Effects of Reduced Energy Intake on Protein Utilization in Obese Children

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Dietary treatment of pediatric obesity is a challenge given the need for adequate nutrients to support the maintenance of lean tissue and growth. The primary purpose of this investigation was to assess the effects of reduced energy intake on protein turnover in obese children aged 8 to 10 years. Following a 2-week baseline period, 16 subjects reduced energy intake during a 6-week intervention period. At baseline and following the intervention, ^{15}N-glycine methodology was used to measure nitrogen flux (Q), protein synthesis (PS), protein breakdown (PB), and net turnover ([NET] PS − PB). Other criterion measures included resting metabolic rate (RMR), fat mass (FM), fat-free mass (FFM), urinary creatinine to height ratio (Cr:Ht), and nitrogen balance (NB). On average, subjects lost 2.2 ± 0.3 kg, of which greater than 85% was FM. Decreased Q ($P = .03$) indicated downregulation of protein turnover in response to diet-induced weight loss. While PB did not change, NET declined slightly ($P = .06$) as a consequence of reduced PS ($P = .03$). Reductions in FFM ($P = .09$), Cr:Ht ($P = .02$), and NB ($P = .03$) accompanied alterations in protein turnover, but there was no change in the RMR. In conclusion, while short-term therapy promoted the loss of FM and did not compromise RMR, practitioners must be cautious when prescribing diets, given the observed changes in protein utilization and somatic protein status. Longitudinal studies are needed to further characterize the metabolic responses of obese children to long-term diet therapy.

TREATMENT OF PEDIATRIC OBESITY is warranted when considering the health risks conferred by the disease. Excess adiposity predisposes children to "silent" morbidities, including elevations in serum lipids and resting blood pressure.[1-3] Approximately half of all obese children are likely to become obese adults,[4] who are more likely to suffer from chronic diseases (eg, cardiovascular disease, diabetes mellitus, or osteoarthritis) compared with their non-obese counterparts.[5]

Practitioners currently are targeting obese children for weight management programs to minimize risks and enhance health throughout the life span. However, development of dietary intervention strategies for this population presents a challenge given the need for adequate energy to support whole-body protein metabolism for maintenance of lean tissue, growth, and overall health. While reduced energy intake usually is necessary for promoting loss of body fat and ultimately achieving a healthy body weight, diet-induced negative energy balance may adversely affect protein utilization due to energy-sparing downregulation of metabolic processes.[6]

Nitrogen balance (NB) typically becomes negative within 1 week after initiation of hypocaloric therapy in obese adolescents.[7,8] Although NB often is restored to baseline levels by the fourth week of therapy,[7-9] nitrogen loss may persist over an extended period for no apparent reason.[7,10,11] When assessing the long-term effects of intensive hypocaloric therapy in obese adolescents, Stallings et al[11] found that total body nitrogen continued to decline for 9 months following termination of a 3-month intervention program. This finding is disturbing given that net anabolism, reflected by increased nitrogen retention, is the metabolic norm in growing adolescents.

While assessment of nitrogen retention provides information concerning net protein utilization, stable isotope modeling techniques furnish important data regarding the contribution of protein synthesis (PS) and protein breakdown (PB) to net turnover ([NET] PS − PB). Stein et al[6] used ^{15}N-glycine to evaluate changes in whole-body PS and PB in response to 6 weeks of hypocaloric therapy in overweight men. Although NET was not depressed from baseline levels at the end of the intervention period, the rates of PS and PB were downregulated consequent to diet-induced weight loss. These data underscore the importance of incorporating not only measures of net protein utilization but also the rates of PS and PB in studies designed to evaluate the efficacy of treatment strategies. Because amino acid cycling (ie, PS and PB) between the protein and free–amino acid pools is energy-dependent, the findings reported by Stein et al[6] suggest that hypocaloric diets may have adverse effects on energy metabolism and hence weight control.

However, conclusions with regard to protein metabolism during diet therapy in obese children, for whom the provision of nutrients must be adequate to support growth and development, cannot be drawn based on results derived from older subjects. Essentially no data are available concerning the effects of reduced-calorie diets on PS and PB in obese children per se. Thus, the primary purpose of the present investigation was to evaluate changes in protein utilization consequent to reduced energy intake in obese subjects aged 8 to 10 years using stable-isotope methodology. According to the study hypothesis, reduced energy intake would elicit a response characterized by downregulation of protein turnover.

SUBJECTS AND METHODS

Following approval of the study protocol by the Institutional Review Board at the University of Connecticut, written informed consent and assent were obtained from parents and children, respectively. In brief, the study consisted of a 2-week baseline, or control, period followed by a 6-week intervention period. Subjects maintained their usual eating and activity habits during the baseline period and adhered to a reduced-calorie meal plan throughout the intervention period. Nutrient intake

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and physical activity were assessed during alternate weeks throughout the study. Resting metabolic rate (RMR), body composition, and protein utilization (ie, protein turnover, NB, and plasma amino acids) were measured at baseline and at the end of the dietary intervention program (ie, week 6).

Subjects

Sixteen obese but otherwise healthy children aged 8 to 10 years participated in the study (six boys and 10 girls). Obesity was defined as a minimum of 25% and 30% body fat for boys and girls, respectively.[12] The subjects had not previously participated in weight management programs and were not taking any medications known to affect protein metabolism.

Diet Therapy

Energy requirements were estimated based on RMR, baseline dietary intake, and physical activity level for each child. The estimated requirements represented a reduction from baseline caloric intakes for all subjects. To foster the maintenance of lean tissue, daily protein needs were calculated relative to fat-free mass ([FFM] ie, 1.5 g protein/kg FFM). Individualized meal plans were developed with consideration for personal food preferences and presented in the context of *Exchange Lists for Meal Planning.*[13] The intervention was conducted in free-living subjects under "real life" conditions.

Food and Nutrient Intake

Seven-day food records were used to document and monitor the food intake of the children. In addition, a researcher conducted biweekly 24-hour recalls to complement food records. Records and recalls were analyzed using Nutritionist IV software (N-Squared Computing, Salem, OR) to obtain nutrient intake data.

Physical Activity

Daily physical activity was assessed by accelerometry using a Tritrac-R3D activity monitor (Hemokinetics, Madison, WI). Data were obtained at 1-minute intervals and subsequently downloaded from the monitor to a personal computer for analysis and interpretation. Software supplied by the manufacturer was used to calculate activity-induced energy expenditure ([AIEE] in kilocalories). The caloric cost of activities performed when the monitor was not worn (eg, swimming and bathing) was estimated based on the method of Ainsworth et al.[14]

RMR

Energy expenditure at rest was determined by open-circuit indirect calorimetry using a metabolic cart (MedGraphics CPX/D; Medical Graphics, St Paul, MN). Parents brought their children to the University of Connecticut by vehicle early in the morning following a 12-hour overnight fast. RMR was assessed for 15 to 20 minutes with the subject supine in a quiet, temperature-regulated room using a canopy system to collect expired gases.

Body Composition

Prior to assessment of body composition, subjects removed their shoes and all clothing with the exception of undergarments and a light T-shirt. Body weight and height were determined using a balance beam scale equipped with a measuring rod (Health-o-meter, Bridgeview, IL). Triceps and subscapular skinfolds were measured on the right side of the body with Harpenden calipers (British Indicators, West Sussex, UK). All anthropometric measurements were taken according to procedures specified in the *Anthropometric Standardization Reference Manual.*[15] In addition to anthropometry, bioelectrical impedance analysis (BIA) was performed with an RJL analyzer (RJL Systems, Detroit, MI; model BIA-101Q) using an established protocol.[16] Body composition measures were obtained by the same investigator at each time point (ie, baseline and week 6).

The regression equation of Goran et al[17] was used to calculate fat mass (FM) and subsequently FFM (weight − FM) and relative body fat (FM/weight × 100%). This equation was selected given that it (1) was generated using dual-energy x-ray absorptiometry, which currently is emerging as the preferred gold standard for assessment of body composition in children, (2) was developed specifically for young children, and (3) incorporates a resistance index obtained from BIA (height^2/R) in addition to triceps and subscapular skinfold thicknesses, body weight, and gender as independent variables.

Protein Turnover

Whole-body protein turnover was assessed using stable-isotope methodology according to the protocol presented in Fig 1.[18,19] Studies were conducted overnight rather than during the day to eliminate any potentially confounding effects of physical activity on nutrient metabolism, to minimize imposition on the subjects, and to promote adherence.[19] Specifically, the selected methodology incorporated a single oral dose of ^{15}N-glycine (2 mg/kg body weight, 98+ atom percent enrichment; Cambridge Isotope Laboratories, Andover, MA) dissolved in orange juice. Protein turnover studies were initiated at the home of the respective children in the evening (eg, 8 PM) when they were at least 2 hours postabsorptive. Immediately preceding administration of the stable isotope by an investigator, subjects provided baseline spot urine samples for determination of background ^{15}N-ammonia enrichment and then emptied their bladder. For 10 hours following the dose (eg, from 8 PM to 6 AM), the subjects collected cumulative urine samples and refrained from consumption of foods and beverages.

Urinary nitrogen excretion (E) during the 10-hour study and ^{15}N-ammonia enrichment (ie, ratio of tracer to tracee, t:t) were determined in duplicate using the micro-Kjeldahl technique (Tecator Kjeltec System, Hoganas, Sweden) and isotope ratio mass spectrometry (Metabolic Solutions, Merrimack, NH), respectively. The t:t ratio for the cumulative sample was corrected for background ^{15}N-ammonia enrichment. Nitrogen intake (I) during the evening meal was determined based on analysis of food records and recalls. Nitrogen flux (Q), PS, PB, and NET were calculated, where d denotes the oral dose of ^{15}N (d = g gly-

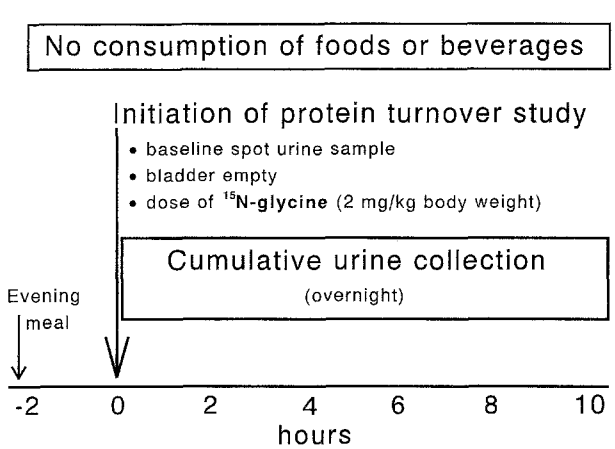

Fig 1. Protocol for assessing whole-body protein turnover using ^{15}N-glycine.

cine × 0.1972).

$$Q(\text{g N/kg/d}) = [\text{d/(corrected t:t)/10 hours} \times 24 \text{ hours/body weight}],$$

$$PS (\text{g/kg/d}) = [Q - (\text{E/10 hours} \times 24 \text{ hours/body weight})] \times 6.25 \text{ g protein/g N},$$

$$PB (\text{g/kg/d}) = [Q - (\text{I/10 hours} \times 24 \text{ hours/body weight})] \times 6.25 \text{ g protein/g N},$$

and $$NET (\text{g/kg/d}) = PS - PB.$$

NB

The subjects collected a 24-hour urine sample for determination of nitrogen excretion. The total nitrogen content of the sample was measured in duplicate using the micro-Kjeldahl technique. Urinary nitrogen excretion (E) and nitrogen intake (I), determined from a food record and recall for the 24 hours corresponding to the urine collection period, were used to calculate apparent NB (NB = I − E).

Urinary Creatinine

The creatinine content of each urine sample was measured in triplicate using a spectrophotometric assay (Sigma Chemical, St Louis, MO). Data were used (1) to evaluate the completeness of urine collections with respect to protein turnover and NB studies and (2) to calculate a creatinine to height ratio (Cr:Ht) using the 24-hour sample. The specified ratio provided a method for evaluating changes in somatic protein status[20,21] and complemented the assessment of body composition based on anthropometry and BIA.

Plasma Amino Acids

Following an overnight (ie, 12-hour) fast, blood was drawn by a physician or phlebotomist using standard venipuncture techniques. Specimens were collected in tubes containing EDTA and centrifuged to obtain plasma. The Pico-tag method (Waters, Milford, MA) was used to determine plasma amino acid concentrations. In brief, samples were analyzed by reversed-phase high-performance liquid chromatography following deproteinization and precolumn derivatization with phenylisothiocyanate.

Statistics

ANOVA was used to evaluate changes in energy intake, expenditure, and balance over time. Paired *t* tests were performed to determine if body composition and protein utilization were compromised in response to reduced energy intake. Statistics were computed using the Statistical Analysis System (version 6.08; SAS Institute, Cary, NC) and Minitab (version 7.2; Minitab, State College, PA). All data are reported as the mean ± SEM.

RESULTS

Baseline Characteristics

Baseline values for age, body weight, height, and body mass index (BMI) were 9.28 ± 0.23 years, 54.5 ± 3.2 kg, 140.4 ± 2.2 cm, and 27.4 ± 1.0 kg/m^2, respectively. All subjects were classified as obese with respect not only to the specified body fat criteria required for participation in the study (≥25% for boys and ≥30% for girls) but also to BMI cutoff points that are commonly used to assess obesity status (ie, age- and gender-specific 95th percentiles).[22,23]

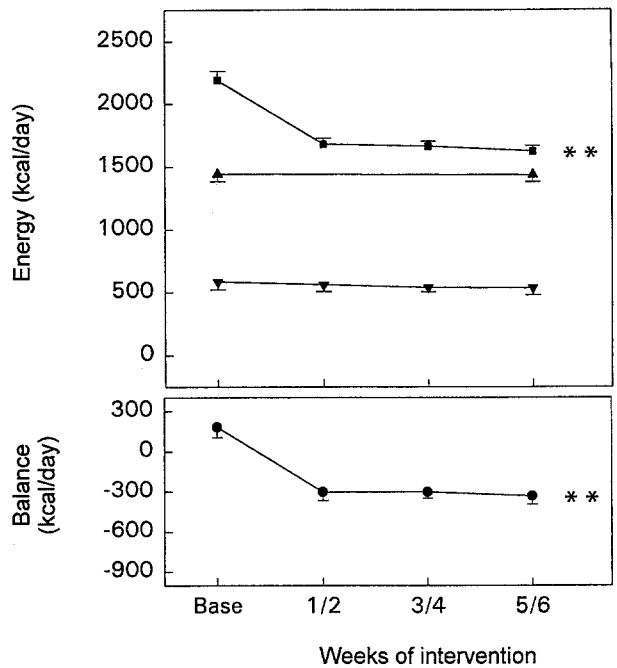

Fig 2. Changes in energy intake (■), RMR (▲), AIEE (▼), and energy balance (●) in response to the intervention (mean ± SEM). **$P < .01$.

Components of Energy Balance

Energy intake, expenditure (ie, RMR and AIEE), and balance data are shown in Fig 2. Findings with regard to AIEE and energy balance represent data obtained with the exclusion of one subject for whom swimming, during which the Tritrac was not worn, accounted for a large proportion of total physical activity. Given that there were no fluctuations in RMR or AIEE over time, energy balance became negative ($P = .0001$) via reductions in caloric intake associated with the prescribed hypocaloric diets rather than changes in energy expenditure.

Macronutrient Intake

Descriptive data for macronutrient intakes based on 7-day food records are presented in Table 1. During the intervention period, the mean contribution of carbohydrate and fat to total energy intake was approximately 55% and 30%, respectively. Protein intakes of all subjects were sufficient to satisfy estimated needs (ie, >1.5 g/kg FFM), accounting for 15% of total energy intake, on average. Dietary recalls provided results that were consistent with information derived from food records (data not shown).

Table 1. Descriptive Data for Average Daily Energy and Macronutrient Intake Based on 7-Day Food Records Obtained at Baseline and During Diet Therapy (mean ± SEM)

Parameter	Energy (kcal)	Carbohydrate (g)	Fat (g)	Protein (g)
Baseline	2,189 ± 74	303.5 ± 12.2	73.2 ± 3.3	79.2 ± 3.6
Diet therapy				
Week 2	1,678 ± 49	232.1 ± 11.4	55.1 ± 3.5	63.3 ± 2.3
Week 4	1,663 ± 40	228.1 ± 12.6	55.4 ± 4.4	63.0 ± 2.0
Week 6	1,622 ± 44	223.5 ± 12.6	53.8 ± 3.7	61.0 ± 2.0

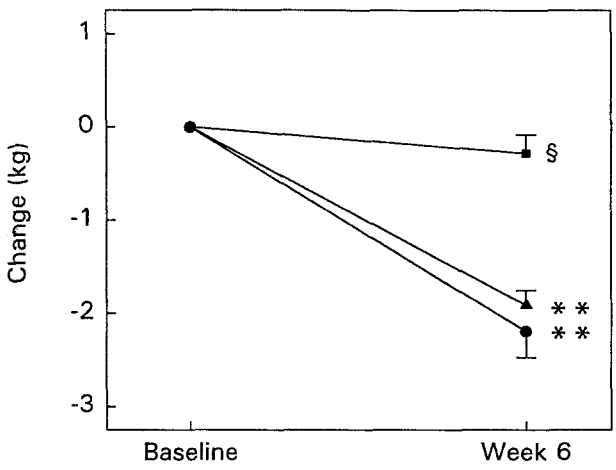

Fig 3. Contributions of FM (▲) and FFM (■) to total weight loss (●) (mean ± SEM). **$P < .01$, §$P = .09$.

Body Composition

Contributions of FM and FFM to total weight loss are presented in Fig 3. In brief, weight loss was attributed to a significant reduction in FM ($P = .00005$) accompanied by a slight decrease in FFM ($P = .09$).

Urinary Creatinine

The baseline 24-hour urine sample for one subject was considered inaccurate because the urinary creatinine concentration differed by more than 20% among collections for this child. Therefore, results with respect to NB and Cr:Ht are based on data from 15 subjects. In response to the intervention, Cr:Ht decreased significantly (-0.59 ± 0.27 mg/cm, $P = .02$).

Protein Utilization

The effects of reduced energy intake on protein utilization are plotted in Fig 4. With hypocaloric therapy, there were significant reductions in Q ($P = .03$) and PS ($P = .03$). Because measurements of PB did not differ between the specified time points, NET declined ($P = .06$) due to reduced PS. Likewise, there was a significant decrease in NB (-1.1 ± 0.51 g/d, $P = .03$).

Amino acid profiles were not determined for one child because plasma was unavailable due to the child's extreme apprehension regarding blood sampling. Plasma concentrations of the respective essential (EAA) and nonessential (NEAA) amino acids based on data from the remaining 15 subjects are listed in Table 2. Changes over time were evaluated for the sum of EAA and NEAA. Both EAA and NEAA remained essentially constant throughout the study.

DISCUSSION

Diet-induced negative energy balance altered protein metabolism in obese children in the present study. Protein intake coincided with the diet prescription (ie, >1.5 g/kg FFM/d) throughout the intervention period. For all subjects, the mean daily intakes equalled or exceeded the recommended protein requirements for boys and girls (0.86 to 0.88 g/kg/d),[24] and were therefore considered adequate. The subjects were likely consuming protein in excess of the threshold levels required to support protein metabolism.[25] With regard to physical activity, AIEE did not fluctuate significantly from baseline levels during the intervention period. Thus, based on these observations, the changes in protein utilization were a response to reduced energy intake as opposed to the corresponding reductions in dietary protein or alterations in physical activity.

The data reported herein support the study hypothesis (ie, protein turnover would be downregulated with reduced energy intake in obese children) given that Q decreased in response to treatment. While PB did not change significantly, NET declined as a consequence of reduced PS. Significant reductions in NB and Cr:Ht accompanied the observed changes in protein turnover. Because urinary creatinine is dependent on muscle protein mass,[20,21] the reduction in Cr:Ht suggests that somatic protein status was compromised secondary to alterations in protein utilization. The slight decrease in FFM is consistent with this contention given that somatic protein is a primary constituent of FFM.

Unlike other measures of protein utilization, plasma amino acid profiles did not change discernibly in response to reduced energy intake. Concentrations of each respective amino acid at baseline and following the intervention period were comparable to the mean values for healthy children reported by Armstrong and Stave.[26] Because flux between multiple protein and free amino acid pools is regulated by several complex homeostatic mechanisms (eg, protein turnover, hormones such as insulin, and transport systems),[27,28] circulating amino acid concentrations typically are maintained within tight limits as observed in

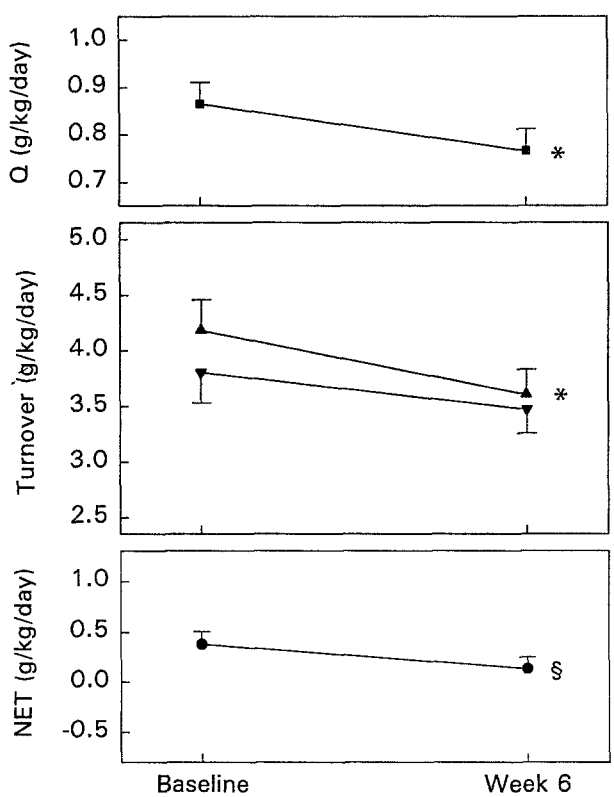

Fig 4. Shifts in Q (■), PS (▲), PB (▼), and NET (●) in response to hypocaloric therapy (mean ± SEM). *$P < .05$, §$P = .06$.

Table 2. Plasma Concentrations (μmol/L) of EAA and NEAA at Baseline and Following 6 Weeks of Diet Therapy (mean ± SEM)

	Amino Acid									
Time	HIS	THR	VAL	MET	ILE	LEU	PHE	TRP	LYS	Sum
EAA										
Baseline	66.8 ± 3.0	104.4 ± 5.9	231.8 ± 15.5	22.2 ± 1.3	60.9 ± 4.7	121.1 ± 8.2	53.1 ± 3.6	55.3 ± 3.4	143.1 ± 7.7	858.7 ± 46.4
Week 6	67.9 ± 2.5	105.7 ± 4.5	217.7 ± 10.5	21.1 ± 0.8	58.7 ± 4.0	113.5 ± 5.5	51.1 ± 2.1	55.9 ± 3.0	144.9 ± 6.0	836.7 ± 30.0
	ASP	GLU	SER	ASN	GLY	ALA	ARG	PRO	TYR	
NEAA										
Baseline	2.3 ± 0.2	30.2 ± 3.5	82.5 ± 3.4	56.7 ± 2.5	177.9 ± 12.2	343.1 ± 22.5	76.7 ± 4.1	165.2 ± 11.3	75.9 ± 6.0	1,010.5 ± 49.4
Week 6	2.1 ± 0.3	23.1 ± 2.9	93.7 ± 3.5	59.5 ± 2.5	193.7 ± 10.6	326.9 ± 16.9	83.9 ± 3.5	146.9 ± 8.6	66.1 ± 3.4	996.1 ± 30.6

the present investigation. Studies using carbon-labeled tracers (eg, 1-^{13}C-leucine) are needed to systematically evaluate the effects of reduced energy intake on amino acid metabolism per se. Evaluation of the relationships among isotopically determined amino acid oxidation and the components of protein turnover that contribute to Q may provide further insight regarding the metabolic consequences of hypocaloric therapy in obese children.

From a clinical perspective, downregulation of protein turnover may have adverse consequences when interpreted in the context of the principles of metabolic control proposed by Crabtree and Newsholme et al.[29-31] Protein turnover is a substrate cycle consisting of PS and PB such that a large protein pool is in equilibrium with a substantially smaller free amino acid pool (Fig 5). Nitrogen flux is a measure of amino acid cycling between the protein and free amino acid pools. In addition to interconversions between these two pools, protein metabolism consists of branch pathways and alternate routes for utilization of free amino acids (eg, purine and pyrimidine biosynthesis and gluconeogenesis).[32] In theory, a reduced rate of protein turnover may decrease sensitivity to changes in cellular and tissue environments, thereby compromising the responsiveness to physiological stimuli associated with processes such as growth and development. However, because essentially no data are available concerning the acceptable limits for protein turnover rates, conclusions regarding the long-term effects of the observed changes in protein utilization on metabolic control in obese children cannot be drawn from this study.

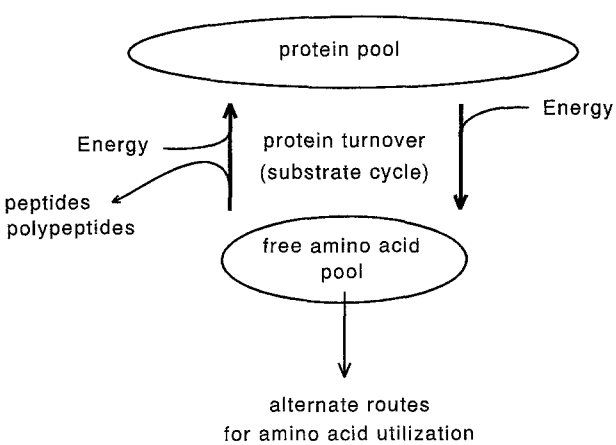

Fig 5. Protein turnover as a substrate cycle with branch pathways and alternate routes for utilization of free amino acids.

The regulatory function of amino acid cycling between protein and free amino acid pools requires energy.[6,30-33] Nevertheless, RMR remained relatively constant in the present study despite decreased rates of cycling (ie, reduced Q). While the relationship between protein turnover and energy expenditure is well documented across a wide range of individuals and species varying in age, size, and nutritional status,[33,34] Clugston and Garlick[34] concluded that interindividual variations in these parameters are not strongly related within homogeneous groups of subjects. The lack of change in RMR may be attributed to homogeneity with respect to age (8 to 10 years), body size (overweight due to excess adiposity), and nutritional status (overnourished) among the children who participated in the present study. Potential changes in energy expenditure associated with reduced rates of protein turnover were likely relatively small and undetectable by indirect calorimetry, given that numerous factors contribute to interindividual variability in the RMR.

Although changes in protein utilization and body composition did not affect RMR, the slight loss of FFM and significant reduction in Cr:Ht are disturbing when interpreted in the context of data reported by Schwingshandl and Borkenstein.[35] These investigators found a significant relationship between loss of lean tissue in obese children during a 3-week dietary intervention program and weight regain within 4 months following completion of the program. That is, greater loss of FFM was associated with a greater regain of body weight, possibly due to suppression of RMR.[35] While short-term therapy promoted loss of FM and did not compromise RMR in the present investigation, practitioners must be cautious when prescribing hypocaloric diets for children given the observed changes in protein utilization and somatic protein status. Even undetectable reductions in RMR associated with depressed rates of protein turnover and loss of somatic protein may have adverse consequences with regard to long-term weight management. Longitudinal studies are warranted to further characterize the effects of changes in protein utilization on energy expenditure, body composition, and health outcomes with long-term therapy in obese children.

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