Nitrogen Balance in Men with Adequate and Deficient Energy Intake at Three Levels of Work[1,2]

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ABSTRACT Two studies were conducted to investigate the effects of mild exercise on nitrogen balance in men given diets supplying adequate or slightly limiting energy. In experiment A the diet supplied 91 mg N/kg body weight (0.57 g protein/kg, the FAO/WHO safe level of intake) as egg white; in experiment B the same source was used to provide the 1980 NRC-RDA for adult males, 128 mg N/kg body weight (0.8 g protein/kg). By adjusting energy intake and activity, periods of energy equilibrium and negative energy balance (−15%) were achieved at three levels of activity (X for exercise): no programmed work (0.85X), 1 hour of treadmill walking (1.0X) and 1 hour each of treadmill and cycle ergometry (1.15X). "True" nitrogen balance (TNbal) was more positive or less negative during periods of energy equilibrium as compared to those of energy deficit. This effect of energy balance on TNbal increased with physical activity. At the lower protein intake the mean difference in TNbal between the period of energy equilibrium and that of energy deficit at 1.0X was 0.19 g N/day (nonsignificant difference) and 0.54 g N/day at 1.15X. When protein intake was increased, the difference in TNbal between periods of equilibrium and deficit was significant at all levels of activity: 0.65 g N/day at 0.85X, 0.93 g N/day at 1.0X and 1.09 g N/day at 1.15X. Physical activity was anabolic when energy balance was maintained. In experiment A the addition of 1 hour of exercise (1.0X to 1.15X) spared 2.5 mg N/kg body weight; reducing activity by 1 hour (1.0X to 0.85X) cost 1.4 mg N/kg body weight. In experiment B, TNbal was more positive with increased activity (by 5.9 mg N/kg body weight) and more negative (by 11.5 mg N/kg body weight) when the men were sedentary. During periods of energy deficit, the anabolic effect of activity was also present, although less markedly. When activity increased from 1 to 2 hours in experiment A, TNbal improved by 2.1 mg N/kg body weight and in experiment B, by 3.5 mg N/kg body weight. Thus, circumstances of negative energy balance with adequate protein intake are better tolerated when the energy deficit is generated by physical activity than when it derives from reduced intake; the picture when protein intake is marginal requires further investigation. J. Nutr. *114*: 2107–2118, 1984.

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International (1) and national (2) recommendations for protein intake are presumed to be safe allowances for persons who meet their energy requirements, regardless of activity level. Different activity patterns must be associated with correspondingly different energy intakes if energy is in balance. The protein-sparing effect of increasing energy intake is well known and well documented (3–7). Recent research in this laboratory (8) indicates that added

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[2]A preliminary report of part of this research was presented at the 65th Annual Meeting of the Federation of American Societies for Experimental Biology, Atlanta, Georgia, April 12-17, 1981. Butterfield, G. & Calloway, D. H. (1981) Protein utilization (PrU) in men fed inadequate energy at three levels of work. Fed. Proc. *40*, 857 (abs. 3565).

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energy improves nitrogen retention even when added energy intake is balanced by an equivalent increase in energy expenditure in the form of steady-state work.

The effect of physical activity on protein and energy utilization has not been sufficiently evaluated to know if it needs to be taken into account in setting requirements. Some believe that protein requirement (9) [or more specifically essential amino acid requirement (10, 11)] is increased in conjunction with increased physical activity, as is energy need. Data from less developed countries, where moderate to heavy manual labor is a way of life and protein intakes are less than generous, indicate, however, that this belief is not well grounded (12). It seems important to know whether or not exercise is anabolic when energy intake is insufficient as might be the condition at some times in developing countries.

Two experiments were conducted to investigate the short-term effects of mild to moderate exercise on nitrogen balance in healthy men. The results now to be reported show that activity is consistently anabolic, but less so under energy-deficient than energy-adequate conditions, and less so with marginal than moderate protein intake.

MATERIALS AND METHODS

Subjects. Twelve male volunteers participated in these studies (table 1). Their health, nutritional status and endocrine function were assessed to be essentially normal by medical examination and history. One man, no. 5007, was moderately obese (33% body fat). Men engaged in endurance training or strenuous physical activity were excluded from selection. Throughout the experiments the men were confined to a metabolic unit[4] with the exception of brief supervised outings of less than 2 hours duration once or twice each month. To standardize energy expenditure, periods of exercise on a treadmill or cycle ergometer were prescribed. At other times, the men were restricted to quiet or light activities. General procedures in the unit were described elsewhere (13).

Study design. Two experiments scheduled 3 years apart are reported here.[5]

Experiment A: The experiment was divided into six periods (table 2). For 6 days prior to the study, to standardize intake, the men were given ordinary food of approximately constant protein and energy content to eat at home. A 9-day in-house preexperimental period allowed for adaptation to diet and the conditions of the metabolic unit. During this time, the men received a defined formula diet supplying 160 mg egg white N (1.0 g protein) per kilogram body weight. At this time the men were required to walk 60 minutes/day on a motor-driven treadmill (3 miles/hour, 10% grade). Energy expenditure for this assigned work represents about 15% of their maintenance energy intake under the conditions specified. This activity pattern, 1 hour of prescribed plus unprogrammed sedentary activities, was considered the standard activity condition (X for exercise: 1.0X). Energy intake determined under these conditions was considered to be the individual's maintenance requirement (1.0E). For the subsequent five periods (each 18 days long), N intake was set at 91 mg (0.57 g protein) per kilogram body weight, the FAO/WHO recommended safe level of intake (1).

Periods of energy equilibrium (0) and marginally negative energy balance (−0.15) were achieved by manipulating energy intake and activity. In test period 1, a period of energy equilibrium, energy intake and expenditure were as in the preexperimental period (1.0E, 1.0X), but at the lower N intake. During periods 2 and 3, additional exercise on the cycle ergometer (50 rpm, 600 kp) was assigned. The time spent (average, 60 minutes/day) at this activity was adjusted according to the individual's rate of energy expenditure on the cycle to provide exercise equivalent to an additional 15% of the 1.0E level of energy intake. Energy equilibrium was achieved in period 2 by increasing energy intake to cover the extra exercise (1.15E, 1.15X). An energy deficit was planned in period 3 by decreasing energy intake to the level fed during standardization while the extra exercise was continued (1.0E, 1.15X). Negative energy balance of a similar magnitude was achieved in period 4 when

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[5] Protocols and procedures for these experiments were approved by the Campus Committee for the Protection of Human Subjects, and all participants were given the opportunity to ask and have answered all questions before signing a written consent form.

TABLE 1

Characteristics of participants

Code	Age	Height	Body wt[1]	Lean mass[2]	Urinary creatinine[3]	Energy need[4]
	yr	*cm*	*kg*	*kg*	*g/day*	*kcal/day*
5002	28	173.0	71.2	56.7	1.47	2848
5003	26	181.0	73.5	61.3	1.46	2870
5004	27	181.0	83.6	64.0	1.62	3093
5007	28	180.0	98.9	66.5	1.68	3545
5008	23	172.0	63.4	56.4	1.32	2536
5009	24	173.0	60.3	52.2	1.21	2408
Mean ± SD	26 ± 2	176.7 ± 4.4	75.1 ± 14.2	59.5 ± 5.4	1.46 ± 0.19	2883 ± 407
6001	35	173.3	65.6	53.8	1.39	2680
6002	29	176.0	72.4	60.5	1.60	2997
6003	29	166.5	71.3	55.9	1.66	2709
6004	23	183.2	73.9	55.1	1.82	2808
6006	28	167.5	59.6	46.5	1.25	2609
6007	31	180.5	76.2	55.2	1.39	3036
Mean ± SD	29 ± 4	174.5 ± 6.8	69.8 ± 6.1	54.5 ± 4.5	1.52 ± 0.21	2761 ± 150

[1]Initial body weight entering study. [2]Calculated from underwater weighing (expt A) or skinfold thickness (expt B) during period 1. [3]Mean for entire study. [4]Energy intake to maintain body weight with 1 hour treadmill exercise daily, i.e., control conditions (1.0E, 1.0X).

the cycle exercise was discontinued, and energy intake was dropped another 15% (0.85E, 1.0X). Energy equilibrium was intended in period 5 when the treadmill, too, was discontinued (0.85E, 0.85X).

All exercise sessions were scheduled on a rotating daily plan to eliminate any effect that the timing of exercise relative to meals

TABLE 2

Experimental design

Period	Days	Energy: Intake	Energy: Expenditure	Energy: Intended balance	Period descriptor[1]
		Expt A — Protein intake 0.57 g/kg, periods 1-5			
Preexpt period	9	1.00[2]	1.00[3]	0	1.0E, 1.0X
1	18	1.00	1.00	0	1.0E, 1.0X
2	18	1.15	1.15[4]	0	1.15E, 1.15X
3	18	1.00	1.15	−0.15	1.0E, 1.15X
4	18	0.85	1.00	−0.15	0.85E, 1.0X
5	18	0.85	0.85[5]	0	0.85E, 0.85X
		Expt B — Protein intake 0.85 g/kg, all periods			
1	18	1.00[2]	1.00[3]	0	1.0E, 1.0X
2	15	0.85	1.00	−0.15	0.85E, 1.0X
3	15	0.85	0.85[5]	0	0.85E, 0.85X
4	15	0.70	0.85	−0.15	0.70E, 0.85X
5	6	1.00	1.00	0	1.0E, 1.0X
6	15	1.15	1.15[4]	0	1.15E, 1.15X
7	15	1.00	1.15	−0.15	1.0E, 1.15X
8	6	1.00	1.00	0	1.0E, 1.0X

[1]E, energy; X, exercise. [2]Energy intake required to maintain stable body weight. [3]Includes 1 hour/day on motor-driven treadmill (3 mph, 10% grade). [4]Includes 1 hour/day on treadmill and approximately 1 hour/day on cycle ergometer (50 rpm, 600 kp). [5]No scheduled exercise.

might have on protein utilization (14). Thus, each man exercised at a different time each day. Twenty-minute periods on the treadmill were scheduled between 0930 and 1330 hours, 1430 and 1830 hours and 1930 and 2300 hours; half-hour periods of cycling were scheduled between 0930 and 1330 hours and between 1430 and 1830 hours. The cycle sessions for each man were usually scheduled within 30 minutes of the end of the treadmill walk.

The data presented here are derived from three men who started the experiment initially (nos. 5002, 5003 and 5004), and three others who began the study on day 55 and followed the same sequence of periods as did the original men (nos. 5007, 5008 and 5009).

Experiment B: The second experiment differed slightly in design (table 2). The constant N intake was higher, 128 mg (0.8 g protein) per kilogram initial body weight, the 1980 NRC Recommended Dietary Allowance for male adults (2). Period 1 (18 days) was an adaptation period. In other periods (15 days each) energy intake was varied from 0.70 to 1.15 of the level fed at the end of period 1. Energy intake and expenditure were varied such that there were periods of energy equilibrium and deficit (−15%) at three levels of work, i.e., no programmed work (0.85X), 1 hour of treadmill walking (1.0X), and 1 hour of treadmill and 1 hour of cycle ergometer work (1.15X). Periods 5 and 8 (6 days each) were restandardization periods and are not included in the statistical analyses. One man left the study before the end of the second period and his replacement, no. 6007, completed 67 days of the experiment by following the treatment sequence described for periods 1 through 5.

Diet. In both experiments a liquid, purified diet (table 3) was fed in three equal portions at 0830, 1330 and 1830 hours. Individual protein and energy requirements were met by adding egg white and/or a protein-free caloric supplement to the basic formula. The diet and the vitamin and mineral supplements were planned to provide all known nutrients in amounts thought to be adequate for healthy men. To add variety to the diet, a few protein-free foods were included. Up to 3 drops of nonnutritive synthetic flavoring were added by some men to the formula at each meal.

Energy intake was initially set at 40 kcal/kg body weight and adjusted as necessary during the first period to maintain constant body weight.

Composites of the total daily intake of the largest and smallest man in each experiment were made each time the diet was changed. The basic formulas and diet composites were analyzed for N content by the micro-Kjeldahl method, as modified by Block and Weiss (15) and by using a selenium catalyst. N intake of each man was computed from these analyzed values.

Procedures. Feces were collected as 3-day pools, frozen immediately, and later were homogenized in a colloid mill with a weighed amount of deionized water. Urine was collected for each 24-hour period after the first morning voiding (0800 to 0815 hours). Urine was refrigerated without preservative during collections and diluted to volume for analysis.

Fecal and urinary samples were analyzed for total N by the micro-Kjeldahl method described above. Creatinine was measured in daily urine samples (automated alkaline picrate method, Autoanalyzer Method N116, Technicon Corp., Chauncey, NY). Urine samples were discarded in the very few instances where creatinine content was exceptional. Three-day pools of urine were analyzed for urea (16).

Skin and sweat N losses were measured for three consecutive days during all test periods except period 6 of experiment B. The method used, involving the wearing of chemically treated clothing, has previously been described (13). The last sweat measurement in experiment B was eliminated to accommodate subject comfort during unseasonably warm weather; values from period 6 have been applied to period 7 as the exercise level was the same.

Blood samples were taken before the study and on the morning of discharge for routine health status evaluation. At regular intervals blood was drawn for determination of other parameters; the results have been published separately (17, 18). The total volume of blood taken during experiment A was 615–707 g/subject, representing an average loss of approximately 0.18–0.19 g N/day (13); in experiment B, 220–240 g of blood was drawn, amounting to 0.06–0.07 g N/day. Body weight

TABLE 3

Diet composition

Component	Expt A	Expt B
	Basic formula[1,2]	
	g/70 kg man day[3]	*g/subject day*[4]
Egg white, dry	48.4	45.2
Maltodextrins	124.5	76.4
Cornstarch	124.5	76.4
Sucrose	30.5	2.8
Cottonseed oil	61.1	30.9
Lecithin	—	13.9
Sodium chloride	4.0	1.9
Potassium hypophosphate	1.9	0.3
Potassium chloride	3.3	3.9
Calcium citrate	2.4	2.5
Magnesium oxide	0.2	0.1
Sodium bicarbonate	2.5	2.0
α-Cellulose	4.0	3.1
Raffinose	2.0	1.3
Pectin	—	1.9
	mg/70 kg man day	*mg/subject day*
Biotin	—	0.2
Vitamin K	1.0	7.0
Sodium molybdate	4.0	0.5
Sodium fluoride	2.2	1.3
	Energy formula[5]	
	Proportion of component	
Maltodextrins	15.5	15.2
Cornstarch	15.5	15.2
Sucrose	3.9	3.9
Cottonseed oil	7.8	7.9
Water	57.4	57.7
	Protein formula[6]	
	mg N/g wet wt	
Egg white in water	31	
Additional diet components[7]		

[1]Diets supplied minerals in the following amounts: expt A, per 70 kg man, in grams/day: sodium, 3.1; potassium, 3.0; calcium, 0.80; magnesium, 0.40; phosphorus, 0.81; chloride, 4.7. Expt B: per man, in grams/day: sodium, 2.42; potassium, 3.21; calcium, 0.91; magnesium, 0.35; phosphorus, 0.92; chloride, 3.0. [2]In both experiments, vitamin supplements (Bronson Insurance Formula) given daily provided in milligrams/day: vitamin A palmitate, 2.25; choline bitartrate, 500; ascorbic acid, 250; 2-amino-α-tocopheryl acetate, 0.40; thiamin mononitrate, 2; riboflavin, 2; pyridoxine, 3; niacinamide, 20; *d*-pantothenic acid, 15; biotin, 0.3; folacin, 0.4; iron (ferrous fumarate), 15; zinc (gluconate), 15; copper (gluconate), 2; iodine (as kelp), 0.15; manganese (gluconate), 5; molybdenum (sodium molybdate), 0.1; chromium (chromic sulfate), 1; selenium (dioxide), 0.02; and in micrograms/day: vitamin B-12 (cobalamin), 9; cholecalciferol, 10. These commercial tablets also included nondietary essentials: inositol, 250; *p*-aminobenzoic acid, 0.3; and rutin, 200. [3]Energy equivalent for 70 kg man, 1800 kcal; fed according to individual body weight at constant levels throughout experiment. [4]Energy equivalent, 1200 kcal; fed at this level to each man throughout experiment. [5]Provided 2 kcal/g formula. Varied as necessary to provide the stipulated energy level. [6]As needed to provide specified protein allowance. [7]Instant decaffeinated coffee powder, 1.0 g; in expt A men had two cups of coffee, one cup of tea on alternate days; two cups of tea, one of coffee on alternate days. Instant tea powder, 0.5 g; in expt B men had two cups of tea each day. In expt B fruit juices (grams) were fed in a 3-day cycle: peach nectar, 145.8; lemonade concentrate, 32.8; grape juice concentrate, 37.8. Lifesaver candies (fed twice a day, expt A), 1 piece. Sugarless gum (expt A), 1 stick.

was recorded daily after voiding at approximately 0800 hours. In experiment A body composition was measured at the end of each period, by hydrostatic weighing (19). During experiment B, skinfold measurements were made twice at the end of each metabolic period, and body composition was calculated by using the method of Durnin and Rahaman (20).

Calculated data. Crude N balance was calculated for each man for each metabolic period by using analyzed N intake (NI), average urinary N excretion (UN) during the last 6 days of each period, and the average fecal excretion (FN) for all but the first 3 days of each metabolic period. "True" N balance (TNbal) was calculated by subtracting from crude balance the individual sweat losses (SN) for each period, N lost in blood, and an allowance for unmeasured miscellaneous losses [0.09 g/day (13)].

Data analysis. Two-way analysis of variance was performed to evaluate the effects of energy balance and exercise on measured parameters. Pairwise comparisons were made between periods of energy equilibrium and deficit at different work levels. Study design does not allow evaluation of possible sequence effects. Tukey's test (21) was used to determine significant difference between means, with $P \leq 0.05$ as the accepted level of significance.[6] The computer program, Statistical Package for the Social Sciences,[7] was used for all determinations.

RESULTS

Health. Excluding minor complaints (occasional headaches, athlete's foot), the men remained healthy and tolerated the procedures, diet and confinement without problems.

Energy intake, body weight and body composition. Energy intake, mean body weight at the end of each metabolic period and body weight change during the last 6 days of each period are given in table 4. In both studies, maintenance of body weight was the criterion of adequate energy intake under the control condition (1.0X). Weight changes of less than 0.5 kg from day to day or 0.2 kg over several days were considered within the range of normal fluctuations and equivalent to no weight change (22).

The men were initially fed 40 kcal/kg body weight, except no. 5007, whose allowance was calculated according to his ideal body weight. Energy intake was adjusted until weight was thought to be steady, but true maintenance requirement was not determined in the case of nos. 6007 and possibly 6006. Mean body weight change during the last 6 days of the control period was -0.02 ± 0.07 kg/day in experiment A, and 0.0 ± 0.06 kg/day (excluding 6007) in experiment B. The final daily mean energy requirement for weight maintenance (1.0E) with standard activity was 38.6 ± 2.2 kcal/kg body weight in experiment A. The mean energy requirement for weight maintenance in experiment B was 40.7 ± 2.6 kcal/kg body weight, a value closer to that found in previous experiments [41 kcal/kg (8, 23)]. The maintenance energy intake was not, however, significantly different for experiments A and B.

With some individual exceptions (nos. 5003 and 6007, particularly), weight changes throughout both experiments were as would be expected according to the energy balance conditions, i.e., more weight was lost or gained during energy deficit periods than during periods of intended balance. Comparison of weight change over the total treatment periods with weight changes the last 6 days of each suggests some variability in the rate of weight change throughout the period. However, a weighted average of the slopes of the regression lines for each individual showed no significant difference in the rates of weight change during any period of study.

The methods for determination of body composition used in these experiments are not sufficiently sensitive to determine the composition of the small amounts of tissue lost or gained. There was no significant change detected in percentage of body fat throughout the experiments.

Nitrogen excretion. Statistical analysis of the FN values showed no effect of energy and activity conditions in experiment A. Although there was wide variation in individual response in experiment B, mean FN

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[6] One participant in experiment B (no. 6007) had fasted several days before beginning the study and appeared to be repleting body stores throughout the study. For statistical analysis, this man's data were not included in the calculation of mean values. His data are presented separately.

[7] Northwestern University, Evanston, IL.

TABLE 4

Energy intake per kilogram of body weight, body weight and body weight change of young men under two conditions of energy balance and three levels of work[1]

Measure	Treatment[2] 0.85E, 0.85X	0.70E, 0.85X	1.0E, 1.0X	0.85E, 1.0X	1.15E, 1.15X	1.0E, 1.15X
			Expt A			
Energy intake, *kcal/kg*	33.9 ± 1.7		38.6 ± 2.2	33.3 ± 1.7	44.3 ± 2.5	38.5 ± 2.0
Body wt, *kg*	72.8 ± 13.9		75.5 ± 15.0	73.2 ± 14.1	75.6 ± 14.7	74.4 ± 14.5
Body wt change, *kg/day*						
5002	0.00 ± 0.19		−0.08 ± 0.16	−0.16 ± 0.21	−0.06 ± 0.05	−0.04 ± 0.19
5003	−0.06 ± 0.09		−0.02 ± 0.40	−0.02 ± 0.18	−0.08 ± 0.31	−0.06 ± 0.19
5004	0.00 ± 0.22		−0.12 ± 0.15	−0.04 ± 0.17	0.04 ± 0.51	−0.12 ± 0.51
5007	−0.04 ± 0.40		−0.06 ± 0.05	−0.06 ± 0.19	−0.04 ± 0.42	−0.20 ± 0.24
5008	−0.08 ± 0.13		0.00 ± 0.10	−0.06 ± 0.21	−0.10 ± 0.07	−0.08 ± 0.11
5009	0.00 ± 0.30		0.02 ± 0.13	−0.10 ± 0.39	0.00 ± 0.19	−0.08 ± 0.22
Group mean	−0.03 ± 0.04		−0.02 ± 0.07	−0.07 ± 0.05	−0.04 ± 0.05	−0.10 ± 0.06
			Expt B			
Energy intake,[3] *kcal/kg*	35.0 ± 2.8	28.9 ± 2.3	40.7 ± 2.6	34.9 ± 3.0	47.7 ± 3.7	40.7 ± 3.1
Body wt, *kg*[3]	67.3 ± 7.1	66.3 ± 7.1	68.2 ± 6.8	67.5 ± 7.1	67.8 ± 7.11	67.4 ± 6.8
Body wt change, *kg/day*						
6001	0.04 ± 0.34	−0.08 ± 0.19	−0.08 ± 0.31	0.02 ± 0.11	−0.04 ± 0.30	−0.04 ± 0.30
6002	0.04 ± 0.13	−0.14 ± 0.21	0.06 ± 0.34	−0.06 ± 0.11	0.04 ± 0.18	−0.06 ± 0.33
6003	0.02 ± 0.29	−0.10 ± 0.20	0.00 ± 0.14	−0.02 ± 0.16	0.08 ± 0.18	−0.02 ± 0.13
6004	0.02 ± 0.47	−0.06 ± 0.27	0.04 ± 0.18	−0.10 ± 0.16	0.06 ± 0.26	−0.08 ± 0.23
6006	0.00 ± 0.29	−0.04 ± 0.30	−0.04 ± 0.09	−0.08 ± 0.26	0.06 ± 0.35	−0.04 ± 0.09
6007	−0.06 ± 0.15	0.10 ± 0.21	−0.08 ± 0.25	0.00 ± 0.22	—	—
Group mean[3]	0.02 ± 0.02	−0.08 ± 0.04	0.00 ± 0.06	−0.05 ± 0.05	0.04 ± 0.05	−0.05 ± 0.02

[1]Values are means ± SD. Energy intake per kilogram of body weight was computed by using mean body weight of days 1–3 of each period. Body weight is mean of last 6 days of each period. Body weight change is from last 6 days of each period. [2]E, energy; X, exercise. See table 2 for full description. [3]Mean of subjects nos. 6001–6006.

decreased significantly with energy deficit without scheduled work (0.70E, 0.85X) and increased significantly with increased physical activity (1.15E, 1.15X; 1.0E, 1.15X). To standardize calculations, the average FN value for each man for each period (less the first 3 days, allowed for adaptation) was used when computing N balances for both experiments.

Energy balance had a significant effect on UN in both experiments (table 5). In experiment A, mean UN during a period of energy equilibrium with 2 hours of work (1.15E, 1.15X) was significantly less than it was with energy deficit at the same level of activity (1.0E, 1.15X). With 1 hour of treadmill walking (1.0X) the same trend was apparent but UN did not differ significantly for different energy balance conditions. The design of experiment B allowed pairwise comparisons between periods of energy equilibrium and deficit at all three levels of work. At all levels the mean UN was significantly lower during periods of energy balance than during the periods of deficit.

In both experiments, activity level also affected UN significantly under conditions of energy balance. UN was significantly lower with 1.15X than with 0.85X. The differences in UN between 1.15X and 1.0X or 1.0X and 0.85X were, however, not significant.

The effect of energy balance and activity on the proportion of UN excreted as urea (U:UN, table 5) varied between the two experiments. In experiment A there were no significant differences between periods of energy balance and deficit with work held constant. When physical activity was performed for 2 hours a significantly smaller

TABLE 5

Nitrogen intake (NI), fecal nitrogen (FN), urinary nitrogen (UN), proportion of UN as urea (U:UN) and sweat nitrogen (SN) in young men under two conditions of energy balance and three levels of work[1,2]

Measure	Treatment[3]					
	0.85E, 0.85X	0.70E, 0.85X	1.0E, 1.0X	0.85E, 1.0X	1.15E, 1.15X	1.0E, 1.15X
	Expt A (NI = 6.91 ± 1.30 g/day)					
FN, *g/day*	0.79 ± 0.36		0.72 ± 0.44	0.70 ± 0.40	0.75 ± 0.43	0.79 ± 0.42
UN, *g/day*	6.12 ± 0.73		6.00 ± 0.81	6.20 ± 0.58	5.77 ± 0.78[ab]	6.27 ± 0.52
U:UN	0.75 ± 0.07		0.77 ± 0.02	0.78 ± 0.03	0.72 ± 0.10	0.72 ± 0.04
SN, *g/day*	0.19 ± 0.05		0.30 ± 0.07	0.28 ± 0.06	0.32 ± 0.08	0.30 ± 0.03
	Expt B (NI = 8.80 ± 0.79 g/day)[4]					
FN	0.80 ± 0.29	0.63 ± 0.18	0.94 ± 0.32	0.82 ± 0.30	0.96 ± 0.23	0.93 ± 0.27
UN	7.93 ± 0.75[c]	8.83 ± 1.00	7.09 ± 0.30[d]	8.11 ± 0.61	6.49 ± 0.63[ab]	7.60 ± 1.15
U:UN	0.79 ± 0.02	0.82 ± 0.01	0.77 ± 0.02[d]	0.80 ± 0.01	0.76 ± 0.03[b]	0.79 ± 0.02
SN	0.25 ± 0.06	0.18 ± 0.04	0.20 ± 0.08	0.22 ± 0.09	0.38 ± 0.06	—

[1]Values are means ± SD. [2]Statistically significant ($P \leq 0.05$) difference: [a]1.15E, 1.15X vs. 1.0E, 1.15X; [b]0.85E, 0.85X vs. 1.15E, 1.15X; [c]0.85E, 0.85X vs. 0.70E, 0.85X; [d]1.0E, 1.0X vs. 0.85E, 1.0X. [3]E, energy; X, exercise. See table 2 for full description. [4]Computed for subjects 6001–6006 only.

proportion of N was excreted as urea both with energy balance and deficit, as compared to excretion during the periods of 1 hour of exercise (1.0X). There was no significant difference in U:UN between 0.85X and other work levels.

In experiment B, with 1 hour of work, U:UN was significantly lower when the men were in energy equilibrium than when energy intake was deficient. Similar differences at other work levels did not reach significance. With energy equilibrium U:UN was significantly higher during the sedentary period than with 2 hours of programmed activity.

In experiment A the mean SN losses were similar from period to period with the exception that SN dropped significantly during the period of no activity (0.85E, 0.85X). In experiment B, SN was lowest with energy deficit and sedentary activity (0.70E, 0.85X), and highest when physical activity was highest (1.15X). Because of the interindividual variability in response to the various treatments, values for each man in each period were used in computing "true" N balance.

Nitrogen balance. Crude N balance (CNbal) and N balance corrected for all measured and suspected sources of loss (TNbal) are included in table 6. Most investigators report CNbal so these values are useful for comparison with published literature, but TNbal is a closer approximation of the actual N economy. In all cases, trends seen in CNbal were present in TNbal.

Only two of the six men fed 0.57 g protein/kg body weight (experiment A) were able to establish N balance with either 1 or 2 hours of programmed activity, even when energy intake was theoretically adequate to cover the activity. The men fed 0.8 g protein/kg body weight (experiment B) were able to maintain N balance with activity provided energy intake was sufficient. All of the men in experiment A and four of the six subjects in experiment B were in negative N balance when sedentary.

In all cases, the mean N balance was more positive (or less negative) during periods of energy equilibrium than during periods of energy deficit. In experiment A, these differences were statistically significant at the higher activity level, and in experiment B, at all three levels of activity. The difference in TNbal due to energy intake increased with increasing physical activity being, in experiment B, 0.65 g/day at 0.85X, 0.93 g/day at 1.0X and 1.09 g/day at 1.15X. With the lower protein intake in experiment A, the difference due to energy was less, 0.19 g/day at 1.0X and 0.54 g/day at 1.15X.

N balance was more positive at higher than at lower work loads, holding energy balance conditions constant. In experiment B all differences were significant, but in

TABLE 6

Crude nitrogen balance (CNbal) and total nitrogen balance (TNbal) in young men under two conditions of energy balance and three levels of work[1,2]

Measure	Treatment[3]					
	0.85E, 0.85X	0.70E, 0.85X	1.0E, 1.0X	0.85E, 1.0X	1.15E, 1.15X	1.0E, 1.15X
			Expt A			
CNbal, *g N/day*	−0.01 ± 0.34		0.19 ± 0.45	−0.01 ± 0.45	0.41 ± 0.38[ab]	−0.15 ± 0.59
TNbal, *g N/day*	−0.48 ± 0.32		−0.38 ± 0.43	−0.57 ± 0.40	−0.19 ± 0.41[a]	−0.73 ± 0.59
			Expt B[4]			
CNbal	0.07 ± 0.40[c]	−0.66 ± 0.35	0.77 ± 0.71[d]	−0.14 ± 0.34	1.35 ± 0.58[ab]	0.26 ± 0.71
TNbal	−0.35 ± 0.44[c]	−1.00 ± 0.34	0.42 ± 0.73[d]	−0.51 ± 0.36	0.82 ± 0.58[ab]	−0.27 ± 0.72

[1]Values are means ± SD. CNbal is computed as (NI − [UN + FN]). TNbal is computed as (NI − [FN + UN + SN + blood N + miscellaneous losses]) where blood N is computed by dividing total blood loss over the study by the number of days, and has the following values in grams/day for each man: 5002, 5003 and 5004: 0.18; 5007, 5008 and 5009: 0.19; 6001, 6004 and 6006: 0.06; 6002, 6003 and 6007: 0.07. Miscellaneous losses are assumed to be 0.09 g N/day. [2]Statistically signficant ($P \leq 0.05$) difference: [a]1.15E, 1.15X vs. 1.0E, 1.15X; [b]0.85E, 0.85X vs. 1.15E, 1.15X; [c]0.85E, 0.85X vs. 0.70E, 0.85X; [d]1.0E, 1.0X vs. 0.85E, 1.0X. [3]E, energy; X, exercise. See table 2 for full description. [4]Statistical analysis computed for subjects nos. 6001–6006, only because the data set for no. 6007 is incomplete.

experiment A only that between 0.85X and 1.15X treatments was significant. With energy balance maintained in experiment B, TNbal was −0.35 g/day at 0.85X, 0.42 g/day at 1.0X and 0.82 g/day at 1.15X. With insufficient energy, respective values for TNbal were −1.00, −0.51 and −0.27 g/day.

DISCUSSION

Results of the present experiments confirm and extend previous observations regarding the effects of energy balance on nitrogen balance (3, 4, 23). Prior studies of this relationship in men have involved constant activity levels, or an assumption that activity patterns were unchanged during periods of altered energy intake. However, two recent lines of evidence suggested a need to reexamine the relationship between energy deficit and N balance while giving consideration to activity level.

First, as noted above, Butterfield and Calloway (8) showed that N retention was promoted by increased energy intake, even though energy balance was maintained by the addition of physical work equivalent in expenditure to the added energy intake. As the assigned work was of the same intensity as that to which the subjects were already conditioned, N retention could not be ascribed to a training-associated increase in muscle mass. This report challenges the concept that energy balance is a physiological constant regardless of the magnitude of the balancing intake and output components. Second, there is evidence that a decrease in energy intake, even of small magnitude, results in a partially compensatory reduction of physical effort (24). Restricted energy intake situations may, thus, involve diminished activity and, perhaps, even physical deconditioning as an unintended variable.

In experiment A, the 1973 FAO/WHO safe level of protein intake [0.57 g/kg (1)] was fed. This level is known to be marginal under our laboratory conditions, in that it will only maintain N balance in about half of the young males studied under conditions of adequate energy intake and some exercise. That only two of the six men in the experiment reported here achieved N balance under control conditions may be due to chance or may be a reflection of their mean maintenance energy intake (1.0E), which was slightly lower (by 2 kcal/kg or 5%) than that fed in previous studies. Energy intake was adjusted to maintain body weight close to constant, but experience indicates that N balance is a more sensitive indicator of energy balance than is body weight (23, 25). With the control treatment (1.0E, 1.0X) mean true N balance in experiment A was −0.38 g/day; a 15% energy deficit (ca. 430 kcal/day, 0.85E) reduced N balance to −0.57 g/day. The change in N balance due to lowered energy intake was 0.44 mg N/kcal eliminated. At the higher work level (1.15E), N balances were −0.19 and −0.74 g/day with intakes of 1.15E and 1.0E, respectively; the difference in balances was 1.25 mg N/kcal eliminated. Energy deficit was not imposed with the sedentary activity condition (0.85X) in experiment A.

In experiment B, protein intake was 0.8 g/kg, the NRC-RDA (2). This higher level was given to allow inclusion of a more inadequate treatment involving no programmed activity with an energy deficit (0.70E, or 830 kcal less daily than under control conditions). Mean true N balance was 0.42 g/day with the control treatment, and four of the six men were in positive balance. Both men whose N balances were negative had a small weight loss during this period. The effect of a 15% reduction in energy intake on N balance was 1.56 mg N/kcal eliminated in sedentary men; comparable values at 1.0X and 1.15X activity levels were 2.24 and 2.63 mg/kcal eliminated, respectively. We conclude from these experiments that changing energy intake from a deficit to an adequate state promotes N retention more effectively in active than in sedentary men, and that the effect is more pronounced with adequate than with marginal protein intake.

Our data show that physical activity is anabolic when energy balance is maintained. With the FAO/WHO safe level of protein intake (experiment A), an additional hour of exercise (1.15X vs. 1.0X) spared about 2.5 mg N/kg body weight, and elimination of exercise (0.85X vs. 1.0X) cost about 1.4 mg N/kg. In experiment B, with the NRC-RDA protein allowance, N balance became more positive with added exercise (5.9 mg N/kg body weight) and clearly negative under sedentary conditions (11.5 mg N/kg).

In our study, physical activity continued to show an anabolic effect when energy intake was reduced (by about 430 kcal/day or 15%), but the effect was less pronounced than when energy balance was maintained. The second hour of work improved N balance by 2.1 mg/kg in experiment A and 3.5 mg/kg in experiment B. With the reduction to sedentary conditions, examined under deficit energy conditions only in experiment B, the difference was 7.2 mg/kg.

Three different responses to work have been reported by others. Polyakov (26) found a beneficial effect of exercise on protein utilization in men prescribed 500 kcal of activity and 3000 kcal intake when compared with a nonexercised group given 3000 kcal; both groups were fed the FAO/WHO protein intake level. N balance was positive in the first group throughout the 30-day study but not until day 15 in the second group. Kraut et al. (27) reported two men to be in positive N balance with a diet supplying 1 g protein/kg and about 4000 kcal/day; N balance was described as being "slightly" positive when 3 hours of daily work and 1000 kcal of intake were eliminated, but balance became negative when protein intake was lowered to 0.84 g/kg. Gontzea and co-workers (28) found that imposing 2 hours of bicycle work with a compensatory increase in intake caused 29 out of 30 previously sedentary men to go from positive to negative N balance with an intake of 1.0 g protein/kg; with 1.5 g protein/kg, N balance became negative in only 2 of 6 men. In a subsequent study (29), these investigators reported that the negative N balance phase was brief, a reduction in N excretion occurring after the first 4 or 5 days; the negative N balance seen in this experiment may well have been a transient response to the stress of intensive and unaccustomed physical effort.

Generally, it is accepted that activity is beneficial. Active rats grow better than inactive ones, even when food intake is severely restricted (30). Goldberg and colleagues have shown that contractile activity is associated with growth and hypertrophy of rat skeletal muscle independent of insulin (31), growth hormone (32) and feeding (33). The biochemical mechanism for this anabolic effect of isometric contraction is not known. However, the same workers have shown that both contraction (33) and passive stretch (34) reduce the rate of muscle tissue catabolism. Millward and co-workers (35) have found, in men, a decrease in muscle protein breakdown during exercise (as determined by muscle 3-methyl histidine content) while total body N turnover was seen to increase.

A key issue in studies of physical activity clearly is whether or not energy balance is maintained; protein intake is an important second variable. The men in this study who received the NRC-RDA level of 0.8 g/kg were better protected, in N balance terms, if a 15% deficit in energy balance was brought about by increasing work with intake held constant, rather than by reducing energy intake 15% and holding work constant (comparing 0.85E, 1.0X with 0.70E, 0.85X and 1.0E, 1.15X with 0.85E, 1.0X, table 6). At the lower protein intake level, 0.57 g/kg, in experiment A, in the one such comparison available (1.0E, 1.15X vs. 0.85E, 1.0X), N balance was slightly more negative with the higher work level. Since low protein intake and energy deficit are the most potentially damaging combination of conditions encountered worldwide where work demands may be seasonally heavy, the subject deserves further study.

Those on slimming diets are probably well advised to be active, if only to minimize the trauma of food deprivation while sustaining an energy deficit. They may also retain more tissue protein if they exercise. Weltman and co-workers (36) found that middle-aged men on a diet coupled with mild exercise experienced significantly less loss of lean body mass than did those who simply restricted their energy intake.

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