Refeeding meal-fed rats increases lipoprotein lipase activity and deposition of dietary [^{14}C]lipid in white adipose tissue and decreases oxidation to $^{14}\text{CO}_2$

The role of undernutrition

Martha L. CRUZ and Dermot H. WILLIAMSON

Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE, U.K.

Meal-fed (3 h) rats had a decreased food intake, body weight and carcass fat compared with rats fed *ad libitum*. On refeeding a chow meal containing [^{14}C]triolein, the production of $^{14}\text{CO}_2$ was lower (45 %) and the accumulation of carcass [^{14}C]lipid higher (37 %) in the meal-fed rats. There was higher lipoprotein lipase activity and greater accumulation of [^{14}C]lipid in the epididymal and subcutaneous adipose-tissue depots of the meal-fed rats. In contrast, heparin-releasable lipoprotein lipase was not increased in perfused hearts of meal-fed rats on refeeding. Return of meal-fed rats to feeding *ad libitum* reversed these changes before the restoration of body weight or carcass fat. Evidence is presented that decreased dietary intake rather than meal pattern is an important determinant of the alterations in adipose lipid metabolism in the meal-fed rat in response to a meal.

INTRODUCTION

The adaptive changes in lipid metabolism in rats trained to eat their food during a limited period were first noted by Dickerson *et al.* (1943) and Tepperman & Tepperman (1958), who reported increased lipogenesis in liver slices of meal-fed rats. Numerous studies *in vitro* showed that this increased lipogenic capacity was not confined to liver, but also occurred in adipose tissue (Hollifield & Parson, 1962; Leveille & Hanson, 1965; Leveille, 1967*a*). The incorporation of [^{14}C]glucose *in vivo* into fatty acids of adipose tissue and liver was also shown to be enhanced in the meal-fed rat (Leveille, 1967*b*; Palmquist *et al.*, 1977). Pallardo & Williamson (1989) measured lipid synthesis *in vivo* with $^3\text{H}_2\text{O}$ on feeding a chow meal to starved rats that had previously been fed *ad libitum* or meal-fed, and found higher rates of lipogenesis in both liver and adipose tissue of the latter. The metabolic adaptations induced by meal feeding have been shown to be accompanied by corresponding increases in activity of enzymes involved in fatty acid synthesis. These include the pentose-phosphate-pathway enzymes (Tepperman & Tepperman, 1958; Cohn & Joseph, 1960; Hollifield & Parson, 1962), as well as pyruvate carboxylase (EC 6.4.1.1), malic enzyme (EC 1.1.1.40; Leveille, 1970) and pyruvate dehydrogenase (EC 1.2.4.1; Holness & Sugden, 1989). An increased activity of pyruvate kinase (EC 2.7.1.40; the glycolytic pathway) and glycerol phosphate dehydrogenase (EC 1.1.1.8; fatty acid esterification) in adipose tissue was also shown by Chakrabarty & Leveille (1968). Although much research has concentrated on the stimulation of fatty acid synthesis *de novo* in adipose tissue of the meal-fed rat, little has been related to the deposition of exogenous preformed fat by adipose tissue. Leveille (1967*b*) found that when a high-fat diet was given to meal-fed rats, lipid synthesis *de novo* was suppressed and the changes in enzyme activities did not occur. In the chow-fed rat the rate of deposition of lipid *in vivo* in white adipose tissue from exogenous sources (chylomicrons) is considerably higher than that of fatty acid synthesis *de novo* (Williamson, 1990). The aim of the present study was therefore to determine whether meal feeding altered the disposal of a chow meal containing [^{14}C]triolein between adipose-tissue [^{14}C]lipid accumulation and oxidation to $^{14}\text{CO}_2$ and to relate this to the

activity of lipoprotein lipase (EC 3.1.1.34), the enzyme responsible for hydrolysis of plasma lipoprotein triacylglycerols before entry of the constituent fatty acids into extrahepatic tissues. Preliminary results have been briefly reported (Tedstone *et al.*, 1991).

EXPERIMENTAL

Materials

Rats. Male Wistar rats of approx. 60 days of age and a starting weight of 210 ± 5 g were used. All animals were housed individually in wire-mesh cages and fed on a chow diet consisting of 52 % carbohydrate, 21 % protein, 4 % fat (all by wt.) and residue of non-digestible material (Special Diet Services, Witham, Essex, U.K.) with free access to water. They were kept at an ambient temperature of 22 ± 2 °C with a 12 h-light/12 h-dark cycle (light from 08:00 h). Rats were either allowed free (*ad libitum* group) or restricted (08:00–11:00 h meal-fed group) access to food for 14–15 days unless otherwise stated. At the end of the feeding period, rats were starved for 21 h and either used in the starved state or refed at 08:00 h.

Chemicals. [^{14}C]Triolein (glycerol tri[^{14}C]oleate), [9,10- ^3H]triolein and $^3\text{H}_2\text{O}$ were obtained from Amersham International. Other biochemicals were from Boehringer Corp. (London).

Measurement of [^{14}C]lipid oxidation and tissue [^{14}C]lipid accumulation *in vivo*

Starved rats were refed with 5 g of chow in which 0.75 g of [^{14}C]triolein (15 % by wt., 0.3 μCi) had been incorporated. Rats usually ate the meal in less than 30 min. The $^{14}\text{CO}_2$ produced after intake of the ^{14}C -labelled diet was collected every 60 min for 5 h by absorption in Lumasorb (May & Baker) (Oller do Nascimento & Williamson, 1986). At the end of the period, rats were anaesthetized with Nembutal (60 mg/kg body wt.). Tissues, carcass and intestinal tract were treated and extracted to determine the [^{14}C]lipid content as previously described (Oller do Nascimento & Williamson, 1986).

Measurement of blood metabolites and plasma insulin

A group of rats, fed *ad libitum* and meal-fed, were killed in the starved state or refed with 5 g of a triolein-rich (15%) chow and killed at 1.5 and 3 h after refeeding. Rats were anaesthetized with pentobarbital, and arterial blood samples were collected with a heparinized syringe. Samples of epididymal and inguinal subcutaneous adipose tissue were rapidly excised, freeze-clamped and stored in liquid nitrogen for determination of lipoprotein lipase. Whole-blood glucose (Slein, 1963), L-lactate and pyruvate (Hohorst, 1963), D-3-hydroxybutyrate and acetoacetate (Williamson *et al.*, 1962) and plasma glycerol and triacylglycerols (Eggstein & Kreutz, 1966) were determined by enzymic methods. Non-esterified fatty acids were measured with an enzymic colorimetric kit (Wako NEFA C test kit; Wako Chemicals G.m.b.H., Neuss, Germany). Plasma insulin was measured by radioimmunoassay with a rat insulin standard (Albano *et al.*, 1972).

Measurement of lipoprotein lipase

Activity of lipoprotein lipase in epididymal and inguinal subcutaneous adipose tissue was measured in acetone/ether-dried preparations of the frozen tissues (Nilsson-Ehle *et al.*, 1972). The dried tissue powders were solubilized with 0.2 M-Tris/HCl buffer, pH 8.1, containing 1 M-ethylene glycol, and the activity was measured with [9,10-³H]triolein as substrate (Nilsson-Ehle & Schotz, 1976). The activity is expressed as nmol of fatty acid released/min per mg of acetone-dried tissue. Heparin-releasable lipoprotein lipase activity in the isolated perfused heart was measured as described by Kuwajima *et al.* (1988). Most of the lipoprotein lipase activity was released into the perfusate within 5 min of heparin addition. After 10 min perfusion the heart was freeze-clamped (Wollenberger *et al.*, 1960) and treated as above.

Measurement of lipogenesis *in vivo*

A group of rats, fed *ad libitum* and meal-fed, were starved for 21 h and then refed with 5 g of triolein-rich (15%) chow. Lipogenesis was measured *in vivo* with ³H₂O as described by Robinson *et al.* (1978). Rats were injected intraperitoneally with 2 mCi (0.2 ml) of ³H₂O at 120 min after refeeding. Rats were anaesthetized with pentobarbital at 55 min after ³H₂O administration. At 60 min, liver, epididymal and subcutaneous adipose tissue were removed and samples were taken for extraction of lipids (Stansbie *et al.*, 1976).

RESULTS AND DISCUSSION

Food intake and body weight change

Meal-fed rats took 3–4 days to adapt to the meal-feeding schedule, and at the end of the experimental period (15 days) had only reached 42% of the food intake and 72% of the body wt.

Table 1. Food intake and body weight of rats fed *ad libitum* and meal-fed rats

For experimental details see the text. The results are means $\pm$ S.E.M. for 6 rats in each group. Values that are statistically different by Student's *t* test from those of rats fed *ad libitum* are shown by ****P* < 0.001.

Days	Food intake (g/day)		Body weight (g)	
	<i>Ad libitum</i>	Meal-fed	<i>Ad libitum</i>	Meal-fed
1–3	30.7 $\pm$ 2.90	6.9 $\pm$ 0.83***	208 $\pm$ 6.1	209 $\pm$ 4.1
4–6	22.5 $\pm$ 1.15	10.0 $\pm$ 1.10***	239 $\pm$ 3.7	197 $\pm$ 5.7***
7–9	22.7 $\pm$ 1.60	10.7 $\pm$ 0.62***	258 $\pm$ 3.9	192 $\pm$ 6.3***
10–12	26.2 $\pm$ 0.37	12.4 $\pm$ 0.69***	266 $\pm$ 4.9	203 $\pm$ 8.1***
13–15	28.1 $\pm$ 0.47	11.7 $\pm$ 0.64***	279 $\pm$ 10.6	202 $\pm$ 6.5***

of rats fed *ad libitum* (Table 1). The findings persisted even in rats meal-fed for up to 8 weeks (results not shown), and are in agreement with the results of Muiruri & Leveille (1970), De Bont *et al.* (1975), Paik & Yearick (1978) and Bittman *et al.* (1984), who used synthetic diets high in carbohydrate.

Weight and percentage of fat in adipose-tissue depots

Although rats fed *ad libitum* had significantly larger adipose-tissue depots and liver, when results were expressed per 100 g carcass wt., differences only persisted in epididymal adipose tissue (Table 2). As expected from previous work (De Bont *et al.*, 1975), carcass total fat was decreased in the meal-fed rat (Table 2) and remained lower in rats meal-fed for up to 8 weeks (results not shown).

¹⁴CO₂ production and [¹⁴C]lipid accumulation in carcass and tissues

The rate of ¹⁴CO₂ production was linear after the first 1 h of refeeding and was significantly decreased in the meal-fed rats (Fig. 1). The absorption of [¹⁴C]lipid during the 5 h period was higher in rats fed *ad libitum* (Table 3). To correct for these differences, results for ¹⁴CO₂ production and tissue [¹⁴C]lipid accumulation were expressed as a percentage of the absorbed dose. There was a decrease (45%) in ¹⁴CO₂ production in meal-fed rats, and this was accompanied by an increase (37%) in carcass [¹⁴C]lipid accumulation compared with rats fed *ad libitum* (Table 3). This indicates a redirection of exogenous lipid (chylomicrons) for deposition (adipose tissue), rather than oxidation (liver, muscle, brown adipose tissue). As might be expected, the differences in [¹⁴C]lipid accumulation in the carcass were accompanied by 7–8 times higher rates of [¹⁴C]lipid accumulation on a g-fresh-wt. basis in epididymal and inguinal subcutaneous adipose tissue of meal-fed rats (Table 3). When rates of uptake were expressed per depot they were still significantly higher (results not shown), indicating that the changes were not simply due to an increased number of adipocytes per g of tissue. Muscle is considered an important site of removal of circulating triacylglycerols for oxidation (Linder *et al.*, 1976; Tan *et al.*, 1977). Meal-fed rats had 4 times greater rates of apparent [¹⁴C]lipid accumulation in hind-leg skeletal muscle than controls. When expressed on a whole-body basis, this represents less than 10% of that found in the carcass. However, individual muscles were not dissected out, and therefore the sample included intermuscular adipose tissue (Mattocks & Pond, 1988). The present finding, together with the lower ¹⁴CO₂ production observed in meal-fed rats (Fig. 1), suggests that a lower percentage of fatty acids are oxidized by muscle of these rats. There were no significant differences in the amount of [¹⁴C]lipid accumulated in liver or brown adipose tissue.

Lipoprotein lipase activity

The increased accumulation of [¹⁴C]lipid in the adipose-tissue depots of meal-fed rats on refeeding suggested an increase in the tissue activity of lipoprotein lipase, the enzyme responsible for the hydrolysis of plasma lipoprotein triacylglycerols (chylomicrons and very-low-density lipoproteins), and which therefore regulates the availability of fatty acids for deposition in tissues (Robinson, 1970; Greenwood, 1985). There were no significant differences in whole-tissue enzyme activity between meal-fed and rats fed *ad libitum* in the starved state. This is not in agreement with the results of Paik & Yearick (1978), who found increased lipoprotein lipase activity in epididymal adipose tissue from meal-fed rats in the starved state. It is possible that the discrepancy could be due to a shorter period of starvation in their experiments (11 h) or to their use of a synthetic diet. The activity of lipoprotein lipase in epididymal adipose tissue after refeeding

Table 2. Final carcass weight and fat content, and weight of adipose tissue depots of rats fed *ad libitum* and meal-fed rats

For experimental details see the text. The results are mean $\pm$ S.E.M. for 6 rats in each group. Values for meal-fed rats that are statistically different by Student's *t* test from those for rats fed *ad libitum* are shown by **P* < 0.05, ****P* < 0.001.

Experimental group	Carcass		Adipose tissue			
	Wt. (g)	Fat (%)	Epididymal		Subcutaneous	
			Wt. (g/100 g carcass wt.)	Fat (%)	Wt. (g/100 g carcass wt.)	Fat (%)
Fed <i>ad libitum</i>	224 $\pm$ 7.0	6.7 $\pm$ 0.28	0.89 $\pm$ 0.09	76.6 $\pm$ 1.4	1.27 $\pm$ 0.11	59.2 $\pm$ 4.8
Meal-fed	163 $\pm$ 4.8***	5.2 $\pm$ 0.56*	0.61 $\pm$ 0.05*	70.2 $\pm$ 2.3*	1.03 $\pm$ 0.09	62.0 $\pm$ 3.8

Fig. 1. Effects of meal-feeding on ¹⁴CO₂ production after refeeding with chow containing [1-¹⁴C]triolein

For experimental details see the text. The results are means $\pm$ S.E.M. for the numbers of rats in parentheses: ●, rats fed *ad libitum* (5); ○, meal-fed rats (6). The meal-fed values are significantly different from the *ad libitum* at 2 h (*P* < 0.05), 3 h and 4 h (*P* < 0.01), and 5 h (*P* < 0.001).

a meal (5 g) of chow containing triolein (15%) was significantly higher in meal-fed rats than in rats fed *ad libitum* at 1.5, 3 and 5 h (Table 4), whereas in the subcutaneous depot the activity was significantly higher at 1.5 and 3 h only. Although the activity increased significantly (*P* < 0.001) by 5 h in epididymal adipose tissue of rats fed *ad libitum* and refeed with chow containing triolein, the absolute increase was about 50% of that of the meal-fed rats at 5 h (Table 4). The subcutaneous adipose tissue was less responsive to refeeding in both groups of rats compared with the epididymal fat-pad. Refeeding meal-fed rats with a normal chow meal resulted in a similar increase in lipoprotein lipase activity in adipose tissue to that found with the triolein-rich chow meal (results not shown).

Table 4. Effects of starvation and refeeding with a triolein-containing chow meal on lipoprotein lipase activity in epididymal and subcutaneous adipose tissue of meal-fed and rats fed *ad libitum*

For experimental details see the text. The results are means $\pm$ S.E.M., with the numbers of rats shown in parentheses. Values that are statistically different by Student's *t* test from those of the corresponding rats fed *ad libitum* are shown by **P* < 0.05, ***P* < 0.001.

Experimental group	Lipoprotein lipase activity (nmol of fatty acid released/min per mg of acetone-dried tissue)	
	Epididymal adipose tissue	Subcutaneous adipose tissue
Fed <i>ad libitum</i>		
Starved (21 h)	1.16 $\pm$ 0.20 (10)	0.90 $\pm$ 0.14 (10)
Refed (1.5 h)	1.12 $\pm$ 0.12 (5)	0.46 $\pm$ 0.06 (6)
Refed (3 h)	1.70 $\pm$ 0.12 (5)	0.61 $\pm$ 0.14 (6)
Refed (5 h)	2.62 $\pm$ 0.28 (5)	0.86 $\pm$ 0.14 (5)
Meal-fed		
Starved (21 h)	1.44 $\pm$ 0.21 (9)	0.63 $\pm$ 0.08 (10)
Refed (1.5 h)	2.21 $\pm$ 0.20 (5)**	1.22 $\pm$ 0.18 (6)**
Refed (3 h)	2.92 $\pm$ 0.28 (6)**	1.30 $\pm$ 0.24 (6)*
Refed (5 h)	4.80 $\pm$ 0.49 (4)**	1.16 $\pm$ 0.18 (5)

Comparison of the results in Tables 3 and 4 indicates that in epididymal adipose tissue the increase (8-fold) in accumulation of [¹⁴C]lipid on refeeding meal-fed rats is considerably greater than the increase in lipoprotein lipase activity (3-fold). It must be emphasized that whole-tissue lipoprotein lipase activity was measured rather than the 'functional' activity on the endothelial-cell surface, and this may explain the difference. It is likely that the [¹⁴C]lipid accumulation is an index of functional activity of the enzyme.

The heparin-releasable activity of lipoprotein lipase from perfused heart was lower in starved meal-fed rats (Table 5).

Table 3. Effect of meal feeding on whole-body ¹⁴CO₂ production and tissue accumulation of [¹⁴C]lipid after refeeding with chow containing [1-¹⁴C]triolein

For experimental details see the text. The results are means $\pm$ S.E.M. for 6 rats in each group. Values that are statistically different by Student's *t* test from those of rats fed *ad libitum* are shown by **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Experimental group	Absorption of [¹⁴ C]lipid (%)	¹⁴ CO ₂ production (% of absorbed dose)	Total carcass (% of absorbed dose)	Tissue [¹⁴ C]lipid accumulation (% of absorbed dose/g of tissue)				
				Epididymal adipose tissue	Subcutaneous adipose tissue	Interscapular brown adipose tissue	Liver	Muscle
Fed <i>ad libitum</i>	68.5 $\pm$ 2.7	46.8 $\pm$ 3.8	38.1 $\pm$ 2.2	0.44 $\pm$ 0.04	0.34 $\pm$ 0.05	9.9 $\pm$ 2.3	0.75 $\pm$ 0.06	0.03 $\pm$ 0.003
Meal-fed	58.9 $\pm$ 2.2*	25.6 $\pm$ 0.91***	60.1 $\pm$ 2.6***	3.30 $\pm$ 0.42***	2.72 $\pm$ 0.54**	10.7 $\pm$ 2.0	0.83 $\pm$ 0.04	0.13 $\pm$ 0.02***

Table 7. Effects of return of meal-fed rats to feeding *ad libitum* on whole-body $^{14}\text{CO}_2$ production and tissue accumulation of [^{14}C]lipid after refeeding with chow containing [1- ^{14}C]triolein

For experimental details see the text. The results are means $\pm$ S.E.M. for the numbers of rats shown in parentheses. Values that are statistically different by Student's *t* test from those of meal-fed rats are shown by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Experimental group		Absorption of [^{14}C]lipid (%)	$^{14}\text{CO}_2$ production (% of absorbed dose)	Total carcass (% of absorbed dose)	Tissue [^{14}C]lipid accumulation (% of absorbed dose/g of tissue)				
					Epididymal adipose tissue	Subcutaneous adipose tissue	Interscapular brown adipose tissue	Liver	Muscle
Meal-fed	(5)	73.4 $\pm$ 4.0	21.5 $\pm$ 4.1	50.6 $\pm$ 3.5	3.00 $\pm$ 0.38	3.00 $\pm$ 0.40	4.10 $\pm$ 0.67	0.60 $\pm$ 0.03	0.088 $\pm$ 0.01
Meal-fed, 3-day returned	(8)	67.4 $\pm$ 3.9	28.4 $\pm$ 1.6	36.9 $\pm$ 2.9*	1.17 $\pm$ 0.16***	0.88 $\pm$ 0.17***	11.2 $\pm$ 2.5	0.65 $\pm$ 0.07	0.054 $\pm$ 0.01*
Meal-fed, 7-day returned	(6)	53.1 $\pm$ 2.3***	38.8 $\pm$ 2.2**	40.3 $\pm$ 1.9*	0.68 $\pm$ 0.12***	0.54 $\pm$ 0.09***	14.9 $\pm$ 2.5***	0.95 $\pm$ 0.08**	0.059 $\pm$ 0.02
Fed <i>ad libitum</i>	(4)	58.7 $\pm$ 3.6*	40.0 $\pm$ 4.5*	34.1 $\pm$ 4.3*	0.25 $\pm$ 0.04***	0.18 $\pm$ 0.03***	—	0.70 $\pm$ 0.09	0.035 $\pm$ 0.005**

carcass fat did not reach values for rats fed *ad libitum* studied at the same time [meal-fed, 6.3 $\pm$ 0.48% (5); meal-fed, 3-day returned, 6.2 $\pm$ 0.48% (8); meal-fed, 7-day returned, 6.6 $\pm$ 0.37 (6); fed *ad libitum*, 9.6 $\pm$ 1.3 (4)]. There were no differences in weights (expressed per 100 g body wt.) or fatty acid content of the subcutaneous adipose tissue. However, the epididymal adipose-tissue weight in the meal-fed/7-day returned rat was significantly higher (25%; $P < 0.01$) than that of the meal-fed group (results not shown).

Despite the unchanged carcass fat content in the rats returned to feeding *ad libitum*, the disposal of exogenous [^{14}C]lipid between $^{14}\text{CO}_2$ production and [^{14}C]lipid accumulation in tissues showed a gradual return (within 7 days) to the pattern of rats fed *ad libitum* (Table 7). Although differences in [^{14}C]lipid accumulation in epididymal and subcutaneous adipose tissue of meal-fed/7-day returned rats were much lower than values obtained for meal-fed rats ($P < 0.001$), they remained slightly higher than those for rats fed *ad libitum* ($P < 0.05$) (Table 7).

Muiruri & Leveille (1970) and De Bont *et al.* (1975) reported that the incorporation of labelled glucose into adipose-tissue lipid *in vitro* remained higher in meal-fed rats than in rats fed *ad libitum*, after 3 weeks and 7 weeks return to feeding *ad libitum* respectively. To determine if the increased rate of lipogenesis in liver and adipose tissue of chow-meal-fed rats *in vivo* (Pallardo & Williamson, 1989) was also refractory to change of meal pattern, we measured lipogenesis *in vivo* by the incorporation of $^3\text{H}_2\text{O}$ in meal-fed rats returned to feeding *ad libitum* for 3 and 7 days (Table 8). The results indicate that lipogenesis in liver, epididymal and inguinal subcutaneous adipose tissue parallels the decrease of exogenous lipid deposition on return to feeding *ad libitum* (Table 8).

Effect of undernutrition

A key question is whether the observed changes in adipose-tissue lipid metabolism are due to the meal-feeding and/or the accompanying undernutrition (Table 1). A recent report indicates that food restriction of Sprague-Dawley rats to give a 20–25% weight deficit compared with rats fed *ad libitum* considerably increased the activity of lipoprotein lipase in epididymal adipose tissue from starved and refed rats (Bessesen *et al.*, 1991). However, these diet-restricted rats were meal-fed (2 h) for 4 weeks. Similarly, lipoprotein lipase activity in adipose tissue has been reported to be increased in food-restricted pair-fed rats refed with a 5 g meal of chow (Hansson *et al.*, 1983). It should, however, be emphasized that 'pair-feeding', as normally carried out, results in meal-feeding because the rats rapidly consume the measured diet. To differentiate between the effects of meal-

Table 8. Effects of return of meal-fed rats to feeding *ad libitum* on rates of lipogenesis *in vivo* in adipose tissue and liver after refeeding with chow containing triolein

For experimental details see the text. The results are means $\pm$ S.E.M., with the numbers of observations in parentheses. Values that are statistically different by Student's *t* test from those for meal-fed rats are shown: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Experimental group		Rate of fatty acid synthesis (μmol of $^3\text{H}_2\text{O}$ incorporated into lipid/h per g fresh wt. of tissue)		
		Epididymal adipose tissue	Subcutaneous adipose tissue	Liver
Meal-fed	(6)	9.6 $\pm$ 2.2	7.0 $\pm$ 1.7	13.8 $\pm$ 1.3
Meal-fed, 3-day returned	(4)	4.9 $\pm$ 0.86	2.5 $\pm$ 0.40	4.7 $\pm$ 0.21***
Meal-fed, 7-day returned	(4)	3.3 $\pm$ 0.96*	2.1 $\pm$ 0.23*	6.0 $\pm$ 0.58**
Fed <i>ad libitum</i>	(6)	2.9 $\pm$ 0.86*	2.7 $\pm$ 0.66*	8.7 $\pm$ 0.90**

feeding and undernutrition, we fed groups of rats for 14 days on either the daily food intake of meal-fed rats or that of rats fed *ad libitum* in four equal portions at 08:00 h, 12:00 h, 16:00 h and 20:00 h. This pattern of food intake is similar to that of the nocturnal feeding of a rat fed *ad libitum* (Le Magnen & Tallon, 1966; Balagura & Coscina, 1968). There was no difference between the results for meal-fed (single meal) rats (Tables 3 and 7) and undernourished rats (multiple meals; $n = 5$) for whole-body $^{14}\text{CO}_2$ production (24.5 $\pm$ 2.8% of absorbed dose/5 h) or for accumulation of [^{14}C]lipid in epididymal or subcutaneous white adipose tissue (3.9 $\pm$ 0.54% and 3.0 $\pm$ 0.23% of absorbed dose/5 h per g respectively). However, rats ($n = 6$) fed on multiple meals equivalent in amount to that for the rats fed *ad libitum* gave values which were not significantly different from those for the latter group ($^{14}\text{CO}_2$ production 42.7 $\pm$ 2.4% of absorbed dose/5 h; epididymal and subcutaneous adipose tissue 0.56 $\pm$ 0.16% and 0.53 $\pm$ 0.16% of absorbed dose/5 h per g respectively). On refeeding, lipoprotein lipase activity in adipose tissue of the undernourished rats increased at 5 h to values [epididymal fat pad, 7.2 $\pm$ 0.42 nmol of fatty acid released/min per mg of acetone-dried powder ($n = 3$); subcutaneous adipose tissue, 1.09 $\pm$ 0.16 ($n = 5$)] similar to those of meal-fed rats,

whereas the activity in adipose tissue of the group given multiple meals *ad libitum* was lower [epididymal, 3.5 ± 0.33 ($n = 5$); subcutaneous, 0.56 ± 0.11 ($n = 6$)]. These results indicate that decreased dietary intake is more important than meal pattern in determining the changes in lipid metabolism found in the meal-fed rat on feeding.

Concluding remarks

The present results extend previous observations that adipose tissue of meal-fed rats has a higher rate of lipogenesis to show that it also has a greater capacity for deposition of dietary fat owing to the increased activity of lipoprotein lipase in response to a meal. This results in a smaller proportion of the dietary fat being immediately oxidized. However, because the meal-fed rats consume less food than do rats fed *ad libitum*, a loss of carcass fat occurs. Clearly, this increased capacity of adipose tissue of the undernourished rat for fat deposition after feeding has a teleological advantage, because it will direct dietary carbohydrate (for conversion into fatty acids) or lipid to adipose tissue for storage rather than immediate oxidation.

Recent work has shown that decreasing the weight of obese Zucker rats (Bessesen *et al.*, 1991) or obese humans (Kern *et al.*, 1990) by food restriction also results in increased lipoprotein lipase activity and mRNA in adipose tissue. The outstanding question is how does food restriction bring about this increase? The most likely signal is the altered hormonal milieu, because food restriction in the rat results in decreased plasma growth hormone and thyroid hormone (Rodriguez & Jolin, 1991) and these alterations may act directly and/or via a change in insulin sensitivity to increase lipoprotein lipase activity.

We thank Mrs. V. Ilic for skilled assistance, Mr. R. Hems for performing the heart perfusions and Mrs. M. Barber for preparation of the manuscript. M. L. C. is a Krebs Memorial Scholar and D. H. W. is a member of the External Scientific Staff of the Medical Research Council (U.K.).

REFERENCES

- Albano, J. D. M., Ekins, R. P., Maritz, G. & Turner, R. C. (1972) *Acta Endocrinol. (Copenhagen)* **70**, 487–509
- Balagura, S. S. & Coscina, D. V. (1968) *Physiol. Behav.* **3**, 641–643
- Bessesen, D. H., Robertson, A. D. & Eckel, R. H. (1991) *Am. J. Physiol.* **261**, E246–E251
- Bittman, B. J., Layman, D. K., Glore, S. R. & Link, J. G. (1984) *Nutr. Res.* **4**, 477–483
- Chakrabarty, K. & Leveille, G. A. (1968) *J. Nutr.* **96**, 76–82
- Cohn, C. & Joseph, D. (1960) *Metab. Clin. Exp.* **9**, 492–500
- Cryer, A., Riley, S. E., Williams, E. R. & Robinson, D. S. (1976) *Clin. Sci. Mol. Med.* **50**, 213–221
- De Bont, A. J., Rosmos, D. R., Tsai, A. C. & Waterman, R. A. (1975) *Proc. Soc. Exp. Biol. Med.* **149**, 849–854
- Dickerson, V. C., Tepperman, J. & Long, C. N. H. (1943) *Yale J. Biol. Med.* **15**, 875–892
- Eggstein, M. A. & Kreutz, F. H. (1966) *Klin. Wochenschr.* **44**, 262–267
- Fried, S. K., Velazquez, N. & Nobel, J. (1990) *J. Nutr.* **120**, 1087–1095
- Greenwood, M. R. C. (1985) *Int. J. Obesity* **9**, 67–70
- Hansson, P., Nordin, G. & Nilsson-Ehle, P. (1983) *Biochim. Biophys. Acta* **753**, 364–371
- Hohorst, H. J. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 134–138, Academic Press, New York and London
- Hollifield, G. & Parson, W. (1962) *J. Clin. Invest.* **41**, 245–249
- Holness, M. J. & Sugden, M. C. (1989) *Biochem. J.* **262**, 321–325
- Kern, P. A., Ong, J. M., Saffari, B. & Carty, J. (1990) *N. Engl. J. Med.* **322**, 1053–1059
- Kuwajima, M., Foster, D. W. & McGarry, J. D. (1988) *Metab. Clin. Exp.* **37**, 597–601
- Le Magnen, J. & Tallon, S. (1966) *J. Physiol. (Paris)* **58**, 323–349
- Leveille, G. A. (1967a) *J. Nutr.* **92**, 460–466
- Leveille, G. A. (1967b) *Proc. Soc. Exp. Biol. Med.* **125**, 85–88
- Leveille, G. A. (1970) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **29**, 1294–1301
- Leveille, G. A. & Hanson, R. W. (1965) *Can. J. Physiol. Pharmacol.* **43**, 857–868
- Linder, C., Chernick, S. S., Fleck, T. R. & Scow, R. O. (1976) *Am. J. Physiol.* **231**, 860–864
- Mattocks, C. A. & Pond, C. M. (1988) *Int. J. Obesity* **12**, 585–597
- Muiruri, K. L. & Leveille, G. A. (1970) *J. Nutr.* **100**, 450–459
- Nilsson-Ehle, P. & Schotz, M. C. (1976) *J. Lipid Res.* **17**, 536–541
- Nilsson-Ehle, P., Tornquist, H. & Belfrage, P. (1972) *Clin. Chim. Acta* **42**, 383–390
- Oller do Nascimento, C. M. & Williamson, D. H. (1986) *Biochem. J.* **254**, 233–236
- Paik, H. S. & Yearick, E. S. (1978) *J. Nutr.* **108**, 1798–1805
- Pallardo, F. V. & Williamson, D. H. (1989) *Biochem. J.* **257**, 607–610
- Palmquist, D. L., Learn, D. B. & Baker, N. (1977) *J. Nutr.* **107**, 502–509
- Robinson, D. S. (1970) *Comp. Biochem.* **18**, 51–117
- Robinson, A. M., Girard, J. R. & Williamson, D. H. (1978) *Biochem. J.* **176**, 343–346
- Rodriguez, F. & Jolin, T. (1991) *Acta Endocrinol. (Copenhagen)* **125**, 186–191
- Slein, M. W. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 117–123, Academic Press, New York and London
- Stansbie, D., Brownsey, R. W., Crettaz, M. & Denton, R. M. (1976) *Biochem. J.* **160**, 413–416
- Tan, M. H., Sata, T. & Havel, R. J. (1977) *J. Lipid Res.* **18**, 363–370
- Tedstone, A. E., Ilic, V. & Williamson, D. H. (1991) *Proc. Nutr. Soc.* **50**, 39A
- Tepperman, J. & Tepperman, H. M. (1958) *Am. J. Physiol.* **193**, 55–64
- Williamson, D. H. (1990) in *The Control of Body Fat Content* (Forbes, J. M. & Hervey, G. R., eds.), pp. 43–61, Smith-Gordon, London
- Williamson, D. H., Mellanby, J. & Krebs, H. A. (1962) *Biochem. J.* **82**, 90–96
- Wollenberger, A., Ristau, O. & Schoffa, G. (1960) *Pflügers Arch. Gesamte Physiol. Menschen Tiere* **270**, 399–412

Received 2 October 1991/6 February 1992; accepted 13 February 1992