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Rapeseed protein inhibits the initiation of insulin resistance by a high-saturated fat, high-sucrose diet in rats

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In contrast to the quality of carbohydrates and lipids, little is known on the influence of the type of dietary protein on the development of the metabolic or insulin resistance syndrome. Cysteine intake has been recently documented to impact insulin sensitivity. The aim of this study was to determine whether rapeseed protein, an emergent cysteine-rich protein, could inhibit the onset of the metabolic syndrome. For 9 weeks, rats were fed a diet rich in saturated fats and sucrose, which also included 20 % protein either as milk protein ('Induction' diet I) or rapeseed protein (diet R). A third, control group received an isoenergetic diet containing milk protein but polyunsaturated fats and starch ('Prudent' diet P). Plasma glucose, insulin, TAG and cholesterol, and blood pressure were monitored during the study, glucose tolerance was tested at week 7 and body composition determined at week 9. Plasma glucose, insulin and TAG increased during the experiment and, at week 9, plasma insulin was significantly 34 % lower in the R group and 56 % lower in P group as compared with the I group. The insulin peak after the glucose load was significantly 28–30 % lower in R and P than in I and the insulin sensitivity index was significantly higher in R than in I. Unexpectedly, peripheral fat deposition was slightly higher in R than in I. In this model, substituting rapeseed protein for milk protein had preventive effects on the early onset of insulin resistance, similar to those achieved by manipulating the types of dietary fat and carbohydrates.

Insulin resistance: Dietary protein: Metabolic syndrome: Body composition: Cysteine: Glutathione

The metabolic syndrome is a corpus of cardiovascular risk factors that cluster together. The clinical criteria used to define metabolic syndrome vary, but it is usually defined – according to National Cholesterol Education Program - Third Adult Treatment Panel – as comprising three or more of the following abnormalities: hypertriacylglycerolaemia, low HDL, high fasting glucose, excessive waist circumference and elevated blood pressure, on the basis of associations with adverse cardiovascular outcomes derived from large research trials[1]. In addition, adults with the metabolic syndrome are at a greater risk of developing diabetes mellitus. The prevalence of the metabolic syndrome is rapidly increasing in Western countries, being about 10 % in France and higher than 25 % in the general population in the USA[2,3]. Beyond its clinical definitions, and from a pathophysiological point of view, the metabolic syndrome is mainly considered as the expression of a complex, multi-tissue insulin resistance syndrome, so has therefore been termed 'insulin resistance syndrome' by many investigators[4–6]. Indeed, notwithstanding the complexity of the pathophysiological interrelations, insulin resistance is central to the onset of the metabolic syndrome, or at least that of most of the clusters grouped under the definition of the metabolic syndrome[1,7–10]. From an experimental point of view, studies in rodents fed Western-type diets rich in saturated fats and refined carbohydrates have shown that diet-induced insulin resistance precedes other aspects of the metabolic syndrome[11].

Strategies targeting the nutritional prevention and treatment of the metabolic syndrome are still under debate. Body-weight control, manipulation of the quantities and quality of carbohydrates and lipids and higher dietary fibre contents are the main strategies considered for metabolic syndrome patients.

A large body of evidence shows that the type of energy macronutrients in the diet, namely, lipids (PUFA, in particular *n*-3, *v.* SFA) and carbohydrates (low *v.* high glycaemic index) has a major impact on insulin sensitivity[12–16]. By contrast, the quality of dietary protein has been overlooked. However, we[17] and others[18] have shown that some types of dietary protein can specifically prevent insulin resistance in rats fed a high-sucrose diet, without any modifications to body weight or composition. The mechanisms are traditionally ascribed to the amino acid pattern of dietary sources[19]. Recently, we demonstrated that a large proportion of the

Abbreviation: HOMA-IR, homeostasis model assessment for insulin resistance.

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acute and chronic beneficial effects of some cysteine-rich proteins on insulin sensitivity could originate in the potential of cysteine to alleviate the detrimental effects of diet on glutathione redox status[20,17].

Rapeseed protein is a main fraction of rapeseed meal, a co-product of the extraction of oil for both edible use and the growing sector of bio-diesel fuel. Interestingly, rapeseed protein has recently been demonstrated to be of high nutritional value in human subjects[21]. Rapeseed protein, being rich in cysteine and a good source of other candidate amino acids such as the nitric oxide precursor arginine[22,23], is an attractive candidate for further study of the potential of dietary protein to prevent insulin resistance and related metabolic/physiological abnormalities in the setting of the metabolic syndrome.

The aim of the present study was therefore to examine whether rapeseed protein, if substituted for standard milk protein in a Western-type diet rich in saturated lipids and sucrose, could affect the onset of insulin resistance and related abnormalities. To compare this potential with the current paradigm concerning the benefits of manipulating the quality of carbohydrates and lipids, we aimed to compare its effects with those of a diet containing the same level of energy-yielding macronutrients but where sucrose and SFA were replaced by starch and *n*-3 PUFA.

Experimental methods

Animals and diets

The '*Principles of Laboratory Animal Care*' (NIH publication no. 85-23, revised 1985) and French guidelines concerning the care and use of laboratory animals were followed. Thirty Wistar–Hanover male rats (Harlan, France), weighing 130–140 g on arrival, were housed individually, with free access to food and tap water, under a 12 h light–dark cycle with the lights going on at 05.00 hours. The experimental diets were prepared under strict laboratory conditions by UPAE (Experimental Food Preparation Unit, INRA, Jouy-en-Josas, France). A rapeseed protein isolate was prepared as previously described[21]. During the first 6 d, animals were fed a habituation diet ('Baseline diet', Table 1), which was a modified AIN-93 diet with a higher protein content (29 % total energy) and a higher lipid content and isoenergetic when compared with the experimental diets. The animals were then divided into three groups (Induction (diet I); Rapeseed (diet R); Prudent (diet P)) with similar body weight (136 (SD 7), 136 (SD 5) and 138 (SD 5) g for groups I, R and P, respectively) and each group was assigned to one experimental diet (Table 1) for 9 weeks. The control experimental diet (diet I) was mainly composed of sucrose as the carbohydrate source and saturated fat (palm oil) as the lipid source. Diet R was similar to diet I but contained rapeseed protein isolate instead of milk protein concentrate as the protein source. By contrast, diet P contained milk protein concentrate as the protein source but starch instead of sucrose and polyunsaturated *n*-3 rich fat (rapeseed oil) instead of saturated fat (palm oil). Because the rapeseed protein isolate contained a higher level of crude protein than the milk protein concentrate, and because the latter also included small quantities of milk fat, we adjusted the amount of rapeseed protein so that the diets would be isonitrogenous and the residual was equilibrated between diets using small quantities of maize starch and milk fat. All diets were isoenergetic, with fat, carbohydrates and protein amounting to 32 %, 50 % and 18 % total energy content, respectively, as calculated on the basis of 37·7 kJ (9 kcal) per g lipid and 16·7 kJ (4 kcal) per g carbohydrates and protein. The weights and food intake of animals were determined weekly.

Table 1. Diet composition*

	Composition (g/100 g)			
		Experimental diets†		
	Baseline diet	I	R	P
Milk protein concentrate – crude protein	19·25	19·25	–	19·25
Milk protein concentrate – non-N residual‡	5·75	5·75	–	5·75
Rapeseed protein isolate – crude protein	–	–	19·25	–
Rapeseed protein isolate – non-N residual‡	–	–	1·45	–
Maize starch	43·6	2·0	5·8	43·6
Sucrose	7·1	48·7	48·7	7·1
Palm oil	–	10·9	10·9	–
Rapeseed oil	–	–	–	10·9
Milk fat	–	–	0·5	–
Lupin oil	–	3·6	3·6	3·6
Oleisol oil	14·5	–	–	–
AIN-93M-MX mineral mix	3·5	3·5	3·5	3·5
AIN-93-VX vitamin mix	1·0	1·0	1·0	1·0
α-Cellulose	5·0	5·0	5·0	5·0
Choline	0·23	0·23	0·23	0·23

I, induction; R, rapeseed; P, prudent.
* For details of diets and procedures, see Experimental methods.
† Experimental diets included the same amount of fat, carbohydrates and protein.
‡ As the crude protein content differed slightly between milk and rapeseed protein source, different amounts were used so that the diets were isonitrogenous (19·25 g protein per 100 g feed) and the non-protein residual was adjusted using small amounts of starch and milk fat.

Experimental design

During the first 2 weeks, rats were adapted to laboratory conditions, handling and blood pressure measurements. Blood was sampled at weeks 0, 3, 5 and 9, in the morning after food had been removed at 19.00 hours. Blood drawn from a tail vein was dropped into pre-chilled tubes containing 35 μl (per 500 μl blood) of a solution of EDTA (0·7 % (w/v)) and aprotinin (0·014 % (w/v)). Plasma was separated (1800 ***g***, 20 min, 4°C) and aliquots were stored at −20°C. At week 9, for whole-blood glutathione determinations, two additional 70 μl blood samples were drawn on ice and immediately deproteinized by the addition of 70 μl TCA (10 % (w/v), 4°C), vortexed, centrifuged (9000 ***g***, 5 min, 4°C). The supernatant, stored at 4°C, was assayed on the same day.

Systolic and diastolic blood pressures were determined on weeks 3, 5 and 9 by volume-pressure recordings using an automated tail-cuff plethysmographic device (XBP 1000; Kent Scientific, Torrington, CT, USA), as previously described[24]. Rats from the three groups were processed in a randomized Latin-square order, on the same day of each week, in the fasted state.

On week 7, all rats underwent an oral glucose test, as previously described[17]. Briefly, an indwelling catheter

(Introcan Certo; B Braun Medical, France) was inserted in a distal lateral tail vein, locked with heparinized saline, secured to the tail with medical adhesive tape and coated with anti-onychophagic bitter varnish. Blood was collected via the catheter before and 10, 30, 60 and 120 min after the rats had received a 1 g/kg body weight D-glucose oral load.

At the end of the experiment (week 9), the rats were administered an overdose of pentobarbital (30 mg/kg body weight, intraperitoneally), exsanguinated and dissected out. The liver, two abdominal fat pads (retroperitoneal and epididymal) and the interscapular brown fat pad were isolated and weighed and subcutaneous fat in the lower half of the animal was carefully dissected out (by the same person) and weighed. The stripped carcass was prepared (by discarding other abdominal and thoracic organs and severing the distal parts of the limbs, head and tail) and weighed.

Biochemical analysis and calculations

Food efficiency was calculated as weight gain (g) divided by food intake (g). Blood glucose concentrations were measured using a Glucometer (Roche Diagnostics, Meylan, France). Insulin was detected by a solid phase two-site enzyme immunoassay (Mercodia Rat Insulin ELISA, Paris, France). Plasma total cholesterol, HDL-cholesterol and TAG concentrations were determined using standard enzymic assays (Cholesterol RTU and triglycerides PAP 150; BioMerieux, Marcy l'Etoile, France). Whole-blood oxidized (GSSG) and total GSH concentrations were measured using the enzymic recycling method described by Anderson[25] on six samples per diet group. The reduction potential of the GSSG/2GSH half-cell (GSH Ehc), an indicator of intracellular redox status, was calculated using the following formula[26]:

$$\text{GSH Ehc (mV)} = -264 + \frac{59{\cdot}1}{2}\log(\text{GSSG}/\text{GSH}^2),$$

where GSH and GSSG are expressed in mol/l. The homeostasis model assessment for insulin resistance (HOMA-IR) was used as an index of insulin resistance[27]. HOMA-IR was calculated as fasting insulin (mU/l) × fasting glucose (mmol/l)/22·5, with 1 mU insulin equal to 6 pmol.

The insulin sensitivity index based on the glucose oral tolerance test[28] was calculated at 60 and 120 min after the load, according to Belfiore *et al.* [29], as:

$$\frac{2}{(\text{AIns}_i/\text{AIns}_p \times \text{AGly}_i/\text{AGly}_p) + 1},$$

where AGly_i and AIns_i are the areas under the plasma glucose and insulin curves above baseline for rat i and AGlyp and AInsp the mean areas under the plasma glucose and insulin curves above baseline for reference diet P.

Statistical analyses

Repeated measurements (i.e. weekly measures or post-glucose challenge measures) were analysed using a mixed model with diet and time (i.e. weeks or min) as independent, fixed factors and the rat as a random factor, nested in the diet factor (SAS institute, Cary, NC, USA). For repeated measurements, when the time–diet interaction was $P<0{\cdot}10$, two pre-planned comparisons between treatment diets (R and P) and the control diet (I) were made at week 9 (for weekly measurements, this being the last time point of the study) or at time 10 min (for post-glucose plasma insulin, this being the peak time) using *ad hoc* contrasts under the mixed models. For selective measurements (at week 9), data were analysed with ANOVA and post-test comparisons between treatment diets (R and P) and the control diet (I) were performed using the Dunnett test. A P value $<0{\cdot}05$ was considered to be significant.

Results

Food consumption, body weight and composition

Food consumption and body weight gain did not differ significantly between the groups throughout the experiment (data not shown). Average food consumption and final body weight are shown in Table 2. However, total food efficiency (Table 2) was significantly higher in group R than in group I. Body composition at week 9 did not differ between groups except for the subcutaneous fat component, which was higher in group R (Table 2).

Metabolic parameters

Plasma glucose increased slightly over time ($P<0{\cdot}001$) but did not vary as a function of diet type (Table 3). Plasma insulin rose markedly over time ($P<0{\cdot}0001$) and was significantly affected by the diet (diet effect, $P<0{\cdot}05$; diet × time interaction, $0{\cdot}05 < P<0{\cdot}10$, Table 3). At week 9, plasma insulin (Table 3) differed between diets ($P<0{\cdot}01$) and was significantly lower in groups R and P than in group I. Accordingly, the HOMA-IR index (Fig. 1) increased over time ($P<0{\cdot}0001$) and was affected by the diet (diet effect, $P<0{\cdot}05$; diet × time interaction, $P<0{\cdot}05$). At week 9, HOMA-IR differed significantly between diets ($P<0{\cdot}01$), being lower in groups R and P than in group I. Systolic and diastolic blood pressures (Table 3) did not vary with time or diet. Plasma TAG concentrations (Table 3) increased significantly with time but were not significantly affected by diet type. Plasma cholesterol decreased, plasma HDL-cholesterol increased (Table 3) and the HDL-:total cholesterol ratio (data not shown) increased slightly over time ($P<0{\cdot}001$), but in a similar manner with all diets (Table 3).

Following the oral glucose challenge, plasma glucose (Fig. 2 (A)) and area under the curve (data not shown) were similar in all groups. By contrast, the increase in plasma insulin (Fig. 2 (B)) was significantly affected by diet type. After the load (10 min) (i.e. at the insulin peak), plasma insulin concentrations were markedly and significantly lower in groups R and P than in group I. The Belfiore index of insulin sensitivity, calculated at both 60 min and 120 min after the glucose load (Fig. 3), was significantly higher in group R than in group I.

Whole-blood glutathione was significantly 30 % higher after diet R than after diet I (Table 4) and GSH in the reduced form tended ($P=0{\cdot}08$) to be higher after diet R (data not shown). However, the half-cell reduction potential of the GSH/GSSG redox couple did not differ between the groups (Table 4).

Discussion

The main finding of the present study is that rapeseed protein substituted for milk protein inhibited the onset of insulin

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Table 2. Body composition, average food intake and total food efficiency in rats fed with the induction diet (rich in saturated fat and sucrose) containing milk protein (diet I), diet I containing rapeseed protein (diet R) or an isoenergetic diet including milk protein but rich in polyunsaturated *n*-3 fat and starch (diet P) for 9 weeks*

(Mean values with their standard errors)

	Diet					
	I		R		P	
	Mean	SE	Mean	SE	Mean	SE
BW (g)	359	8	374	9	354	10
9-week average food intake (g/d)	17·5	0·4	16·9	0·3	16·2	0·4
Food efficiency (‰)	206	7	227†	5	212	5
Brown adipose tissue (g)	0·76	0·03	0·75	0·05	0·76	0·04
Liver (g)	8·6	0·3	8·7	0·2	8·4	0·3
Liver (%BW)	2·4	0·1	2·3	0·04	2·4	0·1
Epididymal fat pad (g)	10·3	0·8	12·2	0·8	10·6	0·6
Epididymal fat pad (%BW)	2·88	0·21	3·28	0·21	2·98	0·13
Retroperitoneal fat pad (g)	10·7	1·1	12·0	0·8	10·3	0·7
Retroperitoneal fat pad (%BW)	2·97	0·25	3·20	0·17	2·91	0·16
Lower subcutaneous fat (g)	13·9	1·0	17·8†	1·3	14·0	1·0
Lower subcutaneous fat (%BW)	3·87	0·25	4·74†	0·30	3·94	0·20
Stripped carcass (g)	161	3	162	4	157	4
Stripped carcass (%BW)	45·1	0·6	43·4	0·7	44·2	0·6

BW, body weight.
* For details of animals and procedures, see Experimental methods.
† Mean values were significantly lower than those of diet I, as tested with Dunnett *post hoc* test; $P<0·05$.

resistance in rats fed the high-saturated, high-sucrose diet. First, rapeseed protein led to a reduction in fasting plasma insulin when compared with milk protein, thereby moderating or slowing down compensatory hyperinsulinaemia (Fig. 1), which was the critical parameter in the present model. Second, the improved insulin sensitivity to glucose homeostasis was further substantiated by the oral glucose tolerance test. During this test, the improved insulin sensitivity index could mostly be explained

Table 3. Metabolic markers before (week 0) and after (weeks 3, 5 and 9) rats were fed with either the induction diet (rich in saturated fat and sucrose) containing milk protein (diet I), diet I containing rapeseed protein (diet R) or an isoenergetic diet including milk protein but rich in polyunsaturated *n*-3 fat and starch (diet P)*

(Mean values with their standard errors)

		Week							
		0		3		5		9	
	Diet	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Blood glucose (g/l)	I	0·99	0·04	1·12	0·08	1·16	0·05	1·12	0·05
	R	0·99	0·07	1·12	0·08	1·16	0·03	0·95	0·02
	P	0·90	0·04	1·00	0·04	1·13	0·04	1·04	0·03
Plasma insulin (pmol/l)	I	41	4	67	10	125	24	224	57
	R	40	3	126	30	126	14	147†	42
	P	35	2	63	11	62	12	99†	14
Diastolic blood pressure (mmHg)	I	ND	ND	92	3	88	5	91	5
	R	ND	ND	95	3	88	4	88	7
	P	ND	ND	84	3	89	3	89	4
Systolic blood pressure (mmHg)	I	ND	ND	126	3	124	5	131	5
	R	ND	ND	129	4	124	5	122	6
	P	ND	ND	118	2	126	4	128	4
Plasma TAG (mmol/l)	I	0·76	0·08	0·94	0·09	0·93	0·10	1·06	0·15
	R	0·74	0·11	0·85	0·06	0·92	0·08	0·90	0·09
	P	0·69	0·07	0·81	0·08	0·87	0·08	0·85	0·13
Plasma total cholesterol (mmol/l)	I	1·65	0·08	1·50	0·08	1·34	0·10	1·42	0·08
	R	1·89	0·08	1·55	0·16	1·40	0·10	1·60	0·10
	P	1·94	0·10	1·65	0·08	1·58	0·08	1·63	0·10
Plasma HDL-cholesterol (mmol/l)	I	0·75	0·08	0·90	0·05	0·72	0·03	0·88	0·08
	R	0·78	0·05	0·96	0·05	0·75	0·08	0·90	0·05
	P	0·72	0·05	1·14	0·05	0·96	0·08	1·03	0·08

ND, not determined.
* For details of animals and procedures, see Experimental methods.
† Mean values were significantly lower than those of diet I, assessed by pre-planned contrast at week 9: $P<0·05$.

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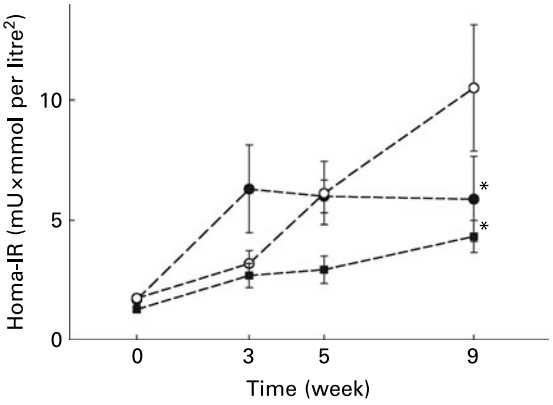

Fig. 1. Mean values and standard errors (n 10) for model assessment for insulin resistance (HOMA-IR) calculated from fasting blood glucose and plasma insulin concentrations in rats fed for 9 weeks with either the induction diet (diet I) (rich in saturated fat and sucrose) containing milk protein (○), diet I containing rapeseed protein (●) or an isoenergetic diet including milk protein but rich polyunsaturated fat and starch (■). The effect of time ($P<0·01$), diet ($P<0·05$) and the diet × time interaction ($P<0·05$) were analysed using repeated-measure ANOVA. Pre-planned comparisons with control diet I were made at week 9; * mean values were significantly different from those of diet I: $P<0·05$.

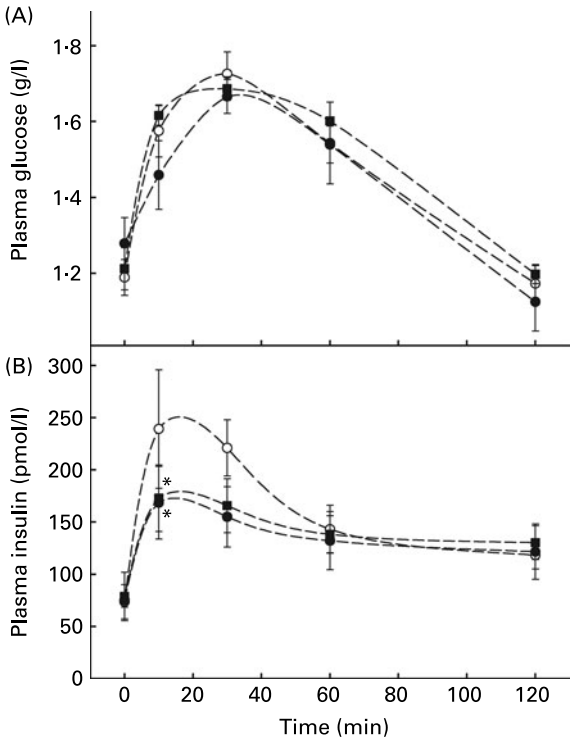

Fig. 2. (A) Mean blood glucose concentrations and standard errors (n 10) during the oral glucose tolerance test in rats fed with either the induction diet (rich in saturated fat and sucrose) containing milk protein (○), the induction diet (diet I) containing rapeseed protein (●) or an isoenergetic diet including milk protein but rich polyunsaturated fat and starch (■) for 7 weeks. The effect of time ($P<0·001$), diet (NS) and the diet × time interaction (NS) were analysed using repeated-measure ANOVA. (B) Mean plasma insulin concentrations and standard errors (n 10) during the oral glucose tolerance test, simultaneously under the same conditions. The effect of time ($P<0·001$), diet (NS) and the diet × time interaction ($P<0·05$) were analysed using repeated-measure ANOVA. Pre-planned comparisons with control diet I were made at week 9; * mean values were significantly different from those of diet I: $P<0·05$.

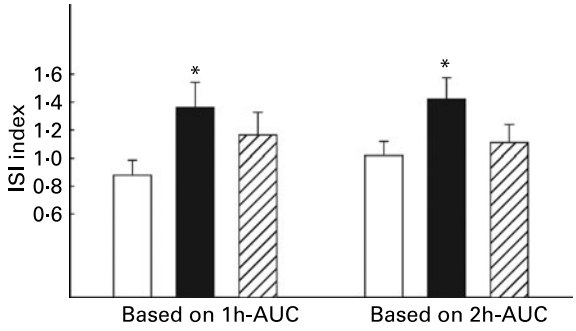

Fig. 3. Mean values for Belfiore's insulin sensitivity index (ISI) and standard errors calculated from glucose and insulin area under the curve (AUC) values 1 h and 2 h after the glucose load in rats fed with either the induction (I) diet (rich in saturated fat and sucrose) containing milk protein (□), the I diet containing rapeseed protein (R; ■) or an isoenergetic diet including milk protein but rich in polyunsaturated fat and starch (P; ▨) for 7 weeks. The effect of diet ($P<0·05$) was tested with ANOVA. *Post-hoc* comparisons between treatment diets R and P and the control diet (I) were performed using the Dunnett test. * Mean values were significantly different from those of the diet I: $P<0·05$. For details of diets and procedures, see Experimental methods.

by a markedly lower plasma insulin excursion after the glucose load, for a comparable plasma glucose excursion (Fig. 2). A similar feature had been reported regarding the effect of cysteine enrichment in a sucrose-rich diet[17] and that of cod or soya protein substitution for casein in a high-saturated fat, high-sucrose diet[18]. When comparing the effects of protein type (rapeseed *v.* milk) with the effect of the type of fat and carbohydrates, it is of note that they were of a similar magnitude. As well as the importance of the overall contribution of the macronutrient to diet energy, the importance of the types of fats and carbohydrates to prevention and, in most cases, to the treatment of the metabolic syndrome has been widely investigated[14,30,31]. The present result thus highlights the importance of the type of protein as a major component of diet quality, in terms of cardiovascular and diabetic risks.

During the current study, the high-saturated fat/high-sucrose diet induced insulin resistance, characterized by a marked increase in fasting plasma insulin values over weeks. Diet I was also associated with a slight increase in TAG, but not with changes to other metabolic or physiological abnormalities. As far as the plasma cholesterol profile is concerned, the increase in HDL:total cholesterol with time resulted from both an increase in HDL-cholesterol and a decrease in total cholesterol. These variations were unexpected, but were observed to a similar extent in the three dietary groups. Although data on the natural variation in plasma cholesterol profile are lacking in the rat, the present changes in total and HDL-cholesterol may have originated from ageing rather than the nutritional conditions. As far as body composition is concerned, it is not possible to draw any definite conclusions from the present study as to the level of obesity (especially of the visceral type) because the experiment did not include a group receiving a standard chow. However, if the findings were compared with those of other studies in our laboratory (e.g. Blouet *et al.* [32]), rats did not exhibit higher total body fat and/or regional fat excess and it is therefore likely that diet I did not (or at least during the time allotted) result in obesity or visceral obesity. Further, it is not surprising that blood pressure values did not rise during the study, in the light of

Table 4. Whole-blood glutathione status in rats fed with either the induction diet (rich in saturated fat and sucrose) containing milk protein (diet I), the induction diet containing rapeseed protein (diet R) or an isoenergetic diet including milk protein but rich in polyunsaturated *n*-3 fat and starch (diet P) for 9 weeks*

(Mean values with their standard errors)

		Diet					
		I		R		P	
		Mean	SE	Mean	SE	Mean	SE
Whole-blood glutathione	Total (μmol/l)	631	118	821†	56	581	61
	Oxidized (μmol/l)	69	14	102	14	87	15
	Ehc (mV)	−199	3	−200	2	−193	3

Ehc, half-cell reduction potential of the oxidised glutathione/2 total glutathione couple (see Biochemical analysis and calculations).
* For details of animals and procedures, see Experimental methods.
† Mean values were significantly lower than those of diet I, as tested with Dunnett *post-hoc* test; $P<0{\cdot}05$.

reports that this parameter lags far behind other abnormalities in diet-induced metabolic syndrome[33]. Finally, our model was found to reflect the initiation of insulin resistance, as illustrated by marked and progressive compensatory hyperinsulinaemia. This model is associated with glucose intolerance and a moderate elevation of triacylglycerolaemia. However, there is little doubt that our dietary model of induction would eventually lead to a typical metabolic syndrome. First, as in the present model, hyperinsulinaemia and glucose intolerance are the first manifestations of diet-induced metabolic syndrome, the former being the first feature of insulin resistance on glucose homeostasis[34]. Furthermore, and as discussed earlier, insulin resistance is central to the clustering of abnormalities in human subjects[10,35,36]. Second, similar diets have been reported as leading eventually to the complete clustering of metabolic abnormalities. The sequences and timing of other abnormalities vary considerably and it is difficult to draw a clear picture from the literature because different studies used different diets, which differ in: (1) the proportion of fat *v.* refined sugar; (2) the type of refined sugar (fructose *v.* sucrose), saturated fat (lard fat or palm oil, as in the present study) or other components[37–39] and also resorted to different rat strains, gender and study duration. In this regard, although the duration of the present study was based on our literature survey, that a longer period of study would have been needed to show all traits of the metabolic syndrome in the present model before the end of the study further illustrates the profound heterogeneity in the conditions and effects reported in the literature. However, the full sequence of abnormalities has been repeatedly and clearly demonstrated in Fisher rats receiving a diet with a composition similar to that used during our study[40,41]. Indeed, use of a high (saturated) fat and refined sugar diet (which is, interestingly, an equilibrated means of combining the deleterious effects of each macronutrient[42]), is probably the most relevant as a model for Westernized diets. Finally, insulin sensitivity is recognized as a very important target function, and glucose tolerance an excellent endpoint, when examining the potential for diet to modulate cardiovascular and diabetic risk[43].

Based on the favourable effects of rapeseed protein on glutathione status, the links between cysteine, glutathione and insulin sensitivity may be involved in the favourable effect of rapeseed protein. In sucrose-fed rats, we previously reported strong, reproducible and dose-dependent associations between glutathione redox status in numerous tissues and insulin sensitivity that were likely to explain the favourable effect of dietary cysteine[17]. In addition, other authors have largely documented that liver glutathione is a critical component in the 'hepatic insulin sensitizing substance', a substance that largely drives peripheral insulin sensitivity[44,45]. Our observation of a higher glutathione content in the whole blood of rapeseed-fed rats, although the redox state was similar, fuels observations that the intake of cysteine in nutritional amounts can impact glutathione status[17,46,47] and also suggests that the effect of rapeseed may be driven by its cysteine content. Other characteristics of the rapeseed protein may have played a role and a synergetic effect of arginine might also be involved[48]. Further dedicated studies are needed to closely address the precise underlying mechanism.

Similarly, the increased food efficiency and higher subcutaneous fat deposition reported in the present study may unravel other complex metabolic features associated with manipulation of the protein source in this induction diet. Some data have shown that, unlike visceral fat, subcutaneous fat may be neutral or even reduce the risk of insulin resistance and related metabolic dysregulation[49–52]. A preferential development of peripheral fat with a similar percentage of body weight as visceral fat may therefore be linked to the reduced development of insulin resistance evidenced in the current study. It would be interesting to determine how rapeseed protein elicits this effect and how it could be related to favourable effects on insulin resistance.

Further studies are required to closely address the underlying mechanisms and to assess the benefits of rapeseed protein regarding later endpoints of the metabolic syndrome. Finally, because in complement to the essential 'preventive' role, diet modification is known to exert some reversal effects in diet-induced insulin resistance[53]. Further studies may interestingly, and complementarily, assess if rapeseed protein can improve insulin sensitivity in animals or human subjects that already display insulin resistance.

In conclusion, in this Western-diet model, substituting rapeseed protein for milk protein slowed down the increase in fasting insulinaemia and lowered insulin secretion after a glucose load, thus demonstrating a preventive effect on the onset of insulin resistance. This effect is important inasmuch as it is of a magnitude similar to that afforded by the dual substitution of *n*-3 polyunsaturated fat for saturated fat and

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starch for sucrose. The quality of dietary protein may be an underestimated parameter in dietary strategies against the metabolic syndrome.

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