The fate of ^{14}C derived from radioactively labelled dietary precursors in young rats of the Zucker strain (*Fa*/– and *fa/fa*)

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The metabolic fate of ^{14}C derived from radioactively labelled dietary precursors was determined in immature (18- and 25-day-old) lean and obese Zucker rats. This included measurement of ^{14}C incorporated into body lipid, non-essential amino acids and expired CO_2 . Before weaning (18 days) there was no phenotypic difference between the fates of [^{14}C]palmitate and [^{14}C]glucose. However, after weaning (25 days) all the precursors studied exhibited an increase in the fraction incorporated into lipid in the obese rat as compared with the lean animal. This was reflected in the fate of acetyl-CoA in the tricarboxylic acid cycle. There was little phenotypic difference in the fraction of leucine or valine catabolized. The results presented here suggest that the high rate of lipogenesis found in the obese rat is supported by carbon from all the dietary precursors studied. It is also argued that the decreased protein deposition found in the obese rat is not caused by the high rate of lipogenesis removing precursors for protein synthesis, as has been suggested elsewhere [Cleary, Vasselli & Greenwood (1980) *Am. J. Physiol.* 238, E284–E292].

INTRODUCTION

Since its identification by Zucker & Zucker (1961), the genetically obese rat of the Zucker strain has been the subject of many investigations. Male rats that are homozygous for the recessive *fa* gene (i.e. *fa/fa*) not only become obese, but also deposit protein at a slower rate than do their lean littermates (*Fa*/–), even when they are allowed to feed *ad libitum* (Pullar & Webster, 1974). This may be due to an alteration in the three-way partition of one or more of the dietary precursors between expired CO_2 and the storage of carbon in carcass protein and lipid. It has been shown previously that at 25 days *post partum* the main feature of obesity in the Zucker rat is a very high rate of lipogenesis (Haggarty, 1983). It has been suggested that this may result in channelling of carbon away from protein synthesis, thus ‘pulling substrate preferentially into adipose tissue, secondarily depriving lean body tissues’ (Cleary *et al.*, 1980). Knowledge of which precursors supply the carbon for the high rate of fat synthesis and how this affects precursors for protein synthesis is important to our understanding of the Zucker syndrome of obesity.

The fate of ^{14}C -labelled compounds has been studied *in vitro* in isolated hepatocytes (Bloxham *et al.*, 1977; McCune *et al.*, 1981; Triscari *et al.*, 1982) and in adipose tissue (Martin, 1974; Martin *et al.*, 1978; Smith & Kaplan, 1980) taken from obese and lean rats, but little work has been performed on the fate of dietary carbon in the intact animal. Notable exceptions include the work of Thenen & Mayer (1977) on glyceryl tri[^{14}C]oleate oxidation, and that of Dunn & Hartsook (1980) on the fate of amino acid carbon. However, these investigations have involved mature Zucker rats, at a stage of development when the alterations in the body composition

of the animals may have come to dominate their metabolism. The present paper reports measurements of the incorporation of carbon from various dietary precursors into expired CO_2 and stored protein and lipid in immature Zucker rats before weaning (18 days), when there is no phenotypic difference in carcass protein and little difference in lipid content, and after weaning (25 days), when there are significant phenotypic differences in both the protein and lipid contents of the carcass (Reeds *et al.*, 1982; Haggarty, 1983).

MATERIALS AND METHODS

Materials

Chemicals were purchased from either B.D.H. or Sigma Chemical Co. (both at Poole, Dorset, U.K.). The ^{14}C -labelled compounds were purchased from Amersham International (Amersham, Bucks., U.K.).

Animals

Zucker rats were obtained from the breeding colony at the Rowett Institute (Pullar & Webster, 1974). Between 16 and 18 days of age a preliminary identification of *fa/fa* rats was made on the basis of their decreased rectal temperatures (Godbole & York, 1978) and, after 21 days of age, visually. The identification was always subsequently verified by measurement of the carcass lipid content (Haggarty, 1983).

The rats were housed in groups of four in rooms at 22 °C with a 12 h-light/12 h-dark cycle. After weaning, the animals were offered, *ad libitum*, Oxoid pasteurized rat breeding diet [Oxoid Herbert C. Styles (Bewdley) Ltd., Bewdley, Worcs., U.K.]. This diet was also made freely available before weaning (21 days *post partum*).

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Experimental procedure

After the determination of phenotype, paired lean and obese rats were tube-fed with 0.5 ml of an agar suspension (5%, w/v) containing a tracer amount of the relevant labelled compound at 11:00 h. The animals were then placed in Perspex chambers and were without food or water for the duration of the experiment. Air was drawn through each chamber at the rate of 500 ml/min and the exhaust from each chamber was bubbled through two CO₂ traps arranged in series (10 ml of 2 M-NaOH in each trap). The traps were pooled and fresh ones substituted every 30 min.

At the end of the experiment the animals were removed from the chambers and killed by cervical dislocation. The stomachs were removed and the carcasses frozen and stored at -20 °C.

Rats were given the following amounts of radioisotope; 1.4 µCi of [1-¹⁴C]palmitate (5.9 mCi/mmol), or 4.1 µCi of [U-¹⁴C]glucose (5.5 mCi/mmol), or 4.1 µCi of [U-¹⁴C]lactate (5 mCi/mmol) or 4.1 µCi of [U-¹⁴C]valine (62.7 mCi/mmol), or 4.1 µCi of [U-¹⁴C]leucine (33.9 mCi/mmol).

Fate of dietary carbon

The amount of activity in expired CO₂ was determined by counting radioactivity of a sample (1 ml) of the pooled contents of CO₂ traps and summing the half-hour values. Background radioactivity was determined by counting a sample from a CO₂ trap which had room air bubbled through it at the rate of 500 ml/min for the duration of the experiment.

The frozen carcasses were freeze-dried and powdered as described by Haggarty (1983). The incorporation of ¹⁴C from labelled leucine, valine and glucose into non-essential amino acids was determined as follows. A portion of the powdered freeze-dried carcass was heated in 5.6 M-HCl at 105 °C for 24 h. The hydrolysate was then evaporated to dryness under reduced pressure, dissolved in 0.1 M-HCl, and the sample applied to a Dowex 50 column (H⁺ form) (Sigma). After the column was washed with distilled water, all amino acids were eluted from the column with 15 ml of NH₃ (20%, v/v). A sample of the effluent was taken for scintillation counting. The remaining sample was neutralized, dried under vacuum and resuspended in sodium citrate (0.2 M; pH 2.2). The amino acids were separated by ion-exchange chromatography on a column (25 cm × 0.9 cm) of LA 49 resin (Locarte Co., London W.12, U.K.). The resin was eluted with sodium citrate buffer (0.2 M; pH 4.1 for separation of leucine and pH 3.67 for the separation of valine) at 35 ml/h at 52 °C. A sample of the valine and leucine peaks was taken for determination of the radioactivity present, and the difference between the radioactive counts in all amino acids and those in the parent amino acids was taken to represent the incorporation of ¹⁴C from leucine and valine into non-essential amino acids. The recovery of amino acids, as checked with radioactively labelled standards, was over 95%.

Saponification of the freeze-dried carcass powder and determination of the label incorporation into fatty acid and cholesterol were performed as described elsewhere (Haggarty, 1983).

The stomachs were removed and weighed at various times after gastric intubation and removal of food. The stomach contents were flushed into universal bottles with

distilled water, frozen and freeze-dried to decrease the volume of fluid. The dry stomach contents were then taken up in 60% (w/v) KOH (15 ml) and transferred to round-bottomed flasks; 95% (v/v) ethanol (30 ml) was then added and the resulting suspension refluxed on a boiling-water bath for 2 h until the stomach contents were digested. A sample of the digest (1 ml) was then taken for scintillation counting to determine the amount of radioactivity still remaining in the stomach at the end of the experiment.

The dose of ¹⁴C effectively absorbed by each animal was calculated by adding the radioactivity in the expired CO₂ to that in the carcass after removal of the stomach. The radioactivity in urine and in faeces at the end of the experiments was negligible.

Scintillation counting

The radioactivity in the samples was determined by counting in a Packard Tri-Carb 460 CD liquid-scintillation spectrometer. Corrections for quenching were made by the external-standard channels-ratio method. ¹⁴C was counted with an efficiency of approx. 75%.

Statistics

Results are expressed as means values ± S.E.M. The statistical significance between means was assessed by Student's *t* test. In the Tables, * *P* < 0.05, ** *P* < 0.01 and *** *P* < 0.001; N.S. means not statistically significant.

RESULTS

Before weaning, at 18 days *post partum*, there was no significant phenotypic difference in the fate of carbon derived from either dietary glucose (Table 1) or palmitate (Table 2). However, at this age approx. 60% of the carbon from glucose was incorporated into expired CO₂, whereas only 10% of the label from palmitate was expired as CO₂. Consistent with this finding is the observation that approx. 70% of the label from palmitate was found in body lipid (Table 2), but only 1–2% of glucose carbon was incorporated into body lipid (Table 1). These findings suggest that, before weaning, oxidation is the major fate of dietary glucose, whereas dietary fatty acid is mainly incorporated into body lipid.

However, after weaning at 25 days *post partum*, there were significant phenotypic differences in the fate of carbon derived from these two precursors. Table 1 shows that the proportion of glucose carbon that was expired as CO₂ was the same in both phenotypes (approx. 60%; the same fraction as was found before weaning), but that the obese rat incorporated a greater fraction of glucose carbon into lipid at the expense of carbon incorporation into non-essential amino acids. The proportion of glucose carbon incorporated into fatty acid in the obese rat was 3 times that in the lean animal.

The fate of ¹⁴C from [1-¹⁴C]palmitate in the obese rat after weaning was remarkably similar to that found in the obese animal before weaning (Table 2). However, in the lean rat the proportion of ¹⁴C from [1-¹⁴C]palmitate which was extracted as total body lipid decreased on weaning, from approx. 70% at 18 days to 50% at 25 days. This decrease after weaning in the lean rat was balanced by an increase in both the proportion of label expired as CO₂ and the fraction unaccounted for (Table 2), whereas the increase in the proportion of fatty acid carbon

Results are expressed as percentages of the radioactive dose absorbed.

Rats	Pre-weaning (18 days)				Post-weaning (25 days)							
	Total lipid	Saponi- fiable lipid	Non- saponi- fiable lipid	Fraction unaccounted for	Total lipid	Saponi- fiable lipid	Non- saponi- fiable lipid	Fraction unaccounted for				
Lean	1.2 ± 0.15 (5)	0.8	0.4	23.3 ± 3.69 (5)	56.6 ± 3.25 (5)	18.9	1.9 ± 0.17 (6)	1.2	0.7	25.5 ± 2.78 (6)	61.5 ± 2.34 (6)	11.1
Obese	1.6 ^{N.S.} ± 0.20 (7)	1.2	0.4	21.0 ^{N.S.} ± 2.07 (7)	59.4 ^{N.S.} ± 2.50 (7)	18.0	6.2* ± 2.05 (5)	6.1	0.1	18.4* ± 1.42 (6)	61.5 ^{N.S.} ± 1.83 (6)	13.9

Tables 1-5 illustrate that the greatest proportion of label in lipid was found in the saponifiable fraction (i.e. fatty acids). When the stomach weights of both phenotypes were plotted against time, it was found that the rate of gastric emptying was not significantly different between obese and lean animals during the course of the experiment (the regression coefficients for obese and lean rats were 0.28 ± 0.024 and 0.24 ± 0.021 g/h respectively).

DISCUSSION

With the transition at weaning from the high-fat mother's milk to the low-fat solid diet, there is a marked change in the pattern of fatty acid metabolism in the lean rat. Before weaning, the main fate of dietary fatty acid is storage in both phenotypes, with approx. 10% of the ingested fatty acid being oxidized. After weaning in the lean animal, the amount of fatty acid stored is decreased and the fraction oxidized to CO_2 is doubled. However, the obese animal maintains the pre-weaning pattern of metabolism where storage is the main fate of fatty acid. These findings agree with the observation that there is no phenotypic difference in the rate of fat deposition at 18 days *post partum*, but that, after weaning at 25 days, the obese rat deposits fatty acid at a rate significantly greater than that of the lean animal (Haggarty, 1983).

Table 2. The proportional fate of ^{14}C derived from $[1-^{14}\text{C}]$ palmitic acid 3 h after gastric intubation in agar in 18- and 25-day-old obese and lean Zucker rats

Results are expressed as percentages of the radioactive dose absorbed.

Rats	Pre-weaning (18 days)					Post-weaning (25 days)		
	Total lipid	Saponifiable lipid	Non-saponifiable lipid	Expired CO_2	Fraction unaccounted for	Saponifiable lipid	Expired CO_2	Fraction unaccounted for
Lean	68.3 ± 2.64 (4)	66.7	1.6	11.7 ± 2.17 (4)	20.0	51.3 ± 3.08 (5)	20.8 ± 3.58 (6)	27.9
Obese	$72.9^{\text{N.S.}} \pm 1.67$ (4)	72.0	0.9	$9.9^{\text{N.S.}} \pm 0.90$ (4)	17.2	$80.8^{**} \pm 6.50$ (5)	$9.8^* \pm 2.39$ (5)	9.4

Table 3. The proportional fate of ^{14}C derived from $[\text{U}-^{14}\text{C}]$ lactate 2 h after gastric intubation in agar in 25-day-old obese and lean Zucker rats

Results are expressed as percentages of the radioactive dose absorbed.

Rats	Total lipid	Saponifiable lipid	Non-saponifiable lipid	Expired CO_2	Fraction unaccounted for
Lean	4.7 ± 0.87 (4)	3.9	0.8	68.0 ± 1.62 (4)	27.3
Obese	$16.8^{**} \pm 2.46$ (4)	16.1	0.7	$61.5^* \pm 2.09$ (4)	21.7

Table 4. The proportional fate of ^{14}C derived from $[\text{U}-^{14}\text{C}]$ leucine 3 h after gastric intubation in agar in 25-day-old obese and lean Zucker rats

Results are expressed as percentages of the radioactive dose absorbed.

Rats	Total lipid	Saponifiable lipid	Non-Saponifiable lipid	Total protein	Parent amino acids	Non-essential amino acids	Expired CO_2	Fraction unaccounted for
Lean	1.7 ± 0.24 (4)	1.1	0.6	82.9 ± 3.33 (4)	65.7	17.2	16.7 ± 4.09 (4)	0†
Obese	$4.2^{**} \pm 0.61$ (4)	3.5	0.7	$72.5^{\text{N.S.}} \pm 5.54$ (4)	62.2	10.3	$21.6^{\text{N.S.}} \pm 0.84$ (4)	1.7

† The calculated recovery of label in lipid, protein and expired CO_2 was 101.3% in the lean rat; therefore the fraction unaccounted for was assumed to be zero.

Table 5. The proportional fate of ^{14}C derived from $[\text{U}-^{14}\text{C}]$ valine 3 h after gastric intubation in 25-day-old obese and lean Zucker rats

Results are expressed as percentages of the radioactive dose absorbed

Rats	Total lipid	Saponifiable lipid	Non-saponifiable lipid	Total protein	Parent amino acids	Non-essential amino acids	Expired CO_2	Fraction unaccounted for
Lean	0.9 ± 0.04 (6)	0.7	0.2	70.6 ± 4.43 (6)	63.6	7.0	8.6 ± 0.65 (6)	19.9
Obese	$1.7^{***} \pm 0.12$ (6)	1.5	0.2	$80.6^{\text{N.S.}} \pm 3.8$ (6)	70.7	9.9	$16.9^{***} \pm 1.40$ (6)	0.8

In contrast with the findings with dietary fatty acid, it was observed that, before weaning, the main fate of glucose was oxidation in both phenotypes. However, as with fatty acid, there was no significant difference between phenotypes in the pattern of glucose metabolism

at this time. After weaning, oxidation remained the main fate of glucose in both phenotypes, but the fraction of glucose carbon incorporated into lipid was increased and the fraction incorporated into amino acids decreased in the obese rat when compared with the lean animal. With

leucine and valine there was a striking similarity between phenotypes in the fraction of dose remaining in the parent amino acid 3 h after intubation in the post-weaning animal. This shows that there was little phenotypic difference in the fraction of these amino acids catabolized. With all the dietary precursors studied, a greater proportion of the carbon was incorporated into lipid in the obese rat than in the lean animal after weaning. This is consistent with previous work (Haggarty, 1983), which suggests that the excessive lipid deposition found in the obese rat is mainly a consequence of an elevated rate of lipogenesis. The carbon to support this very high rate of fatty acid synthesis appears to be derived from a wide range of dietary precursors and is not specific to any type of compound. This might be expected, since the obese rat consumes 12% more food than its lean littermate at this age (Haggarty, 1983) and, given that the obese rat exhibits a decreased protein deposition and has if anything a lower metabolic rate (Pullar & Webster, 1974, 1977), this excess energy must presumably be stored as lipid. However, the rate of gastric emptying and hence supply of labelled precursors was not different between phenotypes throughout the period of measurement. This suggests that the altered partition of carbon in the tricarboxylic acid cycle of the obese rat is not simply a consequence of hyperphagia. Supporting the idea that it is not the higher food intake that drives lipogenesis in the obese animal, but that the lipogenic pathway 'pulls' carbon from the tricarboxylic acid cycle, is the observation by Cleary *et al.* (1980) that obese rats, when pair-fed to the food intake of their lean littermates from weaning until 33 weeks of age, deposit the same amount of lipid as obese rats allowed to feed *ad libitum*.

It has been suggested that an alteration in lipid metabolism causing a 'pulling of substrate preferentially into adipose tissue, secondarily depriving lean body tissues' could explain the lower rate of protein deposition found in the obese rat (Cleary *et al.*, 1980). However, although the two amino acids leucine and valine did donate more of their carbon to fatty acid in the obese than in the lean animal, there was little phenotypic difference

in the fraction of each amino acid catabolized, suggesting that protein synthesis in the obese animal is not limited by precursor supply when compared with the lean animal. This conclusion is supported by the finding that leucine and valine and most of the other amino acids are present in the blood in significantly higher concentrations in the obese rat than in the lean animal at 25 days *post partum* (Haggarty, 1983), when the defect in protein metabolism is already present (Reeds *et al.*, 1982).

The *fa* gene in the obese Zucker rat results in an increased rate of lipid deposition concomitant with a decreased rate of protein deposition. It is postulated here that these consequences of the *fa* gene are not inter-related at the level of metabolic intermediates, but are controlled at a higher level of regulation.

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REFERENCES

- Bloxham, D. P., Fitzsimons, J. T. R. & York, D. A. (1977) *Horm. Metab. Res.* **9**, 304–309
Cleary, M. P., Vasselli, J. R. & Greenwood, M. R. C. (1980) *Am. J. Physiol.* **238**, E284–E292
Dunn, M. A. & Hartsook, E. W. (1980) *J. Nutr.* **110**, 1865–1879
Godbole, V. A. & York, D. A. (1978) *Diabetologia* **14**, 191–197
Haggarty, P. (1983) Ph.D. Thesis, University of Aberdeen
Martin, R. J. (1974) *Life Sci.* **14**, 1447–1453
Martin, R. J., Stolz, D. J. & Buck, D. C. (1978) *J. Nutr.* **109**, 412–417
McCune, S. A., Durant, P. J., Jenkins, P. A. & Harris, R. A. (1981) *Metab. Clin. Exp.* **30**, 1170–1178
Pullar, J. D. & Webster, A. J. F. (1974) *Br. J. Nutr.* **31**, 377–392
Pullar, J. D. & Webster, A. J. F. (1977) *Br. J. Nutr.* **37**, 355–363
Reeds, P. J., Haggarty, P., Wahle, K. W. J. & Fletcher, J. M. (1982) *Biochem. J.* **204**, 393–398
Smith, P. A. & Kaplan, M. L. (1980) *Int. J. Biochem.* **11**, 217–228
Thenen, S. W. & Mayer, J. (1977) *J. Nutr.* **107**, 320–329
Triscari, J., Greenwood, M. R. C. & Sullivan, A. C. (1982) *Metab. Clin. Exp.* **31**, 223–228
Zucker, L. M. & Zucker, T. F. (1961) *J. Hered.* **52**, 275–281

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