Dietary Protein Restriction Alters Glucose But Not Protein Metabolism in Non–Insulin-Dependent Diabetes Mellitus

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We determined whether a customary diet high or low in protein (1) influences postabsorptive amino acid catabolism, nitrogen (N) balance, and hepatic glucose output (HGO) in normal subjects or patients with non–insulin-dependent diabetes mellitus (NIDDM) or (2) alters blood glucose levels in NIDDM. Eight normal young adults and five obese middle-aged persons with NIDDM consumed low-protein (0.8 g/kg lean body mass [LBM]) or high-protein (3.0 g/kg LBM) diets at maintenance energy for consecutive 7-day periods. Fasting and average blood glucose and N balance were measured daily. The level of dietary protein had no effect on the basal plasma leucine rate of appearance (Ra) or urinary 3-methylhistidine excretion in either subject group. Basal leucine oxidation (and by inference, whole-body amino acid catabolism) was reduced on the low-protein diet but basal HGO was not, and although exogenous glucose effectively suppressed HGO, it did not reduce leucine oxidation with either diet. After adaptation to the low-protein diet, N balance in both the normal and NIDDM subjects was close to zero. The low-protein diet reduced the fasting and daily blood glucose of the diabetic subjects by approximately 2 mmol/L ($P < .05$). We conclude that physiologic variation in dietary protein does not affect basal whole-body protein turnover or HGO in either normal young adults or obese middle-aged NIDDM subjects. However, protein restriction to the level of the average daily requirement significantly reduces postabsorptive and average daily blood glucose concentrations in persons with NIDDM.

BASAL GLUCONEOGENESIS and hepatic glucose output (HGO) are increased in non–insulin-dependent diabetes mellitus (NIDDM).[1,2] This observation has suggested that the gluconeogenic precursor supply could affect HGO in normal or diabetic persons.[3] Indeed, infusion of several gluconeogenic precursors increases gluconeogenesis in normal subjects[3-6] or persons with NIDDM,[5] but in every study but one[7] it failed to increase HGO. By contrast, protein consumption increases fed-state HGO in humans[8] and basal HGO in rats,[9] and infusion of a physiologic mixture of amino acids increases HGO in normal and NIDDM humans.[10] Unlike other gluconeogenic substrates, dietary protein[11] and infused mixed amino acids[10] stimulate glucagon release, which stimulates intrahepatic conversion of amino acids to glucose and directs glucose from the liver into the circulation.[12] This raises the possibility that a habitually high-protein intake could increase both basal and fed-state gluconeogenesis and HGO in humans, whereas a diet low in protein could have the reverse effect.[13]

In keeping with this possibility, we recently observed that consumption of a protein-free diet reduced the postabsorptive HGO of normal persons and reduced the insulin requirement of individuals with insulin-dependent diabetes mellitus (IDDM).[14,15] We suggested this reduction in HGO occurred because of a reduction of protein conversion to glucose that extended into the postabsorptive period.[13] However, our observation pertained to an artificial condition of extreme protein restriction that would not occur physiologically. The present study was designed to test whether more physiologic variations in protein intake affect amino acid metabolism and HGO of normal persons or those with NIDDM. Specifically, the following questions were asked: Does basal whole-body leucine oxidation (an index of whole-body amino acid catabolism[16,17] and its coordinate availability for gluconeogenesis) reflect the protein content of the diet? If so, will this high-protein intake, with its associated obligatory oxidation of an equivalent amount of amino acids, render postabsorptive leucine oxidation more resistant to suppression by exogenous glucose? Does a customary diet high in protein increase postabsorptive HGO? To address these questions, we included subjects with mildly hyperglycemic NIDDM, because less effective hepatic glycogen storage, if it occurs in this disease,[1] could increase the importance of the protein component of basal HGO. As well, we wished to extend our earlier observations that protein restriction improves metabolic control in IDDM.[14,15]

SUBJECTS AND METHODS

Subjects and Study Procedure

Eight normal adults (five men and three women) and five subjects with NIDDM (three men and two women) who had never received insulin therapy were admitted to the clinical investigation unit of the Royal Victoria Hospital for a 14-day protocol. Written informed consent was obtained for the study, which was approved by the local institutional review board. Subjects were randomly assigned to either a high- or low-protein diet for 7 days. On the morning after the first diet period, a tracer infusion study was performed, after which each subject was switched to the alternate diet for 7 days commencing that day. On the final morning, a second tracer infusion study was performed. None of the normal subjects received any medication. Before admission, one diabetic subject received captopril (50 mg three times daily) for blood pressure control. One diabetic subject was receiving thyroxine replacement therapy (0.125 mg/d) and had normal serum thyrotropin and thyroxine concentrations. These medications were continued throughout the study. Four diabetic subjects had been treated with oral hypoglycemic drugs. To permit rapid dosage adjustments (which turned out to be unnecessary), these subjects were switched to a stable dose of tolbutamide three times daily at least 1 week before admission for the formal study protocol. The tolbutamide dosage was 500 mg three times daily for one subject, 750 mg three times daily for a second, and 1,000

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mg three times daily for two subjects. All subjects resided in the clinical investigation unit for the duration of the study, where they maintained a constant sedentary activity level. Body weight, blood pressure, and heart rate were measured and urine was collected daily. The resting metabolic rate (RMR) was measured at 2- or 3-day intervals and after the final day of each diet period using the Deltatrac Sensor Medics ventilated-hood indirect calorimeter (Summit Technology, Oakville, Ontario, Canada) as previously described.[18] Lean body mass (LBM) was calculated from bioelectrical impedance[19] measured using the RJL Systems BIA-101A analyzer (Clinton Township, MI).

The energy provision was 150% of the measured RMR. Weight loss or gain was avoided by monitoring body weight on a daily basis and adjusting nonprotein energy intake accordingly. Additional carbohydrate was given at each meal to match urine glucose loss in the NIDDM group (20 to 300 kcal/d). The high-protein diet provided 3.0 g protein/kg LBM · d (equivalent to 2.25 g protein/kg · d of normal adult body weight), a high but physiologic level. The low-protein diet provided 0.8 g protein/kg LBM · d, equivalent to 0.6 g/kg · d of normal adult body weight, which is the normal average minimum protein requirement.[20] The protein for both diets was whole-milk protein (ProtiMax vanilla pudding mix; Bariatrix International, Lachine, Quebec, Canada). Nonprotein energy was provided in 260 kcal "units" combining natural, very–low-protein foods (protein-free bread rolls, low-protein wafers, butter, jam, and apple juice) in a fixed carbohydrate to fat ratio. The protein energy missing from the low-protein diet was replaced with carbohydrate. The "protein-free" component of the diets actually provided approximately 3 g protein/d; this was accounted for in calculating the total protein levels of the two diets. One multiple vitamin-mineral tablet (Centrum Forte, Whitehall-Robins, Mississauga, Ontario, Canada) was consumed each day, and to each day's protein formula were added magnesium chloride hexahydrate (2.11 g), dipotassium hydrogen phosphate (3.79 g), and sodium chloride (2.56 g) (all from Biopharm, Montreal, Quebec, Canada) to meet the recommended daily requirements for vitamins and essential minerals. Meals were consumed at 8:30 AM, 1:00 PM, and 6:30 PM, except on turnover days, when meals were consumed at 4:30, 8:00, and 11:00 PM. Capillary blood glucose was recorded seven times daily (before and 2 hours after each meal and before sleep) using Chemstrip bG glucose oxidase reagent test strips with the Accu-Check III blood glucose monitor (Boehringer Mannheim, Dorval, Quebec, Canada).

Tracer Infusions

The plasma HGO and leucine rate of appearance (Ra) and oxidation were measured in the postabsorptive condition after each 7-day dietary period. Hypoglycemic medication was withheld for the duration of the 7-hour study. After baseline plasma and breath samples were obtained to determine natural isotopic abundance, primed continuous infusions of L-[1-^{13}C]leucine (99% ^{13}C; priming dose, 40 mg; rate, 40 mg/h) and D-[6,6-$^{2}H_2$]glucose (99% ^{2}H; priming dose, 200 mg; rate, 150 mg/h; both tracers from MassTrace, Woburn, MA) were administered for 7 hours using a syringe pump (model 2681; Harvard Apparatus, Milford, MA). Five plasma samples for glucose and α-ketoisocaproic acid (KICA) enrichment and five breath samples for $^{13}CO_2$ enrichment were collected at 15-minute intervals during the third hour of tracer infusion, whereupon unlabeled glucose was infused at the rate of 3 mg/kg LBM · min using a Baxter Flo-Gard 6200 volumetric infusion pump (Montreal, Canada) connected to the tracer infusion catheter. Plasma and breath samples were collected again during the fourth hour of glucose infusion. Total CO_2 production was measured twice during each basal period and twice during each glucose infusion using the Deltatrac indirect calorimeter.

The glucose infusion rate was equivalent to 2.0 mg/kg · min of normal adult body weight. This approximates normal basal HGO and was anticipated to suppress HGO of normal subjects by approximately 70%.[21] Contrary to a previous report that glucose of corn origin infused at this low rate does not affect background $^{13}CO_2$ abundance,[22] the glucose infusion caused a small but measurable increase in breath $^{13}CO_2$ enrichment. To correct for this, glucose without labeled leucine was infused the morning after the second turnover study. The resulting increase in $^{13}CO_2$ enrichment, while small (approximately 20% of the enrichment caused by the [^{13}C]leucine infusion), was appropriately subtracted from the enrichment values obtained during the glucose and [^{13}C]leucine coinfusion studies.

Biochemical Analysis

Glucose enrichment was measured by electron-impact gas chromatography/mass spectrometry (GC/MS) following derivatization to the methyloxime pentatrimethylsilyl derivative.[23] The plasma leucine rate of appearance (Ra) was measured using KICA plasma enrichment, which is believed to be the best indicator of leucine intracellular enrichment.[24] This measurement was conducted as previously described,[25] by first reducing KICA to α-hydroxyisocaproic acid with sodium borohydride and then forming the *t*-butyl-dimethylsilyl derivative[26] for analysis by GC/MS. The Ra was calculated according to the equation, $Ra = i \cdot (E_i/E_p - 1)$, where i is the tracer infusion rate, E_i is the enrichment (in atoms percent excess) of the administered isotope, and E_p is the plasma KICA enrichment at isotopic steady state.[27] ^{13}C enrichment of expired CO_2 was measured with a Europa Tracermass isotope ratio mass spectrometer (Metabolic Solutions, Merrimack, NH). The rate of leucine oxidation was calculated from expired $^{13}CO_2$ enrichment at isotopic steady state according to the equation, $(ECO_2 \cdot VCO_2)/(0.8 \cdot E_p)$, where ECO_2 is $^{13}CO_2$ enrichment in expired air, VCO_2 is the rate of CO_2 production, and 0.8 corrects for the fraction of $^{13}CO_2$ formed on oxidation of [1-^{13}C]leucine but not released from the body bicarbonate pool into expired air.[24,27] Tissue leucine uptake was calculated as leucine Ra − oxidation.

All urine was collected daily in refrigerated 24-hour aliquots to measure daily urea, creatinine, and total N excretion. Urine N levels were determined after micro-Kjeldahl digestion using an automated analysis system.[28] Daily N balance was calculated as intake minus the total of urinary, fecal, and miscellaneous losses; the latter were assumed to be 16 and 8 mg/kg, respectively, for both diets.[20,29] Daily urinary 3-methylhistidine excretion was measured by high-performance liquid chromatography[30] for the final 2 days of each diet and reported as the average of the two measurements. Plasma amino acid levels were measured by high-performance liquid chromatography.[31] Plasma glucose levels during tracer infusion studies were measured using the Beckman II glucose analyzer (Beckman Instruments, Fullerton, CA). Plasma glucagon and insulin levels were measured by kit radioimmunoassay (Linco Research, St Louis, MO).

Statistical Analysis

Data are presented as the mean ± SEM. Repeated-measures ANOVA was used to test differences between repeated readings in the same group. When significance existed, the Newman-Keuls test was used to determine the source. When analyzing data in the same group, a paired Student's *t* test was performed. An unpaired Student's *t* test was used to test differences between different groups. Differences were considered significant at a P level less than .05.

RESULTS

Body Weight and Metabolic Rate

Characteristics of the subjects are listed in Table 1. NIDDM patients were middle-aged and obese, whereas control subjects were younger and lean. This does not bear heavily on the conclusions of the study, since the two protocols were independent of one another and the important comparisons were within rather than between the different subject groups. Details of the

Table 1. Subject Characteristics

Characteristic	Normal	NIDDM
No. of subjects	8	5
Age (yr)	30.4 ± 4.5	55.6 ± 2.2*
Height (cm)	170.5 ± 2.7	167.1 ± 4.0
Weight (kg)	69.9 ± 3.7	109.4 ± 10.0*
Body mass index (kg/m²)	23.9 ± 0.9	39.0 ± 2.6*
LBM (kg)	53.4 ± 3.3	61.3 ± 7.0
% LBM	76.4 ± 2.0	55.6 ± 2.9*
RMR (kcal/24 h)	1,512 ± 62	1,885 ± 67*
RMR/LBM (kcal/kg · 24 h)	28.6 ± 0.8	32.1 ± 2.9
Hemoglobin A_1 (%)	—	10.1 ± 0.45

NOTE. Data are the mean ± SEM. The normal range for hemoglobin A_1 was 4.6% to 8.5%.

*Significantly different *v* nondiabetic group by the Student *t* test, $P < .05$.

study diets are shown in Table 2. The high-protein diet contained nearly four times more protein than the low-protein diet, and correspondingly less carbohydrate. Because of a small error in calculating food energy values that was detected only after the study was completed, total energy intake for the normal subjects was 200 kcal/d more on the high-protein diet than on the low-protein diet ($P < .05$). However, none of the diets had a significant effect on body weight, body composition, or RMR (Table 3).

Protein Metabolism

Plasma leucine concentrations were significantly increased by the high-protein diet in both the control and NIDDM groups (Table 4). The concentration of the other branched-chain amino acids (isoleucine and valine) also increased, whereas alanine, methionine, serine, threonine, glutamate, and aspartate concentrations did not differ between the diets (data not shown). The low-protein diet was associated with a significant reduction of basal leucine oxidation but not of leucine Ra (an indicator of whole-body proteolysis) or leucine uptake into the tissues for protein synthesis (Table 4). These results were therefore insensitive for detecting the small reduction in leucine Ra and/or increase in leucine tissue uptake that must occur when leucine oxidation decreases. Urinary 3-methylhistidine excretion, an indicator of the breakdown rate of myofibrillar protein, was also insensitive to the high- or low-protein diets. Daily 3-methylhistidine excretion of the control subjects was 3.7 ± 0.2 and 3.6 ± 0.2 μmol/kg · LBM on the high- and low-protein diets, respectively. Corresponding 3-methylhistidine excretion for NIDDM subjects was 3.1 ± 0.3 and 3.1 ± 0.3 μmol/kg · LBM.

We had predicted that an intravenous glucose infusion could reduce leucine oxidation only when the prior diet was low in protein, but this proved not to be the case. Despite effectively reducing the plasma leucine Ra, leucine tissue uptake, and plasma leucine concentration in all but the high-protein NIDDM group, an intravenous glucose infusion equivalent to the normal

Table 2. Composition of the Low- and High-Protein Diets

	Normal		NIDDM	
Component	LP	HP	LP	HP
Energy				
kcal/d	2,280 ± 130	2,480 ± 130	2,860 ± 180	2,780 ± 240
kcal/kg LBM	42.9 ± 1.1	46.6 ± 1.1	48.6 ± 3.2	46.4 ± 3.2
Protein				
g/d	41.0 ± 2.8	158.3 ± 9.6	48.4 ± 5.8	185.9 ± 20.9
% total kcal	6.7 ± 0.2	25.6 ± 0.6	7.3 ± 0.5	26.5 ± 2.3
g/kg LBM	0.77 ± 0.01	3.0 ± 0.0	0.79 ± 0.01	3.0 ± 0.0
Fat				
g/d	79.7 ± 4.5	81.3 ± 3.9	91.1 ± 6.3	85.1 ± 7.6
% total kcal	29.3 ± 0.4	29.7 ± 0.3	31.5 ± 1.5	27.4 ± 1.7
g/kg LBM	1.5 ± 0.1	1.5 ± 0.1	1.6 ± 0.2	1.4 ± 0.1
Carbohydrate				
g/d	349 ± 20	277 ± 14	460 ± 27	317 ± 27
% total kcal	57.1 ± 1.2	45.1 ± 0.4	70.9 ± 3.9	45.1 ± 1.4
g/kg LBM	6.6 ± 0.2	5.2 ± 0.1	7.9 ± 1.0	5.3 ± 0.5
Fiber (g/d)	1.5 ± 0.1	5.8 ± 0.4	1.7 ± 0.2	6.8 ± 0.8

NOTE. Data are the mean ± SEM.

Abbreviations: LP, low-protein; HP, high-protein.

Table 3. Metabolic Responses to Low- and High-Protein Diets

	Normal		NIDDM	
Parameter	LP	HP	LP	HP
Weight (kg)	69.1 ± 3.7	69.2 ± 3.7	108.6 ± 9.9*	108.6 ± 10.0*
Body mass index (kg/m²)	23.6 ± 0.8	23.7 ± 0.8	38.7 ± 2.6*	38.7 ± 2.6*
LBM (kg)	51.2 ± 3.1	52.0 ± 3.2	62.7 ± 7.1	62.3 ± 7.1
% LBM	74.2 ± 2.2	75.1 ± 2.1	57.4 ± 3.2*	57.0 ± 3.5*
RMR (kcal)	1,494 ± 52	1,513 ± 61	1,844 ± 95*	1,841 ± 84*

NOTE. Data are the mean ± SEM. Measurements were made in the postabsorptive state after the final day of each diet period.

Abbreviations: LP, low-protein; HP, high-protein.

*Significantly different *v* nondiabetic group, $P < .05$.

Table 4. Plasma Leucine Concentration and Kinetics

	Normal		NIDDM	
Parameter	LP	HP	LP	HP
Concentration (μmol/L)				
Basal	115.4 ± 6.1	131.5 ± 5.1*	121.7 ± 7.6	143.5 ± 9.5*
Glucose infusion	107.4 ± 4.1†	116.6 ± 4.3*†	113.9 ± 10.5	136.9 ± 13.7*
Ra (μmol/kg LBM · h)				
Basal	136.2 ± 7.3	148.1 ± 9.9	156.0 ± 17.0	147.0 ± 10.4
Glucose infusion	126.2 ± 8.2†	130.7 ± 9.5†	128.0 ± 7.9†	139.0 ± 7.6*
Oxidation (μmol/kg LBM · h)				
Basal	18.5 ± 1.9	27.1 ± 2.4*	15.4 ± 1.2	24.3 ± 1.9*
Glucose infusion	19.7 ± 1.7	26.2 ± 2.9*	17.1 ± 0.8	24.8 ± 1.9*
Tissue uptake (μmol/kg LBM · h)				
Basal	117.7 ± 6.2	120.9 ± 8.3	140.6 ± 17.3	122.8 ± 9.8
Glucose infusion	106.5 ± 7.4†	104.4 ± 6.9†	110.9 ± 7.5†	114.2 ± 7.2

NOTE. Data are the mean ± SEM. Glucose infusion refers to values obtained during the fourth hour of intravenous glucose infusion.

Abbreviations: LP, low-protein; HP, high-protein.

*Significantly different *v* LP in the same subject group, $P < .05$.

†Significantly different *v* basal value, $P < .05$.

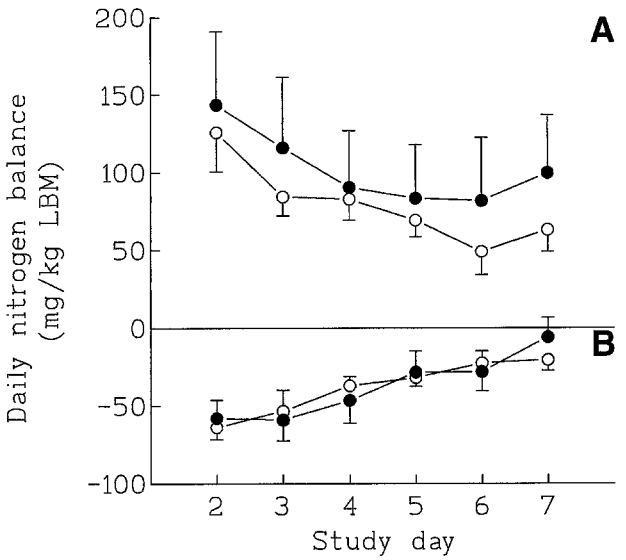

Fig 1. Daily N balance (mean ± SEM) expressed per unit LBM in normal (○) and NIDDM (●) subjects for the last 6 days of a 7-day diet high (A) or low (B) in protein. There is no significant difference between the normal and NIDDM responses.

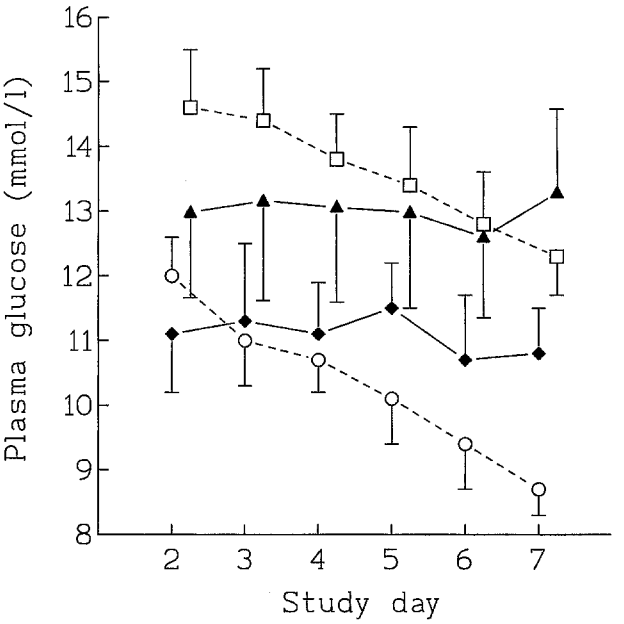

Fig 2. Daily blood glucose concentration in NIDDM subjects for the last 6 days of the diet high (▲, ◆) or low (□, ○) in protein. Average blood glucose was the sum of the 3 premeal values, 3 2-hour postmeal values, and the evening value divided by 7. The high-protein diet did not change fasting (◆) and average daily (▲) blood glucose. Fasting (○) and average daily (□) blood glucose were significantly reduced during the low-protein diet ($P < .05$).

postabsorptive glucose Ra failed to suppress leucine oxidation under all conditions (Table 4). N balance, determined for the final 6 days of each dietary period, was strongly positive on the high-protein diet. It promptly became negative upon introduction of the low-protein diet, but by day 7 was insignificantly different from zero (Fig 1).

Carbohydrate Metabolism

On the high-protein diet, postabsorptive plasma glucose and insulin concentrations in NIDDM subjects were nearly twice normal and the HGO was approximately 40% higher than normal (Table 5). The low-protein diet reduced plasma glucose in NIDDM subjects by nearly 2 mmol/L ($P < .05$) in the absence of any change in HGO or plasma insulin or glucagon concentrations (Table 5). The glucose infusion increased plasma glucose in control subjects by about 1 mmol/L, while increasing plasma insulin by 30% to 50% and reducing glucagon by about the same margin, and it reduced HGO by approximately 85% whether the prior diet was high or low in protein. For NIDDM subjects, glucose infusion increased plasma glucose by approximately 3 mmol/L while increasing plasma insulin by 30% with no change in glucagon (Table 5). The glucose infusion reduced HGO in NIDDM subjects as effectively as in control subjects (Table 5).

Figure 2 depicts the time course of dietary effects on capillary

Table 5. Steady-State Glucose Kinetics and Insulin and Glucagon Concentrations

Parameter	Normal		NIDDM	
	LP	HP	LP	HP
Basal glucose metabolism				
Glucose (mmol/L)	4.8 ± 0.1	4.9 ± 0.1*	7.0 ± 0.5†	8.9 ± 0.8*†
HGO (μmol/kg LBM · min)	16.6 ± 0.8	16.0 ± 0.7	22.1 ± 2.6†	23.0 ± 2.5†
Insulin (pmol/L)	80 ± 7	75 ± 3	163 ± 51	171 ± 52†
Glucagon (pg/mL)	64 ± 3	68 ± 6	82 ± 11	80 ± 13
During glucose infusion				
Glucose (mmol/L)	5.7 ± 0.1‡	6.2 ± 0.2*‡	9.7 ± 0.6†‡	11.5 ± 0.8*†‡
Total glucose Ra (μmol/kg LBM · min)	19.3 ± 1.0‡	19.0 ± 1.0‡	22.6 ± 2.6	21.4 ± 2.6
Infusion rate (μmol/kg LBM · min)	17.0 ± 0.04	17.0 ± 0.08	19.7 ± 2.7	19.6 ± 2.7
HGO (μmol/kg LBM · min)	2.3 ± 1.0‡	2.0 ± 1.0‡	2.9 ± 1.5‡	1.7 ± 1.5‡
Insulin (pmol/L)	105 ± 7‡	114 ± 8‡	215 ± 75	222 ± 63‡
Glucagon (pg/mL)	46 ± 4‡	47 ± 3‡	73 ± 10†	71 ± 11†‡

NOTE. Data are the mean ± SEM.
Abbreviations: LP, low-protein; HP, high-protein.
*Significantly different *v* LP in the same subject group, $P < .05$.
†Significantly different *v* nondiabetic group, $P < .05$.
‡Significantly different *v* basal value, $P < .05$.

blood glucose in the five NIDDM subjects for the final 6 days of each diet period. Neither fasting nor average daily blood glucose (millimoles per liter) changed over the course of the high-protein diet (fasting day 2, 11.1 ± 1.2; fasting day 7, 10.8 ± 0.7; average day 2, 13.0 ± 1.0; average day 7, 13.3 ± 1.3). However, both fasting and average daily glycemia improved over the course of the low-protein diet (fasting day 2, 12.0 ± 0.6; day 7, 8.7 ± 0.4; average day 2, 14.6 ± 0.9; day 7, 12.3 ± 0.6; both $P < .05$).

DISCUSSION

We previously observed that consumption of a maintenance-energy, protein-free diet reduced the basal leucine Ra, leucine oxidation, and HGO of normal subjects.[14,32] We have now tested whether variations in protein intake within the physiologic range modify leucine kinetics and basal HGO in normal subjects or patients with hyperglycemic NIDDM. The latter were studied because NIDDM is associated with increased basal gluconeogenesis and HGO, and it is possible that less effective hepatic glucose storage, which might exist in NIDDM,[1] could increase the importance of dietary protein in determining HGO. In this study, diets high or low in protein did not affect the basal glucose Ra of normal and NIDDM subjects. Additionally, we showed that a modest glucose infusion was equally effective in suppressing HGO when the prior diet was high or low in protein, indicating that a high rate of whole-body amino acid catabolism does not require the body to export the resulting carbons as glucose from the liver under basal conditions. This conclusion is consistent with a recent report that HGO in nondiabetic uremic patients was not reduced after 3 months of a 0.3-g protein/kg diet.[33] However, the blood glucose level and HGO of diabetic subjects in the present study was only modestly increased, and perhaps more importantly, HGO was effectively suppressed by a low-dose glucose infusion equal to the basal endogenous production rate. NIDDM patients whose basal HGO is higher than this or more resistant to exogenous glucose might respond differently to increases or decreases in dietary protein.

An associated aim of this research was to learn whether the metabolic need to catabolize dietary amino acids imposed by a high-protein intake extends into the postabsorptive period. We found that postabsorptive leucine oxidation did indeed reflect the preceding several days of protein intake. This agrees with some[34-37] but not other[38-40] previous measurements of the effect of variations in dietary protein intake on basal leucine oxidation. The reason for this disagreement is unclear. As suggested by Waterlow,[41] it is possible that basal leucine oxidation does indeed reflect habitual protein intake, as shown in the present study, but the measurement is insufficiently precise to distinguish the effects of only moderately different protein intakes. We also probed the extent to which a prior high-protein diet imposes a metabolic obligation to continue catabolizing amino acids in the postabsorptive period, by measuring whether leucine oxidation was more readily suppressed by exogenous glucose infusion when the preceding diet was low in protein. The effect of euglycemic hyperinsulinemia on postabsorptive leucine oxidation has been studied many times, with unclear results.[22,42] However, the persons studied were always adapted to a typical, excess-protein diet. In a more physiologic study (but also of subjects adapted to a standard, high-protein diet), a simple glucose infusion at 2 mg/kg · min (11 μmol/kg · min) did not affect leucine oxidation, whereas glucose infused at 4 mg/kg · min suppressed leucine oxidation considerably.[43] We therefore infused glucose at the lower of these rates to test whether leucine oxidation can be suppressed by glucose after the requirement to catabolize amino acids has been eased by a low-protein diet. The result was contrary to our prediction, for although the glucose infusion effectively increased plasma insulin and reduced the plasma leucine Ra, tissue uptake, and concentration, it failed to suppress leucine oxidation even when the prior diet was low in protein.

As measured using the leucine Ra and urinary 3-methylhistidine excretion and expressed per unit of LBM, whole-body protein proteolysis and myofibrillar proteolysis were unchanged by adaptation to either the high- or low-protein diets for both normal and NIDDM subjects. Consumption of a severely protein-deficient diet for 7 to 10 days clearly reduces basal whole-body protein turnover.[32,44] However, in the present study, widely different protein intakes within the normal physiologic range did not measurably affect basal protein turnover.

The high-protein diet resulted in a strongly positive N balance for both the normal and NIDDM groups. We believe this occurred because the diet our subjects consumed before entering the study almost certainly contained less protein than the very–high-protein diet we studied,[45] and because random assignment of the order of the study diets ensured that half of the subjects were protein-restricted immediately before switching to the high-protein test diet. Switching from a lower to a higher protein intake is normally associated with a period of positive N balance.[46,47] The clinically important conclusion is that the N balance of NIDDM subjects in the present study was close to zero and indistinguishable from that of the normal group after 7 days of adaptation to a protein intake that matched the average minimum protein requirement of normal adults (0.6 g/kg normal body weight[20]). Because of low statistical power, we cannot rule out a true difference in adapted N balance between NIDDM and normal subjects, but evidence for it was not even minimally apparent from this experiment. Unlike what has been suggested for hyperglycemic IDDM,[32,34] this result suggests that persons with mildly hyperglycemic NIDDM are not at a higher than normal risk of protein malnutrition if they restrict their protein intake.

We did observe that a diet providing protein at the level of the average adult requirement reduced basal and average daily blood glucose concentrations in NIDDM patients by approximately 2 mmol/L, whereas a high-protein intake had no effect on blood glucose. This effect became evident only after several days of protein restriction, and hence is not in conflict with evidence from short-term studies that mixed carbohydrate-protein test meals high in protein can blunt postprandial hyperglycemia in NIDDM.[48,49] The gradual reduction in blood glucose we observed is presumably due to reduced insulin resistance, for this has been reported after protein restriction of patients with insulin resistance related to renal insufficiency with[50] or without[33,51] associated IDDM. The present results indicate that persons with NIDDM and normal renal function may experience reduced fasting and average daily blood glucose levels if, as in this study, dietary protein is replaced

isoenergetically by carbohydrate. However, larger studies are required to confirm this.

In conclusion, we found no evidence that dietary protein, when consumed in widely different amounts within the physiologic range, alters basal HGO or its suppression by an exogenous glucose infusion in either normal or mildly hyperglycemic obese NIDDM subjects. A diet providing protein at the level of the average requirement was associated with reduced basal and average daily blood glucose concentrations in patients with NIDDM. This is presumed to be the result of improved insulin sensitivity induced by the low protein intake.

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