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The hypercholesterolaemic effect of caffeine in rats fed on diets with and without supplementary cholesterol

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1. The effect of caffeine (2·5 g/kg diet) on lipid metabolism was examined in rats fed on a stock (low-cholesterol) diet or on a cholesterol plus cholic acid-supplemented (high-cholesterol) semi-synthetic diet.

2. When caffeine was included in the stock diet fed to rats for 7 d, there was a moderate but significant increase in the concentration of serum cholesterol compared to the levels observed in the control rats. This change can be accounted for by the increase that was observed in the rate of cholesterogenesis in the liver.

3. After 25 d of caffeine in the stock diet, hepatic cholesterogenesis was still increased but the concentration of serum cholesterol was now the same as in the control rats. During the experimental period there was a progressive increase in the faecal excretion of neutral sterols in the rats receiving caffeine.

4. When caffeine was added to a cholesterol plus cholic acid-supplemented diet, there was a marked increase in the concentration of serum cholesterol but hepatic cholesterogenesis was now reduced.

5. Caffeine in the high-cholesterol diet appeared to delay, but probably did not reduce, the absorption of an oral dose of radio-labelled cholesterol. This conclusion was confirmed using rats which had not previously received either caffeine or cholesterol in the diet.

6. When the effect of caffeine in the high-cholesterol diet was investigated during a 24 h period, an exacerbation of the hypercholesterolaemia was seen only at certain times.

7. After a 4-month period of feeding rats on the caffeine-supplemented high-cholesterol diet, histological examination did not detect any damage to the heart and aorta.

8. The metabolic regulations involved in the effects of caffeine in the two diets are discussed and the relevance of the present results to observations made with human subjects is considered.

A possible association between the consumption of caffeine-containing beverages and cardiovascular disease has been much discussed during the last decade. Recently, Moore, Guzman, Schilling & Strong (1975) suggested that the frequency of intake of caffeine may be related to the development of atherosclerotic lesions and Mann & Thorogood (1975) found that excessive coffee consumption increased the risk of myocardial infarction. Conversely, Dawber, Kannel & Gordon (1974) and Hennekens, Drolette, Jesse, Davies & Hutchison (1976) maintain that there is little risk of death from coronary heart disease associated with coffee-drinking.

Dietary caffeine is known to increase serum cholesterol in rats receiving a cholesterol-free diet (Naismith, Akinyanju & Yudkin, 1969) and Akinyanju & Yudkin (1967) showed that supplementation with coffee of a diet containing cholesterol exacerbates hypercholesterolaemia in the rat. Contradictory results have been obtained concerning the effect of caffeine on serum cholesterol in human subjects. Originally, Myasnikov (1958) found caffeine to increase serum cholesterol but Naismith, Akinyanju, Szanto & Yudkin (1970) demonstrated the converse. Nichols, Ravenscroft, Lamphier & Ostrander (1976), in a prospective epidemiological survey of 4057 adults in Tecumseh, Michigan, USA, found that frequency of intake of coffee was the only dietary variable to be significantly correlated with serum cholesterol.

It was the purpose of the present experiments to investigate, in more detail, the effect of caffeine on serum cholesterol levels in the rat in the hope of obtaining information about the control of lipid metabolism in hyperlipidaemia and, perhaps, to shed light on the conflicting results in man.

Table 1. *Composition (g/kg) of the cholesterol+cholic acid-supplemented semi-synthetic diet fed to rats*

Ingredient	
Low-vitamin casein	200
Hydrogenated coconut oil	100
Minerals*	27·5
Vitamins*	4·0
$NaH_2PO_4 . 2H_2O$	22·0
Vitamin A beads (500 mg retinol/g)	0·022
Vitamin E oil (735 mg/g D-α-tocopheryl acetate)	0·100
Sodium selenite solution (330 mg/l)	1·0 ml
Sucrose	634
Cholesterol	10
Cholic acid	2

* Diplock, Green, Bunyan, McHale & Muthy (1967).

MATERIALS AND METHODS

Animals and diets. Male rats of the CFY strain (initial body-weight 80–100 g) were obtained from Carworth (Europe) Ltd, Alconbury, Hunts. Animals were fed *ad lib.* either on a stock pelleted diet (Oxoid Breeding Diet; Lillico and Sons, Wonham Mill, Betchworth, Surrey) or on a semi-synthetic diet supplemented with cholesterol and cholic acid (Table 1), for 7 d, after which they were allocated to experimental groups (six or eight rats/group, three or four rats/cage) so that the mean body-weight of each group was the same. Body-weight was recorded throughout the experimental period. Where applicable, faeces were collected, dried at 110°, ground and stored at $-15°$ for subsequent analysis.

Biological measurements. The synthesis of fatty acids (FA) and digitonin-precipitable sterols (DPS) in liver in vitro and in liver and small intestine in vivo was measured by the method of Fears & Morgan (1976). Lipogenesis in vitro in pieces of epididymal adipose tissue (approximately 100 mg/assay) and in vivo in whole fat pads was measured by procedures similar to those used for the other tissues. Aortic total lipogenesis was measured as disintegrations/min in the chloroform phase of the total lipid extract of aorta prepared by the method of Folch, Lees & Sloane-Stanley (1957) as modified by Bligh & Dyer (1959).

Total cholesterol in serum was measured using the Technicon Instruments Corp. (1965) AutoAnalyzer method as modified by Siegel & Bowdoin (1971). Total triacylglycerol (TG) in serum was measured by using the Technicon Instruments Corp. (1965) AutoAnalyzer method. Total free fatty acid (FFA) in the serum was measured by a combination of the methods of Duncombe (1963) and Itaya & Ui (1965). Serum corticosterone was measured by the method of Murphy (1967) as modified by Fears (1973). Liver total cholesterol and TG was measured, using an AutoAnalyzer (Technicon Instruments Corp., Basingstoke, Hants), on the chloroform phase of total lipid extract. Faecal neutral sterols were estimated by the method of Miettinen, Ahrens & Grundy (1965) as modified by Morgan, Heald, Atkin, Green & Chain (1974). Radioactivity in the tissues after an oral dose of cholesterol was measured as disintegrations/min in the chloroform phase of total lipid extracts.

Statistical analysis. Statistical analyses were done using Student's t test or, when small numbers of samples were involved, the Mann & Whitney (1947) U test.

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Table 2. *Effect of caffeine* (2 mM) *on lipogenesis in vitro in rats fed on a stock diet*† *for at least* 2 *weeks*

(Mean values with their standard errors for four assays; results expressed as μmol substrate incorporated/h per g tissue)

Treatment ...	Control		Caffeine	
	Mean	SE	Mean	SE
Liver [1-^{14}C]acetate				
Sterols	80·4	8·2	42·8	5·3*
Fatty acid	162·6	6·4	107·1	8·0*
Liver [2-^{14}C]mevalonate				
Sterols	179·0	13·1	153·7	10·2
Fatty acid	18·1	2·0	17·7	3·1
Adipose tissue [1-^{14}C]acetate				
Sterols	8·8	1·0	3·2	0·5*
Fatty acid	175·5	8·2	40·0	6·1*

Significantly different from corresponding control value: * $P < 0·05$.
† Oxoid Breeding diet (Lillico and Sons, Wonham Mill, Betchworth, Surrey).

RESULTS

Experiments in vitro

The effect of caffeine on the rate of lipogenesis in vitro was examined using liver slices and pieces of epididymal adipose tissue taken from rats maintained on the control diet for at least 2 weeks. Caffeine (2 mM) significantly decreased the incorporation of [1-^{14}C]acetate into DPS and FA in both liver and adipose tissue (Table 2), but did not inhibit the incorporation of [2-^{14}C]mevalonate into DPS or prenoic acid.

Effect of caffeine supplementation of the stock (*low-cholesterol*) *diet*

Lipogenesis. To determine if the inhibitory effects of caffeine on lipogenesis in vitro were maintained when caffeine was given to rats as a supplement in the diet, the over-all rate of biosynthesis of DPS and FA in each tissue was measured in vivo using ^3H_2O.

Groups of eight rats were fed, *ad lib.*, on the stock diet with or without caffeine (2·5 g/kg). After 7 d, the rats which had received caffeine showed a significantly reduced gain in body-weight, a lower concentration of TG in the serum and increased serum cholesterol levels (Table 3). In contrast to the situation in vitro, the rate of cholesterogenesis in the liver was increased by caffeine. The rate in the ileum was unchanged. Caffeine had no effect on the rate of FA synthesis in liver, ileum or epididymal adipose tissue.

In a second experiment, in which the rats were killed after 25 d, caffeine (2·5 g/kg) again significantly decreased the gain in body-weight and the concentration of TG in the serum. However, in contrast to the result obtained after 7 d, the concentration of serum cholesterol was the same in both groups. The concentrations of FFA and corticosterone in the serum were also unchanged. After 25 d, liver cholesterogenesis, as measured with ^3H_2O, was again significantly increased by caffeine and again, there was no change in the rate of cholesterogenesis in the ileum. The rate of synthesis of FA in liver and ileum was not affected. The hepatic content of cholesterol and TG was similar in both experimental groups.

Sterol excretion. During days 8–11, 15–18 and 22–25 of the 25 d experimental period, faeces were collected from each cage of four rats (two cages/experimental group) and faecal sterols were measured. During the first collection period (days 8–11 of the 25 d experimental period), the excretion of cholesterol, coprostanol and coprostanone was similar in both dietary groups (Table 4). Subsequently, however, the excretion of cholesterol and

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Table 3. *Effect of caffeine (2·5 g/kg diet) on serum and liver lipids and lipogenesis from ^3H_2O in rats fed on a stock diet† for 7 or 25 d*

(Mean values with their standard errors for eight rats/treatment)

Experimental period . . .	7 d				25 d			
Treatment . . .	Control		Caffeine		Control		Caffeine	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Body-wt gain (g/rat per experimental period)	54·7	4·9	39·9	2·7**	161·8	4·9	140·1	5·1**
Serum lipids (mmol/l)								
Cholesterol	2·33	0·06	2·58	0·08**	1·96	0·07	1·83	0·06
Triglyceride	1·25	0·18	0·58	0·04***	1·11	0·12	0·70	0·04***
Free fatty acid	—		—		0·49	0·02	0·47	0·02
Liver lipids (g/kg)								
Cholesterol	—		—		3·09	0·15	3·17	0·12
Triglyceride	—		—		4·43	0·35	4·96	0·35
Tissue lipogenesis‡								
Liver sterols	69	6	114	9***	110	8	148	18***
Liver fatty acid	111	10	108	17	172	29	225	45
Ileum sterols	41	3	34	1	40	4	31	4
Ileum fatty acid	72	5	103	14	39	5	40	4
Epididymal adipose tissue fatty acid	7	2	11	4	—		—	
Serum corticosterone (μmol/1)	—		—		0·58	0·14	0·70	0·06

Significantly different from corresponding control value: ** $P < 0·01$, *** $P < 0·001$.
† Oxoid Breeding diet (Lillico and Sons, Wonham Mill, Betchworth, Surrey).
‡ Lipid synthesized (μg/h per g tissue).

coprostanol was increased by 60% in the group receiving caffeine compared to that in the control group. As the faeces were collected from cages of rats and not from individual rats, no statistical analysis is possible.

Effect of caffeine supplementation of cholesterol+bile acid-supplemented diets

When the rat is fed on a cholesterol-supplemented diet, endogenous cholesterogenesis fulfils a less important role and homoeostatic mechanisms may not be so finely controlled as in rats fed on normal (low-cholesterol) diets. It was considered of interest, therefore, to compare the effects of caffeine in cholesterol+bile acid-supplemented diets with the results obtained using the stock diet (which contained only traces of cholesterol).

Lipogenesis. Caffeine, given at 2·5 g/kg in a cholesterol+bile acid-supplemented diet for 21 d, reduced the gain in body-weight without altering the concentration of serum TG (Table 5). There was a marked exacerbation of the hypercholesterolaemia in comparison to control values.

In contrast to the results obtained using the stock diet, inclusion of caffeine in a cholesterol+bile acid-supplemented diet led to a further reduction in the (already low) rate of hepatic cholesterogenesis. There was a significant increase in the content of cholesterol in the liver (not observed on the stock diet). The rate of FA synthesis in the liver was also inhibited by caffeine, but there was no effect of caffeine on cholesterogenesis in the ileum or on total lipogenesis in the aorta.

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Table 4. *Effect of caffeine (2·5 g/kg diet) on neutral sterol excretion (mg/d per rat) in rats fed on a stock diet* for 25 d*

(Mean values from two cages of four rats each)

Treatment . . .	Control			Caffeine		
Period of faecal collection (d) (inclusive) . . .	8–11	15–18	22–25	8–11	15–18	22–25
Cholesterol	2·68	2·58	2·20	2·58	3·07	3·36
Coprostanol	0·98	0·96	1·40	0·85	1·13	2·48
Coprostanone	0·02	0·03	0·03	0·02	0·04	0·04
Total neutral sterols	3·68	3·57	3·63	3·45	4·24	5·88

* Oxoid Breeding diet (Lillico and Sons, Wonham Mill, Betchworth, Surrey).

Table 5. *Effect of caffeine (2·5 g/kg diet) on lipid levels and lipogenesis from ^3H_2O in rats fed on a cholesterol+bile acid-supplemented diet† for 21 d*

(Mean values with their standard errors of six rats/group)

Treatment . . .	Control		Caffeine	
	Mean	SE	Mean	SE
Body-wt gain (g/rat per 21 d)	115·3	3·7	74·8	4·9***
Serum lipids (mmol/l)				
Cholesterol	11·4	0·35	19·4	1·1***
Triglyceride	0·89	0·12	0·51	0·19
Liver cholesterol (g/kg)	7·99	0·73	10·2	0·63***
Tissue lipogenesis‡				
Liver sterols	50	3	38	2**
Liver fatty acid	205	37	112	23***
Ileum sterols	158	10	169	10
Aorta total lipogenesis§	4340	210	4700	290

Significantly different from corresponding control value: ** $P < 0.01$, *** $P < 0.001$.

† For details of composition, see Table 1.

‡ Lipid synthesized (µg/h per g tissue).

§ Lipid synthesized (disintegrations/min per h per g tissue).

Cholesterol absorption

Chronic effects. The increase in serum cholesterol in conjunction with the reduction in cholesterogenesis suggested that caffeine could also be affecting the absorption of cholesterol. The effect of dietary caffeine (2·5 g/kg diet) on cholesterol absorption was measured 4 and 24 h after an oral dose of [4-^{14}C]cholesterol followed, where applicable, by a single oral dose of caffeine at 200 mg/kg body-weight (Table 6). Rats killed 4 h after dosing received a cholesterol+bile acid-supplemented diet; rats killed 24 h after dosing received a cholesterol-supplemented diet. In both experiments, caffeine again reduced the gain in body-weight and increased the concentration of cholesterol in the serum.

At 4 h after an oral dose of cholesterol, the amount of radio-labelled cholesterol found in serum, liver, epididymal adipose tissue and aorta was reduced in the rats given caffeine. The amount of radio-labelled cholesterol remaining in the gut plus contents was increased.

At 24 h after an oral dose of cholesterol, the amount of radio-labelled cholesterol found in the serum and liver was increased in the rats given caffeine, with no difference now observed in epididymal adipose tissue and aorta. Radioactivity in the washed tissue of the small intestine was increased by caffeine, as was the concentration of sterols. There were no

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Table 6. *Effect of caffeine (2·5 g/kg diet) on tissue levels of radioactivity after an oral dose of* [4-^{14}C]*cholesterol in rats fed on a cholesterol+bile acid-supplemented diet*† (4 *h*) *or a cholesterol-supplemented diet* (24 *h*) *for* 21 *d*

(Rats which had been given dietary caffeine also received an oral dose of caffeine (200 mg/kg body-weight) immediately after the dose of [4-^{14}C]cholesterol (20 μCi, 25 mg/kg body-weight). Mean values with their standard errors for six (4 h) or eight (24 h) rats/group)

Period after dosing ...	4 h				24 h			
Treatment ...	Control		Caffeine		Control		Caffeine	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Body-wt gain (g/rat per 21 d)	108·7	5·6	76·0	4·5**	150·0	6·9	129·3	5·7**
Serum lipids (mmol/l)								
Cholesterol	14·5	1·1	19·7	1·4**	1·81	0·11	3·03	0·13***
Triglyceride	0·85	0·10	0·75	0·07	0·70	0·06	0·70	0·09
Tissue radioactivity (disintegrations/min)								
Serum (/ml)	110500	14500	13400	10700***	6369	435	10054	629***
Liver (/g)	68250	14930	4460	3590***	64970	6310	94030**	10900
Adipose tissue (/g)	5300	705	1112	44***	5900	547	4430	509
Aorta (/mg)	7·2	1·1	3·2	0·4***	8·3	0·7	6·9	0·9
Gut+contents (×10^6)	10·5	1·7	14·6	2·6	—	—	—	—
Small intestine	—	—	—	—	131300	9000	569000	88700***
Intestinal contents+faeces (×10^6)	—	—	—	—	3·68	0·7	3·60	0·4

Significantly different from corresponding control value: ** $P < 0·01$, *** $P < 0·001$.
† For details of composition, see Table 1.

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Table 7. *Effect of a single dose of caffeine (200 mg/kg body-weight) on tissue levels of radioactivity after an oral dose of [4-^{14}C]cholesterol (20 μCi, 25 mg/kg body-weight) in rats fed on a stock diet*†

(Mean values with their standard errors for six rats/group)

Period after dosing . . .	4 h				24 h			
Treatment . . .	Control		Caffeine		Control		Caffeine	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Tissue radioactivity (disintegrations/min)								
Serum (/ml)	38400	3498	5800	1330***	46300	2240	43200	5800
Liver (/g)	247000	25000	25000	5700***	149000	17100	132000	16300
Adipose tissue (/g)	7405	656	1058	212***	19900	1750	9600	1590***
Gut+contents ($\times 10^6$)	10·2	1·2	19·9	1·2	—	—	—	—
Small intestine	—	—	—	—	834000	117000	1744000	342000**
Intestinal contents+faeces ($\times 10^6$)	—	—	—	—	3·5	0·4	3·1	0·7

Significantly different from corresponding control value: ** $P < 0·01$, *** $P < 0·001$.
† Oxoid Breeding diet (Lillico and Sons, Wonham Mill, Betchworth, Surrey).

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differences between the experimental groups with respect to the radioactivity remaining in the contents of the gut, plus that in the faeces excreted during the 24 h period.

Acute effects. The effect of caffeine in delaying the absorption of cholesterol was confirmed in an 'acute' experiment where rats maintained on the stock diet received a single oral dose of [4-^{14}C]cholesterol followed, where applicable, by a single oral dose of caffeine at 200 mg/kg body-weight (Table 7).

At 4 h after an oral dose of cholesterol, the amount of radio-labelled cholesterol found in serum, liver and epididymal adipose tissue was reduced in the rats given caffeine. The amount of radio-labelled cholesterol remaining in the gut plus contents was increased.

At 24 h after an oral dose of cholesterol there was now no difference between the two groups in the radio-labelled cholesterol found in the serum and liver, but radioactivity in the adipose tissue was still lower in rats given caffeine. Radioactivity in the washed tissue of small intestine was again increased by caffeine, as was the concentration of total sterols. There were no differences between the experimental groups with respect to the radioactivity remaining in the contents of the gut, plus that in the faeces excreted during the 24 h period.

Caffeine and diurnal rhythms in a cholesterol-supplemented diet

In view of its interference with cholesterol absorption, certain of the effects of caffeine were studied throughout a 24 h period.

Groups of rats were given a cholesterol+bile acid-containing diet, with or without a supplement of caffeine (2·5 g/kg), for 21 d, the rats being killed at 3 h intervals during a 24 h period. In order to standardize environmental conditions as far as possible, rats were maintained on a reversed lighting cycle with a dark period between 04.00 and 16.00 hours.

In control animals, the concentration of cholesterol in the serum showed a clear diurnal rhythm, with a peak at the onset of the dark period (Fig. 1). In general, this diurnal rhythm was exacerbated in the rats receiving caffeine but, for approximately half the dark period (10.00–15.00 hours), there was no significant effect of caffeine. The concentration of TG in the serum also showed a clear diurnal rhythm, of similar magnitude in both dietary groups. The peak in serum TG was separated by approximately 12 h from the peak in serum cholesterol. Serum FFA showed no obvious diurnal rhythm in the control animals but FFA levels were increased during the light (post-absorptive) period in the rats receiving caffeine, although a statistically significant difference was obtained only at 18.00 hours.

The concentration of cholesterol in the liver showed a diurnal variation with a peak at the same time as the peak in serum cholesterol. The hepatic content of cholesterol was increased by caffeine at most times but was decreased by caffeine with respect to control values during that period when the concentration of serum cholesterol was similar in the two groups.

No obvious diurnal rhythms were observed in the weight of the small intestine plus contents, liver weight, or liver TG. In general, the rats receiving caffeine had a lower body-weight than the controls but the most severe effect on growth, as in previous experiments, was during the first week.

Long-term effects of caffeine

To investigate the consequences in rats of an exacerbated hypercholesterolaemia over a longer period than that used previously (21 d), caffeine (2·5 g/kg) was included in a cholesterol-supplemented diet for 4 months.

There was no effect of caffeine on the cholesterol concentration in the aorta or on the total cholesterol content of the body. Histological examination of the aorta and heart disclosed no atherosclerotic changes induced by caffeine.

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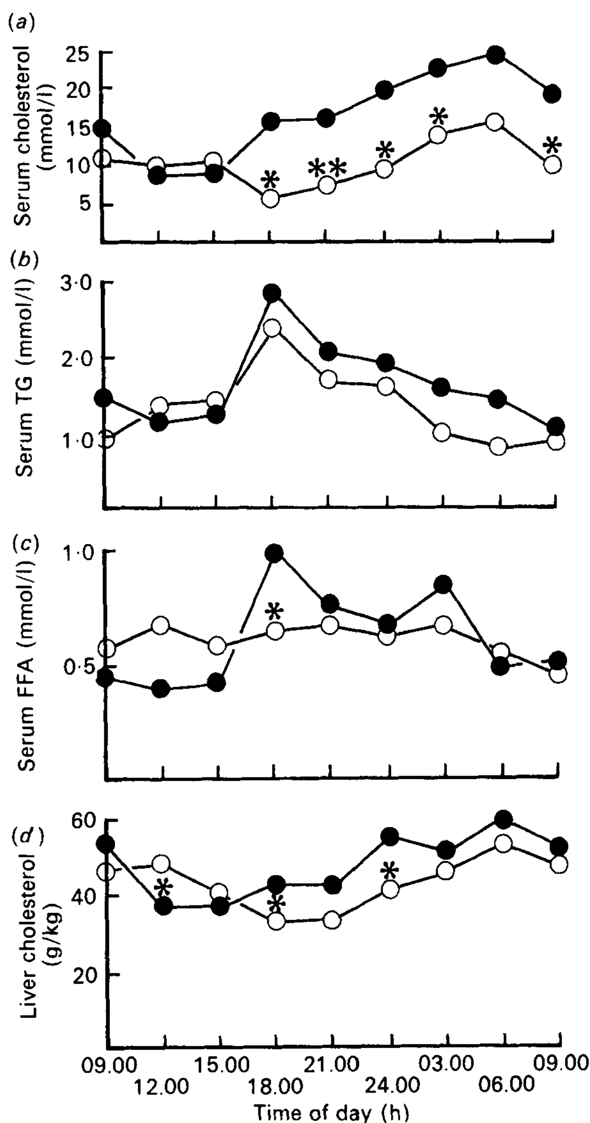

Fig. 1. Variation with time of day of (*a*), serum cholesterol (mmol/l); (*b*), serum triglyceride (TG) (mmol/l); (*c*), serum free fatty acid (FFA) (mmol/l); (*d*), liver cholesterol (g/kg). The rats were fed either a cholesterol+cholic acid-supplemented diet (○) or the same diet supplemented with 2·5 g caffeine/kg diet (●) for 15 d. The results are mean values for five rats/treatment. Values for the caffeine-supplemented group were statistically significantly different from corresponding values for the control group: * $P < 0.05$, ** $P < 0.01$.

DISCUSSION

Caffeine has been observed to have an effect on various aspects of lipid and carbohydrate metabolism (Marks, 1974) but many of the biochemical changes obtained with caffeine have only been demonstrated in isolated tissues or in acute experiments, usually performed using fasted animals.

In the present experiments, caffeine at levels known to affect 3′,5′-cyclic AMP levels and

lipolysis reduced the synthesis of both sterols and fatty acids from acetate in rat liver and adipose tissue in vitro. This observation confirms the work of others who have shown the inhibitory effect of caffeine (Jeanrenaud, 1968) and other methylxanthines such as theophylline (Dominguez & Herrera, 1976) on lipogenesis in adipose tissue.

However, when caffeine is included in the diet of rats for 7 d, its net effects are different from those expected on the basis of the results obtained in vitro. Serum cholesterol was significantly increased, probably as a result of the observed increase in hepatic total cholesterogenesis. Growth and serum TG were reduced, perhaps because of the increase in locomotor activity which can be expected at this intake of caffeine (Waldeck, 1975). After 25 d intake of a caffeine-supplemented diet, hepatic cholesterogenesis remained increased but there was now no difference in serum cholesterol. An explanation for this apparent discrepancy is provided by the progressive increase in excretion of neutral sterols throughout the experimental period in the rats receiving caffeine. Thus, it seems likely that homoeostatic mechanisms prevent hypercholesterolaemia from being maintained for more than short periods of time in rats given a low-cholesterol diet. The unchanged serum FFA concentration is in agreement with other work suggesting that caffeine does not affect lipolysis in the non-fasted state (Truswell, 1974).

Although Naismith *et al.* (1969) found caffeine to be hypercholesterolaemic for a longer period than was observed in the present experiment, those authors used approximately half the level of caffeine used in the present experiments and this may have induced a slower build-up of serum cholesterol followed by a delayed return to homoeostasis.

In both human and animal studies, caffeine has been noted to interfere with epinephrine metabolism either by increasing output of the hormone (Bellet, Roman, DeCastro, Kim & Kershbaum, 1969) or by increasing receptor sensitivity (Frantsuzova, 1975; Waldeck, 1975). As epinephrine has similar effects on cholesterol and TG metabolism (Shafrir, Sussman & Steinberg, 1959; Bortz, 1968; Edwards, 1975) to those described here for caffeine, it seems likely that at least some of the effects of caffeine on lipids can be explained in terms of epinephrine. An effect of caffeine on the levels of other hormones, for example the corticosteroids, has been discussed (Oberman, Herzberg, Jaskolka, Harell, Hoerer & Laurian, 1975) but no influence on serum corticosterone was observed in the present work. Whatever the mediating factor, the effect of caffeine in diets containing no supplementary cholesterol appears to be relatively moderate and transient.

In a number of experiments using cholesterol-supplemented diets the effect of caffeine was much more noticeable, giving an exacerbation of the hypercholesterolaemia commonly obtained with such diets. In contrast to the results obtained using a stock diet, a further reduction in the already low level of hepatic cholesterogenesis was obtained. Under such conditions, caffeine delayed, but probably did not reduce, the absorption of an oral dose of radio-labelled cholesterol. Other workers have described an effect of caffeine on events in the small intestine (Wald & Bayless, 1975). In view of the effect of caffeine in delaying the absorption of cholesterol, it seemed possible that, if the concentration of cholesterol in the serum exhibited a diurnal rhythm in rats fed on cholesterol-supplemented diets, then caffeine might be found to exacerbate the hypercholesterolaemia only at certain times of the day. Such a transient effect would then explain the apparently paradoxical effect on cholesterogenesis and might be interpreted in terms of the known effects of methylxanthines as circadian *zeitgebers* in species including the rat (Feldman, 1975; Ehret, Potter & Dobra, 1975). In fact, serum cholesterol does undergo a diurnal rhythm in rats fed on a cholesterol-supplemented diet and caffeine did exacerbate the hypercholesterolaemia only at certain times of the day. As far as is known, this is the first reported observation of diurnal changes in serum cholesterol. No such changes have been found in rats maintained on a stock diet (Fears, unpublished results).

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The changes in serum cholesterol cannot be related directly to temporal changes in absorption, because the concentration of cholesterol in the serum is a composite of several processes, including, for example, the equilibrium between very-low-density lipoproteins-chylomicrons and high-density lipoproteins (Naidoo, Lossow & Chaikoff, 1962). Nonetheless, the response to caffeine is phasic, may well be related to a prime effect on absorption and can explain the observed changes in the content of cholesterol in the liver and in liver cholesterogenesis (which is often inversely correlated with the liver cholesterol concentration). The possible involvement of epinephrine in producing these effects in cholesterol-supplemented diets is less clear (Shafrir *et al.* 1959; Shrivastava, 1965) but cannot be discounted.

It is interesting to note that, in a cholesterol-supplemented diet, liver cholesterogenesis can be suppressed to even lower rates by further increasing the concentration of cholesterol in the serum and liver. This does not occur in peripheral tissues such as the small intestine and aorta, although, in fact, most of the activity associated with the aorta may be attributed to that of periaortic brown fat (Vost & Hollenberg, 1969). The absence of an effect of increasing serum cholesterol on peripheral tissue cholesterogenesis may be explained by the possibility that their rates are already completely repressed, as suggested by the lipoprotein-receptor theory of Brown & Goldstein (Balasubramaniam, Goldstein, Faust & Brown, 1976).

The levels of dietary caffeine and cholesterol used in the present experiments were high compared to the average intake of human populations although the period of intake was, of course, much less. The concentration of caffeine in the serum of the rats used in the present experiments (cholesterol-supplemented diets) was found to be approximately 40 mg/l, whereas the level measured in the plasma of a randomly-selected group of ten human subjects ranged from 1·5 to 6·0 mg/l in reasonable agreement with results drawn from the literature (Axelrod & Reichenthal, 1953).

However, if a more moderate intake of caffeine and cholesterol did influence serum cholesterol in man, then, by analogy with the rat experiments, hypercholesterolaemia might only be expected outside of the feeding period, i.e. at night. Thus, the results of Naismith *et al.* (1969) might be reconciled with results obtained from laboratory animals.

The fact that caffeine produces exacerbation of hypercholesterolaemia suggests that atherosclerosis might be promoted over a period of time. However, an experiment lasting several months failed to disclose any damage exerted by caffeine on the aorta or heart or any increase in the cholesterol content of aorta and other peripheral tissues. Although the rat is known to be particularly resistant to the effect of hypercholesterolaemia, similar negative findings have been obtained by other workers using the rabbit (Cholewa, 1973).

Thus, although it is felt that the present experiments have gone some way to explaining the effects of caffeine in rats maintained on various diets and to reconciling the results from animal studies with the findings in man, the possibility of an association between consumption of caffeine-containing beverages and cardiovascular disease remains to be decided.

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