

# Splanchnic and Leg Substrate Exchange After Ingestion of a Natural Mixed Meal in Humans

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The disposal of a mixed meal was examined in 11 male subjects by multiple (splanchnic and femoral) catheterization combined with double-isotope technique (intravenous [ $2\text{-}^3\text{H}$ ]glucose plus oral U- $^{14}\text{C}$ starch). Glucose kinetics and organ substrate balance were measured basally and for 5 h after eating pizza (600 kcal) containing carbohydrates 75 g as starch, proteins 37 g, and lipids 17 g. The portal appearance of ingested carbohydrate was maximal (1.0 mmol/min) between 30 and 60 min after the meal and gradually declined thereafter, but was still incomplete at 300 min ( $0.46 \pm 0.08$  mmol/min). The total amount of glucose absorbed by the gut over the 5 h of the study was  $247 \pm 26$  mmol ( $45 \pm 6$  g), corresponding to  $60 \pm 6\%$  of the ingested starch. Net splanchnic glucose balance ( $-6.7 \pm 0.5$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , basal) rose by 250–300% between 30 and 60 min and then returned to baseline. Hepatic glucose production (HGP) was suppressed slightly and only tardily in response to meal ingestion ( $\sim 30\%$  between 120 and 300 min). Splanchnic glucose uptake ( $3.7 \pm 0.6$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , basal) peaked to  $9.8 \pm 2.0$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  ( $P < 0.001$ ) at 120 min and then returned slowly to baseline. Leg glucose uptake ( $34 \pm 5$   $\mu\text{mol} \cdot \text{leg}^{-1} \cdot \text{min}^{-1}$ , basal) rose to  $151 \pm 29$   $\mu\text{mol} \cdot \text{leg}^{-1} \cdot \text{min}^{-1}$  at 30 min ( $P < 0.001$ ) and remained above baseline until the end of the study, despite no increase in leg blood flow. The total amount of glucose taken up by the splanchnic area and total muscle mass was  $161 \pm 16$  mmol ( $29 \pm 3$  g) and  $128$  mmol (23 g), respectively, which represent 39 and 30% of the ingested starch. Arterial blood lactate increased by 30% after meal ingestion. Net splanchnic lactate balance switched from a basal net uptake ( $3.2 \pm 0.6$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) to a net output between 60 and 120 min and tended to zero thereafter. Leg lactate release ( $25 \pm 11$   $\mu\text{mol} \cdot \text{leg}^{-1} \cdot \text{min}^{-1}$ , basal) drastically decreased postprandially. Arterial concentration of both branched-chain amino acids (BCAA) and non-branched-chain amino acids (N-BCAA) increased significantly after meal ingestion ( $P < 0.001$ ). The splanchnic area switched from a basal net amino acid uptake ( $31 \pm 16$  and  $92 \pm 48$   $\mu\text{mol}/\text{min}$  for

BCAA and N-BCAA, respectively) to a net amino acid release postprandially. The net splanchnic amino acid release over 5 h was  $11.3 \pm 4.2$  mmol for BCAA and  $37.8 \pm 9.7$  mmol for N-BCAA. Basally, the net leg balance of BCAA was neutral ( $-3 \pm 5$   $\mu\text{mol} \cdot \text{leg}^{-1} \cdot \text{min}^{-1}$ ), whereas that of N-BCAA indicated a net release ( $54 \pm 14$   $\mu\text{mol} \cdot \text{leg}^{-1} \cdot \text{min}^{-1}$ ). After meal ingestion, there was a net leg uptake of BCAA ( $20 \pm 6$   $\mu\text{mol} \cdot \text{leg}^{-1} \cdot \text{min}^{-1}$ ), whereas leg release of N-BCAA decreased by 50%. It is concluded that in human subjects, 1) the absorption of a natural mixed meal is still incomplete at 5 h after ingestion; 2) HGP is only marginally and tardily inhibited; 3) splanchnic and peripheral tissues contribute to the disposal of meal carbohydrate to approximately the same extent; 4) the splanchnic area transfers  $>30\%$  of the ingested proteins to the systemic circulation; and 5) after meal ingestion, skeletal muscle takes up BCAA to replenish muscle protein stores. *Diabetes* 48:958–966, 1999

**A**ctivation of glucose uptake by body tissues and inhibition of hepatic glucose production are of major importance in the control of postprandial glucose regulation (1–6). It is now recognized that splanchnic and peripheral tissues contribute to the disposal of a glucose load to approximately the same extent (7). On the other hand, the postprandial inhibition of endogenous glucose production varied greatly in previous studies, ranging from almost complete to modest suppression (1–9).

Postprandial glucose regulation has been explored in most studies by administering a pure glucose load, the rationale being that glucose is almost completely absorbed within 4–5 h and its kinetics may be carefully monitored by labeled glucose. However, a mixed meal differs from pure glucose in that it delays gastric emptying (10), stimulates glucagon secretion (11), and enhances the response of insulin (12) and gastrointestinal hormones (13). In addition, a mixed meal contains other nutrients, such as fat and protein, that may interfere with glucose absorption and metabolism. Finally, the ideal test meal should consist of natural food so as to reproduce as closely as possible the psychological setting of everyday feeding.

In the dog, the majority (58%) of the mixed meal-derived glucose is disposed of by nonsplanchnic tissues, whereas only 19% is taken up by the liver (14,15). Few studies have focused on the disposal of a mixed meal in humans (16–20), and in none of them was the role of the splanchnic and muscle tissues evaluated simultaneously. In one study, splanchnic glucose metabolism was similar after ingestion of pure glucose and after a mixed meal (19), whereas in

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A, arterial; BCAA, branched-chain amino acid; EHBF, estimated hepatic blood flow; F, femoral venous; FE, fractional extraction; FFA, free fatty acid; HGP, hepatic glucose production; HV, hepatic venous; LBF, leg plasma flow; N-BCAA, non-branched-chain amino acid; NSGB, net splanchnic balance of glucose; SGP, splanchnic glucose production; SGU, splanchnic glucose uptake.

another, muscle glucose uptake was greater after a mixed meal than after a glucose load (18). Using [ $^{13}\text{C}$ ]glucose spectroscopy, it has recently been shown that 20% of the carbohydrate content of a mixed meal is deposited as glycogen in the liver, and 26–35% is stored as muscle glycogen (8,20). However, the mixed meal given in these studies was a liquid formula, with the carbohydrate content consisting entirely of glucose. In addition, little is known about the fluxes of nutrients other than glucose and their interorgan exchange after ingestion of a mixed meal. For instance, the relative contribution of splanchnic and nonsplanchnic tissues to amino acid disposal has not been assessed in humans.

In the present study, we evaluated the interorgan fluxes of the major metabolic substrates after ingestion of a natural mixed meal in humans by combining a double-isotope approach with the splanchnic and leg catheter technique. Pizza was chosen as a natural, mixed meal because its carbohydrate content is entirely in the form of starch, i.e., complex carbohydrate, which represents the recommended carbohydrate form for diabetic patients. Moreover, given its worldwide popularity, this food is ideal to simulate one of the most common postprandial physiologic conditions in modern society.

## RESEARCH DESIGN AND METHODS

**Subjects.** Eleven male subjects aged 40–58 years were studied. All subjects were within 20% of their ideal body weight (Metropolitan Life Insurance Tables, 1959) and exhibited normal glucose tolerance. Their plasma glucose was  $5.7 \pm 0.3$  mmol/l in the fasting state and  $6.0 \pm 0.3$  mmol/l 120 min after a 75-g oral glucose load. None had a history or laboratory evidence of liver or renal disease. All subjects were admitted to the Department of Internal Medicine of the University of Naples for elective cardiac catheterization for diagnostic purposes (atypical chest pain or valve disease). Subjects with an abnormal cardiac output or elevated pulmonary artery pressure were excluded from the study. Three patients took  $\beta$ -blockers that were discontinued 48 h before the study. The nature, purpose, and possible risks of the additional procedures were fully explained to each subject before his written consent to participate was obtained before cardiac catheterization. The experimental protocol was approved by the Ethics Committee of the University Federico II of Naples. The use of tracers for research purpose in human subjects was approved by the National Health Authority.

**Procedures.** All subjects were studied in the postabsorptive state after a 15–17 overnight fast. At least 30 min after the diagnostic procedures were completed, a 6-French Gensini catheter was advanced through the femoral vein sheath into a right-sided hepatic vein and its tip placed 2–3 cm from the wedge position. The catheter was used to sample hepatic venous blood, and the femoral sheath was used to sample femoral venous blood. A 5-French Gensini catheter was advanced through the ipsilateral femoral artery until its tip was in the common iliac artery. This catheter was used to sample arterial blood, whereas its sheath was used to infuse indocyanine green dye. A saline solution free of anticoagulant was infused through the sheaths and the catheters to maintain their patency throughout the study.

TABLE 1

Arterial blood glucose, plasma insulin, plasma glucagon, estimated hepatic blood flow, and leg blood flow after ingestion of a mixed meal

	Minutes								
	-15	0	30	60	90	120	180	240	300
Blood glucose (mmol/l)	4.4 ± 0.1	4.4 ± 0.1	6.0 ± 0.2	6.9 ± 0.2	7.0 ± 0.3	6.8 ± 0.3	6.0 ± 0.4	5.5 ± 0.2	4.8 ± 0.3
Plasma insulin (pmol/l)	54 ± 6	54 ± 6	180 ± 42	294 ± 48	294 ± 36	306 ± 48	264 ± 48	174 ± 42	96 ± 30
Plasma glucagon (ng/ml)	120 ± 20	125 ± 24	—	151 ± 30	—	164 ± 33	154 ± 35	141 ± 32	130 ± 25
Estimated hepatic blood flow (ml/min)	1,278 ± 147	1,297 ± 140	1,650 ± 201	1,733 ± 264	1,499 ± 163	1,601 ± 124	1,572 ± 108	1,426 ± 143	1,169 ± 71
Leg blood flow (ml · leg <sup>-1</sup> · min <sup>-1</sup> )	358 ± 42	356 ± 45	308 ± 27	325 ± 34	335 ± 38	346 ± 42	326 ± 39	336 ± 38	347 ± 51

Data are means ± SE.

At -120 min, a primed (50  $\mu\text{Ci}$ ) continuous (0.5  $\mu\text{Ci}/\text{min}$ ) infusion of [ $^2\text{-}^3\text{H}$ ]glucose (New England Nuclear, Cambridge, MA) was started and continued throughout the study via an antecubital vein. At -60 min, indocyanine green dye (Cardio-Green; Hynson, Westcott and Dunning, Baltimore, MD) was infused into the femoral artery at a rate of 0.5 mg/min for measurement of splanchnic and femoral blood flow. Then, each subject ate the test meal in about 10 min, which consisted of pizza enriched with tomato, olive oil, and bresaola (lean prosciutto). The meal contained carbohydrate 75 g (47% of calories), lipids 17 g (27%), and proteins 37 g (26%), for a total amount of 600 kcal. The carbohydrate provided in the meal was entirely in the form of starch, which was labeled with 80  $\mu\text{Ci}$  of U- $^{14}\text{C}$ starch (New England Nuclear). To ensure a uniform distribution of labeled starch, the tracer was dissolved in the water used to make the pizza dough. The loss of radioactivity due to the preparation procedure amounted to <1%. Pizza was cooked according to the Neapolitan tradition—that is, at high temperature (300–350°C) for as short as 1.5–2 min—and served immediately. Olive oil and bresaola were added after the pizza was cooked. During the study, subjects were in bed in a comfortable semi-upright position. Blood samples were simultaneously drawn from the arterial (A), hepatic venous (HV), and femoral venous (F) catheters for chemical analyses and regional blood flow measurements in the basal state and at 30- to 60-min intervals for 5 h postprandially.

**Analytical methods.** Blood glucose was determined by the glucose oxidase method (YSI glucose analyzer; Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin and glucagon were assayed by radioimmunoassay. The plasma concentration of indocyanine green was measured spectrophotometrically. Although the plasma samples obtained during the absorption period were clear, in two subjects the hepatic vein plasma samples were treated with a lipid clarifying solution (Lipoclear; Boehringer Mannheim, Mannheim, Germany). The values of dye concentration and blood flow were identical to those of untreated samples. Blood lactate was measured in perchloric extracts of whole blood by using an enzymatic method. Amino acid concentration was measured in perchloric extracts of whole blood by the automated ion-exchange chromatographic method (21). Plasma free fatty acids (FFAs) were assayed using a colorimetric method. The assay of [ $^2\text{-}^3\text{H}$ ]glucose radioactivity was performed on deproteinized blood samples (22). A combined chromatographic-enzymatic procedure was used for determination of [ $^{14}\text{C}$ ]glucose specific activity, as previously described (22).

**Calculations.** Estimated hepatic blood flow (EHBF, milliliters per minute) was calculated according to Leevy et al. (23). The net splanchnic balance of glucose (NSGB, micromoles per kilogram per minute) and substrates was calculated as the product of the substrate hepatic venous-arterial (HV – A) difference and EHBF. The rate of portal glucose appearance ( $\text{Ra}_{\text{port}}$ ) was calculated using the following:

$$\{ \text{HV} [^{14}\text{C}] \text{glucose} \times 1 / (1 - \text{FE}) - \text{A} [^{14}\text{C}] \text{glucose} \} \times \text{EHBF} \times 1 / \text{SA}_{\text{ing}}$$

where  $\text{HV} [^{14}\text{C}] \text{glucose} \times 1 / (1 - \text{FE})$  represents the theoretical [ $^{14}\text{C}$ ]glucose concentration at prehepatic level reconstructed from the [ $^{14}\text{C}$ ]glucose in the hepatic vein corrected by the hepatic fractional extraction (FE), and  $\text{SA}_{\text{ing}}$  represents the specific activity of the ingested starch. FE was calculated from the tritiated glucose data as

$$\text{FE}(\%) = ([^2\text{-}^3\text{H}] \text{glucose HV} - [^2\text{-}^3\text{H}] \text{glucose A}) / [^2\text{-}^3\text{H}] \text{glucose A}$$

We assumed that FE expresses glucose extraction by the liver since glucose extraction by splanchnic nonhepatic tissues is known to be negligible (24). The splanchnic glucose clearance (milliliters per kilogram per minute) was calculated as the product of FE and EHBF. NSGB represents the sum of two independent processes that occur simultaneously within the splanchnic area, production and uptake. These were dissected out using the balance of unlabeled and [ $^{14}\text{C}$ ] and

[2-<sup>3</sup>H]glucose data. After meal ingestion, splanchnic glucose uptake (SGU) includes the uptake of glucose from the systemic arterial circulation and the uptake of meal-derived glucose. Therefore, SGU was calculated as follows:

$$SGU = FE[(EHB \times \text{arterial blood glucose concentration}) + Ra_{\text{port}}]$$

Once NSGB and SGU are known, splanchnic glucose production (SGP) is obtained by difference:

$$SGP = NSGB - SGU.$$

Hepatic glucose production (HGP) is then calculated by subtracting  $Ra_{\text{port}}$  from SGP. HGP was also measured by the tracer method using the classic dilution equation in the basal state (tracer infusate divided by the glucose specific activity) and by Steele's approach thereafter. Postprandially, circulating blood glucose is made up of two components. One derives from the meal (meal-derived blood glucose) and the other from the liver (endogenous blood glucose). Meal-derived blood glucose was calculated by dividing the arterial concentration of [<sup>14</sup>C]glucose by the specific activity of starch. Endogenous blood glucose is the difference between the latter component and total blood glucose. HGP was quantified by Steele's equation using the infusion rate of tritiated glucose, endogenous glucose blood concentration, and the specific activity of endogenous glucose.

We measured glucose kinetics from [2-<sup>3</sup>H]glucose, since it does not recycle through glycogen (25). Thus, it is the tracer of choice under conditions in which liver glycogen deposition occurs simultaneously with glycogen breakdown. However, [2-<sup>3</sup>H]glucose might undergo detritiation through the futile cycle of hexose-6-phosphates, which may lead to overestimation of the true rate of splanchnic glucose uptake. Therefore, in two subjects, splanchnic glucose fractional extraction was measured by means of [6-<sup>3</sup>H]glucose, a tracer that is detritiated downstream of the futile cycles. Data on splanchnic glucose and substrate metabolism are not available in two subjects because of malfunction of the hepatic vein catheter. Thus, for this part of the study we have data from nine subjects, whereas the leg data are based on eleven subjects.

Leg plasma flow (LBF) was estimated by dividing the dye infusion rate by its concentration in the femoral venous plasma and was converted to blood flow according to the hematocrit. Substrate balance across the leg was calculated as the product of the arterial-venous femoral substrate concentration difference and the LBF.

The cumulative balance for each substrate was obtained from the area under the curve of organ balance versus time with the trapezoidal method. Branched chain amino acid (BCAA) concentration is the sum of the blood concentrations of leucine, isoleucine, and valine. The remaining amino acids are included in the group of non-branched-chain amino acids (N-BCAA).

To minimize the potential source of error in our estimates under conditions of non-steady state, we delayed sampling from the hepatic and femoral vein by 0.5 and 3 min, respectively, to compensate for the transit time of blood through the splanchnic and the femoral regions. Furthermore, the changes in circulating substrate concentration were in general quite slow, and a quasi-steady state prevailed for extended periods of time. In addition, for most substrates, including glucose, there was a symmetrical pattern (a rise in the first part of the study and a fall in the second one), so that potential over- and underestimation cancel each other out.

Data are presented as the mean  $\pm$  SE. Statistical analysis was performed by the two-way analysis of variance (ANOVA) using the least significant difference test for multiple comparisons.

**RESULTS**

Arterial blood glucose ( $4.4 \pm 0.1$  mmol/l, basal) reached the peak level of  $7.0 \pm 0.3$  mmol/l 90 min after meal ingestion and then gradually declined. Arterial plasma insulin increased from the fasting value of  $54 \pm 6$  pmol/l to  $306 \pm 48$  pmol/l 120 min after the meal and then returned slowly to baseline. Arterial plasma glucagon rose by 20% at 120 min and then returned to basal levels. EHB (1,287  $\pm$  142 ml/min, basal) increased by 30% ( $P < 0.005$ ) in response to the mixed meal and returned to basal levels by the end of the study. Leg blood flow was  $357 \pm 42$  ml  $\cdot$  kg<sup>-1</sup>  $\cdot$  min<sup>-1</sup> in the basal state and remained substantially unchanged throughout the postprandial period (Table 1).

Net splanchnic glucose balance ( $-6.74 \pm 0.53$   $\mu$ mol  $\cdot$  kg<sup>-1</sup>  $\cdot$  min<sup>-1</sup>, basal) rose by 270% between 30 and 60 min ( $P < 0.001$ ) and then returned slowly to baseline (Fig. 1). The portal appearance of oral glucose reached the peak value of 1.03  $\pm$

0.20 mmol/min between 30 and 60 min and then gradually declined, but still continued at a rate of  $0.46 \pm 0.08$  mmol/min at the 5th h of the study. The total amount of glucose absorbed by the gut over the 5-h study period was  $247 \pm 26$  mmol ( $45 \pm 6$  g), a value corresponding to  $60 \pm 6\%$  of the ingested starch. HGP ( $10.4 \pm 1$   $\mu$ mol  $\cdot$  kg<sup>-1</sup>  $\cdot$  min<sup>-1</sup>, basal) was unaffected by meal ingestion during the first 90 min of the postprandial period. Thereafter, HGP declined slightly and only at the end of the study period was it suppressed by 30% (Table 2). As described in METHODS, HGP was calculated by two independent methods, Steele's approach and the state dilution equation. The time courses of endogenous glucose and endogenous specific activity are shown in Table 2. Both methods yielded estimates of endogenous glucose release similar to those obtained with catheter measurements (Table 2). The maximal suppression averaged 30% and occurred in the last 3 h of the postprandial period. In the subjects who received [2-<sup>3</sup>H]glucose, splanchnic glucose clearance was

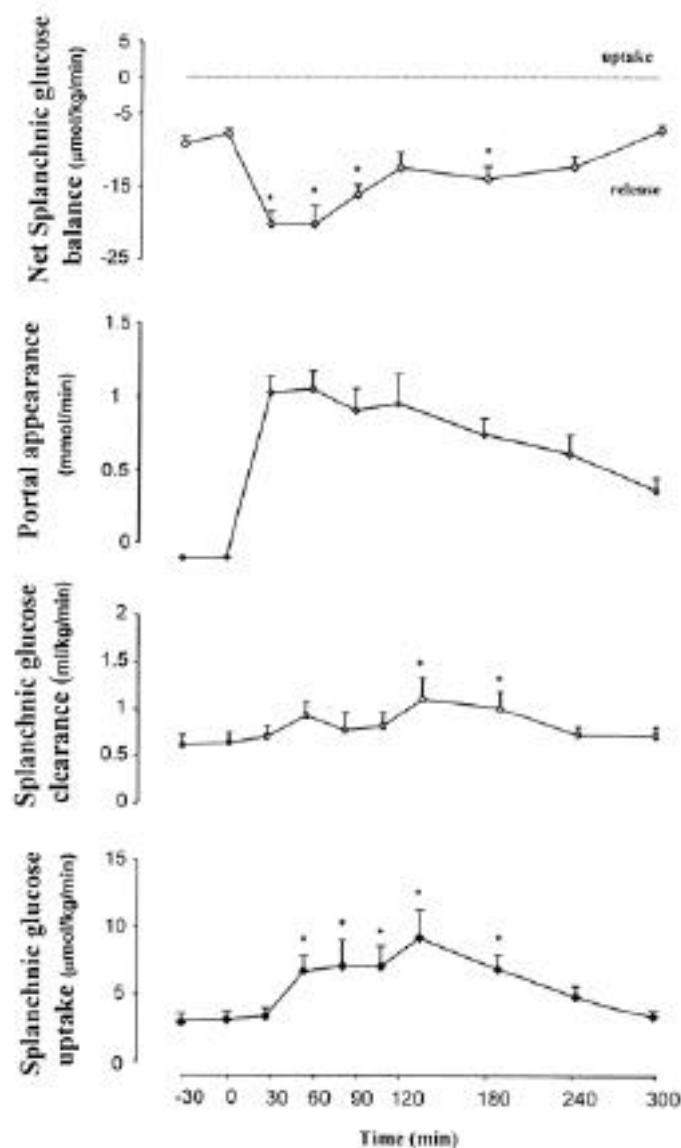


FIG. 1. Time course of net splanchnic glucose balance, portal appearance, splanchnic glucose clearance, and splanchnic glucose uptake after ingestion of a mixed meal.

TABLE 2

Endogenous glucose, percentage of total glucose, endogenous glucose specific activity, and hepatic glucose production calculated with the organ balance technique, Steele's approach, or steady-state equation

	Minutes								
	-15	0	30	60	90	120	180	240	300
Endogenous glucose (mmol/l)	4.4 ± 0.1	4.4 ± 0.1	4.1 ± 0.3	4.3 ± 0.3	4.1 ± 0.2	3.9 ± 0.3	3.1 ± 0.2	2.9 ± 0.2	2.6 ± 0.2
Percent of total glucose	100 ± 0	100 ± 0	72 ± 5	64 ± 3	60 ± 3	58 ± 3	54 ± 3	54 ± 3	55 ± 3
Endogenous glucose specific activity (dpm/mg)	7,013 ± 609	7,145 ± 580	8,814 ± 1315	8,196 ± 835	8,643 ± 878	9,541 ± 1422	11,479 ± 1,846	11,085 ± 1,411	11,023 ± 1,421
Hepatic glucose production (μmol · kg <sup>-1</sup> · min <sup>-1</sup> )									
Organ balance	10.9 ± 1.4	9.9 ± 0.8	11.4 ± 1.6	11.1 ± 2.4	9.3 ± 2.9	7.0 ± 2.8	9.1 ± 2.2	7.3 ± 2.1	6.9 ± 1.6
Steele's approach	12.0 ± 1.0	12.0 ± 1.0	10.9 ± 0.9	10.3 ± 1.1	9.8 ± 1.1	9.4 ± 1.2	8.5 ± 0.9	7.9 ± 0.9	7.6 ± 0.9
Steady-state equation	12.0 ± 1.0	11.7 ± 1.0	10.1 ± 1.0	10.4 ± 0.8	9.9 ± 0.9	9.4 ± 1.1	8.2 ± 1.2	8.2 ± 0.9	7.9 ± 0.8

Data are means ± SE.

0.83 ± 0.14 ml · kg<sup>-1</sup> · min<sup>-1</sup> in the fasting state and increased by 20–40% during the postprandial period ( $P < 0.05$  at 120–180 min). A similar pattern was observed in the two subjects who received [6-<sup>3</sup>H]glucose. Since the values in splanchnic glucose clearance were similar in the two groups, the data obtained with [2-<sup>3</sup>H]glucose and [6-<sup>3</sup>H]glucose were pooled. Splanchnic glucose uptake (3.7 ± 0.6 μmol · kg<sup>-1</sup> · min<sup>-1</sup>, basal) peaked to 9.8 ± 2.0 μmol · kg<sup>-1</sup> · min<sup>-1</sup> ( $P < 0.001$ ) at 120 min and then returned to baseline. The amount of glucose taken up by the splanchnic area during the 5 h postprandial period was 161 ± 16 mmol (29 ± 3 g), corresponding to 39% of the ingested starch (Fig. 1).

Leg glucose uptake rose from the basal value of 33.8 ± 5.3 μmol · leg<sup>-1</sup> · min<sup>-1</sup> to 151 ± 29 μmol · leg<sup>-1</sup> · min<sup>-1</sup> at 30 min ( $P < 0.001$ ) and remained above basal until the end of the study (Fig. 2). The total amount of glucose taken up by the leg during the entire experimental period was 32 mmol/l (5.8 g). Assuming that leg muscle represents 25% of total muscle mass, the amount of glucose taken up by the skeletal muscle was 128 mmol (23 g). This was equivalent to 30% of the ingested starch.

Arterial lactate (0.71 ± 0.07 mmol/l, basal) rose to 1.05 ± 0.08 mmol/l at 60 min ( $P < 0.001$ ) and returned to baseline by 120 min. The splanchnic lactate balance switched from the basal situation of a net uptake (3.2 ± 0.6 μmol · kg<sup>-1</sup> · min<sup>-1</sup>) to one of net output (-2.7 ± 0.2 μmol · kg<sup>-1</sup> · min<sup>-1</sup>,  $P < 0.001$ ) at 60 min. Subsequently, the splanchnic lactate balance tended to zero. In the basal state, the leg produced lactate at a rate of 25 ± 11 μmol · leg<sup>-1</sup> · min<sup>-1</sup>. During meal absorption, the leg lactate balance decreased to reach almost zero (0.97 ± 0.08 μmol · kg<sup>-1</sup> · min<sup>-1</sup>) at 90 min, and remained suppressed by 50% until the end of the study (Fig. 3).

Arterial FFAs (0.76 ± 0.14 mmol/l, basal) fell rapidly and remained at very low levels throughout the study period. The splanchnic FFA balance, which in the basal state indicated a net uptake (2.51 ± 0.69 μmol · kg<sup>-1</sup> · min<sup>-1</sup>), was profoundly suppressed in parallel with the fall in arterial FFA concentration. The leg FFA release (12.03 ± 16.6 μmol · leg<sup>-1</sup> · min<sup>-1</sup>, basal) was markedly inhibited by meal ingestion (Fig. 4).

Arterial BCAA concentration (408 ± 24 mmol/l in the basal state) increased by 37% at 60 min, and remained significantly

elevated ( $P < 0.001$ ) throughout the postprandial period (Fig. 5). The same pattern was observed for N-BCAA, which increased by 15% over the basal values (2247 ± 90 mmol/l) and remained elevated ( $P < 0.001$ ) until the end of the study (Fig. 6). In the postabsorptive state, the splanchnic area was a site of net uptake of both BCAA (31 ± 16 μmol/min) and N-BCAA (92 ± 48 μmol/min). After meal ingestion, the splanchnic balance of both BCAA and N-BCAA rapidly switched to a net release, which tended to decline at the 5th h of the study. The cumulative release of BCAA by the splanchnic area over the 5-h study period was 11.3 ± 4.2 mmol/l, whereas that of N-BCAA was 36 ± 10 mmol/l. Assuming that splanchnic amino acid uptake remained constant during the postprandial period, the total release of amino acid from the splanchnic area was 20.6 ± 6.1 mmol/l for BCAA and 68.7 ± 12.7 mmol/l for N-BCAA, equivalent to 38 and 29%, respectively, of the ingested proteins. In the basal state, the leg neither produced nor consumed BCAA (-3 ± 5 μmol · leg<sup>-1</sup> · min<sup>-1</sup>) whereas it released N-BCAA at a rate of 54 ± 14 μmol · leg<sup>-1</sup> · min<sup>-1</sup>. After meal ingestion, the leg became a site of transient BCAA uptake (20 ± 6 μmol · leg<sup>-1</sup> · min<sup>-1</sup> at 60 min,  $P < 0.01$ ). The leg release of N-BCAA tended to decrease in response to meal ingestion. During the whole postprandial period, the uptake by the leg was 3.8 ± 1.4 mmol for BCAA, whereas N-BCAA release decreased by 50%.

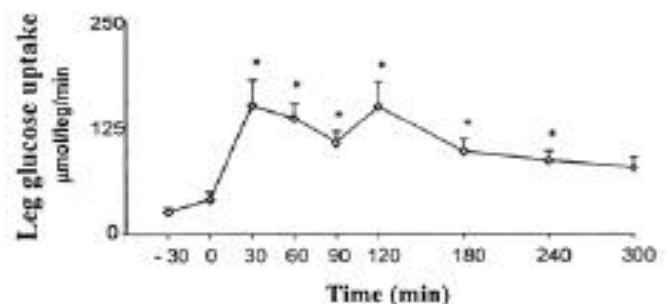


FIG. 2. Time course of leg glucose uptake after ingestion of a mixed meal.

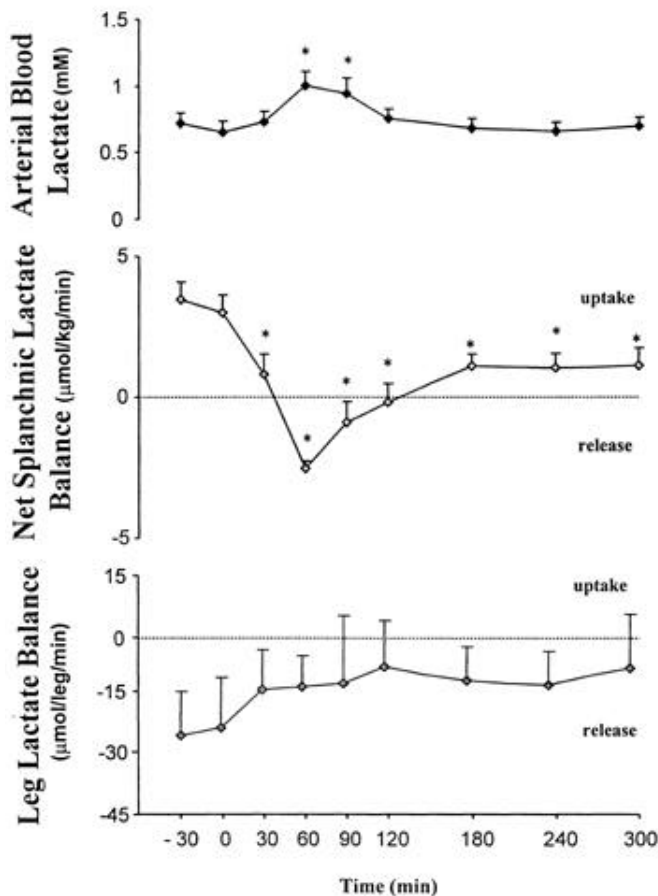


FIG. 3. Arterial lactate concentration and regional lactate balance after ingestion of a mixed meal.

DISCUSSION

This study is the first attempt to quantify simultaneously the contribution of splanchnic and peripheral tissues to the disposal of a mixed meal composed of natural foodstuff, in which the sugar content consists entirely of complex carbohydrate.

In our study, only 60% of the meal carbohydrate was absorbed as glucose, indicating that the process of digestion and absorption of complex carbohydrate is largely incomplete even after 5 h. This finding is strikingly different from that obtained with orally administered glucose. In the latter condition, as much as 90% of the load is absorbed in the same length of time. In all likelihood, the prolonged absorption of a mixed meal results from slowed gastric emptying, with a consequent delay in carbohydrate delivery to the small bowel. Scintigraphic techniques showed that the emptying time for a mixed meal, similar in composition to that of our study, is >4 h (26). Therefore, it is not surprising that meal absorption in our study was largely incomplete at 5 h.

Our finding of prolonged absorption of mixed-meal carbohydrate is in line with the results obtained in experimental animals. In the portal catheterized dog, after meal ingestion, glucose appearance in the portal vein ranged from 44 to 70% of the amount administered (14,15). Similar results were obtained in fed rats gavaged with additional glucose (27). Thus, it seems evident that in both animal models and humans, a large portion of ingested glucose remains unaccounted for. Had we prolonged the observation time beyond 5 h, carbohydrate recovery would probably have been

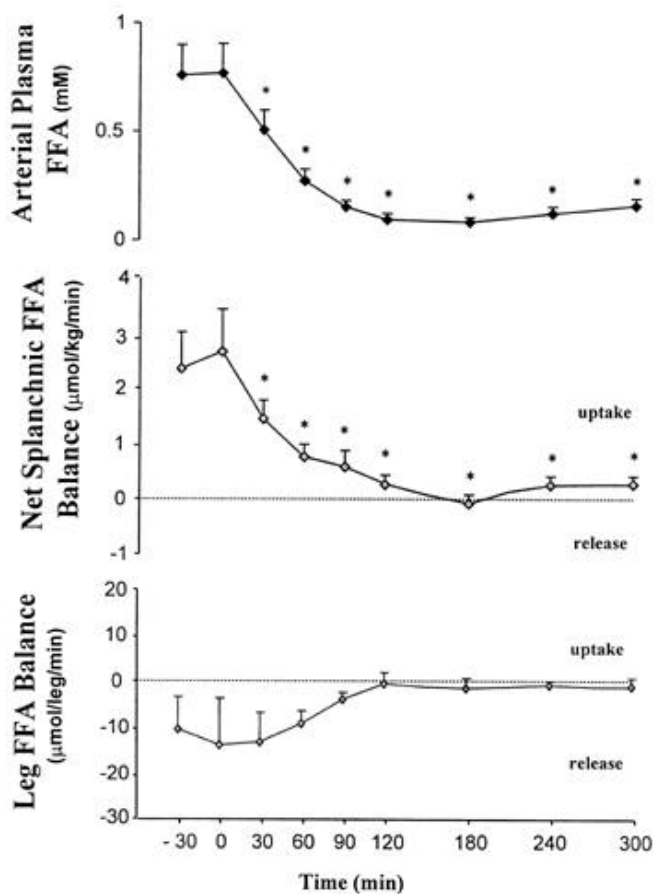


FIG. 4. Arterial FFA concentration and regional FFA balance after ingestion of a mixed meal.

greater. However, even assuming that [<sup>14</sup>C]glucose appearance in the following 2 h had continued at the same rate as that of the 5th h, the amount of glucose absorbed would have been 72% of the ingested starch, still leaving nearly 30% of meal carbohydrate unaccounted for.

Although the fate of unabsorbed glucose cannot be determined from the present data, some insight may be gained from previous experiments in the dog. There is evidence that the gut itself can dispose of ingested nutrients, as indicated by the fact that 10–15% of an oral glucose load is metabolized within the gut wall, where it undergoes complete oxidation (24). A certain amount of unabsorbed glucose may serve as a substrate for the production of volatile fatty acids, namely acetate and butyrate (28). Part of the glucose may be transported through the lymph; however, this mechanism seems to be irrelevant in the dog where less than 1% of the administered [<sup>14</sup>C]glucose was found in the thoracic duct (15). Thus, one could speculate that most of the unabsorbed carbohydrate is still in the gut lumen after 5 h. However, only 0.2% of ingested glucose was found in the gut lumen of the dog after 8 h (15). In addition, studies in humans have shown that the fecal excretion of starch after a meal rich in digestible starch amounts to 1–2% of the administered amount (29). In all likelihood, all these factors taken together could account for the incomplete carbohydrate absorption. On the other hand, it is also feasible that one of these pathways may be more active in humans than in experimental animals.

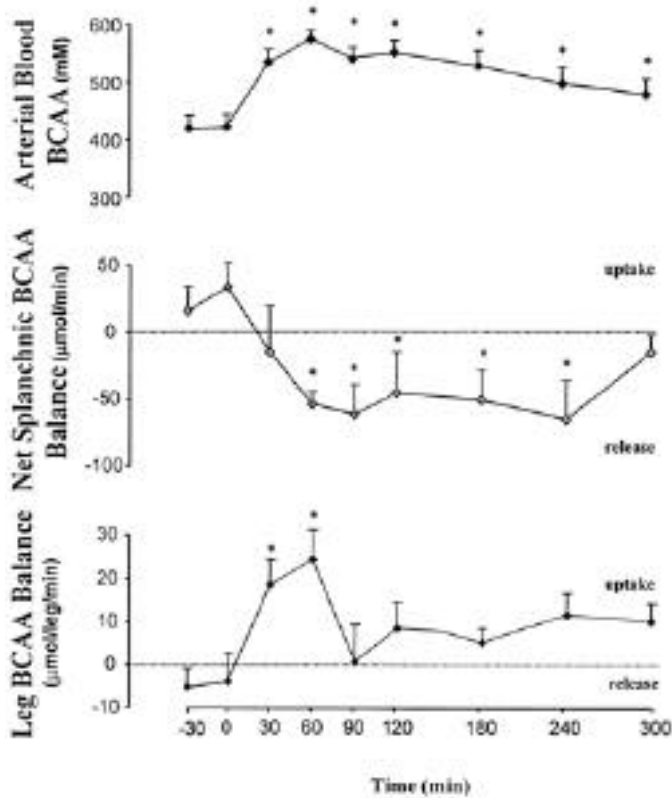


FIG. 5. Arterial BCAA concentration and regional BCAA balance after ingestion of a mixed meal.

The estimate of carbohydrate absorption based on the appearance of  $^{14}\text{C}$ -glucose in the hepatic vein is correct to the extent that  $^{14}\text{C}$ -glucose originates from the ingested starch and there is no other source of  $^{14}\text{C}$ -glucose (glycogen cycling and glucose recycling through three-carbon compounds). We cannot exclude that some glucose may have cycled through hepatic glycogen. However, liver glycogen cycling is known to be higher in the fed than in the fasted state (30,31). Since our subjects were studied after a 16–18-h fast (a condition in which glycogen stores are nearly depleted), it is likely that glycogen turnover may not have been particularly active. Usually, glucose recycling is estimated by determining the degree of randomization of  $^{14}\text{C}$  in the glucose molecule. We could not do this because the only form of labeled starch available is uniformly labeled. However, we measured [ $^{14}\text{C}$ ]lactate concentration in our study, and it accounted for approximately 8% of circulating [ $^{14}\text{C}$ ]glucose. However, the important point is that potential inaccuracies in the splanchnic [ $^{14}\text{C}$ ]glucose release would result in an overestimation of the portal appearance of meal-derived carbohydrate. Therefore, the 60% glucose absorption subsequent to starch ingestion reported here probably represents an upper-bound estimate rather than an underestimation.

An interesting finding of this study is the slight and late fall in endogenous glucose production, which reached 30% maximal suppression during the last 3 h of the postprandial period. This pattern was confirmed with the tracer method using not only Steele's approach but, because of the slow kinetic pattern and the prolonged quasi-steady state, also the dilution equation. Previous estimates of the degree of postprandial inhibition of endogenous glucose release ranged from 40 to 80%

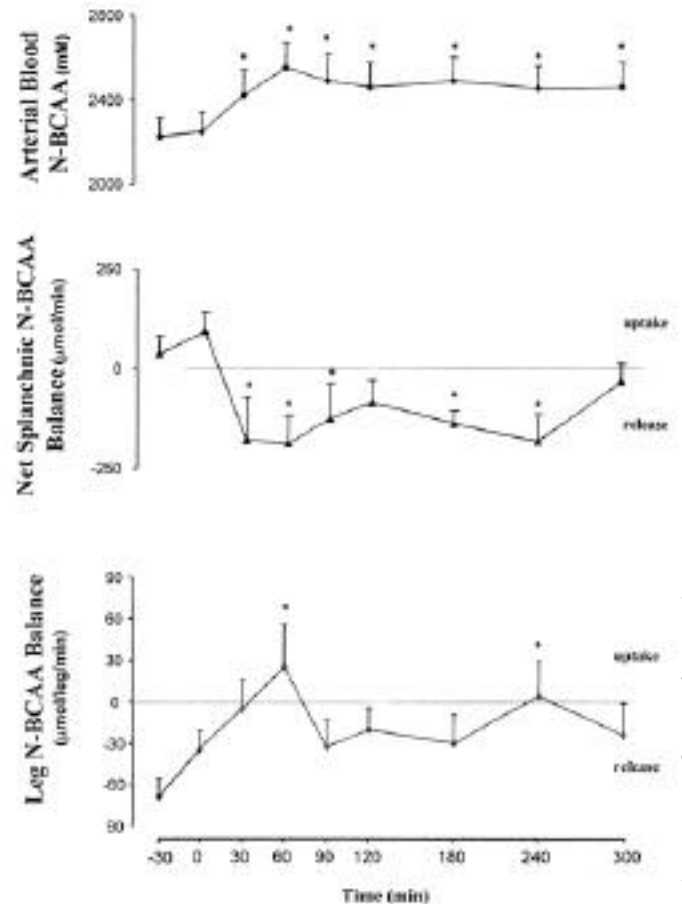


FIG. 6. Arterial N-BCAA concentration and regional N-BCAA balance after ingestion of a mixed meal.

(8,9). Using a variable tracer infusion, Taylor et al. (8) observed a rapid and profound suppression of hepatic glucose release after meal ingestion. However, the meal administered in that study contained a higher carbohydrate content (140 g) in the form of glucose, which resulted in a greater increase in glucose and insulin concentration. In addition, the slight increase in plasma glucagon observed in our study may have prevented an early fall in endogenous glucose production.

Recently, the importance of the so-called "portal signal" in the regulation of splanchnic glucose uptake has been underlined (32–34). This signal, which is activated by the arterial-portal glucose gradient, has an important physiologic significance since it permits adequate removal of glucose by the liver without requiring an excessive increase in blood glucose and insulin concentrations. The activation of such a signal is even more critical in the context of a mixed meal because it enhances hepatic glucose uptake even though blood glucose is only modestly elevated and glucagon level is increased. In this study, splanchnic glucose uptake increased substantially above the basal level, reaching a peak value of  $\sim 10 \mu\text{mol} \cdot \text{l}^{-1} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  ( $\sim 1.8 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) at 120 min. A similar rate of splanchnic glucose uptake was obtained in humans during peripheral glucose infusion, which produced a rise in insulin level similar to that of our study (40–55 U/l) but a much higher increase in glucose concentration (10–15 mmol/l) (32,35). Our results confirm that even a modest rise in arterial glucose concentration, in the presence of the portal signal, results in stimulation of splanchnic glucose uptake. The

amount of glucose removed by the splanchnic area during the 5-h postprandial period was  $161 \pm 16$  mmol/l ( $29 \pm 3$  g). Considering that in the same interval the appearance of glucose (endogenous and exogenous) in the systemic circulation was 442 mmol/l (79 g), it follows that splanchnic tissues are responsible for the disposal of 36% of the glucose delivered.

Over the 5-h experimental period, the leg extracted 32.4 mmol/l of glucose from the circulation; extrapolation of this value to whole-body muscle, assuming that the leg represents 25% of total muscle mass, yields an estimate of 128 mmol/l (23 g) for whole-body muscle glucose uptake. This value corresponds to 29% of the glucose delivered to the systemic circulation over the 5-h absorptive period.

Although these estimates are not strictly quantitative, they do illustrate the relative importance of splanchnic and peripheral tissues to glucose disposal after a natural mixed meal. It appears that splanchnic tissues contribute slightly more than skeletal muscle to glucose disposal. This conclusion is supported by the finding that the splanchnic area is the major site of oxygen consumption after meal ingestion, accounting for 50% of postprandial thermogenesis (versus 30–35% in the skeletal muscle) (36).

In the postprandial state, the splanchnic area rapidly switched from a condition of net lactate uptake to one of net release. From previous studies in the dog, it can be surmised that most of the lactate released from the splanchnic tissues originates from the liver, and only a small fraction is produced by the gut (37–39). In our study, another source of lactate may have been the meal proteins. This hypothesis coincides with the finding that intravenous amino acid infusion is associated with a 30% increase in lactate concentration (40).

Nonsplanchnic tissues may also contribute to postprandial elevation of arterial lactate, since lactate is also produced in skeletal muscle, erythrocytes, skin, brain, and adipose tissue. There is evidence that adipose tissue, but not the skin and brain (41,42), increases lactate release during an oral glucose load (43) or insulin stimulation (44). However, because glucose uptake by the adipose tissue in response to a glucose load is marginal, the contribution of fat-derived lactate to overall postprandial lactate generation seems of limited importance. Regarding skeletal muscle, no increase in lactate release from muscle was observed after glucose administration (2,45) or meal ingestion (14). Rather, a transient lactate uptake has been reported in some cases (46). In the present study, lactate release from leg tissues was suppressed by 50% during meal absorption, which confirms previous reports. The inhibition of muscle lactate release is also consistent with the finding that the majority (50%) of glucose taken up by muscle after a glucose load is oxidized, and only 15% undergoes complete glycolysis to lactate (7).

The present study provides novel data on the regional flux of amino acids after ingestion of a natural mixed meal. After meal ingestion, the splanchnic area released a large part of the ingested amino acid to the systemic circulation, thus increasing circulating amino acid concentrations. The increase in arterial concentration of BCAA was greater than that of N-BCAA (42 vs. 12%), indicating a lower splanchnic retention of BCAA. Assuming that in the postprandial period the splanchnic area continues to take up amino acid at a rate similar to the basal rate, the cumulative amino acid release over the 5-h study would be  $\sim 21$  mmol/l for BCAA and  $\sim 69$  mmol/l for N-BCAA. Those rates imply that 38 and 29% of the

ingested load of BCAA and N-BCAA, respectively, is transferred from the splanchnic area to peripheral tissues, and that a considerable amount of meal protein is removed by the splanchnic bed. Although the gut metabolizes some amino acids, mainly BCAA (47,48), there is no doubt that the liver dominates in the splanchnic disposal of ingested protein. Studies in the dog have shown that the liver removes most of the amino acids ingested, with a predominant uptake of gluconeogenic amino acids (47,48).

After the meal, BCAA were actively taken up by the leg tissues, whereas the leg release of N-BCAA was significantly reduced, suggesting that these substrates are retained by muscle tissues. A similar pattern has been observed after ingestion of a protein meal (49). In this condition, the uptake of BCAA by the leg accounted for more than half the total muscle amino acid uptake, whereas alanine and glutamine were continuously released (although at a reduced rate) from muscle and actively taken up by the splanchnic tissues throughout the absorptive period (49). Thus, after ingestion of a mixed meal or a protein load, BCAA are the major source of nitrogen repletion in muscle. The uptake of BCAA by muscle tissue is stimulated by a combined increase in amino acid and insulin concentration, which is essential to fully activate BCAA transport in muscle (50).

Overall, after a mixed meal, BCAA are transferred from the gut through the liver to peripheral tissues, where they are used to replete protein stores. The other amino acids are retained to a greater extent by the splanchnic area (conceivably in the liver), where they may be oxidized or used to produce glucose, lactate, or glycogen. In this regard, Moore et al. (15) have shown in the dog that 34% of the glycogen formed after ingestion of a mixed meal derives from gluconeogenic precursors. The stimulation of postprandial protein anabolism is promoted by both enhanced protein synthesis and inhibited proteolysis (51,52). Hyperinsulinemia and hyperaminoacidemia cooperate in suppressing proteolysis, whereas hyperaminoacidemia, more than insulin elevation, is effective in stimulating protein synthesis (53,54). These mechanisms operate both at whole-body level (53,54) and in skeletal muscle (55), where a net protein deposition has been demonstrated during ingestion of a mixed meal (55).

The hemodynamic response to ingestion of a mixed meal merits some comment. The flow increase in the splanchnic bed is probably due to the carbohydrate component of the meal, since the ingestion of protein or fat does not induce any variation in regional blood flow (49). The lack of increase in leg blood flow after a mixed meal is at variance with previous studies showing a significant increase in limb blood during euglycemic-hyperinsulinemic clamp or oral glucose tolerance test (56). This is not a marginal issue because, based on previous studies (56), the vasodilating effect of insulin has been viewed as an integral part of its metabolic action.

The results of this study do not negate the vasodilatory effect of insulin. Rather, in keeping with other studies (57), they suggest that under physiologic conditions of meal ingestion additional factors come into play to offset the hemodynamic effect of insulin on muscle vasculature, with the result that muscle blood flow remains unchanged. Thus, it appears that in the postprandial state the stimulatory action of insulin on muscle glucose uptake relies primarily on its effect on cellular permeability to glucose rather than on muscle perfusion, and that the vasodilatory ability of insulin is not essential for



its metabolic effects. Studies showing that insulin's hemodynamic and metabolic effects are not coupled led to similar conclusions (58,59).

In conclusion, the current studies provide a full picture of glucose and substrate fluxes after ingestion of a mixed meal. The main findings are 1) the absorption of a natural mixed meal is largely incomplete until 5 h after ingestion; 2) endogenous glucose production is only marginally and tardily inhibited; 3) splanchnic and peripheral tissues contribute to the disposal of ingested starch to approximately the same extent; 4) the splanchnic area transfers >30% of the ingested proteins to the systemic circulation; and 5) after meal ingestion, skeletal muscle takes up BCAA to replenish muscle protein stores.

## REFERENCES

- Felig P, Wahren J, Hender R: Influences of oral glucose ingestion on splanchnic glucose and gluconeogenic substrate metabolism in man. *Diabetes* 24:1954-1962, 1975
- Radziuk J, McDonald TJ, Rubenstein D, Dupre J: Initial splanchnic extraction of ingested glucose in normal man. *Metab Clin Exp* 27:657-669, 1978
- Katz LD, Glickman MG, Rapaport S, Ferrannini E, DeFronzo RA: Splanchnic and peripheral disposal of oral glucose in man. *Diabetes* 32:675-679, 1983
- Waldhauser WK, Gasic S, Bratusch-Marrain P, Nowotny P: The 75-g oral glucose tolerance test: effect on splanchnic metabolism of substrates and pancreatic hormone release in healthy man. *Diabetologia* 25:489-495, 1983
- Ferrannini E, Bjorkman O, Reichard GA, Pilo A, Olsson M, Warhen J: The disposal of an oral glucose load in healthy subjects: a quantitative study. *Diabetes* 34:580-588, 1985
- Jackson RA, Roshania RD, Hawa MI, Sim BM, Di Silvio L: Impact of glucose ingestion on hepatic and peripheral glucose metabolism in man: an analysis based on simultaneous use of the forearm and double isotope techniques. *J Clin Endocrinol Metab* 63:541-549, 1986
- Kelley D, Mitrakou A, Marsh H, Schwenk F, Benn J, Sonnenberg G, Arcangeli M, Aoki T, Sorensen J, Berger M, Sonksen P, Gerich J: Skeletal muscle glycolysis, oxidation, and storage of an oral glucose load. *J Clin Invest* 81:1563-1471, 1988
- Taylor R, Magnusson I, Rothman DL, Cline GW, Caumo A, Cobelli C, Shulman GI: Direct assessment of liver glycogen storage by  $^{13}\text{C}$  nuclear magnetic resonance spectroscopy and regulation of glucose homeostasis after a mixed meal in normal subjects. *J Clin Invest* 97:126-132, 1996
- Bell P, Firth R, Rizza R: Assessment of the postprandial pattern of glucose metabolism in nondiabetic subjects and in patients with non insulin-dependent diabetes mellitus using simultaneous infusion of  $2\text{-}^3\text{H}$  and  $3\text{-}^3\text{H}$  glucose. *Metabolism* 38:38-45, 1989
- Malagelada J: Quantification of gastric solid-liquid discrimination during digestion of ordinary meals. *Gastroenterology* 72:1264-1267, 1977
- Ahmed M, Nuttal F, Gannon M, Lamusga R: Plasma glucagon and  $\alpha$ -amino acid nitrogen response to various diets in normal humans. *Am J Clin Nutr* 33:1917-1924, 1980
- Rabinowitz D, Maffezzoli R, Merimie T, Murgess J: Patterns of hormone release after glucose, protein, and glucose plus protein. *Lancet* 11:454-456, 1966
- Jones IR, Owens DR, Luzio S, Williams S, Hayes TM: The glucose dependent insulinotropic polypeptide response to oral glucose and mixed meals is increased in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 32:668-677, 1986
- Davis MA, Williams PE, Cherrington AD: Effect of a mixed meal on hepatic lactate and gluconeogenic precursor metabolism in dogs. *Am J Physiol* 247:E362-E369, 1984
- Moore MC, Pagliassotti MJ, Swift LL, Asher J, Murrell J, Neal D, Cherrington AL: Disposition of a mixed meal by the conscious dog. *Am J Physiol* 266:E666-E675, 1994
- Jackson RA, Blix P, Matthews J, Morgan L, Rubenstein A, Nabarro J: Comparison of peripheral glucose uptake after oral glucose loading and a mixed meal. *Metabolism* 32:706-710, 1983
- Pehling G, Tessari P, Gerich JE, Haymond MW, Service FJ, Rizza RA: Abnormal meal carbohydrate disposition in insulin-dependent diabetes. Relative contributions of endogenous glucose production and initial splanchnic uptake and effect of intensive insulin therapy. *J Clin Invest* 74:985-991, 1984
- Elia M, Livesey G: Effects of ingested steak and infused leucine on forelimb metabolism in man and the fate of the carbon skeletons and amino groups of branched-chain amino acids. *Clin Sci* 64:517-526, 1983
- McMahon M, Marsh H, Rizza RA: Comparison of the pattern of postprandial carbohydrate metabolism after ingestion of a glucose drink or a mixed meal. *J Clin Endocrinol Metab* 68:647-653, 1989
- Taylor R, Price TB, Katz LD, Shulman RG, Shulman GI: Direct measurement of change in muscle glycogen concentration after a mixed meal in normal subjects. *Am J Physiol* 265:E224-E229, 1993
- Buzzigoli G, Lanzone L, Ciociaro D, Frascerra S, Cerri M, Scandroglio A, Coldani R, Ferrannini E: Characterization of a reversed-phase high-performance liquid chromatographic system for the determination of blood amino acids. *J Chromatography* 507:85-93, 1990
- Saccà L, Vigorito C, Cicala M, Corso G, Sherwin RS: Role of gluconeogenesis in epinephrine-stimulated hepatic glucose production in humans. *Am J Physiol* 245:E294-E302, 1983
- Leevy CM, Mendenhall CL, Lesko W, Howard MM: Estimation of hepatic blood flow with indocyanine green. *J Clin Invest* 41:1169-1179, 1962
- Abumrad NN, Cherrington AD, Williams PE, Lacy WW, Rabin D: Absorption and disposition of a glucose load in the conscious dog. *Am J Physiol* 242:E397-E406, 1982
- Katz J, Rogstad R: Futile cycles in the metabolism of glucose. *Curr Top Cell Regul* 10:237-289, 1976
- Frank JW, Camilleri M, Thomforde GM, Dinneen SF, Rizza RA: Effects of glucagon on postprandial carbohydrate metabolism in nondiabetic humans. *Metabolism* 47:7-12, 1998
- Niewoehner CB, Nuttall FQ: Disposition of a glucose load in fed rats and rats adapted to a high carbohydrate diet. *Am J Physiol* 265:E811-E817, 1989
- Bleiberg B, Beers TR, Persson M, Miles JM: Systemic and regional acetate kinetics in dogs. *Am J Physiol* 262:E197-E202, 1992
- Phillips J, Muir JG, Birkett A, Lu ZX, Jones GP, O'Dea K, Young GP: Effect of resistant starch on fecal bulk and fermentation-dependent events in humans. *Am J Clin Nutr* 62:121-130, 1995
- David M, Petit WA, Laughlin MR, Shulman RG, King JE, Barrett EJ: Simultaneous synthesis and degradation of rat liver glycogen. *J Clin Invest* 86:612-617, 1990
- Magnusson I, Rothman DL, Jucker B, Cline GW, Shulman RG, Shulman GI: Liver glycogen turnover in fed and fasted humans. *Am J Physiol* 266:E796-E803, 1994
- DeFronzo RA, Ferrannini E, Hender R, Wahren J, Felig P: Influence of hyperinsulinemia, hyperglycemia, and the route of glucose administration on splanchnic glucose exchange. *Proc Natl Acad Sci U S A* 75:5173-5177, 1978
- Pagliassotti MJ, Cherrington AD: Regulation of net hepatic glucose uptake in vivo. *Annu Rev Physiol* 54:847-860, 1992
- Pagliassotti MJ, Holste LC, Moore MC, Neal DW, Cherrington AD: Comparison of the time courses of insulin and the portal signal on hepatic glucose and glycogen metabolism in the conscious dog. *J Clin Invest* 97:81-91, 1996
- Saccà L, Cicala M, Trimarco B, Ungaro B, Vigorito C: Differential effects of insulin on splanchnic and peripheral glucose disposal after an intravenous glucose load in man. *J Clin Invest* 70:117-126, 1982
- Jensen MD, Johnson CM, Cryer PE, Murray MJ: Thermogenesis after a mixed meal: role of leg and splanchnic tissues in men and women. *Am J Physiol* 268:E433-E438, 1995
- Mittrakou A, Jones R, Okuda Y, Pena J, Nurjhan N, Field JB, Gerich JE: Pathway and carbon sources for hepatic glycogen repletion in dogs. *Am J Physiol* 260:E194-E202, 1991
- Moore MC, Cherrington AD, Cline G, Pagliassotti MJ, Jones EM, Neal DW, Badet C, Shulman GI: Sources of carbon for hepatic glycogen synthesis in the conscious dog. *J Clin Invest* 88:578-587, 1991
- Bjorkman O, Eriksson LS, Nyberg B, Wahren J: Gut exchange of glucose and lactate in basal state and after oral glucose ingestion in postoperative patients. *Diabetes* 39:747-751, 1990
- Gelfand RA, Glickman MG, Jacob R, Sherwin RS, DeFronzo RA: Removal of infused amino acids by splanchnic and leg tissues in humans. *Am J Physiol* 250:E407-E413, 1986
- Mittrakou A, Milde J, Michenfelder J, Gerich J: Rates of lactate appearance and disappearance and brain lactate balance after oral glucose in the dog. *Horm Metab Res* 21:236-239, 1989
- Jansson PA, Krogstad AL, Lonnroth P: Microdialysis measurements in skin: evidence for significant lactate release in healthy humans. *Am J Physiol* 271:E138-E142, 1996
- Jansson PA, Larsson A, Smith U, Lonnroth P: Lactate release from the subcutaneous tissue in lean and obese men. *J Clin Invest* 93:240-246, 1994
- Hagstrom-Toft E, Enoksson S, Moberg E, Bolinder J, Arner P: Absolute concentrations of glycerol and lactate in human skeletal muscle, adipose tissue, and blood. *Am J Physiol* 273:E584-E592, 1997
- Jackson RA, Hamling JB, Sim BM, Hawa MI, Blix PM, Nabarro JDN: Peripheral lactate and oxygen metabolism in man: the influence of oral glucose loading. *Metab Clin Exp* 36:144-150, 1987



46. Elia M, Folmer P, Schlatmann A, Goren A, Austin S: Carbohydrate, fat and protein metabolism in muscle and in the whole body after mixed meal ingestion. *Metab Clin Exp* 37:542-551, 1988
47. Barrett EJ, Gusberg R, Ferrannini E, Tepler J, Felig P, Jacob R, Smith D, DeFronzo RA: Amino acid and glucose metabolism in the postabsorptive state and following amino acid ingestion in the dog. *Metabolism* 35:709-717, 1986
48. Ferrannini E, DeFronzo RA, Gusberg R, Tepler J, Jacob R, Aaron M, Smith D, Barrett EJ: Splanchnic amino acid and glucose metabolism during amino acid infusion in dogs. *Diabetes* 37:237-245, 1988
49. Wahren J, Felig P, Hagenfeldt L: Effect of protein ingestion on splanchnic and leg metabolism in normal man and in patients with diabetes mellitus. *J Clin Invest* 57:987-999, 1976
50. Bennet WM, Connacher AA, Scrimgeour CM, Jung RT, Rennie MJ: Euglycemic hyperinsulinemia augments amino acid uptake by human leg tissues during hyperaminoacidemia. *Am J Physiol* 259:E185-E190, 1990
51. Hoffer LJ, Yang RD, Matthews DE, Bistran BR, Bier DM, Young VR: Effects of meal consumption on whole body leucine and alanine kinetics in young adult man. *Br J Nutr* 53:31-38, 1985
52. Tessari P, Pehling G, Nissen SL, Gerich JE, Service FJ, Rizza RA, Haymond MW: Regulation of whole body leucine metabolism with insulin during mixed meal absorption in normal and diabetic humans. *Diabetes* 37:512-516, 1988
53. Tessari P, Inchiostro S, Biolo G, Trevisan R, Fantin G, Marescotti MC, Iori E, Tiengo A, Crepaldi G: Differential effects of hyperinsulinemia and hyperaminoacidemia on leucine-carbon metabolism in vivo. Evidence for distinct mechanisms in regulation of net amino acid deposition. *J Clin Invest* 79:1062-1069, 1987
54. Castellino P, Luzi L, Simonson DC, Haymond M, DeFronzo RA: Effect of insulin and plasma amino acid concentrations on leucine metabolism in man. Role of substrate availability on estimates of whole body protein synthesis. *J Clin Invest* 80:1784-1793, 1987
55. Tessari P, Zanetta M, Barazzoni M, Vettore M, Michielan F: Mechanisms of postprandial protein accretion in human skeletal muscle. Insight from leucine and phenylalanine forearm kinetics. *J Clin Invest* 98:1361-1372, 1996
56. Baron AD: Hemodynamic actions of insulin. *Am J Physiol* 267:E187-E202, 1994
57. Mijares AH, Jensen MD: Contribution of blood flow to leg glucose uptake during a mixed meal. *Diabetes* 44:1165-1169, 1995
58. Natali A, Bonadonna R, Santoro D, Galvan AQ, Baldi S, Frascerra S, Palombo C, Ghione S, Ferrannini E: Insulin resistance and vasodilatation in essential hypertension: studies with adenosine. *J Clin Invest* 94:1570-1576, 1994
59. Raitakari M, Nuutila P, Ruotsalainen U, Laine H, Teras M, Iida H, Makimattila S, Utriainen T, Oikonen V, Sipilä H, Haaparanta M, Solin O, Wegelius U, Knuuti J, Yki-Jarvinen H: Evidence for dissociation of insulin stimulation of blood flow and glucose uptake in human skeletal muscle. Studies using (<sup>15</sup>O)H<sub>2</sub>O, (<sup>18</sup>F)fluoro-2-deoxy-D-glucose, and positron emission tomography. *Diabetes* 45:1471-1477, 1996