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comment

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

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ABSTRACT

Metabolic disorders induced by high-fat feeding in rodents evoke some, if not all, of the features of 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, in the present study, we investigated the long-term obesogenic and diabetogenic effects of three high-fat diets differing by their fat/carbohydrate ratios. Sprague–Dawley rats were fed a control high-carbohydrate and low-fat diet [HCD; 3:16:6 ratio of fat/carbohydrate/protein; 15.48 kJ/g (3.7 kcal/g)], a high-fat and medium-carbohydrate diet [HFD1; 53:30:17 ratio of fat/carbohydrate/protein; 19.66 kJ/g (4.7 kcal/g)], a very-high-fat and low-carbohydrate diet [HFD2; 67:9:24 ratio of fat/carbohydrate/protein; 21.76 kJ/g (5.2 kcal/g)] or a very-high-fat and carbohydrate-free diet [HFD3; 75:0:25 ratio of fat/carbohydrate/protein; 24.69 kJ/g (5.9 kcal/g)] for 10 weeks. Compared with the control diet (HCD), rats fed with high-fat combined with more (HFD1) or less (HFD2) carbohydrate exhibited higher BMI (body mass index; + 13 and + 10% 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 had insulin resistance. By contrast, a very-high-fat diet devoid of carbohydrate (HFD3) led to impaired glucose tolerance, insulin resistance and hypoadiponectinaemia (− 50%; $P < 0.05$), whereas BMI, adiposity and plasma leptin did not differ from respective values in animals fed the control diet. We conclude that increasing the 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 in 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

Key words: adiponectin, high-fat diet, insulin resistance, leptin, metabolic syndrome, obesity, Type 2 diabetes.

Abbreviations: a.u., arbitrary units; BMI, body mass index; CV, coefficient of variation; HCD, low-fat and high-carbohydrate diet; HDL-C, high-density lipoprotein cholesterol; HFD1, high-fat and medium-carbohydrate diet; HFD2, very-high-fat and low-carbohydrate diet; HFD3, very-high-fat and carbohydrate-free diet; HOMA, homoeostatic model assessment; HOMA-IR, HOMA of insulin resistance; LDL-C, low-density lipoprotein cholesterol; NEFA, non-esterified fatty acid.

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Table 1 Composition of the diets used in the present study

All diets contained cellulose (5–10 % in mass), minerals (3–11 % in mass) and vitamins (1 % in mass). All of the diets were obtained from Scientific Animal Food and Engineering.

	Diet			
Composition	HCD	HFD1	HFD2	HFD3
Energy content (kJ/g)	15.48 (3.7 kcal/g)	19.66 (4.7 kcal/g)	21.76 (5.2 kcal/g)	24.69 (5.9 kcal/g)
Protein (%)	24	17	24	25
Carbohydrate (%)	64	30	9	0
Fat (%)	12	53	67	75
Corn oil	12	6	12	21
Lard	—	47	55	54

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

MATERIALS AND METHODS

Animals

A total of 48 male Sprague–Dawley rats (Charles River Laboratories), weighing approx. 120 g upon their arrival, were housed individually, kept at 21 ± 1 °C under a 12 h light/dark cycle (lights on at 07.00 hours) and had *ad libitum* access to food (standard low-fat diet; Scientific Animal Food and Engineering) and tap water for 2 weeks.

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 no. 67-88).

Experimental design

Rats were then divided into four groups: (i) 12 control rats were fed a standard pelleted high-carbohydrate and low-fat diet [HCD; 15.48 kJ/g (3.7 kcal/g)]; (ii) 12 rats received a pelleted high-fat and medium-carbohydrate diet [HFD1; 19.66 kJ/g (4.7 kcal/g)]; (iii) 12 rats were fed a pelleted very-high-fat and low-carbohydrate diet [HFD2; 21.76 kJ/g (5.2 kcal/g)]; and (iv) 12 rats received a pelleted very-high-fat and carbohydrate-free diet [HFD3; 24.69 kJ/g (5.9 kcal/g)]. Each diet was obtained from Scientific Animal Food and Engineering, and the diet compositions are shown in Table 1. Diets were vacuum-packed and stored at 8 °C before use. Animals were given fresh food once a week. Body mass was measured every week. Due to considerable wasting (especially in rats fed HFD2 and HFD3), 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 removed from the experiment, leaving $n = 11$ control animals.

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

Analytical methods

To test oral glucose tolerance, blood glucose was assessed in rats fasted overnight just before, 30 min, 1 h and 2 h following oral administration of glucose (2 g/kg of body weight; Sigma) or water. Blood samples were collected via tail veins, and blood glucose was immediately

determined (Glucotrend premium kit; Roche Diagnostics). To optimize the timing of blood sampling and to avoid any time-of-day effect, 24 rats were sampled, half of them receiving either glucose or water. For each condition, three rats/diet were tested.

To assess insulin-induced hypoglycaemia, overnight-fasted rats received a subcutaneous injection of human insulin (1 international unit/kg of body weight; Umuline NPH; Lilly France) 2 h after the lights were turned on. Blood glucose collected as above was assessed just before, 30 min, 1 h and 2 h after insulin treatment. During the sampling period, rats had no access to food. Again, to optimize the timing of blood sampling and avoid any time-of-day effect, 24 rats ($n = 6$/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 NEFAs (non-esterified fatty acids; 'free fatty acids') with a RxL Dade Boehring analyser (Diamond Diagnostics) and plasma triacylglycerols (triglycerides), total cholesterol and HDL-C (high-density lipoprotein cholesterol) using an Advia 1650 analyser (Bayer Diagnostics). LDL-C (low-density lipoprotein cholesterol) was calculated as follows:

$$\text{LDL-C} = \text{total cholesterol} - (\text{HDL-C}) - (\text{triacylglycerols}/2.2). \quad (1)$$

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

HOMA (homoeostatic model assessment) was used to assess β-cell function, and insulin resistance (HOMA-IR) was calculated, as described by Matthews et al. [18], as follows:

$$\text{HOMA-IR} = (\text{fasting plasma glucose} \times \text{fasting plasma insulin})/22.5. \quad (2)$$

Statistical analysis

To test the effects of diet (HCD, HFD1, HFD2 or HFD3) and time (weeks), data were processed by ANOVA with or without repeated measures, depending on the parameter considered. If significant main effects or significant interactions were detected ($P < 0.05$), post-hoc comparisons were performed with a Tukey HSD test. Values are means $\pm$ S.E.M. For assessment of correlations, data were fitted to the following equation (SigmaPlot software; Jandel Scientific): $y = y0 + ax$, where a is the slope and $y0$ the intercept.

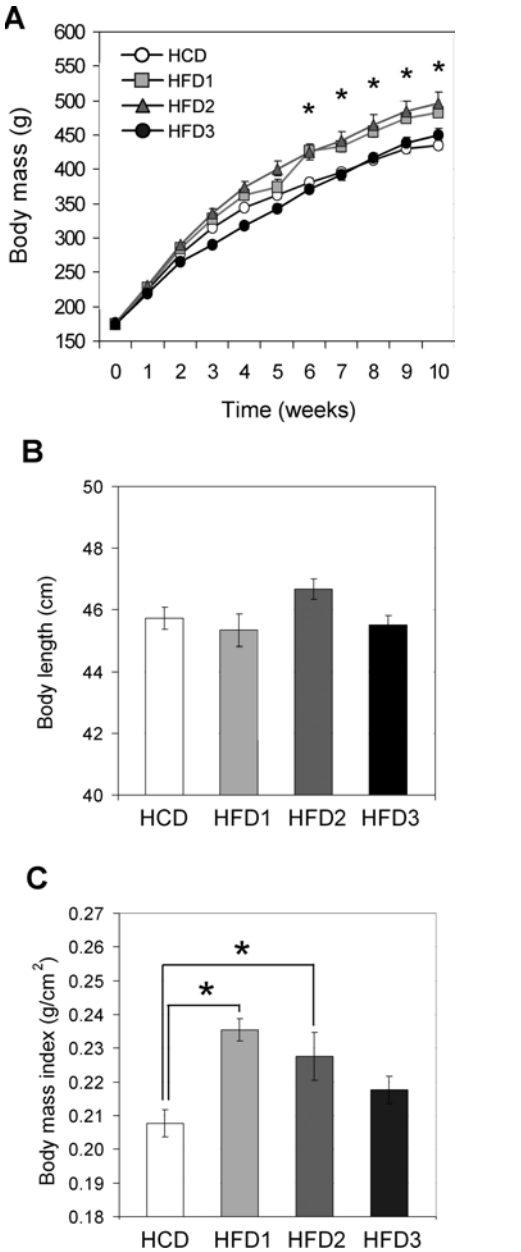

Figure 1 Changes in body mass (A), body length (B) and BMI (C) in HCD-, HFD1-, HFD2- or HFD3-fed rats

*$P < 0.05$ compared with the HCD group.

RESULTS

Body mass and adiposity

As shown in Figure 1(A), both HFD1 and HFD2 led to increased body mass compared with HCD 10 weeks

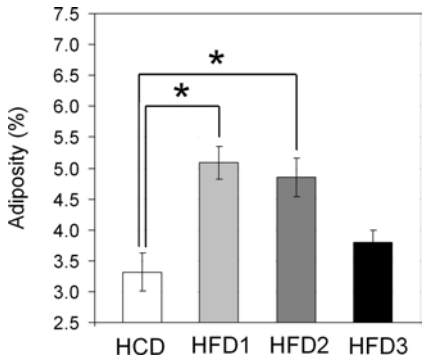

Figure 2 Adiposity in HCD-, HFD1-, HFD2- or HFD3-fed rats
Adiposity was measured as the ratio of abdominal (i.e. epididymal and retroperitoneal) white fat and body mass. $^{*}P < 0.05$ compared with control HCD group.

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

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

Glucose tolerance

Fasting blood glucose, determined in the morning (i.e. 2 h after the lights were turned on), did not differ significantly between the dietary conditions ($P > 0.05$; Figure 3A). Whichever diet was considered, handling and oral administration of water did not modify basal blood glucose significantly during the 2 h period of blood sampling ($P > 0.05$; Figure 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 (Figure 3A). Blood glucose was increased almost 2-fold in either HFD2- or HFD3-fed rats (Figure 3A), whereas only a trend to an increase was seen in the HFD1 group (Figure 3A). In addition, 2 h after glucose administration, in contrast with the control group, blood glucose in the three groups of animals fed high-fat diets (HFD1, HFD2 and HFD3) was still greater than the basal values ($P < 0.05$; Figure 3A), indicating a reduction in glucose tolerance.

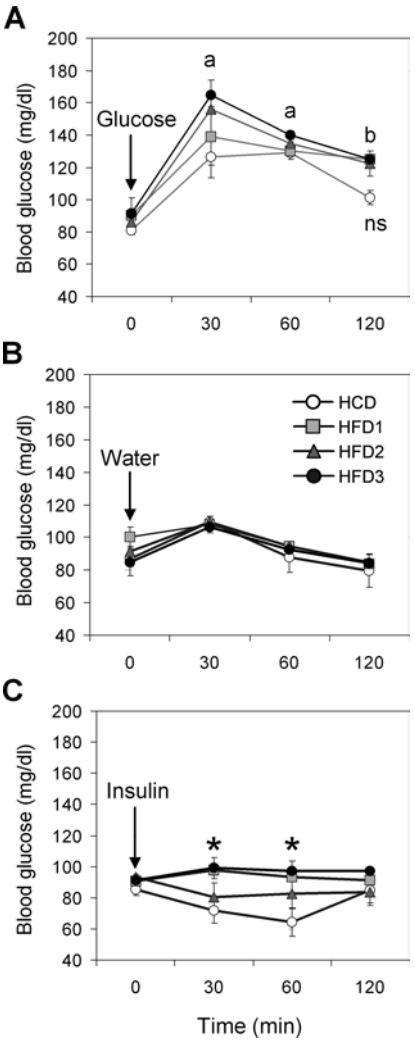

Figure 3 Time course of glucose tolerance following administration of glucose (A), and the time course of changes in plasma glucose after administration of water (B) or insulin (C) in HCD-, HFD1-, HFD2- or HFD3-fed rats
(A) Glucose tolerance was determined following the oral administration of glucose (2 g/kg of body weight) $^{a}P < 0.05$ for all groups compared with 0 min; $^{b}P < 0.05$ for all high-fat groups compared with 0 min; ns, non-significant (HCD group at 120 min compared with 0 min). (B) Time course of changes in plasma glucose. No significant effect was detected in the time course of plasma glucose after an oral administration of water. (C) Changes in plasma glucose after insulin (1 international unit/kg of body weight). $^{*}P < 0.05$ in the HFD1 and HFD3 groups compared with the control HCD group at the same time point.

Insulin resistance

In response to an acute administration of insulin after an overnight fast, control HCD-fed rats displayed the expected lowering in blood glucose, which returned to normal values 2 h after the treatment (Figure 3C). Albeit reduced, insulin-induced hypoglycaemia was not significantly impaired in HFD2-fed rats compared with control rats, whichever time point was considered ($P > 0.05$; Figure 3C). Unlike these limited alterations

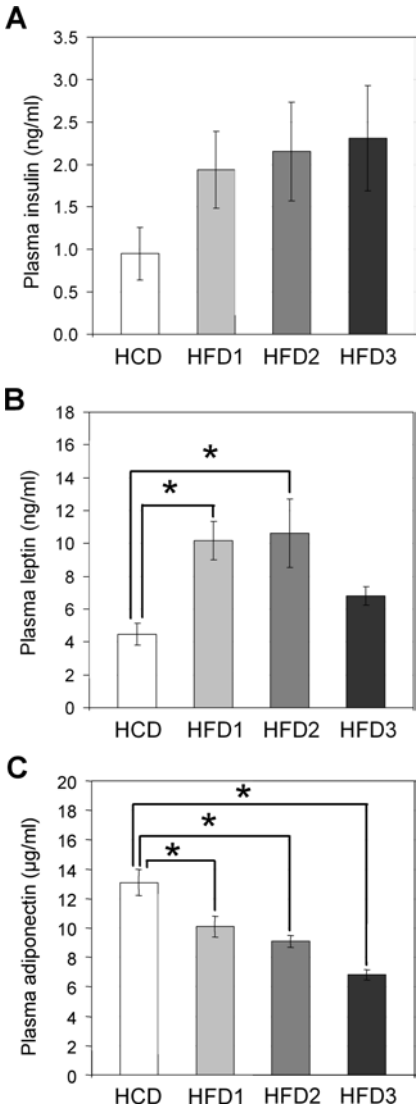

Figure 4 Plasma insulin (A), leptin (B) and adiponectin (C) levels in HCD-, HFD1-, HFD2- or HFD3-fed rats

*$P < 0.05$ compared with the control HCD group.

in the HFD2 group, blood glucose in HFD1- and HFD3-fed rats did not change during the testing period, leading to higher glucose levels 30 min and 1 h after insulin treatment in comparison with those in control rats ($P < 0.05$; Figure 3C), suggesting a reduced whole-body sensitivity to insulin.

Hormonal changes

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

HOMA-IR and correlations with adipokines

HOMA-IR tended to be higher in HFD1 [8.6 ± 1.8 a.u. (arbitrary units)], HFD2 (9.1 ± 2.5 a.u.) and HFD3 (8.9 ± 2.5 a.u.) groups compared with the control group (HCD, 3.5 ± 0.9 a.u.), but these differences did not reach significance ($P > 0.05$). Plasma adiponectin did not correlate with either fat mass (Figure 5A) or HOMA-IR (Figure 5B). By contrast, plasma leptin was significantly correlated with both fat mass (Figure 5C) and HOMA-IR (Figure 5D).

Circulating lipids

With regard to total cholesterol, there was an apparent increase in all rats fed high-fat diets, but the differences compared with control values were only significant in HFD2- and HFD3-fed rats ($P < 0.05$; Figure 6A). Furthermore, LDL-C was clearly altered in HFD3-fed rats, but not with in HFD1-fed rats (Figure 6B). Although not statistically significant, LDL-C levels tended to be increased in HFD2-fed rats.

Plasma triacylglycerols were not markedly modified by the diets, except a higher level in HFD1-fed rats compared with that in the HFD3 group was observed (Figure 6C). Moreover, NEFAs were increased in HFD2-fed rats ($P < 0.05$), but this higher level did not reach statistical significance in HFD1- and HFD3-fed animals (Figure 6D).

DISCUSSION

The present study demonstrates that, depending on the 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), whereas the high-fat and carbohydrate-free diet (HFD3) did not produce abdominal obesity, but led to insulin resistance and, by increasing LDL-C, also led to a higher risk of cardiovascular disease.

High-fat diets have been widely used in rodents to provide experimental models of human metabolic syndrome that simultaneously associate obesity, dyslipidaemia, diabetes-related alterations and, eventually,

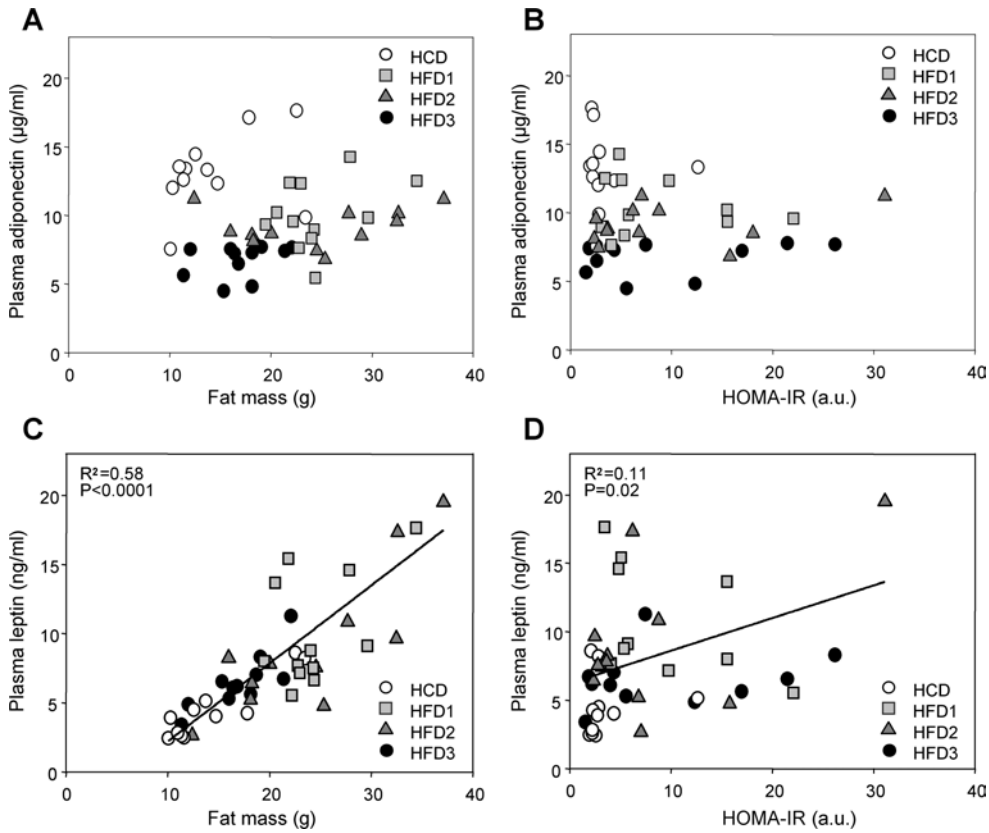

Figure 5 Plasma adiponectin (A and B) and leptin (C and D) expressed according to fat mass (A and C) and HOMA-IR (B and D) in HCD-, HFD1-, HFD2- or HFD3-fed rats

Only plasma leptin was positively correlated with fat mass and HOMA-IR.

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] and the present study), Wistar (e.g. [15]) or Long–Evans (e.g. [14,17]) rats. The increase in body mass obtained in the present study with both HFD1 (i.e. +11 %) and HFD2 (i.e. +13 %) is approximately consistent with previous experiments considering all of the experimental animals (i.e. with no post-hoc selection according to body mass gain) and having the same duration (e.g. +10 % after 10 weeks [17], and +10 % after 12 weeks [15]). The accretion of body fat in HFD1- and HFD2-fed rats was even clearer when considering BMI and adiposity values.

Moreover, we aimed to investigate 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 in their fat/carbohydrate ratio, while their protein content remained largely unchanged (range 17–25 % of metabolizable energy). The most pronounced manifestations of abdominal obesity and insulin intolerance were observed after feeding with HFD1, which is a diet containing high-fat and medium-carbohydrate proportions (in energy). Rats fed this diet gained more body mass, had a larger BMI, were fattier with high leptinaemia and low adiponectinaemia, and displayed physiological signs not only of impaired glucose tolerance, but also of whole-body insulin resistance compared with animals fed a standard high-carbohydrate diet. HFD2, the second diet in which the metabolic consequences were evaluated, contained very high fat (>50 %) and low carbohydrate. HFD2 led to approximately the same metabolic abnormalities as HFD1, except that the glycaemic responses to insulin treatment were less altered, being close to the values in the control group (HCD). Dyslipidaemia has been difficult to obtain in rats, even after modifications in the fat/carbohydrate ratio. In the present study, only HFD2- and HFD3-fed rats had altered profiles of circulating lipids. The increase in plasma NEFAs was only significant with HFD2. Nevertheless, except for dyslipidaemia, the results of the present study suggest that HFD1 is a better experimental model of human metabolic syndrome than the two other high-fat diets tested (i.e. HFD2 and HFD3).

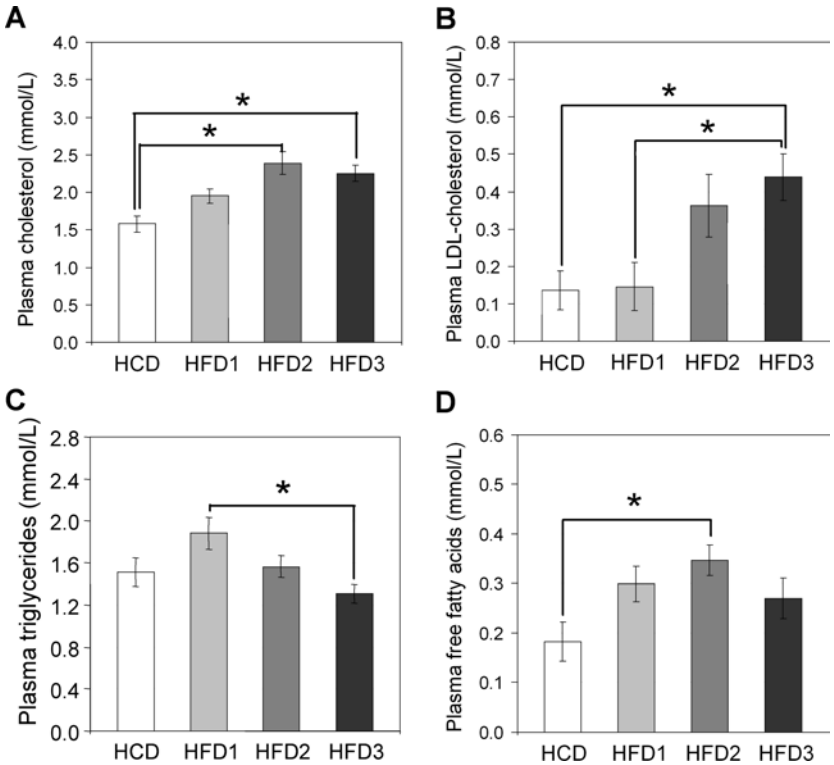

Figure 6 **Plasma total cholesterol (A), LDL-C (B), triacylglycerols (C) and NEFAs (D) in HCD-, HFD1-, HFD2- or HFD3-fed rats**

*$P < 0.05$ compared with the control HCD group (in A, B and D) or compared with HFD3 (in C). Free fatty acids, NEFAs; triglycerides, triacyclycerols.

Diets with different fat/carbohydrate ratios have been studied previously (e.g. in mice [19] and in rats [20–23]). The overall conclusion from these studies is as follows: the more the caloric content derives from dietary saturated fat, the fattier and less glucose-tolerant the animals become. To our knowledge, however, the previous studies have not investigated directly a carbohydrate-free/high-fat diet compared with a high-fat diet with variable amounts of carbohydrate, while keeping the 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 was 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 as the greatest BMI and adiposity were found in rats fed with the lowest energy content (i.e. HFD1 with 19.66 kJ/g) among the three high-fat diets studied. Conversely, the highest caloric diet (i.e. HFD3 with 24.69 kJ/g) did not lead to a significant increase in BMI or adiposity compared with control animals. Therefore these opposite responses clearly indicate that the different caloric content of the diets used in the present study is not the main factor causing fat accretion. Alternatively, even though 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 a resulting increase in adiposity and insulin resistance [24]. In contrast, dietary unsaturated fats, particularly $n-3$ (omega-3) fatty acids from fish, have been demonstrated to limit fat accretion and hyperleptinaemia [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 $n-6$ (omega 6) polyunsaturated fatty acids, provided in a semi-synthetic diet [21] have beneficial consequences on plasma cholesterol and insulin resistance. Therefore a potential bias in the effects of HFD3 is that it contains a larger proportion of corn oil (21 %) than the other (including control) groups (6–12 %). Because the HFD3-fed rats were insulin-resistant, displayed impaired glucose tolerance and had the highest plasma total cholesterol and LDL-C, these effects are actually opposite to those expected from the known benefits of (poly)unsaturated fat consumption. First, it should be highlighted that the larger proportion of mono- and poly-unsaturated fats in HFD3 comes from a vegetable (i.e. corn) and not a fish source. Secondly, corn

oil contains mainly $n-6$ fatty acids (linoleic acid), and this oil is not recognized as a major source of $n-3$ 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 [23] has shown that a carbohydrate-free and high-fat diet also leads to a reduction in body mass gain and the development of body fat, even if in this study [23] the lipid/protein ratio was close to 0.8 compared with 3 in the present study. Rather than a lack of dietary carbohydrate-induced thermogenesis, enzymatic activities measured by Pichon et al. [23] in white adipose tissue and liver suggested that such a reduced gain of body fat in carbohydrate-free diets can be attributed to reduced hepatic lipogenesis.

When rats were challenged with exogenous insulin, there was whole-body insulin resistance, indicative of compromised β-cell function, in both HFD1- and HFD3-fed rats, whereas only a trend in the alteration was observed in HFD2-fed rats. Probably, as a consequence of insulin resistance, all of the high-fat diets tended to produce hyperinsulinaemia 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, which did not display increased adiposity, leptinaemia was not significantly different from animals fed 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 adipocytes. Its role and regulation differ greatly from leptin, because the development of the metabolic syndrome and diabetes is associated with a down-regulation of adiponectinaemia. Furthermore, adiponectin is now known to have potent insulin-sensitizing effects [29,30]. It is noteworthy that low levels of plasma adiponectin were observed not only in HFD1- and HFD2-fed animals (i.e. those with increased body fat), but also in HFD3-fed rats (i.e. those with an adiposity close to normal values). This observation raises doubts as to whether low plasma adiponectin can be used as a reliable marker of the metabolic syndrome [31]. In humans, it has been proposed that hypoadiponectinaemia is more closely related to insulin resistance than adiposity [32,33]. The relationship was not clearly confirmed by the findings in the present study in rats.

In conclusion, we propose that the carbohydrate/fat ratio of the diet may be important in the aetiology of the metabolic syndrome linked to high-fat diets. Nevertheless, we do not mean that certain high-carbohydrate and low-fat diets also may not predispose and/or generate the metabolic syndrome, but the present study was specifically focused on the carbohydrate/fat ratio in a high-fat diet. The two diets combining high-fat and significant amounts of carbohydrate led to a prediabetic state associated with an increase in both adiposity and leptinaemia. Of interest, the high-fat and carbohydrate-free diet (HFD3) had only diabetogenic properties, but no obvious obesogenic features. Further experiments will be required to understand the mechanisms underlying such a limited fat accretion (possibly reduced hepatic lipogenesis).

Even if it is clearly premature to consider the results of the present study from 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, and new dietary strategies using low-carbohydrate diets are emerging [34,35].

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