The effect of dietary lipids on lipolysis in rat adipose tissue

By P. W. LARKING and E. R. NYE

Department of Medicine, University of Otago Medical School, Dunedin, New Zealand

(*Received* 6 *August* 1974 – *Accepted* 10 *October* 1974)

1. Rats were fed for 8 weeks on one of five diets differing in the amount of fatty acids 18:1, 18:2 and 18:3. Lipolysis, in vitro, of epididymal fat from fed and fasted rats was measured both basally and in the presence of noradrenaline with and without prostaglandin E_1.

2. Lipolysis was markedly influenced by the type of dietary fat. In particular, lipolysis in adipose tissue from rats given diets rich in the fatty acid 18:3 was higher than in the rats given diets containing 18:2.

3. Results showing the effects of fasting on adipose tissue lipolysis are also presented.

4. The results are discussed in relation to the known effects of unsaturated fats on hyperplasia and protein synthesis in adipose tissue and on the possible role of prostaglandins.

Changes in the fatty acid composition of diets affect the rate of lipolysis in rat epididymal fat when enzyme activity is estimated by the amount of glycerol released in vitro. When rats weighing 150–170 g were given one of two diets containing maize oil or lard (200 g/kg diet) for 2 weeks, the basal level of glycerol released/g wet weight of tissue from pieces of fat pad incubated in vitro was significantly less in the rats given the unsaturated maize oil than in those given lard (Pawar & Tidwell, 1968). Carreau, Loriette, Counis & Ketevi (1972) found that, for rats given diets containing sunflower oil or lard (200 g/kg diet) for 3 weeks from weaning, the glycerol released/mg protein from the fat pads incubated in vitro, when stimulated by noradrenaline (5 μg/ml) was significantly higher in the group given sunflower oil, contrary to the findings of Pawar & Tidwell (1968).

Dietary lipids may influence the level of lipolysis in adipose tissue through the potent antilipolytic effect of prostaglandins E_1 and E_2 (PGE_1 and PGE_2), since the precursors of these compounds are the essential fatty acids (van Dorp, Beerthuis, Nugteren & Vonkeman, 1964). Therefore, giving diets rich in linoleic acid might result in lower levels of adipose tissue lipolysis because of increased availability of prostaglandin precursors (Shaw & Ramwell, 1968). We have attempted a further evaluation of the effect of dietary lipids on lipolysis in rat adipose tissue in vitro.

MATERIALS AND METHODS

Animals and diets. Sixty Wistar rats 7–9 weeks old (average weight, 190 g) were divided into five groups, each of twelve animals, and each group was fed *ad lib.* on one of five diets (see Table 1) for 8 weeks.

Experimental procedures. At the end of this 8-week period, food was withdrawn from all the animals at 09.00 hours. Immediately, three animals from each group were

Table 1. *Fat source and fatty acid composition of diets given to rats*

	Diet*				
	A	B	C	D	E
Dietary fat (g/kg):					
Linseed oil	200	—	—	—	—
Sunflower oil	—	200	150	50	—
Beef fat	—	—	50	150	200
Principal fatty acids (percentage by weight):					
14:0	0·1	0·2	1·0	1·7	3·5
16:0	7·2	8·3	9·8	20·3	24·8
16:1	0·1	0·2	0·9	2·3	2·4
18:0	4·5	6·0	11·3	17·7	23·6
18:1	17·3	29·6	34·5	36·0	38·0
18:2	20·3	53·3	39·0	13·6	1·8
18:3	50·1	1·8	1·2	1·1	0·8

* Each diet also contained (g/kg): casein 250, wheat bran 60, mineral salt mix 40, vitamin mix 20 and sucrose 430.

taken, weighed and anaesthetized with sodium pentobarbitone (1 ml/kg body-weight). Blood samples were taken from the abdominal aorta, transferred to ice-cold heparinized tubes, centrifuged, and the plasma taken and stored at −20°. The epididymal fat pads were removed and immersed in warm saline solution. Ten pieces of fat (each approximately 50 mg) from each rat were separately incubated at 37° in 1·2 ml Krebs bicarbonate buffer (pH 7·4) containing albumin (40 g/l) and glucose (0·54 mg/l). The following five incubation mixtures were used and each incubation was done in duplicate: (1) no drugs, (2) noradrenaline 0·1 μg/ml, (3) noradrenaline 0·2 μg/ml, (4) noradrenaline 0·2 μg/ml and PGE_1 0·01 μg/ml, and (5) noradrenaline 0·2 μg/ml and PGE_1 0·1 μg/ml. Thus on day 1, fifteen rats were killed (five diet groups, three rats/group) and 150 separate pieces of tissue incubated (ten pieces per rat). On day 2 (24 h fasting), day 3 (48 h fasting) and day 4 (72 h fasting) the procedure for day 1 was repeated.

Glycerol released into the incubation medium was determined by the method of Chernick (1969). The fatty acid composition of the diets and of adipose tissue triglycerides was determined using gas–liquid chromatography (Hunter, Buchanan & Nye, 1970). Free fatty acids (FFA) in plasma were determined by the method of Laurell & Tibbling (1967). The results were analysed statistically by an analysis of variance.

RESULTS

The mean weight of animals at the start of the in vitro experiments was 377 g. Differences between the mean weights for rats in groups given different diets were not significant. The composition of the fatty acids in the diets and those of adipose tissue of rats given the diets for 8 weeks are shown in Tables 1 and 2. A summary of the analysis of variance of the results of in vitro incubation of adipose tissue is presented in Table 3. The differences between levels of lipolysis in tissues from fed and fasted animals and differences between rats fed on different diets was highly significant

Table 2. *Principal fatty acids (percentage by weight) in the epididymal fat pads of rats given diets containing different amounts of linseed oil, sunflower oil and beef fat for 8 weeks*

(Mean values for duplicate determinations for three rats/group)

	Dietary group*				
	A	B	C	D	E
Principal fatty acids					
16:0	15	16	17	22	23
16:1	4	3	4	5	7
18:0	2	2	3	3	5
18:1	27	32	36	45	50
18:2	19	39	34	17	6
18:3	29	2	0·5	0·8	0·7

* For details of diets, see Table 1.

Table 3. *Analysis of variance of the results (glycerol release) from the in vitro incubation* of adipose tissue from rats given diets containing different amounts of linseed oil, sunflower oil and beef fat for 8 weeks, then fasted for 0, 24, 48 and 72 h*

Source of variance	Degrees of freedom	Mean square	Statistical significance of interaction
Nutritional states†	3	0·1942	$P < 0·001$
Diets‡	4	0·1484	$0·001 < P < 0·01$
Nutritional states *v.* diets	12	0·0369	NS
Between rats within (diet *v.* nutritional state)	40	0·0284	—
Drugs§	4	2·3240	$P < 0·001$
Nutritional states *v.* drugs	12	0·0223	$P < 0·001$
Diets *v.* drugs	16	0·0093	NS
Nutritional states *v.* diets *v.* drugs	48	0·0078	NS
Rat within (diet *v.* nutritional state) *v.* drugs	160	0·0072	—
Between duplicate measurements	300	0·0052	—

NS, not significant ($P > 0·05$).

* For details of incubation procedure, see p. 292.

† Fasted for 0, 24, 48 and 72 h.

‡ For details, see Table 1.

§ Noradrenaline and prostaglandin E_1 included either together or alone and at different concentrations in the incubation mixture, see p. 292.

($P < 0·001$ and $0·01 < P < 0·001$ respectively (Table 4)). The effect of fasting on lipolysis was most noticeable after 72 h rather than after shorter periods of fasting. Further analysis of the differences between mean lipolysis rates for the various dietary groups by Scheffé's test (Scheffé, 1959) indicated that there were significant differences ($P < 0·1$) between group A (linseed oil) and groups B, C and D, in which all or part of the dietary fat was sunflower oil. The differences between A and C, or D were highly significant ($P < 0·05$). There was no significant difference between groups A and E or between E and B, C or D.

As expected, the differences between the effects of the various levels of drugs used were highly significant ($P < 0·001$) (Table 4). There was also a highly significant

Table 4. *Average lipolysis rates (nmol/mg per h) for pieces of epididymal fat pads* from rats given diets containing different amounts of linseed oil, sunflower oil and beef fat for 8 weeks, then fasted for 0, 24, 48 and 72 h*

(Mean values for duplicate determinations for three animals/group)

(*a*) Mean values for different dietary groups

	Diet†				
	A	B	C	D	E
Lipolysis rate	3·183	2·525	2·331	2·418	2·847

Analysis between dietary groups by Scheffé's (1959) test showed dietary group A to differ significantly from groups B, C and D. No other differences were significant (SEM 0·0154).

(*b*) Mean values for different nutritional states, drug combinations and their interactions

Drug combinations* (μg/ml)		Period of fasting (h)				Mean values for drug combinations
Noradrenaline	PGE_1	0	24	48	72	
0	0	0·764	1·232	1·054	1·327	1·095
0·1	0	3·179	3·353	3·206	4·521	3·565
0·2	0	4·509	4·005	4·056	5·168	4·435
0·2	0·01	2·353	2·510	2·679	3·263	2·701
0·2	0·1	1·388	1·540	1·400	1·708	1·508
Mean values for nutritional states		2·439	2·528	2·478	3·198	

The analysis of variance showed a highly significant nutritional state *v.* drug interaction and highly significant differences between drug combinations and between nutritional states (SEM for drugs 0·0077, SEM for nutritional states 0·0138).

PGE_1, prostaglandin E_1.
* For details of incubation procedure, see p. 292.
† For details of diets, see Table 1.

nutritional state *v.* drug interaction. The pattern of differences between nutritional states was generally similar for each drug and the interactions mainly reflect differences in the magnitude of the responses. The mean lipolysis rate in the presence of noradrenaline + PGE_1 (0·2 and 0·1 μg/ml respectively), expressed as a percentage of that in the presence of 0·2 μg noradrenaline/ml is an indication of the changes in the effectiveness of PGE_1 as an inhibitor of stimulated lipolysis, as the period of fasting increases. At 0 h of fasting, PGE_1 reduced the lipolysis rate to 52% of the stimulated rate and at 24, 48 and 72 h, lipolysis was reduced to 63, 66 and 63% respectively.

Differences between diets in the levels of plasma glycerol and FFA were not significant. However, plasma FFA levels for the four nutritional states differed significantly ($P < 0·001$, Table 5).

DISCUSSION

Plasma FFA and glycerol levels. The increased release of FFA from adipose tissue into the blood stream on fasting is mainly due to decreased esterification resulting from decreased glucose uptake into adipose tissue followed by increased rates of

Table 5. *The effect of fasting and diet on glycerol and free fatty acid (FFA) (nmol/l) in plasma from rats given diets containing different amounts of linseed oil, sunflower oil and beef fat for 8 weeks, then fasted for 0, 24, 48 and 72 h*

(Mean values calculated from an analysis of variance)

	Period of fasting (h)				
	0	24	48	72	SEM
Glycerol	0·181	0·170	0·182	0·177	0·0082
FFA	0·335	0·227	0·267	0·628	0·0274

	Dietary group*					
	A	B	C	D	E	
Glycerol	0·184	9·149	0·188	0·188	0·179	0·009
FFA	0·378	0·363	0·358	0·352	0·369	0·031

* For details of diets, see Table 1.

lipolysis as fasting continues (Scow & Chernick, 1970). Our results (Table 5) suggested that plasma FFA levels decreased during mild fasting, but increased markedly after 48–72 h of fasting. Basal rates of lipolysis, in vitro (Table 4*b*) were higher 24 and 48 h after fasting than at 0 h, and were still increasing at 72 h, when plasma FFA levels were also increasing.

The highly significant differences in vitro between different dietary treatments were not reflected in circulating plasma FFA or glycerol levels as there were no significant differences between dietary groups in these measurements. Another anomaly was associated with the lower levels of plasma triglycerides in animals given diets containing linseed oil compared to animals given diets containing saturated fats (Nye & Larking, 1970); in the present experiment the highest levels of lipolysis in vitro were found in the animals given the diet containing linseed oil. Possibly lipolysis or re-esterification in adipose tissue in vivo may also be controlled by additional mechanisms that are affected by diet, or by increased removal of FFA.

Adipose tissue lipolysis in vitro. Our results were similar to those of Pawar & Tidwell (1968). Rats given a diet rich in the fatty acid 18:2 (sunflower oil) had lower rates of lipolysis in vitro than those given beef fat, although the difference was not significant. However, when a diet rich in 18:3 (linseed oil) was given, the rates of lipolysis in adipose tissue were significantly higher than those for rats given diets containing sunflower oil. These differences are apparent both when lipolysis was stimulated by noradrenaline, and at the basal level. There were no significant diet *v.* drug or diet *v.* nutritional state interactions.

The effect of changes in fat-cell size and number with different dietary treatments has not been determined. It is known that giving weanling rats a diet rich in linoleic acid, compared to a diet containing lard, results in differences in adipocyte size and rates of incorporation of precursors into adipocyte RNA and DNA (Launay, Vodovar & Raulin, 1968; Launay, Richard, Alavoine & Raulin, 1972). Our animals were, however, 8–9 weeks old at the start of the experiment, a time when hyperplasia in the

epididymal fat pad is almost complete and further growth occurs mainly by a process of cellular hypertrophy (Hubbard & Matthew, 1971). The diets were therefore given when hyperplasia was probably of decreasing importance compared to hypertrophy and the effect on adipocyte number and size of a diet containing unsaturated fat might also have been of less significance. This suggestion was supported by the finding that the weights of rats for each dietary treatment after 8 weeks on the diet did not differ significantly. We have previously observed that giving a linseed-oil diet to rats from weaning for 13 months resulted in higher body-weights and epididymal-fat-pad weights compared to those of rats fed on mutton fat or hydrogenated coconut oil (Nye & Larking, 1970). If diet had not affected adipocyte number, dietary unsaturated fats could possibly change the rates of RNA and DNA synthesis, thereby changing other metabolic processes. In comparative studies of the effects of dietary fat on size of adipocytes and protein synthesis in these cells, the fatty acid 18:2 has been used (Launay *et al.* 1968; Launay *et al.* 1972). Our experiments indicate the need for comparative studies in which 18:3 is included in the diet.

A feedback hypothesis relating increased lipolysis to increased synthesis of prostaglandin has been proposed (Shaw & Ramwell, 1968). According to this hypothesis, diets which increase levels of prostaglandin precursors in adipose tissue should lower rates of lipolysis. Our results did not support a direct relationship, as lipolysis rates for rats given diets containing sunflower oil were not significantly lower than those for the rats fed on beef fat, which contains principally the fatty acid 18:1. With respect to the availability of prostaglandin precursors, all our diets should have contained adequate levels of linoleic acid, with the possible exception of diet E.

The highest lipolysis rates were associated with the diet containing linseed oil and these may be accounted for by mechanisms suggested by other workers. There is competition by 18:3 for the enzymes involved in chain elongation and desaturation of 18:2 to 20:3 and 20:4 acids, the precursors of PGE_1 and PGE_2 (Brenner & Peluffo, 1966). Also, prostaglandin synthesis is markedly inhibited by some unsaturated fatty acids, particularly 18:3ω3 (Pace-Asciak & Wolfe, 1968). Some of the increased lipolysis rates found in our rats fed on linseed oil may, therefore, result from these mechanisms. Further investigations comparing the effects of giving 18:3ω6 (a precursor of 8, 11, 14, 20:3 and 5, 8, 11, 14, 20:4 and thus of PGE_1 and PGE_2) and 18:3ω3 on lipolysis would now seem appropriate.

The effect of fasting on the antilipolytic effect of PGE_1. While PGE_1 is a potent inhibitor of lipolysis in vitro in adipose tissue from fed rats, under certain experimental conditions minimal inhibition is found when adipose tissue from 24 h-fasted animals is incubated (Carlson & Micheli, 1970). The results indicate that the effectiveness of PGE_1 is decreased during fasting, although a considerable antilipolytic effect on stimulated lipolysis is still present. This in part accounts for the highly significant nutritional state *v.* drug interaction. Carlson & Micheli (1970) showed that injection of glucose into the fasted rat 1 h before removal of the fat pads for incubation, restored the antilipolytic effect of PGE_1 on basal lipolysis rates. Therefore, the decreased effectiveness of added PGE_1 may be due to the increased synthesis within the adipocyte resulting from an increased supply of fatty-acid pre-

cursors derived from decreased re-esterification and increased lipolysis during fasting. The increased supply of prostaglandin within the adipocyte would then reduce the effect of any additional prostaglandin. Alternatively, the increased amounts of FFA having a prostaglandin-like configuration found during fasting may block some prostaglandin receptor sites.

The authors thank Dr J. E. Pike of the Upjohn Company for the gift of PGE_1 and Mr G. F. S. Spears of the Department of Social and Preventive Medicine, University of Otago Medical School for the statistical analysis. This work was supported by the Medical Research Council of New Zealand.

REFERENCES

Brenner, R. R. & Peluffo, R. O. (1966). *J. biol. Chem.* **241,** 5213.
Carlson, L. A. & Micheli, H. (1970). *Acta physiol. scand.* **80,** 289.
Carreau, J. P., Loriette, C., Counis, R. & Ketevi, P. (1972). *Biochim. biophys. Acta* **280,** 440.
Chernick, S. S. (1969). *Meth. Enzym.* **14,** 628.
Hubbard, R. W. & Matthew, W. T. (1971). *J. Lipid Res.* **12,** 286.
Hunter, J. D., Buchanan, H. & Nye, E. R. (1970). *J. Lipid Res.* **11,** 259.
Launay, M., Richard, M., Alavoine, R. & Raulin, J. (1972). *Nutr. Rep. Int.* **5,** 339.
Launay, M., Vodovar, N. & Raulin, J. (1968). *Bull. Soc. Chim. biol.* **50,** 439.
Laurell, S. & Tibbling, G. (1967). *Clinica chim. Acta* **16,** 57.
Nye, E. R. & Larking, P. W. (1970). *Gerontologia* **16,** 266.
Pace-Asciak, C. & Wolfe, L. S. (1968). *Biochim. biophys. Acta* **152,** 784.
Pawar, S. S. & Tidwell, H. C. (1968). *Biochim. biophys. Acta* **164,** 167.
Scheffé, H. (1959). *The Analysis of Variance.* New York: Wiley.
Scow, R. O. & Chernick, S. S. (1970). In *Comprehensive Biochemistry* Vol. 18, p. 23 [M. Florkin and E. H. Stotz, editors]. Amsterdam: Elsevier.
Shaw, J. E. & Ramwell, P. W. (1968). *J. biol. Chem.* **243,** 1498.
van Dorp, D. A., Beerthuis, R. K., Nugteren, D. H. & Vonkeman, H. (1964). *Biochim. biophys. Acta* **90,** 204.

Printed in Great Britain