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Impact of high-protein diets with either moderate or low carbohydrate on weight loss, body composition, blood pressure and glucose tolerance in rats

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One approach to achieve weight loss and decrease both obesity and associated morbidities involves high-protein, low-carbohydrate (HPLC) diets. This study compares the impact on metabolic health of HPLC and high-protein, medium-carbohydrate (HPMC) diets offered to diet-induced obese (DIO) rats. Weanling male rats were fed either a 37 % fat diet (n 48) or stock pellets (n 12) for 22 weeks. Rats fed the 37 % fat diet accumulated more body fat (26·6 *versus* 14·8 % body weight, $P<0·001$) compared with those on stock diet. The DIO rats had higher systolic blood pressure (+6·6 mmHg, $P=0·002$), fasting insulin (+63 % $P=0·006$) and areas under the glucose (+21 %, $P<0·001$) and insulin (+81 %, $P<0·001$) curves following an oral glucose tolerance test. DIO rats were then separated into four groups and offered for 8 weeks either: (1) the 37 % fat diet; (2) an HPLC or (3) HPMC diet; or (4) fed the 37 % fat diet to the intake of the HPMC group. Rats offered the 37 % fat or HPLC diets gained while those on HPMC lost body fat. Blood pressure was not altered by the dietary switch. Both HPLC and HPMC rats had lowered fasting insulin ($P=0·027$) and improved homeostatic assessment (HOMA; $P=0·011$) that was not different from those of stock animals. These improvements occurred despite differences in fat gain, and indicate that both weight loss and macronutrient intake can impact favourably on obesity-associated morbidities.

Low-carbohydrate diets: Rats: Obesity: Insulin sensitivity

Obesity is associated with co-morbidities, including dyslipidaemia, hypertension, cardiovascular disorders, and insulin insensitivity leading to type 2 diabetes. Combinations of these co-morbidities, loosely grouped under the term metabolic syndrome (Kahn *et al.* 2005), impact on longevity and quality of life. Therefore, the inexorable rise in obesity in more affluent countries (World Health Organization, 2000) has associated burgeoning costs of health care (Avenell *et al.* 2004; Kuller, 2006), In consequence, a large number of behavioural, surgical and nutritional strategies have been proposed to either halt or reverse the obesogenic trend. One popular approach with the general public involves use of low-carbohydrate (ketogenic) diets as these appear to induce satiety at intakes below energy maintenance (Stadler *et al.* 2003; Johnstone *et al.* 2006). Such diets have provoked fierce debate amongst nutritionists, however, as the low carbohydrate intake is partly substituted by fat and this may increase the dyslipidaemia often observed in the obese (Grundy, 2005) and contribute to hypertension, plaque formation and other CVD (Lorenzo *et al.* 2006; Truesdale *et al.* 2006). Nonetheless, in human trials, the effects of such diets on blood lipid parameters have been either favourable or neutral (e.g. Foster *et al.* 2003; Wood *et al.* 2006). This is probably because either the enforced (Foster *et al.* 2003) or voluntary (Stadler *et al.* 2003; Johnstone *et al.* 2006) reduced intake results in negative energy balance and oxidation of both dietary and stored fat.

This raises the question – how do such low-carbohydrate diets lead to reduced voluntary intake? Often such diets contain high amounts of protein, the macronutrient with the greatest impact on satiety (e.g. Weigle *et al.* 2005). Alternatively, low carbohydrate supply may alter insulin status and sensitivity, with impacts on appetite (Hellstrom *et al.* 2004; Wynne *et al.* 2005). Finally, during ketosis, regions of the brain switch from glucose as the primary fuel to oxidation of ketone bodies (Hawkins *et al.* 1986). These regions include those critically involved in regulation of appetite (Archer *et al.* 2004), and thus alteration in their fuel use may influence intake. A second question relates to the reported beneficial effects on metabolic health of low-carbohydrate diets (Foster *et al.* 2003; Samaha *et al.* 2003; Johnstone *et al.* 2006) – are these due to the associated weight loss or are the improvements linked to the pattern of macronutrient intake (Volek & Feinman, 2005)? For example, a recent report (Boden *et al.* 2005) showed that insulin sensitivity in the obese, as determined by homeostatic assessment (HOMA), improved

Abbreviations: AUC, area under the curve; DIO, diet-induced obese; HOMA, homeostatic assessment; HPMC, high-protein, medium-carbohydrate; HPLC, high-protein, low-carbohydrate; IAUC, incremental area under the curve; ME, metabolisable energy; OGTT, oral glucose tolerance test; tAUC, total area under the curve.

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within 2 weeks on a low-carbohydrate diet, before there were appreciable changes in body weight or fat mass.

While these questions may be partly answered by direct studies in obese humans, some of the more detailed information requires invasive approaches and use of appropriate animal models that mimic the human situation. A number of dietary-induced obese (DIO) rodent models have been developed to examine impacts on either blood pressure (e.g. Velkoska *et al.* 2005) or insulin sensitivity (e.g. Madsen *et al.* 2002), but rarely have both morbidities been studied at the same time in rodents. Furthermore, technical constraints have limited the ability to monitor longitudinal changes in body composition in rats or mice, and thus to link such changes with metabolic responses. Finally, many studies of rodent models have described the effect of rate of body fat gain, but few have attempted to look at the loss of lipid stores from obese animals under different dietary conditions. Yet this is exactly the situation that faces many dieters' having become obese, how do they lose weight and what is the impact on overall metabolic health?

The current study describes a DIO rat model, based on offering a diet similar in macronutrient energy composition to the average UK human diet, and then quantification of the gain in body fat. The impact of the resultant obesity on blood pressure and aspects of the glucose insulin axis (HOMA and an oral glucose tolerance test (OGTT)) was then assessed. This was followed by offering two high-protein diets, one moderate and the other low in carbohydrate, with changes in body weight and body composition monitored over an 8-week period, with intermediate measures of blood pressure and glucose tolerance.

Materials and methods

Rats and diets

Sixty male Rowett strain Hooded Lister rats were weaned at 19 d of age onto CRM (P) (Special Diet Services, Witham, Essex, UK) stock cubes, with measured digestible and estimated metabolisable energy (ME) of 13·20 and 12·13 MJ/kg, partitioned as Atwater fuel energy, protein 22 %, fat 9 %, carbohydrate 69 %. At 4 weeks of age, the rats were randomly separated into two groups of similar average weight. One group (*n* 12) were maintained on stock while the others (*n* 48) were transferred to a semi-synthetic 37 % fat diet (Table 1), with measured digestible energy of 18·96 MJ/kg and estimated ME of 17·98 MJ/kg as fed, partitioned by Atwater coefficients as protein 16 %, fat 37 %, carbohydrate 47 %. While this is similar in energy composition to the average UK human diet, the maize oil used (MP Biochemicals Inc., Solon, OH, USA) contains less saturated fat (10–12 %) and more MUFA plus PUFA (39–42 and 43–47 %, respectively) than the average human fat intake. Animals were housed four to a cage for the next 18 weeks and then were housed singly for the remainder of the study (total 30 weeks). Throughout the study, individual rats were weighed twice weekly, with food intake per cage measured continuously throughout. All procedures were approved by the Animal Ethics Committee of the Rowett Research Institute under the auspices of the UK Animals (Scientific Procedures) Act 1986.

Table 1. Compositions, as g/kg as fed, of the various diets used in this study

	Stock*	37 % fat	HPLC	HPMC
Casein	(184)	180	474	418
Methionine + cysteine		3	3	3
Sugar	(39)	193		
Cornstarch	(758)	325	24	279
Cellulose		50	50	50
Maize oil	(34)	200	200	200
Suet			200	
Vitamins and minerals†		50	50	50
% Energy				
Protein	22·0	16·4	36·6	36·9
Carbohydrate	68·9	46·8	7·6	27
Fat	9·1	36·8	55·8	36·1
Energy MJ/kg AF				
Actual DE	13·20	18·96	24·91	19·83
Calculated ME	12·13	17·98	22·22	17·56
% DM	89·9	93·6	95·0	91·8
ME MJ/kg DM‡		19·98	23·26	20·37

HPLC, high-protein. low-carbohydrate; HPMC, high-protein, medium-carbohydrate; AF, as fed; DE, digestible energy; ME, metabolisable energy.
* Values in parentheses are for stock diet, CRM (P), provided by the manufacturer
† Contains 10 g of AIN-93G vitamin mixture, 35 g of AIN-93 VX minerals mixture (both from MP Biomedicals Europe, Illkirch, France), 2 g of choline chloride and 3 g of potassium phosphate.
‡ Based on assumed N in urine (digested N − retained N) × 40 kJ/gN.

Measurements

Body composition. Whole body composition (as total fat and lean) of conscious animals was quantified by MRI (EchoMRI, Houston, TX, USA) at intervals during the trial. The machine was originally calibrated by the manufacturer based on chicken breast (for lean) and canola oil (for fat). The lean calibration was checked with weights (from 75 to 600 g) of chicken breast analysed for dry matter plus lean and fat content (the latter approximately 2 %), by Dumas combustion and extraction with petroleum spirit, respectively, and appropriate corrections applied. The relationship against the manufacturer's lean calibration was $0{\cdot}947x + 6{\cdot}0$, $R^2 = 0{\cdot}999$. The lipid calibration was tested against freshly dissected rat fat (from 5 to 375 g), also analysed for N, fat and dry matter. The relationship between measured and observed calibration (based on the canola oil calibration) was $0{\cdot}959x + 2{\cdot}31$, $R^2 = 0{\cdot}999$. These calibration factors were then used to correct the MRI output to obtain body composition as fat and lean mass (g).

First measurements on the rats were made at 14 weeks, and from 18 weeks onwards the animals (six rats per day) were scanned at 2-weekly intervals until the end of the study.

Blood pressure. At 19 weeks, blood pressure was measured using the tail cuff procedure (Rees *et al.* 2006). Rats were left in a cage over a plate heated to 28°C for 10 min and lightly restrained manually during the measurement. Data were recorded using an MP35 BSL system (BioPac Systems, Inc, Goleta, CA, USA) with software BSL Pro 7. Systolic pressure was determined from the pressure at which a pulse was first detected. The procedure was repeated until three acceptable traces were obtained; this required the

rat to be motionless. The rats were measured again on the following 2 d and the composite data analysed.

Oral glucose tolerance test. Starting at week 20, over a 2-week period, six rats were selected daily at random for the 2 h measurement of OGTT. On the previous day, body composition was quantified by MRI scan and then the rats were fasted overnight (18.00 h onwards). At 08.00 h the next morning, 0·25 ml of blood was taken from the tail tip into Eppendorf tubes containing 10 μl of heparin solution (15 IU/ml). Animals were then given a gavage of a 50 % (w/v) glucose solution equivalent to 2 g of glucose per kg body weight. Samples of blood (150 ml) were then taken from the tail tip at 7, 15, 30, 60, 90 and 120 min. Animals were then returned to their normal cages and allowed free access to food and water. Insulin was assayed using a Mercodia enzyme-linked immunosorbent assay (Mercodia AB, Uppsala, Sweden) and glucose was quantified by an enzyme-linked procedure based on a glucose oxidase/peroxidase method with 4-amino-antipyrine as dye (Maloney *et al.* 2003). Both analyses were quantified spectrophotometrically by plate reader. Areas under the curve (AUC) for both plasma insulin and glucose were calculated for both total (tAUC) and incremental (iAUC; Wolever & Jenkins, 1986, 1997) values. Various indices were used to assess glucose tolerance, insulin sensitivity or insulin resistance, and these were based on either the fasting glucose and insulin concentrations or the responses to the oral glucose load. These methods included fasting insulin resistance index, quantitative insulin sensitivity check index, simplified formula for the HOMA method, insulin sensitivity index, insulin sensitivity index of glycaemia; insulin sensitivity index, oral glucose insulin sensitivity; and insulin sensitivity index (composite), composite insulin sensitivity index for the hepatic and peripheral tissues. These are all described and discussed for rats in Tran *et al.* (2003). Many gave similar conclusions and, for simplicity, therefore, HOMA, calculated as (fasting insulin × fasting glucose)/22·5, and iAUC and tAUC, was used as the main indices.

Diet switches for rats fed the 37 % fat diet

At week 22, the 48 rats on the 37 % fat diet were allocated to four matched groups (each n 12), based on percentage body fat as assessed by the MRI scans. Each group was then allocated to one of four dietary treatments (Table 1), either: (1) remaining on the 37 % fat diet; (2) a high-protein, low-carbohydrate (HPLC) diet; (3) a high-protein, medium-carbohydrate (HPMC) diet; or (4) the 37 % fat diet but with each animal fed the ME intake of the weekly group average of the HPLC rats (GF). In practice, within the first week of the dietary switch, it became apparent that the GF animals could not match the energy intake of those on HPLC. This was because of the higher energy density of the HPLC diet compared with either the 37 % fat or HPMC diets (Table 1). In consequence, after 7 d, the GF rats were then matched to the ME intake of the HPMC group. The remaining twelve animals (stock) continued to be fed CRM pellets. Because only six animals could be measured by the OGTT each day, the starts for these dietary regimes were staggered over a 2-week (2 × 5 d) period. For the first 8 d, animals were allocated on a random basis from each of the stock, 37 % fat, HPLC and HPMC groups (i.e. one rat from two groups and two rats from the other two groups). Within each group, the start dates for the rats were based on descending order of percentage body fat. The GF animals were started on days 9 and 10. Time on treatment was set from the day of allocation and so all main measurements (MRI scans and OGTT) were staggered over 2-week periods, with six animals measured per day, and with the MRI scans performed the day before each OGTT. Animals remained on these diet allocations between weeks 22 and 30.

Faeces were collected over a 4 d interval during weeks 26 and 27 from stock (n 6) and 37 % fat, HPLC and HPMC (all n 8) animals so that dry matter, energy (bomb calorimetry) and N (Dumas combustion) digestibilities could be calculated (Table 1). These were used to estimate energy intakes throughout the trial. At the end of week 27, blood pressure was again measured on three successive days.

At the start of week 28, rats were fasted overnight and an OGTT was performed as for the pre-diet allocation procedure. At week 30 (i.e. 8 weeks after diet switch), animals were anaesthetised using isofluorane inhalation at 10.00 h, decapitated and trunk blood taken into two tubes containing either EDTA or heparin. Plasma samples (EDTA) were assayed for insulin and leptin using a Lincoplex dual assay kit (Linco Research, Inc., St Charles, MO, USA) measured on a Luminex 100 system (Luminex Corporation, Austin, TX, USA). Analyses were by enzyme-linked assays for plasma glucose, cholesterol, triglycerides (Thermo Scientific kits 981304, 981813, 981301, respectively; Labmedics Ltd, Salford, Manchester, UK), NEFA (kit 999–75 406; Wako Chemicals GmbH, Neuss, Germany) and 3-hydroxybutyrate (based on Li *et al.* 1980) using a Konelab 30 discrete clinical analyser (Thermo Electron Corp, Vantaa, Finland).

Statistical analysis

Data from the first stage of the study (stock *versus* 37 % fat diet) were analysed by a two-sample t test. For OGTT data, two-way ANOVA was conducted, with effects of diet, time and their interactions as fixed effects. Results from the five dietary groups during the last 8 weeks of the study were analysed by one-way ANOVA, as were differences between the four high-fat groups (37 % fat, HPMC, HPLC and GF). The status of the animals at the start of this second phase (e.g. fat content, blood pressures or OGTT) was also included as an appropriate covariate to allow for any differences established in the pre-diet switch period (weeks 1–22). For a number of variables, the covariate regression was significant but, despite this, inclusion in the analysis rarely altered the main findings. For simplicity, therefore, only the results of the one-way ANOVA are reported unless inclusion of a covariate altered the findings. Any significant differences in main effects were further assessed on group means by a *post hoc* t test. All analyses were performed with Genstat 8th Edition, Release 8·1 (VSN International Ltd., Hemel Hempstead, Herts, UK).

Results

Chow versus *37 % fat dietary treatments*

Food intake and weight gain. Body weights and body weight gains were either greater or equal for stock compared with the

37 % fat diet for the first 6 weeks, but from 8 weeks onwards the latter rats were heavier ($P<0{\cdot}05$). By week 22 (diet switch), the 37 % fat diet animals weighed, on average, 80 g more than rats fed the stock diet ($P<0{\cdot}001$; Table 2). Average intakes over weeks 1–22 were 24·6 and 18·2 g/d for stock and 37 % fat diets ($P<0{\cdot}001$) with corresponding ME intakes of 298 and 327 kJ/d ($P=0{\cdot}004$), respectively.

Body composition. By week 14, the stock and 37 % fat rats had mean weights of 505 and 549 g ($P<0{\cdot}001$); this included 70 and 145 g of fat ($P<0{\cdot}001$), respectively. Lean tissue was not significantly different (425 *versus* 417 g). At week 20, the time of the first OGTT, respective mean body weights had increased to 577 and 644 g ($P<0{\cdot}001$), fat to 86 and 172 g ($P<0{\cdot}001$) and lean tissue to 441 and 436 g (not significant; Table 2). Together, these accounted for 0·91–0·95 of live weight, with the remainder attributed to bone mass (which gives a low signal in the MRI scanner), glycogen and gut fill. Fat comprised 14·8 and 26·6 % of live weight (15·4 and 27·1 % of fasted weight) for stock and 37 % fat groups, with lean tissue as 76·5 and 67·7 % (79·5 and 68·9 % of overnight fasted weight).

Blood pressure. There was a significant day effect ($P=0{\cdot}003$) for systolic pressures, associated mainly with higher values for the first day; this may involve an animal 'training' effect. There were no differences between days 2 and 3, and these were then averaged to test for diet effects. Rats on the 37 % fat diet had higher systolic pressure (6·6 mmHg, $P=0{\cdot}002$; Table 2).

OGTT (Table 3 and Fig. 1) . Fasting glucose was not different between the two diets (Table 3). Within 60 min after the oral glucose dosing, plasma values had returned ($P>0{\cdot}05$) to pre-dose concentrations for rats fed stock diet (Fig. 1a). In contrast, the post-dose values for the 37 % fat group remained higher ($P<0{\cdot}001$) than pre-dose concentrations throughout, and even at 120 min were still 24 % greater. From 30 to 120 min, the values for the 37 % fat group exceed those for the stock-fed animals by 24–31 % ($P<0{\cdot}005$). In consequence, both iAUC ($P=0{\cdot}009$) and tAUC ($P<0{\cdot}001$; Fig. 1a, Table 3) were greater for the 37 % fat group.

Fasting plasma insulin was 63 % higher ($P=0{\cdot}006$, Table 3, Fig. 1b) for rats fed the 37 % fat diet compared with those offered stock pellets, and this also resulted in higher HOMA values ($P=0{\cdot}005$). Rats on stock increased ($P<0{\cdot}05$) plasma insulin 15 and 30 min post-dose, but at all other times the values were not different from fasting concentrations. In contrast, for rats on 37 % fat, insulin concentrations remained above fasting from 15 min onwards ($P<0{\cdot}001$), and even at 120 min were still more than double the pre-dose values. Furthermore, in comparison with the stock group, insulin values for the 37 % fat rats were greater ($P<0{\cdot}001$) from 60 min onwards. Consequently, the AUC were greater for the 37 % fat group compared with stock-fed animals for both the incremental (+88 %; $P=0{\cdot}007$) and total (+80 %; $P<0{\cdot}001$) areas. The maximal insulin concentration (at 30 min) was greater for 37 % fat rats than those on stock; this indicates that the inability to lower glucose concentration to fasting concentrations when animals were fed the 37 % fat diet was not due to less pancreatic release of insulin.

The main parameters of the glucose–insulin axis correlated ($P<0{\cdot}001$) with body fat (either in absolute terms or as a percentage of body weight), e.g. HOMA ($R=0{\cdot}56$), glucose tAUC ($R=0{\cdot}50$), fasting insulin ($R=0{\cdot}51$), insulin iAUC ($R=0{\cdot}42$) and insulin tAUC ($R=0{\cdot}54$). Weaker relationships ($P<0{\cdot}01$) were observed between systolic blood pressure and body fat ($R=0{\cdot}36$) and between systolic blood pressure and fasting insulin ($R=0{\cdot}42$).

Table 2. Body composition and blood pressure data from Hooded Lister rats (25 weeks old) fed for 20 weeks on either stock pellets (*n* 12) or a 37 % fat diet (15 % protein; 48 % carbohydrate; 37 % fat; *n* 48)

	Stock	37 % fat	SED	*P*
Body composition				
Live weight (g)	567·4	644·0	10·78	<0·001
Fat g	85·9	171·5	6·81	<0·001
Lean g	441·2	435·6	7·01	NS
% Fat BW	14·8	26·6	0·84	<0·001
% Lean BW	76·5	67·7	0·78	<0·001
Systolic blood pressure (mmHg)*	136·0	142·6	2·04	0·002

* Average data for days 2 and 3 of measurement, minimum of three measurements per day.

Responses of obese rats to dietary interventions

Food intake, weight gain, body composition and blood pressure (Table 4 and Fig. 2). After the 8 weeks on the new diet allocations, all groups were heavier, except for rats on the HPMC diet who lost weight (average 25 g), and this was different ($P<0{\cdot}001$) from the other groups that originated from animals fed the 37 % fat diet. Rats on the HPLC diet ate less food (g/d, $P<0{\cdot}001$) than those on the 37 % fat and HPMC diets but, due to the greater energy density of this diet, they had the largest daily ME intake (kJ/d, $P<0{\cdot}001$). In contrast, the HPMC rats had the lowest ME intake. Protein intakes were more than doubled for the two high-protein groups compared with 37 % fat rats, while for the HPLC animals carbohydrate consumption was only approximately 16 %. Changes in body weight and fat gain were approximately linear during the 8 weeks following diet switch (Fig. 2). Over this period, most of the differences in body weight reflected changes in fat gain (groups 37 % fat, GF and HPLC) or fat loss (HPMC). The GF animals lost the most lean tissue, probably related to the restricted intake of the 16 % protein diet. While the ME intakes of the GF and HPMC groups were similar, energy retention was positive in the former and negative in the latter (11·0 *versus* −15·4 kJ/d, respectively). This may reflect either differences in physical activity between the two groups or a higher thermic effect of high protein intake as observed in humans (Westerterp-Plantenga *et al.* 1999). There were no changes in systolic pressure, although this remained lower ($P=0{\cdot}02$) for those rats offered stock compared with all other groups.

Glycaemia, insulinaemia and OGTT (Table 5) . At diet switch, animals originally fed the 37 % fat diet were allocated to the four matched groups based on percentage body fat and not on responses to the first OGTT. In practice, this selection introduced a bias between groups with lower initial glucose iAUC (197 *versus* 440 mM/2 h, $P<0{\cdot}016$) and tAUC (1003 *versus* 1242 mM/2 h) for rats maintained on the 37 % fat

Table 3. Oral glucose tolerance test data from Hooded Lister rats (25 weeks old) fed for 20 weeks on either stock pellets (*n* 12) or a 37 % fat diet (15 % protein; 48 % carbohydrate; 37 % fat; *n* 48)

	Stock	37 % fat	SED	*P*
Fasted weight (g)	555·2	632·4	10·64	<0·001
Glucose (mM) time 0	7·144	7·491	0·345	NS
Glucose (mM) time 120*	7·486	9·269***	0·360	<0·001
Insulin (mU/l) time 0	37·2	60·9	8·25	0·006
Insulin (mU/l) time 120*	41·9	121·4***	19·24	<0·001
HOMA (mU × l^{-1} × mM)	12·0	20·2	2·84	0·005
iAUC glucose (mM/2 h)	197	320	45·5	0·009
tAUC glucose (mM/2 h)	1003	1215	45·2	<0·001
iAUC insulin (mU/l per 2 h)	4898	9236	1536	0·007
tAUC insulin (mU/l per 2 h)	8892	16 090	1971	<0·001
Glucose/insulin iAUC (mM/mU per l)	0·0445	0·0209	0·00495	<0·001
Glucose/insulin tAUC (mM/mU per l)	5·13	2·06	0·967	0·002

NS, not significant; HOMA, homeostatic assessment; iAUC, incremental area under the curve; tAUC, total area under the curve.
* Data also compared within the variable for effect of time (***P<0·001).

compared with the other three groups. Other parameters, including fasting glucose, all insulin parameters and HOMA, were not different between the original 37 % fat groups at diet switch. Nonetheless, all parameters were also tested with inclusion of a covariate to allow for any differences in starting values. Comparisons were made with results from rats fed stock both included and excluded; the latter allowed the impact of diet switch from 37 % fat to be analysed in more detail.

At 6 weeks post-diet switch (week 28), rats fed stock diet had lower fasting glucose (6·44 *versus* 7·10 mM, P=0·002), iAUC (190 *versus* 288 mM/2 h, P=0·006) and tAUC (960 *versus* 1137 mM/2 h, P<0·001) for glucose than animals on the other diets, similar to the findings at week 20. In contrast, for insulin parameters, including fasting values, iAUC and tAUC, the stock animals were not significantly different from the other four groups combined, neither was HOMA.

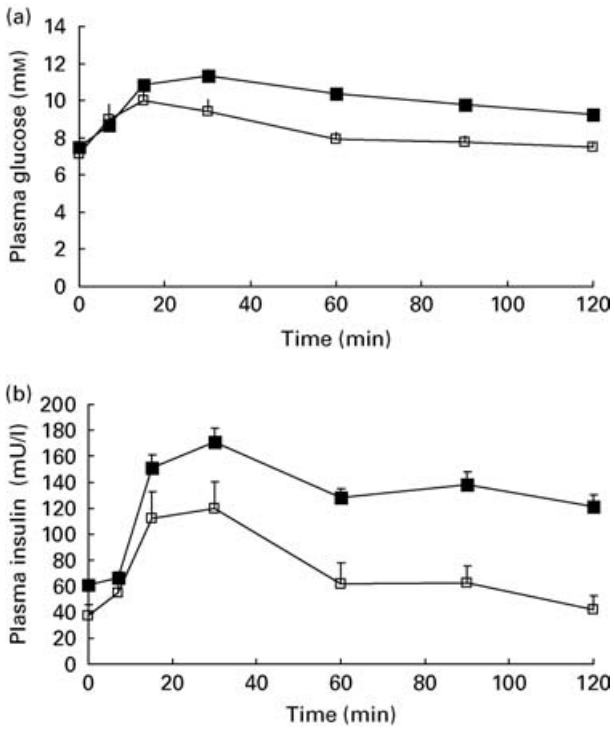

Fig. 1. Temporal changes in plasma (a) glucose and (b) insulin in overnight fasted rats given an oral dose of glucose (2 g/kg body weight). Rats were offered either stock pellets (□) or a 37 % fat diet (■). Data are means with their standard error.

These differences compared with findings at 20 weeks were related to changes within the four groups following the diet switch from 37 % fat. For example, fasting insulin concentrations were lower (P=0·011) for the GF, HPLC and HPMC groups compared with 37 % fat. This difference remained even after covariate inclusion.

Furthermore, the GF, HPLC and HPMC groups were not different from rats that continued on the stock diet. In contrast, fasting glucose concentrations were similar for the 37 % fat, GF and HPMC diets and greater than for the HPLC diet (P<0·05). HOMA (based on the product of insulin and glucose concentrations) reflected these responses, with greater values for rats maintained on 37 % fat diet compared with the GF, HPMC and HPLC groups (P≤0·006).

Following the oral glucose dose, insulin iAUC and tAUC were lower for rats fed HPMC (P<0·05) compared with those that continued on 37 % fat. This was due to higher insulin concentrations for the latter throughout the post-dose period, as reflected in the difference between the diets at 120 min (P<0·05; Table 5). For glucose, again iAUC and tAUC were similar for 37 % fat, HPMC and HPLC, but the GF values were lower (P<0·05) than for those that remained on the 37 % fat diet.

Several of the parameters that showed correlations with body fat (either absolute or as percentage body weight) prior to the diet switch were maintained (P<0·01); these included HOMA (R = 0·34), glucose tAUC (R = 0·43), fasting insulin (R = 0·43), insulin iAUC (R = 0·49) and tAUC (R = 0·57). Again, a weaker relationship was maintained with systolic blood pressure (R = 0·37).

Terminal biochemical measurements (Table 6). The terminal measurements were performed on non-fasted animals, except for the GF rats that were deprived of food for 9 h. Plasma leptin was lower (P<0·001) in the stock-fed rats compared with the other groups, but there were no differences between plasma insulin. Differences in plasma glucose concentrations (P<0·001) were due to the lower values for the fasted GF rats; the other groups of fed animals showed no

Table 4. Body weight, composition, food intake, macronutrient intake (protein, carbohydrate and fat), metabolisable energy intake and blood pressure in obese rats following 8 weeks on diets of either 37 % fat, HPLC, HPMC or the 37 % fat diet fed to the group intake mean of HPMC (GF)

	37 % fat	GF	HPLC	HPMC	Stock	SED	P_{all}*	P_{HF}†
Body composition								
Start‡								
BW (g)	651·2	649·9	635·8	658·6	579·7	13·26	<0·001	NS
Fat (g)	173·1	164·1	168·0	171·4	86·4	10·36	<0·001	NS
Lean (g)	444·6	448·5	433·0	449·9	447·4	9·40	NS	NS
End (gain)§								
BW (g)	25·0	6·3	15·2	−25·5	10·6	6·09	<0·001	<0·001
Fat (g)	18·8	18·2	20·6	−21·4	−2·9	4·55	<0·001	<0·001
Lean (g)	1·0	−13·9	−8·4	−9·1	7·9	3·62	0·002	<0·001
FI (g/d)	17·00	15·92	15·54	16·54	23·30	0·359	<0·001	<0·001
Protein (g/d)	3·08	2·88	7·41	6·96	4·29	0·137	<0·001	<0·001
CHO (g/d)	9·66	9·04	1·54	5·44	18·57	0·203	<0·001	<0·001
Fat (g/d)	3·40	3·18	5·80	3·31	0·79	0·095	<0·001	<0·001
MEI (kJ/d)	305·6	286·2	345·4	290·4	282·7	7·05	<0·001	<0·001
Systolic BP (mmHg)	134·21	130·07	134·13	134·61	127·65	2·55	0·075	NS

HPLC, high-protein low-carbohydate; HPMC, high-protein, medium-carbohydrate; HF, high fat; BW, body weight; NS, not significant; FI, food intake; CHO, carbohydrate; MEI, metabolisable energy intake; BP, blood pressure.
* Analysis for all groups, including rats fed stock (SED refers to this analysis).
† Analysed for four groups selected from rats fed on the original 37 % fat diet.
‡ Data at the time (22 weeks) the rats fed from weaning on the 37 % fat diet were re-allocated to subsequent treatment diets.
§ Changes after 8 weeks on diets (difference between week 30 and week 22 data).
An additional group of animals was maintained on stock pellets. For all groups, *n* 12 except for stock and GF (*n* 11 in both cases).

differences. Plasma 3-hydroxybutyrate was greatest in the fasted GF rats, followed by those on the HPLC diet (both $P<0·001$ compared with each other and the remaining groups). For lipid parameters, there were no differences in plasma cholesterol or NEFA. Stock animals had higher triacylglycerols ($P<0·001$) than any of the other groups, with values for the 37 % fat rats also greater than those for the remaining groups, while the GF rats were the lowest ($P<0·05$).

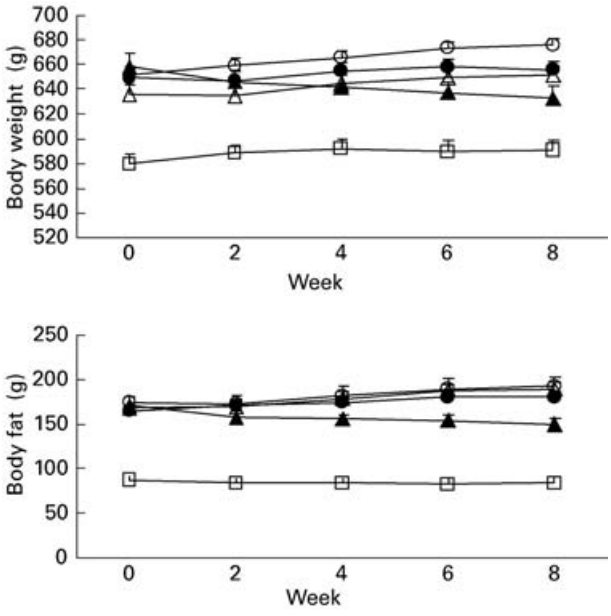

Fig. 2. Body weight (g) and body fat (g) during the last 8 weeks of the study after diet switch. Rats remained on either ther 37 % fat diet (○) or stock pellets (□), or were switched to high-protein, low-carbohydrate (Δ), high protein-moderate carbohydrate (▲) or fed the 37 % fat diet to intake of the HPMC diet (●).

Discussion

The first objective of this study was to make rats obese through feeding a diet similar to the average macronutrient energy composition consumed by humans in the UK and to see if this resulted in aspects of metabolic syndrome, including raised systolic blood pressure and impaired insulin sensitivity. The second objective was to offer these obese rats diets high in protein, with either moderate or low carbohydrate, and assess impacts on food intake, body composition and parameters of metabolic syndrome. These results would be compared with published human data to assess the reliability of the rodent model for studies on underlying mechanisms in obese humans.

DIO rats

A large number of studies have shown that feeding rats or mice high-fat diets results in increased weight gain and adiposity (e.g. Levin *et al.* 1997; Archer *et al.* 2003; Alexander *et al.* 2006). In the current study, body fat was approximately doubled from week 14 onwards and, in view of observations that early changes in fatness occur before differences in weight gain are apparent (Archer *et al.* 2004), an obesogenic condition was probably prolonged over much of the current study. The slow development of obesity with the current protocol matches the situation in the human population.

Blood pressure. It has long been recognised that hypertension in both men and women correlates with obesity (Kannel *et al.* 1967) and forms an important component of the health risks contained within the definition of metabolic syndrome (Kahn *et al.* 2005). A number of rodent studies have also shown that obese animals have increased systolic blood pressure (e.g. Dobrian *et al.* 2000; Wilde *et al.* 2000; Yoshioka *et al.* 2000; Velkoska *et al.* 2005), although this has not always been observed (Beltowski *et al.* 2006). Such changes have also

Table 5. Fasting glucose and insulin concentrations, HOMA calculation and OGTT data in obese rats following 6 weeks on diets of either 37 % fat, HPLC, HPMC or the 37 % fat diet fed to the group intake mean of HPMC (GF)

	37 % fat	GF	HPLC	HPMC	Stock	SED	P_{all}*	P_{HF}†
Fasted weight (g)	662·1	651·2	652·8	617·5	574·2	12·96	<0·001	<0·001
Glucose (mM) time 0	7·242	7·117	6·772	7·277	6·436	0·2482	0·005	NS
Glucose (mM) time 120	9·816	8·872	9·254	9·449	7·653	0·364	<0·001	NS
Insulin time 0 (mU/l)	75·1	56·3	51·4	47·8	48·1	9·28	0·027	0·011
Insulin time 120 (mU/l)	148·6	120·8	111·5	100·3	75·1	18·92	0·006	0·054
iAUC glucose (mM/2 h)	322	220	309	291	190	42·3	0·010	NS
tAUC glucose (mM/2 h)	1189	1065	1120	1163	960	46·8	<0·001	0·092
iAUC insulin (mU/l per 2 h)	7624	8497	6900	5649	6407	1302·7	NS	NS
tAUC insulin (mU/l per /2 h)	16 443	15 321	13 070	11 358	12 173	2164·5	NS	0·062
HOMA (mU × l^{-1} × mM)	24·29	17·97	15·50	15·41	14·06	3·032	0·011	0·006

HOMA, homeostatic assessment; OGTT, oral glucose tolerance test; HPLC, high-protein low-carbohydate; HPMC, high-protein, medium-carbohydrate; HF, high fat; NS, not significant; iAUC, incremental area under the curve; tAUC, total area under the curve.
* Analysis for all groups (including rats fed stock); SED refers to this analysis.
† Analysed for four groups selected from rats fed on the original 37 % fat diet.
An additional group of animals was maintained on stock pellets. For all groups, *n* 12 except for stock and GF (*n* 11 in both cases).

correlated with the higher concentrations of plasma leptin and insulin (Velkoska *et al.* 2005), but the correlation with fasting insulin was weak in the current study. Such comparisons may be confounded by inclusion of maize oil in the current diets, because this can lower blood pressure (Langley-Evans *et al.* 1996; Truett *et al.* 1998) and may reduce the impact of obesity on hypertension.

OGTT and insulin sensitivity. Decreased insulin sensitivity is a characteristic feature of metabolic syndrome in humans, usually first detected as increased plasma glucose concentrations despite elevated insulin following an overnight fast. In the current study, the simpler approaches based on either fasting glucose and insulin concentrations (e.g. HOMA), or the OGTT were used rather than surgically invasive hyperinsulinaemic–euglycaemic clamps (Madsen *et al.* 2002; Tran *et al.* 2003). These simplified approaches are not perfect substitutes but, in rats, approximately 50 % of the variability (R^2) with glucose infusion rate during an insulin clamp could be accounted for by the insulin tAUC (taken as a measure of insulin resistance) or the tAUC for glucose/insulin (a marker of insulin sensitivity) during an OGTT (Tran *et al.* 2003). The HOMA approach has also been shown to correlate well with clamp data in humans ($R = 0{\cdot}62$; Yeni-Komshian *et al.* 2000).

The current results support the myriad of observations that demonstrate obese rodents to have elevated fasting plasma insulin (e.g. Gao *et al.* 2002; Madsen *et al.* 2002; Catalano *et al.* 2005; Clegg *et al.* 2005; Morris *et al.* 2005) occasionally in combination with elevated fasting glucose (Molnar *et al.* 2005; Thim *et al.* 2006). Obese rats require lower glucose infusion to maintain euglycaemia during hyperinsulinaemic clamps (Chang *et al.* 1990; Tran *et al.* 2003; Catalano *et al.* 2005) or have a greater AUC for insulin during OGTT (Madsen *et al.* 2002; Tran *et al.* 2003). This was also observed here, but only 10–35 % of the observed variation in OGTT was explained by the overall animal fatness. Although this study had the advantage of longitudinal measures of body fat content, the MRI scan did not discriminate regional fat distribution and, in both humans (Coon *et al.* 1992) and rodents (Barzilai & Rossetti, 1996; Catalano *et al.* 2005), decreased susceptibility to insulin was associated with increased intra-abdominal fat.

Effect of diet switch

Intake and body composition. Rats switched to the two high-protein diets (HPLC and HPMC) showed modest reduced food intake, by 9 and 3 % as g/d, less than the 13–16 % observed in other studies (Jean *et al.* 2001; Mithieux *et al.* 2005) with diets that included an even higher proportion of gross energy as protein (50 %, compared with 36 % in the current experiment). The modest responses in rodents compares with reductions

Table 6. Plasma concentrations of insulin, leptin, glucose, cholesterol, NEFA, triacylglycerols and 3-hydroxybutyrate after 8 weeks of diet switch

	37 % fat	GF	HPLC	HPMC	Stock	SED	P_{all}*	P_{HF}†
Leptin (pM)	740	802	733	712	290	128·1	0·001	NS
Insulin (pM)	299	241	470	247	401	161·2	NS	NS
Glucose (mM)	11·17	9·43	10·72	10·72	11·11	0·446	0·002	<0·001
Cholesterol (mM)	2·306	2·114	2·273	2·205	2·463	0·1203	0·069	NS
NEFA (mM)	0·515	0·562	0·591	0·519	0·504	0·047	NS	NS
Triacylglycerols (mM)	1·222	0·633	0·993	0·853	2·107	0·099	<0·001	<0·001
3-OHB (mM)	0·430	2·492	1·391	0·872	0·587	0·1713	<0·001	<0·001

HPLC, high-protein low-carbohydate; HPMC, high-protein, medium-carbohydrate; HF, high fat; NS, not significant; 3-OHB, 3-hydroxybutyrate.
* Analysis for all groups (including rats fed stock); SED refers to this analysis.
† Analysed for four groups selected from rats fed on the original 37 % fat diet.
These animals were not fasted, except for 9 h of food withdrawal for the GF rats. Animals were fed 37 % fat, *ad libitum* or pair fed (GF) to the HPMC group, HPLC or HPMC, with *n* 12 per group except for stock and GF (*n* 11 in both cases).

of energy intake up to 35 % below maintenance requirements observed in obese humans offered high-protein diets (Skov *et al.* 1999; Stadler *et al.* 2003; Weigle *et al.* 2005; Johnstone *et al.* 2006). These reductions led to substantial loss of body weight (Skov *et al.* 1999; Stadler *et al.* 2003; Johnstone *et al.* 2006), much of this as body fat (Johnstone *et al.* 2006).

Nonetheless, even small changes in intake (or metabolism) produce substantial changes over the long term. For example, for the rats fed the HPMC diet, body fat was reduced by 12 % over 8 weeks; this represents a difference in energy retention of 14 kJ/d, similar to the 5 % decrease in ME intake observed. Rats fed the HPLC diet had the lowest absolute intake (g/d) but gained the most fat due to the higher energy density of the ration. This raises the possibility that a combination of high protein and low carbohydrate in the diet has synergistic effects in terms of lowering appetite, at least for the absolute weight of food eaten. Recent data from humans offered isoenergetic rations (Johnstone *et al.* 2006) indicate that diets supplying 30 % of energy as protein with low (20 g/d) as opposed to moderate (170 g/d) carbohydrate intake produce an additional modest reduction in intake (approximately 0·7 MJ/d), coupled with feeling less hungry. Such findings may explain why HPLC diets can be effective in achieving weight loss.

Health. It is well established that risks associated with obesity-related metabolic syndrome e.g. insulin resistance, dyslipaemia, hypertension and CVD, are all improved by weight loss (Pasanisi *et al.* 2001). In the current study, the dietary changes did not improve systolic blood pressure, while, in human studies, effects of short-term weight loss induced by high-protein diets on blood pressure have been equivocal (Elliott *et al.* 2006). Reduced plasma cholesterol has been a specific target towards reducing cardiovascular risk, and concerns have been expressed about the consequences of high-fat diets (e.g. Nordmann *et al.* 2006). In practice, for many human trials with low-carbohydrate diets, the total fat consumption is equal or below normal due to either the enforced (Foster *et al.* 2003; Samaha *et al.* 2003) or the voluntary (Stadler *et al.* 2003; Johnstone *et al.* 2006) reduction of intake. Consequently, blood lipid parameters remain unchanged or improve with such diets (Foster *et al.* 2003; Volek *et al.* 2003; Crowe, 2005; Johnstone *et al.* 2006). This was also observed with the rats fed the HPLC diet that comprised 56 % of the dietary energy supplied as fat, much in saturated form, and where there was no increase in plasma cholesterol. Such comparisons need to be treated with caution, however, because there were no differences in cholesterol concentrations between the stock and 37 % fat groups either, and this may simply reflect the differences in fat metabolism between rodents and humans (Siguel, 1983; Nishina *et al.* 1991).

The current study did show changes in the indirect measures of insulin sensitivity and resistance for rats switched from the 37 % fat diet. This was most clear for fasting plasma insulin concentrations that were reduced by 32–37 % in the HPLC and HPMC groups, and were not different from those of rats fed the stock diet. These changes were reflected in the 37 % lowering in calculated HOMA values for both the HPLC and HPMC groups and where, 2 h after the oral glucose load, plasma insulin concentrations or tAUCs were not different from those of rats fed stock pellets. Despite these insulin responses, plasma glucose concentrations 2 h after the oral dose were greater for all rats initially offered the 37 % fat diet compared with those on stock pellets, as were the incremental and total AUCs for glucose. Therefore, the apparent improvement under basal (fasting) conditions for both high-protein groups did not fully extend to the induced hyperglycaemic state and, therefore, full restoration of glucose tolerance (and thus insulin sensitivity) was not achieved. This is not too surprising when the relatively modest fat loss (12 %) is considered for the HPMC group. Nonetheless, in humans, even a relatively small weight loss, assumed to be mainly as fat, is associated with improvements in indices of insulin sensitivity (Diabetes Prevention Program Research Group, 2002; Foster *et al.* 2003; Samaha *et al.* 2003; Johnstone *et al.* 2006).

Although fat loss is the preferred route to improve indices of insulin sensitivity in the obese, other options exist. For example, in the rats fed the HPLC diet, and where body fat increased by 12 % over the 8 weeks of dietary intervention, fasting insulin and HOMA improved. This probably relates to the low carbohydrate intake, because reduced fasting plasma glucose and insulin concentrations have been observed for humans in response to such diets within 4 d (Fery *et al.* 1982), with improved insulin sensitivity for obese patients by 2 weeks (Boden *et al.* 2005), before any substantial loss of weight. Furthermore, in obese volunteers on high-protein weight loss diets, the HOMA values were lowered more with low than moderate carbohydrate intakes (Johnstone *et al.* 2006). Together, these data suggest that insulin sensitivity may be improved independently by either fat loss or markedly lowered carbohydrate supply. Indeed, the current rodent data suggest that this latter improvement can occur in the face of increased fat deposition. While improvements in metabolic syndrome through diet alone but in the absence of weight loss is not the ideal scenario, there may be situations where improvements induced by changes in macronutrient supply are better than none at all, and may provide short-term approaches to reducing certain health risks.

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