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Response of male and female rats to undernutrition

1. Changes in energy utilization, body composition and tissue turnover during undernutrition

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1. In two separate trials male and female Wistar rats, 12 weeks of age, were either killed as a preliminary control group, *ad lib.*-fed or undernourished for 4 weeks until one-third of their 12-week body-weight was lost.

2. Food intakes, urinary and faecal collections and measurements of standard metabolic rate were made at one-weekly intervals on both the *ad lib.*-fed and undernourished animals of both sexes.

3. The bodies of the preliminary controls, the *ad lib.*-fed and the undernourished animals of both sexes were analysed for protein and fat, and the weights of four fat depots, two muscles and the major organs of all groups were determined.

4. Measurements of lipid synthesis rate (LSR) and lipoprotein lipase (*EC* 3.1.1.34) (LPL) activity in the four fat depots and measurements of whole-body protein synthesis rates were carried out on animals of both sexes in each group.

5. Although both sexes lost the same proportion of body-weight the females required more food on a body-weight basis than the males during the undernutrition period. The females absorbed significantly more energy on a body-weight basis during undernutrition and so were less efficient than the males at withstanding nutritional stress.

6. There were no significant differences between males and females, on a body-weight basis, in the excretion of nitrogenous waste products (urinary nitrogen, creatinine, hydroxyproline or N^{τ}-methylhystidine) suggesting that there were no differences between the sexes in protein sparing during undernutrition.

7. There were no differences between males and females in the proportions of body fat and protein used during the period of undernutrition or in the sites of the body from which the protein and fat were mobilized.

8. There were no differences between males and females in the way they responded to undernutrition by altering LSR, LPL activity or whole-body protein synthesis rates. Both undernourished males and undernourished females maintained synthesis of lipid, on a per g tissue basis and whole-body protein synthesis at the level found in well-nourished animals of the same sex.

It is commonly accepted that after puberty female and male animals of most species differ in both their body composition and their efficiency of energy utilization. Females are generally supposed to have higher proportions of body fat than males (e.g. Keys & Brozek, 1953) and to be more efficient, primarily as a result of having a lower basal metabolic rate for their body size (e.g. Durnin, 1976).

During periods of undernutrition it would be expected that higher levels of carcass fat would confer on females a greater ability to withstand deficiencies of energy. This, combined with lower energy requirements for basal metabolism, should enable females to withstand a deficiency of energy better than males.

It has been suggested (Widdowson, 1976) that, after allowing for differences in body composition between the sexes, males and females respond differently to the same degree of undernutrition; both in the proportion of body protein and fat utilized and in the physiological sources of this protein and fat. Widdowson (1976) suggested a greater susceptibility of the male to nutritional stress due to greater catabolism of body protein, especially catabolism of vital organs such as the liver and heart. However, this suggestion is based on little direct evidence.

The work presented in this present paper was undertaken to look at the response of both

Table 1. *Trials* 1 *and* 2. *Measurements and calculations carried out on the treatment groups*

	Trial 1	Trial 2
Wt gain and food intakes	+	+
Protein and energy digestibility	−	+
Urinary metabolites	+	+
Respiratory quotient	+	−
Standard metabolic rate	−	+
Body composition	+	+
Lipid synthesis rate	−	+
Lipoprotein lipase (*EC* 3.1.1.34) activity	−	+
Whole-body protein synthesis rates	+	+

+ Measurement or calculation carried out.
− Measurement or calculation not carried out.

males and females to equivalent levels of undernutrition; firstly, as a comparison of well-nourished and undernourished animals using indirect measurements of the turnover of body components and, secondly, as a comparison of the same animals using direct measurements of changes in body composition and protein and fat synthesis rates.

EXPERIMENTAL

Two feeding trials were carried out. The only difference in the feeding of the animals was the incorporation of chromic oxide at a level of 3 g/kg in the diet of trial 2. Results from the two trials were analysed separately as different sources of Wistar rats were used and there were corresponding differences in the growth rates of well-nourished animals of the same age (Fig. 1, see p. 294). Measurements made on groups of animals in each trial are shown in Table 1. As the majority of results reported are corrected for body size, where the same measurements were carried out for both trials only the results from trial 2 are reported unless there were significant differences between trials.

Experimental animals

All animals were individually caged in a room maintained at 21° with a 12 h light–dark cycle. In both trials, daily food intakes and body-weights were recorded for all animals. At one-weekly intervals throughout both trials 24 h urine collections were made. At the same weekly intervals in trial 1 a measurement of respiratory quotient (RQ) was taken using an open-circuit technique (Kleiber, 1961). In trial 2 a complete 24 h faeces collection was made at the same time as the weekly urine collection and the standard metabolic rate (SMR) of all animals was measured under fasting and resting conditions using an open-circuit technique (Kleiber, 1961). The respiratory measurements carried out in both trials were performed between 20 and 24 h after the last period of meal feeding.

Trial 1. Twenty-one male and twenty-one female Wistar rats were randomly allocated to three groups of seven animals of each sex at 10 weeks of age. All animals were transferred to a powdered ration (diet MM77; Farm Products, Palmerston North) which was made available for only 4 h daily from 13.00 to 17.00 hours. After an initial weight loss the rats adapted to single-meal feeding and had regained all lost weight by the commencement of the trial at 12 weeks of age.

At 12 weeks of age one group each of males and females were killed (sodium pentobarbitone (60 mg/ml) by intraperitoneal injection at 10 ml/kg body-weight) to act as

preliminary controls (PreM and PreF respectively). The skin was removed, the gut was removed, cleaned and replaced in the carcass. The major body organs (liver, kidneys, heart, lungs and brain), two muscles (gluteus maximus, pectoralis major) and four fat depots (abdominal, scapular, perirenal and either parametrial or epididymal) were completely dissected, weighed and frozen at −20° together with the weighed skin and empty carcass. A further group of 12-week-old males and another group of 12-week-old females were allowed *ad lib.* access to the powdered ration for the 4 h feeding period throughout the 4-week trial. These groups were the well-nourished controls (groups WNM and WNF).

The remaining groups of animals were weighed daily and given access to amounts of food calculated from the previous day's weight loss to produce an overall decrease of one-third from their 12-week body-weights. This decrease was achieved evenly over the entire 4-week treatment period. These animals formed the undernourished treatment groups, UNM and UNF.

At the end of the 4-week treatment period, all animals were infused via a tail vein with L-[carboxyl-^{14}C]tyrosine (Amersham International, Amersham, Bucks) for 6 h at 1μCi/h commencing 16 h after the last meal. Open-circuit respiration apparatus, including an ionization chamber, was used for measurement of expired radioactivity. At the end of infusion a blood sample was taken by heart puncture and animals were killed and dissected as described for the preliminary groups. Samples of organs and muscles were rapidly frozen in liquid nitrogen and stored at −70°.

Trial 2. Thirty-five male and thirty-five female Wistar rats were randomly allocated to five groups of seven animals at 10 weeks of age and trained in meal feeding as described for trial 1. At 12 weeks of age one group each of males and females were meal-fed 4 h before slaughter. At 1 h before slaughter they were injected intravenously with 1 mCi ^3H_2O (Amersham International). They were then killed and dissected as described for trial 1, except that the four fat sites were halved; half was weighed and frozen at −20° and half weighed and placed in chloroform–methanol (3:1 v/v) and stored at −20°. These groups of animals formed the preliminary controls (PreM and PreF) for trial 2. Two further groups of males and two further groups of females were allowed *ad lib.* access to the powdered ration throughout the trial. These groups formed the well-nourished controls (WNMa, WNMb, WNFa and WNFb). The remaining groups of animals were given amounts of food calculated to reduce their 12-week-old body-weights by one-third over the 4-week feeding period. These animals formed the undernourished treatment groups (UNMa, UNMb, UNFa and UNFb).

At the end of the experiment animals in groups WNMa, WNFa, UNMa and UNFa were injected with ^3H_2O and killed as described for PreM and PreF in this trial. Animals in groups WNMb, WNFb, UNMb and UNFb were infused with L-[carboxyl-^{14}C]tyrosine and killed as described for animals in trial 1.

Analytical procedures

Urine samples (24 h) were analysed for total N (microKjeldahl digestion using a selenium catalyst followed by automated colorimetric determination of N by the method of Munro & Fleck (1969)), creatinine (Technicon Instruments Co. Ltd (1965)), hydroxyproline (automated modification of Kivirikko *et al.* (1967)), N^{τ}-methylhistidine (hydrolysis in 6 M-hydrochloric acid at 121° for 24 h followed by separation and quantitative determination using a Beckman 120 C amino acid analyser) and gross energy (freeze-dried samples measured in a Gallenkamp adiabatic bomb calorimeter against benzoic acid standards). The presence of ketone bodies in the urine was investigated by Rothera's test (Wooton, 1975). Samples of diet and freeze-dried ground faeces were analysed for total N and gross energy, by the methods used for the urine samples, and for Cr_2O_3 (Fisher & Lee, 1982).

The frozen carcasses were forced twice through a power-driven mincer resulting in a

uniform mince of tissue and bone. The skins were also minced twice to produce a uniform mince of tissue and fur. Organ and muscle samples were finely chopped with a scalpel. Subsampled, weighed samples of carcass, skin, organs and muscles were oven-dried at 105° to allow dry-matter determinations to be carried out, and then used to determine crude protein (N × 6·25) and crude fat content. N was determined as described for urine samples. Total crude fat was determined by the method of Southgate (1971). The total weight and the proportions of fat and protein in the carcass were corrected by adding back in the weights of fat and protein determined in the organs, muscles and deep body fat depots. The total weights and the proportions of fat and protein in the skin were corrected by adding back in the weights determined for the subcutaneous fat depots. The use of ^3H_2O to study rates of fatty acid synthesis is based on the work of Lowenstein (1971), who showed that for the first 90 min after an intravenous injection of tritiated water the rate of incorporation of ^3H into lipid is directly proportional to time. Thus, because all the animals in trial 2 were killed at the same time interval after intravenous injection, and within 90 min, the rate of ^3H incorporation is directly comparable between treatment groups. Synthesis rates were measured on the samples stored in chloroform–methanol (3:1 v/v). The samples were homogenized, evaporated to dryness, saponified, acidified, extracted and counted as described by Spencer & Lowenstein (1962). Because all animals were injected with the same absolute dose (1 mCi) of ^3H_2O, a correction for differences in body size was made on the radioactivity (disintegrations/min) incorporated for each tissue.

Lipoprotein lipase (LPL; *EC* 3.1.1.34) activity was assessed in the other half of each fat sample using the technique of Nilsson-Ehle & Schotz (1976). In the undernourished animals not all depots were sufficiently large to allow both lipid synthesis rates and LPL activity to be determined. In such cases the entire sample was used to determine lipid synthesis rates.

The rate of catabolism of L[carboxyl-^{14}C]tyrosine was estimated from the $^{14}CO_2$ production during infusion. Whole-body protein synthesis was calculated from the $^{14}CO_2$ production in combination with measurements of plateau plasma specific activity of free [^{14}C]tyrosine (Waterlow *et al.* 1978).

Calculations

Estimates of apparent digestible protein and apparent digestible energy were calculated from the measurements of crude protein and energy in both the diet and faeces corrected by the recovery of Cr_2O_3 in the faeces. Calculations of N-free RQ and the resultant percentage fat metabolized were made using the measured values of RQ in association with measurements of urinary N (Kleiber, 1961).

The 'energy output' was calculated as the difference between the energy retained or lost from the carcass of the animals and the apparent digestible energy intake. Calculations similar to these have been made by Forbes *et al.* (1946) and Schemmel & Mickelson (1974) and these workers suggest that this measure of energy component is related to lean body mass (LBM; the carcass weight minus the gut contents and body fat). The carcass energy deposited or lost during the experimental period was calculated by subtracting the carcass energy (weight of protein × 22·60 J + weight of fat × 38·93 J) in the preliminary control groups of each sex from that in the treatment groups of each sex.

Statistical methods

Results were analysed using analysis of variance. However, in several cases the error variances of well-nourished and undernourished animals were found to be unequal using Bartlett's test. In these cases the data were transformed using natural logarithms. In this

form the differences in variance were greatly reduced. Some differences still remained but since analysis of variance is a 'robust' test it will not have given a wrong answer, merely an insensitive one.

RESULTS

Changes in body-weight and availability of nutrients

In both trial 1 and trial 2, group UNM lost the same proportion of their initial body-weight as group UNF (Fig. 1*a*). Although group WNF consumed less food than group WNM (Fig. 1*b*) when the food intakes were expressed on a metabolic body size (body-weight $(BW)^{0.75}$) basis these differences disappeared (Fig. 1*c*) and the two WN groups consumed the same amount of food ($P > 0.05$). By the second half of both trial 1 and trial 2 it was clear that group UNF required the same amount of food as the heavier group UNM to prevent them losing a disproportionate amount of body-weight ($P > 0.05$). When the intakes of groups UNM and UNF are considered on a $BW^{0.75}$ basis, group UNF had a higher requirement for food than group UNM ($P < 0.05$) over the second half of both trials 1 and 2.

Table 2 shows the mean weekly values for apparent digestibility of protein and energy. Apparent digestibility of both protein and energy was the same throughout trial 2 for all treatment groups (WNM, WNF, UNM, UNF) except that by the fourth week both groups UNM and UNF showed a significant decline ($P < 0.01$) in their apparent ability to digest protein compared with the WN animals. Calculated values for the amounts of both protein and energy apparently digested during the trial and corrected for $BW^{0.75}$ are also shown in Table 2. There were no significant interactions ($P > 0.05$) between sex and level of feeding in the amounts of protein apparently digested. However, there was a significant interaction ($P < 0.05$) between sex and feeding on the amount of energy apparently digested, i.e. on a metabolic body-weight basis the females apparently digested more energy from the diet while losing the same proportion of body-weight as the males.

Excretion of urinary metabolites

The 24 h excretions of N, creatinine, hydroxyproline and N^{τ}-methylhistidine in the urine of all groups is illustrated in Fig. 2. Both groups UNM and UNF conserved N by excreting significantly less than their WN controls, both on an absolute basis and when corrected for metabolic body-weight. There was no significant difference ($P > 0.05$) between groups UNM and UNF in excretion of N on a $BW^{0.75}$ basis. There was a greater decline in the 24 h excretion of creatinine by group UNF (relative to group WNF) than group UNM (relative to group WNM) both on an absolute basis and when corrected for body-weight ($P < 0.05$). The rapid decline shown in hydroxyproline excretion, both on an absolute basis and corrected for body size, was the same for both groups UNM and UNF relative to groups WNM and WNF respectively. There was no significant change in the excretion of N^{τ}-methylhistidine as a result of undernutrition, although both groups WNF and UNF excreted less N^{τ}-methylhistidine than groups WNM and UNM ($P < 0.01$).

Ketone bodies were not detected in the urine of any group at any stage of either trial 1 or 2.

Indirect calorimetry

Table 3 contains the weekly changes in RQ, the proportion of fat metabolized calculated from the RQ values, and the mean SMR of each treatment group over the entire trial. As the RQ measurements were made on fasting animals they reflect the utilization of body fat, protein and carbohydrate stores for energy, rather than the use of dietary sources. The

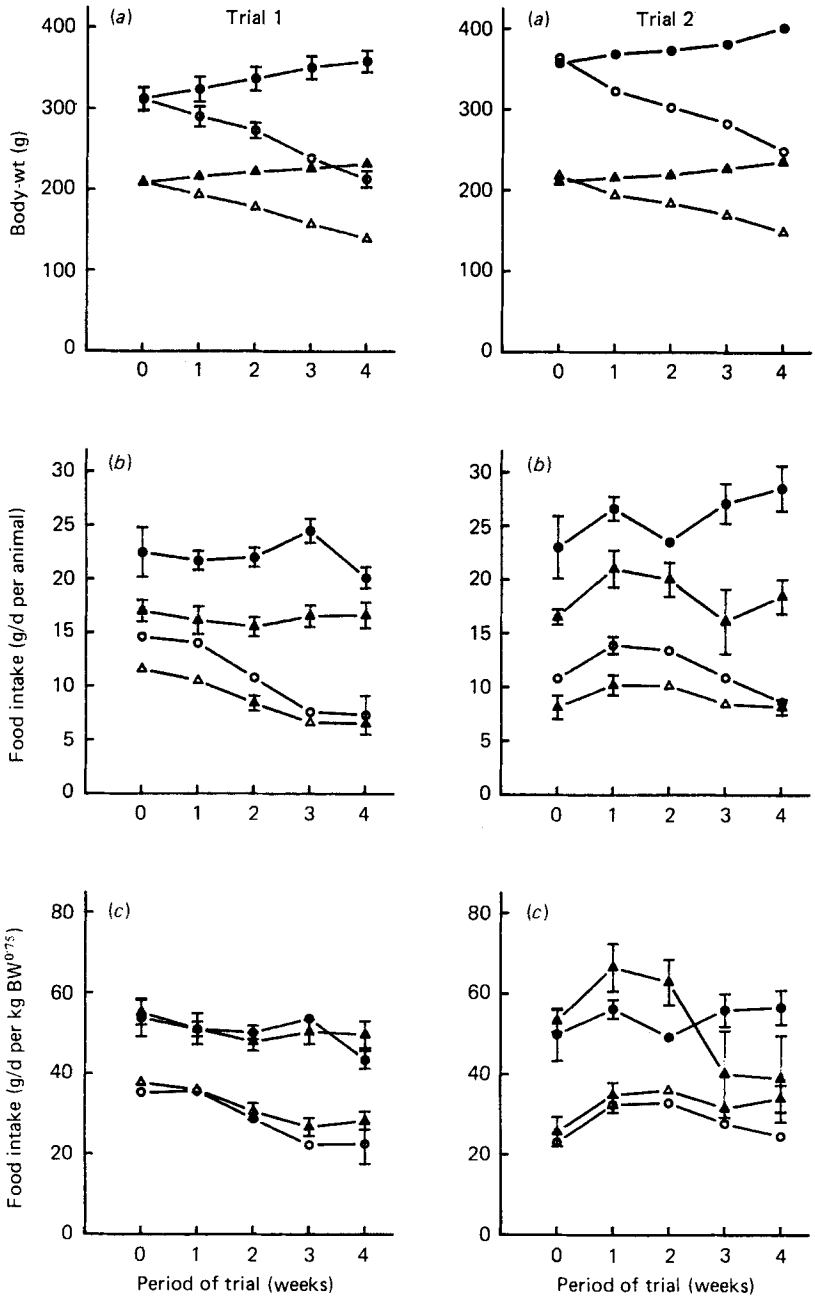

Fig. 1. Trials 1 and 2. Curves of (*a*) growth (points are mean values with their standard errors > 9·5), (*b*) daily food intake (points are mean values with their SE > 0·7) and (*c*) daily food intake corrected for body-weight $(BW)^{0.75}$ (points are mean values with their SE > 2·0) for *ad lib.*-fed males (WNM, ●), *ad lib.*-fed females (WNF, ▲), undernourished males (UNM, ○) and undernourished females (UNF, △) from 12 weeks of age.

Table 2. *Trial* 2. *Apparent digestibility of protein and energy in the diet and intakes of digestible protein and energy of Wistar rats either* ad lib.*-fed* (*WNM and WNF*) *or undernourished* (*UNM and UNF*)*

(Mean values with their standard errors for seven rats)

Group	Week of trial	Apparent protein digestibility (%) Mean	SE	Apparent energy digestibility (%) Mean	SE	Intake of digestible protein (g/week per kg $BW^{0.75}$) Mean	SE	Intake of digestible energy (kJ/week per kg $BW^{0.75}$) Mean	SE
WNM	1	76	1	76	1	46·9	1·0	4655	121
	2	74	1	75	1	42·1	1·2	4255	151
	3	73	1	74	1	41·6	0·7	4148	50
	4	72	1	73	1	40·3	1·5	4081	167
UNM	1	77	1	75	2	26·9	1·4	2600	134
	2	74	2	75	2	30·2	1·4	2888	163
	3	71	3	76	1	26·2	0·9	2746	75
	4	64	4	75	1	20·4	1·5	2382	126
WNF	1	72	1	75	1	47·5	2·1	4860	197
	2	75	1	75	1	44·6	1·1	4412	129
	3	69	1	70	1	39·3	1·4	3901	142
	4	70	2	73	1	42·9	2·5	4475	239
UNF	1	74	3	76	1	28·8	0·4	2942	129
	2	71	3	75	1	31·5	1·2	3294	105
	3	68	3	73	1	27·1	1·1	2893	75
	4	58	2	73	1	22·6	1·9	2787	142

BW, body-weight.

* For details of feeding regimens, see p. 290.

proportion of fat metabolized in the UN animals showed a rise relative to the WN controls for the first 2 weeks of undernutrition and then a decline to levels below those found for WN animals. The UNF animals began to mobilize a higher proportion of body fat more rapidly than the UNM animals. There were no significant differences in SMR, on the basis of $BW^{0.75}$, between the sexes or as a result of undernutrition.

Changes in body composition

The gross body composition of all groups of animals in trials 1 and 2 (PreM, PreF, WNM, WNF, UNM, UNF) are shown in Figs 3 (percentage fat levels) and 4 (percentage protein levels). Although the PreF had a higher percentage of fat associated with the skin than did the PreM, overall the preliminary groups had the same ($P > 0.05$) body composition as each other on a gross percentage basis. Although both groups WNM and WNF gained body fat and protein compared with their preliminary controls, there were no final differences ($P > 0.05$) between groups WNM and WNF in the proportion of the body which was fat or protein. There were also no differences between groups WNM and WNF in the ratio, fat in the carcass:fat in the skin. This is also shown by the changes in individual fat depots (Figs. 5 and 6). Although group WNF had significantly less fat in each depot by weight ($P < 0.01$) than group WNM, when the depots are compared as a percentage of total body-weight these differences disappear at the scapular and parametrial–epididymal depots (Fig. 6).

All groups of animals had a higher percentage of both protein and fat in the skin than in the carcass. After 4 weeks undernutrition the percentage of skin fat had markedly

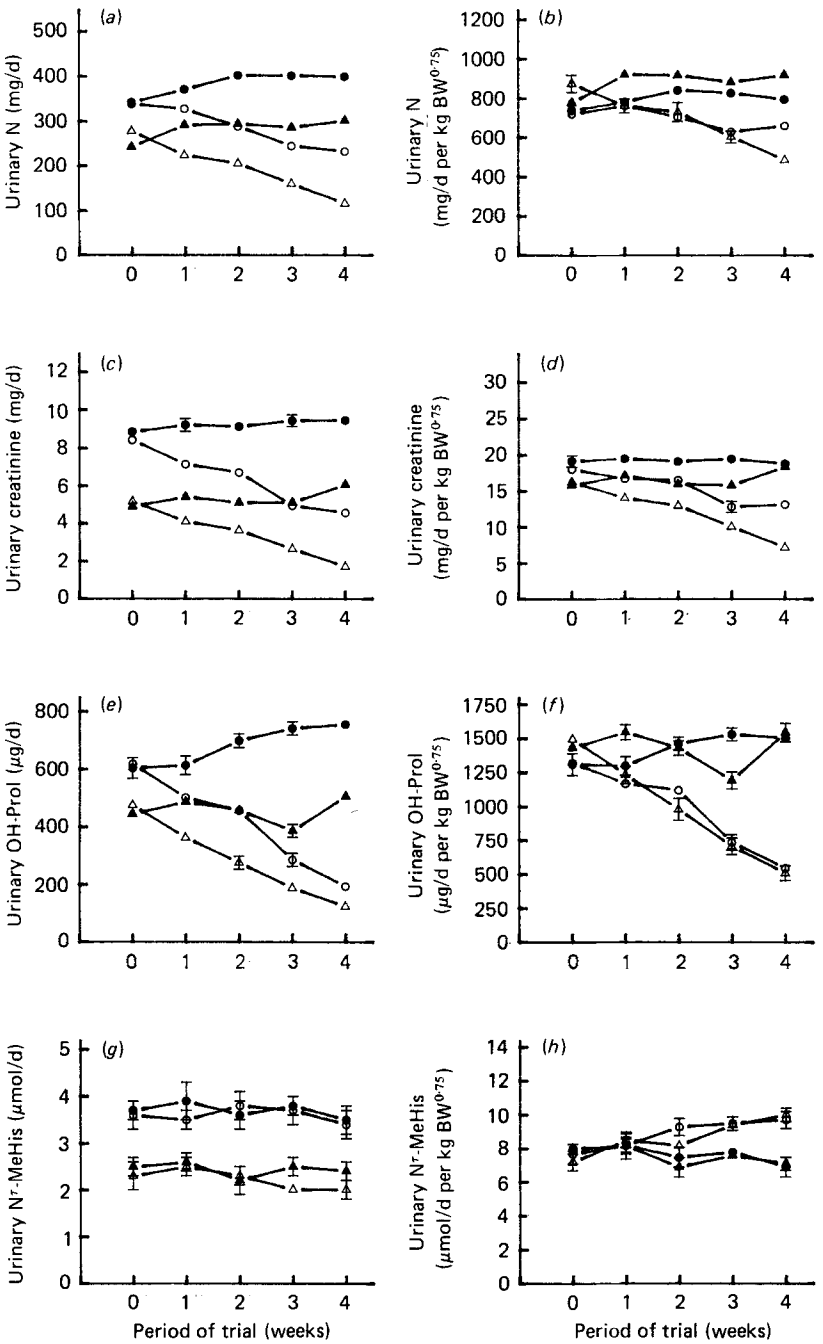

Fig. 2. Daily excretion of (*a*) total nitrogen (points are mean values with their standard errors > 25), (*b*) total N corrected for body-weight (BW)$^{0.75}$ (points are mean values with their SE > 30), (*c*) creatinine (points are mean values with their SE > 0·30), (*d*) creatinine corrected for BW$^{0.75}$ (points are mean values with their SE > 0·80), (*e*) hydroxyproline (OH-Prol) (points are mean values with their SE > 25), (*f*) OH-Prol, corrected for BW$^{0.75}$ (points are mean values with their SE > 50), (*g*) N$^{\tau}$-methylhistidine (N$^{\tau}$-MeHis) (points are mean values with their SE > 0·2) and (*h*) N$^{\tau}$-MeHis corrected for BW$^{0.75}$ (points are mean values with their SE > 0·4). Values shown for *ad lib.*-fed males (WNM, ●), *ad lib.*-fed females (WNF, ▲), undernourished males (UNM, ○) and undernourished females (UNF, △) from 12 weeks of age.

Table 3. *Trials* 1 *and* 2. *Nitrogen-free respiratory quotient* (*RQ*), *calculated fat metabolism as a percentage of total N-free metabolism and standard metabolic rate* (*SMR*) *of Wistar rats either* ad lib.*-fed* (*WNM and WNF*) *or undernourished* (*UNM and UNF*)*

(Mean values with their standard errors)

Group	Week of trial	Trial 1				Trial 2	
		N-free RQ (*n* 7)		Fat metabolized (%) (*n* 7)		SMR (*n* 28) (litres O_2/h per kg $BW^{0.75}$)	
		Mean	SE	Mean	SE	Mean	SE
WNM	1	0·84	0·04	34	3		
	2	0·84	0·02	35	2	1·04	0·04
	3	0·84	0·02	34	1		
	4	0·86	0·03	26	2		
UNM	1	0·80	0·05	45	7		
	2	0·75	0·04	78	7	1·12	0·04
	3	0·86	0·02	28	3		
	4	0·94	0·01	8	2		
WNF	1	0·83	0·02	35	1		
	2	0·84	0·01	34	1	1·23	0·04
	3	0·85	0·02	31	1		
	4	0·85	0·02	31	1		
UNF	1	0·74	0·05	73	7		
	2	0·75	0·04	69	8	1·15	0·04
	3	0·89	0·02	17	4		
	4	0·94	0·03	8	4		

BW, body-weight.

* For details of feeding regimens, see p. 290.

decreased but there was still a higher percentage of fat in the skin than in the carcass. While there was a decrease in the weight of skin protein as a result of undernutrition, because of the very large loss of skin fat there was an increase in the percentage of protein in the skin of groups UNM and UNF compared with preliminary controls. There were no significant differences ($P > 0{\cdot}05$) in the percentage of fat in the skin of group UNM compared with group UNF. There were significant differences ($P < 0{\cdot}05$) between groups UNM and UNF in the percentage of fat in the carcass and total body. In trial 1, group UNM had greater percentages of carcass and total body fat than group UNF, while in trial 2 group UNM had lower percentages of carcass and total body fat than group UNF. However, the difference in percentage composition between groups UNM and UNF in either trial was less than 1%. This is reflected in Figs. 5 and 6 because although group UNM had a greater weight of fat than group UNF at every depot sampled, except at the parametrial–epididymal depot, there were no significant differences between groups UNM and UNF at any fat depot when the depot weights were expressed as a percentage of body-weight.

The effect of undernutrition on the fat depots varies more with depot than sex. The most extreme losses of fat, both on a depot weight basis and on a percentage of body-weight basis, occurred in the deep body depots (perirenal and parametrial–epididymal). The abdominal depot was less affected by undernutrition than the deep body depots, whereas the scapular depot was close in weight in both sexes to the weight found for preliminary control animals when compared as a percentage of total body-weight.

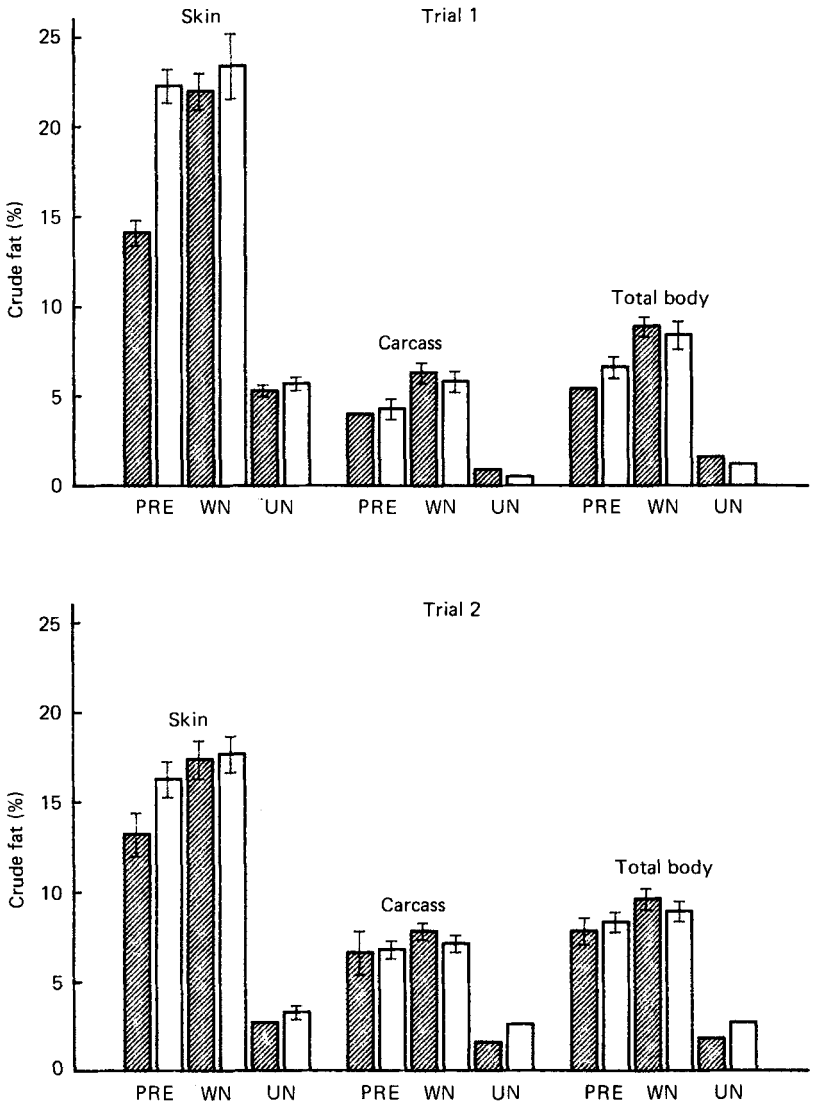

Fig. 3. Trials 1 and 2. Percentage crude fat in the skin, carcass and total body of male (▨) and female (□) rats; either preliminary controls (PRE), *ad lib.*-fed for 4 weeks (WN) or undernourished to lose one-third of body-weight over 4 weeks (UN). Values are means with their standard errors > 0·3% represented by vertical bars.

Fig. 7 shows the mean weights of the muscles and organs dissected from each treatment group, while Fig. 8 shows these muscle and organ weights as a percentage of total body-weight. PreF and WNF animals had smaller muscles and organs than PreM and WNM animals respectively, but as a percentage of body size groups PreF and WNF had the same size (or in the case of brain significantly larger ($P < 0{\cdot}01$)) muscles and organs as groups PreM and WNM. Undernutrition affects some organs severely (e.g. the liver) while others (e.g. the brain) lose little or no weight over the period of food deprivation. There were few consistent differences in percentage protein within individual muscles or organs between the various treatment groups. Thus, the differences between the treatment groups

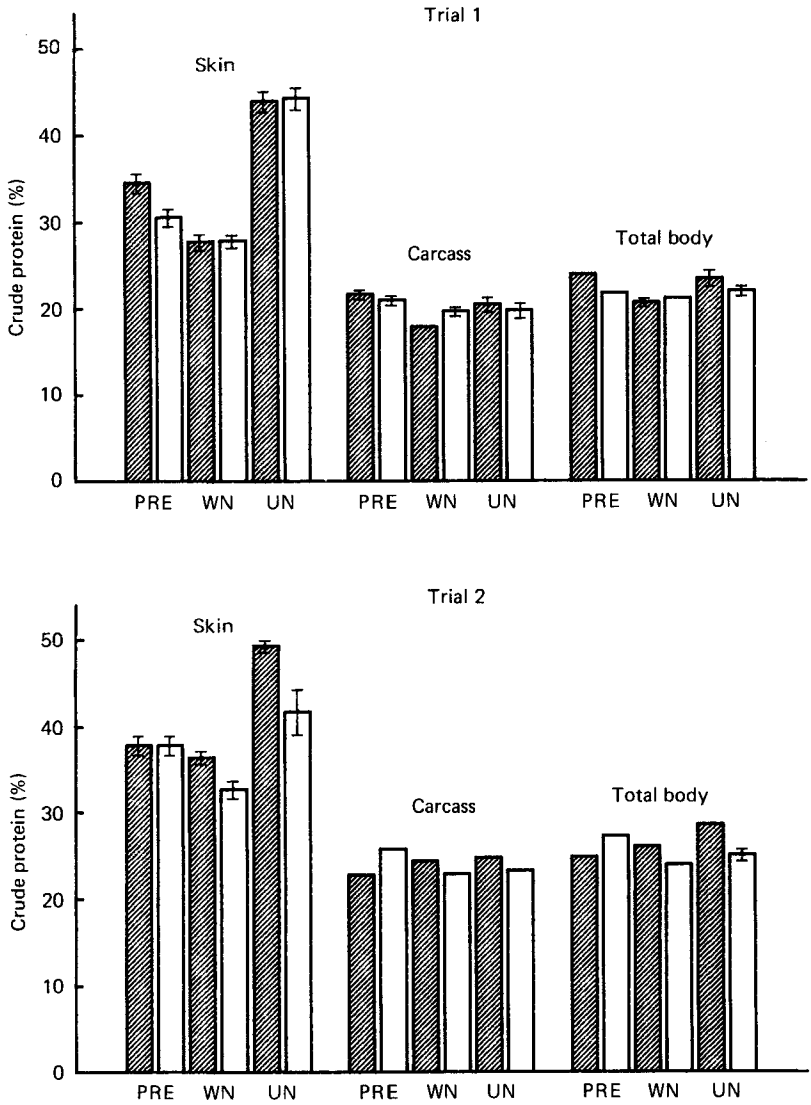

Fig. 4. Trials 1 and 2. Percentage crude protein in the skin, carcass and total body of male (▨) and female (□) rats; either preliminary controls (PRE), *ad lib.*-fed for 4 weeks (WN) or undernourished to lose one-third of body-weight over 4 weeks (UN). Values are means with their standard errors > 0·5% represented by vertical bars.

in muscles and organs were an effect of changes in tissue weight rather than composition. The UNF animals showed a tendency to lose a slightly greater proportion of tissue from the muscles dissected than did the UNM animals, but UNF animals lost a lower proportion of tissue from the organs than the UNM animals.

Total energy output

An estimate of total 'energy output' during the whole experiment is shown in Table 4, i.e. an estimate of the energy required to sustain the animals throughout the experimental

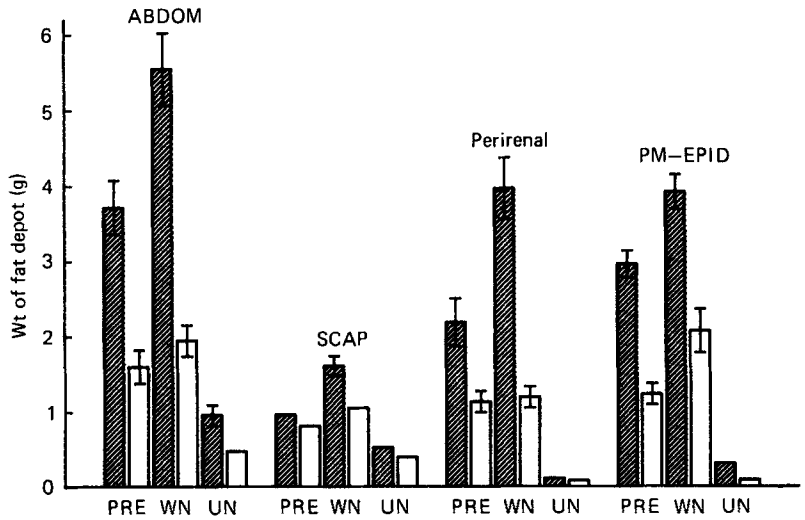

Fig. 5. Trial 2. Weights of adipose tissue (g) at the subcutaneous abdominal (ABDOM), scapular (SCAP), perirenal and parametrial (PM) or epididymal (EPID) depots of male (▨) and female (□) rats; either preliminary controls (PRE), *ad lib.*-fed for 4 weeks (WN) or undernourished to lose one-third of body-weight over 4 weeks (UN). Values are means with their standard errors > 0·07 g represented by vertical bars.

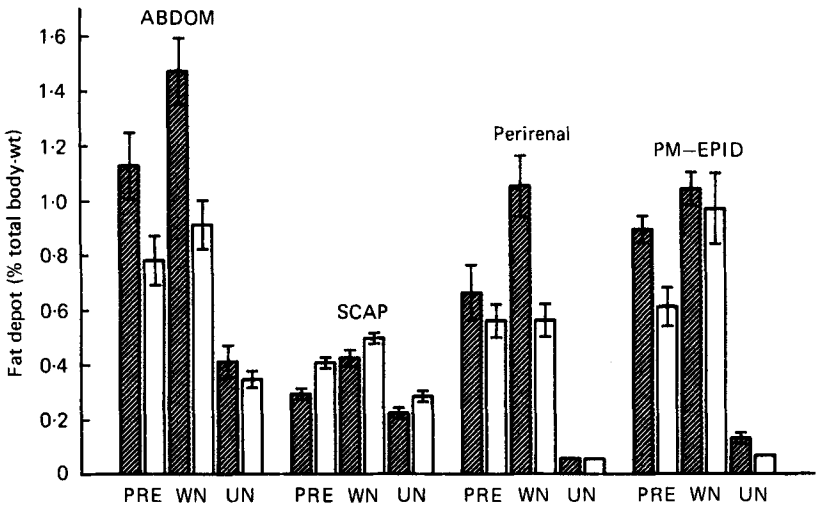

Fig. 6. Trial 2. Weights of adipose tissue (g) at the subcutaneous abdominal (ABDOM), scapular (SCAP), perirenal and parametrial (PM) or epididymal (EPID) depots expressed as a percentage of total body weight for male (▨) and female (□) rats; either preliminary controls (PRE), *ad lib.*-fed for 4 weeks (WN) or undernourished to lose one-third of body-weight over 4 weeks (UN). Values are means with their standard errors > 0·02% represented by vertical bars.

period. Although the energy output of well-nourished animals was greater than that for undernourished animals, when the energy output was corrected for lean body mass there was no difference between well-nourished and undernourished animals of the same sex ($P > 0·05$). However, both groups WNF and UNF required significantly more ($P < 0·05$) energy than that required by groups WNM and UNM.

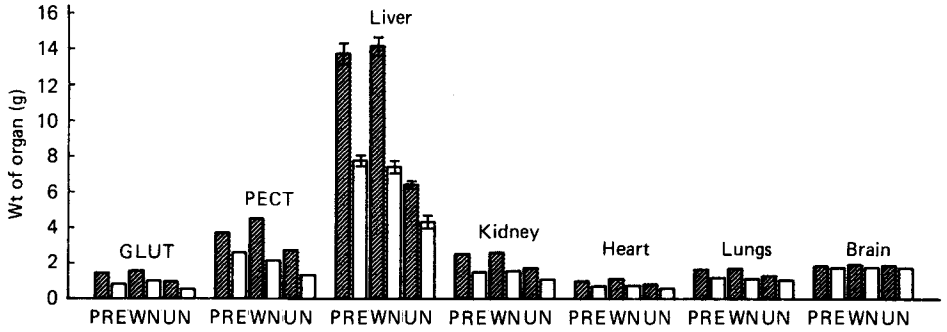

Fig. 7. Trial 2. Weights of tissue (g) in the gluteus maximus (GLUT) pectoralis major (PECT), liver, kidney, heart, lungs and brains of male (▨) and female (□) rats; either preliminary controls (PRE), *ad lib.*-fed for 4 weeks (WN) or undernourished to lose one-third of body-weight over 4 weeks (UN). Values are means with their standard errors > 0·2 g represented by vertical bars.

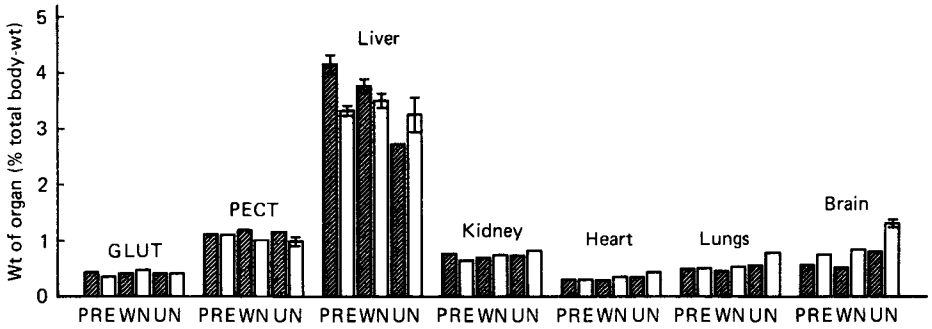

Fig. 8. Trial 2. Weights of tissue expressed as a percentage of total body-weight in the gluteus maximus (GLUT), pectoralis major (PECT), liver, kidney, heart, lungs, and brains of male (▨) and female (□) rats; either preliminary controls (PRE), *ad lib.*-fed for 4 weeks (WN) or undernourished to lose one-third of body-weight over 4 weeks (UN). Values are means with their standard errors > 0·7% represented by vertical bars.

Lipid synthesis rates and lipoprotein lipase activity

Table 5 shows the relative rates of lipid synthesis (LSR) in each depot as measured by incorporation of ^{3}H. Except for the male perirenal tissue there were no changes in relative LSR between the preliminary and well-nourished animals of each sex at any depot. There were also no consistent differences in rate on a whole depot basis between well-nourished males and females. There was a very large decline in LSR in both males and females as a result of undernutrition although this was less obvious at the scapular site, especially in group UNF. Although there was a very large decline in LSR in both undernourished groups, because there was also a large decline in the fat weight at each depot (Fig. 3), the LSR on a per g tissue basis was maintained at the same or greater rates in the undernourished animals than in the well-nourished controls.

Table 6 shows the activity of LPL for all groups able to be measured. Except for the scapular depot there was lower LPL activity in the fat depots of group WNF compared with group WNM. Although not all sites were able to be measured, due to insufficient tissue being available, both groups UNM and UNF showed a decline in LPL activity relative to well-nourished controls except at the scapular depot.

Table 4. *Trials* 1 *and* 2. *Total energy output** (*J*) *and energy output corrected for lean body mass* (*J/g*) *for Wistar rats either* ad lib.*-fed* (*WNM and WNF*) *or undernourished* (*UNM and UNF*) *for* 28 *d*†

(Values are means with standard errors for seven rats)

		Total energy output (J)		Final lean body mass (g)		Energy output / Lean body mass (J/g)	
Group	Trial no.	Mean	SE	Mean	SE	Mean	SE
WNM	1	8296	410	298	12	27·8	0·9
	2	7610	92	340	6	22·4	0·9
UNM	1	5379	213	186	8	28·9	0·8
	2	5517	175	230	6	23·9	0·7
WNF	1	6630	179	193	4	34·4	1·0
	2	5680	230	192	5	29·5	1·0
UNF	1	4294	96	123	3	35·0	1·0
	2	4219	125	130	6	32·6	1·5

* For calculation of energy output, see p. 292.
† For details of feeding regimens, see p. 290.

Table 5. *Trial* 2. *Estimates of lipid synthesis rate* (*incorporation of* ^3H) *on the basis of total rate per site* (*disintegrations/min* $\times 10^3$), *with rates per g lipid in parentheses* (*disintegrations/min* $\times 10^3$ *per g*), *for every site sampled from Wistar rats either at the start of the trial* (*PreM*, *PreF*), ad lib.*-fed* (*WNM*, *WNF*) *or undernourished* (*UNM*, *UNF*)*

(Mean values with their standard errors for seven rats)

	Abdominal		Scapular		Perirenal		Parametrial–epididymal	
Group	Mean	SE	Mean	SE	Mean	SE	Mean	SE
PreM	67·3	7·5	96·0	15·6	83·8	9·4	52·4	6·2
	(34·7)	(5·5)	(211·7)	(36·4)	(49·3)	(6·6)	(20·0)	(2·4)
WNM	97·4	16·4	113·6	12·8	156·2	21·9	76·9	7·8
	(29·4)	(5·2)	(137·9)	(19·6)	(46·7)	(5·8)	(25·2)	(3·6)
UNM	5·6	0·7	9·2	1·9	2·7	5·6	4·2	0·8
	(110·9)	(12·5)	(189·6)	(48·4)	(139·1)	(36·5)	(53·8)	(12·3)
PreF	38·9	3·3	102·9	10·3	59·7	6·6	50·2	1·9
	(62·7)	(16·8)	(284·3)	(42·7)	(75·9)	(17·3)	(60·5)	(13·3)
WNF	56·1	10·6	116·6	16·1	63·6	16·0	82·6	15·1
	(56·1)	(9·6)	(229·3)	(42·2)	(62·7)	(11·5)	(47·8)	(3·6)
UNF	6·5	4·2	38·6	21·6	4·1	2·4	2·3	1·2
	(109·7)	(35·8)	(478·6)	(222·9)	(235·7)	(98·4)	(136·1)	(61·8)

* For details of feeding regimens, see p. 290.

Whole-body protein synthesis rate

Table 7 shows the flux and oxidation of tyrosine together with calculated values for rates of whole-body protein synthesis obtained from the uptake of [^{14}C]tyrosine during continuous infusion. While group WNM had a higher daily rate of protein synthesis than group WNF ($P < 0·05$), on the basis of equal body-weight there was no difference in synthesis rate

Table 6. *Trial* 2. *Activity of lipoprotein lipase* (*LPL*, EC *3.1.1.34*) *on the basis of activity per site* (*mU*), *with activity per g of lipid in parentheses* (*mU/g*), *for every site able to be measured from Wistar rats either at the start of the trial* (*PreM*, *PreF*), ad lib.*-fed* (*WNM*, *WNF*) *or undernourished* (*UNM*, *UNF*)*

(Mean values with their standard errors for seven rats)

	Abdominal		Scapular		Perirenal		Parametrial–epididymal	
Group	Mean	SE	Mean	SE	Mean	SE	Mean	SE
PreM	11·1	1·2	9·4	0·8	14·2	1·7	18·5	0·9
	(5·6)	(0·7)	(20·4)	(3·1)	(7·8)	(1·2)	(7·1)	(0·8)
WNM	24·7	4·5	14·1	2·0	24·6	2·3	44·1	3·9
	(4·4)	(0·6)	(8·9)	(1·1)	(6·4)	(0·5)	(11·4)	(0·9)
UNM	3·6	0·3	12·2	0·7	—	—	8·9	1·4
	(4·0)	(0·3)	(24·8)	(3·2)	—	—	(25·6)	(4·0)
PreF	6·3	0·9	13·2	1·4	6·5	1·5	4·3	1·2
	(4·2)	(0·6)	(16·2)	(1·5)	(5·7)	(1·2)	(4·9)	(1·7)
WNF	7·8	1·9	17·2	2·3	11·5	1·9	19·3	4·0
	(3·7)	(0·6)	(16·2)	(2·2)	(9·7)	(1·2)	(9·5)	(1·5)
UNF	1·2	0·5	13·2	0·6	—	—	—	—
	(2·4)	(1·0)	(35·1)	(3·6)	—	—	—	—

* For details of feeding regimens, see p. 290.

Table 7. *Trials* 1 *and* 2. *Tyrosine flux and oxidation rates with calculated rates of whole-body protein synthesis and whole-body degradation rates on an absolute basis* (*g/d*) *and on a body-weight basis* (*g/kg per d*) *for Wistar rats* ad lib.*-fed* (*WNM*, *WNF*) *or undernourished* (*UNM*, *UNF*)*

(Mean values with their standard errors for seven rats)

	Trial no.	WNM		UNM		WNF		UNF	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Tyrosine flux	1	8·9	2·0	4·9	0·5	3·3	0·6	3·3	0·2
(mg/h)	2	7·2	0·5	4·4	0·4	6·1	0·6	2·5	0·9
Tyrosine oxidation	1	37	5	29	6	30	3	28	4
(%)	2	34	3	33	4	39	4	31	5
Protein synthesis:									
g/d	1	5·5	1·4	3·4	0·4	2·3	0·4	2·3	0·2
	2	4·8	0·6	3·0	0·4	3·7	0·4	1·7	0·7
g/kg per d	1	15·2	4·1	14·8	1·4	9·8	1·5	15·4	1·4
	2	13·2	1·5	12·8	1·8	16·7	2·4	12·4	5·2

* For details of feeding regimens, see p. 290.

between the sexes. There was also no difference in the rate of whole-body protein synthesis as a result of undernutrition when comparisons were made on the basis of equal body-weight.

DISCUSSION

It is well established that there are major differences in the metabolic adaptions made by an animal to undernutrition as compared with starvation (Cahill, 1976). During starvation there is a change, especially by the brain, to the utilization of ketone bodies instead of

glucose as the primary fuel. In this situation amino acids are conserved for the maintenance of tissue integrity rather than used as a substrate for glucose production by the liver. Although there is also a requirement for conservation of nitrogenous compounds during undernutrition, adaption to utilization of lipid fuels does not occur because some non-endogenous protein and energy is available. A detailed study of the effects of starvation on the male rat by Goodman & Ruderman (1980) and Goodman *et al.* (1980) shows that after only 3 d of complete starvation 16-week-old animals show a marked decrease in the excretion of urinary N and this excretion remains low for the remainder of the fast. In contrast, in the present trials a decrease of one-third of total body-weight was achieved over 4 weeks but it was not until the end of the second week of undernutrition of both males and females that there was a significant decline ($P < 0{\cdot}05$) in excretion of N below that of WN control animals. Both creatinine and hydroxyproline excretion of UNM and UNF animals showed similar patterns to that of N excretion. Thus, when undernourished, both male and female rats conserved nitrogenous compounds relative to well-nourished controls, but were both slower and possibly less efficient at conservation than animals undergoing complete starvation.

Changes in N excretion during undernutrition reflect changes in protein synthesis, protein degradation or the recycling of amino acids. The decline in creatinine excretion by the UN animals suggested either a smaller muscle mass or a slower turnover of creatinine in the muscle mass in both UN groups but especially in the UNF animals. A smaller muscle mass was confirmed by carcass analyses of the UN animals of both sexes and so the maintenance of N^{τ}-methylhistidine excretion suggested an increase in muscle-protein degradation rate by the UN animals. Such an increase in muscle-protein degradation rate was supported by the measurements made of whole-body protein synthesis using [^{14}C]tyrosine infusions. However, although the N^{τ}-methylhistidine measurements suggested an increased degradation of the muscle mass, the rapid decline of hydroxyproline excretion indicates a marked decline in the degradation of body collagen by the UN animals. Turnover of collagen is slow relative to that of mixed muscle protein (Waterlow *et al.* 1978) consequently, although collagen comprises a large proportion of body protein, it does not contribute significantly to whole-body protein synthesis.

Tyrosine was chosen as the tracer amino acid to measure rates of whole-body protein synthesis because most comparable measurements of whole-body flux have been made using tyrosine or lysine (Waterlow *et al.* 1978). There is some species variability in the flux of tyrosine relative to other amino acids (e.g. Lobeley et al. 1980) but within a species useful comparative results can be obtained. Garlick *et al.* (1973) demonstrated that there is little change in synthesis rate of previously well-fed rats until 24–36 h after their last meal. Accordingly, infusions were carried out on animals in the post-absorptive, but not fasting, state (16–22 h after the last meal), to allow comparisons of true adaptive changes rather than metabolism of dietary amino acids. However, the timing of the infusion relative to feeding may account for the low levels of whole-body protein synthesis determined for all treatments groups.

The errors found when measuring whole-body protein synthesis rates are high and daily rates of synthesis were very large compared with the small daily losses which cumulate into the total loss over the 28-d period. With these relatively small daily losses it would seem that the sensitivity of the technique used to measure protein synthesis rate was not adequate under the conditions of the present trials. On an absolute basis both well-nourished and undernourished males had significantly higher rates of protein synthesis than well-nourished and undernourished females respectively. However, when a correction was made for body-weight there was no difference in whole-body protein synthesis between males and females and no changes as a result of undernutrition. Waterlow *et al.* (1978) suggested that

there are three stages of response to extreme nutritional stress in muscles and that the third, and final, stage in adult rats is a relatively minor change in synthesis rate in association with a marked change in breakdown rate. The undernourished animals in the present trial were losing body protein while maintaining protein synthesis rates which supports the suggestion that it is protein breakdown, rather than synthesis, which contributes most markedly to the changes in N balance during terminal undernutrition.

Obviously the loss of one-third of starting body-weight caused a loss of both protein and fat from male and female rats. However, undernutrition which caused the loss of the same proportion of the body-weight resulted in no major differences between males and females either in the gross use of body protein or fat stores, or in the relative effects on individual fat depots, muscles or organs.

Net losses of body protein and fat involve changes in the synthesis rate or the degradation rate, or both. Measurements of LSR in the present work showed that synthesis occurred at a greater rate on a per g tissue basis during undernutrition but at a lower rate on the basis of the whole depot. Measurements of LPL activity showed similar trends. Thus, both *de novo* synthesis of fatty acids and clearing of plasma fatty acids were decreased overall at the depots measured in both sexes. It is not possible from the present work to say whether there was a concomitant increase in the breakdown of stored triglyceride. As there was an elevated rate of synthesis of fatty acids on a per g tissue basis it is probable that an increase in breakdown rate would have been required to achieve the large losses of body fat that occurred.

There are major differences between the design of the present trials and the work referred to by Widdowson (1976) when she postulated a difference in response between the sexes. Widdowson & McCance (1956) used fully-grown animals with a correspondingly higher level of body fat than the sexually-mature but still-growing animals used in the present trials. The undernutrition was less severe in the work of Widdowson & McCance (1956); equivalent to a loss of 15% of adult weight compared with one-third in the present trials.

Despite differences in design, several points from the results of Widdowson & McCance (1956) are comparable with results from the present work. While there were losses in the absolute amounts of body protein of both the undernourished males and females there were no large changes in the percentage protein in the carcasses (well-nourished males 22·6 *v.* undernourished males 21·5, well-nourished females 17·5 *v.* undernourished females 18·8). Similarly, there was little change in the percentage protein of the livers in either sex, or in the proportion of body-weight taken by the liver as a result of the undernutrition inflicted in the study by Widdowson & McCance (1956). Thus, while the undernourished adult males may have mobilized a greater weight of protein than the undernourished adult females on an absolute basis, both sexes maintained the normal proportions of both body protein and organs in the work of Widdowson & McCance (1956) as in the present work.

In the second half of both trials 1 and 2 the UNF group required more food on a body-weight basis than the UNM group to ensure that both sexes lost the same proportion of body-weight. Because there were no differences between UNM and UNF animals in apparent digestibility of dietary energy this increased intake of energy means that the UNF animals were less efficient in their utilization of energy during the period of undernutrition. This was confirmed by calculations of 'total energy output' where the female groups consistently had a significantly higher level of energy output on a body-weight basis regardless of their nutritional state. Schemmel & Mickelson (1974) reported a similar inefficiency of energy utilization by well-nourished female rats compared with male rats given the same diets, and these workers noted that such results were 'contrary to many popular statements'.

It seems that inefficient utilization of available energy by female rats in comparison with

males extends beyond the situation of *ad lib.* feeding to occur during periods of food restriction. When allowance was made for body-weight no differences were found between males and females in their absorption of body protein, conservation of body N, whole-body protein synthesis rate or in their SMR during undernutrition. Consequently the differences found between the sexes in efficiency of energy utilization are probably the result of differences in either body temperature or activity.

Overall the present work suggests that there are no major differences between the sexes in gross utilization of fat or protein, either in the depots used to provide energy or the way these depots are utilized with respect to alterations in synthesis or degradation rates. Female rats are less efficient in their utilization of available energy and so under conditions of equivalent energy availability would probably not survive a period of deprivation as well as male rats.

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