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Dietary cholesterol reduces lipoprotein lipase activity in the atherosclerosis-susceptible Bio F_1B hamster

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We have compared lipoprotein metabolism in, and susceptibility to atherosclerosis of, two strains of male Golden Syrian hamster, the Bio F_1B hybrid and the dominant spot normal inbred (DSNI) strain. When fed a normal low-fat diet containing approximately 40 g fat and 0·3 g cholesterol/kg, triacylglycerol-rich lipoprotein (chylomicron + VLDL) and HDL-cholesterol were significantly higher ($P<0·001$) in Bio F_1B hamsters than DSNI hamsters. When this diet was supplemented with 150 g coconut oil and either 0·5 or 5·0 g cholesterol/kg, significant differences were seen in response. In particular, the high-cholesterol diet produced significantly greater increases in plasma cholesterol and triacylglycerol in the Bio F_1B compared with the DSNI animals ($P=0·002$ and $P<0·001$ for cholesterol and triacylglycerol, respectively). This was particularly dramatic in non-fasting animals, suggesting an accumulation of chylomicrons. In a second experiment, animals were fed 150 g coconut oil/kg and 5·0 g cholesterol/kg for 6 and 12 months. Again, the Bio F_1B animals showed dramatic increases in plasma cholesterol and triacylglycerol, and this was confirmed as primarily due to a rise in chylomicron concentration. Post-heparin lipoprotein lipase activity was significantly reduced ($P<0·001$) in the Bio F_1B compared with the DSNI animals at 6 months, and virtually absent at 12 months. Bio F_1B animals were also shown to develop significantly more ($P<0·001$) atherosclerosis. These results indicate that, in the Bio F_1B hybrid hamster, cholesterol feeding reduces lipoprotein lipase activity, thereby causing the accumulation of chylomicrons that may be associated with their increased susceptibility to atherosclerosis.

Lipoprotein lipase: Hepatic lipase: Dominant spot normal inbred strain: Bio F_1B: Dietary cholesterol: Atherosclerosis: Hamster

The male Golden Syrian hamster has been used extensively in studies of lipoprotein metabolism because it is more similar in this respect to man than other rodent species. It has also proven to be a useful model in studying pharmacological (Kowala *et al.* 1991, 1993; Foxall *et al.* 1992; Otto *et al.* 1995; Nicolosi *et al.* 1998*a*; Pitman *et al.* 1998) and nutritional influences on the development of atherosclerosis (Parker *et al.* 1995; Kahlon *et al.* 1996; Nicolosi *et al.* 1997, 1998*b*,*c*; Xu *et al.* 1998; Mangiapane *et al.* 1999). Previous studies in our laboratory (Mangiapane *et al.* 1999) showed that one strain of hamster (dominant spot normal inbred, DSNI) required high levels of dietary cholesterol (30 g/kg diet) to induce aortic lesions. This is in agreement with earlier findings of Nistor *et al.* (1987). However, the hybrid Bio F_1B strain of hamster has been shown to develop aortic atherosclerosis at much lower dietary cholesterol concentrations (Kowala *et al.* 1991). The biochemical and physiological basis of this response to dietary cholesterol remains to be established.

The aim of the present study was to compare the lipoprotein responses of DSNI and Bio F_1B hamsters to dietary cholesterol. Plasma lipoprotein profiles and hepatic cholesterol concentrations of DSNI and Bio F_1B hamsters fed normal rodent diet or the same diet but supplemented with

Abbreviations: Apo, apolipoprotein; D, diet; DNSI, dominant spot normal inbred; F, fed or fasted; HFHC, high-fat, high-cholesterol diet; HFLC, high-fat, low-cholesterol diet; LPL, lipoprotein lipase; S, strain; T, length of time on diet; TAG, triacylglycerol.

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150 g coconut oil/kg and either 0·5 or 5·0 g cholesterol/kg were compared. In a further experiment, we investigated the effects of feeding 150 g coconut oil and 5·0 g cholesterol/kg for 6 and 12 months on lipoprotein concentrations, heparin-releasable plasma lipase activities and atherosclerosis in the two strains of hamster.

Materials and methods

Animals and diets

All procedures involving animals in these studies were subject to UK Home Office regulations

Study 1. Twenty-four 12-week-old DSNI hamsters (Joint Animal Breeding Unit, University of Nottingham, Nottingham, UK) and twenty-four 12-week-old Bio F_1B hamsters (Biobreeders, Fitchburg, MA, USA) were housed individually at the University of Nottingham. Following acclimatisation, eight hamsters of each strain, selected by stratified randomisation of body weight, were fed normal RM3 diet containing approximately 40 g fat and 0·3 g cholesterol/kg (Special Diet Supplies, Chelmsford, Essex, UK) that had been ground to a powder. A further eight animals of each strain were fed the same diet supplemented with 150 g coconut oil (ICN Biomedicals Ltd, Thame, Oxon., UK) and 0·5 g cholesterol/kg (high-fat, low-cholesterol diet; HFLC). The remaining eight animals per strain were fed RM3 diet supplemented with 150 g coconut oil and 5·0 g cholesterol/kg (high-fat, high-cholesterol diet; HFHC). After 4 weeks, four hamsters from each group were fasted overnight, while the remaining animals were allowed free access to food until killing. The next morning, animals were anaesthetised with sodium pentabarbitone (Sagatal, 1 ml/kg), and 3–4 ml blood collected by cardiac puncture and placed into EDTA tubes. The liver was flushed with 10 ml ice-cold 0·15 M-NaCl, removed immediately, frozen in liquid N_2 and stored at −20°C awaiting analysis.

Study 2. Sixteen 12-week-old Bio F_1B and sixteen 12-week-old DSNI male hamsters were housed at the University of Nottingham as described earlier. Following acclimatisation, they were fed RM3 diet (Special Diet Supplies) supplemented with 150 g coconut oil and 5·0 g cholesterol/kg. Animals from each strain were allocated to two groups of eight by stratified randomisation for body weight. One group from each strain was killed at 6 months and the remaining animals after 12 months. Animals were fed *ad libitum* until they were killed. At killing, four animals from each group were anaesthetised and blood collected as described earlier. The remaining animals were anaesthetised and injected with 200 μl heparin (Sigma, Poole, Dorset, UK) in 0·15 M-NaCl (approximately 7500 units/kg body weight) via the vena cava. After 5 min, blood was collected from the heart by cardiac puncture and plasma separated and stored at −20°C awaiting analysis for post-heparin lipase activity. The hearts and aortae were then removed and the aortae cut approximately 1 mm from the base of the heart. The aortae and hearts were then fixed in neutral-buffered formalin (100 ml/l) and stored at 4°C awaiting further analysis.

Lipoprotein analysis

In study 1, lipoprotein fractions were separated from plasma (normally 1 ml) by sequential ultracentrifugation and corrected for recovery (typically >85 %) as previously described (Salter *et al.* 1998). VLDL, intermediate-density lipoproteins, LDL and HDL were separated within the density ranges <1·006, 1·006–1·020, 1·020–1·060 and >1·060 g/ml respectively. In non-fasted animals, the <1·006 g/ml fraction also contained chylomicrons and so has been termed the triacylglycerol (TAG)-rich lipoprotein fraction. In study 2, chylomicrons were first isolated from the plasma by spinning at 15 500 ***g*** for 20 min at 12°C. The remaining lipoproteins were then separated as described earlier. Cholesterol and TAG concentration in whole plasma and lipoprotein fractions were determined using diagnostic kits from Wako (Alpha Laboratories, Eastleigh, Hants., UK).

To confirm the identity of the TAG-rich lipoproteins, the apolipoprotein (Apo) B composition was examined by Western blotting. Lipoprotein fractions were delipidated (sample–ice-cold methanol–diethyl ether (1:12:28, by vol.)) and the remaining proteins subjected to SDS–PAGE on 4–20 % gradient gels. Separated proteins were then transferred on to nitrocellulose membrane by Western blotting. Blots were then incubated with human polyclonal antibody to ApoB (Calbiochem, Nottingham, UK) at a dilution of 1:10 000. ApoB-48 and -100 were visualised using a secondary monoclonal anti-goat antibody conjugated to horseradish (*Armoracia rusticana*) peroxidase (1:5000 dilution) with enhanced chemiluminescence detection reagent (Amersham International, Amersham, Bucks., UK).

Post-heparin lipase activity

Lipoprotein lipase (LPL) and hepatic lipase activities were measured using the method of Corey & Zilversmit (1977) as modified by Groot *et al.* (1992).

Samples were analysed in triplicate. Total lipase activity was measured in the presence of 0·15 M-NaCl while hepatic lipase activity was measured by inhibiting LPL with 3·55 M-NaCl. LPL was calculated as the difference between the two values. Activity was expressed as nmol fatty acid released/ml plasma per min.

Hepatic free and esterified cholesterol

Hepatic free and esterified cholesterol were separated by TLC, extracted and assayed enzymatically as described previously by Bennett *et al.* (1995).

Measurement of atherosclerosis

The heart was bisected beneath the atria and the base of the heart, which had approximately 1 mm of the ascending aorta protruding, and then submerged in optimum cutting temperature compound (Bayer Diagnostics, Leverkusen, Germany) for 24 h prior to sectioning. The heart was frozen on a cryostat chuck (Bright Instrument Company Ltd, Huntingdon, Cambs., UK) and 10 μm sections of the

aortic sinus were taken from where the three aortic valve leaflets first appeared (Paigen *et al.* 1987; Groot *et al.* 1996). Sections were stained with Oil Red O for neutral lipid and counterstained with Cole's haematoxylin for nuclei and basophilic tissue.

Images of the sections were acquired using an Olympus Zoom Stereo microscope (model SZCTV; Olympus Optical Co. Ltd, Southall, Middlesex, UK) and the area of Oil Red O lipid-staining in the sections was quantified using the Optimas Imaging software (version 5.2; Media Cybernetics Inc., Silver Spring, MD, USA) as previously described (Mangiapane *et al.* 1999). Ten alternate sections were examined and the average lesion area calculated.

In addition, the remaining portions of the fixed aortae were stained with Oil Red O and the stained areas from the aortic arch to the abdominal aorta were quantified as described previously (Mangiapane *et al.* 1999).

Statistical analysis

All data were checked for normal and equal distribution and those variables not normally distributed were converted to log values before statistical analyses. Such results are presented graphically as the geometric mean values and standard deviations. Data for animals fed on RM3 diet alone were analysed independently (two-way ANOVA) from that for the animals on the HFLC and HFHC diets. This was because the energy and nutrient density of the basic diet was considerably less than that of the high-fat diets. The remaining data from this trial (HFLC and HFHC groups) were analysed by three-way ANOVA, using the Genstat Computer Package (Lawes Agricultural Trust, Rothamstead, Herts., UK). The three factors in this analysis were defined as strain (S), diet (D) and whether the animals were fasted or fed (F). Significant P values ($<0{\cdot}05$) for the effect of these factors or the interactions between them are shown in the tables. For the second trial, data was analysed by two-way ANOVA with S and length of time on the diet (T) as factors.

Results

DSNI hamsters had a significantly greater starting body weight than Bio F_1B hamsters (145·9 (SD 12·16) *v.* 124·5 (SD 15·73) g respectively, $P<0{\cdot}001$). This weight difference increased during the course of the trial (final body weight 157·6 (SD 12·78) *v.* 124·3 (SD 15·78) g respectively, $P<0{\cdot}001$). However, no significant effects of D or D × S interaction were observed.

Table 1 shows the lipoprotein profiles of the two strains of hamsters fed the normal diet. Animals were killed either after an overnight fast or allowed constant access to food. Bio F_1B hamsters had significantly higher plasma cholesterol concentrations than DSNI strain and this was significant in both the TAG-rich lipoprotein and HDL fractions ($P<0{\cdot}001$). LDL-cholesterol did not differ between the two strains; intermediate-density cholesterol, although a very minor fraction, was significantly greater in the DSNI strain ($P=0{\cdot}001$). Plasma cholesterol was greater in fed animals of both strains than those that were fasted overnight. Plasma total and TAG-rich lipoprotein-TAG did not differ significantly between the strains. Plasma total TAG was reduced on fasting in Bio F_1B animals, but not DSNI animals; however, the interaction between strain and fasting failed to reach statistical significance (S × F interaction, $P=0{\cdot}075$).

Table 1. Plasma lipoprotein concentrations (mM) in dominant spot normal inbred (DSNI) and Bio F_1B hamsters fed a normal diet*

(Mean values and standard deviations for four hamsters per group)

					Statistical significance of effect (two-way ANOVA)	
			Mean	SD	Factor	P†
Total chol	DSNI	Fasted	1·83	0·16	S	<0·001
		Fed	2·08	0·12	F	0·003
	F_1B	Fasted	2·79	0·25		
		Fed	3·21	0·16		
Total TAG	DSNI	Fasted	1·10	0·20		
		Fed	1·13	0·32		
	F_1B	Fasted	0·82	0·18		
		Fed	1·33	0·43		
TRL-chol	DSNI	Fasted	0·34	0·14	S	<0·001
		Fed	0·25	0·10		
	F_1B	Fasted	0·88	0·21		
		Fed	0·96	0·17		
TRL-TAG	DSNI	Fasted	0·91	0·34		
		Fed	0·92	0·42		
	F_1B	Fasted	0·79	0·14		
		Fed	1·19	0·25		
IDL-chol	DSNI	Fasted	0·20	0·14	S	0·002
		Fed	0·18	0·10		
	F_1B	Fasted	0·02	0·01		
		Fed	0·03	0·02		
LDL-chol	DSNI	Fasted	0·38	0·18		
		Fed	0·74	0·45		
	F_1B	Fasted	0·29	0·13		
		Fed	0·44	0·05		
HDL-chol	DSNI	Fasted	0·91	0·10	S	<0·001
		Fed	0·91	0·29		
	F_1B	Fasted	1·60	0·21		
		Fed	1·79	0·20		

chol, cholesterol; TAG, triacylglycerol; TRL, triacylglycerol-rich lipoprotein; IDL, intermediate-density lipoprotein; S, strain; F, fasted or fed.

* Hamsters were killed either after an overnight fast (fasted) or allowed constant access to food (fed); for details of diet and procedures, see p.342.

† P values are shown for significant ($P<0{\cdot}05$) effects of S and F. No significant interactions were seen between the two factors.

Total plasma cholesterol concentrations were higher in animals fed HFHC diet for 4 weeks than in those fed the HFLC diet, with a greater increase seen in the Bio F_1B animals (D × S interaction, $P=0{\cdot}002$; Fig. 1). Fasting caused a marked decrease (58 %) in plasma cholesterol in F_1B compared with DSNI animals (F × S interaction, $P=0{\cdot}039$). Fasting decreased plasma TAG in both strains, on both diets, but this was most pronounced on the HFHC diet where an 80 % decrease was seen (D × F interaction, $P=0{\cdot}002$). Bio F_1B animals showed a significantly more pronounced increase in TAG in response to the HFHC diet (S × D interaction, $P<0{\cdot}001$). The lipoproteins were analysed and the results for TAG-rich lipoproteins are shown in Fig. 2, whilst the results for the other lipoproteins are shown in Table 2. Fig. 2 indicates that most of the differences in total plasma lipids can be accounted for by changes in the TAG-rich lipoprotein fraction.

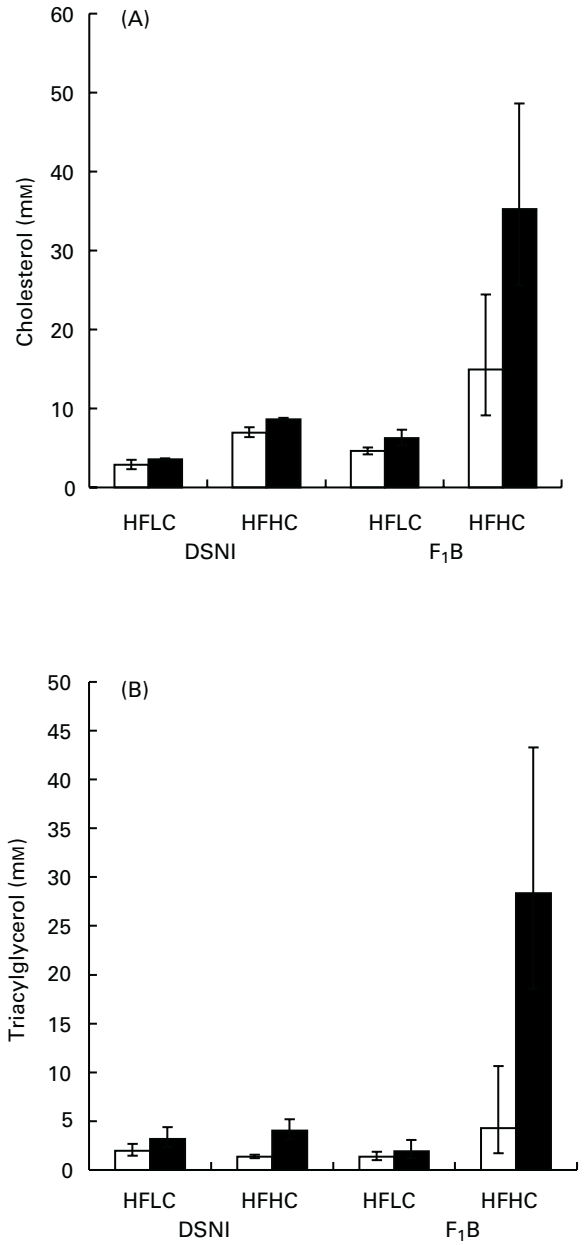

Fig. 1. Total plasma cholesterol (A) and triacylglycerol (B) concentrations in dominant spot normal inbred (DSNI) and Bio F_1B hamsters fed diets containing 150 g coconut oil/kg and either 0·5 g (high-fat, low-cholesterol; HFLC) or 5·0 g (high-fat, high-cholesterol; HFHC) added cholesterol/kg for 4 weeks. Four animals from each group were allowed constant access to food up to time of killing (■), while the remaining four were fasted overnight (□). Lipids were analysed as described on p. 342; for details of diets and procedures, see p. 342. Values are geometric means for eight animals with standard deviations shown by vertical bars.

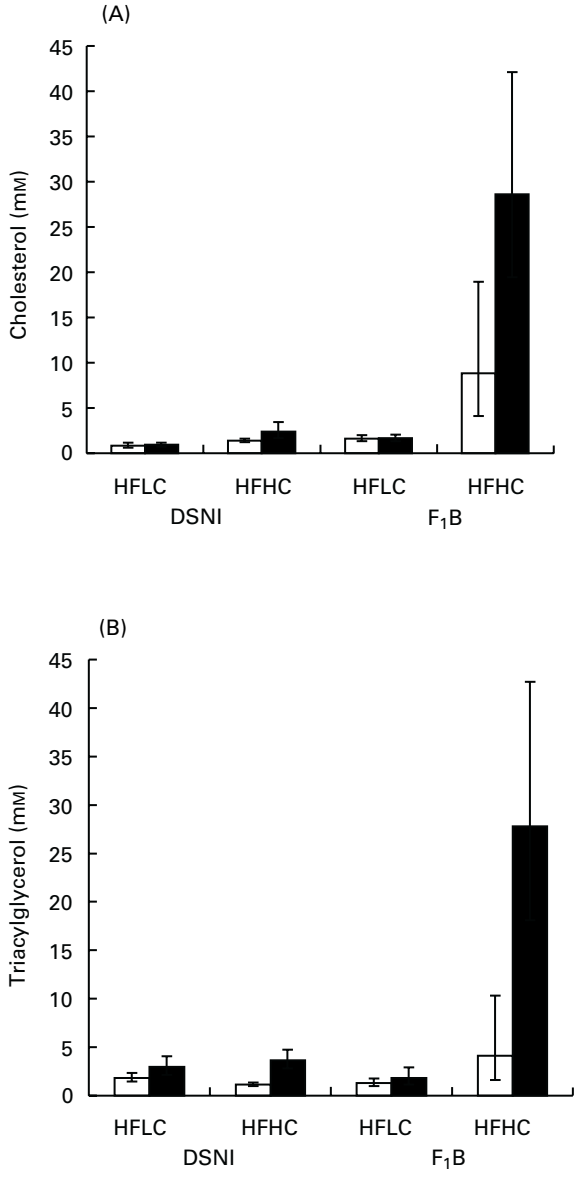

Fig. 2. Triacylglycerol-rich lipoprotein-cholesterol (A) and -triacylglycerol (B) concentrations in dominant spot normal inbred (DSNI) and Bio F_1B hamsters fed diets containing 150 g coconut oil/kg and either 0·5 g (high-fat, low-cholesterol; HFLC) or 5·0 g (high-fat, low-cholesterol; HFHC) added cholesterol/kg for 4 weeks. Four animals from each group were allowed constant access to food up to time of killing (■), while the remaining four were fasted overnight (□). Lipids were analysed as described on p. 342; for details of diets and procedures, see p. 342. Values are geometric means for eight animals with standard deviations shown by vertical bars.

The HFHC diet increased TAG-rich lipoprotein, TAG and cholesterol to a greater extent in Bio F_1B animals (S × D interaction, $P<0{\cdot}001$ for both lipids). While fasting had little effect on TAG-rich lipoprotein lipids in animals on the HFLC diet, both were reduced in animals fed the HFHC diet (D × F interaction, $P=0{\cdot}007$ and $P=0{\cdot}002$ for cholesterol and TAG respectively).

Hamsters fed HFHC diet had a greater amount of cholesterol in intermediate-density lipoprotein compared with those fed HFLC diet (Table 2). This effect was more pronounced in Bio F_1B animals as illustrated by a significant S × D interaction ($P<0{\cdot}001$). Intermediate-density-lipoprotein cholesterol was reduced by fasting, but to a greater extent in animals fed the HFHC than in those fed HFLC diet (D × F interaction, $P=0{\cdot}002$). LDL was higher in fed than fasted animals ($P=0{\cdot}013$) and DSNI animals had a

Table 2. Intermediate-density lipoprotein (IDL)-, LDL- and HDL- cholesterol concentration (mM) in dominant spot normal inbred (DSNI) and Bio F_1B hamsters after feeding diet supplemented with 150 g coconut oil and 5 g cholesterol/kg (HFLC) or diet supplemented with 150 g coconut oil and 5 g cholesterol (HFHC)*

(Mean values and standard deviations for four hamsters per group)

			HFLC		HFHC		Statistical significance of effect (three-way ANOVA)	
			Mean	SD	Mean	SD	Factor	P†
IDL	DSNI	Fasted	0·10	0·04	0·63	0·30	S × D	<0·001
		Fed	0·22	0·07	0·97	0·20	D × F	0·002
	F_1B	Fasted	0·04	0·01	1·15	0·14		
		Fed	0·08	0·04	1·75	0·20		
LDL	DSNI	Fasted	0·55	0·31	3·39	0·21	F	0·013
		Fed	1·24	0·33	3·52	0·79	S × D	<0·001
	F_1B	Fasted	0·91	0·28	2·34	0·50		
		Fed	1·92	0·47	2·64	1·05		
HDL	DSNI	Fasted	1·38	0·10	1·54	0·20	S × D	0·002
		Fed	1·16	0·26	1·67	0·72		
	F_1B	Fasted	2·05	0·13	1·74	0·27		
		Fed	2·60	0·23	1·95	0·28		

S, strain; D diet; F, fasted or fed.
* Blood was collected following an overnight fast (fasted or following free access to food (fed)); for details of diets and procedures, see p. 342.
† *P* values are shown for significant ($P<0·05$) effects of each variable on interactions between them.

greater increase in LDL-cholesterol in response to the higher dietary cholesterol level ($P<0·001$). HDL tended to rise in response to the high-cholesterol diet in DSNI animals, but fell in Bio F_1B animals, causing a significant S × D interaction for HDL-cholesterol ($P=0·002$).

The livers of DSNI animals were significantly heavier than those from Bio F_1B animals ($P<0·001$, Table 3). However, this was not significant when expressed as % body weight (results not shown). Livers from fed animals were heavier than those from fasted animals and the high-cholesterol diet also produced heavier livers. Hepatic free cholesterol (Table 3) was significantly higher in animals fed the HFHC diet ($P<0·001$) and was higher in fed than fasted animals ($P=0·001$). Interestingly, Bio F1B hamsters had a lower hepatic cholesteryl ester content than DSNI animals. Fasting did not influence hepatic cholesteryl ester, but levels increased significantly more in the DSNI than the Bio F_1B strain fed HFHC diet ($P=0·002$).

In the second study, animals were fed diets containing 150 g coconut oil and 5·0 g cholesterol/kg for 6 or 12 months.

Table 3. Liver weight, hepatic free cholesterol and cholesteryl ester concentrations in dominant spot normal inbred (DSNI) and Bio F_1B hamsters after feeding a normal diet supplemented with 150 g coconut oil and 0·5 g cholesterol/kg (HFLC) or diet supplemented with 150 g coconut oil and 5 g cholesterol/kg (HFHC)*

(Mean values and standard deviations for four hamsters per group)

			HFLC		HFHC		Statistical significance of effect (three-way ANOVA)	
			Mean	SD	Mean	SD	Factor	P†
Liver weight (g)	DSNI	Fasted	5·65	0·06	6·80	0·77	S	<0·001
		Fasted	7·57	0·33	9·18	0·40	D	<0·001
	F1B	Fasted	4·75	0·79	5·53	0·96	F	<0·001
		Fed	5·73	0·92	7·23	0·73		
Cholesterol (mg/liver)	DSNI	Fasted	9	5	20	4	D	<0·001
		Fed	12	2	23	4	F	0·001
	F_1B	Fasted	9	2	118	3		
		Fed	10	1	27	3		
Cholesteryl ester (mg/liver)	DSNI	Fasted	13	8	266	18	S × D	0·002
		Fed	9	3	264	102		
	F_1B	Fasted	10	3	131	11		
		Fed	9	2	189	56		

S, strain; D, diet; F, fed or fasted.
* Livers were removed following an overnight fast (fasted) or following free access to food (fed); for details of diets and procedures, see p. 342.
† *P* Values are shown for significant ($P<0·05$) effects of each variable or interactions between them.

As with the first trial, Bio F_1B animals were smaller than DSNI animals at equivalent ages. The diet was generally well tolerated by both strains, although the Bio F_1B animals tended to gain more weight, and this was significant by 12 months (25·0 (SD 2·7) *v.* 13·5 (SD 8·3) g body-weight gain, $P=0·002$).

The chylomicron results are shown in Fig. 3 and the results for the other lipoproteins are shown in Table 4. Chylomicron-cholesterol and -TAG were much higher in Bio F_1B than DSNI animals and rose more dramatically between the two time points in the Bio F_1B animals ($T \times S$ interaction, $P=0·002$ and $P=0·001$ respectively, Fig. 3). The identity of this fraction as chylomicrons was confirmed by Western blotting using a polyclonal antibody against human ApoB, which indicated that virtually all of the ApoB was of the ApoB-48 rather than -100 isoform (results not shown).

Similar increases in VLDL-cholesterol ($P=0·005$) and -TAG ($P=0·002$) were also seen with time in Bio F_1B hamsters (Table 4). However, this fraction contained both ApoB-38 and -100 (results not shown) and was likely to be contaminated with chylomicrons. Intermediate-density-lipoprotein cholesterol was higher in Bio F_1B than DSNI animals but tended to decrease with time. By contrast, LDL-cholesterol did not change significantly with time and did not differ between strains. Although both strains had similar levels of HDL-cholesterol after 6 months, they tended to rise between 6 and 12 months in DSNI hamsters and fall in Bio F_1B hamsters causing a significant $S \times T$ interaction ($P=0·012$).

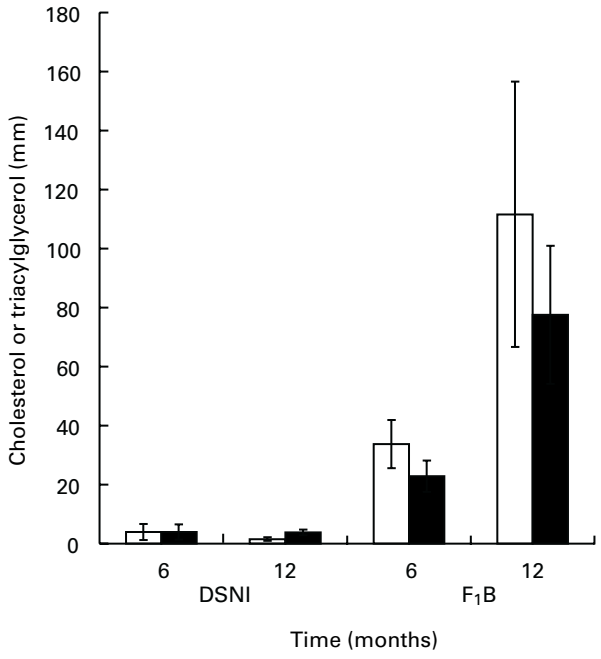

Fig. 3. Total chylomicron-cholesterol (■) and -triacylglycerol (□) concentrations in dominant spot normal inbred (DSNI) and Bio F_1B hamsters fed diets containing 150 g coconut oil/kg and 5·0 g added cholesterol/kg for 6 or 12 months. Animals were allowed constant access to food until time of killing. Chylomicrons were isolated and lipids analysed as outlined on p. 342; for details of diets and procedures, see p. 342. Values are geometric means for four animals with standard deviations shown by vertical bars.

LPL was higher in DSNI animals than Bio F_1B ($P<0·001$, Fig. 4) and was significantly reduced with time in both strains ($P=0·006$). As a result, activity was negligible in the Bio F_1B animals after 12 months of feeding. Hepatic lipase tended to increase with time in DSNI animals, but not Bio F_1B animals ($S \times T$ interaction, $P=0·017$).

Atherosclerosis was determined in both the aortic sinus and, by *en face* staining, throughout the length of the aorta (Fig. 5). No significant difference could be seen in staining in the aortic sinus between the two strains at 6 months. However, by 12 months much more staining was seen in the aortic sinus of the Bio F_1B animals ($S \times T$ interaction, $P=0·001$). In the rest of the aorta, Bio F_1B animals showed significantly more atherosclerosis at both time points ($P<0·001$), with the amount of staining increasing with time ($P<0·001$). Lesions were generally restricted to the inner curvature of the aortic arch. By 12 months, atherosclerosis had spread to both the thoracic and abdominal aorta in the Bio F_1B, although this was not seen in the DSNI hamsters.

Discussion

Previous work has indicated that the Bio F_1B hybrid hamster is more susceptible to atherosclerosis than other non-hybrid strains (Kowala *et al*. 1991). The aim of the present studies was to compare the lipoprotein response of Bio F_1B hamster to dietary saturated fat and cholesterol with that of the more atherosclerosis-resistant DSNI strain of hamster.

Initially, we compared the lipoprotein profile of the two strains fed a normal diet with no added cholesterol or fat. No dramatic differences between the two strains were seen, although TAG-rich lipoproteins and HDL-cholesterol were both found to be higher in the Bio F_1B than the DSNI strain. HDL was the predominant cholesterol-carrying lipoprotein in both strains, which confirms our previous findings (Sessions & Salter, 1994) and those of other investigators (Trautwein *et al.* 1993).

The addition of fat and cholesterol to the diet, particularly at the higher level of 5·0 g cholesterol/kg, elicited different responses in the two strains. Bio F_1B animals responded with a marked increase in TAG-rich lipoprotein-cholesterol and -TAG, which was most pronounced in animals on the high-cholesterol diet and in the fed state. We have previously shown an increase in VLDL-cholesterol and -TAG in other strains of hamster in response to dietary cholesterol (Sessions *et al.* 1994; Billett *et al.* 2000). However, the magnitude of the changes seen in the Bio F_1B hamsters was considerably greater than in other strains. The response seen in the present study is also considerably greater than that found by Trautwein *et al.* (1993), who compared the response to 4·0 or 8·0 g cholesterol/kg in Bio F_1B hamsters with that of two other strains However, these workers used a purified diet containing significantly less saturated fat (50 g butter/kg) than was used in the present study.

Elevated concentrations of TAG-rich lipoprotein, particularly in the fed state, and reduced hepatic cholesteryl ester storage in the Bio F_1B animals is consistent with

Table 4. Lipoprotein cholesterol concentration in dominant spot normal inbred (DSNI) and Bio F_1B hamsters fed 150 g coconut oil and 5 g cholesterol/kg for 6 or 12 months*

(Mean values and standard deviations for four animals per group)

		Time (months)					
		6		12		Statistical significance of effect (two-way ANOVA)	
		Mean	SD	Mean	SD	Factor	P†
VLDL-TAG	DSNI	2·63	0·98	1·78	0·44	S × T	0·002
	F_1B	10·01	2·51	21·44	5·56		
VLDL-chol	DSNI	2·60	0·58	1·90	0·76	S × T	0·005
	F_1B	10·64	1·55	22·87	7·27		
IDL-chol	DSNI	2·20	0·37	1·18	0·61	S	0·028
	F_1B	2·63	0·35	1·88	0·40	T	0·002
LDL-chol	DSNI	3·89	0·85	4·39	0·94		
	F_1B	3·49	0·29	3·53	0·86		
HDL-chol	DSNI	2·27	0·46	3·32	0·61	S × T	0·012
	F_1B	1·96	0·24	1·80	0·20		

chol, cholesterol; IDL, intermediate-density lipoprotein; S, strain; T, length of time on the diet.

* At each time point, animals were bled by cardiac puncture following free access to food and plasma lipoproteins prepared; for details of diet and procedures, see p. 342.

† *P* Values are shown for significant ($P<0·05$) effects of each variable or interactions between the variables.

a reduced activity of LPL on these lipoproteins, and hence a reduced production and clearance of cholesterol-rich remnants by the liver. To test this hypothesis, a further experiment was designed to determine the effect of the HFHC diet on post-heparin LPL activity. The present trial was also designed to compare the development of atherosclerotic lesions in the two strains over time.

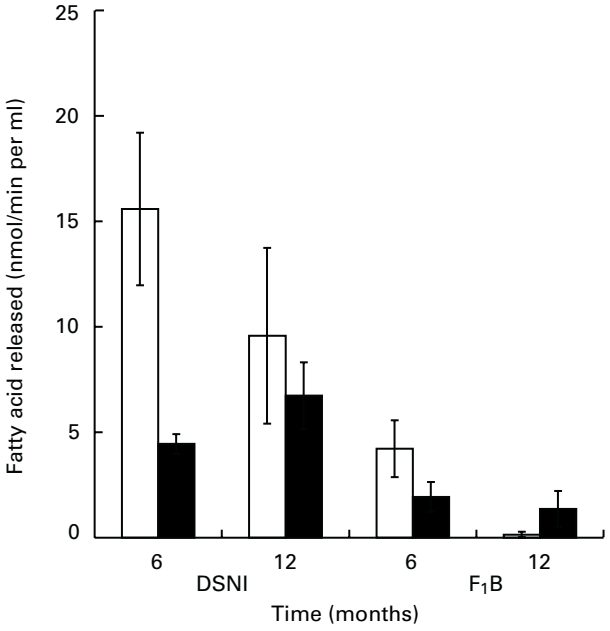

Fig. 4. Post-heparin lipoprotein lipase (□) and hepatic lipase (■) activities in dominant spot normal inbred (DSNI) and Bio F_1B hamsters fed a diet containing 150 g coconut oil/kg and 5·0 g added cholesterol/kg for 6 or 12 months. Animals were allowed constant access to food until time of killing. Animals were injected with heparin, blood collected and plasma lipase activity determined as described on p. 342; for details of diets and procedures, see p. 342. Values are geometric means for four animals with standard deviations shown by vertical bars.

After 6 months on a diet containing 150 g coconut oil and 5·0 g cholesterol/kg, Bio F_1B hamsters had much greater plasma total cholesterol and TAG concentrations than DSNI hamsters, and this was largely due to an accumulation of chylomicrons (as identified by density and presence of ApoB-48). By 12 months, chylomicron-TAG was more than 50-fold greater in the Bio F_1B compared with the DSNI animals. This accumulation of chylomicrons was shown to be associated in Bio F_1B animals with much lower activity of heparin-releasable LPL, which declined almost to zero by 12 months. Thus, the massive hyperlipidaemia associated with cholesterol feeding in Bio F_1B hamsters is associated with an accumulation of chylomicrons, and to a lesser extent VLDL, due to a reduction in LPL activity.

At the present time, we do not know why LPL responds so differently to dietary cholesterol in these two strains. Heparin releases LPL from the vascular endothelium of many tissues in the body, with adipose tissue and skeletal muscle being quantitatively the most important. In the fed state, due to the actions of insulin, LPL activity in adipose tissue would be expected to be up-regulated and thus make the greatest contribution to the released pool. However, the fact that by 12 months virtually no activity could be detected in Bio F_1B hamsters suggested that all tissues of the body might be affected. Absence of functional LPL is one of the underlying causes of type 1 hyperlipoproteinaemia in man. Models of such hyperlipidaemia have been described in the cat (Ginziger *et al.* 1996) and mink (Savonen *et al.* 1999). However, these are a direct result of mutations within the gene resulting in a failure to produce functional LPL. The apparent deficiency of LPL activity in the Bio F_1B animals was dependent on feeding dietary cholesterol. It is possible that cholesterol may exert direct effects on LPL gene expression. Sterol regulatory element binding proteins (Kim & Spiegelman, 1996; Shimano *et al.* 1996, 1997; Yang & Deeb, 1998) may regulate the transcription of the LPL gene. It has been shown that depletion of cholesterol, or over-expression of

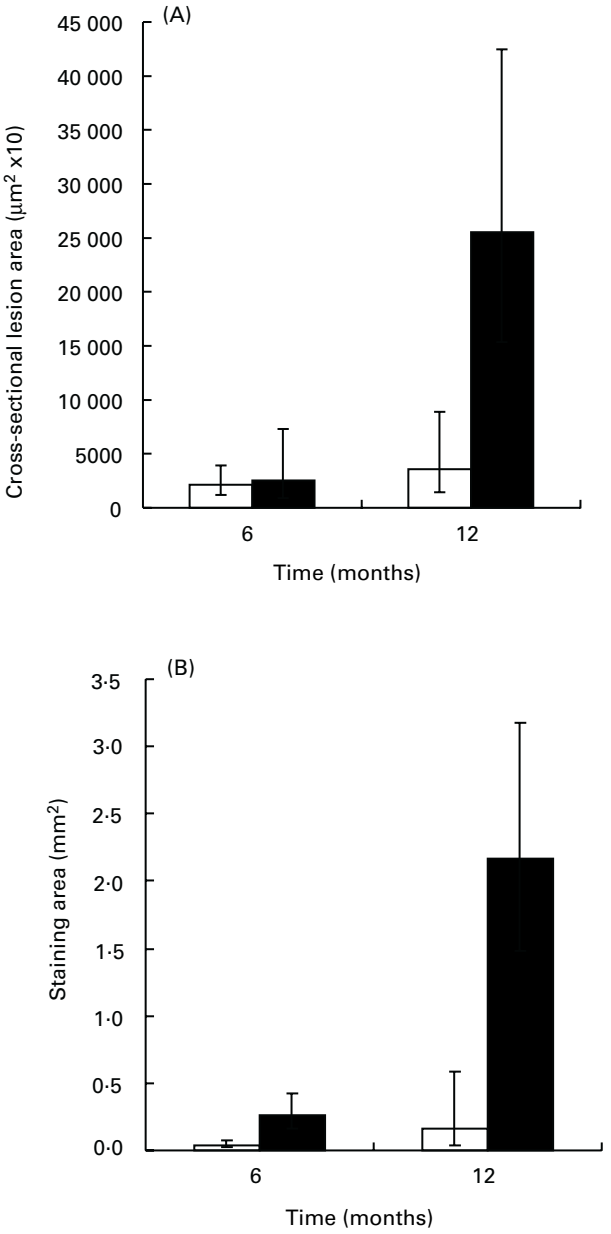

Fig. 5. Atherosclerosis in dominant spot normal inbred (□) and Bio F_1B (■) hamsters fed a diet containing 150 g coconut oil/kg and 5·0 g added cholesterol/kg for 6 or 12 months. Atherosclerosis was quantified as Oil Red O-staining of either sections taken from the aortic sinus (A) or *en face* staining of the aorta from the aortic arch to the abdominal aorta (B) as described on p. 343; for details of diets and procedures, see p. 342. Values are geometric means for eight animals with standard deviations shown by vertical bars.

sterol regulatory element binding proteins 1 or 2 induces LPL gene expression in 3T3-L1 adipocytes (Schoonjans *et al.* 2000). It is interesting to speculate that polymorphisms in the sterol response element of the LPL promoter may influence the response of the two strains to dietary cholesterol. However, at present, the molecular basis of this strain-specific response to dietary cholesterol remains to be established.

It is also not clear whether this phenotype is restricted to the Bio F_1B hybrid or may be associated with one of the parent strains. The Bio F_1B hybrid is derived from two highly inbred strains, namely Bio 87.20 female and Bio 1.5 male. Little is known about lipid metabolism in either of the parent strains, although there are some reports of altered cholesterol metabolism in the Bio 87.20 strain (Schaffer *et al.* 1981; Singhal *et al.* 1983). We are not aware, however, of any reports of the effect of cholesterol feeding on plasma TAG concentrations in the parent strains. It is understood that the Bio F_1B hybrid was originally favoured due to its relative longevity and general robustness (C. VanDongen, personal communication) prior to the discovery of an increased susceptibility to atherosclerosis (Kowala *et al.* 1991). Further investigation of the parental strains should provide useful information as to the genetic basis of the increased susceptibility to hyperlipidaemia.

In general, we confirm that Bio F_1B animals are more susceptible to atherosclerosis than DSNI animals. While at 6 months no significant difference was seen in the amount of lipid staining seen at the aortic sinus between the two strains of animal, significantly more staining was seen in the aortic arch of Bio F_1B animals ($P<0{\cdot}001$). By 12 months, little change was seen in the amount of atherosclerosis in the DSNI animals, but lipid staining at the aortic sinus and throughout the rest of the aorta increased dramatically, and was 5–6-fold greater in the Bio F_1B compared with the DSNI animals. Thus, the increased susceptibility of the Bio F_1B hamster to atherosclerosis became increasingly apparent the longer the animals were fed an atherogenic diet.

It remains to be established which lipoproteins are responsible for this increased susceptibility to atherosclerosis. It has generally been assumed that familial LPL deficiency is not associated with increased risk of atherosclerosis in man. However, Benlian *et al.* (1996) reported premature atherosclerosis in four patients suffering from this condition. Mice that are heterozygous for LPL deficiency actually show a decreased susceptibility to atherosclerosis (Semenkovich *et al.* 1998). Semenkovich *et al.* (1998) suggested that a deficiency of LPL in the vessel wall could prevent the retention of atherogenic lipoproteins. However, in the ApoE knockout mouse, over-expression of LPL appears to protect against atherosclerosis (Yaghu *et al.* 1999). Over-expression in these animals was found in a number of tissues including the aorta. In the cholesterol-fed Bio F_1B hamster, a deficiency in LPL activity was associated with an increased susceptibility to atherosclerosis. This could not be attributed to changes in LDL-cholesterol, which was not significantly different between the two strains. Compared with the heterozygous LPL-deficient mice (Semenkovich *et al.* 1998), plasma TAG and cholesterol levels were much greater in the Bio F_1B hamster. Feeding cholesterol to these animals resulted in the accumulation of cholesterol-enriched chylomicrons that may be atherogenic. Furthermore, we do not know the relative activity of LPL in different tissues. Recent evidence suggests that, in mice, cholesterol feeding actually induces LPL expression in macrophages, but not in adipose tissue or muscle (Zhang *et al.* 2001). This was shown to be mediated through the oxysterol liver X receptor class of transcription factors. Thus, the reduction

in heparin-releasable LPL may not reflect a similar change in LPL activity within the artery wall. Further study of the cholesterol-fed Bio F_1B hamster should yield some useful insights into the possible role of TAG-rich lipoproteins and LPL in the development of atherosclerosis.

In conclusion, we confirm the susceptibility of Bio F_1B hamsters to atherosclerosis at relatively modest dietary cholesterol concentrations. Prolonged consumption of an atherogenic diet results in the development of lesions throughout the aorta. This increase in susceptibility to the effects of dietary cholesterol is associated with a dramatic accumulation of chylomicrons in the plasma as a result of reduced LPL activity. The mechanisms underlying this strain difference remain to be established.

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