Effect of sex hormones on fasting serum triglycerides in baboons given high-sucrose diets

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1. The incorporation of [^{14}C]sucrose into serum triglyceride was shown to increase in both male and female baboons after a period of high sucrose intake. During the same period of high sucrose intake there was an increase in the fasting serum triglyceride concentration of the male baboons but not of the females.

2. When the male baboons were given a parenteral oestrogen preparation in addition to the sucrose diet the increase in fasting serum triglycerides was greatly reduced but there was little alteration in the extent of the incorporation of sucrose into serum triglyceride compared to that with the diet and no oestrogen.

3. A parenteral testosterone preparation given to the female baboons in addition to the sucrose diet had no effect on either the extent of incorporation of sucrose into triglyceride or the fasting serum concentrations of triglyceride.

4. The findings suggest that the differing patterns of fasting triglyceride response in the male and female baboons to the sucrose diet may have resulted from oestrogen enhancing the removal of triglyceride from the serum of the female animals.

Diets containing a high percentage of the total calories (70% or more) as sucrose have been shown to increase the concentration of triglyceride in the fasting serum of healthy young men (Macdonald & Braithwaite, 1964; Hodges & Krehl, 1965). In premenopausal women of the same age, however, this hypertriglyceridaemic effect of sucrose diets is much less apparent (Macdonald, 1965; Klugh & Irwin, 1966).

The present investigation concerns the effects of a sucrose-enriched diet on the fasting serum triglycerides of male and female baboons. Initially, untreated animals were studied and subsequently the males received oestrogen parenterally and the females testosterone. The degree of incorporation of sucrose into serum triglycerides was assessed by giving a sucrose meal containing [U-^{14}C]sucrose, before the diet commenced and again after 13 weeks.

Baboons were chosen rather than human subjects because, firstly high-sucrose–fat-free diets administered for a long time are unpalatable to man, but avidly consumed by baboons, and because the administration of sex hormones to human subjects produces unpleasant side-effects, the complete reversibility of which cannot be guaranteed. In addition, the use of radioisotopes was only possible with animals.

MATERIAL AND METHODS

Experimental animals

It was essential to ensure that the baboons, six of each sex, had attained full sexual maturity. This was confirmed by the presence in all animals of erupted canine teeth,

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a feature which indicated a minimum age of 4 years (Reed, 1963), and since sexual maturity in the baboon has been shown to occur by the age of 3 years (Kummer & Kurt, 1963), the animals were considered suitable for the experiment. Furthermore, the females menstruated regularly.

Diets

The sucrose diets were all qualitatively identical and consisted on a weight basis of 75 parts sucrose, 18 parts calcium caseinate and 7 parts dried yeast. This was in contrast to the control diet, which was 65 parts starch, 4 parts fat, 26 parts protein and 5 parts fibre, derived mainly from cereals and milk powder. The solid constituents of the diet were mixed with water in the concentration of 100 g solids/500 ml water, and given to the animals to drink three times a day in equal doses. The quantity given was such as to maintain the body-weight of each animal constant. This amounted to about 1500 ml (= 1125 kcal) per d for a 15 kg male baboon, and 1000 ml (= 750 kcal) per d for a 10 kg female baboon. While on the sucrose diets the animals were given daily, in the gruel, salts in the amounts described by Hegsted, Mills, Elvehjem & Hart (1941) and vitamins (0·5 ml Abidec; Parke Davis).

Blood sampling

Before blood samples were taken each animal was tranquillized with an intramuscular injection of phencyclidine hydrochloride (Sernylan; Parke Davis) at a level of 1 mg/kg body-weight. Fasting venous blood samples were obtained from the femoral vein before the diets were introduced and at intervals during the dietary periods.

Analytical procedures

The serum triglycerides were estimated by a semi-automated technique (Lofland, 1964).

The concentration of serum triglyceride was also estimated gravimetrically after separation of the lipid fractions by thin-layer chromatography. The radioactivity in the triglycerides was thus estimated in a Beckmann scintillation counter (for details see Macdonald, 1968).

Expt 1

The experimental animals were each fed on the sucrose-enriched–fat-free diet for 17 weeks; 5 ml of fasting venous blood were taken from each animal while on the control diet, and at intervals while on the experimental diet.

After an overnight fast, at the commencement of the experimental dietary period, each animal was given, by stomach tube, a sucrose meal at the level of 4 g/kg body-weight made up as a 50% solution in distilled water, to which was added 25 μCi [U-^{14}C]sucrose. Venous blood samples were obtained from each baboon before the meal and at 0·5, 1, 1·5, 2, 3, 4 and 5 h afterwards.

After the animals had been eating the sucrose diet for 13 weeks, each baboon was given a second [U-^{14}C]sucrose meal similar to that administered before the diet. Venous blood samples were collected at the same intervals of time as for the first

meal, and again the serum concentration and specific activity of the serum triglycerides were estimated.

The animals in this and the subsequent experiments were weighed on the occasions at which blood samples were taken in order to ensure that there was no significant weight change (Table 1).

Table 1. *Mean weights (kg) with their standard errors of the baboons while being given the high-sucrose diets*

Weeks on diet	0	1	2	3	4	5	7	9	11	13	15	17
Males $\bar{x}$	15·4		15·0			14·8	15·1	15·0	15·6	15·7	16·0	16·1
SE	0·97		0·99			0·99	0·94	0·94	1·05	1·06	0·97	1·04
Females $\bar{x}$	10·8	10·8	10·5	10·7	10·8	10·4	10·4	10·4	10·7	11·2	10·9	11·1
SE	0·36	0·34	0·35	0·41	0·32	0·42	0·44	0·39	0·38	0·43	0·42	0·41
Males given oestrogen $\bar{x}$	17·9	17·4	17·5	17·3		17·4	17·2	17·2	17·2	17·2	17·0	17·0
SE	0·92	0·73	0·81	0·77		0·83	0·79	0·82	0·94	1·06	1·02	1·01
Females given testosterone $\bar{x}$	11·7	11·7	11·8	11·9	11·8	11·6	12·0	11·9	12·0	12·0	11·8	11·8
SE	0·47	0·39	0·36	0·37	0·31	0·36	0·36	0·35	0·35	0·31	0·35	0·37

Expt 2

The same animals were used in this experiment as in Expt 1. Eleven weeks after they had reverted to the control diet the hormone treatments began. The male baboons were given oestradiol monobenzoate (Organon) twice weekly by intramuscular injection. Evidence of adequate 'oestrogenization' was considered to be the presence of a perineal 'bloom' similar to that observed in the females, and the absence of penile erections. In five out of six animals 1 mg oestrogen twice weekly was sufficient to produce these effects within 3 weeks of starting the injections. In the remaining animal, however, the requirement was 2 mg twice weekly.

The female baboons were injected with testosterone phenyl propionate (BP) once a week. In all animals, 20 mg were sufficient to inhibit the menstrual cycle, a feature that was considered to indicate an adequate dose of the hormone. Tests for liver function (bilirubin, zinc sulphate turbidity, alkaline phosphatase and aspartate aminotransferase activities), performed on each animal before and immediately after the course of testosterone, confirmed that there was no hepatic damage (Varley, 1960).

The hormone treatments continued for 23 weeks, and for the last 17 weeks of this period the control diet was replaced by the sucrose diet.

The same procedure of administering a [^{14}C]sucrose meal to each animal at 0 and 13 weeks as in Expt 1 was carried out during the period on the second sucrose diet. The same measurements were also made.

It was essential before starting Expt 2 to ensure that the interval between the two periods on the sucrose diet was sufficient to allow the metabolism of both male and female baboons to return to normal. Evidence to this effect was obtained from the observation that, following the [^{14}C]sucrose meal given before the second dietary period, the increased specific activity of serum triglyceride which occurred during the first period of high sucrose intake had reverted to normal levels.

RESULTS

Fasting serum triglyceride concentration

Males (Fig. 1). There was a significant increase in the mean fasting triglyceride concentration from an initial value of 39 mg/100 ml to a peak level of 63 mg/100 ml after 3 weeks on the sucrose diet ($P < 0.005$). From 4 weeks until the end of the dietary period at 17 weeks, the concentration steadily declined from a value of 62 mg/100 ml
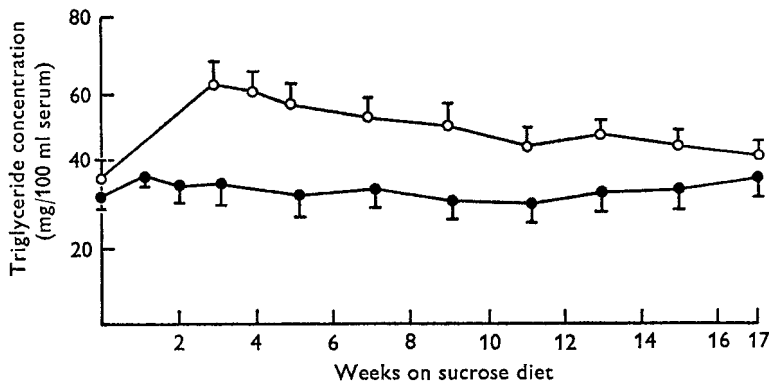

Fig. 1. Concentration of triglycerides in the fasting serum of the male baboons during the first period on a high-sucrose diet (○—○), and of the male baboons receiving oestrogen during the second period on the sucrose diet (●—●). The vertical lines indicate the standard error of the mean.
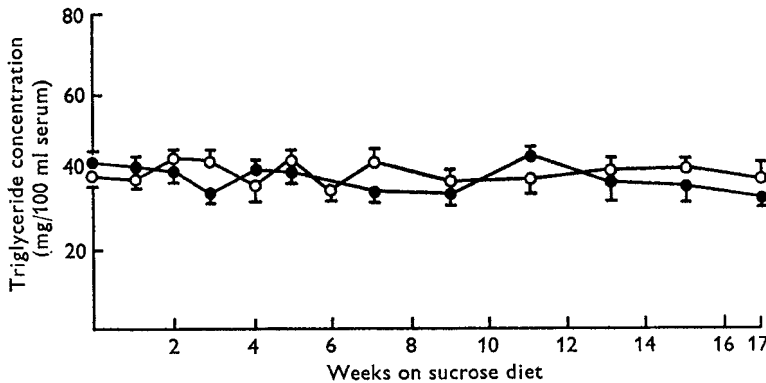

Fig. 2. Concentration of triglycerides in the fasting serum of the female baboons during the first period on a high-sucrose diet (○—○), and after receiving testosterone during the second period on the sucrose diet (●—●). The vertical lines indicate the standard error of the mean.

to one of 46 mg/ml. When the mean increase for each week was assessed by comparing the value for each animal with the corresponding fasting value, with the exception of that for week 11, the increase was significantly greater than zero on each occasion that samples were obtained ($P < 0.025$).

When the high-sucrose diet was given to the males receiving oestrogen, there was a small but significant increase in triglycerides after 1 week ($0.05 > P > 0.025$) and the

Table 2. *Mean serum triglyceride concentrations (mg/100 ml) at various times after a [U-¹⁴C]sucrose meal given to male and female baboons before and after 13 weeks on a high-sucrose diet, without and with hormonal treatment*

Time after meal (h) …	0		0·5		1		1·5		2		3		4		5	
	x̄	SE	x̄	SE	x̄	SE	x̄	SE	x̄	SE	x̄	SE	x̄	SE	x̄	SE
Males:																
Before	35	7·6	40	4·9	33	6·4	41	5·8	35	4·7	38	3·6	35	4·5	42	4·0
After 13 weeks	46	5·6	43	5·5	39	6·2	39	6·4	40	5·7	36	4·7	40	6·6	44	5·4
Females:																
Before	43	3·6	37	5·3	36	4·2	35	3·5	31	3·3	31	3·7	36	1·9	38	2·0
After 13 weeks	46	5·6	38	2·6	40	1·9	39	2·9	40	3·8	40	6·0	46	6·3	41	6·1
Males given oestrogen:																
Before	30	4·4	31	4·6	31	3·2	37	3·6	34	4·0	37	3·6	36	2·7	37	2·5
After 13 weeks	42	4·0	41	2·3	42	3·7	38	4·1	41	3·3	41	2·2	44	2·3	45	2·5
Females given testosterone:																
Before	44	1·7	40	4·6	39	3·9	40	4·2	38	3·5	44	2·6	42	2·8	45	1·3
After 13 weeks	38	5·0	36	5·4	42	7·0	42	5·8	35	5·7	41	6·7	45	4·6	47	6·3

concentration tended to remain slightly higher than the initial level. There was no particular trend in the pattern of triglyceride response during the 17-week dietary period, although at weeks 13, 15 and 17 the mean values were significantly greater than zero ($0.05 > P > 0.025$).

When the corresponding weekly values for the first and second diets were compared, it was found on the first four occasions at which sampling coincided (weeks 3, 5, 7 and 9) that the mean triglyceride concentrations were significantly different ($P < 0.025$), the values always being lower for the male animals when receiving oestrogen. After 9 weeks on the diet the corresponding weekly values were no longer significantly different.

Females (Fig. 2). No significant change was observed in the triglyceride concentration of these animals at any time while on the sucrose diet. As a consequence, when the results for the female baboons during the sucrose diet without hormone injection were compared with the values for the males, it was found that at 3, 4, 5 and 7 weeks after the start of the sucrose diet the mean changes in serum triglyceride concentration of the two sexes were significantly different ($P < 0.025$).

Concentration of serum triglyceride following sucrose meals (Table 2)

Males. There was no significant alteration in serum triglyceride concentration during the 5 h following the first sucrose meal. Following the sucrose meal after 13 weeks on the sucrose diet, there was a fall in the mean concentration of serum triglyceride. When the individual values for each animal were compared with the corresponding fasting values, the mean decreases in triglyceride concentration at 1, 2 and 3 h were significant ($P < 0.05$).

After oestrogen treatment there was no significant alteration in the mean concentration of triglyceride following the sucrose meals, before or after 13 weeks on the sucrose diet.

Females. Following the first sucrose meal there was a fall in the mean concentration of serum triglyceride. When the individual values for each animal were compared with the corresponding fasting values, the mean decrease in triglyceride concentration at 2 and 3 h was significant ($P < 0.05$). Following the second sucrose meal after 13 weeks on the sucrose diet there was no significant alteration in the mean concentration of serum triglyceride during the 5 h period.

When the female baboons received testosterone there was no significant alteration in the mean concentration of triglyceride following the sucrose meals on either occasion.

[^{14}C]sucrose incorporation into serum triglycerides (Fig. 3)

There was no difference, before the sucrose diets were introduced, between the male and female animals regarding the degree of incorporation of [^{14}C]sucrose into serum triglyceride. This also applied to both sets of animals receiving hormones. In all four groups, the effect after 13 weeks on the high-sucrose diet was an increase in the incorporation of [^{14}C]sucrose into serum triglyceride, the increase being more marked in the male baboons than in the females. When the male animals were given

the oestrogen preparation, there was a suggestion of decreased incorporation of [^{14}C]-sucrose into triglyceride, although this was not significant. The females given testosterone exhibited a specific activity curve for triglyceride that was almost identical to that found when the hormone was not administered.
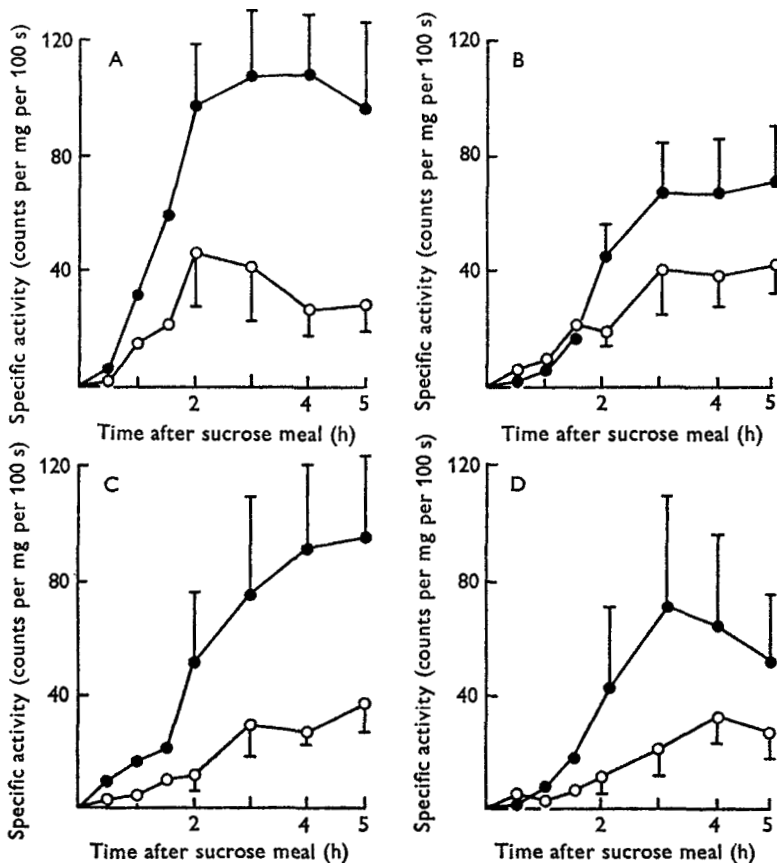

Fig. 3. Specific activities in the serum triglycerides of baboons after a meal of [U-^{14}C]sucrose, before the sucrose diet (○—○) and after 13 weeks on the sucrose diet (●—●). The vertical lines indicate the standard error of the mean. A, male baboons; B, female baboons; C, male baboons given oestrogen; D, female baboons given testosterone.

DISCUSSION

Dietary sucrose is converted into its constituent monosaccharides, glucose and fructose, in the brush border of the intestinal wall (Miller & Crane, 1961) and some of the fructose is converted into glucose in the liver and possibly in the gut wall. Fructose can be used for the formation of triglycerides, as can glucose, and it has been suggested (Macdonald, 1970) that the same pathways are used by each monosaccharide, but that quantitative differences occur and that these quantitative differences may be influenced by the sex of the consumer (Macdonald, 1966). When ^{14}C-labelled glucose or fructose is given by mouth, it has been shown that more of the label is found in the glycerol

moiety of the triglyceride than in the fatty acid moiety (Maruhama & Macdonald, submitted for publication).

An increase in triglyceride concentration in the male baboons during the early stages of the dietary period was not observed in the female animals. Furthermore, when the male animals received oestrogen in addition to sucrose, the triglyceride response was greatly reduced. On the other hand, the absence of any significant change in triglyceride concentration in the female baboons given testosterone in addition to the sucrose diet would seem to absolve testosterone from having stimulated the increase in triglyceride concentration in the male animals during the first period on the sucrose diet. It may be inferred that the hypertriglyceridaemic effect of sucrose was probably independent of any control by male sex hormone, and that the difference in triglyceride response between the male and female baboons depended largely, if not entirely, on the inhibitory effect of oestrogens.

The concentrations of triglyceride in the serum following the sucrose meals showed different patterns of response in the males and in the females. Before the sucrose diet there was no change in the concentration of serum triglyceride in the male baboons, but in the female baboons it fell significantly. One explanation for this is that the female animals metabolized sucrose like glucose, since it has been shown that feeding with glucose results in a fall in serum triglyceride concentration over the ensuing few hours (Havel, 1957; Macdonald & Roberts, 1967). It would seem unlikely that the different response was due to the male animals forming more triglyceride from sucrose than the females, since the concentrations of specific activity following the sucrose meals were the same in the two sexes. Another possibility is that under the stimulus of lipogenesis the female baboons cleared triglyceride from the serum more rapidly than did the males, resulting in a transient fall in serum concentration. Were this so, it could account for the fasting concentration of serum triglyceride in the females remaining unchanged during the period on the sucrose diet, in spite of the increase in synthesis, as suggested by the raised level of specific activity.

After 13 weeks on the sucrose diet, the picture was reversed and in response to the second sucrose meals there was a fall in the serum concentration of triglycerides in the male baboons, but no change in the females. This would suggest that after a period of high sucrose intake the male baboon had increased the rate of removal of triglyceride from the serum in response to a lipogenic stimulus, a situation that could possibly account for the steady fall in fasting concentration of serum triglyceride during the latter stage of the dietary period.

The studies with [^{14}C]sucrose suggest that sucrose had the effect of increasing the extent of triglyceride synthesis in both male and female baboons during the dietary period, with the increase more marked in the male than in the female baboons. It is possible that this difference was due to the higher levels of endogenous oestrogens in the female animals. That this effect was not convincingly reproduced in the male baboons receiving oestrogen may have been due, in part at least, to the type of oestrogen administered, there being several endogenous hormones as opposed to the one 'exogenous'. Marked variations between baboons in the extent to which sucrose was incorporated into triglyceride also contributed towards the difficulty in assessing small

changes in levels of specific activity. The major effect of the oestrogen was to curtail the rise in fasting serum triglyceride concentration that occurred early in the dietary period, and had the labelled sucrose been given at this time it is possible that the effect of the oestrogen on triglyceride specific activity might have been more striking. The similar values for triglyceride specific activity in the females after 13 weeks on the two diets, irrespective of whether they were receiving testosterone or not, is further evidence that the testosterone had no effect on this measurement.

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