < 0.05$ compared to the control HCD group.

Figure 5: Plasma adiponectin expressed according to fat mass (panel A) and homeostatic model assessment and insulin resistance (HOMA-IR; panel B). Plasma leptin plotted to fat mass (panel C) and HOMA-IR (panel D). Only plasma leptin was positively correlated with fat mass and HOMA-IR. Rats were fed either with low-fat, high carbohydrate diet (HCD, white circles), high-fat, medium-carbohydrate diet (HFD1, light grey squares), very high-fat, low-carbohydrate diet (HFD2, dark grey triangles) or very high-fat, carbohydrate-free diet (HFD3, black circles). a.u., arbitrary unit.

Figure 6: Plasma total cholesterol (A), Low-density lipoprotein (LDL)-cholesterol (B), triglycerides (C) and free fatty acids (D) in rats fed either with low-fat, high carbohydrate diet (HCD, white bar), high-fat, medium-carbohydrate diet (HFD1, light grey bar), very high-fat, low-carbohydrate diet (HFD2, dark grey bar) or very high-fat, carbohydrate-free diet (HFD3, black bar). * $P < 0.05$ compared to the control HCD group. # $P < 0.05$ between HFD1 and HFD3.

TABLE LEGENDS

Table 1. Diets composition

HCD: low-fat, high carbohydrate diet

HFD1: high-fat, medium-carbohydrate diet

HFD2: very high-fat, low-carbohydrate diet

HFD3: very high-fat, carbohydrate-free diet

All diets contain cellulose (5-10% in mass), minerals (3-11% in mass) and vitamins (1 % in mass). CHO, carbohydrate.

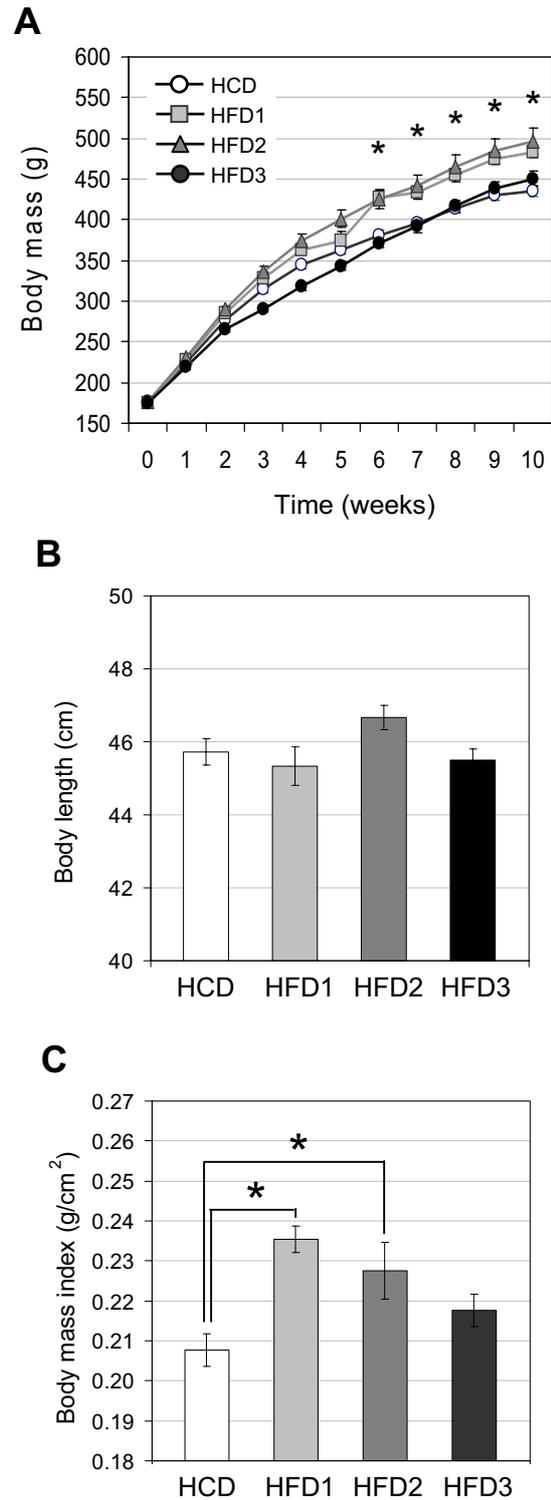


Fig. 1

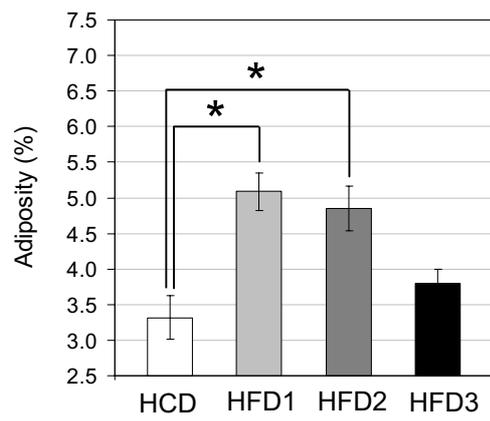


Fig. 2

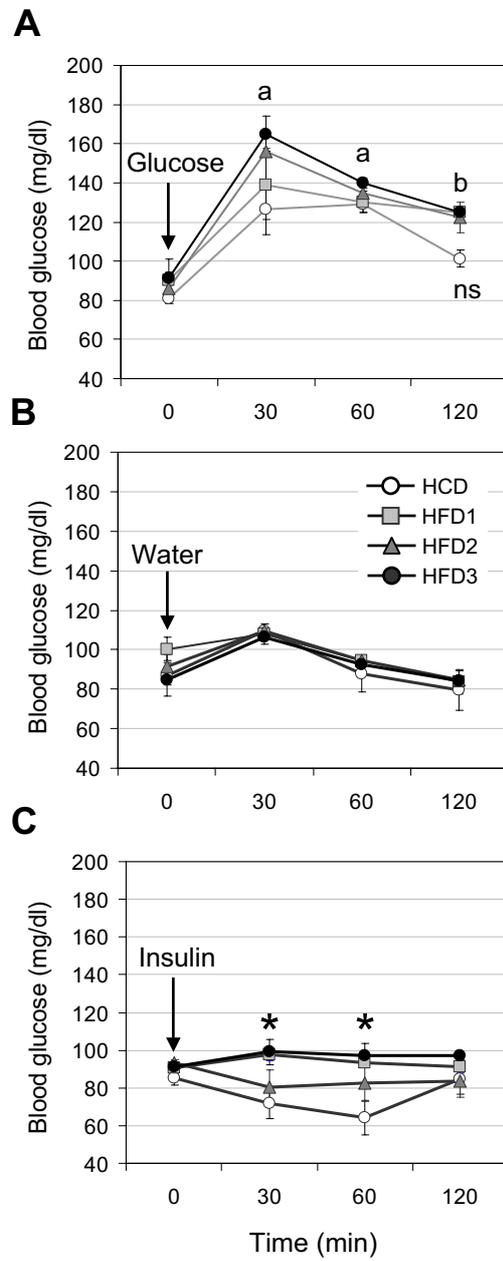


Fig. 3

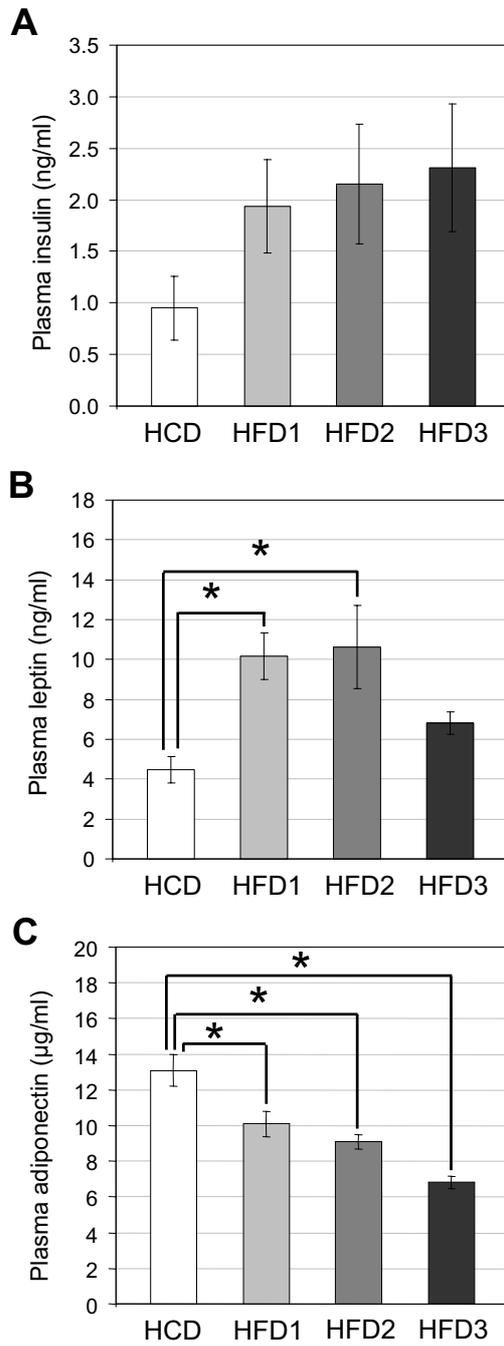


Fig. 4

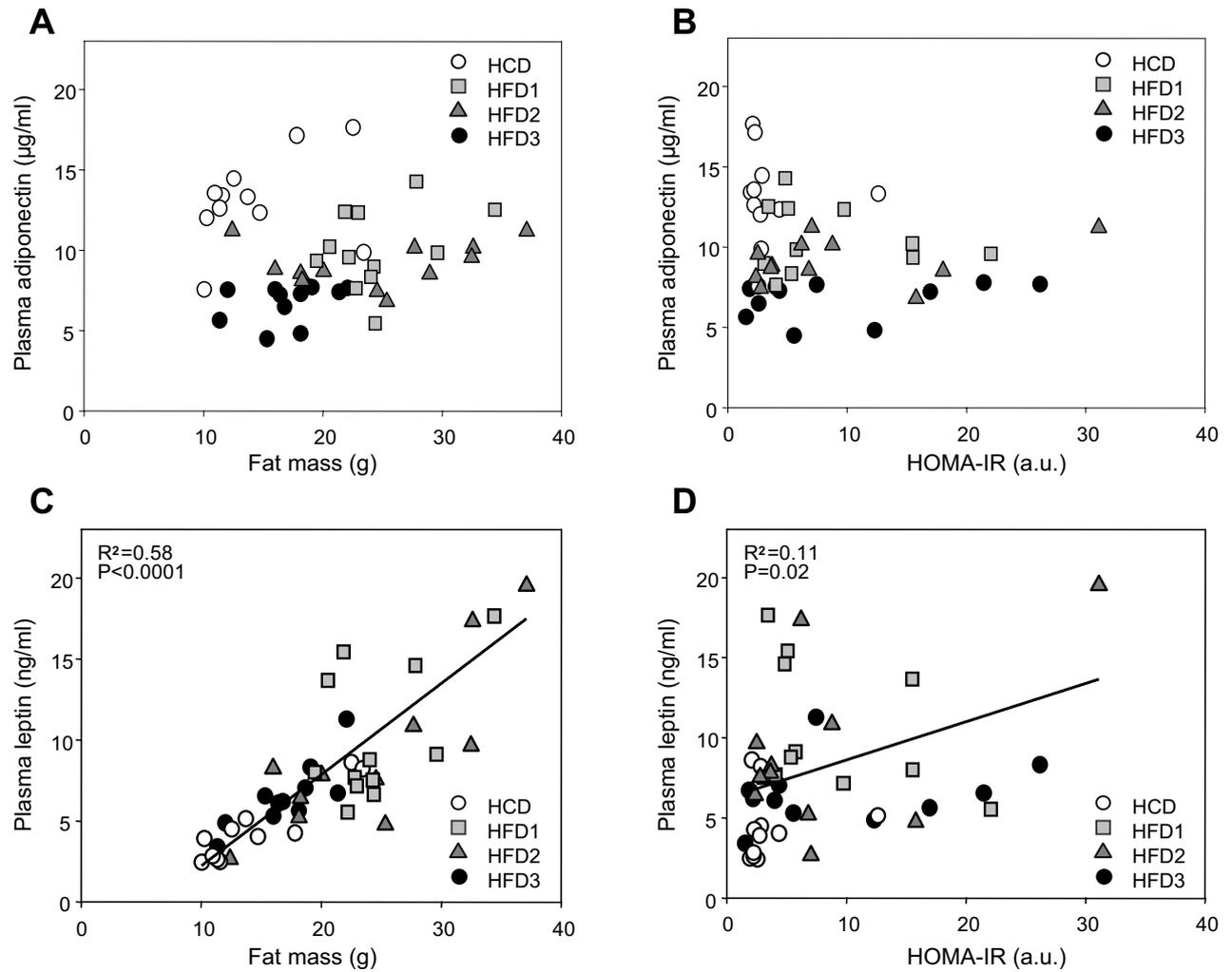


Fig. 5

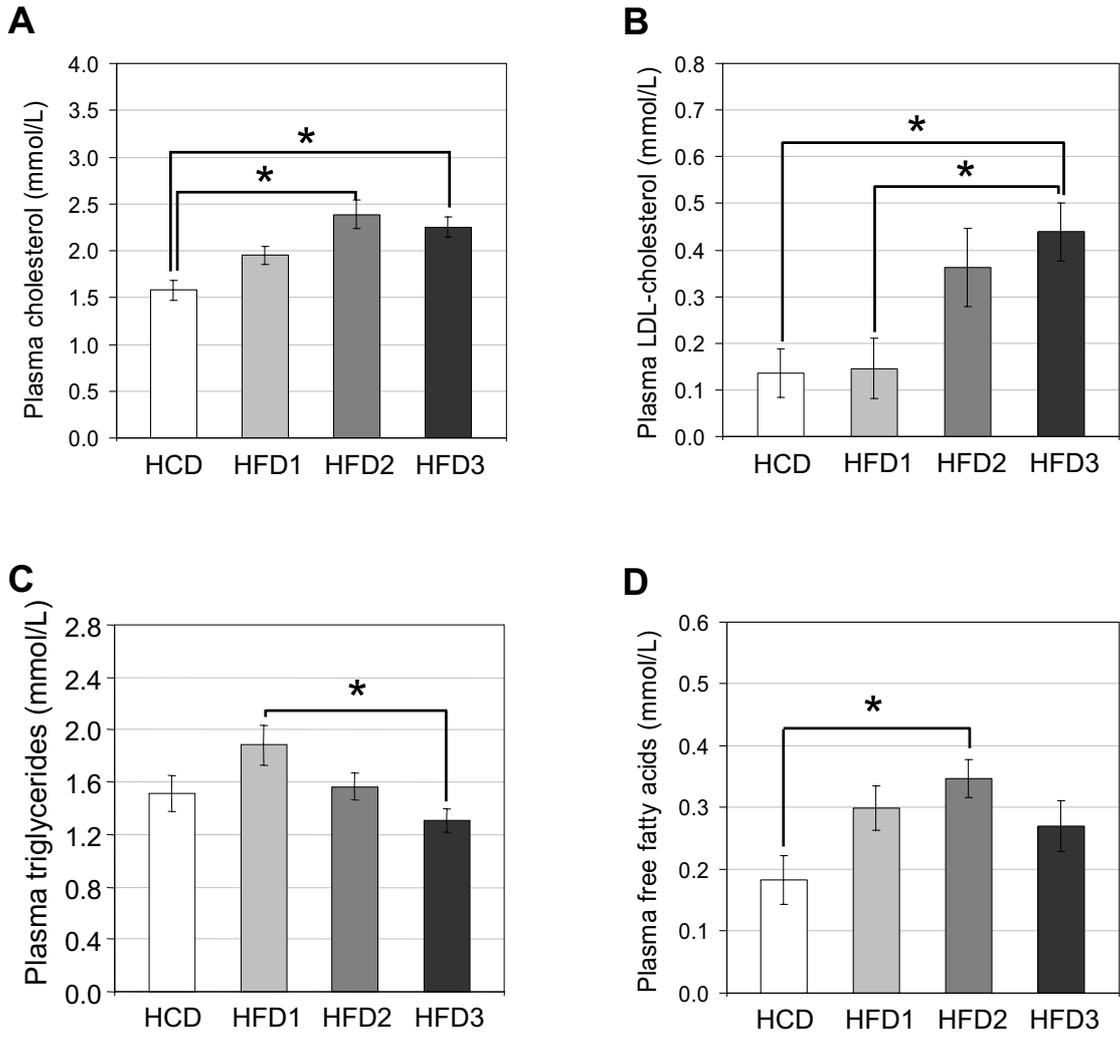


Fig. 6

Table 1. Composition of the diets

| | HCD | HFD1 | HFD2 | HFD3 |
|-------------------------|---------------------|---------------------------------|----------------------------------|----------------------------------|
| Energy content (kcal/g) | 3.7 | 4.7 | 5.2 | 5.9 |
| Protein (%) | 24 | 17 | 24 | 25 |
| CHO (%) | 64 | 30 | 9 | 0 |
| Fat (%) | 12 (corn oil 12) | 53 (corn oil 6) (lard 47) | 67 (corn oil 12) (lard 55) | 75 (corn oil 21) (lard 54) |