Effect of Protein Ingestion on Splanchnic and Leg Metabolism in Normal Man and in Patients with Diabetes Mellitus

JOHN WAHREN, PHILIP FELIG, and LARS HAGENFELDT

From the Department of Clinical Physiology, Serafimer Hospital, S-112 83 Stockholm; the Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut 06510; and the Department of Clinical Chemistry, Karolinska Hospital, S-104 01 Stockholm, Sweden

ABSTRACT The inter-organ flux of substrates after a protein-rich meal was studied in seven healthy subjects and in eight patients with diabetes mellitus. Arterial concentrations as well as leg and splanchnic exchange of amino acids, carbohydate substrates, free fatty acids (FFA), and ketone bodies were examined in the basal state and for 3 h after the ingestion of lean beef (3 g/kg body wt). Insulin was withheld for 24 h before the study in the diabetic patients.

In the normal subjects, after protein ingestion, there was a large amino acid release from the splanchnic bed predominantly involving the branched chain amino acids. Valine, isoleucine, and leucine accounted together for more than half of total splanchnic amino acid output. Large increments were seen in the arterial concentrations of the branched chain amino acids (100-200%) and to a smaller extent for other amino acids. Leg exchange of most amino acids reverted from a basal net output to a net uptake after protein feeding which was most marked for the branched chain amino acids. The latter accounted for more than half of total peripheral amino acid uptake. Alanine and glutamine were continuously taken up by the splanchnic tissues and released by the leg tissues after the protein meal, although their rate of output from the leg declined transiently at 30-60 min.

In the diabetics, the splanchnic exchange of amino acids after protein ingestion was comparable to controls. The positive change in total amino acid balance across the leg in diabetics tended to be greater than in controls at 30 min but thereafter was comparable in the two groups. In contrast, the rise in branched chain amino acid concentrations in arterial blood was 30-50% greater than in controls. Net uptake of branched chain amino acids by the leg was comparable to controls at 30-60 min. However, in contrast to the ongoing leg uptake observed in controls, no significant change from basal in the flux of branched chain amino acids across the leg was observed in the diabetics at 90-180 min. Leg output of alanine and glutamine was also unaffected by protein feeding in the diabetics.

In the normal subjects, the arterial glucose concentration remained within 10% of basal levels and splanchnic glucose output was unchanged after protein intake. In contrast, a greater rise in blood glucose, and a three-to-fourfold increment in splanchnic glucose output was observed at 30-90 min in the diabetics. Protein intake resulted in a doubling of arterial insulin and glucagon in the controls, and in a doubling of arterial glucagon in the diabetics.

It is concluded that (*a*) repletion of muscle nitrogen occurs after protein ingestion by selective splanchnic escape and muscle uptake of branched chain amino acids despite ongoing peripheral release of alanine and glutamine; (*b*) in patients with diabetes mellitus, leg uptake of total amino acids is normal or transiently

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Reprint orders may be obtained from Dr. Felig, Department of Internal Medicine, Yale University School of Medicine, New Haven, Conn. 06510.

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TABLE I

Clinical Data for Diabetic Patients and Healthy Subjects

	Age	Height	Weight	History of diabetes	Dose of lente insulin*	Arterial glucose concentration
	yr	*cm*	*kg*	*yr*	*IU*	*mmol/liter*
Patients						
K. M.	44	183	74	19	44+8	7.5
T. B.	31	172	63	12	60+56	15.6
H. L.	34	181	63	3	36	17.0
K. W.	30	180	82	8	24+20	18.9
L. B.	28	176	64	11	44	8.6
M. L.	23	184	69	8	40+24	20.6
J. G.	23	178	64	5	40+16	12.5
J. B.	30	179	74	7	20+20	8.8
Controls						
A. W.	27	184	70			4.9
G. E.	22	178	66			4.2
G. W.	22	181	72			4.9
B. I.	23	185	70			4.5
C. G.	23	190	68			4.1
P. L.	28	176	65			4.8
N. L.	32	180	83			5.0

* Where two numbers are given they represent morning and evening doses of insulin, respectively.

increased; however, uptake of branched chain amino acids is less persistent and augmented arterial increments of these amino acids are observed; (*c*) protein intake has a stimulatory effect on splanchnic glucose output in diabetes which is not observed in normal man.

INTRODUCTION

It is well established that in normal, postabsorptive man, amino acids, particularly alanine and glutamine, are continuously released by muscle tissue (1–3). Muscle output of alanine is further increased during periods of exercise (2), and persists in spite of hyperinsulinemia (4). Both in vivo and in vitro studies indicate that glucose-derived pyruvate is the major source of the carbon skeletons required for alanine synthesis in muscle (2, 5, 6). On the other hand, the pattern and mechanism of nitrogen repletion in muscle tissue and the source of the amino groups for alanine and glutamine synthesis have not been established. The branched chain amino acids have been suggested as the origin of the amino groups for alanine synthesis (2, 5–7) inasmuch as extrahepatic tissues, particularly muscle, have been implicated as the site of their oxidation (8, 9), and physiologic increments in these amino acids stimulate alanine output from isolated muscle tissue (6, 7). Since in the postabsorptive state total alpha amino nitrogen balance across muscle is negative (2, 3, 5) and no consistent uptake of branched chain amino acids is observed (2, 3, 5), repletion of muscle nitrogen is likely to occur mainly in the fed state after a protein-containing meal.

The metabolic response to protein ingestion has received relatively little attention. Early experiments by Van Slyke and Meyer (10) demonstrated that protein ingestion in dogs resulted in increased levels of total circulating amino acids. Subsquent studies in man (11, 12) have indicated that the greatest and most prolonged increments in systemic amino acid concentration induced by protein involve the branched chain amino acids. In addition, protein ingestion is known to be associated with a significant rise in the concentration of circulating insulin (13) as well as glucagon (14). It has been suggested that the rise in insulin concentration after a protein meal serves to facilitate disposal and utilization of absorbed amino acids by peripheral tissues (13). Protein-induced hyperglucagonemia has been proposed as a stimulus of hepatic glucose output, thereby counteracting insulin-induced augmentation of peripheral glucose utilization (15) and preventing hypoglycemia after protein feeding (15). No data are available, however, on splanchnic and muscle exchange of substrates, particularly amino acids and glucose, after the ingestion of a protein-rich meal in normal man. Moreover, the effect of diabetes mellitus with its attendant alterations in insulin and glucagon secretion (14) on the metabolic response to a protein meal has not been established. It may be speculated that an altered hormonal response to protein ingestion in diabetes may influence regional exchange of glucose and amino acids. To test this hypothesis and to examine in normal man the inter-organ flux of substrates after protein ingestion, healthy subjects and patients with diabetes mellitus were studied after the ingestion of a standard protein meal. Arterial concentrations as well as splanchnic and leg exchange of substrates were examined in the basal state and for 3 h after protein intake.

METHODS

Subjects. Two groups of subjects were studied. The control group consisted of seven healthy nonobese male volunteers. Data on age and body dimensions are given in Table I. None of the subjects participated in competitive athletics on a regular basis. The diabetic group consisted of eight male nonobese patients within insulin-dependent diabetes mellitus of several years duration. All patients were receiving insulin therapy. Clinical data are given in Table I. The patients were in good nutritional balance and ingested a weight-maintaining diet made up of approximately 40% carbohydrate and 25–35% each of protein and fat. All patients were followed at regular intervals of 2–3 mo in the outpatient department. None had periods of weight loss or episodes of hypoglycemia during the year preceding the study. There was no history or evidence of liver disease. No patients had signs or symptoms of peripheral vascular disease. At the time of the study all patients were actively

employed. The studies were carried out at the Serafimer Hospital, Stockholm. The nature, purpose, and possible risks involved in the study were carefully explained to all patients and control subjects before obtaining their voluntary consent to participate. Five of the diabetic patients (K. M., T. B., M. L., K. W., and L. B.) had participated in previous studies (16) and thus were completely familiar with the procedures.

Procedure. The studies were performed with the subjects in the recumbent position after an overnight fast (12–14 h). Insulin was withheld for 24 h before the study. Teflon catheters with an outer diameter of 1.2 mm were inserted percutaneously into a femoral artery, both femoral veins, and an antecubital vein. A Cournand catheter (no. 7 or 8) was introduced percutaneously into another antecubital vein and manipulated under fluoroscopic control to a right-sided main hepatic vein. The tip of the catheter was placed 3–4 cm from the wedge position. Patency of the catheters was maintained by intermittent flushing with saline; the hepatic venous catheter was flushed with 1.5% sodium citrate solution; a total of less than 0.3 g was administered to each subject. Heparin was not employed in the study.

When the catheters had been introduced, the subjects were studied in the basal state and for 3 h after protein ingestion. They consumed within a period of 15 min a protein meal in the form of boiled lean beef (raw composition, 22% protein, 5% fat) administered in a dose of 3 g (raw weight) per kg body weight. All subjects consumed the entire amount of lean beef. Blood samples for determination of substrate and hormone concentrations were collected simultaneously from the femoral artery and vein and from the hepatic vein repeatedly at timed intervals during a 30-min basal period and for 3 h after protein ingestion. Blood flow to the splanchnic area and the leg was determined in the basal state and repeatedly after protein ingestion. Expired air was collected before and twice after protein intake for measurement of pulmonary oxygen uptake.

Hepatic blood flow was estimated by the continuous infusion technique (17) using indocyanine green dye (18). Leg blood flow was determined using the indicator dilution procedure described by Jorfeldt and Wahren (19).

Analytical methods. Glucose was analyzed in whole blood using the glucose oxidase reaction (20). Lactate (21), pyruvate (22), glycerol (23), 3-hydroxy-butyrate, and acetoacetate (24) were determined enzymatically in whole blood. Individual amino acids were measured in whole blood by the automated ion-exchange chromatographic technique (25). By this technique, glutamine and asparagine emerge as a single peak (25), and are referred to collectively in the Results section and tables as glutamine plus asparagine (inter-organ exchange of asparagine is negligible [3]). Glutamate values, on the other hand, are often artifactually elevated with the chromatographic technique (3, 26) and therefore are not reported. Before analysis, the whole blood samples were deproteinized and treated with sodium sulfite to remove glutathione as described previously (25, 26). This procedure results in loss of the cystine and methionine peaks (25, 26) which are consequently not reported. The basic amino acids were measured in the control group only, in the basal state, and at 60, 120, and 180 min. Plasma glucagon was analyzed by radioimmunoassay using Unger antibody 30K (27). Plasma-free fatty acids (FFA) were determined using a gas chromatographic method (28). Indocyanine green dye was determined spectrophotometrically at 805 nm in serum samples. Oxygen saturation was measured spectrophotometrically (29) and hemoglobin concentration was determined by the cyanmethemoglobin technique (30). Hematocrit was measured using a microcapillary hematocrit centrifuge and corrected for trapped plasma. Expired air was analyzed using the Scholander microtechnique.

Data in the text, tables, and figures are given as mean

TABLE II

*Oxygen Uptake and Regional Blood Flows in the Basal State and after Protein Ingestion in Diabetic Patients (D) and Healthy Controls (C)**

		Basal	After protein ingestion 60 min	After protein ingestion 180 min
Pulmonary O_2 uptake, *ml/min*	D	278±8	301±10‡	310±6‡
	C	297±7	338±9‡	336±13‡
Respiratory exchange ratio	D	0.77±0.01	0.78±0.01	0.77±0.01
	C	0.73±0.01	0.76±0.02	0.75±0.01
Estimated hepatic Blood flow, *liter/min*	D	1.32±0.03	1.46±0.09§	1.35±0.08
	C	1.54±0.05	1.50±0.08	1.55±0.11
Splanchnic O_2 uptake, *ml/min*	D	56±4	76±9‖	73±8‖
	C	55±3	65±5‖	73±4‡
Leg blood flow, *liter/min*	D	0.55±0.04	0.53±0.05	0.63±0.05
	C	0.72±0.10	0.69±0.09	0.75±0.11
Leg O_2 uptake, *ml/min*	D	26±3	26±2	27±3
	C	33±1	34±1	35±1

* Data are presented as mean±SEM.
‡ Significantly different from the corresponding value in the basal state, $P < 0.005$.
‖ Significantly different from the corresponding value in the basal state, $P < 0.05$.
§ Significantly different from the corresponding value in the basal state, $P < 0.01$.

TABLE III

*Arterial Concentrations of Substrates, Insulin, and Glucagon in the Basal State and after Protein Ingestion in Diabetic Patients (D) and Control Subjects (C)**

			Protein ingestion					
		Basal‡	30 min	60 min	90 min	120 min	150 min	180 min
Glucose, *mmol/liter*	D	12.48±1.00	13.03±2.07§	14.01±2.02‖	13.71±1.96‖	14.06±1.93‖	14.05±1.91‖	12.97±1.62§
	C	4.63±0.07	4.47±0.18§	4.64±0.16	4.72±0.13	4.77±0.09§	4.92±0.14‖	5.05±0.15‖
Lactate, *mmol/liter*	D	0.55±0.04	0.54±0.07	0.57±0.06	0.56±0.05	0.56±0.06	0.62±0.08	0.60±0.08
	C	0.60±0.06	0.55±0.08	0.60±0.11	0.61±0.11	0.58±0.10	0.54±0.07	0.51±0.06
Glycerol, *μmol/liter*	D	68±4	77±9	78±10	78±9	84±10	91±14	92±14
	C	52±4	45±5	43±4	44±5	46±4	55±5	59±7
3-hydroxybut. *mmol/liter*	D	0.96±0.15	1.0±0.23	1.15±0.27	1.14±0.33	1.34±0.40	1.54±0.46§	1.59±0.52§
	C	0.19±0.05	0.17±0.07	0.12±0.04	0.11±0.03	0.10±0.03	0.12±0.04	0.16±0.05
Acetoacetate, *μmol/liter*	D	240±33	275±55	319±62‖	316±69§	349±74§	358±72‖	336±70‖
	C	78±19	93±31	79±17	81±19	80±16	89±23	105±25
FFA, *μmol/liter*	D	826±74	—	993±109§	—	1,103±137‖	—	1,274±154‖
	C	572±69	—	331±45‖	—	368±49‖	—	623±41
Glucagon, *pg/ml*	D	131±33	202±41§	241±46‖	317±54‖	300±71‖	267±55‖	270±72‖
	C	76±9	134±29‖	161±32‖	165±26‖	149±18‖	145±18‖	138±24‖
Insulin, *μU/ml*	C	8.9±0.7	15.9±2.7‖	16.0±3.8§	17.9±3.9§	13.5±1.6‖	13.4±1.5‖	13.5±2.0§

* Data are given as mean±SEM.

‡ Data for the basal state represent the mean of two–four observations at 5–10 min intervals.

§ Significantly different from the corresponding value for the basal state, $P < 0.025$.

‖ Significantly different from the corresponding value for the basal state, $P < 0.01$.

±SE. Standard statistical methods (31) have been employed, using the paired *t* test when applicable.

RESULTS

Oxygen uptake and regional blood flow. Pulmonary oxygen uptake in the basal state was similar in the diabetic patients and the healthy subjects (Table II). Both groups showed an 8-14% rise in oxygen uptake at 1 h after protein ingestion ($P < 0.005$), this increment being demonstrable also after 3 h. The respiratory exchange ratio was slightly higher for the diabetics in the basal state ($P < 0.05$) but did not differ between the groups after protein ingestion. Estimated hepatic blood flow for the diabetics was 15% lower than control in the basal state but rose to the same level after protein ingestion; estimated hepatic blood flow remained unchanged in the control group after the protein meal. Splanchnic oxygen uptake was similar in the two groups in the basal state and increased by 20-35% ($P < 0.01$) in both groups after protein ingestion. The rise in splanchnic oxygen uptake in the diabetic group could account for, on the average, 55-58% of the simultaneous increase in pulmonary oxygen uptake. The corresponding values for the controls were 25-45%. Leg blood flow in the basal state was no different in the two groups and was not significantly influenced by the protein meal. Calculated leg oxygen uptake was approximately 25% smaller for the diabetics ($P < 0.05$) and was uninfluenced by the protein meal in both groups.

Arterial concentrations. (Tables III and IV) In agreement with the findings from previous studies involving patients with diabetes mellitus (16, 32), a relative steady state could be established with regard to the concentrations of circulating metabolites and hormones during the basal observation period. Thus, the mean arterial glucose concentration varied less than 1% between the four measurements obtained during the 30-min basal period. Corresponding variations for lactate, glycerol, 3-hydroxybutyrate, and FFA were 1-7% for both groups of individuals.

The arterial glucose concentration in the basal state was two- to threefold greater in the diabetic group as compared to controls (Table III). During the 1st h after protein ingestion, the diabetics showed a progressive rise in arterial glucose level ($P < 0.025$-0.01). In contrast, healthy subjects displayed a small (3-5%) initial decrease in arterial glucose 15 and 30 min after protein intake ($P < 0.025$-0.01). After this, a slow gradual rise was seen so that 3 h after the protein meal, the arterial glucose concentration exceeded the basal level by 9% ($P < 0.001$). Although the relative increments were comparable in the two groups, the absolute increase was three to fourfold greater for the diabetics (1.57 mmol/liter) than for the healthy subjects (0.42 mmol/liter, $P < 0.02$).

Lactate and glycerol concentrations were not significantly influenced by protein ingestion in either group (Table III), although the diabetics showed consistently higher levels of glycerol than did controls

TABLE IV

*Arterial Concentrations of Amino Acids in Whole Blood in the Basal State and after Protein Ingestion in Diabetic Patients (D) and Control Subjects (C)**

			After protein ingestion					
		Basal	30 min	60 min	90 min	120 min	150 min	180 min
TAU	D	149±10	190±7‡	190±6‡	200±7§	200±10§	218±14§	198±21‖
	C	133±6	164±6‡	155±6‡	177±6§	171±7‖	201±10§	179±9‡
ASP	D	104±14	124±25	108±11	120±20	105±14	127±29	105±20
	C	174±28	150±14	147±18‖	160±17	153±19	184±23	149±22
THR	D	84±6	124±12‖	133±18‡	144±18§	135±14§	127±16‖	114±11‖
	C	105±6	134±8	162±11§	185±9§	180±8§	173±11§	159±9§
SER	D	121±15	144±9‖	139±11‖	142±11‖	128±13	129±9	124±15
	C	137±12	158±8‡	162±14‡	185±13‡	175±13§	169±13‡	156±14‖
GLN + ASN	D	589±28	528±49	—	522±41‖	—	503±54‖	—
	C	624±32	644±34	—	691±41	—	663±46	—
PRO	D	152±23	185±18	213±25‡	220±13‡	216±36‖	183±19	160±18
	C	170±10	181±12	199±16	210±15‡	214±13‡	219±11‡	216±10‡
CIT	D	31±9	27±2	37±9	37±13	40±9	28±7	39±16
	C	28±6	28±4	25±3	33±2	25±5	39±6	29±5
GLY	D	260±18	334±13‡	344±16‡	329±25‡	304±23	292±13	283±21
	C	274±19	327±15‡	342±22§	365±20§	330±18‡	342±21§	295±19
ALA	D	160±11	209±15§	202±17§	209±19‖	177±21	183±15	167±10
	C	240±27	300±29‖	298±32‖	340±24‡	298±30‡	312±22‖	258±16
α-AB	D	22±3	25±5	36±5‡	31±5	34±5‡	30±2‖	36±4‡
	C	20±3	28±5‖	30±6‡	32±5‡	30±6‡	38±4§	35±5‡
VAL	D	231±14	393±41§	399±24§	513±55§	438±30§	515±59§	408±43§
	C	203±10	276±17‡	318±23§	381±24§	387±14§	396±11§	364±10§
ILE	D	69±6	134±16§	173±11§	205±14§	203±17§	211±20§	177±25§
	C	42±3	86±8§	114±13§	141±13§	132±7§	139±6§	123±4§
LEU	D	140±10	255±21§	330±20§	369±21§	375±31§	368±26§	320±41§
	C	103±10	186±14§	227±20§	279±22§	260±16§	269±11§	233±6§
TYR	D	40±2	59±4‡	67±4§	75±6§	72±5§	70±5§	60±5‡
	C	46±3	59±7‖	71±6‡	82±6§	79±6§	79±6§	71±6‡
PHE	D	40±3	63±3‡	66±3§	71±5§	70±4§	68±6§	62±6‡
	C	39±1	54±3‡	65±3§	69±3§	68±4§	63±2§	58±3§
ORN	D							
	C	80±5	—	85±14	—	109±9‖	—	102±12
LYS	D							
	C	169±13	—	211±28‡	—	221±51‖	—	245±36‡
HIS	D							
	C	71±4	—	80±8	—	95±11	—	76±10
ARG	D							
	C	56±3	—	86±16	—	93±15‡	—	94±11‖

* Data are presented as means±SEM in micromoles per liter; the basic amino acids (orinithine, lysine, histidine, and arginine) were measured in the control group only.

‡ Significantly different from the corresponding value in the basal state, $P < 0.01$.

§ Significantly different from the corresponding value in the basal state, $P < 0.005$.

‖ Significantly different from the corresponding value in the basal state, $P < 0.025$.

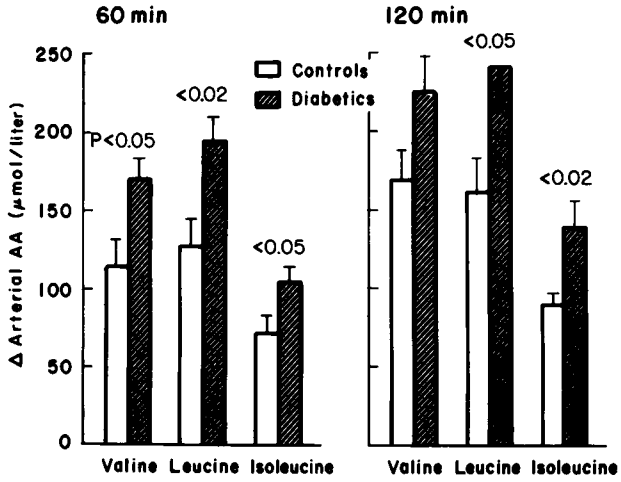

FIGURE 1 The increase in branched chain amino acid concentrations in arterial blood after protein ingestion in normal and diabetic subjects. Mean values±SEM are shown. P values refer to the significance of the differences between the two groups. The increments at 30 and 90 min (not shown) were also significantly greater ($P < 0.05$) in the diabetic group (see Table IV).

($P < 0.05$–0.01). Arterial levels of ketone acids and FFA were higher in the diabetic group in the basal state ($P < 0.025$), and throughout the 3-h observation period ($P < 0.025$–0.01) (Table III). In healthy controls, an initial fall in arterial FFA was seen (55% at 60 min, $P < 0.001$), and was followed by a gradual return to the basal level during the remaining observation period (Table III).

Arterial concentrations of amino acids in whole blood are presented in Table IV. In agreement with previous observations for plasma concentrations (32–34), in the diabetic group there were increments in the concentrations of isoleucine (65%, $P < 0.01$), leucine (35%, $P < 0.025$), and valine (15%, $P < 0.1$). In addition, the diabetic patients showed lower levels of alanine (35%, $P < 0.01$), threonine (20%, $P < 0.05$), and aspartate (40%, $P < 0.05$). After the ingestion of protein, the most pronounced rises in arterial concentration were seen for the branched chain amino acids in both diabetic patients and healthy controls. In the control group, arterial valine, leucine, and isoleucine increased by 100, 170, and 230%, respectively. Smaller increments (20–85%) were seen for taurine, threonine, serine, glycine, alanine, α-aminobutyrate, tyrosine, phenylalanine, ornithine, lysine, and arginine. The mean maximal increments for the branched chain amino acids were 224±23 μmol/liter (valine), 196±21 μmol/liter (leucine), and 112±10 μmol/liter (isoleucine); for the remaining amino acids, the maximal increments were less than 100 μmol/liter. In the diabetics the absolute levels of valine, isoleucine, and leucine as well as their rise above basal at 30–120 min after protein ingestion were 30–50% greater than for the controls (Fig. 1). As in the basal state, the arterial levels of both alanine and threonine were lower in the diabetics than in controls throughout the observation period ($P < 0.01$–0.05) (Table IV).

The diabetic patients showed a 50% higher arterial glucagon concentration in the basal state in relation to controls ($P < 0.05$) (Fig. 2). After protein ingestion there was a prompt rise in arterial glucagon to approximately twice the basal concentration in both groups ($P < 0.01$). These levels were then largely maintained during the remainder of the observation period. After protein ingestion the arterial insulin concentration rose in the healthy controls and after 60 and 90 min reached values approximately twice the basal level ($P < 0.025$–0.05) (Table III).

Splanchnic exchange. Splanchnic glucose output in the basal state was relatively stable in both diabetics and controls (Fig. 2) and there was no significant difference between the groups. After protein ingestion the diabetic patients showed a rise in splanchnic glucose output above basal which persisted for 90 min ($P <$ 0.05–0.01). The peak was threefold above basal level and occurred 45 min after protein ingestion (Fig. 2). Glucose output then declined gradually to the basal level. Values obtained at 30, 45, and 60 min after protein were significantly greater than the corresponding data for controls ($P < 0.05$–0.01). In contrast to the

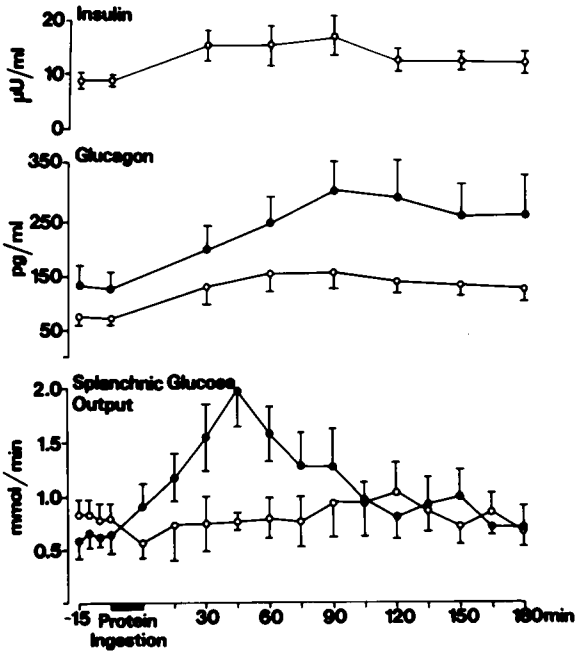

FIGURE 2 Arterial concentrations of insulin and glucagon and splanchnic glucose output in the basal state and after protein ingestion in diabetic patients (●) and healthy controls (○). Mean values±SEM are indicated.

TABLE V

*Splanchnic Exchange of Substrates in the Basal State and after Protein Ingestion in Diabetic Patients (D) and Control Subjects (C)**

			Protein ingestion					
		Basal	30 min	60 min	90 min	120 min	150 min	180 min
Glucose output, *mmol/min*	D	0.64±0.07	1.55±0.31‡	1.58±0.25‡	1.27±0.35§	0.81±0.21	0.72±0.08	0.71±0.20
	C	0.79±0.13	0.75±0.26	0.80±0.19	0.95±0.33	1.05±0.27	0.73±0.18	0.68±0.15
Lactate uptake, *mmol/min*	D	0.33±0.04	0.27±0.07	0.31±0.06	0.27±0.04	0.29±0.07	0.29±0.05	0.26±0.03
	C	0.21±0.03	0.12±0.04‖	0.05±0.09§	−0.01±0.11§	−0.01±0.13	0.05±0.12	0.12±0.09§
Glycerol uptake, *μmol/min*	D	43±4	66±13‖	56±11‡	70±9‡	71±12§	70±12§	61±12
	C	35±7	29±5	25±4	28±5	28±5	41±7	46±10
3-hydroxybut. output, *μmol/min*	D	285±31	303±68	352±93	441±106	437±96	663±143‖	535±103‖
	C	110±48	40±16	29±10	38±22	43±17	50±20	96±39
Acetoacetate, output *μmol/min*	D	199±23	201±38	205±31	191±36	208±37	189±27	155±30
	C	78±16	75±13	69±18	69±22	83±27	86±23	108±34
FFA uptake, *μmol/min*	D	146±21		192±39		197±40§		247±30‖
	C	148±22		42±8‡		60±8‡		123±22

* Data are presented as mean±SEM.
‡ Significantly different from the corresponding value in the basal state, $P < 0.01$.
§ Significantly different from the corresponding value in the basal state, $P < 0.05$.
‖ Significantly different from the corresponding value in the basal state, $P < 0.025$.

changes seen in the diabetics, the healthy subjects showed a transient 25% reduction in splanchnic glucose output immediately after protein ingestion ($P < 0.1$) whereupon the glucose output rose and remained at the basal level during the rest of the observation period.

Splanchnic uptake of lactate in the basal state was approximately 55% greater in the diabetics than in the controls (Table V). In the former group lactate uptake was not significantly influenced by the protein meal, while in the latter, a significant reduction to approximately 60% of the basal was seen 30 min after protein intake ($P < 0.025$). At no time during the remainder of the observation period was significant lactate uptake demonstrable in the control group. Glycerol uptake by the splanchnic area in the basal state was similar in the two groups and remained stable in the controls. In the diabetics a 30–65% increase in glycerol uptake was observed over the 3-h period. (Table V).

Table VI presents the splanchnic exchange of amino acids in diabetics and controls. In the basal state the quantitatively most important amino acids taken up were alanine and glutamine. In addition, threonine, serine, glycine, tryosine, phenylalanine, lysine, and histidine were taken up. In both groups the ingestion of protein resulted in a large release of amino acids from the splanchnic area, particularly the branched chain amino acids. In controls as well as diabetics, valine, isoleucine, and leucine together accounted for more than half of the total amino acid release at 60 min after the protein meal (200–225 μmol/min) and were the only amino acids consistently released at 120–180 min. The average output of branched chain amino acids from the splanchnic area was not significantly different in diabetics and controls at any time during the observation period. Besides the branched chain amino acids, smaller outputs were observed at 30–60 min for taurine, threonine, glycine, citrulline, α-aminobutyrate, tyrosine, phenylalanine, ornithine, lysine, and histidine. In contrast to all other amino acids, consistent net splanchnic uptakes of alanine and glutamine were observed in both groups at all times, albeit at transiently reduced rates at 30–60 min.

Net splanchnic exchange of ketone acids and FFA are shown in Table V. In the controls splanchnic output of ketone acids remained stable throughout the 180-min observation period. Net uptake of FFA fell 60–70% during the first 2 h after the protein meal, coincident with the fall in arterial FFA levels. In the diabetics there was a progressive rise in splanchnic output of ketone acids and uptake of FFA. The latter paralleled the rising arterial concentration of FFA. It should be noted, however, that the progressive ketogenesis and rising arterial levels and splanchnic uptake of FFA observed in the diabetics may reflect progressive insulin deficiency rather than an effect of protein ingestion (see Discussion, below).

Leg exchange. As indicated in Table VII there was, in the basal state, a significant output of alanine as well as glutamine from the leg tissues, the release being of similar magnitude in the two groups. A small release of valine, isoleucine, and leucine ($P < 0.025$–0.05) was detectable in the controls. In the diabetics net exchange of these amino acids was of such variability that the mean value was not significantly different from zero.

After protein ingestion this pattern was markedly altered. In the control group net uptake by the leg was observed within 30–60 min of protein ingestion for valine, leucine, and isoleucine, and to a lesser extent for threonine, serine, glycine, tyrosine, phenylalanine, lysine, histidine, and arginine. The uptake of the branched chain amino acids accounted for more than half of total leg amino acid uptake at 30–60 min, and for virtually all of the amino acid uptake at 90–180 min. Throughout the 3-h period of observation after protein intake, a continuous net release of alanine and glutamine was observed. A transient 65% decline in the release of alanine was noted, however, at 60 min in the controls ($P < 0.01$) (Table VII).

Leg exchange of individual amino acids in the diabetic group is shown in Table VII. To take into ac-

TABLE VI

*Splanchnic Exchange of Amino Acids in the Basal State and after Protein Ingestion in Diabetic Patients (D) and Control Subjects (C)**

			After protein ingestion					
		Basal	30 min	60 min	90 min	120 min	150 min	180 min
TAU	D	0±5.3	−5.0±7.9	6.7±5.6	20.2±31.2	−11.5±11.2	8.7±7.4	−12.7±7.6
	C	3.3±5.3	−17.0±8.4	10.0±11.4	4.1±17.2	11.9±8.9	−10.0±4.6	−10.8±9.0
ASP	D	−22.3±12.1	−19.1±23.7	−19.3±9.7	17.5±34.7	−1.7±16.2	6.2±9.1	−20.0±12.7
	C	37.4±15.2	−25.7±23.5	11.2±16.9	−27.3±26.0	−2.4±11.4	43.7±13.2	−2.6±19.8
THR	D	9.4±3.2	−23.1±9.7‡	−11.0±12.6	10.7±26.9	2.2±8.7	22.5±7.3	11.0±8.6
	C	15.3±4.9	−20.6±10.7‡	−12.1±10.0‡	−11.0±15.4	5.6±11.1	22.4±28.5	4.4±11.4
SER	D	8.0±9.7	0±20.0	9.4±11.4	18.5±13.8	25.5±7.4	31.3±7.9	19.8±7.2
	C	22.7±10.7	−2.0±10.5	0.5±9.9	17.3±15.9	27.7±8.7	31.4±15.1	18.2±11.6
GLN + ASN	D	74.2±22.0	43.1±41.7	—	40.4±17.5	—	136.6±26.0	—
	C	67.7±19.0	58.1±22.8	—	131.3±61.9	—	126.0±40.0‡	—
PRO	D	14.0±13.2	−43.7±23.9§	−18.4±7.9‡	24.4±60.5	−4.0±10.9	16.5±21.1	−9.8±19.5
	C	20.2±15.3	−38.0±12.0§	4.5±5.8	−36.2±24.3	−11.2±31.2	−20.3±13.4	−16.1±17.3
CIT	D	−37.2±12.7	−14.6±7.2	−22.9±10.5	−17.5±10.6	−14.5±16.0	−13.2±6.8	−21.8±7.0
	C	−18.0±7.7	−13.8±3.8	−10.8±5.7	−2.8±13.1	−6.5±3.7	−12.6±6.3	−2.5±4.1
GLY	D	−7.6±13.4	−3.0±41.9	−23.0±15.1	21.2±51.8	−11.8±19.7	26.7±8.6	7.0±21.8
	C	26.0±10.2	−66.6±15.7‡	−26.2±10.4‡	−14.9±23.3	5.6±15.2	7.1±17.6	6.8±18.7
ALA	D	95±15.1	63.6±20.0	60.9±15.1	82.7±29.3	75.5±14.2	94.0±13.9	100.7±20.2
	C	97.3±16.8	40.3±18.7	58.3±12.6	60.4±28.7	108.7±29.8	107.4±19.2	113.1±16.3
α-AB	D	0.4±1.2	−1.2±2.1	−2.7±5.3	1.7±6.1	2.0±4.2	−1.3±5.3	5.3±7.9
	C	−2.6±1.7	−7.0±2.3	0±4.9	−2.2±2.5	−3.9±6.8	4.0±3.9	9.1±6.0
VAL	D	−0.1±12.1	−65.3±24.8‡	−58.6±11.3‡	−30.2±17.3§	−76.7±18.9‡	−11.2±7.4	−62.3±16.7
	C	−7.0±7.1	−74.9±14.4‡	−64.6±14.4‡	−67.4±17.3‡	−45.7±13.4§	−69.7±19.5‡	−46.5±12.6
ILE	D	0.4±3.6	−49.0±16.8§	−38.0±11.3§	−86.0±14.5‖	−71.2±23.3§	−20.3±9.0	−46.2±20.3§
	C	−6.6±2.6	−32.1±7.7‡	−32.0±13.4‡	−62.5±13.5‖	−43.2±8.8‖	−47.3±16.5‡	−27.8±7.5
LEU	D	−2.3±6.1	−76.1±21.7§	−68.0±17.8§	−146.2±30.2‡	−93.0±24.5§	−34.7±12.9	−69.8±24.3§
	C	−18.0±14.3	−63.0±14.6§	−63.6±20.8§	−97.3±18.8‡	−71.2±16.3‡	−89.6±18.6‡	−44.5±14.8
TYR	D	11.0±2.1	−10.9±7.1§	−1.1±4.6§	7.7±12.7	0.7±3.8	14.0±2.2	7.3±5.2
	C	13.7±1.6	−9.1±3.0§	7.9±7.7	−1.9±7.5	6.0±8.1	7.7±7.7	3.1±5.3
PHE	D	5.7±2.5	−12.3±14.3	0.9±4.9	0±11.7	2.0±3.9	8.7±2.0	10.5±5.8
	C	6.4±2.1	−8.3±3.5	8.3±7.1	−5.5±6.1	2.8±5.2	−1.1±6.2	−5.9±2.5§
ORN	D	—	—	—	—	—	—	—
	C	4.2±4.9	—	−12.0±3.6§	—	6.3±5.2	—	−24.8±3.1‡
LYS	D	—	—	—	—	—	—	—
	C	28.7±14.1	—	−33.0±2.8§	—	17.3±29.2	—	−20.2±15.1
HIS	D	—	—	—	—	—	—	—
	C	12.5±5.1	—	−8.8±5.3§	—	27.3±11.9	—	1.7±8.5
ARG	D	—	—	—	—	—	—	—
	C	4.5±6.2	—	−8.8±10.3	—	6.5±27.2	—	1.6±3.2

* Data are presented as means±SEM in micromoles per minute; The basic amino acids (ornithine, lysine, histidine, arginine) were measured in the control group only.

‡ Significantly different from the corresponding value in the basal state, $P < 0.01$.

§ Significantly different from the corresponding value in the basal state, $P < 0.025$.

‖ Significantly different from the corresponding value in the basal state, $P < 0.005$.

TABLE VII

*Leg Exchange of Amino Acids in the Basal State and after Protein Ingestion in Diabetic Patients (D) and Control Subjects (C)**

			After protein ingestion					
		Basal	30 min	60 min	90 min	120 min	150 min	180 min
TAU	D	−6.3±4.4	3.7±2.4	1.4±1.6	−11.5±6.0	−6.3±5.2	−0.8±2.0	−1.3±6.0
	C	0.2±3.4	−1.4±4.6	4.2±3.3	−3.3±4.2	2.9±2.4	−1.9±5.0	−1.9±6.3
ASP	D	−8.4±4.2	8.5±11.1	3.0±3.0	−13.2±8.8	−1.7±9.5	5.3±7.4	−0.3±8.7
	C	18.0±6.8	−3.6±4.0	2.0±4.9	−18.0±21.5	3.6±7.0	26.1±10.8	4.4±9.9
THR	D	−13.1±3.4	4.7±5.2	2.4±3.5	−5.0±4.0	−4.8±7.4	−6.0±2.1	−6.0±5.0
	C	−2.4±2.8	0.7±4.3	9.1±4.0‡	6.0±4.8	5.9±3.0	0.3±5.3	−3.1±2.7
SER	D	−6.9±3.6	6.0±5.1	3.1±2.4‡	−2.7±5.2	−5.3±4.5	−1.3±1.6	1.0±5.2
	C	4.7±2.5	7.1±3.2§	6.9±8.1	8.9±5.6	7.2±1.6	4.4±4.0	3.4±2.9
GLN + ASN	D	−50.9±16.7	−31.3±13.0	—	−60.7±12.5	—	−40.4±12.0	—
	C	−41.8±10.5	−39.7±14.7	—	−67.8±20.7	—	−36.4±10.5	—
PRO	D	−10.6±14.0	−3.0±10.8	−6.2±7.0	0±9.0	−30.3±25.5	7.0±7.0	−15.8±10.0
	C	−6.0±6.0	11.0±2.0	43.0±30.0	6.0±8.0	10±18.0	12.0±7.2	2.0±1.5
CIT	D	−8.5±6.2	0.8±3.0	2.4±2.7	−0.3±0.3	−0.8±8.3	−9.8±9.1	−12.3±5.0
	C	3.6±3.0	−1.0±1.4	2.0±1.3	1.2±1.4	−8.0±7.0	8.3±2.6	0.8±3.3
GLY	D	−36.0±16.4	−0.3±10.0	−2.1±5.2	−4.2±11.8	−15.5±11.9	−18.0±11.2	−24.3±4.9
	C	−6.1±5.8	14.7±4.5§	23.3±6.9‖	−0.6±9.1	−4.6±10.5	2.9±11.0	−9.7±7.1
ALA	D	−37.6±9.1	−23.9±8.3	−24.3±5.4	−25.7±7.7	−38.5±7.2	−30.5±9.5	−26.5±8.5
	C	−33.5±6.9	−22.3±7.2	−12.0±5.2‡	−39.4±11.0	−33.7±6.2	−30.6±8.5	−35.6±3.2
α-AB	D	0±1.4	−1.9±2.2	2.7±0.8	−1.5±1.3	1.5±1.3	−1.7±1.5	1.7±3.8
	C	−4.2±5.2	1.0±2.0	−0.3±2.5	0.6±1.7	−9.7±6.1	0.9±2.1	−0.7±1.6
VAL	D	−9.7±10.6	32.0±6.7‡	21.0±4.3§	7.3±13.4	−20.8±12.4	−4.2±9.8	−7.0±4.9
	C	−9.4±3.6	25.0±6.8	30.4±8.2‡	22.0±8.6‡	30.1±7.1	19.6±8.2§	11.1±4.7§
ILE	D	−4.1±3.5	21.6±8.5§	13.1±2.2‖	−2.5±11.1	−2.4±7.2	−0.3±5.3	−0.2±2.8
	C	−2.7±1.5	11.4±3.5‡	15.4±3.5‖	18.5±3.5‡	10.5±2.1§	8.7±3.2	8.9±2.4‡
LEU	D	−8.6±60	33.9±9.0‡	24.1±4.4‖	6.3±9.4	0.2±10.2	1.0±3.6	−4.6±3.5
	C	−5.1±1.5	21.9±7.6§	28.7±5.3‖	23.2±7.5§	18.1±5.2§	14.3±5.9§	13.1±4.2‡
TYR	D	−3.4±1.3	3.7±1.3‡	2.0±1.1§	0.1±0.9	−2.7±2.0	−0.5±1.0	−3.5±2.5
	C	−1.1±1.6	2.6±1.4‡	5.0±1.9‖	2.4±2.7	4.1±2.6‡	1.3±2.5	−0.6±1.5
PHE	D	−4.4±1.6	3.3±1.3§	0.3±1.2	−1.3±1.1	−3.7±1.8	−1.5±1.1	−1.3±2.2
	C	−2.1±1.3	1.3±1.4	5.4±1.8‖	1.4±2.1	3.4±1.9‡	−0.4±2.4	−0.7±2.0
ORN	D	—	—	—	—	—	—	—
	C	4.4±4.8	—	3.8±5.8	—	0.7±5.8	—	−3.5±2.6
LYS	D	—	—	—	—	—	—	—
	C	−19.0±7.9	—	12.4±7.7§	—	15.8±24.2	—	−3.0±6.5§
HIS	D	—	—	—	—	—	—	—
	C	2.2±3.6	—	9.3±3.1§	—	9.3±2.9§	—	−5.0±4.8
ARG	D	—	—	—	—	—	—	—
	C	−1.5±3.3	—	9.0±2.9§	—	9.3±5.8	—	1.8±5.6

* Data are presented as means±SEM in micromoles per minute; the basic amino acids (orinithine, lysine, histidine, and arginine) were measured in the control group only.

‡ Significantly different from the corresponding value in the basal state, $P < 0.01$.

§ Significantly different from the corresponding value in the basal state, $P < 0.025$.

‖ Significantly different from the corresponding value in the basal state, $P < 0.005$.

count basal differences between diabetic and control subjects, the change in amino acid flux across the leg induced by protein feeding was compared in the two groups (Fig. 3). The rise in uptake of total amino acids tended to be greater in the diabetics than in controls at 30 min ($0.05 < P < 0.1$), but was comparable in the two groups throughout the remainder of the 3-h observation period (Fig. 3). With respect to the branched chain amino acids, the diabetic group showed a significant change in flux to a net uptake at 30 and 60 min which was comparable to controls (Fig. 3). However, in contrast to the ongoing leg uptake observed in controls, no significant change from the basal flux of branched chain amino acids was demonstrable in the diabetics at 90–180 min (Fig. 3). Furthermore, the change in flux in the control group was significantly

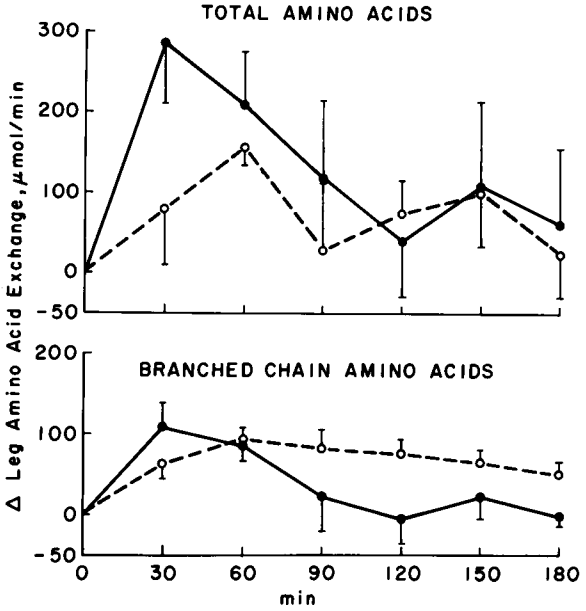

FIGURE 3 Changes in amino acid exchange across the leg in normal controls (dashed line) and in diabetic patients (solid line) after a protein meal. The changes in flux were determined by comparing net exchange after protein feeding with that observed in the basal state (Table VII). A positive value indicates a greater net uptake or reduced output. Mean values±SEM are shown. In the upper panel, total acidic and neutral amino acids are shown. The change in total amino acid flux was significantly different from basal in the diabetics at 30 min ($P < 0.005$) and 60 min ($P < 0.01$), and in the controls at 60 min ($P < 0.005$). In the lower panel the changes in flux of branched chain amino acids (valine, leucine, and isoleucine) are shown. The change in flux in the controls was significantly different from basal at all time points (30–180 min, $P < 0.02$–$P < 0.01$). In the diabetics it was significantly different from basal at 30 and 60 min ($P < 0.005$) only.

greater than in the diabetics at 120 and 180 min ($P < 0.05$). This tendency to a less persistent uptake of branched chain amino acids in the diabetics is also reflected in the absolute net exchange of these amino acids across the leg. In the diabetic group net exchange of valine, leucine, and isoleucine was not significantly different from zero at 90–180 min, while in the controls, consistent leg uptakes were observed (Table VII). Net release of alanine and glutamine from the leg continued throughout the 3-h period of observation after protein feeding, showing a small but not statistically significantly decline at 30 and 60 min.

DISCUSSION

The present study provides data on the effects of protein ingestion on splanchnic and peripheral amino acid exchange in normal postabsorptive man and human diabetes. The findings in normal subjects indicate a special role for the branched chain amino acids in providing for repletion of amino acid nitrogen in muscle tissue after protein feeding. After protein ingestion, the branched chain amino acids exceeded all others with respect to their escape from the splanchnic bed, their increase in arterial concentration, and their uptake by peripheral leg tissues. In contrast, only transient splanchnic escape and peripheral uptake was observed for a variety of other amino acids. Inasmuch as the branched chain amino acids account for only 20% of the total amino acid residues in ingested beef (muscle) proteins (6, 35), their primacy in protein-stimulated amino acid output from the splanchnic bed indicates a unique tendency for these amino acids to escape hepatic uptake and/or metabolism after intestinal absorption. A similar pattern of hepatic amino acid output involving the branched chain amino acids has been noted in a variety of experimental animals (dogs, rats, and sheep) after protein feeding (36–38). These observations are in keeping with the relative unimportance of the liver in branched chain amino acid metabolism (8). In contrast, the relatively minor increments in arterial levels and splanchnic escape of other amino acids suggest a major role for the liver in the uptake and utilization of most amino acids ingested in a protein meal.

Complementing the pattern of splanchnic amino acid output was the key role of valine, leucine, and isoleucine in peripheral nitrogen repletion after protein ingestion. The branched chain amino acids were responsible for more than half of leg amino acid uptake over the 3-h period of protein feeding. Underscoring this finding is the observation that alanine and glutamine are continuously released from the leg (albeit at a transiently reduced rate) and transported to the splanchnic bed in the protein-fed as well as in the fasted state. In a previous report on muscle amino acid uptake after protein feeding involving a single obese subject studied after a 6-wk fast, a predominance of branched chain amino acid extraction was also noted (39). It is thus clear that the branched chain amino acids are the major source for repletion of muscle nitrogen after protein intake. Furthermore, since the branched chain amino acids comprise only 20% of muscle amino acid residues (6, 35) but are responsible for the majority of protein-induced amino acid uptake, it is likely that these amino acids are not solely utilized for protein synthesis but are largely catabolized in muscle. These data are in keeping with the conclusion that the branched amino acids are the major source of nitrogen for the glucose-alanine cycle, (2, 5, 6, 7, 9) and possibly for muscle glutamine synthesis.

The findings in the normal subjects thus suggest the existence of a nitrogen "shuttle" involving muscle, liver, kidney, and the gut, in which alanine, glutamine, and the branched chain amino acids are of major impor-

tance. In the fasted as well as the fed state α-amino nitrogen is continuously released from muscle in the form of alanine and glutamine. The liver is the site of alanine uptake while the gut and kidney are the sites of glutamine utilization (26, 40). After protein feeding, repletion of α-amino nitrogen in muscle tissue occurs primarily in the form of transfer from the gut to muscle of branched chain amino acids contained in ingested protein. In contrast, most of the remaining amino acids in the protein meal are retained within the splanchnic bed and are presumably metabolized within the liver.

In the diabetic group splanchnic output of total and individual amino acids after protein ingestion was comparable to controls. The change in flux of total amino acids across the leg to a net uptake tended to be greater in the diabetics than in controls at 30 min, but thereafter (60–180 min) was comparable in the two groups (Fig. 3). These findings are in keeping with previous observations in which comparable plasma levels of total α-amino nitrogen were observed in diabetic and control subjects after a protein meal (14, 41). In contrast to the changes in total amino acids, the arterial increments in the branched chain amino acids were 30–50% greater in the diabetics than in controls. It should be noted in this respect that in the absence of protein ingestion no increment is observed in circulating valine, leucine, or isoleucine in postabsorptive diabetic patients studied over a 3-h period 24 h after their last insulin dose.[1] Furthermore, in contrast to the persistent positive change from basal in the flux across the leg of branched chain amino acids observed in the controls, in the diabetics a change in flux occurred only at 30 and 60 min (Fig. 3). These observations of increased arterial levels and less persistent peripheral uptake of branched chain amino acids in the diabetics are in keeping with the known effects of insulin on muscle and amino acid metabolism. Previous studies have shown that physiologic increments in insulin stimulate muscle amino acid uptake involving particularly leucine and isoleucine (4). Furthermore, the decline in concentration of circulating amino acids accompanying glucose-stimulated insulin secretion is most marked for the branched chain amino acids (42–44). The lack of a persistent leg uptake of the branched chain amino acids and the arterial accumulation observed in the diabetic group thus may be related to insulin deficiency. On the other hand, the transiently normal leg extractions of these amino acids observed at 30 and 60 min may reflect a residual effect of previously injected, intermediate acting insulin, and/or transient stimulation of endogenous insulin secretion (45).

[1] Felig, P., and R. Sherwin. In preparation.

In addition to the effects of protein ingestion on branched chain amino acid metabolism, a transient but significant reduction in leg alanine output was observed in the normal subjects but not in the diabetics. A variety of studies have indicated that the rate of alanine synthesis in muscle is related to the availability of glucose-derived pyruvate (2, 5, 6) as well as the rate of protein degradation and *in situ* catabolism of amino acids which provide the amino groups for alanine formation (6, 7, 9). The increase in peripheral insulin levels accompanying protein ingestion (90–100%; Fig. 2) would not be considered of sufficient magnitude to stimulate peripheral glucose uptake (4, 43, 46, 47). (This conclusion is supported by the failure to observe a progressive decline in arterial blood glucose levels in the normal subjects in whom splanchnic glucose production showed no increment after protein feeding [Tables III and V]). Thus the availability of glucose-derived pyruvate for alanine synthesis in muscle is unlikely to increase after a protein meal. The transient but significant decline in arterial glucose may in fact result in decreased pyruvate availability and thereby contribute to the fall in alanine output. In addition, to the extent that insulin reduces protein catabolism in muscle (48), an over-all decrease in the availability of amino groups for alanine synthesis might be expected in association with protein-induced hyperinsulinemia. The failure to observe a significant fall in leg alanine output in the diabetics thus may be related to the rising arterial glucose level and/or insulin deficiency.

It should be noted that in view of the changing arterial levels of amino acids induced by the protein meal, the observations on inter-organ exchange were obtained under nonsteady state conditions. Accordingly, the differences noted above between diabetics and controls with respect to branched chain amino acid and alanine metabolism should be considered as indicative of qualitative trends rather than quantitatively definitive.

In addition to the observed effects on amino acid metabolism, the present study provides data on the influence of protein intake on splanchnic glucose exchange. In the control group, splanchnic glucose output tended to remain at basal levels, save for a very transient initial decline. Previous studies from our laboratories have shown that when increments in arterial insulin comparable to those observed with the protein meal (60–100% above basal) are induced in normal subjects by glucose administration, splanchnic glucose output falls to values 80–85% below basal (43, 47). The failure to observe a similar decline in splanchnic glucose output after a protein meal may well be a consequence of the accompanying hyperglucagonemia. A variety of studies have suggested that net rates of hepatic glucose balance are determined by the ratios

of insulin and glucagon (49, 50). The current data thus provide further evidence of the role of glucagon in the maintenance of hepatic glucose output and in the prevention of hypoglycemia after protein administration in normal man (15). On the other hand, it should be recalled that most of the amino acids in the protein meal (other than the branched chain amino acids) are retained within the splanchnic bed. A possible role for ingested gluconeogenic substrate in the maintenance of splanchnic glucose output after protein feeding thus cannot be excluded.

A striking finding in the present study was the stimulatory effect of protein feeding on splanchnic glucose output in the diabetics. Splanchnic glucose production rose by 150% in the diabetics and was accompanied by a 1.5-mmol/liter rise in blood glucose, changes which were not observed in the control group (Table V, Fig. 2). While a progressive rise in splanchnic glucose output might be anticipated in insulin-deprived diabetics, the return to base line at 2 h (Fig. 2) suggests that the protein meal rather than insulin deficiency per se was responsible for the augmentation in glucose production observed in the diabetics. Several factors may have contributed to this divergence of responses with respect to splanchnic glucose balance. Firstly, although arterial glucagon levels increased in both groups, the effect of the rise in glucagon may have been counteracted by the simultaneous increment in insulin observed in the healthy subjects. Although some residual β-cell function may be retained by long-standing diabetics (45), it is unlikely that comparable insulin increments occurred in the diabetic subjects. On the other hand, it should be noted that the peak increment in splanchnic glucose output preceded the peak increase in arterial glucagon by 45 min, and returned to base line despite ongoing hyperglucagonemia (Fig. 2). Among the other factors which may influence the splanchnic glucose response, augmented hepatic availability and disposal of glucogenic amino acids must be considered. As noted above, the splanchnic balance data indicate that in both groups most of the glucogenic amino acids in the protein meal are retained within the splanchnic bed with little escape to peripheral tissues. Inasmuch as portal vein catheterization was not feasible in the present study, no conclusions can be drawn however regarding the effects of diabetes on the rates of intestinal absorption and hepatic uptake of glucogenic amino acids released from ingested proteins. An additional factor which may have contributed to the increase in splanchnic glucose release in the diabetics is the greater availability and splanchnic uptake of FFA (Tables III, V). Augmented utilization of FFA by the liver would be expected to enhance gluconeogenesis (51) and hence might contribute to the rise in splanchnic glucose output. The greater glycemic response to protein ingestion in the diabetic group may thus reflect the combined effects of altered levels of hormones, as well as changes in availability and uptake of glycogenic precursors and energy-yielding substrates.

Finally, it should be emphasized that while the diabetics differed from the controls with respect to the progressive rise in arterial FFA and ketones (Table III), these changes may not be a consequence of protein ingestion but may reflect progressive insulin deficiency independent of protein feeding.

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