Lipid metabolism in the obese Zucker rat

Disposal of an oral [^{14}C]triolein load and lipoprotein lipase activity

Francisco J. LÓPEZ-SORIANO, Neus CARBÓ and Josep M. ARGILÉS*

Departament de Bioquímica i Fisiologia, Facultat de Biologia, Universitat de Barcelona, Diagonal 645, 08071-Barcelona, Spain

Oxidation *in vivo* of [^{14}C]triolein to $^{14}\text{CO}_2$ was significantly lower in obese (*fa/fa*) Zucker rats as compared with their lean (+/?) controls. In response to a 24 h starvation period, both lean and obese rats showed an enhanced rate of [^{14}C]triolein oxidation. There were, however, no changes in the rate of intestinal absorption of [^{14}C]triolein between the lean and obese animals. Conversely, the total tissular [^{14}C]lipid accumulation was significantly higher in white adipose tissue, carcass and plasma in the obese animals, whereas that of brown adipose tissue was lower. This was associated with a marked hyperinsulinaemia and hypertriglyceridaemia in the *fa/fa* animals. Starvation dramatically decreased [^{14}C]lipid accumulation in white adipose tissue of the lean Zucker rats, but had no effect in the obese rats. The lipogenic rate of the obese rats was significantly higher than that of lean rats in liver, white adipose tissue, skeletal muscle and carcass. Lipoprotein lipase activity (per g of tissue) was significantly lower in both white and brown adipose tissue of obese versus lean rats; however, total activity was higher in both tissues. Starvation significantly lowered perigenital-adipose-tissue lipoprotein lipase activity in the lean groups, and had no effect in the obese ones. These results demonstrate that the tissue capacity of exogenous lipid uptake is involved, but cannot be the only factor influencing the maintenance of obesity in these animals. Thus, in the adult *fa/fa* rat, the large increase in obesity is not solely dependent on a deviation of energy-producing substrate metabolism towards the storage of lipids in white fat. Other factors, such as a low rate of oxidation, a high lipogenic rate and decreased brown-adipose-tissue activity are involved in the perseverance of the obesity syndrome.

INTRODUCTION

The obesity of the fatty rat is inherited as an autosomal recessive gene mutation (Zucker & Zucker, 1961) which has proved to be a reasonable animal model for early-onset human hypertrophic-hyperplastic obesity (Johnson *et al.*, 1978) and is characterized by the accumulation of excessive quantities of fat in both subcutaneous and intraperitoneal fat stores (Zucker & Zucker, 1963; Bray & York, 1971; Argilés, 1989). This is mainly due to an adipocyte enlargement, which may be dependent on lipoprotein lipase activity (Hartman, 1981). Indeed, the activity of the enzyme, which hydrolyses plasma lipoprotein triacylglycerols before their entry into extrahepatic tissues (Robinson, 1963), may play a significant role in the regulation of lipid deposition (Garfinkel *et al.*, 1967; Cryer *et al.*, 1976; Greenwood & Vasselli, 1981). In the young genetically obese rat, elevated lipoprotein lipase activity is associated with enlarged fat-cell size (Boulangé *et al.*, 1978) and increased carcass lipid content (Bell & Stern, 1977). Although the fate of dietary carbon in the intact animal has been previously studied (Thenen & Mayer, 1977; Dunn & Hartsook, 1980; Haggarty *et al.*, 1986), no attempt has been made to relate dietary lipid uptake with lipoprotein lipase activities. The influence of fasting on several metabolic parameters in this animal model has been previously reported. Fasting resulted in increased plasma non-esterified fatty acids and glycerol (Zucker, 1972) and decreased plasma insulin levels (Zucker & Antoniadis, 1972). Triscari *et al.* (1980) concluded that fasting results in qualitatively similar metabolic and hormonal changes in both lean and obese rats, but that abnormalities in carbohydrate and lipid metabolism persist in obese rats even after a 12-day starvation period. The aims of the present study were to relate lipid deposition (lipid uptake and lipid synthesis *de novo*) to lipoprotein lipase activities and to examine whether starvation (24 h) altered the disposal of an oral

load of [^{14}C]triolein between tissue accumulation and production of $^{14}\text{CO}_2$ in lean (+/?) and obese (*fa/fa*) Zucker rats.

EXPERIMENTAL

Animals

Female lean (+/?) and obese (*fa/fa*) Zucker rats, aged 12 weeks, were fed *ad libitum* on a chow diet (Panlab, Barcelona, Spain) consisting of (by wt.) 54% carbohydrate, 17% protein and 5% fat (the residue was non-digestible material), with free access to drinking water, and were kept in polypropylene cages fitted with wire-mesh bottoms at an ambient temperature of $22 \pm 2^\circ\text{C}$ with a 12 h-light/12 h-dark cycle (lights on from 08:00 h). Four groups of Zucker rats were studied. Groups I and II were fed and starved (24 h) lean (+/?) respectively. Groups III and IV were fed and starved (24 h) obese (*fa/fa*) respectively.

Biochemicals and radioactive compounds

All enzymes and coenzymes were purchased from either Boehringer Mannheim S.A. (Barcelona, Spain) or Sigma Chemical Co. (St. Louis, MO, U.S.A.). $^3\text{H}_2\text{O}$, [^{14}C]triolein (glycerol tri[^{14}C]oleate) and glycerol tri[9,10(n)- ^3H]oleate were purchased from Amersham International (Amersham, Bucks., U.K.).

Measurement of lipid oxidation and tissue lipid accumulation

The metabolic fate of an orally administered [^{14}C]lipid load was examined as described by Oller do Nascimento & Williamson (1986). About 0.7 g (0.33 μCi) of [^{14}C]triolein per rat was given enterally by gastric intubation, without anaesthetic but with minimal stress to the animal. Expired CO_2 was then collected every 60 min for 5 h by absorption in Lumasorb (Lumac, Landgraaf, Holland), and the rate of $^{14}\text{CO}_2$ production was estimated

* To whom correspondence should be addressed.

by counting radioactivity in a sample of Lumasorb. After the collection period, the animals were killed. Arterial blood was collected in a heparinized syringe. The gastrointestinal tract (plus contents) was homogenized in 150 ml of 3% (w/v) HClO_4 . Samples were taken of liver, perigenital adipose tissue, interscapular brown adipose tissue, hind-leg muscle (consisting of rectus femoris, vastus medialis and fasciae latae) and carcass. Carcass (minus liver and intestinal tract) was finely minced with an electric blender. Samples of tissues and plasma were saponified and the lipid was extracted (Stansbie *et al.*, 1976). The extracted fatty acids were dissolved in 8 ml of liquid-scintillation fluid for determination of [^{14}C]lipid formation. The amount of lipid extracted was determined gravimetrically. [$1\text{-}^{14}\text{C}$]Triolein absorption was calculated by subtracting total gastrointestinal radioactivity from that administered.

Blood metabolites and plasma insulin

Whole-blood glucose was determined by the method of Slein (1963) and lactate by the method of Hohorst (1963). Acetoacetate and 3-hydroxybutyrate were determined fluorimetrically by the method of Williamson *et al.* (1962). Plasma insulin was determined by radioimmunoassay, with a rat insulin standard (Albano *et al.*, 1972). Plasma triacylglycerols were measured by the method of Eggstein & Kreutz (1966). Plasma glycerol was measured by the method of Garland & Randle (1962), and plasma non-esterified fatty acids were measured by the method of Shimizu *et al.* (1979).

Lipoprotein lipase

Perigenital-adipose-tissue, heart and brown-adipose-tissue lipoprotein lipase activities were measured by a modification of the technique of Nilsson-Ehle & Ekman (1977). Tissue samples were dried to a powder with acetone/ether and then resolubilized and used in an assay system containing [^3H]triolein as substrate; [^3H]fatty acids released after a 60 min incubation period were extracted and determined by the method of Nilsson-Ehle & Schotz (1976). Lipoprotein lipase activity is expressed as nmol of fatty acid released/min per mg of acetone-dried powder.

Lipogenic rate *in vivo*

An intragastric glucose load (4 mmol) to stimulate lipogenesis (Agius & Williamson, 1980) was given 90 min before the death of the animal. Lipogenesis was measured by using $^3\text{H}_2\text{O}$ as previously described (Robinson *et al.*, 1978). At 1 h before being killed, animals were injected with 0.3 ml (3 mCi) of $^3\text{H}_2\text{O}$ intraperitoneally; 5 min before death the animals were anaesthetized with pentobarbital (60 mg/kg body wt.). Tissues were saponified and fatty acids extracted by the method of Stansbie *et al.* (1976).

RESULTS AND DISCUSSION

Food intake, body weight and body fat content

The food intake of the obese animals was higher than that of the lean ones (Table 1). This parameter has been claimed to be a major factor in the deposition of excess lipid in the *fa/fa* animals (Bray & York, 1971; Bray *et al.*, 1973). However, it is not solely responsible for the adiposity, since obese rats pair-fed together with lean rats still gain excess quantities of lipid (Bray *et al.*, 1973; Pullar & Webster, 1974). The tissue fat content of the obese animals was significantly higher in all the tissues studied, except for white adipose tissue (Table 1). In response to starvation there was an increase in the hepatic lipid percentage in the lean animals, but this was not observed in the obese ones (Table 1).

Absorption, oxidation and tissue accumulation of an oral lipid load

There were no marked differences in intestinal absorption of the tracer between the lean and obese groups (Table 2), nor did starvation affect this parameter. However, to correct for differences in absorption between experimental groups, all results for $^{14}\text{CO}_2$ production and [^{14}C]lipid accumulation in tissues are expressed as a percentage of the absorbed dose (see Argilés *et al.*, 1989). The oxidation of [^{14}C]triolein to $^{14}\text{CO}_2$ was significantly lower in the obese animals. Haggarty *et al.* (1986) found similar oxidation for [$1\text{-}^{14}\text{C}$]palmitic acid in obese Zucker rats. Our

Table 1. Food intake, body weight and body fat content in fed and starved Zucker rats

For further details, see the Experimental section. Carcass mass is body mass minus liver and gastrointestinal-tract mass. The results are mean values $\pm$ S.E.M. for four different animals. Values that are significantly different by Student's *t* test from those of the lean groups are indicated by: $\dagger P < 0.05$, $\dagger\dagger P < 0.01$, $\dagger\dagger\dagger P < 0.001$. Those that are significantly different from the corresponding fed group are shown by: $** P < 0.01$.

	State of rats			
	Lean (+/?)		Obese (<i>fa/fa</i>)	
	Fed	Starved	Fed	Starved
Food intake (g/day)	18.0 $\pm$ 0.61	—	29.8 $\pm$ 1.59 $\dagger\dagger\dagger$	—
Body wt. (g)	206 $\pm$ 4.00	211 $\pm$ 5.70	413 $\pm$ 25.3 $\dagger\dagger\dagger$	437 $\pm$ 32.0 $\dagger\dagger\dagger$
Tissue mass (g)				
Carcass	172 $\pm$ 8.40	172 $\pm$ 3.70	342 $\pm$ 22.2 $\dagger\dagger\dagger$	364 $\pm$ 28.4 $\dagger\dagger\dagger$
Liver	7.55 $\pm$ 0.17	6.53 $\pm$ 0.10	14.7 $\pm$ 0.95 $\dagger\dagger\dagger$	12.3 $\pm$ 1.73 $\dagger$
Fat content (%)				
Carcass	9.87 $\pm$ 0.53	9.13 $\pm$ 0.38	47.4 $\pm$ 2.62 $\dagger\dagger\dagger$	45.3 $\pm$ 1.77 $\dagger\dagger\dagger$
Liver	4.22 $\pm$ 0.30	6.26 $\pm$ 0.76 $**$	12.4 $\pm$ 0.32 $\dagger\dagger\dagger$	13.9 $\pm$ 2.41 $\dagger$
Skeletal muscle	2.19 $\pm$ 0.27	3.67 $\pm$ 0.81	11.9 $\pm$ 0.52 $\dagger\dagger\dagger$	12.0 $\pm$ 1.67 $\dagger\dagger$
White adipose tissue	84.6 $\pm$ 0.46	83.5 $\pm$ 2.84	92.8 $\pm$ 4.16	92.4 $\pm$ 3.40
Brown adipose tissue	52.1 $\pm$ 4.84	49.7 $\pm$ 1.45	89.3 $\pm$ 2.17 $\dagger\dagger\dagger$	86.5 $\pm$ 2.39 $\dagger\dagger\dagger$
Plasma	2.50 $\pm$ 0.73	1.54 $\pm$ 0.06	2.67 $\pm$ 0.27	2.36 $\pm$ 0.33 $\dagger$

Table 2. Absorption, whole-body oxidation and metabolic fate of orally administered [1-¹⁴C]triolein in lean and obese Zucker rats

For full details see the Experimental section. ¹⁴CO₂ production was calculated during the course of the 5 h after triolein administration and expressed as (a) % of absorbed dose/5 h per g or per ml or (b) % of absorbed dose/5 h per total tissue. The results are mean values ± S.E.M. for four different animals. Total incorporations into skeletal muscle (Glore *et al.*, 1984), brown adipose tissue (Smith & Roberts, 1964) and plasma (Chaves & Herrera, 1980) were calculated by assuming previously reported values. Total estimated adipose-tissue accumulation was calculated by assuming that all carcass fat corresponded to adipose tissue. Values that are significantly different by Student's *t* test from their corresponding lean values are indicated by: † *P* < 0.05, †† *P* < 0.01, ††† *P* < 0.001. Values that are significantly different between fed and starved groups are indicated by * *P* < 0.05, ** *P* < 0.01

Parameter		State of rats			
		Lean (+/?)		Obese (fa/fa)	
		Fed	Starved	Fed	Starved
Absorption of [¹⁴ C]lipid (% of administered dose)		79.0 ± 6.21	76.4 ± 7.45	77.7 ± 4.26	73.9 ± 3.84
¹⁴ CO ₂ production (% of absorbed dose)		23.0 ± 1.52	30.8 ± 2.56*	7.52 ± 1.24†††	14.4 ± 1.32††**
Tissue [¹⁴ C]lipid accumulation					
Carcass	<i>a</i>	0.157 ± 0.026	0.125 ± 0.013	0.140 ± 0.016	0.103 ± 0.010
	<i>b</i>	27.5 ± 5.35	22.1 ± 2.06	46.9 ± 2.47†	36.8 ± 2.84*††
Skeletal muscle	<i>a</i>	0.047 ± 0.007	0.065 ± 0.011	0.055 ± 0.004	0.055 ± 0.005
	<i>b</i>	6.21 ± 0.99	7.40 ± 1.29	4.57 ± 0.30	4.56 ± 0.41†
Liver	<i>a</i>	0.740 ± 0.097	0.715 ± 0.159	0.542 ± 0.044	0.532 ± 0.103
	<i>b</i>	4.56 ± 1.47	3.94 ± 1.54	7.94 ± 0.82	6.14 ± 0.88
White adipose tissue	<i>a</i>	0.528 ± 0.115	0.089 ± 0.018**	0.182 ± 0.024†	0.128 ± 0.019
	<i>b</i>	10.9 ± 2.87	1.68 ± 0.37*	31.3 ± 4.10††	22.3 ± 3.32†††
Brown adipose tissue	<i>a</i>	2.967 ± 0.535	2.973 ± 0.219	0.193 ± 0.011††	0.184 ± 0.036†††
	<i>b</i>	4.14 ± 0.75	3.98 ± 0.26	0.53 ± 0.03†	0.51 ± 0.08†††
Plasma	<i>a</i>	0.097 ± 0.014	0.064 ± 0.010	0.349 ± 0.090†	0.456 ± 0.107†
	<i>b</i>	0.61 ± 0.09	0.38 ± 0.06*	3.58 ± 1.01†	5.70 ± 1.34††

observations contrast with studies where the oxidation of amino acid carbon was increased or those where the oxidation of glucose was not altered (Haggarty *et al.*, 1986). In response to starvation (24 h), both lean (34%) and obese rats (91%) significantly increased the oxidation of the tracer (Table 2).

The increase in ¹⁴CO₂ production was accompanied by a decrease in [¹⁴C]lipid uptake by white adipose tissue of the lean rats, without any other changes in the rest of the tissues considered (Table 2). The fa/fa rats showed no changes in [¹⁴C]lipid uptake as a result of starvation. These had a higher lipid deposition in white adipose tissue in the fed state, as compared with the lean ones (Table 2), but a lower incorporation in brown adipose tissue which persisted in the starved state. They also showed higher levels of circulating [¹⁴C]lipid.

The total estimated adipose tissue incorporation, assuming that all carcass fat corresponded to adipose tissue, was significantly higher in the obese animals than in the lean ones. Starvation caused a dramatic decrease (6.5-fold) in this parameter in the lean animals, but did not alter it in the obese group.

The recovery of the absorbed radioactivity (¹⁴CO₂ + [¹⁴C]lipid in liver + [¹⁴C]lipid in carcass) ranged from 56% to 64% and did not differ significantly within each experimental group of rats. The radioactivity not recovered was likely to be present in water-soluble compounds (Leyton *et al.*, 1987).

Blood metabolites

Although starvation (24 h) induced a decrease in glycaemia in the lean rats (Table 3), it did not affect the circulating concentration of glucose in the obese animals. These were normoglycaemic in both the fed and starved states, despite the hyperinsulinaemia present in these animals (Table 3). Indeed, there was a 4-fold decrease in the insulin/glucose ratio in the lean

rats but no significant changes were observed in the obese ones (Table 3). Pancreatic islets from obese rats are characterized by hypertrophy and hyperplasia of the β-cell, which result in a major hypersecretion of insulin with subsequent hyperinsulinaemia (Zucker & Antoniadis, 1972), which coexists with glucose intolerance, denoting a state of resistance to insulin (Leturque *et al.*, 1984). Starvation induced a rise in the concentrations of acetoacetate and 3-hydroxybutyrate in both lean and obese groups (Table 3).

Lipaemia and lipoprotein lipase

Obese Zucker rats showed a very high concentration of circulating triacylglycerols, but a normal concentration of non-esterified fatty acids (Table 4). Since the [¹⁴C]lipid accumulation (expressed as % of absorbed dose/5 h per g) was lower in the white and brown adipose tissue of obese rats in the fed state (Table 2), we decided to assay the activity of lipoprotein lipase, the enzyme responsible for the hydrolysis of plasma triacylglycerols (see Robinson, 1970). Although obese rats showed a lower lipoprotein lipase activity (expressed per g of tissue) in both white (79%) and brown adipose tissue (83%) (Table 4), total estimated whole-tissue lipoprotein lipase activity was higher in adipose tissue of the obese animals (Table 5). These results do not agree with those of Maggio & Greenwood (1982) and Horwitz *et al.* (1984), who showed that white-adipose-tissue lipoprotein lipase activity was higher (even when expressed per mg of protein or per g of tissue) in the obese animals. In contrast, other studies have reported similar values to those found here (Bertin *et al.*, 1985; Dugail *et al.*, 1988). In the obese animals, because of their hyperinsulinaemia, an increased lipoprotein lipase activity could be expected from the previously documented regulator role of insulin on lipoprotein lipase synthesis in adipose

Table 3. Blood metabolite and insulin concentrations in lean and obese Zucker rats

For full details see the Experimental section. The results are mean values $\pm$ S.E.M. of four different animals. Blood glucose and lactate concentrations are expressed as $\mu\text{mol/ml}$. Ketone-body concentrations are expressed as nmol/ml . Insulin concentration is expressed as $\mu\text{units/ml}$ of plasma. Values that are significantly different by Student's *t* test from lean values are indicated by: $\dagger P < 0.05$, $\dagger\dagger P < 0.01$. Those that are significantly different from the corresponding fed group are shown by $* P < 0.05$, $** P < 0.01$, $*** P < 0.001$.

Parameter	State of rats			
	Lean (+/?)		Obese (fa/fa)	
	Fed	Starved	Fed	Starved
Glucose	5.71 $\pm$ 0.40	4.01 $\pm$ 0.31**	4.54 $\pm$ 0.5	5.10 $\pm$ 0.40
Lactate	1.90 $\pm$ 0.41	1.56 $\pm$ 0.06	3.14 $\pm$ 0.30 $\dagger$	1.43 $\pm$ 0.30**
Insulin	36.9 $\pm$ 4.60	6.50 $\pm$ 1.10***	304 $\pm$ 62.0 $\dagger\dagger$	220 $\pm$ 93.0
Insulin/glucose ratio	6.64 $\pm$ 1.19	1.62 $\pm$ 0.28**	70.4 $\pm$ 21.4 $\dagger\dagger$	41.3 $\pm$ 15.7
Acetoacetate	42.6 $\pm$ 2.76	461 $\pm$ 111**	41.0 $\pm$ 4.65	374 $\pm$ 99.1*
3-Hydroxybutyrate	65.7 $\pm$ 3.15	999 $\pm$ 297*	65.5 $\pm$ 4.88	668 $\pm$ 127**
Total ketone bodies	108 $\pm$ 5.65	1460 $\pm$ 405*	106 $\pm$ 9.02	1040 $\pm$ 219**
3-Hydroxybutyrate/acetoacetate ratio	1.55 $\pm$ 0.06	2.12 $\pm$ 0.20*	1.64 $\pm$ 0.13	1.88 $\pm$ 0.22

Table 4. Tissue lipoprotein lipase (LPL) activity and plasma triacylglycerols, non-esterified fatty acids and glycerol concentrations in lean and obese Zucker rats

For full details see the Experimental section. The results are mean values $\pm$ S.E.M. for four different animals. LPL activity is expressed as nmol of fatty acid released/min per mg of acetone-dried tissue. Values that are significantly different by Student's *t* test from those of the lean groups are indicated by: $\dagger P < 0.05$, $\dagger\dagger P < 0.01$, $\dagger\dagger\dagger P < 0.001$. Those that are significantly different from the corresponding fed group are shown by: $* P < 0.05$, $*** P < 0.001$.

Parameter	State of rats			
	Lean (+/?)		Obese (fa/fa)	
	Fed	Starved	Fed	Starved
LPL activity				
White adipose tissue	0.322 $\pm$ 0.036	0.079 $\pm$ 0.022***	0.069 $\pm$ 0.013 $\dagger\dagger\dagger$	0.067 $\pm$ 0.025
Brown adipose tissue	0.397 $\pm$ 0.060	0.409 $\pm$ 0.113	0.067 $\pm$ 0.020 $\dagger\dagger$	0.081 $\pm$ 0.040 $\dagger$
Heart	0.241 $\pm$ 0.063	0.189 $\pm$ 0.032	0.134 $\pm$ 0.030	0.121 $\pm$ 0.038
Plasma triacylglycerols (mg/100 ml)	71.8 $\pm$ 5.26	82.4 $\pm$ 10.2	282 $\pm$ 59.2 $\dagger\dagger$	286 $\pm$ 51.2 $\dagger\dagger$
Plasma non-esterified fatty acids (n-equiv./ml)	549 $\pm$ 71.0	1007 $\pm$ 277	514 $\pm$ 143	990 $\pm$ 78.0*
Plasma glycerol (nmol/ml)	101 $\pm$ 29.9	143 $\pm$ 20.1	303 $\pm$ 75.8 $\dagger$	326 $\pm$ 121

Table 5. Total tissue activity of lipoprotein lipase (LPL) and the total tissue accumulation of [^{14}C]lipid in white and brown adipose tissue of lean (+/?) and obese (fa/fa) Zucker rats

The results for tissue accumulation of [^{14}C]lipid (% of absorbed dose) from Table 2 and for LPL (nmol of fatty acid released/min) from Table 4 have been calculated on a total-tissue basis. The white-adipose-tissue mass was calculated from the carcass fat content, taking into account the lipid composition of this tissue in each experimental group; the brown-adipose-tissue mass was calculated by assuming the value of 0.67% of body weight (Smith & Roberts, 1964). The results are means values for four rats.

State of rats	White adipose tissue		Brown adipose tissue	
	LPL	[^{14}C]Lipid	LPL	[^{14}C]Lipid
Lean (+/?)				
Fed	90.8	10.6	54.8	4.09
Starved	20.8	1.67	57.8	4.20
Obese (fa/fa)				
Fed	168	31.7	18.5	0.53
Starved	167	22.8	23.7	0.54

tissue of normal rats (Speake *et al.*, 1985; Vydelingum *et al.*, 1983). Starvation caused a sharp decrease in white-adipose-tissue lipoprotein lipase activity in lean rats (4-fold), but had no effect in the obese animals (Tables 4 and 5). A similar observation had previously been made by Maggio & Greenwood (1982), who found that fasting did not decrease white-adipose-tissue lipoprotein lipase activity or lipid uptake into the tissue in the fa/fa animals. The higher total lipoprotein lipase activity found in the white adipose tissue of the obese animals (Table 5) does not agree with either the high levels of circulating [^{14}C]lipid (Table 2) or the high plasma triacylglycerol concentration found in the fa/fa rats (Table 4). It must be pointed out, however, that the low rate of oxidation of the tracer found in the obese animals (Table 2) would certainly tend to increase the levels of circulating [^{14}C]lipid found in these animals. As for the hyperlipidaemia, it can be accounted for by taking into consideration the high rate of hepatic lipogenesis (Table 6), linked to an enhanced overproduction of very-low-density lipoproteins (Azain *et al.*, 1985). The low lipoprotein lipase activity found in brown adipose tissue of obese rats can be related to the lower [^{14}C]lipid uptake found (Table 2). This fact, together with a lower fatty acid oxidation (Stern *et al.*,

Table 6. Tissue lipogenesis in lean and obese Zucker rats

For further details see the Experimental section. Total skeletal-muscle (Glore *et al.*, 1984) and brown-adipose-tissue (Smith & Roberts, 1964) lipogenic rates were calculated by assuming previously reported values for total tissue mass. Total estimated adipose-tissue lipogenic rate was calculated by assuming that all carcass fat corresponded to adipose tissue. The results are mean values $\pm$ S.E.M. for four different animals. Values that are significantly different by Student's *t* test from those of the lean groups are indicated by: $\dagger P < 0.05$, $\dagger\dagger P < 0.01$, $\dagger\dagger\dagger P < 0.001$. Those that are significantly different from the corresponding fed group are shown by: $* P < 0.05$, $** P < 0.01$, $*** P < 0.001$.

State of rats	$^3\text{H}_2\text{O}$ incorporation into saponified lipid (μmol of $^3\text{H}_2\text{O}/\text{h}$ per total weight of tissue)				
	Carcass	Liver	White adipose tissue	Brown adipose tissue	Skeletal muscle
Lean (+/?)					
Fed	437 $\pm$ 61.9	61.9 $\pm$ 12.7	256 $\pm$ 64.8	37.1 $\pm$ 9.75	72.7 $\pm$ 2.29
Starved	490 $\pm$ 36.1	24.9 $\pm$ 0.79*	7.73 $\pm$ 1.10***	98.6 $\pm$ 17.2*	40.2 $\pm$ 6.88**
Obese (fa/fa)					
Fed	3591 $\pm$ 157 $\dagger\dagger\dagger$	913 $\pm$ 162 $\dagger\dagger\dagger$	7100 $\pm$ 173 $\dagger\dagger\dagger$	53.7 $\pm$ 5.44	188 $\pm$ 24.8 $\dagger\dagger$
Starved	1001 $\pm$ 153*** $\dagger$	198 $\pm$ 30.4*** $\dagger\dagger\dagger$	176 $\pm$ 37.9*** $\dagger\dagger\dagger$	13.9 $\pm$ 3.52*** $\dagger\dagger$	67.6 $\pm$ 7.51***

1984), suggests that brown adipocytes may not utilize their fat stores as readily as do the brown adipocytes from lean animals, with a consequent decrease in cell thermogenesis (Bazin *et al.*, 1983).

Tissue lipogenesis *in vivo*

The rate of lipogenesis, as measured as the incorporation of $^3\text{H}_2\text{O}$ into saponified lipid, was significantly higher in the liver (15-fold), white adipose tissue (28-fold), skeletal muscle (2.6-fold) and carcass (8.2-fold) of fed obese rats than in lean ones (Table 6). Brown-adipose-tissue lipogenesis showed no differences between groups. The effects of starvation on lipogenesis were more marked in the obese rats, with decreases in the lipogenic rate in liver (78%), white adipose tissue (97%), brown adipose tissue (74%), skeletal muscle (64%) and carcass (72%). The high rate of hepatic lipogenesis observed in the obese Zucker rat is a consequence of both high activities of lipogenic enzymes (Lavau *et al.*, 1982) and a decreased fatty acid oxidation based on the physiological inhibition by malonyl-CoA (the initial metabolite of the lipogenic pathway via acetyl-CoA carboxylase) of mitochondrial long-chain carnitine acyltransferase (EC 2.3.1.21) (Clouet *et al.*, 1985), which, in turn, is the rate-limiting enzyme of hepatic fatty acid oxidation, since it allows the entry of long-chain fatty acids into the mitochondrial compartment. This effect is reinforced by a high malonyl-CoA concentration and eventually by the low carnitine content of mitochondria (Horwitz *et al.*, 1984).

Concluding remarks

The present work confirms some of the findings by several investigators concerning the role of white-adipose-tissue lipoprotein lipase activity and lipid uptake in the maintenance of obesity in the adult obese Zucker rat. These two parameters are tightly linked as shown in Table 5, where an approximate calculation has been made involving total tissue lipoprotein lipase activity and triacylglycerol uptake. Concerning white adipose tissue, in the fed state, the fa/fa group showed a 1.9-fold increased total lipoprotein lipase activity, whereas total [^{14}C]lipid uptake was increased by nearly 3-fold (Table 5), relative to the fed lean animals. Starvation caused a decrease in the lipoprotein lipase activity of the lean animals (77%) as well as in their [^{14}C]lipid uptake (84%); however, this nutritional status had no effect on lipoprotein lipase in the obese animals and caused a decrease in lipid uptake (31%). It must be pointed out that the obese Zucker rat is hyperlipidaemic (Table 4), although white

adipose tissue seems to remove most of the dietary lipid. This can be tentatively explained by taking into account the high rate of fatty acid synthesis *de novo* that takes place in the liver of the obese animals (Table 6) and which has been demonstrated to be linked to the hypersecretion of triacylglycerol-rich lipoproteins by the liver (Azain *et al.*, 1985). In brown adipose tissue, total lipoprotein lipase activity and [^{14}C]lipid uptake were higher in the lean animals (2.7- and 7.9-fold respectively). Total brown-adipose-tissue lipoprotein lipase activity and [^{14}C]lipid uptake were insensitive to starvation in both lean and obese groups (Table 5).

It must be pointed out that both white-adipose-tissue lipoprotein lipase activity and [^{14}C]lipid uptake (per g of tissue) are actually lower in the adult obese Zucker rat than in the lean one, this fact supporting the view that, in spite of being partially responsible for the maintenance of the obesity, these parameters alone cannot explain the large weight and lipid content of the fa/fa rats. A decreased exogenous [^{14}C]lipid oxidation observed in our study (Table 2), also reported by other authors (Haggarty *et al.*, 1986), could partially explain a 'pulling of substrate preferentially into adipose tissue, secondarily depriving lean body tissues' (Cleary *et al.*, 1980). Conversely, it is quite clear that the hyperphagia alone cannot be taken as a factor contributing to the maintenance of the obesity syndrome, since pair-feeding of the obese rats does not prevent the development of white adipose tissue (Cleary *et al.*, 1980). In addition to a decreased lipid oxidation, tissue lipogenesis (Table 6), which is linked to hyperinsulinaemia, must be another factor contributing to the development and maintenance of obesity in the Zucker rats.

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REFERENCES

- Agius, L. & Williamson, D. H. (1980) *Biochem. J.* **190**, 477–480
- Albano, J. D. M., Ekins, R. P., Maritz, G. & Turner, R. (1972) *Acta Endocrinol. (Copenhagen)* **70**, 487–509
- Argilés, J. M. (1989) *Prog. Lipid Res.* **28**, 53–66
- Argilés, J. M., López-Soriano, F. J., Evans, R. D. & Williamson, D. H. (1989) *Biochem. J.* **259**, 673–678
- Azain, M. J., Fukuda, N., Chao, F.-F., Yamamoto, M. & Ontko, J. A. (1985) *J. Biol. Chem.* **260**, 174–181
- Bazin, R., Lavau, M. & Guichard, C. (1983) *Biochem. J.* **216**, 543–549

- Bell, G. E. & Stern, J. S. (1977) *Growth* **41**, 63–80
- Bertin, R., Triconnet, M. & Portet, R. (1985) *Comp. Biochem. Physiol.* **81B**, 797–801
- Boulangé, A., Planche, E., de Gasquet, P. & Leliepure, X. (1978) *Int. J. Obes.* **2**, 354
- Bray, G. A. & York, D. A. (1971) *Physiol. Rev.* **51**, 598–646
- Bray, G. A., York, D. A. & Swerdloff, R. S. (1973) *Metab. Clin. Exp.* **22**, 435–442
- Chaves, J. M. & Herrera, E. (1980) *Biol. Neonate* **37**, 172–179
- Cleary, M. P., Vaselli, J. R. & Greenwood, M. R. C. (1980) *Am. J. Physiol.* **238**, E284–E292
- Clouet, P., Henninger, C., Pascal, M. & Bézard, J. (1985) *FEBS Lett.* **182**, 331–334
- Cryer, A., Riley, E., Williams, E. R. & Robinson, D. S. (1976) *Clin. Sci. Mol. Med.* **50**, 213–221
- Dugail, I., Quignard-Boulange, A., Brigant, L., Etienne, J., Noe, L. & Lavau, M. (1988) *Biochem. J.* **249**, 45–49
- Dunn, M. A. & Hartsook, E. W. (1980) *J. Nutr.* **110**, 1865–1879
- Eggstein, M. & Kreutz, F. H. (1966) *Klin. Wochenschr.* **44**, 262–267
- Garfinkel, A. S., Baker, N. & Schotz, M. C. (1967) *J. Lipid Res.* **8**, 274–280
- Garland, P. B. & Randle, P. J. (1962) *Nature (London)* **196**, 987–988
- Glore, S. R., Layman, D. K. & Bechtel, P. J. (1984) *Nutr. Rep. Int.* **29**, 797–805
- Greenwood, M. C. R. & Vasselli, J. R. (1981) in *Nutritional Factors: Modulating Effects on Metabolic Processes* (Beers, R. F. & Bassett, E. G., eds.), pp. 323–335, Raven Press, New York
- Haggarty, P., Reeds, P. J., Fletcher, J. M. & Wahle, K. W. J. (1986) *Biochem. J.* **235**, 323–327
- Hartman, A. D. (1981) *Am. J. Physiol.* **241**, E108–E115
- Hohorst, H. J. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 215–219, Academic Press, New York and London
- Horwitz, B. A., Inokuchi, T., Wickler, S. J. & Stern, J. S. (1984) *Metab. Clin. Exp.* **33**, 354–357
- Johnson, P. R., Stern, J. S., Greenwood, M. R. C., Zucker, L. M. & Hirsch, J. (1978) *Metab. Clin. Exp.* **27**, 1941–1954
- Lavau, M., Bazin, R., Karaoghlanian, Z. & Guichard, C. (1982) *Biochem. J.* **204**, 503–507
- Leturque, A., Burnol, A. F., Ferré, P. & Girard, J. (1984) *Am. J. Physiol.* **246**, E25–E31
- Leyton, J., Drury, P. J. & Crawford, M. A. (1987) *Br. J. Nutr.* **57**, 383–393
- Maggio, C. A. & Greenwood, M. R. C. (1982) *Physiol. Behav.* **29**, 1147–1152
- Nilsson-Ehle, P. & Ekman, R. (1977) *Artery* **3**, 197–209
- Nilsson-Ehle, P. & Schotz, M. C. (1976) *J. Lipid Res.* **17**, 536–541
- Oller do Nascimento, C. M. & Williamson, D. H. (1986) *Biochem. J.* **239**, 233–236
- Pullar, J. D. & Webster, B. (1974) *Br. J. Nutr.* **31**, 377–392
- Robinson, A. M., Girard, J. R. & Williamson, D. H. (1978) *Biochem. J.* **176**, 343–346
- Robinson, D. S. (1963) *Adv. Lipid. Res.* **1**, 133–182
- Robinson, D. S. (1970) *Compr. Biochem.* **18**, 51–116
- Shimizu, S., Inoue, K., Tani, Y. & Yamada, H. (1979) *Anal. Biochem.* **98**, 341–345
- Slein, M. W. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 117–123, Academic Press, New York and London
- Smith, R. E. & Roberts, J. C. (1964) *Am. J. Physiol.* **206**, 143–148
- Speake, B. K., Parkinson, C. & Robinson, D. S. (1985) *Horm. Metab. Res.* **17**, 637–640
- Stansbie, D., Brownsey, R. W., Crettaz, M. & Denton, R. M. (1976) *Biochem. J.* **160**, 413–416
- Stern, J. S., Inokuchi, T., Castonguay, T. W., Wickler, S. J. & Horwitz, B. A. (1984) *Am. J. Physiol.* **247**, R918–R926
- Thenen, S. W. & Mayer, J. (1977) *J. Nutr.* **107**, 320–329
- Triscari, J., Bryce, G. F. & Sullivan, A. C. (1980) *Metab. Clin. Exp.* **29**, 377–385
- Vydelingum, N., Drake, R. L., Etienne, J. & Kissebah, A. (1983) *Am. J. Physiol.* **245**, E121–E131
- Williamson, D. H., Mellanby, J. & Krebs, H. A. (1962) *Biochem. J.* **228**, 727–733
- Zucker, L. M. (1972) *J. Lipid Res.* **13**, 234–243
- Zucker, L. M. & Antoniades, H. N. (1972) *Endocrinology (Baltimore)* **90**, 1320–1330
- Zucker, L. M. & Zucker, T. F. (1961) *J. Hered.* **52**, 275–281
- Zucker, T. F. & Zucker, L. M. (1963) *J. Nutr.* **80**, 6–19

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