Biochem. J. (1982) **204**, 393–398
Printed in Great Britain

Tissue and whole-body protein synthesis in immature Zucker rats and their relationship to protein deposition

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(*Received 12 November 1981/Accepted 9 February 1982*)

The rates of protein synthesis in skeletal muscle, intestine, liver and in the whole body of immature (18 and 25 days old) lean and obese male Zucker rats were measured. In addition, the rate of deposition of whole-body and skeletal-muscle protein over the period 16–27 days *post partum* was measured by comparative slaughter and analysis of the composition of the body. At 16 days *post partum*, lean and obese rats had similar body protein contents, but thereafter the rate of protein deposition in the body and skeletal-muscle mass was decreased in the obese rats. The decrease was particularly marked before 21 days of age, and between 23 and 27 days *post partum* the fractional rate of protein deposition was the same in lean and obese rats. Of the tissues that were studied, only skeletal muscle had a lower fractional rate of protein synthesis in the obese rats. At 18 days *post partum*, the decrease in the absolute rate of protein synthesis in skeletal muscle accounted for at least 80% of the decline in protein synthesis in the whole body. After weaning, phenotypic differences in protein synthesis were less marked than at 18 days of age, and skeletal muscle accounted for only 50% of the difference in body protein synthesis between phenotypes. The possibility that a change in the function of the adrenal cortex contributes to differences in protein metabolism between lean and obese Zucker rats is discussed.

Since its identification by Zucker & Zucker (1961), the genetically obese Zucker strain of rats has been the subject of many investigations. Male rats that are homozygous for the *fa/fa* gene not only become obese, but also deposit protein at a slower rate even when they are allowed to feed *ad libitum* (Pullar & Webster, 1974). Many of the investigations of the growth and metabolism of Zucker rats have involved sexually mature animals, at which stage of development the alterations in the body composition of the animals may have come to dominate their metabolism and to obscure thereby the essential phenotypic differences. Thus Lobley *et al.* (1978) found that, at 200g body wt., differences in the amounts of protein synthesized by male lean or fat Zucker rats appeared to be related to the differences in their body-protein mass rather than to differences in the fractional rate of protein synthesis. There is little information on the age at which differences in body protein of lean and obese Zucker rats become manifest. Body fat is increased by at least 16 days *post partum* (Bell & Stern, 1977), but there appears to be no differences between the protein mass of the two phenotypes at this age.

The present paper describes measurements of the rate of protein synthesis in the whole body as well as in some major tissues of Zucker rats at two ages (18 and 25 days), together with observations on the rate of protein deposition in the whole body and in skeletal muscle over the period 16–27 days *post partum*.

Materials and methods

Chemicals were purchased either from B.D.H. or from Sigma Chemical Co. (both at Poole, Dorset, U.K.). L-[2,6-^{3}H]Phenylalanine (TRK 552; 40–60Ci/mmol) was 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 21 days of age a preliminary identification of *fa/fa* rats was made on the basis of their rectal temperatures (Godbole *et al.*, 1978) and, after 21 days of age, visually. The identification was always subsequently checked by measurement of the body fat of the animals.

The rats were housed in groups in rooms at 22°C

0306-3283/82/050393-06$01.50/1

with a 12h-light/12h-dark cycle, and the measurements of protein synthesis were made between 11:30 and 12:15h. After weaning, the animals were offered, *ad libitum*, a commercial pelleted diet [Oxoid; Herbert C. Styles (Bewdley) Ltd., Bewdley, Worcs., U.K.]. This diet was also made freely available before weaning (21 days *post partum*), but observations of the stomach contents of 18-day-old animals suggested that they had ingested little of this diet while they were housed with their dams. At death the stomachs of all the animals contained undigested food.

Body composition

The animals were killed by cervical dislocation, the contents of the intestinal tract were removed and the animals were weighed. The carcases were freeze-dried and ground to a homogeneous powder. The nitrogen in the dried samples was measured by a micro-Kjeldahl procedure (McKenzie & Wallace, 1954). A second portion of the carcase powder was homogenized in 20vol. of 0.5M-$HClO_4$, centrifuged at 3000*g* for 15min, and the supernatant was neutralized with 2M-KOH. A portion (200μl) of the neutral supernatant was made acid with 5.6M-HCl and autoclaved for 1h at 15lb/in^2 (103kPa) to convert the creatine into creatinine. The creatinine in the sample was measured by the Jaffé reaction with alkaline picric acid (Picou *et al.*, 1976). Standards were prepared by autoclaving acid solutions of creatine. $HClO_4$ extracts of skeletal muscle, skin and gastrointestinal tract were treated in a similar manner, and the creatine contents of these constituents of the body (Table 1) together with the creatine content of the whole body were used to calculate the skeletal-muscle mass of the body (Waterlow *et al.*, 1972; Picou *et al.*, 1976).

Protein synthesis

A modification of the technique described by Garlick *et al.* (1980) was used. Animals were injected intraperitoneally with a solution of L-[2,6-^{3}H]phenylalanine (50μCi and 150μmol/ml in 0.9% NaCl; 2ml/100g body wt.). After known intervals of between 5 and 15min the animals were killed by cervical dislocation, and a blood sample was removed from the brachial artery and mixed with an equal volume of 1M-$HClO_4$ at 4°C. While the blood sample was being obtained, the abdominal cavity was opened and flushed thoroughly with 40ml of ice-cold 0.9% NaCl to remove any radioisotope that had not been absorbed. At least 80% of the intestine (excluding the stomach), the whole liver and all the muscles of one thigh were frozen with aluminium blocks pre-cooled in solid CO_2. The frozen samples were placed in pre-weighed bottles and stored at −20°C until analysed. The carcase was cooled in ice/water and frozen. The time at which each sample was obtained was noted, but never more than 1.5min elapsed between kiling the animal and freezing the carcase.

Samples of skeletal muscle (200–400mg) and liver (1–2g) were homogenized (Ystral G.m.b.H., Dottingen, Germany) in 5vol. of 0.5M-$HClO_4$ while still frozen. Samples of the intestine were allowed to warm to 4°C, and their contents were removed by flushing with ice-cold 0.9% NaCl. The sample was rapidly blotted, weighed and homogenized in 7–10vol. of 0.5M-$HClO_4$ at 4°C. Frozen carcases were freeze-dried, ground and homogenized in 15vol. of ice-cold 0.5M-$HClO_4$. The homogenates were centrifuged at 3000*g* at 4°C for 15min.

The $HClO_4$ supernatants were neutralized (pH4–6) with 2M-KOH and samples were taken for analysis of the specific radioactivity of free phenylalanine. The $HClO_4$ precipitates were washed with 0.5M-$HClO_4$ and extracted at least five times with ethanol, dried at room temperature, and weighed samples were taken for hydrolysis in 5.6M-HCl at 105°C for 24h and for nitrogen analysis. The acid hydrolysates were dried under vacuum and resuspended in sodium citrate (0.2M), pH2.2, for analysis of phenylalanine.

Labelled phenylalanine was separated by ion-exchange chromatography on a column (25cm × 0.9cm) of LA 49 resin (Locarte Co., London W.12, U.K.). The resin was eluted with sodium citrate buffer (0.2M; pH4.1) at 25ml/h at 52°C. The contents of the tubes containing radioactivity were mixed together and phenylalanine in the sample so obtained was estimated in an automatic amino acid analyser (Locarte). Contamination of the phenylalanine with tyrosine did not exceed 5%.

Radioactivity was measured by scintillation

Table 1. *Concentration of creatine (μg/mg of fresh wt.) in tissues of Zucker rats*

The creatine content was measured after its conversion into creatinine by autoclaving acid extracts of the tissues at 15lb/in^2 (103kPa) for 1h. The creatinine was then estimated from the colour formed with alkaline picric acid. Mean values ± 1 S.E.M. for the numbers of samples in parentheses are shown. There were no statistically significant phenotypic differences, and the values for lean and obese rats were combined.

Animal age	Muscle	Intestine	Skin
18 days	3.04 ± 0.13 (9)	0.44 ± 0.01 (12)	0.33 ± 0.05 (3)
25 days	3.74 ± 0.15 (9)	0.53 ± 0.05 (9)	0.39 ± 0.04 (3)

counting with NE 260 scintillant (A. and J. Beveridge, Edinburgh, Scotland, U.K.) in a Packard model 460 CD liquid-scintillation spectrometer. Corrections for quenching were made by the external-standard channels-ratio method. ^3H radioactivity was counted with an efficiency of approx. 30%.

Protein synthesis (k_s, day^{-1}) was calculated as:

$$k_s = \frac{S_b}{S_h} \times \frac{1440}{t}$$

where S_b and S_h are the specific radioactivities of protein-bound and tissue-free phenylalanine respectively and t is the time of labelling (min), varying between 10 (10 animals) and 15 (11 animals) min. S_h was calculated as a mean value for the period of labelling, taking account of its changes with time (Table 3). Total protein synthesis was calculated as $k_s \times$ protein content. In the calculations of whole-body protein synthesis, due allowance was made for the contribution of the tissue samples that had been removed for separate analyses. As recommended by Garlick *et al.* (1980), the time of labelling included the time taken to freeze the tissues.

Statistics

The statistical significance of differences between means was assessed by Student's t test, and rates of deposition of body and muscle protein were calculated by linear-regression analysis of protein content against time (Diem & Lentner, 1970). For simplicity of presentation in Tables 2 and 3, a common estimate of variance (residual standard deviation) was given, although the significance of the differences between means was assessed with the standard deviations for the individual groups.

Results

The values for body and total skeletal-muscle protein are shown in Table 2. At 16 days *post partum* there was little difference in whole-body protein between the phenotypes, but the body protein content of the phenotypes was significantly different by 25 days of age. The rates of protein deposition of the two phenotypes between 16 and 21 days *post partum* were significantly different (0.64 ± 0.04 g/day for lean rats and 0.53 ± 0.05 g/day for fat rats; $P < 0.05$), and they remained different between 23 and 27 days (0.74 ± 0.02 for lean rats and 0.65 ± 0.06 for obese rats; $P < 0.01$). Similarly there was no phenotypic difference in total skeletal-muscle protein at 16 days of age, but, by 21 days of age, the two phenotypes had significantly different amounts of protein in their skeletal-muscle mass. Although the rate of deposition of skeletal-muscle protein between 16 and 21 days was significantly lower ($P < 0.05$) in the fat rats (0.13 ± 0.04 g/day) than in the lean animals (0.22 ± 0.04 g/day), the rates were no longer significantly different between 23 and 27 days (obese 0.22 ± 0.06 g/day; lean 0.28 ± 0.08). Between 16 and 21 days of age the fractional rate of deposition of both body and skeletal-muscle protein was lower in the fat rats, but between 23 and 27 days of age the fractional rates of deposition in the two phenotypes were virtually identical (Table 6).

The specific radioactivity of free phenylalanine at different times after injection is shown in Table 3.

Table 2. *Changes in body and skeletal-muscle protein with age*

Carcases were prepared for analysis as described in the Materials and methods section. Total body protein was calculated as body nitrogen $\times$ 6.25. Total muscle protein was calculated from the body creatine content (adjusted for the contributions of skin and intestine to body creatine) and the creatine concentration in samples of mixed thigh muscle. In so doing it was assumed that the creatine concentration in muscle varied in a linear fashion between 16 and 27 days of age. Numbers of observations are given in parentheses. The significances of phenotypic differences are indicated: $^*P < 0.05$; $^{**}P < 0.01$. Abbreviation: RSD, residual standard deviation.

Age (days)	Body protein (g)		Muscle protein (g)	
	Lean	Obese	Lean	Obese
16	4.35 (5)	4.51 (3)	1.67 (4)	1.80 (4)
18	5.64 (12)	5.65 (11)	2.20 (12)	2.14 (11)
21	7.56 (5)	7.12 (5)	2.83 (5)	2.46 (4)*
23	8.25 (3)	7.27 (3)*	3.05 (3)	2.57 (3)*
25	9.73 (11)	8.74 (7)**	3.29 (11)	2.88 (9)**
27	11.05 (5)	9.92 (3)**	4.10 (4)	3.43 (3)*
RSD	0.142	0.117	0.183	0.188

Table 3. *Changes with time of the specific radioactivity (d.p.m./nmol) of free phenylalanine*

Animals (approx. 35 and 55g body wt) were injected intraperitoneally with 100μCi (300μmol) of [^3H]phenylalanine (actual sp. radioactivity 850 d.p.m./nmol)/100g body wt. The tissues were prepared for analysis and free phenylalanine was measured as described in the Materials and methods section. There were no phenotypic differences, and the results for both lean and obese rats have been combined. Abbreviation: RSD, residual standard deviation.

Nominal time after injection (min) ...	5	10	15	RSD
n ...	4	10	11	
Blood	806	841	808	82
Muscle	728	829	784	77
Liver	726	744	722	45
Intestine	511	526	485	53
Carcase	740	820	787	80

Table 4. *Fractional rates of protein synthesis (day^{-1}) in the whole body and in skeletal muscle, intestinal tract and liver*
The rate of protein synthesis was calculated from the incorporation into protein of L-[^{3}H]phenylalanine administered intraperitoneally [100μCi (300μmol)/100g body wt.]. Values are means ± 1 S.E.M. for the numbers of animals in parentheses. Statistical significance of phenotypic differences at the same age: $^*P<0.05$; $^{**}P<0.01$. Statistical significance of differences with age within a phenotype: †$P<0.05$; ††$P<0.01$.

	18-day-old animals		25-day-old animals	
	Lean (7)	Obese (5)	Lean (5)	Obese (4)
Whole body	0.258 ± 0.019	0.207 ± 0.008*	0.224 ± 0.015	0.219 ± 0.016
Muscle	0.224 ± 0.011	0.125 ± 0.016**	0.153 ± 0.006††	0.117 ± 0.007**
Intestine	0.995 ± 0.036	1.000 ± 0.076	1.228 ± 0.121†	1.280 ± 0.152
Liver	0.699 ± 0.044	0.662 ± 0.049	0.610 ± 0.032	0.685 ± 0.045

The values reached a maximum at 10min, but the variation with time was only marked in skeletal muscle and the whole carcase. On average the ratio of the specific radioactivity of tissue-free phenylalanine to that in the blood was 0.93 for muscle, 0.89 for liver, 0.94 for carcase and 0.62 for intestine.

Fractional rates of protein synthesis are shown in Table 4. The only tissue that showed a significant phenotypic difference was skeletal muscle. At 18 days the fractional rate of protein synthesis in this tissue was decreased by 44% ($P<0.01$) in the *fa/fa* rats, and this was reflected in a decrease in the fractional rate of synthesis of whole-body protein (−20%; $P<0.05$). At 25 days of age the fractional rate of muscle protein synthesis was still decreased by 24% in the obese rats. It is noteworthy that, although between 18 and 25 days the fractional rate of protein synthesis in the skeletal muscle of the lean rats fell from 22.4 to 15.3%/day ($P<0.01$), the rate altered little in the fat animals. In both phenotypes the rate of protein synthesis in the intestine was higher after weaning than before.

At both ages the total amount of protein synthesized in skeletal muscle and in the whole body was lower in the obese rats (Table 5). At 18 days, skeletal muscle made a significantly smaller contribution to whole-body protein synthesis in the obese animals, but this was not so at 25 days. The proportion of total protein synthesis that could be ascribed to protein synthesis in the intestine was elevated in the obese rats of both ages.

Discussion

Measurements of protein synthesis

The method that was adopted for the measurement of protein synthesis was related to that used by Garlick *et al.* (1980). Because of difficulties owing to the excessive pigmentation of the skin of the tails of Zucker rats, intravenous injection proved very difficult and the radioisotope was administered intraperitoneally. The reasoning underlying the use of a flooding dose of radioisotope has been discussed previously (McNurlan & Garlick, 1980; Garlick *et al.*, 1980), but an additional precaution is necessary when the dose is administered intraperitoneally, as there exists the possibility that radioisotope remaining in the peritoneal cavity may adhere to the surface of both the intestine and the liver and thereby lead to inaccuracies in the estimate of the tissue-free phenylalanine specific radioactivity. In the present work this was avoided by washing the peritoneal cavity with 40ml of ice-cold 0.9% NaCl, a procedure that also served to accelerate the cooling of the animal after death. The values calculated for the rate of protein synthesis in the intestine and liver are similar to those calculated by McNurlan & Garlick (1980) and Garlick *et al.* (1980).

Growth and protein synthesis

It is now well established that, irrespective of intake, both the body content of protein and the rate of protein deposition are lower in sexually mature male obese (*fa/fa*) Zucker rats (Pullar & Webster, 1974, 1977) than in their lean littermates. The same does not appear to be true of female Zucker rats (Radcliffe & Webster, 1978). Nevertheless, further consideration of the results of measurements of nitrogen retention and carcase composition (Pullar & Webster, 1977) suggests that the fractional rates of protein deposition in pair-fed lean and obese rats are the same at both 200 and 350g body wt. This begs the question as to how the differences in body protein at these ages could have arisen. One possible reason is a growth check from which the animals cannot recover fully. It appears that a check in protein deposition does occur in male obese Zucker rats between 16 and 21 days *post partum* and that, after weaning, they are able only to restore the fractional rate of protein deposition to that of their lean littermates and not to restore the deficit in body protein by a further acceleration in protein deposition. Thus by 25 days *post partum* the pattern of growth seen in older Zucker rats, i.e. similar fractional rates but different absolute rates of protein deposition, is established.

The growth check appears to occur initially in

Table 5. *Absolute rates of protein synthesis in the whole body and in skeletal muscle, intestine and liver*

The fractional rates of protein synthesis were measured as described in the Materials and methods section. Absolute rates were calculated as the product of the protein mass and the fractional rate of protein synthesis. The rate of whole-body protein synthesis was adjusted to take account of the contribution of the tissues that were removed for separate analysis. Results are mean values ± 1 S.E.M. for the numbers of animals in parentheses. Statistical significance of differences between phenotypes: $*P < 0.05$; $**P < 0.01$.

	18-day-old animals				25-day-old animals			
	Lean (7)		Obese (5)		Lean (5)		Obese (4)	
	(g/day)	(% of total)	(g/day)	(% of total)	(g/day)	(% of total)	(g/day)	(% of total)
Whole body	1.502 ± 0.094	—	1.211 ± 0.058*	—	2.264 ± 0.130	—	1.914 ± 0.137	—
Skeletal muscle	0.493 ± 0.039	32.8 ± 2.2	0.263 ± 0.039*	21.7 ± 2.3*	0.512 ± 0.038	22.6 ± 2.1	0.336 ± 0.033*	18.0 ± 2.1
Intestine	0.243 ± 0.021	16.2 ± 0.9	0.288 ± 0.050	23.7 ± 3.5*	0.610 ± 0.061	26.9 ± 4.1	0.785 ± 0.056*	41.0 ± 1.8**
Liver	0.093 ± 0.010	5.8 ± 0.5	0.082 ± 0.003	7.2 ± 0.6*	0.157 ± 0.008	6.8 ± 1.0	0.189 ± 0.012	9.9 ± 0.4

skeletal muscle, and this tissue shows a greater decrease in fractional growth rate than that of the whole body. At 18 days 80% of the difference between phenotypes in the synthesis of body protein can be ascribed to differences in the synthesis of muscle protein. At 25 days, however, only about 50% of the phenotypic difference in body protein synthesis can be accounted for by the decrease in protein synthesis in skeletal muscle, and it would appear that protein synthesis in other, presumably skeletal and integumental, tissues is also affected. Nevertheless, at 25 days of age, differences in the fractional rate of protein synthesis are less marked than at 18 days, and it should be noted that previous measurements of the fractional rate of protein synthesis in various tissues of sexually mature Zucker rats did not reveal significant phenotypic differences (Lobley *et al.*, 1978). It should be noted that in the lean animals the rate of protein synthesis in skeletal muscle was similar to that measured by the constant-infusion technique (Bates & Millward, 1981) and by the method of Garlick *et al.* (1980).

We can only speculate about the mechanism responsible for these changes in obese rats. Interpretation of the results is complicated by the differences that exist in food intake between the phenotypes. Indeed, Webster and his co-workers (Pullar & Webster, 1977; Radcliffe & Webster, 1978) have proposed that the hyperphagia, which is a characteristic of the obese animals after weaning, represents an attempt by the organism to achieve a normal rate of protein deposition. The changes with time between protein deposition and protein synthesis in the obese rats are different from those that occur in their lean littermates, and demonstrate some degree of 'catch-up' growth. Thus, in the lean animals the fractional rates of protein deposition and protein synthesis in skeletal muscle fell significantly between 18 and 25 days (cf. Bates & Millward, 1981), but in the obese rats there was not only little change with age in the fractional rate of whole-body protein deposition but also some acceleration in the fractional rate of growth of their skeletal-muscle mass after weaning.

It is noteworthy that, in addition to exhibiting hyperinsulinaemia (Martin *et al.*, 1978; Godbole & York, 1979), sexually mature genetically obese Zucker rats have increased concentrations of corticosterone in their blood (Martin *et al.*, 1978), and there is evidence to suggest that, in the rat, the full maturation of the function of the adrenal cortex occurs at between 14 and 20 days after birth (Allen & Kendall, 1967; Henning, 1978). Furthermore, it has been reported that adrenalectomy of 42-day-old obese Zucker rats appears to restore, at least partially, their food intake and body composition to normal (Yukimura & Bray, 1978).

The literature on the effects of corticosterone on

Table 6. *Fractional rates of protein synthesis (k_s), protein deposition (k_g) and protein degradation (k_d)*
The fractional rate of protein synthesis was calculated as described in the Materials and methods section. The fractional rate of growth was calculated from linear-regression analysis of the natural logarithm of protein content (16–21 days and 23–27 days respectively) against time. Mean values (day^{-1}) ± 1 S.E.M. are shown. Numbers of observations are shown in parentheses.

		18-day-old animals		25-day-old animals	
		Lean (7)	Obese (5)	Lean (5)	Obese (4)
Whole body	k_s	0.258 ± 0.019	0.207 ± 0.008	0.224 ± 0.015	0.219 ± 0.016
	k_g	0.105 ± 0.016	0.089 ± 0.013	0.072 ± 0.008	0.076 ± 0.009
	k_d	0.153	0.118	0.150	0.143
Skeletal muscle	k_s	0.224 ± 0.011	0.125 ± 0.016	0.153 ± 0.006	0.117 ± 0.007
	k_g	0.101 ± 0.022	0.060 ± 0.013	0.074 ± 0.021	0.072 ± 0.008
	k_d	0.123	0.065	0.079	0.045

protein degradation in rats is equivocal [for example, see Tomas *et al.* (1979), Santidrian *et al.* (1981) and McGrath & Goldspink (1978)]. Some of this uncertainty is due to the fact that the methods for estimating the rate of protein degradation are less satisfactory than those used for measuring protein synthesis. In the present work the rate of protein degradation was calculated from the difference between the rates of growth and of protein synthesis. This approach is associated with considerable error, which in the present study could not be estimated. Nevertheless it seemed that the decrease in protein deposition was not associated with an increase in protein degradation, and may have involved a decrease in the rate of this process. In contrast, it has been found consistently both *in vivo* (Millward *et al.*, 1976*a*,*b*) and in the perfused hemicorpus (Rannels & Jefferson, 1980) that the injection of rats with corticosterone is followed by a decrease in protein synthesis and an inhibition of the stimulatory effects of insulin (Odedra *et al.*, 1982). The present experiments demonstrate both these effects: (1) inhibition of protein synthesis before weaning, when the obese rats are not hyperphagic (Godbole *et al.*, 1981), and (2) intolerance, after weaning, to the stimulatory effects excessive food intake and elevated concentrations of insulin. It is possible that alterations in the activity of the adrenal cortex may underly the aberrations in protein metabolism in genetically obese Zucker rats.

We acknowledge the skilled technical assistance of Susan Forbes and the statistical advice of Mr. A. W. Boyne.

References

Allen, C. & Kendall, J. W. (1967) *Endocrinology* **80**, 926–930
Bates, P. C. & Millward, D. J. (1981) *Br. J. Nutr.* **46**, 7–13
Bell, G. E. & Stern, J. S. (1977) *Growth* **41**, 63–80
Diem, K. & Lentner, C. (1970) Statistical Methods in *Documenta Geigy Scientific Tables* (Diem, K. & Lentner, C., eds.), 7th edn., pp. 172–180, Ciba–Geigy Ltd., Basle
Garlick, P. J., McNurlan, M. A. & Preedy, V. R. (1980) *Biochem. J.* **192**, 719–723
Godbole, V. & York, D. A. (1979) *Horm. Metab. Res.* **11**, 646
Godbole, V., York, D. A. & Bloxham, D. P. (1978) *Diabetologia* **15**, 41–44
Godbole, V. Y., Grundleger, M. L., Pasquine, T. A. & Thenen, S. W. (1981) *J. Nutr.* **111**, 480–487
Henning, S. (1978) *Am. J. Physiol.* **235**, E451–E459
Lobley, G. E., Reeds, P. J. & Webster, A. J. F. (1978) *Proc. Nutr. Soc.* **37**, 20A
Martin, R. J., Wangsness, P. J. & Gahagan, J. H. (1978) *Horm. Metab. Res.* **10**, 187–192
McGrath, J. A. & Goldspink, D. F. (1978) *Biochem. Soc. Trans.* **6**, 1017–1019
McKenzie, H. A. & Wallace, H. S. (1954) *Aust. J. Chem.* **7**, 55–59
McNurlan, M. A. & Garlick, P. J. (1980) *Biochem. J.* **186**, 381–383
Millward, D. J., Garlick, P. J., Nnanyelugo, D. O. & Waterlow, J. C. (1976*a*) *Biochem. J.* **156**, 185–188
Millward, D. J., Nnanyelugo, D. O., Bates, P. & Heard, C. R. C. (1976*b*) *Proc. Nutr. Soc.* **35**, 47A
Odedra, B. R., Dalal, S.-D. & Millward, D. J. (1982) *Biochem. J.* **202**, 363–368
Picou, D., Reeds, P. J., Jackson, A. A. & Poulter, N. (1976) *Pediatr. Res.* **10**, 184–188
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
Radcliffe, J. D. & Webster, A. J. F. (1978) *Br. J. Nutr.* **39**, 483–492
Rannels, G. R. & Jefferson, L. S. (1980) *Am. J. Physiol.* **238**, E564–E572
Santidrian, S., Moreyra, M., Munro, H. N. & Young, V. R. (1981) *Metab. Clin. Exp.* **30**, 798–804
Tomas, F. M., Munro, H. N. & Young, V. R. (1979) *Biochem. J.* **178**, 139–146
Waterlow, J. C., Neale, R. J., Rome, L. & Palin, T. (1972) *Am. J. Clin. Nutr.* **25**, 371–375
Yukimura, Y. & Bray, G. A. (1978) *Endocr. Res. Commun.* **5**, 189–198
Zucker, L. M. & Zucker, T. F. (1961) *J. Hered.* **52**, 275–281