Free amino acids present in various stages of moulting in the haemolymph of the Indian scorpion *P. bengalensis*

Amino acid	Stage 1	Stage 2	Stage 3			Stage 4
			a	b	c	
Alanine	++	+++	++	+	+	++
Arginine	+	++	++	++	+	+
Aspartic acid	+	+	+	+	+	+
Glutamic acid	+	+++	++	++	++	+
Glutamine	++	+++	+	+	+	+
Glycine	+	++	++	++	++	+
Leucine	+	++	+	—	+	+
Lysine	+	++	—	—	++	—
Phenylalanine	—	+	+	+	+	—
Proline	—	—	—	—	++	+
Serine	+	++	+	—	+	+
Threonine	+	++	—	—	+	+
Tryptophan	—	+	—	—	+	—
Tyrosine	+ (weak)	+	++	+++	++	+
Valine	+	++	—	—	+	+
Total amino acid present	12	14	10	8	15	12

Stage 1, normal stage; 2, premoulting or pharate stage; 3a, animal just started moulting; 3b, animal in which ecdysis was in progress; 3c, animal in which ecdysis was at the end; 4, animal in which tanning was in progress.

Alanine's decline might be due to its incorporation in the new cuticle while decrease of glutamic acid level indicated an increased nervous tissue metabolism during moulting. Sharp decrease in serine could have been likewise explained by its conversion into methionine and incorporation in the new cuticle, had there not been evidence contrary to this[2] and also that contrary to *Buthus tamulus*[4]. Methionine was not found in *P. bengalensis*. It was also reported to be absent in *Androctonus australis*[5]. Tyrosine at the premoulting stage accumulated up to the end of ecdysis but decreased sharply as the ecdysis was over. Similar observations with respect to tyrosine are reported for *Bombyx mori*[9]. Proline was present only in the haemolymph of the *P. bengalensis* in which ecdysis was coming to an end. It may be incorporated in the developing cuticle. Insect cuticular proteins are known to have a high concentration of proline[10]. Taurine reported in high concentration in *Androctonus australis*[5] was not found in *P. bengalensis*. Taurine is also reported to be absent in *Limulus polyphemus*[8].

[9] G. Duchâteau-Bosson, C. Jeuniaux and M. Florkin, Archs int. Physiol. Biochim. *70*, 287 (1962).
[10] R. H. Hackman, Biochem. J. *54*, 362 (1953).

Dietary Linoleic Acid and Lipogenesis in Rat Adipose Tissue

C. Loriette[1] and D. Lapous[2]

Equipe de Lipophysiologie du C.N.R.S., U56 I.N.S.E.R.M., Hôpital Bicêtre, F–94270 Bicêtre (France), 1 December 1975.

Summary. Weanling rats were fed diets in which linoleic content varied from 0.4 to 11%. The changes of epididymal adipose tissue lipogenesis from glucose do not depend upon the linoleic content of the diets.

It is well known that adipose tissue lipogenesis is reduced in response to feeding high fat diet[3,4]. However, our previous investigations showed that sunflower oil (67% linoleic acid) is less effective than lard (7% linoleic acid) to depress in vitro adipose tissue lipogenesis[5,6]. These results do not agree with those of Du and Kruger[7] who reported that linoleate is more efficient than oleate to suppress lipogenic activity in adipocytes. Hence we attempted to establish whether or not an essential fatty acid can play an inhibiting role on the de novo lipogenesis. The rats which were used in the Du and Kruger' studies received a fat-free diet for 4 to 6 months after weaning and the effect of an additional dose of linoleic acid was tested when animals showed every symptom of an essential fatty acid deficiency. The present study was carried out on healthy young rats. In order to evaluate the importance of the diet linoleic content on the in vitro adipose tissue lipogenesis from glucose, they received different vegetable oils and in addition to determine whether other oil components can mask linoleic effect, pure methyl esters were also given to rats.

Materials and methods. Several fratries of weanling male Wistar CF rats (40–50 g) were divided into 6 groups of 5 rats (experiment 1) or 6 rats (experiment 2). They were supplied diets (Table I) and water ad libitum for 3 weeks. In a 3rd experiment, 2 groups of 8 rats were fed A5 diet for 10 days after weaning and then they received

Table I. Nomenclature and composition (% by wt.) of the synthetic diets

Diets	Fat composition			Sucrose
	Oil		C18:2	
LP	0.7	sunflower	0.4	63
A5	5	peanut	1.3	58
O5	5	olive	0.4	58
M5	5	corn oil	2.8	58
O20	20	olive	1.4	43
M20	20	corn oil	11.1	43

In addition each diet contained: NBC, vitamin free casein: 29%; NBC, salt mixture: 5; UAR, vitamin mixture: 1; cellulose: 2.

[1] Present address: Laboratoire de Biochimie, 96, boulevard Raspail, F–75006 Paris, France.
[2] Present address: Laboratoire de Pathologie Cellulaire, 21, rue de l'Ecole de Médecine, F–75006 Paris, France.
[3] F. X. Hausberger and S. W. Milstein, J. biol. Chem. *214*, 483 (1955).
[4] G. A. Leveillé, J. Nutrition *91*, 25 (1967).
[5] C. Loriette, M. Jomain-Baum, I. Macaire and J. Raulin, Eur. J. clin. Biol. Res. *16*, 366 (1971).
[6] C. Loriette and J. Raulin, Biochimie *54*, 1467 (1972).
[7] J. T. Du and F. A. Kruger, J. Nutrition *102*, 1033 (1972).

Table II. Incorporation into fatty acids and glyceride-glycerol and oxydation to CO_2 of $U^{14}C$ glucose by rat epididymal adipose tissue (μmoles of glucose converted/mg azote/3 h incubation period)

Diets	%C18:2	Conversion to					
		Fatty acids		Glyceride-glycerol		CO_2	
LP	0.4	3.83 ± 0.36	5.36 ± 0.38	0.55 ± 0.05	0.89 ± 0.06	2.74 ± 0.26	4.05 ± 0.31
A5	1.3	2.53 ± 0.42 ($p < 0.05$)	3.89 ± 0.27 ($p < 0.01$)	0.58 ± 0.07 (NS)	1.00 ± 0.05 ($p < 0.2$)	1.99 ± 0.34 ($p < 0.1$)	3.12 ± 0.24 ($p < 0.05$)
O5	0.4	3.83 ± 0.47 (NS)	4.92 ± 0.67 (NS)	0.66 ± 0.07 ($p < 0.2$)	0.94 ± 0.06 (NS)	2.65 ± 0.27 (NS)	3.71 ± 0.37 (NS)
M5	2.8	3.66 ± 0.47 (NS)	7.43 ± 0.51 ($p < 0.01$)	0.73 ± 0.07 ($p < 0.05$)	1.23 ± 0.08 ($p < 0.01$)	2.70 ± 0.26 (NS)	5.57 ± 0.46 ($p < 0.02$)
O20	1.4	1.39 ± 0.26 ($p < 0.01$)	2.66 ± 0.45 ($p < 0.01$)	0.57 ± 0.06 (NS)	1.26 ± 0.07 ($p < 0.01$)	1.52 ± 0.18 ($p < 0.01$)	2.51 ± 0.25 ($p < 0.01$)
M20	11.1	1.68 ± 0.23 ($p < 0.01$)	2.20 ± 0.59 ($p < 0.01$)	0.85 ± 0.06 ($p < 0.01$)	1.45 ± 0.10 ($p < 0.01$)	1.69 ± 0.05 ($p < 0.01$)	2.52 ± 0.44 ($p \simeq 0.01$)

Data are means of duplicate incubations from 5 or 6 rats ± SEM. p-values are relative to LP diet. Compared to LP rats weights are not statistically different.

Table III. Incorporation into fatty acids and glyceride-glycerol and oxidation to CO_2 of $U^{-14}C$ glucose by rat epididymal adipose tissue (μmoles of glucose converted/mg azote/3 h incubation period)

Diets	%C18:2	Conversion to		
		Fatty acids	Glyceride-Glycerol	CO_2
MeOI	0.4	4.47 ± 0.21	2.08 ± 0.23	4.54 ± 0.21
MeLi	4.0	4.17 ± 0.32	1.96 ± 0.13	4.21 ± 0.20

Rats weights are not statistically different.

for the last 10 days a diet containing 4% methyl linoleate (MeLi group) or 3.6% methyl oleate[8] plus 0.4% methyl linoleate (MeOl group). The rats were fasted for 48 h and refed for 48 h before sacrifice.

Details concerning handling of the tissues, preparation of the flasks for incubation (25 μmoles of glucose, 0.5 μCi of glucose $U^{14}C$ and 0.1 unit of insulin in each flask) $^{14}CO_2$ collection, extraction, washing and saponification of tissue lipids, fatty acids and glycerol extraction, counting of the radioactivity in these different fractions and azote measurements have been described previously[5].

The results are expressed as μmoles $U^{14}C$ glucose oxidated or incorporated per mg azote in a 3 h incubation period. They are presented as the mean ± SEM and the statistical analysis was performed according to Student's t-test.

Results. These are reported in Table II (experiments 1 and 2) and Table III (experiment 3).

Fatty acid synthesis. As expected, fatty acid synthesis is significantly reduced in O20 and M20 groups when compared to the other groups. No significant difference appears between O5 and M5 groups, while in A5 group the smallest synthesis is observed. In experiment 2, the highest incorporation of glucose into fatty acids occurs in epididymal adipose tissue from rats fed M5 diet.

Glyceride-glycerol synthesis. The lowest synthesis is observed in adipose tissue of LP rats and the highest one in the M20 group.

$^{14}CO_2$ production. The conversion of glucose to carbon dioxide is very similar when rats were fed LP, O5 or M5 diet, whereas feeding A5 produces a small decrease. On the other hand, O20 and M20 feeding produces a significant decrease of $^{14}CO_2$ production.

Effect of methyl ester diets. Fatty acid and glyceride-glycerol synthesis and $^{14}CO_2$ production are slightly more elevated in MeOl group than in MeLi group. However, differences are not statistically significant.

Conclusion. The purpose of the present study was to evaluate the relative importance of linoleic acid on in vitro rat epididymal adipose tissue lipogenesis from glucose. The most striking features of these results are the following: 1. Increasing the level of fat from 0.7% to 5% does not significantly depress fatty acid synthesis from glucose, but diets containing 20% of fat always decrease it. Fatty acid synthesis is not in inverse ratio to the level of C18:2 in the diet: M5 diet is not less lipogenetic than O5 one and M20 diet is not less lipogenetic than O20 one. 2. It is shown here that A5, O5, M5, O20 and M20 diets increase glyceride-glycerol formation, the most efficient being M20 diet. 3. Concerning $^{14}CO_2$ production, the percentage of fat in the diet is the most important parameter while the level of C18:2 and the kind of oil used appeared to be far less important.

As previously reported[5], our present results show that linoleic acid is not really involved in the in vitro lipogenesis regulation. In fact, linoleic acid, which is an essential fatty acid, cannot act in any feedback process.

Our observations are in contrast with those made by DU and KRUGER[7]. This discrepancy can be explained by differences between the preparation of animals, as already stated. The nature of the fats given to the rats may also be of importance: we compared the effects of natural oils whereas DU and KRUGER used corn oil or hydrogenated coconut oil, the latter certainly contains *trans* fatty acid in a large proportion. Nevertheless our present results obtained with 20% olive oil (1.4% C18:2) and 20% corn oil (11.1% C18:2) are slightly different from our previous results obtained with 20% lard (2% C18:2) and 20% sunflower oil (11.6% C18:2). We showed that lard is a better lipogenic inhibitor than sunflower oil.

Here, there is no difference between O20 and M20. Therefore, we suggest that unsaponifiables of these oils and fats could also interfere in the fatty acid synthesis and could reduce or increase exogenous fatty acids effectiveness. This is also supported by results obtained with peanut oil (A5).

Finally, our observations made with pure methyl esters strengthen our earlier suggestion that, in rat epididymal adipose tissue, among the fatty acids, linoleic acid is not the best inhibitor of the de novo lipogenesis.

[8] Methyl esters were purchased from Merck.