# Contribution of Blood Flow to Leg Glucose Uptake During a Mixed Meal

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**Insulin has important effects to increase skeletal muscle (leg) blood flow under euglycemic hyperinsulinemic clamp conditions and after oral glucose tolerance testing. The present studies examined the effects of mixed meal consumption on the components of leg glucose uptake (LGU) in lean, healthy adults. Seventeen men and women underwent measures of leg plasma flow and arteriovenous (AV) glucose difference before and for 6 h after a mixed meal providing one-third of daily energy expenditure. Another eight men and women underwent the same studies before and during the consumption of the same-size meal administered in small frequent feedings over 6 h. After the bolus meal, peak leg AV glucose gradient increased approximately fivefold ($P < 0.001$), whereas the peak increase in leg plasma flow was 20% (NS). No significant contribution of increased leg blood flow to the increase in postprandial LGU was apparent. Over the last 100 min of the frequent-feedings meal, the leg AV difference increased approximately fourfold ($P < 0.001$ vs. basal), whereas leg blood flow increased only by 16% (NS vs. basal). We conclude that after a mixed meal, leg (primarily skeletal muscle) blood flow does not increase enough for blood flow to be a major contributor to glucose uptake. These findings raise questions regarding the relative importance of insulin's hemodynamic effects in modulating glucose tolerance under more usual conditions.** ***Diabetes*** **44:1165–1169, 1995**

Much attention has been focused on understanding the cellular actions of insulin to stimulate skeletal muscle glucose uptake via glucose transporters (1). It has recently been pointed out, however, that insulin-mediated glucose transport into muscle could be limited if glucose delivery were inadequate (2). Modulation of skeletal muscle blood flow seems to be an important mechanism to assure optimal glucose delivery. Numerous studies have documented that insulin can increase skeletal muscle blood flow (3–8), perhaps via nitric oxide (9). It has been suggested that as much as 40% of leg glucose uptake (LGU) can be attributed to the hemodynamic effects of insulin under some conditions (3).

These impressive effects of insulin on skeletal muscle (leg) blood flow led to the natural assumption that the hemodynamic effects of postprandial insulinemia would be important for normal glucose tolerance. Supportive of this hypothesis, leg blood flow increased in lean, but not obese, volunteers after an oral glucose tolerance test (10).

It is not always possible to extrapolate results from euglycemic hyperinsulinemic clamp studies (3–9) or glucose tolerance studies (10) to more usual postprandial circumstances, however. More complex changes in the substrate and hormonal milieu occur after a mixed meal than after an oral glucose tolerance test or during an insulin clamp. These differences could exaggerate or obscure the hemodynamic effects of insulin.

The present studies were undertaken to determine whether leg blood flow, primarily to skeletal muscle, increases after the ingestion of a physiological meal chosen for its size and nutrient composition to be compatible with the usual diet. Because little effect on leg blood flow was noted, additional studies were carried out to assess whether longer duration of meal delivery would result in the expected hemodynamic responses.

## RESEARCH DESIGN AND METHODS

**Subjects.** Informed consent was obtained from 14 nonobese men and 11 premenopausal women; their ages ranged from 19 to 37 years (mean age 26 years). All volunteers were in good health, were taking no medications, and had maintained a stable weight for >2 months before the studies. Body mass index values ranged from 20.9 to 26.9 kg/m$^2$ (mean ± SE 23.8 ± 0.5) in the men and from 18.7 to 23.1 kg/m$^2$ (22.2 ± 0.6) in the women (Table 1). Body fat was 18 ± 1% in men (range 12–25%) and 29 ± 1% in women (range 26–35%).

**Materials and assays.** Plasma glucose concentrations were measured using a glucose oxidase method with a glucose analyzer (YSI, Yellow Springs, OH) and were converted to whole blood glucose concentrations by multiplying the plasma value by 1 − (0.30 × hematocrit) (11). Plasma insulin concentrations were measured by double antibody radioimmunoassay (12).

Indocyanine green (Cardio-Green, Becton Dickinson, Cockeysville, MD) was used for these studies. Arterial and venous plasma indocyanine green concentrations were measured using a spectrophotometer (Spectronic Genesis 5, Milton Roy). Analysis of plasma samples for indocyanine green was performed on the day of the study using each volunteer's baseline plasma to create a six-point (0–3.25 μg/ml) linear ($R^2$ >0.99) standard curve to predict plasma indocyanine green concentrations.

Body fat was measured using dual-energy X-ray absorptiometry (Lunar Radiation, Madison, WI) (13).

**Protocol.** Two groups of volunteers were studied as part of studies of fatty acid and glucose metabolism. Group 1 included eight nonobese men and nine premenopausal women (age 26 ± 2 years, range 20–37). Group 2 was composed of six nonobese men and two premenopausal women (age 30 ± 4 years, range 19–44).

Group 1 subjects consumed all meals as provided by the Mayo Clinic General Clinical Research Center (GCRC) for 2 weeks before the study. The diet provided 40% of energy intake as fat, 40% as carbohydrate, and 20% as protein. Each volunteer was weighed to within 0.1 kg every morning and energy intake was adjusted, if needed, to achieve weight maintenance over the 2-week interval. Group 2 subjects consumed all meals at the GCRC for 3 days before the study; the diet composition was the same as for group 1.

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AV, arteriovenous; GCRC, Mayo Clinic General Clinical Research Center; LGU, leg glucose uptake.

TABLE 1
Subject characteristics

| | Men | Women |
|---|---|---|
| *n* | 14 | 11 |
| Age (years) | 27.7 ± 2.2 | 27.5 ± 2.5 |
| Weight (kg) | 78.2 ± 2.5 | 61.6 ± 1.9 |
| Height (cm) | 179 ± 2 | 166 ± 1 |
| 24-h energy expenditure (kcal) | 2,862 ± 148 | 2,159 ± 325 |

Data are means ± SE. With the exception of age, all variables are significantly ($P < 0.01$) different in men and women.

Each subject was then admitted to the GCRC the evening before the study, and an 18-gauge infusion catheter was placed in a forearm vein and infused with 0.45% NaCl at 20 ml/h. Blood was sampled before the indocyanine green infusions were started to be used for construction of the indocyanine green calibration curve.

The morning of the study, volunteers were transferred to the Vascular Radiology Laboratory where a 10-cm-long 5-Fr Terumo sheath was introduced into the right femoral artery using a standard percutaneous technique. The tip of the sheath was ~6 cm proximal to the inguinal ligament. A 20-cm-long, 4-Fr straight catheter with six distally placed side holes (special order Cook) was placed through the sheath with the catheter tip in the common iliac artery (10 cm above the site of indocyanine green infusion); the catheter was used for blood sampling, and the sheath was used to infuse the indocyanine green. The right femoral vein was then punctured in a similar manner and a 10-cm-long, 5-Fr Terumo sheath was introduced for blood sampling purposes. The distal tip of the sheath was placed in the external iliac vein ~6 cm above the inguinal ligament. A solution of 0.45% NaCl was infused through the sheaths and the catheters to maintain patency. The volunteers were then transferred back to the GCRC for completion of the study. Thirty minutes before the first baseline blood sample, a continuous (270 μg/min) infusion of indocyanine green was begun and continued throughout the remainder of the study. Arterial and femoral venous blood samples were taken in triplicate before meal consumption for measurement of plasma glucose, insulin (arterial only), and indocyanine green concentrations.

The meal (Ensure Plus, Ross) was measured to within 2 ml for each individual and calculated to provide one-third of daily energy needs. The meal contained 53% of energy as carbohydrate, 32% as fat, and 15% as protein. The carbohydrate provided in Ensure Plus is 23% from sucrose and 77% from regular corn syrup. Fructose therefore provides ~14% of the carbohydrate energy, whereas glucose accounts for ~86%. Subjects in group 1 consumed the liquid meal over a 10- to 20-min interval; blood samples were obtained at 30-min intervals for the 6 h after the meal consumption.

Subjects in group 2 underwent the same study except that they consumed the liquid meal in small frequent feedings (1/18 of prescribed calories) every 20 min throughout 6 h of the study. The first two feedings were doubled as a "priming dose." Blood was sampled at 20-min intervals over the last 100 min of the study. In this study, the indocyanine green infusion was stopped and then restarted 30 min before the second series of blood samples. After completion of the study, all catheters were removed, and local hemostasis was obtained. The subjects remained in the hospital under observation until the following morning.

**Calculations and statistics.** Leg plasma flow was calculated as described by Jorfeldt and Wahren (14). To account for recirculation of indocyanine green, the concentration in the arterial blood sampled from the catheter in the common iliac artery was subtracted from the concentration of indocyanine green in the femoral vein. The indocyanine green infusion rate was then divided by the difference between the femoral venous and arterial indocyanine green concentrations to predict leg plasma flow (14). The differences averaged ~1 μg/ml. The sites of catheter placement for these studies have previously been shown to result in adequate mixing of dye and blood (14). Leg blood flow was determined using the plasma flow and hematocrit values for each individual (14).

LGU was calculated by the Fick principle (6) as the product of the arteriovenous (AV) difference for blood glucose and leg blood flow, using the following formula:

$$\text{LGU (mg/min)} = \text{AV glucose difference (mg/dl)/100} \times \text{leg blood flow (ml/min)}$$

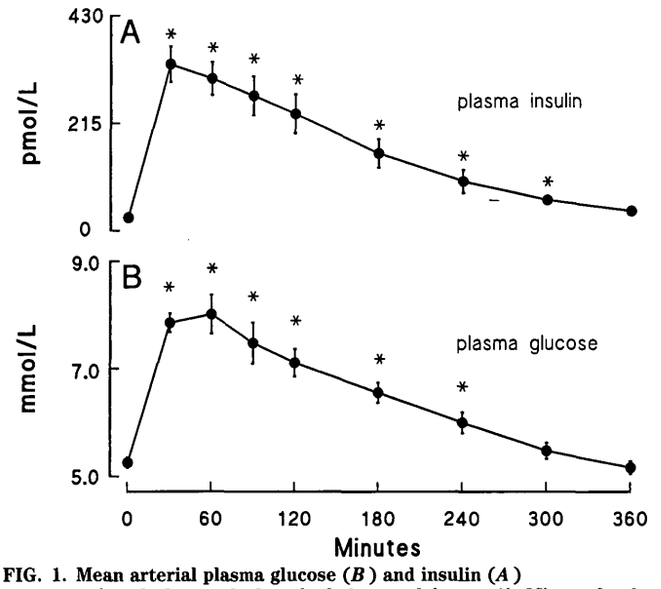


**FIG. 1. Mean arterial plasma glucose (*B*) and insulin (*A*) concentrations before and after the bolus meal (group 1). Minute 0 value is the average of premeal samples. *$P < 0.05$ vs. basal.**

For group 1, total LGU over 6 h was assessed by determining the area under the glucose uptake curve between 0 and 360 min; integration was performed by trapezoidal rule. In addition, we calculated what LGU would have been if leg blood flow had not changed from baseline rates ($F_{fixed}$) as described by Laakso et al. (3).

All results are expressed as means ± SE. Comparisons between groups were made using analysis of variance and nonpaired Student's *t* test. Statistical significance was defined as $P < 0.05$.

## RESULTS

**Group 1.** Men and women were analyzed together because there were no significant differences in glucose, insulin, or blood flow responses between the two groups. The basal values are the averages of the premeal samples. The arterial plasma glucose and insulin concentrations are shown in Fig. 1. The arterial glucose concentrations increased from 5.3 ± 0.1 to a peak of 8.0 ± 0.4 mmol/l 60 min after the meal ($P < 0.001$) and then returned to baseline over the next 5 h (5.1 ± 0.1 mmol/l at 360 min, NS vs. basal). Arterial plasma insulin concentrations increased ($P < 0.001$) from 26 ± 2 pmol/l during fasting to a maximum of 333 ± 36 pmol/l at 30 min after the meal. Plasma insulin concentrations returned to baseline at 360 min after the meal (39 ± 6 pmol/l, NS vs. basal).

AV blood glucose differences (Fig. 2) rose from 0.13 ± 0.02 mmol/l to a peak of 0.67 ± 0.1 mmol/l at 60 min ($P < 0.001$) and returned to baseline values by 300 min (0.15 ± 0.02 mmol/l, NS vs. basal).

Basal leg blood flow was 390 ± 35 ml/min. The maximal postprandial leg blood flow was 469 ± 59 ml/min at 120 min (20% above baseline values). The changes in leg plasma flow after the meal were not statistically significant at any time point. Leg blood flow returned to baseline 270 min after the meal (390 ± 34 ml/min) (Fig. 2).

Basal LGU averaged 0.52 ± 0.10 mmol/min; there was a significant increase in LGU, which was maximal at 2.73 ± 0.70 mmol/min 30 min after the meal (Fig. 3) and remained greater than basal values until time 300 min. There appeared

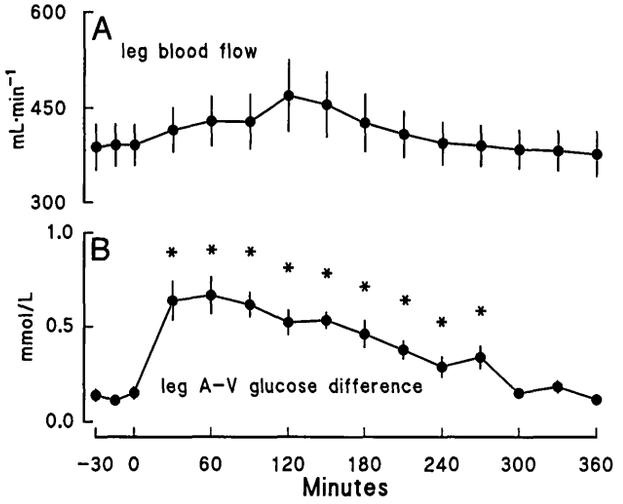


FIG. 2. Leg AV blood glucose differences (*B*) and leg blood flow (*A*) before and after the bolus meal (group 1). *$P < 0.05$ vs. basal.

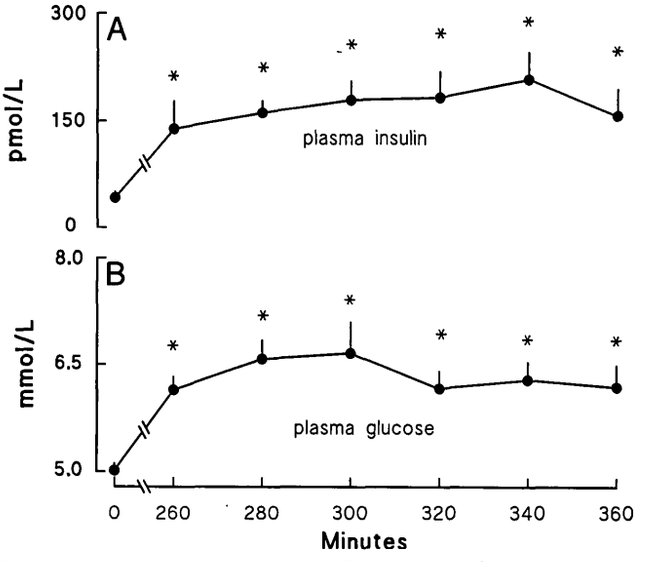


FIG. 4. Mean arterial plasma glucose (*B*) and insulin (*A*) concentrations before and during the frequent-feedings meal (group 2). *$P < 0.005$ vs. basal.

to be a strong temporal association between LGU and both plasma glucose and insulin concentrations (Figs. 1 and 3), but not between plasma flow and glucose uptake.

The observed LGU was compared with the uptake that might have occurred if leg plasma flow had remained constant at fasting levels ($F_{fixed}$) (Fig. 3). There was no significant difference in 6-h LGU (area under the curve) if leg plasma flow had remained constant at basal values (55.4 ± 6.1 mmol), compared with the observed glucose uptake (55.8 ± 5.0 mmol).

**Group 2.** Data from men and women were combined for this analysis, as was done for group 1. Arterial plasma glucose concentrations increased ($P < 0.05$) from 5.0 ± 0.1 mmol/l to a near steady state between 240 and 360 min of 6.2 ± 0.2 mmol/l (Fig. 4). Plasma insulin concentrations increased ($P < 0.01$) from 41 ± 8 to 179 ± 22 pmol/l over the last ~2 h of the study. There were no significant changes in plasma glucose or insulin concentrations between 260 and 360 min ($P = 0.9$ by repeated-measures analysis of variance).

The basal leg AV blood glucose difference was 0.12 ± 0.04 mmol/l and reached a steady state of 0.50 ± 0.16 mmol/l between time 260 and 360 min (Fig. 5). Basal leg blood flow averaged 311 ± 24 ml/min. Leg blood flow averaged 361 ± 32 ml/min over the final 100 min of the study and was not significantly different from basal values.

Basal leg blood glucose uptake was 0.42 ± 0.18 mmol/min, increasing to an average of 1.98 ± 0.51 mmol/min over the final 100 min of the study ($P = 0.005$ vs. basal).

## DISCUSSION

These studies were undertaken to evaluate the components of LGU (AV glucose difference and blood flow) after the consumption of a mixed meal in lean, healthy adults. Subnormal hemodynamic responses to insulin have been suggested as a potential mediator of reduced muscle glucose uptake (3,4) and, therefore, postprandial hyperglycemia (10). We measured leg blood flow and AV glucose differences in healthy adults for 6 h after a mixed meal containing a physiological energy content. After meal consumption the peak increase in LGU was almost sixfold; however, the peak

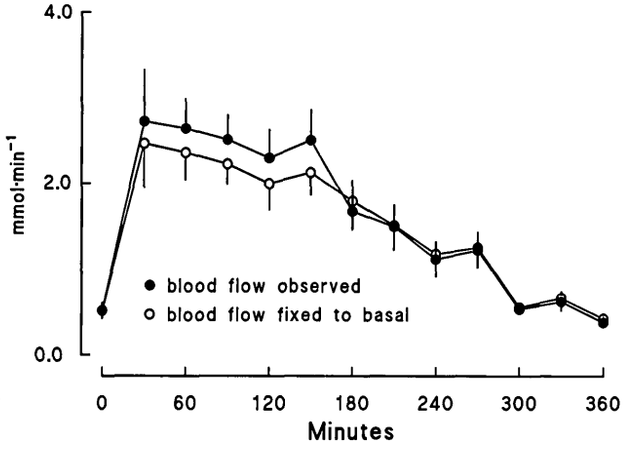


FIG. 3. Rates of LGU before and after the meal using the observed values of leg blood flow (●) and the rates predicted to occur if leg blood flow were fixed at constant (basal) values (○) for group 1 subjects.

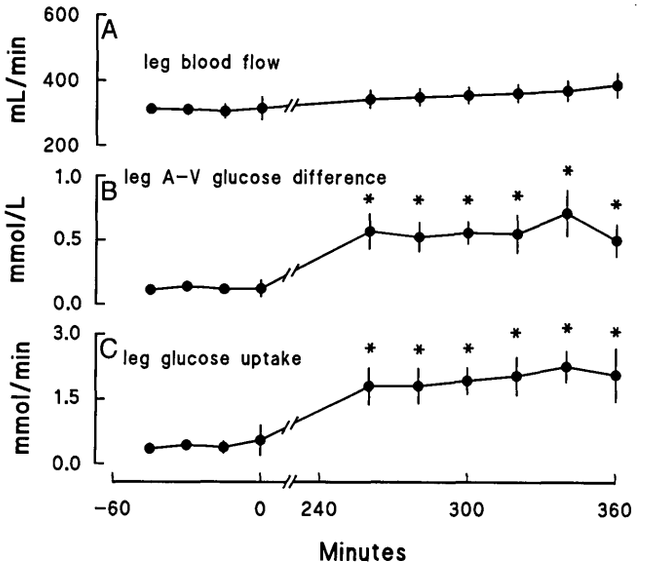


FIG. 5. Leg blood flow (*A*), AV blood glucose differences (*B*), and LGU (*C*) before and during the frequent-feedings meal (group 2). *$P < 0.005$ vs. basal.

increase in leg blood flow was only 20%. The changes in leg blood flow after meal consumption were not statistically significant at any point in time. We conclude that an increase in leg (primarily skeletal muscle) blood flow after consumption of a mixed meal is not necessary to avoid postprandial hyperglycemia and that increases in the AV glucose difference are the primary determinant of glucose uptake after a physiological meal. These data raise questions regarding the relative importance of insulin's hemodynamic effects on postprandial glucose tolerance in diabetes and obesity.

Many (3–10) but not all (15,16) previous studies have found a significant increase in extremity blood flow during hyperinsulinemia. It could be argued that pharmacological hyperinsulinemia obtained during a euglycemic hyperinsulinemic clamp is needed to stimulate leg blood flow. Insulin dose-response studies (3), however, have observed an increase in leg blood flow at plasma insulin concentrations comparable to those observed in our volunteers after meal consumption. Although the time delay for the hemodynamic response to insulin is greater than that required for the AV glucose difference response (3), the duration of our observations suggests that time delay was not a factor in our failure to observe an increase in blood flow. In addition, the continuous delivery of the meal resulted in sustained hyperinsulinemia and hyperglycemia, yet no significant increase in blood flow was observed. We cannot exclude the possibility that if a higher degree of physiological hyperinsulinemia had been achieved, significant increases in blood flow might have occurred. This would have required the ingestion of even larger meals, however. It is possible that the hemodynamic responses to insulin are altered if glucose and insulin are given intravenously, as opposed to the nutrients being provided by mouth and the insulin secreted endogenously. This seems unlikely in light of the observations of Baron et al. (10), who found leg blood flow increased after an oral glucose load in lean but not obese subjects.

A more likely explanation for our failure to observe a change in blood flow is the nature of the meal consumed. Insulin clamp studies or oral glucose tolerance tests provide glucose as the only nutrient. Tissue beds may display differential hemodynamic responses to carbohydrate versus mixed-nutrient challenges. For example, although the consumption of mixed meals or glucose substantially increases cardiac output and splanchnic blood flow (17–19), a meal consisting of fructose does not (19). Protein ingestion does not increase leg blood flow (20). Whether dietary fat influences the blood flow response to a meal is unknown. It seems unlikely that the small amount of fructose present in the meal used in this study would substantially affect the hemodynamic responses, as occurs when pure fructose is ingested (19). We have observed increased splanchnic blood flow when this same type of liquid meal is consumed (20).

We evaluated the potential of the indocyanine green infusion method for detecting changes in leg plasma flow. The coefficient of variation of the three baseline leg plasma flow measurements for the individuals in our study was 6 ± 1%. This represents the combination of measurement error and physiological variation over 30 min. At the peak increase in blood flow after the bolus meal, the 95% confidence intervals for changes in blood flow from baseline were −10 to +53%. For the frequent-feeding study, the 95% confidence intervals for changes in blood flow from baseline were −30 to +63%. We conclude that the measurement precision for leg blood flow using an indocyanine green infusion is high and that there is substantial interindividual variability in leg blood flow responses after meal ingestion.

We also considered the possibility that indocyanine green itself might inhibit the hemodynamic responses to events that increase leg blood flow. This appears not to be the case; both Vierhapper et al. (8) and Biolo et al. (21) report that euglycemic hyperinsulinemia increases leg blood flow by ~30% ($P < 0.01$) as measured by the femoral arterial indocyanine green infusion technique. Thus, the technique used in this study allows the detection of increased leg blood flow in response to insulin.

The AV difference technique accurately reflects glucose extraction only under steady-state conditions (22). As pointed out by Baron et al. (10), there is a significant delay before the increase in arterial glucose is completely reflected on the glucose side; therefore, glucose uptake will be overestimated as plasma glucose concentrations increase and underestimated as they decrease. Our study encompassed the entire postprandial glycemic excursion; therefore, total LGU over 6 h as measured in our study should be accurate. No significant decrease in LGU would be predicted if leg blood flow was unchanged after meal consumption. The steady-state data from continuous meal consumption studies are supportive of our conclusions from the bolus meal study.

Although we saw no significant increase in leg blood flow in our subject in response to a physiological meal, this does not completely exclude a hemodynamic role of insulin as regards glucose uptake in obese/diabetic humans. Insulin has been clearly demonstrated to increase leg blood flow (3–10), but we found that the levels of insulin observed after a mixed meal do not. This implies there may be factors present after a mixed meal that offset the hemodynamic effects of insulin. These same factors may act to decrease postprandial skeletal muscle blood flow in obesity or diabetes and are not offset by insulin's hemodynamic effects. This possibility remains to be investigated.

In summary, after the consumption of a mixed meal providing one-third of daily energy expenditure, leg blood flow did not increase significantly in lean, healthy men and women. Virtually all LGU could be accounted for by changes in the AV glucose difference. Results of a continuous meal delivery experiment were consistent with our findings after the bolus meal. These findings raise significant questions regarding the hemodynamic role of postprandial hyperinsulinemia in regulating postprandial glucose disposal in diabetes and obesity.

## ACKNOWLEDGMENTS

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