

Modulation of the regression of atherosclerosis in the hamster by dietary lipids: comparison of coconut oil and olive oil

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The Golden Syrian hamster (*Mesocricetus auratus*) has been shown to be a useful model of both human lipoprotein metabolism and the development of atherosclerosis. We report the effects of dietary lipids on the progression and regression of atherosclerosis in this model. In the first study, hamsters fed on coconut oil (150 g/kg diet) and cholesterol (30 g/kg diet) developed lipid-rich lesions in the ascending aorta (0.28 (SD 0.14) mm²) and aortic arch (0.01 (SD 0.01) mm²) after 4 weeks that continued to progress over the next 8 weeks (0.75 (SD 0.41) mm² and 0.12 (SD 0.11) mm² for the ascending aorta and aortic arch respectively). Removal of cholesterol from the diet halted this progression. Furthermore, in animals fed on olive oil in the absence of added cholesterol, plasma LDL-cholesterol concentrations were lower ($P < 0.05$) and the extent of atherosclerotic lesions was reduced ($P < 0.001$ for both regions of the aorta) compared with animals fed on coconut oil (with no added cholesterol). In a second study, animals were fed on the atherogenic diet for 10 weeks, transferred to diets containing either coconut oil (150 g/kg diet) or olive oil (150 g/kg diet) without added cholesterol and monitored for up to 16 weeks. In the ascending aorta, lesion size doubled in animals fed on coconut oil but stabilized in those fed on olive oil. In the aortic arch, lesion size decreased linearly ($P < 0.05$, $P < 0.001$ for coconut oil and olive oil respectively) with the greatest reduction being seen in the olive-oil-fed animals ($P < 0.05$). Again, progression and regression of atherosclerosis appeared to reflect the relative concentrations of LDL-cholesterol and HDL-cholesterol in the plasma. We conclude that the male Golden Syrian hamster represents a useful model of dietary induced regression as well as progression of atherosclerosis.

Hamster: Atherosclerosis: Coconut oil: Olive oil: Dietary fat

The Golden Syrian hamster (*Mesocricetus auratus*) has been used extensively in studies of lipoprotein metabolism. We (Sessions & Salter, 1994; Bennett *et al.* 1995; White *et al.* 1997; Salter *et al.* 1998) and others (Spady & Dietschy, 1988; Woollett *et al.* 1989, 1992; Lindsey *et al.* 1990) have shown this species to be useful in studying the lipoprotein response to dietary cholesterol and lipids. The extensive studies of Spady, Dietschy and co-workers clearly demonstrated changes in the kinetics of LDL metabolism in response to different dietary fats (Spady *et al.* 1993). We have recently shown that these changes may, at least in part, be due to changes in the hepatic expression of genes such as LDL receptor, apolipoprotein B and the microsomal triacylglycerol (TAG) transfer protein (White *et al.* 1997; Salter *et al.* 1998). The hamster has also been used for the

study of development of atherosclerosis (Nistor *et al.* 1987; Sima *et al.* 1990). Nistor *et al.* (1987) performed detailed histological studies of the development of aortic atherosclerosis in response to diets enriched in cholesterol and saturated fat. Lesions appeared after only 3 weeks. Human-like lesions, displaying Ca deposits and necrosis, had developed by 10 months. Other workers have demonstrated inhibition of the development of atherosclerosis using a range of pharmacological agents including: hydroxymethyl-glutaryl-CoA reductase (EC 1.1.1.34) inhibitors (Otto *et al.* 1995), bile-acid-binding resins (Kowala *et al.* 1991), prostacyclin agonists (Kowala *et al.* 1993) and α_1 adrenergic inhibitors (Kowala *et al.* 1991; Foxall *et al.* 1992). These studies have used various strains of hamster and a range of dietary interventions to induce atherosclerosis. Some strains

Abbreviations: IDL, intermediate-density lipoprotein; TAG, triacylglycerol.

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require high levels of dietary cholesterol (up to 30 g/kg diet) to develop atherosclerotic lesions (Nistor *et al.* 1987; Sima *et al.* 1990), although the amount of cholesterol required may depend on the amount of saturated fatty acid in the diet (Kahlon *et al.* 1996). By contrast, the F₁B hybrid strain of hamster has been shown to develop aortic fatty streaks when fed on diets with cholesterol concentrations as low as 0.5 g/kg (Kowala *et al.* 1991). In the DSNI strain we have found that a minimum of 5 g cholesterol/kg diet is required to induce aortic lesions (MA McAteer, GM Benson and AM Salter, unpublished results).

Considerable evidence exists to suggest that atherosclerotic lesions can regress in human subjects (Mahler, 1995). However, detailed studies of regression are frequently hampered by choice of animal model and the length of the experiments required. Studies with swine and non-human primates have clearly shown that reduction in plasma lipids, by dietary or pharmacological means, can result in the regression of advanced atherosclerotic plaques (Wissler & Vesselinovitch, 1990). However, such studies can take many years to perform (Strong *et al.* 1994). The normal rabbit (Vesselinovitch *et al.* 1974; Zhu *et al.* 1990, 1994) and the Watanabe heritable hyperlipidaemic rabbit (Oshima *et al.* 1998) have been used in regression studies. However, a smaller animal model of regression would be very valuable for further studies of the mechanisms of regression and the efficacy of potential pharmacological and dietary interventions. Regression of atherosclerosis was recently reported in the hamster (Pitman *et al.* 1998). Such a small animal model of regression could be extremely useful in furthering our understanding of the mechanisms of regression and the potential effects of dietary and pharmacological interventions. In the present study we performed two trials. The first, lasting a total of 12 weeks, was primarily designed to determine whether by removing cholesterol from the diet and changing from a saturated-fatty-acid-rich to a monounsaturated-fatty-acid-rich diet differences in the progression of atherosclerosis could be detected. A second longer (total of 26 weeks) trial was performed to look at the effect of these diets on the regression of atherosclerotic lesions.

Materials and methods

Animals and diets

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

Study 1. Thirty-two 16–24-week-old (weight 134–175 g) male DSNI Golden Syrian Hamsters (Biomedical Services Unit, University of Nottingham, Notts., UK) were housed individually and provided free access to food and water. They were maintained in a controlled environment

(21°, 55% humidity) and were subjected to a 12 h light–dark cycle. On arrival at our facility animals were allowed a 2-week acclimatization period during which they were fed on a commercial rodent chow (Rat and Mouse Breeding Diet No. 3, Special Diet Services, Chelmsford, Essex, UK). During the first week this diet was provided in pelleted form and in the second week as a ground meal. Following the acclimatization period all animals were transferred to an atherogenic diet consisting of (g/kg): Rat and Mouse Breeding Diet No. 3 820, coconut oil (Sigma, Poole, Dorset, UK) 150 and cholesterol (Sigma) 30. All animals were checked daily for signs of ill-health throughout the trial and body weights were checked weekly. No animal lost more than 10% of its initial body weight. After 4 weeks on the atherogenic diet, eight animals were randomly selected, (group A), fasted overnight and killed the following morning. They were anaesthetized using sodium pentobarbitone (Sagatal, 1 ml/kg) and 3–4 ml blood collected by cardiac puncture and placed into EDTA-tubes. The aortas were then dissected from the animals, flushed with saline and fixed in 10% neutral buffered formalin (Sigma).

The remaining animals were randomly allocated to three groups of eight. Group B continued to be fed on the atherogenic diet, group C was fed on (g/kg diet): chow 850, coconut oil (no added cholesterol) 150 and group D was fed on (g/kg diet): chow 850, extra virgin olive oil (Filippo Berio, Lucca, Italy) with no added cholesterol 150. After a further 8 weeks all animals were killed and samples taken as described earlier.

Study 2. Thirty male, 12–18-week-old (weight 100–140 g), DSNI hamsters were housed as described earlier. During the acclimatization period they were fed on Rat and Mouse Breeding Diet 422 (Pilsbury, Northants, UK) and this chow was also used in the experimental diets. Both this chow and that used in study 1 are rodent breeding diets with similar overall compositions (gross energy, 15.3 v. 16.0 MJ/kg; crude oil, 43 v. 44 g/kg; crude protein, 223 v. 209 g/kg; crude fibre, 45 v. 41 g/kg and starch, 358 v. 362 g/kg for the Special Diet Services and Pilsbury diets respectively). For the first 10 weeks all animals were fed on an atherogenic diet similar to that described earlier, except that hydrogenated coconut oil (ICN Biomedicals Ltd, Thame, Oxon., UK) rather than coconut oil was used. This was used to enhance the atherogenicity of the diet as it is even richer in saturated fatty acids (see Table 1). After 10 weeks, a group of six animals was randomly selected and killed as before (group A). The remaining twenty-four animals were allocated to two groups of twelve, one of which was fed on a diet containing (g/kg diet): chow 850 and hydrogenated coconut oil 150 and the other a diet containing chow 850 and olive oil 150 (both with no added cholesterol). After a further 8 weeks, six coconut-oil-fed animals and six

Table 1. Fatty acid composition of dietary oils (g/100 g total fatty acids recovered)

Fatty acid...	8:0	10:0	12:0	14:0	16:0	18:0	18:1	18:2
Coconut oil	4.5	7.9	32.8	24.0	13.3	4.2	10.3	3.1
Hydrogenated coconut oil	5.0	7.5	32.4	24.5	13.6	15.0	0.8	0.7
Olive oil	ND	ND	ND	ND	11.8	3.7	73.2	8.2

ND, not detected.

olive-oil-fed animals (groups B and C respectively) were killed. After another 8 weeks the remaining six coconut-oil-fed (group D) and six olive-oil-fed (group E) animals were killed.

Fatty acid analysis of dietary fats

Coconut oil, hydrogenated coconut oil and olive oil were extracted, transmethylated and analysed by GLC as previously described (Bennett *et al.* 1995).

Separation of lipoproteins

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 lipoprotein (IDL), LDL and HDL were separated with the density ranges <1.006, 1.006–1.020, 1.020–1.060 and >1.060 g/ml respectively. Cholesterol and TAG concentrations in whole plasma and lipoprotein fractions were determined using diagnostic kits from Wako (Alpha Laboratories, Eastleigh, Hants., UK).

Determination of the extent of atherosclerosis

The fixed aortas were cleared of adventitial tissue and rinsed in distilled water. They were then rinsed in propan-2-ol (600 ml/l) before staining with Oil Red O–propan-2-ol (10 : 90, v/v) for 15 min. The tissue was destained with propan-2-ol and then distilled water before returning to neutral buffered formalin. For quantification of lipid staining,

the aorta was cut open along the outer curvature, laid flat on a glass slide and held in place with a cover slip. The aorta was then examined under a stereo microscope and separate images of the ascending aorta and aortic arch captured using Optimas Imaging System Software (version 5.2, Data Cell Ltd, Maidenhead, Berks., UK). Essentially all of the lipid staining in the ascending aorta was located in proximity to the aortic valve. The arch was identified as the region between the left and right subclavian arteries. The total area of Oil-Red-O staining was determined for both regions of the aorta.

Statistical analysis

Data for each variable in each study were checked for Gaussian distribution. Plasma lipid and lipoprotein concentrations were found generally not to be normally distributed, in many cases even after log-transformation, and were therefore analysed by non-parametric tests. Data were analysed by either: one-way ANOVA (followed by either Tukey-Kramer multiple comparisons test or Bonferroni (selected pairs) test as appropriate) or Kruskal-Wallis non-parametric ANOVA test (followed by Dunn's multiple comparisons test) using In Stat software (GraphPad, San Diego, CA, USA). The specific statistical test applied is indicated in the legends to tables and figures. Correlations were calculated using the non-parametric Spearman rank correlations using In Stat software.

Results

Study 1

Animals tolerated each of the diets well and no animal lost more than 10 % of its initial body weight. Fasting final body

Table 2. Plasma total lipids and lipoprotein cholesterol concentrations (mmol/l) in animals from study 1†
(Values are medians and interquartile ranges for eight animals per group)

Group...	A		B		C		D		Statistical significance of difference between means‡; P
	Time from start (weeks)...	4	12	12	12	CO	OO	12	
Diet...	CO + Chol		CO + Chol		CO		OO		
	Median	Interquartile range	Median	Interquartile range	Median	Interquartile range	Median	Interquartile range	
Total cholesterol	11.18	8.89–12.08	19.47	18.58–21.02	8.98	7.94–11.48	4.91	4.62–5.14	Group C v. B, * Group D v. A, * Group D v. B, ***
Total TAG	2.37	1.70–3.17	3.47	2.76–4.23	2.08	1.67–2.26	1.67	1.57–1.79	Group D v. B, **
VLDL-cholesterol	2.54	1.92–3.60	3.84	2.94–5.37	0.96	0.72–1.23	0.87	0.75–0.99	Group C v. B, ** Group D v. A, ** Group D v. B, ***
IDL-cholesterol	2.67	1.32–2.99	4.29	3.78–5.34	0.87	0.69–1.64	0.05	0–0.24	Group C v. B, * Group D v. A, ** Group D v. B, ***
LDL-cholesterol	2.19	0.77–3.85	6.99	5.36–8.64	4.68	3.22–5.16	0.48	0.42–0.60	Group B v. A, ** Group D v. B, *** Group D v. C, *
HDL-cholesterol	2.78	2.50–3.68	3.92	3.35–4.35	3.14	2.75–3.43	3.30	3.00–3.72	

CO, coconut oil; Chol, cholesterol; OO, olive oil; TAG, triacylglycerol; IDL, intermediate-density lipoprotein.

* P<0.05; ** P<0.01; *** P<0.001.

† For details of diets and procedures see pp. 402–403.

‡ Statistical analysis performed by Kruskal-Wallis non-parametric ANOVA test followed by Dunn's multiple comparisons test.

weights for each of the dietary groups were (g): group A 159.7 (SD 13.1), group B 156.8 (SD 8.7), group C 154.5 (SD 12.4) and group D 172.4 (SD 9.8). Groups C and D were significantly different ($P < 0.05$). Plasma and lipoprotein lipid concentrations in the animals from study 1 are shown in Table 2. After 4 weeks on the atherogenic diet (group A)

the total plasma cholesterol and TAG concentrations were approximately 5- and 2.5-times higher than values obtained from animals of the same strain fed on chow alone (cholesterol 2.19 (SD 0.27) mmol/l, TAG 1.09 (SD 0.27) mmol/l, n 4; AM Salter, unpublished results). After another 8 weeks on the high-fat, high-cholesterol diet (group B) there was a

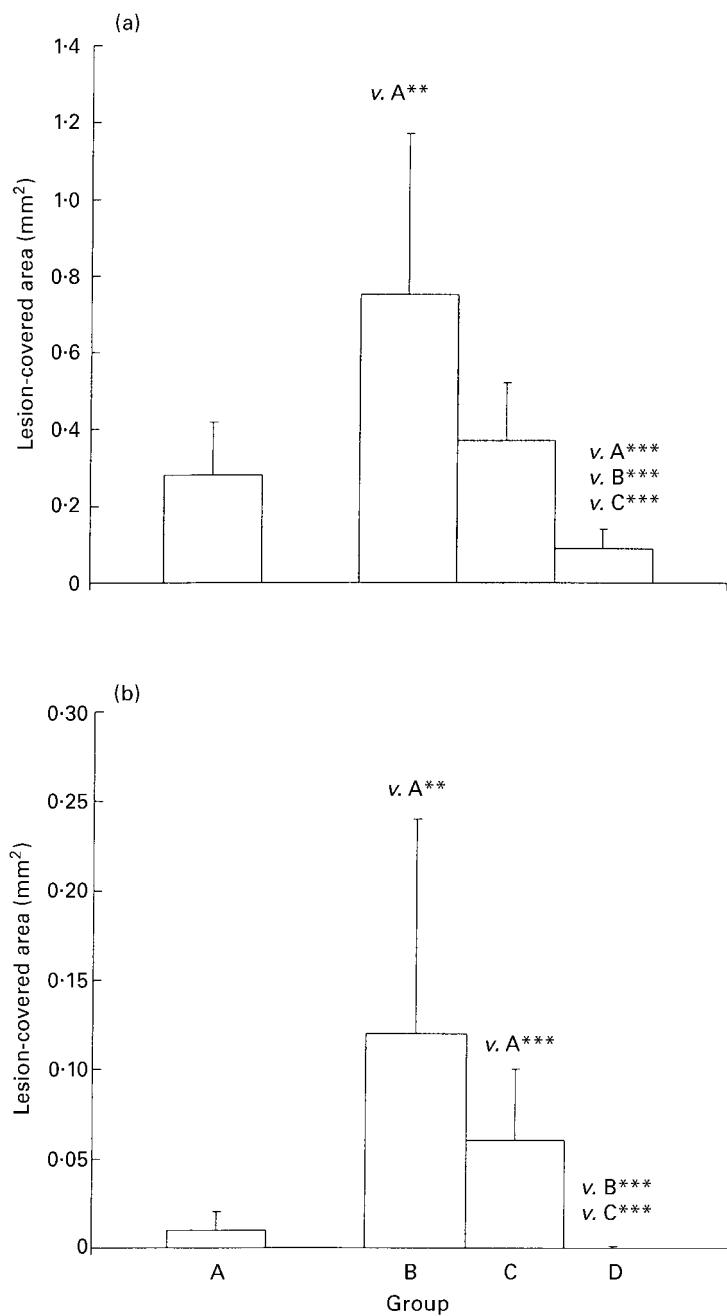


Fig. 1. Atherosclerosis in (a) ascending aorta and (b) aortic arch in hamsters from study 1. Animals were fed on 150 g coconut oil/kg diet and 30 g cholesterol/kg diet for 4 weeks (group A, n 8) or 12 weeks (group B, n 8), or were transferred after 4 weeks onto cholesterol-free diets containing either 150 g coconut oil/kg diet (group C, n 8) or 150 g olive oil/kg diet (group D, n 8) and fed for a further 8 weeks. On killing the aortas were removed and stained with Oil Red O as described on p. 403. Data are presented as mean values with standard deviations represented by vertical bars. Significant differences between groups were determined by ANOVA followed by Bonferroni multiple comparisons test: ** $P < 0.01$, *** $P < 0.001$.

Table 3. Spearman rank correlations between plasma lipoprotein concentration and extent of aortic lipid staining in animals fed on experimental diets for 12 weeks (groups B, C and D) in study 1*

	Study 1 (n 24)			
	Ascending aorta		Aortic arch	
	r	P	r	P
VLDL-cholesterol	0.541	0.008	0.478	0.018
IDL-cholesterol	0.644	< 0.001	0.789	< 0.001
LDL-cholesterol	0.568	0.005	0.713	< 0.001
HDL-cholesterol	-0.045	0.837	0.189	0.375
IDL : HDL	0.671	< 0.001	0.796	< 0.001
LDL : HDL	0.664	< 0.001	0.688	< 0.001

IDL, intermediate-density lipoprotein.

* For details of diets and procedures see pp. 402–403.

further 3-fold rise in the LDL-cholesterol concentration. However, when coconut oil was fed in the absence of added dietary cholesterol (group C), there was a highly significant ($P < 0.01$) drop in the cholesterol concentration in the VLDL fraction compared with groups A and B. Total plasma cholesterol and IDL-cholesterol were also reduced compared with the animals which had been on the atherogenic diet for the full 12 weeks (group B). Replacing the coconut oil with olive oil (with no added cholesterol) reduced the mean cholesterol content in each of the lipoprotein fractions, except HDL, compared with group A. The only statistically significant difference between groups C and D was a 7-fold difference in the LDL fraction.

Fig. 1 shows the extent of positive lipid staining in the ascending aorta and aortic arch of animals in each group. All animals in group A showed some signs of atherosclerosis in both regions of the vessel. However, area of positive lipid staining was considerably greater in the ascending aorta (0.28 (SD 0.14) mm²) than in the arch (0.01 (SD 0.01) mm²). After a further 8 weeks on the atherogenic diet the animals in group B showed a significant increase in area of positive lipid staining in both regions of the aorta (0.75 (SD 0.41) and 0.12 (SD 0.11) mm²) for the ascending aorta and aortic arch respectively. This increase was prevented by feeding the coconut-oil-rich diet without added cholesterol (group C). Feeding olive oil without added cholesterol (group D) reduced the area covered by lesions in the ascending aorta (0.09 (SD 0.05) mm²) compared with the other three groups ($P < 0.001$).

To investigate whether the extent of atherosclerosis could be associated with the plasma concentrations of one particular lipoprotein fraction, correlations were performed on data from all of the animals fed for 12 weeks. Table 2 shows strong correlations between atherosclerosis in both regions of the aorta and VLDL-, IDL- and LDL-cholesterol. While there is some indication that this correlation may be stronger for IDL and LDL than VLDL, the data should be interpreted with caution because of the high degree of inter-correlation between the fractions. HDL-cholesterol was not significantly correlated to the degree of atherosclerosis and expressing IDL or LDL as a ratio against HDL did not improve the correlations significantly.

Table 4. Plasma total lipids and lipoprotein cholesterol concentrations (mmol/l) in animals from study 2†
(Values are medians and interquartile ranges for six animals per group)

Group ...	A		B		C		D		E		Statistical significance of difference between means; P	
	Time from start (weeks) ...	10	CO + Chol	18	CO	Median	Interquartile range	Median	Interquartile range	Median	Interquartile range	
		CO		CO								
Total cholesterol	27.24	22.58–30.44	13.07	12.20–15.33	6.34	5.69–7.38	9.14	7.96–9.88	6.19	5.60–7.37	Group C v. A, *** Group E v. A, ***	
Total TAG	10.76	7.92–13.63	3.01	2.51–3.11	2.20	2.07–2.43	1.97	1.70–2.10	1.21	1.10–1.62	Group D v. A, ** Group E v. A, ***	
VLDL-cholesterol	10.40	10.16–13.96	1.47	1.16–1.73	0.86	0.64–0.91	1.39	1.05–1.47	0.82	0.72–0.87	Group C v. A, ** Group E v. A, ***	
IDL-cholesterol	7.05	6.95–7.77	1.75	1.68–2.35	0.22	0.21–0.43	1.14	0.63–1.58	0.22	0.17–0.48	Group C v. A, ** Group E v. A, ***	
LDL-cholesterol	6.59	6.34–6.94	6.62	5.86–7.77	1.79	1.57–1.87	4.19	3.22–4.32	1.54	1.10–1.90	Group C v. A, * Group C v. B, ** Group E v. A, **	
HDL-cholesterol	2.56	2.50–2.73	3.29	2.72–3.71	3.24	2.90–3.56	2.70	2.17–2.80	3.48	3.37–4.58	Group E v. A, *	

CO, coconut oil; Chol, cholesterol; OO, olive oil; TAG, triacylglycerol; IDL, intermediate-density lipoprotein.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† For details of diets and procedures see pp. 402–403.

‡ Statistical analysis performed by Kruskal-Wallis non-parametric ANOVA test followed by Dunn's multiple comparisons test.

Study 2

In order to confirm the ability of the olive-oil-rich diets to induce regression of the atherosclerotic lesions, a second study was performed in which animals were fed on an atherogenic diet for longer (10 as opposed to 4 weeks) before transfer to the low-cholesterol diets. Animals in this

study were somewhat younger than those used in the first study and this was reflected in starting body weights. No significant difference was seen in fasting final body weight between any of the dietary groups (g): group A 120.2 (SD 4.7), group B 128.5 (SD 10.1), group C 135.9 (SD 10.8), group D 134.2 (SD 7.8) and group E 131.1 (SD 16.8). However, it is of note that the animals never achieved the

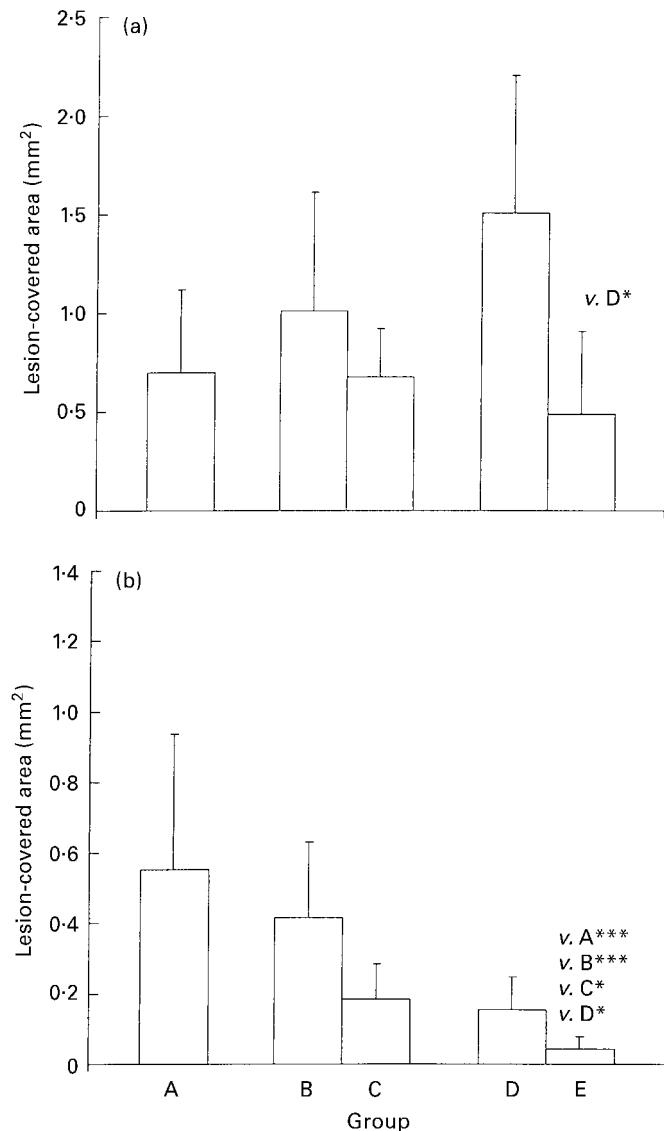


Fig. 2. Atherosclerosis in (a) ascending aorta and (b) aortic arch in hamsters from study 2. Thirty male hamsters were fed on a diet containing 150 g hydrogenated coconut oil/kg diet and 30 g cholesterol/kg diet for 10 weeks. At the end of this period six animals were killed (group A) and the remainder were transferred onto cholesterol-free diets containing either 150 g hydrogenated coconut oil/kg (groups B and D) or 150 g olive oil/kg (groups C and E). Groups of six animals were killed after a further 8 weeks (groups B and C) and 16 weeks (groups D and E). The aortas were removed and stained with Oil Red O as described on pp. 402–403. Data are presented as mean values with standard deviations represented by vertical bars. For data from the ascending aorta significant differences between groups were determined by ANOVA followed by Bonferroni multiple comparisons test. Data from the aortic arch were not normally distributed and were first log-transformed before statistical analysis: *P<0.05, ***P<0.001.

body weights seen in study 1, despite the fact that the trial lasted up to 14 weeks longer. Total plasma cholesterol and TAG (Table 3) were considerably higher in the animals fed on the atherogenic diet for 10 weeks (group A) than for animals fed on the same diet for 12 weeks in the previous study (group B). This was almost entirely due to a 2.5-fold higher concentration of VLDL-cholesterol in the second trial. VLDL- and IDL-cholesterol dropped dramatically when cholesterol was omitted from the diet, with the fall being greatest in the olive-oil-fed animals (groups C and E) (Table 4). After feeding the diets with no added cholesterol for 8 weeks, plasma LDL-cholesterol concentrations remained high in coconut-oil-fed animals (group B) but were markedly reduced in olive-oil-fed animals (group C). After a further 8 weeks LDL-cholesterol was reduced in both dietary groups but still remained lower in those fed on olive oil. HDL-cholesterol was significantly increased after 16 weeks of olive-oil feeding (group E) compared with group A.

Fig. 2 shows the development of atherosclerosis in these animals. In the ascending aorta the extent of atherosclerosis continued to increase in coconut-oil-fed animals even when cholesterol was omitted from the diet. By contrast, the amount of atherosclerosis in the olive-oil-fed animals remained fairly constant. The pattern of lesion development in the aortic arch was different, with evidence of regression in both coconut-oil and olive-oil-fed animals.

Discussion

The male hamster has proved a useful model of lipoprotein metabolism and displays some important advantages over other small animal species (Spady *et al.* 1993). Coupled with its small size and ease of handling the hamster has become a popular model of dietary effects on lipoprotein metabolism.

Significantly less work has been done on the hamster as a potential model of the development of atherosclerosis. Nistor *et al.* (1987) have presented a detailed study of the progression of atherosclerosis in the cholesterol-fed hamster. Feeding on a diet containing 30 g cholesterol/kg and 150 g butter/kg resulted in dramatic increases in LDL-cholesterol which were accompanied by the development of atherosclerosis in the aortic arch. After 4 weeks of feeding, lipid deposits were visible in the aortic arch due to the appearance of lipid-laden macrophages in the sub-endothelium. By 4–5 months, fatty streaks were clearly visible and by 10–12 months, lesions were proliferative with necrotic zones and Ca deposits. These lesions were reported to occlude up to 30% of the lumen. Extensive lesions have also been reported to develop in the coronary arteries of hamsters fed on diets rich in saturated fat and cholesterol for extended periods of time (Sima *et al.* 1990).

The development of atherosclerosis in the hamster has since been shown to be susceptible to a variety of pharmacological interventions (Kowala *et al.* 1991, 1993; Foxall *et al.* 1992; Otto *et al.* 1995) and is responsive to the anti-oxidant content of the diet (Parker *et al.* 1995). However, only one recent paper (Pitman *et al.* 1998) has reported regression of atherosclerosis in the hamster.

The animals used in the present study were bred within our own facility and were of the DSNI strain. We have previously shown that, in the presence of very low concentrations of dietary cholesterol (0.05 g/kg), this strain of hamster responds to dietary saturated fats with an increase in the LDL fraction (Bennett *et al.* 1995; Salter *et al.* 1998). In the present study a diet containing a very high concentration of cholesterol (30 g/kg) was used to induce atherosclerosis. This was done to ensure that lesions covered a significant area of the aorta such that regression might be detected on changing the diet. This atherogenic diet resulted in a dramatic increase in the plasma concentrations of VLDL-, IDL-, and LDL-cholesterol. Plasma TAG was also dramatically increased. In comparing the two studies the increase in VLDL was considerably greater in study 2 than in study 1. The reason for this is unclear, but the use of coconut oil in the first trial and hydrogenated coconut oil in the second may have had some influence. While coconut oil contains approximately 130 g unsaturated fatty acids/kg this is reduced to about 15 g/kg in the hydrogenated form. This is due to the conversion of both oleic acid and linoleic acid to stearic acid (Table 1). The basic rodent chow also differed between the two experiments but both were rodent breeding diets of similar overall nutrient and fatty acid composition. The other difference was the starting age and weight of the hamsters which were significantly less in the animals used in study 2. Thus, one or more of these differences may have contributed to the difference in response seen in the two studies. The hyperlipidaemia and extent of atherosclerosis seen in the present study were not as great as in the commonly used F₁B hybrid strain of hamster which has been shown to develop lesions after feeding cholesterol at only 0.5 g/kg diet (Otto *et al.* 1995). However, we have found that the F₁B strain exhibits even greater increases in cholesterol and TAG content of the VLDL fraction on cholesterol feeding (MA McAtee, GM Benson and AM Salter, unpublished results).

The presence of atherosclerotic lesions, as demonstrated by Oil-Red-O staining was shown in both the ascending aorta, in the area of the aortic valves and on the inner curvature of the aortic arch. Generally lesions appeared earlier in the ascending aorta and were more extensive. The development of lesions at the base of the aortic sinus is also characteristic of dietary induced atherosclerosis in certain inbred strains of mice but only rarely do such lesions extend to the aortic arch in this species (Breslow, 1996). Much more widely distributed lesions are, however, seen in transgenic mouse models such as the apoE-knockout and LDL receptor-deficient mouse (Ishibashi *et al.* 1994; Nakashima *et al.* 1994).

Stein *et al.* (1996) have suggested that on low-cholesterol diets the high concentration of HDL relative to LDL in hamsters makes the animals resistant to atherosclerosis. We have also found that on diets containing less than about 5 g cholesterol/kg plasma HDL-cholesterol concentrations remain higher than LDL-cholesterol and there is little evidence of the development of atherosclerotic lesions (AM Salter and EH Mangiapane, unpublished results). However, as can be seen in the present study, higher concentrations of dietary cholesterol induce increases in VLDL-, IDL- and LDL-cholesterol relative to

HDL-cholesterol and lipid-rich lesions develop. It remains to be established which of the less dense lipoprotein fractions are contributing to the atherosclerosis. It is of note, however, that in study 2, when cholesterol was removed from the diet, VLDL-cholesterol and IDL-cholesterol concentrations fell at similar rates in both the coconut- and olive-oil-fed animals but LDL-cholesterol dropped much faster in those fed on olive oil. During this period, in the coconut-oil-fed animals, atherosclerosis continued to develop in the ascending aorta and remained relatively constant in the arch. This appears to suggest a difference in susceptibility to atherosclerosis in these regions with the ascending aorta being more sensitive to LDL-cholesterol concentrations than the aortic arch. By contrast, substantial regression was seen in both regions in those fed on olive oil, providing indirect evidence that it is the lowering of LDL-cholesterol concentrations that is important. Lowering of LDL may prevent the further development of lesions and allow the HDL to promote regression.

The results of the present experiments suggest that dietary manipulation of lipoprotein concentrations can lead to regression of atherosclerosis in the hamster. At the present time we cannot say whether it is only the change in fatty acid composition of the diet which induces the reduction in atherosclerosis or whether other components of the olive oil may also have an effect. For example, the antioxidant profile of the two oils is very different, with olive oil containing over twenty times as much α -tocopherol as coconut oil (Padley *et al.* 1994) and this may contribute to the regression of atherosclerosis. It would also be interesting to know whether oils rich in polyunsaturated fatty acids are more potent at reducing atherosclerosis as seen in African Green monkeys (Rudel *et al.* 1995). However, the present study and the recent report by Pitman *et al.* (1998) suggest that the hamster is potentially a very useful animal model for the further study of both the progression and regression of atherosclerosis.

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