The relationship between protein turnover and energy balance in lean and genetically obese (*ob/ob*) mice

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1. Groups of lean and genetically obese (*ob/ob*) mice were adapted to varying energy intakes and the rates of total protein turnover in liver, gut and kidney were measured.

2. Lean mice gained less weight when fed above maintenance and lost less weight when fed below maintenance than obese mice.

3. Hepatic protein turnover (mg/d) was sigmoidally related to digestible energy intake in lean mice but showed no significant changes with dietary intake in obese mice.

4. The changes in protein turnover resulted from changes in both the half-lives of protein synthesis and catabolism and in tissue protein content.

5. In the lean mice, protein turnover in kidney and gut was not significantly changed with increasing energy intake until the highest level was reached.

6. The findings suggest that protein turnover may be an important cycle for the regulation of energy balance in mice and that this cycle is impaired in the genetically obese (*ob/ob*) mice.

For energy balance to be maintained energy intake must exactly balance energy expenditure. It is also known that energy expenditure affects energy intake (Masterton *et al.* 1957) and vice versa (Bray, 1972). Early investigations into the control of energy balance centred around the control of food intake. In small animals, energy intake is finely controlled, so that it can meet the requirements of energy expenditure (Garrow, 1974), whereas social and psychological factors tend to over-ride any physiological control of food intake in man (Silverstone, 1974). It is therefore probable that the primary control of energy balance in man involves a control of energy expenditure.

Several classic (Neuman, 1902; Gulick, 1922) and more recent studies (Miller & Payne, 1962; Miller & Mumford, 1967; Sims *et al.* 1968; Apfelbaum *et al.* 1971) have indicated that rats, pigs and man may eat considerable quantities of food in excess of their metabolic requirements without suffering the predictable gain in body-weight. Indeed, some subjects may even lose weight during such overfeeding regimens (Miller & Mumford, 1967). No evidence has been found that changes in body composition could account for more than a fraction of the surplus energy (Miller & Mumford, 1967; Sims *et al.* 1968). The loss of the extra energy occurs through increased heat production, termed either 'luxuskonsumption' (Neuman, 1902) or 'dietary-induced thermogenesis (DIT)' (Miller & Mumford, 1967).

The biochemical basis for the phenomenon of DIT has still to be elucidated. Recent reviews (Stirling & Stock, 1973; Garrow, 1974; Himms-Hagen, 1976) have suggested several pathways which could be of potential importance in the control of energy balance but as yet there is no clear evidence of a positive relationship between energy intake and the activity of these pathways. It has been suggested that the energy loss associated with protein turnover is involved in non-shivering thermogenesis (NST) (Yousef & Chaffee, 1970), the thermic effect of food (Grisolia & Kennedy, 1965; Ashworth, 1969) and DIT

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(Garrow, 1974). However, direct evidence for the involvement of protein turnover in either cold adaptation or the maintenance of energy balance is poor.

The aim of this study was to define the relationship between protein turnover, DIT and energy balance. Both lean (*ob*/?) and obese (*ob*/*ob*) mice were used as experimental models, since it is known that the obese mouse has a defective thermogenesis (Trayhurn *et al.* 1977) and has an increased efficiency of energy utilization (Alonzo & Maren, 1955).

EXPERIMENTAL

Animals

The male mice used in these experiments were bred in the Southampton colony, which originated from the Institute of Animal Genetics, Edinburgh, Scotland. Lean (*ob*/?) and obese (*ob*/*ob*) mice were selected at 7 weeks of age and housed individually with free access to tap-water in a controlled environment at 22° (except for two groups of lean animals that were left at 4°). A 12 h light–12 h dark (07.00–19.00 hours) cycle operated.

Energy balance and protein turnover studies

Feeding regimen. Groups of twenty lean and twenty obese (*ob*/*ob*) mice (protein turnover study), and groups of ten lean and ten obese (*ob*/*ob*) mice (energy balance study) were adapted to a range of food intakes over a 2-week period, as shown in Table 1. Mice were offered powdered Porton Mouse Diet (Christopher Hill Group, Poole, Dorset), either *ad lib.* (24 h/d) or daily for a 4 h period (12.00–16.00 hours). Animals fed 4 h/d had either unrestricted or restricted access to food during this period. The *ad lib.* food intakes of one lean group in both studies were increased by lowering the environmental temperature to 4° (± 1°). Food intake was estimated daily with allowance being made for all spillage, and all mice were weighed regularly.

Energy balance study

After adaptation to the feeding regimen for 2 weeks, five mice from each dietary group were killed by cervical dislocation for an estimation of 'zero-time' body composition. The remaining five mice in each group were maintained on the same regimens for a further 3-week period after which they were killed. During the second week, a 5 d faecal collection was made for each mouse. Carcasses were analysed as follows:

Body composition

At death the animals were weighed, the intestines were removed, rinsed through with water and then returned to the body which was rapidly frozen in liquid nitrogen and stored at −18° until analysed.

Water determination. Water contents were determined gravimetrically by heating the weighed carcasses on aluminium foil at 105° until no further loss in weight occurred. This drying facilitated subsequent mincing. The carcass was refrozen.

Carcass mincing. The frozen carcass was placed in a steel round-bottomed canister (500 ml) together with twice (lean) or three times (obese) its weight of anhydrous sodium sulphate. The canister was fastened to a vertical-axis electric motor on the drive-shaft of which was welded a multiple hammer blade. The canister was immersed in liquid N_2 or a dry ice – acetone mixture. Complete homogenization of the carcass was possible in 5 min, and recovery of the sample powder was in excess of 95%. The ground carcasses were stored in air-tight glass jars at room temperature until analysed.

Table 1. *Digestible energy* (DE) *intake, feeding period and ambient temperature of mice used in the energy balance and protein turnover experiments*

(Mean values with their standard errors for no. of animals shown in parentheses. Animals restricted to a 4 h feeding period were offered food daily between 12.00–16.00 hours. All mice were maintained in a 12 h light–12 h dark cycle (07.00–19.00 hours) with the exception of lean mice exposed to an ambient temperature of 4°. These were maintained under constant lighting)

Phenotype	Ambient temperature (°)	Feeding period (h/d)	DE intake (kJ/d)			
			Energy balance study (5)		Protein turnover study (20)	
			Mean	SE	Mean	SE
Lean (*ob*/?)	4	24	107·6	3·0	107·6	3·0
	22	24	—	—	69·9	2·2
		4	72·8	1·4	62·8	0·6
		4	55·3	0·6	53·1	0·0*
		4	45·2	0·5	43·4	0·0*
Obese (*ob*/*ob*)	22	24	126·1	4·2	121·1	3·1
		4	73·7	2·1	73·7	1·9
		4	56·3	0·2	—	—
		4	41·4	0·8	43·4	0·0*
		4	33·6	0·1	—	—

* Mice ate all the food offered in that time period.

Lipid analysis. Duplicate 1 or 2 g samples of homogenized carcass were extracted twice with 5 ml chloroform – methanol (2:1, v/v). Non-lipid solids were sedimented by centrifugation at 2000 *g*, and the supernatant fractions decanted into tared glass scintillation vials. A further extraction was performed using 5 ml diethyl ether, and this was added to initial extract, which was evaporated to dryness and weighed.

Protein analysis. The sedimented non-lipid solids from the lipid analyses were dissolved in 5 ml 2 M-potassium hydroxide. The concentration of protein in the resultant solutions was estimated by a semi-automated Biuret technique (Technicon Instruments Co. Ltd, 1970) using an AutoAnalyzer II (Technicon Instrument Co. Ltd, Basingstoke, Hants). Protein concentrations were based on a bovine serum albumin fraction V standard (Sigma Chemical Co., Kingston-on-Thames).

Energy contents. Carcass energy was calculated from the sum of the energy in the lipid and protein fractions, assuming the energy density of lipid to be 39·6 kJ/g and of protein to be 22·6 kJ/g (Passmore & Durnin, 1967). Samples checked at random by adiabatic bomb calorimetry showed good agreement. Gross energy values of food and faeces were determined by burning triplicate samples in an adiabatic bomb calorimeter (Autobomb; Gallenkamp). The gross energy value of the diet was $20{\cdot}15 \pm 0{\cdot}13$ kJ/g.

Calculation of results. Since urinary losses were not measured, energy balance was based on the intake of digestible energy (DE), calculated from the difference between daily ingested and faecal energy. The 'zero-time' body energy of each experimental mouse was calculated from the results for animals slaughtered at zero-time and their energy balance taken as the difference in energy content at zero time and at termination.

Protein turnover study

Protein turnover was estimated by the method of Miller *et al.* (1978). Lean mice were adapted to five differing feeding regimens and obese mice to three differing regimens as shown in Table 1. After 14 d adaptation period, all mice were injected intraperitoneally with 100 μCi DL-[2-^{3}H]glutamic acid (specific radioactivity 4·5 Ci/mmol) (Radiochemical Centre, Amersham, Bucks). At 3, 6, 9, 12 and 15 d after injection, four mice from each dietary group were killed by cervical dislocation and both the total and specific activities of ^{3}H-labelled protein in liver, intestine (pylorus to rectum) and both kidneys were determined (Miller *et al.* 1977, 1978). The decay curves for specific and total activities of ^{3}H-labelled protein were analysed to obtain half-lives for protein synthesis and catabolism respectively. For each tissue at each nutritional state, the total protein turnover (PT; mg/d) was calculated from:

$$\text{PT} = \text{total tissue protein} \times \frac{\ln 2}{\text{half-life for synthesis (d)}}.$$

The DE intakes of these mice (kJ/d) were calculated from the regressions obtained in the energy balance study which were:

$$ob/ob \text{ DE} = 15{\cdot}87 \text{ DM} - 0{\cdot}63,$$
$$\text{lean DE} = 19{\cdot}23 \text{ DM} - 0{\cdot}05,$$

where DM is the dry-matter intake (g/d). The correlation coefficients for both regressions were 0·99.

RESULTS

Energy balance study

The body-weights of mice adapted to the feeding regimen are presented in Fig. 1. After adaptation for 2 weeks, all mice maintained or increased their body-weights over the 3-week experimental period with the exception of the two severely restricted obese groups.

The relationship between changes in body energy and energy intake (Fig. 2) showed that lean mice were capable of adapting to wide fluctuations in intake without substantial changes in body energy. Indeed, over the range of intakes from 55 to 73 kJ/d, lean mice showed no change in body energy over the 3-week period. The minimum daily energy requirement for maintenance was estimated from Fig. 2 to be 55 kJ/d.

In sharp contrast to lean mice, obese mice showed no capacity to regulate body energy over the range of intakes offered (Fig. 2), although the daily energy requirement for maintenance (53 kJ/d) was very similar to that for lean mice. However, if dietary energy intake is expressed per unit metabolic body-weight ($kg^{0·75}$) then the obese mice have a lower maintenance requirement (lean 963 kJ/$kg^{0·75}$; obese 735 kJ/$kg^{0·75}$).

Protein turnover study

Liver. The total hepatic protein turnover in lean mice varied greatly with energy intake (Fig. 2). Over the range of DE intakes 53–70 kJ/d, the relationship was linear in lean mice. Liver protein turnover was highest in cold-adapted lean mice eating 108 kJ/d and lowest in mice restricted to 43 kJ/d. In sharp contrast to lean mice, the amount of protein turnover in obese livers showed little change as energy intake increased (Fig. 2). Even when DE intake was restricted to 43 or 53 kJ/d, the amount of hepatic protein turnover was not significantly different ($P > 0{\cdot}1$) from that at *ad lib.* intake.

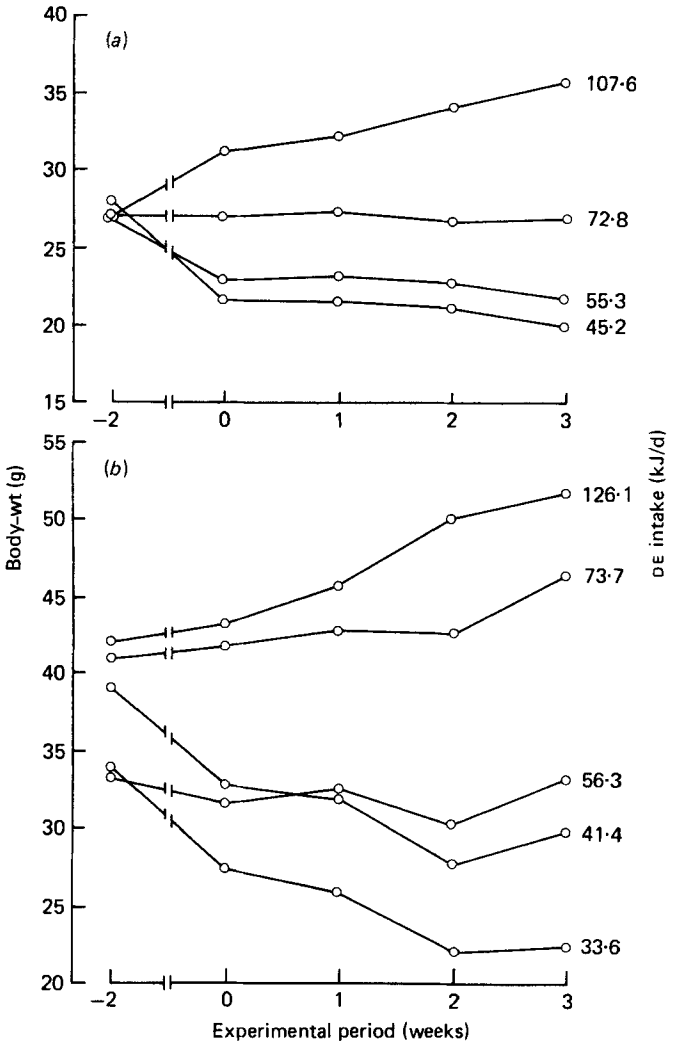

Fig. 1. Body-weight (g) of (*a*) lean (*ob*/?) and (*b*) obese (*ob*/*ob*) mice given variable digestible energy (DE; kJ/d) intakes. Animals were adapted to each DE intake over a 2-week period (-2–0 weeks). Changes in body composition and protein turnover were assessed from week 0 to week 3 as described on p. 186.

The variation in protein turnover (mg/d) observed after dietary manipulation was a reflection of changes in both the half-life of turnover (Table 2) and the amount of total liver protein (Table 3). For instance, the marked increase in protein turnover in all tissues of *ad lib.*-fed cold-exposed animals was associated with a reduction in the half-life of turnover but no change in tissue protein whereas the decrease in hepatic protein turnover in lean mice absorbing 53·1 kJ/d reflected both a loss of tissue protein and an increase in the half-life of turnover. In obese mice given 43·5 kJ/d DE tissue protein (318 mg) was less than in obese mice fed *ad lib.* (464 mg), yet the amount of protein turnover (mg/d) was not significantly different due to compensatory changes in the rate of turnover (half-lives were 4·25 and 3·69 d for *ad lib.* and restricted animals respectively).

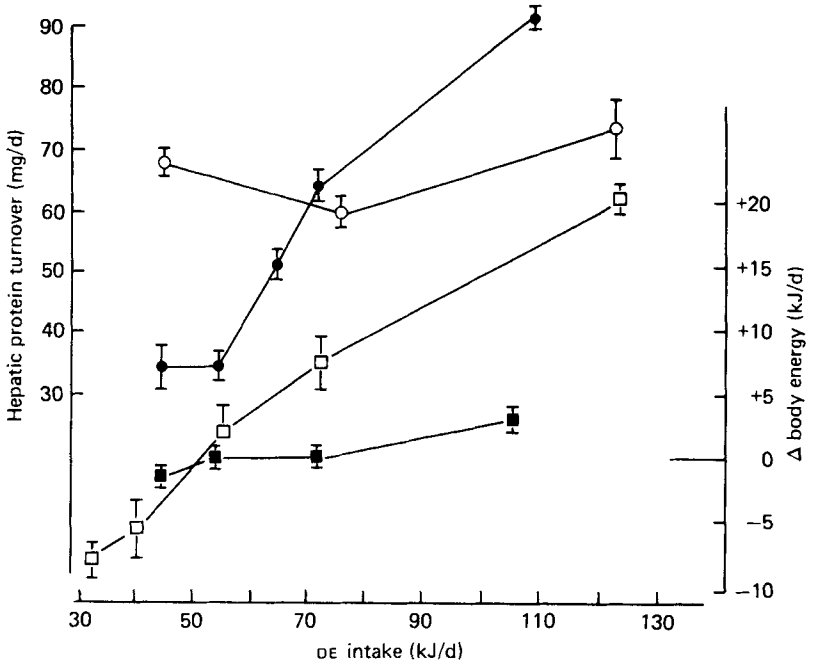

Fig. 2. The relationship between hepatic protein turnover (mg/d) (○, ●) and change in energy balance (△, body energy; kJ/d) (□, ■) in lean (*ob*/? ●, ■) and obese (*ob*/*ob*; ○, □) mice adapted to differing digestible energy (DE) intakes (kJ/d). Hepatic protein turnover was measured after injection of DL-[2-^{3}H]glutamate as described on p. 188. Points represent mean values, with their standard errors represented by vertical bars, for five observations.

Calculation of the liver protein turnover in the lean mice restricted to 43 kJ/d was complicated by the large variation in liver protein over the time-course of the experiment (Table 3). Even though these mice maintained body-weight at 43 kJ/d the protein content of individual livers increased with time, presumably reflecting earlier liver wastage and subsequent 'catch-up' growth. This growth did not invalidate the method of calculating the half-life for synthesis or catabolism, but it may have created an error in the calculation of total protein turnover (mg/d), since the quantity of hepatic protein was not constant.

Gut. In the lean mice (Fig. 3) no major increase in the rate of protein turnover was observed until the animals ingested very high intakes of food as an adaptive response to cold (4°) exposure. A slight increase was observed, however, in the lean mice receiving 62·8 kJ/d in a 4 h meal. Protein turnover (mg/d) in the gut of obese mice increased as dietary energy increased, but was always less than in lean mice. Tissue protein content decreased with dietary restriction in both genotypes, but was most marked in the obese mice (Table 4).

Kidney. The amount of protein turnover in the kidneys of lean animals (Fig. 3) showed a similar pattern to that observed in the gut from the same animals. In lean mice it was found to be increased only at the high food intakes obtained after exposure to 4°. The amount of kidney protein turnover (mg/d) in obese mice showed no change when DE was increased from 73·7 to 121·1 kJ/d. Kidney protein turnover (mg/d) of lean and obese mice were similar at all nutritional states in animals kept at 22°.

Comparative contribution of liver, gut and kidney protein turnover to whole body protein turnover. The contribution of kidney protein turnover to whole body turnover was small

Table 2. *The half-lives* ($t_{\frac{1}{2}}$; *d*) *of protein synthesis and catabolism in liver, gut and kidney in lean* (ob/?) *and obese* (ob/ob) *mice at different nutritional states*

(Mean values with their standard errors for no. of animals shown in parentheses. Each value for $t_{\frac{1}{2}}$ was calculated from analysis of the decay curves of log total and log specific radioactivity of tissue protein. All decay curves were linear with a significance of $P < 0.001$. The curves were obtained by killing four animals at 3, 6, 9, 12 and 15 d after intraperitoneal injection of 100 μCi DL-[2-^{3}H]glutamate. The animals were previously adapted to these food intakes over a 2-week period)

Phenotype	Digestible energy absorbed (kJ/d)	Liver $t_{\frac{1}{2}}$				Gut $t_{\frac{1}{2}}$				Kidney $t_{\frac{1}{2}}$			
		Synthesis		Catabolism		Synthesis		Catabolism		Synthesis		Catabolism	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
ob/?	43·5	4·42	0·33	5·82	0·61	3·49	0·19	3·50	0·19	4·54	0·18	4·88	0·29
(20)	53·1	5·26	0·62	6·01	1·0	3·59	0·17	3·79	0·18	4·22	0·15	4·67	0·38
	62·8	4·08	0·31	3·91	0·34	2·52	0·19	2·59	0·21	5·05	0·22	5·51	0·34
	69·9	3·74	0·18	3·81	0·16	3·99	0·25	4·00	0·24	4·99	0·24	5·55	0·37
	107·6	2·46	0·17	2·43	0·17	1·80	0·12	1·85	0·12	3·45	0·38	3·77	0·54
ob/*ob*	43·5	3·26	0·1	3·69	0·1	4·10	0·20	4·25	0·19	5·21	0·28	6·51	0·77
(20)	73·7	4·25	0·2	4·58	0·25	4·09	0·5	4·29	0·70	4·61	0·13	5·45	0·25
	126·1	4·25	0·39	3·92	0·2	3·39	0·24	3·37	0·20	5·42	0·73	5·84	0·9

Table 3. *Total liver protein content (mg) of lean* (ob/?) *and obese* (ob/ob) *mice fed at differing energy intakes*

(Mean values with their standard errors for four animals in each group at each time period)

Phenotype	Digestible energy intake (kJ/d)	Total liver protein (mg)											
		Period from start of experimental period (d)										Over-all mean	SE
		3		6		9		12		15			
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE		
ob/? (20)	107·6	318	6	326	9	324	8	330	7	322	11	324	2
	69·9	355	5	352	8	341	7	348	4	349	6	349	3
	62·8	289	12	302	9	289	7	295	11	310	6	297	4
	53·1	231	8	265	5	255	13	260	9	269	7	256	7
	43·5	161	11	176	8	240	8	235	1	268	6	216	20
ob/*ob* (20)	121·1	486	14	510	15	441	15	441	14	446	23	464	14
	73·7	383	12	357	13	364	10	358	4	387	6	369	6
	43·5	325	6	268	10	327	6	334	5	331	10	318	5

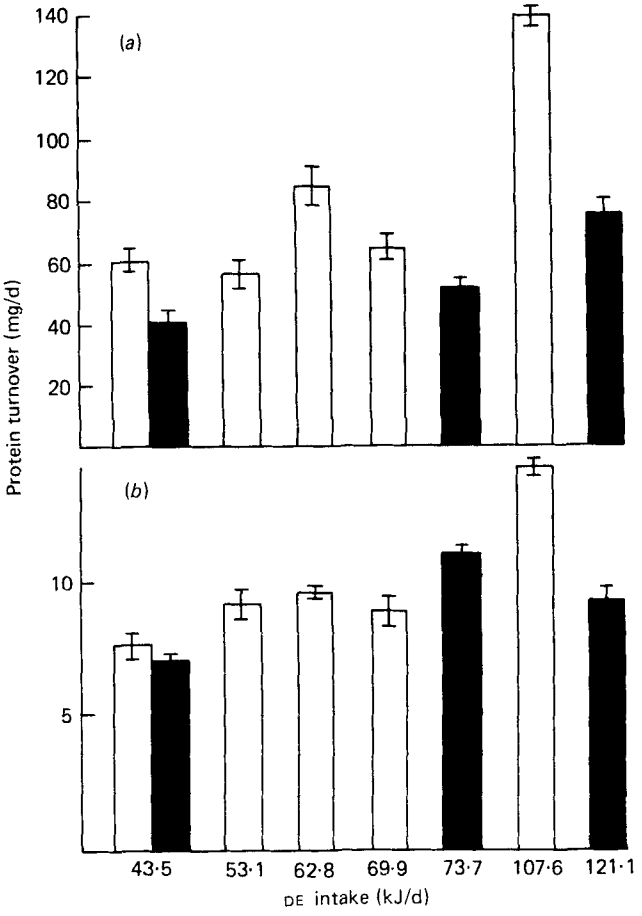

Fig. 3. The variation in total protein turnover (mg/d) in (*a*) gut and (*b*) kidney of lean (*ob*/?) (□) and obese (*ob*/*ob*) (■) mice fed at differing digestible energy (DE) intakes (kJ/d). Points represent mean values, with their standard errors represented by vertical bars, for five observations.

when compared with that of the gut and liver. Under all the regimens studied protein turnover was > 40 mg/d in the gut and > 30 mg/d in the liver, whereas kidney protein turnover was < 15 mg/d.

Comparison of observed and theoretical protein deposition rates

The theoretical rate of protein deposition may be calculated from the difference between the products of mean tissue protein content and fractional synthetic rate and mean tissue protein content and fractional breakdown rate.

There was good agreement between the theoretical rate of protein deposition and the observed rate of deposition in both groups of lean mice that showed an increase in liver protein content during the time course of the experiment.

For the group eating 43 kJ/day, the observed rate of deposition was 8·9 mg/d, compared with a theoretical value of 8·2 mg/d. The group eating 53 kJ/d had an observed value of 2·9 mg/d and a theoretical value of 4·3 mg/d.

Table 4. *Total gut protein content (mg) of lean* (ob/?) *and obese* (ob/ob) *mice fed at differing energy intakes*

(Mean values with their standard errors for four animals in each group at each time period)

		Total gut protein (mg)											
		Period from start of experimental period (d)											
		3		6		9		12		15		Over-all mean	
Phenotype	Digestible energy intake (kJ/d)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE		SE
ob/? (20)	107·6	347	13	370	5	362	6	361	9	398	10	366	1
	69·9	356	6	372	9	393	10	396	5	370	11	363	12
	62·8	269	11	299	7	313	7	293	3	325	9	300	9
	53·1	297	5	278	7	302	17	306	8	292	5	295	9
	43·5	283	7	309	5	297	6	296	4	296	7	301	9
ob/ob (20)	121·1	376	11	385	7	368	8	388	6	367	7	376	13
	73·7	310	4	304	6	290	12	315	6	325	12	309	6
	43·5	241	19	221	3	271	11	271	20	241	10	247	8

Table 5. *Total kidney protein content (mg) of lean* (ob/?) *and obese* (ob/ob) *mice fed at differing energy intakes*

(Mean values with their standard errors for four animals in each group at each time period)

		Total kidney protein (mg)*											
		Period from start of experimental period (d)											
		3		6		9		12		15		Over-all mean	
Phenotype	Digestible energy intake (kJ/d)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE		SE
ob/? (20)	107·6	74	3	65	4	71	2	63	6	78	7	70	2
	69·9	62	6	66	4	59	3	68	1	75	3	65	2
	62·8	63	2	72	3	67	1	69	2	79	4	70	1
	53·1	58	2	43	2	56	4	58	3	65	7	56	2
	43·5	48	4	48	3	47	2	52	3	53	6	49	2
ob/ob (20)	121·1	68	3	73	4	72	3	67	2	71	2	70	2
	73·7	65	4	70	5	69	3	70	4	70	5	67	2
	43·5	54	3	54	3	52	5	54	1	55	2	53	1

* The two kidneys were homogenized together and subsequently treated as one.

For the kidney and intestine the half-lives of synthesis and catabolism of protein were very similar, although they did vary with energy intake (Table 2). These values agree with the observation that no net changes in tissue protein content were observed in either tissue at any energy intake during the time-course of the experiment (Tables 4 and 5). These values provide further validation of the experimental technique.

DISCUSSION

Maintenance energy requirement. The daily energy requirement for maintenance of body-weight, as calculated from the relationship of body energy content and energy intake in Fig. 2 was very similar for lean (55 kJ/d) and obese (53 kJ/d) mice. Although the obese mice were heavier than the lean mice at this time (Fig. 1), it is not appropriate to relate these maintenance energy requirements to body-weight or body-weight$^{0.75}$ since over 90% of the excess weight of the obese mouse may be accounted for solely by the weight of the extra triglyceride stored (Bates *et al.* 1955; Dubuc, 1976). It would be more appropriate to relate the maintenance energy requirements to the lean body mass of the animals. In this study (results not presented) and in several other published studies, it has been shown that the lean body mass of the obese mouse is normal or slightly decreased when compared to that of its age-matched lean litter-mate despite the large increase in body-weight (York, Otto *et al.* 1978; Thurlbey & Trayhurn, 1978; Dubuc, 1976; Lin *et al.* 1977). Thus the same conclusion, i.e. the maintenance energy requirement of lean and *ob/ob* mice are similar, would be reached if the values were expressed on the basis of lean body-weight rather than whole animal. Despite the similarity in maintenance energy requirements, it is clear that at energy intakes above the maintenance level the *ob/ob* mouse is more efficient at storing energy than the lean mouse, confirming similar results of many previous studies (Bray & York, 1971; Lin *et al.* 1977; Coleman, 1978).

Definition of protein turnover and its relationship with protein synthesis, protein catabolism and tissue protein content. Considerable confusion exists in the literature as to the precise definition of protein turnover (Schimke, 1970). In this paper, protein turnover is regarded as the replacement of an amount of protein by an equal quantity of the same material but newly synthesized from its metabolic precursors which are themselves synthesized or transported into the system from outside (Munro, 1964). According to this definition the rate (half-life) of protein turnover is dependent upon the rate-limiting component which may be either protein synthesis or catabolism. It is, therefore, desirable in the measurement of protein turnover to measure protein synthesis and catabolism simultaneously (Millward & Garlick, 1972).

In this study, neither the rate of protein turnover ($t_{\frac{1}{2}}$) nor the amount of protein turnover was correlated with the net deposition of protein, as has been claimed previously (Pullar & Webster, 1974). In the livers of lean mice absorbing 43·5 and 53·1 kJ/d, the livers were undergoing a rapid 'catch-up' growth with net protein deposition, yet both the half-life of turnover and total protein turnover were low. In contrast, in the livers of lean mice absorbing 69·9 and 107 kJ/d there was a high rate of protein turnover (mg/d) although no net deposition of protein occurred during the time-course of the experiment. These results suggest that the control of protein turnover is independent of the balance between protein synthesis and catabolism. A similar conclusion can be drawn from the study of intestinal protein turnover.

Changes in protein turnover in animals fed at differing intakes were a function of changes either in the rate-limiting half-life ($t_{\frac{1}{2}}$) or in the tissue protein content, or both. The decrease

in tissue protein in dietary-restricted animals may reflect the requirements for mobilization of tissue protein for gluconeogenesis during the early adaptation to the reduced energy intake. However, since the livers of mice in the most severely restricted groups underwent catch-up growth after the initial adaptation period, and other tissues showed no significant net growth, this mobilization of tissue protein is unlikely to have been significant during the experimental period. It is therefore possible that the decrease in tissue protein content associated with changes in the rate of turnover in the tissues studied was an adaptation to dietary restriction.

Metabolism of protein in a tissue is therefore likely to be influenced by at least three considerations: the need to (1) control tissue protein content by balancing the rate of protein synthesis and catabolism; (2) provide substrate for gluconeogenesis in the post-adsorptive phase (Felig & Wahren, 1974); (3) control the rate of protein turnover independent of the balance between synthesis and catabolism.

Energy balance and protein turnover

Lean mice were more efficient than obese mice in maintaining energy balance when food intake was decreased below or increased above the maintenance energy requirements. Indeed, over the range of energy absorbed from 55 to 73 kJ/d lean mice dissipated all of the energy ingested in excess of their basal requirement and showed no storage of energy, whereas obese mice displayed no comparable control. This range over which the lean animals maintained neutral energy balance was very similar to the range over which the curve for hepatic protein turnover was linear. An increase in protein turnover could stimulate intracellular respiration and thermogenesis by causing a more rapid conversion of ATP to ADP and AMP (Yousef & Chaffee, 1970). These results would support the hypothesis that the energy cost of protein turnover is an important mechanism for the control of energy loss (Garrow, 1974). If this hypothesis is correct, it could explain the rapid weight loss shown by the obese mice in comparison to lean controls when subjected to dietary restriction (Fig. 4), since the obese mice did not depress their protein turnover after dietary restriction, this component of their energy expenditure must have remained unchanged. These results also suggest that protein turnover in intestine and kidney may be increased at high food intakes when the energy balance control mechanisms are severely stressed by the increased food intake associated with cold adaptation.

Animals presented with restricted amounts of food inevitably eat 'meals' whereas mice fed *ad lib.* (24 h/d) nibble their food. Cohn & Joseph (1959) have shown that animals adapted to a meal-eating routine when compared to animals pair fed on a nibbling routine show increased body-weight due to increased storage of body fat. However, other than the intestine in lean animals digesting 62·9 kJ/d which had increased protein turnover for such a food intake, no evidence was found for the feeding pattern influencing protein turnover. All other changes in protein turnover in liver, intestine and kidney are explicable entirely in terms of DE intake. Nassett (1964) has suggested that gastrointestinal secretions and mucosal shedding by the alimentary canal may act as a homoeostatic device in the prevention of wide fluctuations in plasma amino acid levels between meals. The increase in protein turnover of the alimentary canal in the meal-fed unrestricted mice may be a reflection of such a homoeostatic mechanism. In the meal-fed restricted animals, the response was not apparent, possibly due to substrate levels for intestinal protein synthesis being rate-limiting.

Nettleton & Hegsted (1975) in a similar study on normal rats have reported that varying energy intake has no influence on the half-life for catabolism of liver or gastrocnemius muscle proteins. However, the total amount of protein turnover (mg/d) is a function of both the rate-limiting half-life and total tissue protein, and is only meaningful in energetic

terms as such. Nettleton & Hegsted (1975) showed that the half-life for catabolism of liver protein was constant. However, since the hepatic protein content of their rats fed at restricted energy intakes decreased by up to 50%, this means that total liver protein turnover (mg/d) also decreased significantly as energy intake was reduced. These results, when interpreted in this manner, support the findings presented here. The work of Nettleton & Hegsted (1975) may be further criticized since they only measured the rate of protein catabolism, and assumed it to be indicative of the rate of protein turnover, an assumption which as discussed earlier is not always true.

It has been suggested (Yousef & Luick, 1969) that the increase in protein turnover after cold adaptation might provide a significant contribution to the increased thermogenesis. Although they attempted to determine the extent of the importance of an increased food intake to the cold-induced thermogenesis by comparison of cold-adapted with non-adapted animals, they did not control either weight or age in their two experimental groups which made interpretation of the results on protein turnover very difficult. In our studies, the highest food intakes in lean mice were obtained after adaptation to cold. The results suggest that the increased thermogenesis in cold animals is merely a reflection of the increased food intake. This supports the hypothesis that DIT and non-shivering thermogenesis (NST) may be different expressions of the same phenomenon (Stirling & Stock, 1973). The observations that hyperthermia is associated with a reduction of food intake and protein turnover (Yousef & Johnson, 1970) give further support to this concept. Since muscle protein turnover has been shown to contribute significantly to whole-body protein turnover (Millward & Garlick, 1972), and skeletal muscle contributes 50% to NST (Jansky, 1973), it is probable that DIT should also be associated with changes in muscle protein turnover. This possibility is currently under investigation.

The hormonal mechanism controlling tissue protein turnover is not clear. Neither adult (Davis & Mayer, 1954) nor 2-week-old (Trayhurn *et al.* 1977) *ob/ob* mice can survive cold stress. After exposure to 4° for 1 h, obese mice fail to display the normal increment in the incorporation of [^{3}H]leucine into liver slices (Miller *et al.* 1977). Furthermore, obese (*ob/ob*) mice display peripheral resistance to thyroid hormones, which may be improved by prolonged treatment with triiodothyrinine (T_3) (Otto *et al.* 1976; York, Otto *et al.* 1978) and such treatment improves homoeothermia upon cold exposure in *ob/ob* mice (Ohtake *et al.* 1977). The rate of net protein deposition and the heat production of *ob/ob* mice both improve after T_3 therapy (York, Otto *et al.* 1978). Thyroidectomy in rats decreases protein turnover (Yousef & Johnson, 1970) and lean (*ob/?*) mice show a comparable rate of decrease in body temperature to their obese (*ob/ob*) siblings if made hypothyroid by treatment with propylthiouracil (Ohtake *et al.* 1977). It has also been demonstrated that thyroid activity increases during cold adaptation (Hoch, 1974), and that thyroid hormones increase the rate of incorporation of amino acids into microsomal (Tata *et al.* 1963) and mitochondrial (Bronk, 1963*a*,*b*) proteins. These observations suggest that thyroid hormones may be implicated in the loss of control of protein turnover in *ob/ob* mice.

The results presented here suggest that the amount of protein turnover is closely associated with the level of dietary intake. However, the amount of hepatic protein turnover in *ad lib.* feeding *ob/ob* mice is less than in lean mice at the same dietary intake. These results suggest the possibility that a poorly-controlled protein turnover in *ob/ob* mice significantly contributes both to their positive energy balance at room temperature and to their death when placed in the cold. The relationship of these findings, if any, to the proposed defect in [$Na^+ + K^+$] ATPase (*EC* 3.6.1.3) in *ob/ob* mice (York, Bray *et al.* 1978) awaits clarification.

The relevance of the results reported in this paper to the aetiology of human obesity is

at present unclear. Garrow (1974) has calculated that approximately half the resting energy expenditure in a normal adult is associated with protein turnover, providing a potential range for the control of energy expenditure of ± 1600 kJ/d. Waterlow (1968) has in fact demonstrated a good correlation between protein turnover and basal metabolic rate. Also, in malnourished children, good correlations between protein synthesis and the thermic effect of food (Ashworth, 1969) and between dietary energy intake and protein synthesis (Golden *et al.* 1977) have been observed.

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