

The energy cost of fat and protein deposition in the rat

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1. Measurements were made of energy balance by direct calorimetry, and of nitrogen balance in groups of lean and congenitally obese ('fatty') Zucker rats at body-weights of 200 and 350 g given a highly digestible semisynthetic diet at 14.0 or 18.4 g/rat per 24 h.
2. Losses of food energy and N in faeces were very small. The fatty rats lost much more N in urine than did lean rats. Despite this the proportion of gross energy that was metabolized was 0.92 for both fatty and lean rats.
3. In all trials, fatty rats lost a smaller proportion of metabolizable energy (ME) as heat and deposited less as protein than thin rats but deposited much more as fat.
4. The amounts of ME required to deposit 1 kJ of protein and 1 kJ of fat respectively were shown by regression analysis to be 2.25 (± 0.16) and 1.36 (± 0.06) kJ respectively. These values agree extremely closely with recent, more tentative, estimates based on assumptions as to maintenance requirement which the present experiments were able to circumvent. It may be concluded with confidence that the energy costs of depositing 1 g of protein or fat are almost identical at 53 kJ ME/g.

The energy cost of fat and protein deposition is simply the increment of food energy (usually expressed as metabolizable energy (ME)) required to promote a defined increment in body protein or fat.

The energy cost of fat deposition can be measured with precision in adult animals since, in these circumstances, energy retention as protein is small and the amount of ME required to maintain energy balance (so-called 'maintenance requirement') does not differ much between successive measurements made of metabolic heat production at different levels of ME intake. There is general agreement that in simple-stomached species such as the rat and the pig the energy cost of fat deposition ranges from about 1.4 kJ ME/kJ fat deposited for foods consisting predominantly of carbohydrate to 1.15 for foods rich in triglycerides (ARC/MRC Committee, 1974).

The energy cost of protein deposition has been more difficult to assess. First, even during rapid growth the amount of energy deposited as protein is small relative to that deposited as fat or dissipated as heat. Second, the division of ME between maintenance requirement and that for protein and fat deposition changes continuously during growth and these changes are linked in such a way that changes in maintenance, protein and fat deposition show marked autocorrelation. Kielanowski (1965) recognized this when first he used multiple regression analysis in an attempt to partition ME intake between maintenance, protein and fat deposition and since then a series of reports has used his approach to describe the efficiencies of protein and fat deposition in pigs (Kielanowski & Kotarbinska, 1970; Gädeken, Oslage & Fliegel, 1973; Close, Verstegen & Mount, 1973; Thorbek, 1970, 1975) and in rats (Schiemann, 1970; McCracken & Weatherup, 1973). All these analyses required a priori assumptions to be made concerning the ME requirement for maintenance and since this was

always the largest single component of the multiple regression equation, small variations in assumed maintenance were shown to lead to bizarre differences in the apparent energy cost of protein deposition (Thorbeck, 1970; McCracken & Weatherup, 1973). Kielanowski (1976), in a critical review of these experiments, concluded recently, however, that a majority view favours a value of about 2.3 kJ ME/kJ protein deposited.

In a previous experiment (Pullar & Webster, 1974) we made use of the large phenotypic difference between congenitally obese (fatty) and lean rats of the Zucker strain (Zucker & Zucker, 1961) in order to examine the energy costs of deposition of protein, and fat in a way that was not inextricably linked to differences in maintenance requirement. Our estimate of the energy cost of protein deposition was 2.32 kJ ME/kJ protein, very similar to the best estimate given by Kielanowski (1976). However, this experiment still depended to some extent on a priori assumptions as to the energy requirement for maintenance.

The present study was designed to confirm (or otherwise) estimates of the energy cost of protein and fat deposition in the rat by an experiment that enabled these costs to be measured in a way that was free from assumptions as to the energy cost of maintenance.

EXPERIMENTAL

Animals and diet

A total of thirty-two fatty and forty lean male Zucker rats was used. Fatties could usually be recognized at about 24 d of age, soon after weaning. In the breeding colony the rats were given a commercial pelleted diet (Oxoid, Herbert C. Styles (Bewdley) Ltd, Bewdley, Worcs.).

Over a period of about 12 months, eight groups of four fatty rats and ten groups of four lean rats were selected at about 28 d of age to be as similar in weight as possible. The groups of four fatty rats could not usually be taken from the same litter but birth dates of individuals within a group never differed by more than 2 d.

Each group of four rats was kept in a single cage with a wire mesh floor and remained there for the duration of the experiment. Ambient temperature was maintained at 22° and a 12 h (06.00–18.00 hours) light–dark cycle was operated.

During the experiment the rats were given a highly digestible semisynthetic diet, the composition of which appears in Table 1. This diet was designed to minimize losses of energy and nitrogen in faeces and thereby to minimize uncertainties in balance trials. The rats were weaned from the Oxoid diet to this diet over a period of about 10 d and were eating nothing but the experimental diet by about 40 d of age when they weighed on average about 150 g. Fresh water was available at all times.

Plan of experiments

The experimental design emerged from our previous observation that *ad lib.* intake in Zucker rats remains remarkably constant throughout growth from about 150 to 400 g body-weight (Pullar & Webster, 1974).

Table 1. *Composition (g/kg) of the semisynthetic diet given to rats*

Casein	225.0		
White flour (Bero)	325.0	Dry matter (DM)	93.8
Sucrose	292.5	Composition of DM (g/kg)	
Maize oil (Mazola)	90.0	Nitrogen	39.8
Inorganic salts*	38.0	Ash	40.5
Trace elements†	2.0	Gross energy (KJ/g)	19.9
KI in sucrose‡	2.5		
Vitamin mix§	25.0		

* Contained (g): Na_2HPO_4 8.3, KH_2PO_4 7.9, KCl 5.8, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 3.3, CaCO_3 12.7.

† Contained (mg): $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ 210, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 176, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 260, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 98, made up to 2 g with sucrose.

‡ Containing 32.5 mg KI.

§ Contained (mg): thiamin 5.0, riboflavin 10, nicotinic acid 20, pyridoxine 5.0, pteroylmonoglutamic acid 5.0, cyanocobalamin 2.5, calcium pantothenate 80, biotin 10, *myo*-inositol 400, *p*-aminobenzoic acid 10, choline chloride 10000, ascorbic acid 38, *DL*- α -tocopheryl acetate 200, retinyl acetate 4.2, cholecalciferol 0.2, menaphthone sodium bisulphite 0.5, made up to 25 g with sucrose (Spencer's Feed Supplements, Aberdeen).

Groups of fatty (F) and lean (L) rats were 'pair-fed' either 18.4 g/rat per 24 h (FH and LH groups) or 14.0 g/rat per 24 h (FL and LL groups). The LH group did not eat all their food during the first few days, but all groups were consuming their entire ration by the time of the first balance trials.

Measurements of energy and N balance were made for each group when their average body-weights were 200 and 350 g. The design of the experiment was therefore such that two phenotypes (fatty or lean) given two rations (high or low) were tested at two stages of growth (200 and 350 g).

The cage containing each group of four rats was moved into a gradient-layer calorimeter (Pullar, 1969) in order to measure heat loss over three or four successive periods of 24 h.

The rats were kept in the calorimeter at 22° for 24 h before any measurements or collections were begun so that they could adapt to any small change in their environment. It was necessary to use groups of four rats at a time in order to generate sufficient heat (500–1000 kJ/24 h, or 6–12 W) to obtain acceptable precision from the calorimeter.

Urine was separated from the small amount of faeces and any spilled food using a grid assembly and collected under 2 M- H_2SO_4 . Faeces were removed daily at 11.00 hours when the rats were weighed and fed. Acidified urine was removed at the end of the collection period. The energy contents of food and excreta were determined by adiabatic bomb calorimetry. N contents of food, urine and faeces were determined by the macro-Kjeldahl procedure. Food spillage was extremely small. It was greatest with the FH groups at 8.3 g/kg food offered, and least with the LH groups at 5.3 g/kg. Suitable corrections were made to gross energy (GE) intakes. Faecal collections were not contaminated by spilled food.

When the groups receiving the low ration had completed their balance trials at 200 g, their ration was increased to 18.4 g/rat per 24 h until they reached about 300 g, whereupon it was brought back to the low level of 14.0 g until the rats reached

Table 2. *Losses of food gross energy (GE) and nitrogen in the faeces and urine of fatty and lean Zucker rats at body-weights of 200 and 350 g*

Body-wt (g) ...	Fatty rats				Lean rats				Residual SD
	200		350		200		350		
	High	Low	High	Low	High	Low	High	Low	
Level of feeding ...	High	Low	High	Low	High	Low	High	Low	
GE loss (J/kJ)									
Faeces	26	27	30	29	33	31	42	32	3.5
Urine	47	44	48	50	37	41	44	48	3.7
N loss (mg/g)									
Faeces	56	61	66	64	73	64	90	69	5.6
Urine	703	731	780	863	523	612	678	786	39.6
Ratio, urinary energy:N (kJ/g)	33	30	31	29	36	34	33	31	—
Metabolizability of GE (J/kJ)	926	929	922	921	930	928	914	920	4.2

350 g body-weight. On average the rats took about the same length of time to grow from 300 to 350 g on 14.0 g food/d as they did to grow from 200 to 300 g on 18.4 g/d (FL about 19 d, LL about 26 d for each stage).

RESULTS

Losses of GE and N in the urine and faeces of rats are summarized in Table 2. Faecal energy losses were very small, 28 and 34 J/kJ GE for the fatty and lean rats respectively. This difference was statistically significant ($P < 0.01$). Faecal energy losses were higher at 350 g body-weight and the interaction term was also significant, so that losses were highest in the lean rats at 350 g ($P < 0.01$). Urine energy losses increased with increasing body-weight ($P < 0.01$) but there were no significant differences between phenotypes or rations.

Faecal N losses showed the same pattern as for faecal losses of energy, being greatest for lean rats at 350 g. Urinary N losses were significantly greater for the fatty rats ($P < 0.01$) and at the higher body-weight ($P < 0.01$). This was as expected, but Table 2 also shows that the increased urinary N loss from the fatties was not associated with a comparable increase in urinary energy loss. In other words, the ratio, urinary energy:urinary N was lower in fatties, which indicates that the composition of nitrogenous compounds in the urine must have differed between the phenotypes.

Although the small differences between the groups in faecal and urinary losses of energy and N were statistically significant the effect of these differences on the proportion of GE that was metabolized by the different groups (metabolizability, Table 2) was negligible, the means ranging from 914 to 930 J/kJ.

The actual body-weights of the rats at the times of the two balance trials were all extremely close to those planned. At 200 and 350 g planned weights, actual weights were 201 (SD 2.6) and 350 (SD 1.3) respectively.

Table 3 summarizes the results of the energy and N balance trials made with each group of rats. Again the residual variation within any group was small. As expected,

Table 3. Results of energy and nitrogen balance trials made with lean and fatty Zucker rats kept in groups of four at mean body-weights of 200 and 350 g

Body-wt (g) ...	Fatty rats				Lean rats				Residual SD
	200		350		200		350		
Level of feeding ...	High	Low	High	Low	High	Low	High	Low	
Mid-trial age (d)	46	49	74	87	49	54	80	107	3.8
Metabolizable energy intake (kJ/24 h)	316	241	315	239	318	241	313	239	1.4
Heat loss (kJ/24 h)	161	136	185	155	206	175	243	213	5.2
Energy retention (kJ/24 h)	155	105	129	84	112	66	70	26	5.8
N retention (mg/24 h)	163	107	105	38	275	167	158	76	25.9
Energy retained as protein* (kJ/24 h)	23.9	15.8	15.4	5.6	40.5	24.5	23.3	11.2	3.8
Energy retained as fat† (kJ/24 h)	131.6	89.1	113.6	78.3	71.7	41.7	47.1	15.0	6.8

* N retention (mg) $\times 0.147$

† Energy retention - energy retained as protein.

when ME intake was fixed at two levels, 315 and 240 kJ/24 h, heat loss was significantly greater ($P < 0.001$) for the high ration, greater body-weight and lean phenotype, and N retention greater ($P < 0.01$) for the high ration, lower body-weight and lean phenotype. Thus the experimental design was achieved in so far that there were significant differences between all groups in the way that they partitioned the same two amounts of ME between heat, protein and fat.

In order to calculate the true energy costs of protein and fat deposition it is necessary first to make the reasonable assumption that they are absolute values independent of phenotype or stage of maturity. Then for each phenotype at each body-weight,

$$M_E = A + bR_{E,p} + cR_{E,f}, \quad (1)$$

where M_E is ME intake and $R_{E,p}$ and $R_{E,f}$ are energy retention as protein and fat respectively, all values being expressed in kJ/24 h. Then b and c become the ME required to deposit 1 kJ of protein and fat respectively and the solution for A is the so-called maintenance requirement for each group at each body-weight.

From the results listed in Table 3, the solution to equation 1 becomes:

$$M_E = A + 2.25 R_{E,p} + 1.36 R_{E,f} \quad (\text{RSD } 6.02). \quad (2)$$

(± 0.16) (± 0.06)

The following were the values obtained for A (kJ/24 h):

$$\text{fatty, 200 g, } A = 84.0;$$

$$\text{lean, 200 g, } A = 129.5;$$

$$\text{fatty, 350 g, } A = 122.7;$$

$$\text{lean, 350 g, } A = 192.1.$$

If the assumption that the energy costs of protein and fat deposition are the same for both body-weights and phenotypes is valid, then the observed wide range of values for heat loss (Table 3) should correspond in all circumstances with those predicted from a modification of equation 2, namely,

$$H = A + 1.25 R_{E,p} + 0.36 R_{E,f}. \quad (3)$$

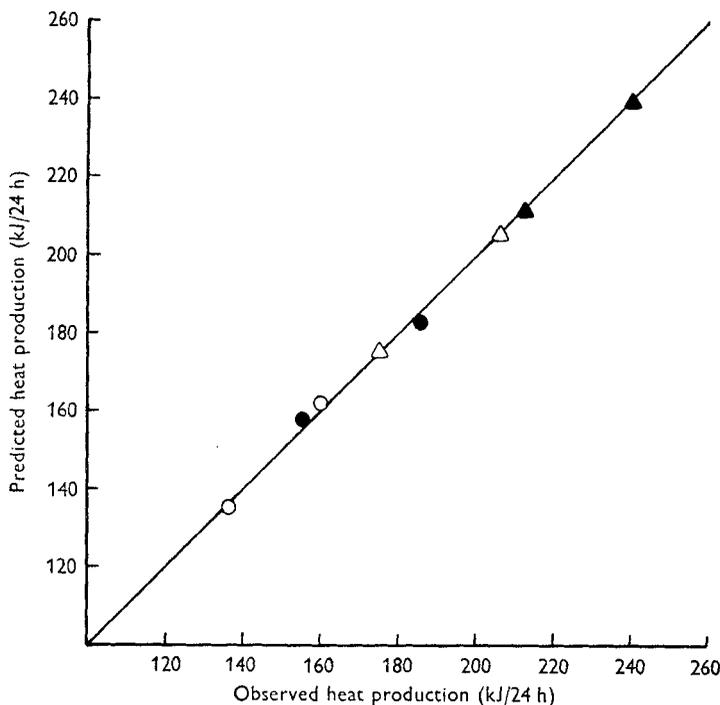


Fig. 1. The relationship between observed heat production in lean and congenitally obese (Zucker) rats and that predicted from the equation:

$$H = A + 1.25 R_{E,p} + 0.36 R_{E,f} \text{ (see p. 359).}$$

○, Fatty rats at 200 g; ●, at 350 g; △, lean rats at 200 g; ▲, at 350 g.

Fig. 1 shows that this is so. The agreement at all stages of growth, levels of intake and for both phenotypes is remarkable and we may confidently conclude that the ME requirements for protein and fat deposition in the Zucker rat given a highly digestible diet are 2.25 and 1.36 respectively. Assuming an energy content of 23.5 and 39.3 kJ/g for protein and fat this corresponds to an ME requirement of 52.9 and 53.4 kJ respectively per g protein and fat deposited, i.e. the ME requirement for deposition of 1 g protein and 1 g fat are almost identical.

DISCUSSION

Table 4 lists the most recent estimates of the energy costs of protein and fat deposition in pigs and rats. A more comprehensive list, which includes earlier estimates from the same workers, is given by Kielanowski (1976), who, as indicated earlier, suggested a preferred value of 2.32 kJ ME/kJ protein deposition, a value almost identical to that obtained in the present experiment.

All the values listed, with the exception of those from the present experiment, depended on certain assumptions as to the relationship between body-weight and ME requirement for maintenance. It was uncertainties attached to these assumptions that gave rise to the wide discrepancies between earlier estimates of the energy cost of protein deposition (not listed in Table 4). Thorbek (1970), for example, estimated the energy cost of protein deposition to be 2.32 using an empirical estimate of main-

Table 4. Recent estimates of the metabolizable energy (ME) requirements for protein and fat deposition in pigs and rats

Species	Diet	ME content of diet (kJ/g DM)	Energy requirement (kJ ME/kJ tissue)		Assumed maintenance requirement (kJ/kg W per 24 h)	Source of values
			Protein deposition	Fat deposition		
Pigs	—	—	2.80	1.36	424 W ^{0.75}	Kielanowski & Kotarbinska (1970)
	—	16.2	1.85	1.42	375-460 W ^{0.75}	Gädeken, Oslage & Fliegel (1973)
	—	14.1	1.75	1.45	418 W ^{0.75}	Close, Verstegen & Mount (1973)
	Barley*	13.4	2.05	1.33	7029 + 33.4 W	Thorbeck (1975)
	Maize*	14.6	2.19	1.20		
	Sorghum*	14.4	1.98	1.37		
—	—	—	2.06	1.40	548 W	Schiemann (1970)
Rats	—	—	1.32	1.32	468 W ^{0.75}	McCracken & Weatherup (1973)
	—	13.4	2.32	1.53	Lean, 63.9 + 310 W } Fat, 19.6 + 380 W }	Pullar & Webster (1974)
	—	18.3	2.25	1.36	Not assumed	Present experiment

* Diets were supplemented with proteins, vitamins and minerals.

tenance requirement at the body-weight of the pigs in her experiments, but obtained a value of 1.06 using the then preferred formula of Breirem (1939) which states that heat production of pigs at maintenance is 821 kJ/kg body-weight (W)^{0.56}.

Kielanowski & Kotarbinska (1970), Close, Verstegen & Mount (1973) and McCracken & Weatherup (1973) all assumed that the maintenance energy requirement of the growing pig was proportional to W^{0.75}. More recent studies with the pig (Close & Mount, 1975) and with cattle (Blaxter & Wainman, 1966; Webster, Brockway & Smith, 1974) have confirmed that basal metabolism, and thus maintenance requirement during growth, are related to an exponent of body-weight slightly below, but not significantly different from, the traditional expression for 'metabolic body size' or W^{0.75}. This was, of course, not originally proposed as a description of the relationship between heat loss and body size in the growing animal, but as an expression which permitted comparison between measurements of basal metabolism in adults of different species differing widely in body size (Kleiber, 1961).

In the experiments of Gädeken *et al.* (1973), Schiemann (1970) and Thorbeck (1975), maintenance energy requirement was assessed empirically from the measurements made of energy balance. However, body-weight still had to be included as a major factor in the multiple regression analysis, and one which was inevitably auto-correlated with the partition of retained energy between fat and protein.

The present study with the Zucker rat did not require that body-weight be included as a variable in regression analysis, but instead made use of the extreme difference between the fatty and lean phenotypes to adjust the proportion of energy retained

as protein from, for example, 0.064 (FL, 350 g) to 0.431 (LL, 350 g) at the same ME intake and body-weight. The value of 1.36 kJ for the ME required to deposit 1 kJ energy as fat is very close to the preferred value (ARC/MRC Committee 1974) for simple-stomached species given diets rich in carbohydrate. The value of 2.25 kJ ME/kJ protein is effectively the same as that recommended, with caution, by Kielanowski (1976).

The majority of the estimates given in Table 4 are rather close to the present values of 2.25 and 1.36 for protein and fat respectively, which suggests that the assumptions made in estimating maintenance requirement were more or less correct. The good agreement between most of the different estimates of the energy costs of protein and fat deposition indicates that values of 2.3 and 1.4 kJ ME/kJ tissue may be used with confidence as predictors of the ME requirements for protein and fat deposition in simple-stomached species given high-quality diets.

The increments of heat production per kJ protein and fat deposited, namely about 1.3 and 0.4 (equation 3) indicate the amount of work done, or the amount of high-energy phosphate bonds used in their deposition under standard dietary conditions.

Since diets differ in the efficiency with which they can make high-energy phosphate bonds available to support the work of anabolism, one would, a priori, expect to see differences between diets in the ME required to deposit protein and fat. Unfortunately the diets used in the experiments quoted in Table 4 were rather similar in composition and do not permit more detailed analysis of this point. Blaxter (1967) has shown that the efficiency with which the wide range of diets given to ruminants is used for fat deposition is proportional to the ME content of the ration. The results from work with pigs (Table 4) cannot be used to establish whether this concept can be used for simple-stomached species, although there is a suggestion from our previous (Pullar & Webster, 1974) and present work with rats that the efficiency of both protein and fat deposition was slightly improved by increasing the ME content of the diet.

The values given in equation 2 for A , the ME requirement associated with zero protein and fat deposition, or so-called maintenance requirement, confirmed our earlier observation that heat losses from fatty Zucker rats were much lower than from lean rats given the same ME intake, and that the difference was much greater than can be accounted for simply in terms of the reduced rate of protein deposition in the fatties. Carcass analysis was not performed on the rats in the present experiments but our previous results (Pullar & Webster, 1974) and other work in this laboratory (J. D. Radcliffe, unpublished results) indicate that the body protein and fat contents of male fatty and lean Zucker rats at 200 and 350 g body-weight and receiving diets similar to those given in these experiments are approximately as follows:

	Weight (g)			Maintenance (A, kJ)	A/Unit protein (kJ/g)
	Body	Protein	Fat		
Fatty	200	25	90	84.0	3.4
	350	45	160	129.5	2.9
Lean	200	40	20	122.7	3.1
	350	70	35	192.1	2.7

These values indicate that the maintenance requirement of both fatty and lean rats is more closely related to body protein content (or to lean body-weight) than to total body-weight. Fatties have, however, a slightly higher requirement per g body protein, and in both phenotypes *A*/Unit protein declines with increasing maturity. Thus it is not possible, from these experiments, to derive a simple expression that will predict maintenance energy requirement in both fatty and lean rats from one or more measurements of body-weight and composition.

It is not really surprising that the multiplicity of energy-demanding processes that are arbitrarily included within the definition of maintenance requirement should not lend themselves to a simple solution.

It is equally true that the values for the energy costs of protein and fat deposition are arbitrary in nature since they do not describe the total energy costs of synthesis, but simply relate deposition (or the difference between total synthesis and degradation) to increments of ME. The energy cost of synthesizing the protein and fat additional to that which is deposited in the growing body is included within the maintenance requirement. Until reliable measurements can be made of total protein and fat synthesis in the Zucker rat it will not be possible to assess the true contribution of the energy costs of protein and fat synthesis to the energy requirements for growth. Nevertheless, the good agreement that now exists between different estimates of the energy costs of protein and fat deposition in simple-stomached species like the rat and the pig does indicate that these values can now be used with confidence for the important practical business of assessing and providing the nutrient requirements of growing animals.

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