Portal glucose infusion-glucose clamp measures hepatic influence on postprandial systemic glucose appearance as well as whole body glucose disposal

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Zheng D, Ionut V, Mooradian V, Stefanovski D, Bergman RN. Portal glucose infusion-glucose clamp measures hepatic influence on postprandial systemic glucose appearance as well as whole body glucose disposal. Am J Physiol Endocrinol Metab 298: E346–E353, 2010. First published November 24, 2009; doi:10.1152/ajpendo.00280.2009.—The full impact of the liver, through both glucose production and uptake, on systemic glucose appearance cannot be readily studied in a classical glucose clamp because hepatic glucose metabolism is regulated not only by portal insulin and glucose levels but also portal glucose delivery (the portal signal). In the present study, we modified the classical glucose clamp by giving exogenous glucose through portal vein, the “portal glucose infusion (PoG)-glucose clamp,” to determine the net hepatic effect on postprandial systemic glucose supply along with the measurement of whole body glucose disposal. By comparing systemic rate of glucose appearance (Ra) with portal glucose infusion rate (PoGinf), we quantified “net hepatic glucose addition (NHGA)” in the place of endogenous glucose production determined in a regular clamp. When PoG-glucose clamps (n = 6) were performed in dogs at basal insulinemia and hyperglycemia (∼150 mg/dl, portal and systemic), we measured consistently higher Rh than PoGinf (4.2 ± 0.6 vs. 2.9 ± 0.6 mg·kg⁻¹·min⁻¹ at steady state, P < 0.001) and thus positive NHGA at 1.3 ± 0.1 mg·kg⁻¹·min⁻¹, identifying net hepatic addition of glucose to portal exogenous glucose. In contrast, when PoG-glucose clamps (n = 6) were performed at hyperinsulinemia (∼250 pmol/l) and systemic euglycemia (portal hyperglycemia due to portal glucose infusion), we measured consistently lower Rh than PoGinf (13.1 ± 2.4 vs. 14.3 ± 2.4 mg·kg⁻¹·min⁻¹, P < 0.001), and therefore negative NHGA at −1.1 ± 0.1 mg·kg⁻¹·min⁻¹, identifying a switch of the liver from net production to net uptake of portal exogenous glucose. Steady-state whole body glucose disposal was 4.1 ± 0.5 and 13.0 ± 2.4 mg·kg⁻¹·min⁻¹, respectively, determined as in a classical glucose clamp. We conclude that the PoG-glucose clamp, simulating postprandial glucose entry and metabolism, enables simultaneous assessment of the net hepatic effect on postprandial systemic glucose supply as well as whole body glucose disposal in various animal models (rodents, dogs, and pigs) with established portal vein catheterization.

net hepatic glucose addition

FOLLOWING A MEAL, PLASMA GLUCOSE RISES as the gastrointestinal tract digests and absorbs carbohydrates. A dramatic increase in postprandial glyceremia is prevented when the systemic supply of the absorbed glucose is matched to the glucose disposal capacity of the body. Various tissues participate in clearing glucose from the bloodstream, including skeletal muscle, adipose tissue, the liver, and non-insulin-dependent tissues (40, 41, 46). In contrast, the liver, uniquely situated to receive the absorbed glucose through the portal vein, plays a pivotal role in regulating the systemic glucose supply by adjusting the balance between hepatic glucose production and uptake of newly appearing portal glucose (34). It has been challenging to address both the supply and disposal aspects of postprandial glucose metabolism in a single method, especially the full impact of the liver on systemic glucose appearance through both hepatic glucose production and uptake.

By establishing a new steady-state insulin level above its fasting value, the hyperinsulinemic euglycemic clamp remains the gold standard for measuring insulin stimulation of whole body glucose disposal and suppression of endogenous (mostly hepatic) glucose production (6, 17). However, since exogenous glucose is infused via a peripheral vein, there is a lack of information in a classical glucose clamp study on how initial first-pass hepatic glucose uptake contributes to hepatic regulation of postprandial systemic glucose appearance. Initial splanchnic glucose uptake, a substitute for hepatic glucose uptake, accounts for ∼10% of an oral glucose load (1, 20, 42, 48, 52), imposing a potentially significant impact on postprandial glyceremia. Several methods are available to specifically evaluate splanchnic glucose uptake, including hepatic vein catheterization (15), the double-tracer approach (16), and the oral glucose clamp method (36), although these methods are limited by the uncertainty of the extent and duration associated with the absorption of an oral glucose load (1, 4).

In the present study, we established a modified glucose clamp in which the maintenance of systemic glyceremia is achieved by exogenous glucose infusion through the portal vein, the “portal glucose infusion (PoG)-glucose clamp.” In doing so, we were to simulate postprandial glucose entry and measure the net hepatic effect (production and uptake) on systemic glucose appearance at different combinations of insulinemia and glyceremia. The net hepatic effect in relation to systemic glucose appearance was defined as net hepatic glucose addition (NHGA) and calculated by comparing portal glucose infusion and tracer-derived systemic glucose appearance. Using NHGA, the PoG-glucose clamp is more sensitive to hepatic changes induced by many metabolic regulators, such as free fatty acids, gut hormones, and adipocytokines, than the widely used classical glucose clamp. In addition, when endogenous glucose production is completely suppressed, the PoG-glucose clamp can estimate first-pass hepatic glucose uptake, comparable with splanchnic glucose uptake derived from hepatic vein catheterization, the oral glucose clamp, or the double-tracer method, without the limitations associated with oral...
MATERIALS AND METHODS

Animals. Experiments were conducted in male mongrel dogs (n = 12; Harlan, Indianapolis, IN) housed under controlled conditions and fed once daily (Labdiet; PMI Nutrition International, Richmond, IN). Animals were used for experiments only if they were judged to be in good health as determined by body weight, food intake and stools, temperature, hematocrit, and direct observation. All surgical and experimental procedures were approved by University of Southern California Institutional Animal Care and Use Committee.

Surgical procedures. At least 1 wk prior to the first experiments, chronic catheters (Tygon, 0.05 in. id; Norton Plastics, Akron, OH) were implanted under general anesthesia. One catheter was placed in the jugular vein (with the tip advanced near the right atrium) and the other in the portal vein (4 cm upstream from porta hepatis). All catheters, filled with heparinized saline (10 U/ml), were led subcutaneously to the back of the neck, exteriorized, and secured in place.

Experimental design. Experiments were performed on animals in a conscious, relaxed state. Animals were fasted for 14–16 h, with free access to water. With such a fasting regime, no difference was found between portal and systemic fasting glucose levels in our previous study (25), which is important because the residual gut glucose absorption can affect the estimation of the balance between hepatic glucose production and uptake. For all experiments, animals were brought to the laboratory at ~6 AM and placed into a Pavlov sling. The PoG-glucose clamp resembles the classical glucose clamp in all aspects, except that exogenous glucose is infused through the portal vein. Briefly, 1) somatostatin and insulin were infused peripherally to produce desired insulinemia uniformly in systemic and portal circulations, 2) exogenous glucose was given through the portal vein, different from the peripheral entry in a classical glucose clamp, and 3) glucose tracer infusions were administered peripherally to achieve constant plasma glucose-specific activity. We examined the ability of the PoG-glucose clamp to assess the net hepatic effect on systemic glucose appearance as well as whole body glucose disposal in the following two conditions: 1) basal insulinemia and hyperglycemia (both systemic and portal) and 2) hyperinsulinemia and euglycemia (systemic euglycemia but portal hyperglycemia due to portal glucose infusion). Part of the data obtained from the above studies were used as the control data in a previously published paper (54).

The basal insulinemic hyperglycemic clamp study. Experiments were conducted in six animals (body weight = 29.5 ± 0.8 kg). The PoG-glucose clamp was composed of two periods, a 2-h tracer equilibrium period (−120 to 0 min) and a 4-h experimental period (0–240 min; Fig. 1). At −120 min, immediately following a systemic injection of 25 μCi bolus of [3-3H]glucose (DuPont-NEN, Boston, MA), a continuous infusion of the glucose tracer, at 0.25 μCi/min, was initiated via a peripheral venous catheter. Ninety minutes later, blood samples for the basal period were taken every 10 min from −30 to 0 min. At 0 min, peripheral infusions of somatostatin (Bachem California, Torrance, CA), at 1 μg·kg⁻¹·min⁻¹, and porcine insulin (Eli Lilly, Indianapolis, IN), at 0.15 mU·kg⁻¹·min⁻¹, were initiated to maintain basal insulin levels. An intraportal infusion of porcine glucagon (Sigma, St. Louis, MO), at 0.65 ng·kg⁻¹·min⁻¹, was started simultaneously to maintain plasma glucagon at basal levels. During the experimental phase (0–240 min), blood samples were taken from another peripheral venous catheter every 10 min, with plasma glucose measured immediately. Exogenous glucose (50% dextrose, 454.5 mg/ml; B Braun, Irvine, CA) was given via the portal vein catheter at variable rates to maintain systemic glucose at 150 mg/dl. When being infused into the lumen of the portal flow, the glucose concentration is mixed with exogenous glucose and the blood may not be thorough, but exogenous glucose was assumed to be evenly distributed to hepatic lobes across study animals, since mixing errors were random. An additional tracer infusion was given at variable rates via the jugular vein catheter to minimize fluctuations in systemic plasma-specific activity; the ratio between the rate of the additional systemic tracer infusion and that of portal glucose infusion was kept at 1.8 μCi/g. The average of the four samples taken from 150 to 180 min was defined as the clamp steady state when plasma glucose, insulin, and exogenous glucose infusion rate were among the least variable. The length of the classical hyperinsulinemic euglycemic clamp conducted in our canine model is 3 h, and the steady state will have usually reached over the period of 150–180 min. We conducted the PoG-glucose clamps for 4 h in case a longer time period needed to reach steady state. In fact, the average coefficients of variation for glucose, insulin, and portal glucose infusion of 150–180 vs. 210–240 min are almost identical. We chose to use average measurements over the 150- to 180-min period so that we can readily compare them with those obtained in our previous classical glucose clamps.

Experiments were conducted in six animals (body eight = 28.6 ± 0.8 kg). The PoG-glucose clamp was conducted as described above, except that porcine insulin was infused at 0.75 mU·kg⁻¹·min⁻¹ to obtain hyperinsulinemia of ~250 pmol/l, whereas systemic glucose was maintained at euglycemia (Fig. 1).

Blood sampling. Samples for the determination of glucose, free fatty acids (FFAs), insulin, C-peptide, glucagon, cortisol, and glucose tracer were collected as described previously (24, 30). All samples were centrifuged immediately, and plasma was separated and stored at −80°C until analysis. To prevent triglyceride breakdown, plasma samples for FFA analysis were kept on ice and either assayed immediately or kept at −80°C for a limited time before assay, as described previously (24).

Assays. Glucose was measured with a YSI 2700 autoanalyzer (Yellow Springs Instruments, Yellow Springs, OH). Insulin was measured using a human insulin ELISA kit (Linco-Millipore, Billerica, MA) adapted for dog plasma (53). C-peptide, glucagon, and cortisol were measured using radioimmunoassay kits (Linco-Millipore). FFAs were determined using an enzymatic colorimetric assay (NEFA C; Wako Pure Chemical Industries, Richmond, VA). Samples for [3-3H]glucose were deproteinized, dried, and resuspended in scintillation fluid (ReadySafe; Beckman, Fullerton, CA) and then read in a β-scintillation counter.

Calculations. Two metabolic parameters are calculated from a PoG-glucose clamp, 1) whole body glucose disposal, i.e., rate of glucose disappearance (RGl), and 2) a new parameter, NHGA, in place of endogenous glucose production in a classical glucose clamp. The time course of whole body glucose disposal is calculated using

![Fig. 1. Portal glucose infusion-glucose clamp. Basal and clamp steady state (SS) indicate the SS during the basal and clamp periods, respectively. Porcine insulin was infused at either 0.15 mU·kg⁻¹·min⁻¹ in the basal insulinemic hyperglycemic clamp study or 0.75 mU·kg⁻¹·min⁻¹ in the hyperinsulinemic euglycemic clamp study. Exogenous glucose was infused via the portal vein to achieve systemic hyperglycemia or maintain systemic euglycemia, respectively, in the 2 studies.](http://ajpendo.physiology.org/DownloadedFrom/10.1152/ajpendo.00565.2009)
Steele’s equation with a labeled glucose infusion (17) after data smoothing using OOPSEG (8) as in a classical glucose clamp. OOPSEG is a data-smoothing program for quantitation and isolation of random measurement error. On the other hand, since exogenous glucose is infused directly into the portal vein in a PoG-glucose clamp, a fraction of portally given glucose could be taken up by the liver without entering the systemic circulation, referred to as first-pass hepatic glucose uptake. Thus, rate of glucose appearance (Ra) equals portal glucose infusion rate (PoGinf) plus endogenous glucose production (EGP) minus first-pass hepatic glucose uptake (HGU; Eq. 1). The difference between EGP and first-pass HGU, defined as NHGA, is then derived from the difference between Ra and PoGinf (Eq. 2, Fig. 2A). In contrast, in a classical glucose clamp where exogenous glucose is infused through a peripheral vein, the difference between rate of glucose appearance and peripheral glucose infusion gives rise to EGP (Fig. 2B). Ra was calculated in both cases using Steele’s equation, similar to Rd:

\[ Ra = PoGinf + EGP - 1^{st}-pass HGU \]  
(1)

\[ Ra = PoGinf = EGP - 1^{st}-pass HGU = NHGA \]  
(2)

\[ Ra = PoGinf + (EGP - 1^{st}-pass HGU) = PoGinf + NHGA \]  
(3)

**RESULTS**

**Basal insulinemic hyperglycemic clamp study.** A basal insulinemic hyperglycemic clamp was conducted to examine the net hepatic effect on systemic glucose appearance in the presence of basal insulin levels and hyperglycemia in the portal circulation. Plasma insulin was maintained at basal levels of 44.4 ± 1.8 pmol/l, plasma glucose was moderately elevated to 151.1 ± 2.8 mg/dl (P < 0.0001 vs. basal), and plasma FFAs were suppressed by ~50%, to 0.23 ± 0.04 mmol/l (P = 0.005 vs. basal), during the clamp steady state (Fig. 3). Plasma glucagon and cortisol were maintained near their basal values (Table 1). Since endogenous insulin secretion was suppressed with somatostatin, as reflected by plasma C-peptide (Table 1), portal insulin, more specifically, was maintained at systemic basal but lower than portal basal level.

To maintain hyperglycemia in the face of basal insulin levels, portal exogenous glucose (PoGinf) was infused at a moderate rate of 2.9 ± 0.6 mg·kg^{-1}·min^{-1} during the clamp steady state (Fig. 4A). The systemic Ra increased with the start of PoGinf, stayed significantly above PoGinf during the entire clamp period (P < 0.0001), and stabilized at 4.2 ± 0.6 mg·kg^{-1}·min^{-1} at steady state (P < 0.001; Fig. 4A). Thus, the difference between Ra and PoGinf, NHGA, remained positive throughout the clamp and was at 1.3 ± 0.1 mg·kg^{-1}·min^{-1} at steady state, indicating a net addition of extra glucose into the systemic circulation, albeit at a lower level (~50%) than the basal EGP (Fig. 5A). The systemic Ra was slightly elevated, to 4.1 ± 0.5 mg·kg^{-1}·min^{-1} (P = 0.01 vs. basal), at steady state in the presence of basal insulinemia and systemic hyperglycemia (Fig. 5B).

The immediate drop in plasma FFA levels at the onset of the basal insulinemic hyperglycemic clamp was most likely a result of portal and systemic hyperglycemia induced by portal...
glucose infusion. It has been shown that hyperglycemia per se can reduce plasma FFA levels in the acutely insulin-deficient (with somatostatin) dogs (47). In our previous study, we saw a similar suppression of plasma FFA levels with hyperglycemia, although it was induced by peripheral glucose infusion, at basal insulinemia (2).

Hyperinsulinemic euglycemic clamp study. A hyperinsulinemic euglycemic clamp was conducted to examine the net hepatic effect on systemic glucose appearance in the presence of hyperinsulinemia and hyperglycemia in the portal circulation. Plasma insulin was raised significantly from basal levels to 242.1 ± 11.1 pmol/l (P < 0.0001 vs. basal), plasma glucose was maintained at euglycemia of 89.4 ± 3.5 mg/dl, and plasma FFAs were 0.05 ± 0.01 mmol/l (P < 0.001 vs. basal) at the clamp steady state (Fig. 3). Plasma glucagon and cortisol were maintained at about their basal values (Table 1). Endogenous insulin secretion was suppressed, shown by minimally detectable plasma C-peptide (Table 1).

Hyperinsulinemia significantly stimulated whole body glucose disposal and demanded a high PoGinf of 14.3 ± 2.4

Table 1. Basal and clamp SS C-peptide, glucagon, and cortisol in the BIHF and HIEG

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<th>BIHG</th>
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<th>HIEG</th>
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<td>Basal</td>
<td>Clamp SS</td>
<td>Basal</td>
<td>Clamp SS</td>
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<tr>
<td>C-peptide, ng/ml</td>
<td>0.20 ± 0.03</td>
<td>0.01 ± 0.01*</td>
<td>0.20 ± 0.06</td>
<td>0.05 ± 0.03*</td>
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<tr>
<td>Glucagon, ng/l</td>
<td>45.3 ± 7.3</td>
<td>38.1 ± 6.3*</td>
<td>47.1 ± 6.5</td>
<td>33.4 ± 5.1</td>
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<tr>
<td>Cortisol, µg/dl</td>
<td>2.5 ± 0.4</td>
<td>2.7 ± 0.6</td>
<td>3.7 ± 1.4</td>
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Data are means ± SE. BIHG, basal insulinemic hyperglycemic clamp study; HIEG, hyperinsulinemic euglycemic clamp study; SS, steady state. *P < 0.05, significantly different from basal in each study.

Fig. 3. Plasma insulin (A), glucose (B), and free fatty acids (FFAs; C) in the basal insulinemic hyperglycemic clamp study (BIHG; ○) and the hyperinsulinemic euglycemic clamp study (HIEG; ●).

Fig. 4. The time course data of PoGinf (○) and the Ra (●) in the BIHG (A) and HIEG (B).

Fig. 5. The time course (left) and clamp SS (right) data of NHGA (A) and the Ra (B) in the BIHG (○) and the HIEG (●).
mg·kg⁻¹·min⁻¹ during the clamp steady state (Fig. 4B). The systemic \( R_s \) was accordingly increased significantly to 13.1 ± 2.4 mg·kg⁻¹·min⁻¹ at steady state (Fig. 4B). However, in contrast to the basal insulinemic hyperglycemic clamp study, the \( R_s \) became less than PoGinf during the 1st hour of the clamp and stayed significantly below PoGinf for the rest of clamp \((P < 0.0001)\) and at steady state \((P < 0.001)\). Thus, NHGA showed a rapid decrease during the 1st hour of the clamp, remained negative thereafter, and was at -1.1 ± 0.1 mg·kg⁻¹·min⁻¹ during the steady state, indicating a switch of the liver to predominantly take up portal exogenous glucose and limitation of its entry into the systemic circulation (Fig. 5A). The systemic \( R_s \) was enhanced significantly in the presence of hyperinsulinemia and systemic euglycemia, to 13.0 ± 2.4 mg·kg⁻¹·min⁻¹ \((P < 0.01\) vs. basal), at steady state (Fig. 5B).

DISCUSSION

Postprandial glycemia is normally prevented from deviating dramatically from basal levels because the rate of glucose appearance is tightly and timely matched to the rate of glucose disappearance in the systemic circulation. Although multiple organs are involved in the process of removing glucose from the bloodstream (skeletal muscle, adipose tissue, the liver, and the brain), the systemic glucose appearance is regulated largely by the dual actions of the liver, i.e., hepatic glucose production and hepatic uptake of portal delivered exogenous glucose. In the present study, we modified the classical glucose clamp by infusing exogenous glucose through the portal vein, intending to simulate postprandial glucose entry and thus capture the combined effect of the liver on systemic glucose appearance through both glucose production and uptake in addition to the measurement of whole body glucose disposal.

It is now known that the failure of the liver to suppress its glucose production is an additional defect besides peripheral insulin resistance in diet-induced metabolic syndrome and type 2 diabetes (31, 32), and it can occur even before the development of frank peripheral insulin resistance (31). Yet when hepatic glucose uptake is taken into account, it appears that the liver has an even bigger impact on postprandial glucose homeostasis by affecting the systemic supply of exogenous glucose. Many studies have shown that the splanchnic bed takes up ~10% of an orally delivered glucose load during the initial extraction (1, 20, 42, 48, 52). Initial splanchnic glucose uptake has also been indicated to be normal in type 1 diabetes (18), increased in an obese state (36), or decreased in type 2 diabetes (37). Thus, when postprandial glucose metabolism is studied, it will be informative to look at the combined effect of the liver to suppress endogenous glucose production as well as take up newly appearing portal glucose in relation to systemic glucose appearance. Enhanced by radiolabeled glucose tracer methodology, the classical hyperinsulinemic euglycemic clamp is reliable in measuring endogenous (hepatic) glucose production (17). However, hepatic glucose uptake cannot be readily studied in a classical glucose clamp, where exogenous glucose is given peripherally, lacking of a portal signal, and at euglycemia (even with elevated insulin levels) hepatic glucose uptake is not stimulated sufficiently (12, 14, 38, 46). In contrast, under hyperinsulinemia and systemic euglycemia, the PoG-glucose clamp can present the liver with elevated insulin and glucose levels in the portal circulation, as happens postprandially, and then allow the study of the net hepatic effect (glucose production and uptake) on systemic glucose appearance.

We first conducted a series of PoG-glucose clamps in the presence of basal insulinemia and hyperglycemia (systemic and portal), in which hepatic glucose load was increased from 14.8 mg·kg⁻¹·min⁻¹ at basal to 27.8 mg·kg⁻¹·min⁻¹ at steady state. NHGA at basal insulin levels and portal hyperglycemia remained positive but was ~50% of the basal endogenous glucose production during steady state, indicating net glucose production and adding extra glucose by the liver into the systemic circulation. Had portal insulin been maintained at the portal basal level (higher than the present 44.4 pmol/l), the percent reduction in endogenous glucose production induced by portal glucose infusion would have been greater since it is known that portal insulin deficiency has a positive effect on hepatic glucose production (51). In contrast, a lower (~20%) reduction in endogenous glucose production was observed in our previous study when exogenous glucose was given peripherally to maintain similar systemic hyperglycemia of ~150 mg/dl at basal insulinemia (2). Since NHGA in the PoG-glucose clamp responds to changes in both hepatic glucose production and uptake (first-pass), it revealed a larger reduction from basal endogenous glucose production and thus a bigger impact of the liver on systemic glucose appearance.

Another series of PoG-glucose clamps were conducted in the presence of hyperinsulinemia and systemic euglycemia (portal hyperglycemia), in which hepatic glucose load was similarly raised from 15.4 mg·kg⁻¹·min⁻¹ at basal to 28.3 mg·kg⁻¹·min⁻¹ at steady state. With hyperinsulinemia and portal hyperglycemia, NHGA quickly became and stayed negative, indicating a switch of the liver to predominantly take up newly appearing portal glucose, and thus limited the amount of exogenous glucose entering the systemic circulation. At similar hyperinsulinemia of ~250 pmol/l, endogenous glucose production is largely suppressed in our canine model, as shown previously by the classical hyperinsulinemic euglycemic clamp (30, 35). The negative NHGA provides an estimation of the additional hepatic effect on systemic glucose appearance through first-pass hepatic glucose uptake, which accounts for 8% of portal glucose infusion in our study and is in line with initial splanchnic glucose uptake (~10% of an oral glucose load) measured by other methods (1, 20, 42, 48, 52). The hepatic glucose load of 28 mg·kg⁻¹·min⁻¹, brought about by portal glucose infusion at 14 mg·kg⁻¹·min⁻¹ at systemic euglycemia, is relevant to the study of postprandial hepatic glucose metabolism in normal, glucose-impaired, and diabetic conditions, where such a load can easily be reached at moderate hyperglycemia (e.g., 150 mg/dl) with even lower portal glucose appearance (e.g., 3 mg·kg⁻¹·min⁻¹) (13, 27). Briefly, the PoG-glucose clamp achieves hyperinsulinemia and euglycemia in the periphery and calculates peripheral glucose disposal similarly to the classical glucose clamp. The PoG-glucose clamp simulates elevated insulin and glucose levels simultaneously in the portal circulation, as happens postprandially, and calculates the net hepatic effect on systemic glucose appearance. In contrast, the classical glucose clamp, using peripheral glucose delivery, measures the glucose production side of hepatic effect in response to insulin.

It is noteworthy that renal glucose production has been shown to account for 10–25% of endogenous glucose produc-
tion and is subject to insulin suppression similarly to hepatic glucose production (39). Thus, there is renal contribution to NHGA in a PoG-glucose clamp, just as there is a renal component in EGP in a classical hyperinsulinemic euglycemic clamp. A failure of NHGA to switch from positive (net production) to negative (net uptake) in the presence of postprandial hyperinsulinemia and hyperglycemia is quantitatively indicative of hepatic defects. However, a concurrent renal defect can be assumed and a definitive conclusion could be derived by combining net organ balance with tracer methodology (11).

The PoG-glucose clamp provides an additional advantage to the classical glucose clamp in terms of the entry of exogenous glucose through the portal vein. It is known that portal vein is not simply a conduit for the absorbed nutrients but also harbors an intrinsic nutrient- and hormone-sensing mechanism. Anatomically, abundant vagal and spinal afferent fibers have been found in the portal vein and the porta hepatitis area (7). Functionally, it has long been established that postprandial hepatic glucose metabolism is controlled not only by portal insulin and glucose levels but also a negative arterial-portal glucose gradient induced by portal entry of exogenous glucose (12, 46). The significance of portal glucose delivery has clearly been shown by its enhancement of net hepatic glucose uptake at any given insulin or glucose level (43, 44). Recent findings have implicated portal nutrient/hormone sensing in an even broader spectrum of events. For example, a portal glucose sensor has been found to be essential for the sympathetic response to systemic hypoglycemia (21, 22), portal glucose infusion increases peripheral glucose clearance (9), and the hepatoportal area appears to mediate the effects of glucagon-like peptide-1 (GLP-1), a gut incretin hormone, on pancreatic insulin secretion and glucose disposal (24, 45). However, there appears to be a species difference in portal glucose sensing in that portal glucose infusion alone has been shown to lower systemic glucose in mice (9), whereas similar results were brought about by portal coinfusion of glucose and GLP-1 in dogs (24, 27, 28).

Nonetheless, portal glucose delivery seems to be the common factor in these studies, and the PoG-glucose clamp is thus beneficial to the study of postprandial hepatic and peripheral metabolic effects that might be initiated or modified by the putative hepatoportal nutrient/hormone-sensing mechanism (54). In addition, it has been indicated that the critical locus for hypoglycemic detection shifts away from the portal-mesenteric vein to other loci (e.g., the brain) when hypoglycemia develops rapidly (49). Although it is not clear whether the portal sensing of glucose excess vs. deficiency is mediated by the same molecular entity and mechanism, increasing evidence also supports that glucose influx is sensed both peripherally, such as in the hepatoportal region, and centrally in the brain (9, 33, 46).

The PoG-glucose clamp setup can thus be combined with methods to manipulate local glucose levels in the brain to determine the relative significance of portal vein vs. brain glucose sensing in many postprandial metabolic events, such as pancreatic insulin secretion and peripheral and hepatic glucose disposal. Several methods are available to specifically measure splanchnic glucose uptake, a surrogate of hepatic glucose uptake, in response to an oral glucose load, including hepatic vein catheterization (HVC) (15), the oral glucose clamp (36), and the double-tracer approach (16). HVC measures net hepatic glucose balance and derives total splanchnic glucose uptake by assuming endogenous glucose production equal to zero (15). The HVC technique is limited by the uncertainty of the extent of gut absorption and the length of time required for complete absorption. We have shown previously that glucose absorption is the single most important factor that significantly affects oral glucose tolerance test-derived insulin sensitivity values (23). It is also known that a portion of ingested glucose is metabolized by the gut, and it takes more than a few hours for glucose absorption to complete (1, 4, 5). When the HVC technique is combined with portal glucose infusion, the limitations associated with oral glucose absorption can be prevented. However, the complexity of arterial and hepatic venous catheterization and the necessity of measuring hepatic blood flow have prevented the wide use of the HVC technique.

The PoG-glucose clamp introduces a simple modification of the widely used classical glucose clamp method to study net hepatic changes through either glucose production or uptake in response to many established and newly discovered metabolic players, such as free fatty acids, gut hormones, and adipocytokines. It is noteworthy that NHGA measured by the PoG-glucose clamp is the difference between endogenous glucose production and first-pass hepatic uptake and thus the better parameter representing the net hepatic effect on systemic glucose appearance.

The oral glucose clamp first establishes a steady state of glucose turnover at supraphysiological hyperinsulinemia, completely suppresses endogenous glucose production, and then calculates the oral glucose appearance by reducing systemic glucose infusion required to maintain euglycemia (36). Therefore, the oral glucose clamp measures first-pass splanchnic glucose uptake, comparable with NHGA measured by the PoG-glucose clamp when endogenous glucose production is also suppressed. Although limited by the extent and duration of oral glucose absorption, the oral glucose clamp, the HVC technique, and the double-tracer method are alternatives to study the glucose uptake side of hepatic effect in human subjects in whom portal vein catheterization is not available.

The double-tracer method labels the systemic glucose and oral glucose load separately and thus determines oral glucose appearance and hepatic glucose retention (16). As mentioned above, the extent and duration of oral glucose absorption affects the accuracy of the double-tracer method. However, one can potentially utilize the double-glucose-labeling approach in the PoG-glucose clamp setup to obtain endogenous glucose production and first-pass hepatic glucose uptake, respectively. We chose not to incorporate the double-tracer method mainly because hepatic glucose uptake is greatly enhanced with portal glucose delivery, and so is hepatic uptake of glucose tracers (46). Concurrent glycogenesis and glycogenolysis and/or glucose/glucose 6-phosphate cycling may lead to over- or underestimated estimation of tracer-derived measurements (10). As the PoG-glucose clamp delivers cold, unlabeled exogenous glucose via portal vein, the liver retains less of the glucose tracer than it would with double-glucose labeling, and thus tracer-derived measurements are less affected by glucose cycling in the liver.

In conclusion, the PoG-glucose clamp delivers known portal glucose doses and thus accurately and directly measures the full impact of the liver through glucose production and uptake on postprandial systemic supply of exogenous glucose in the
term of net hepatic glucose addition. Meanwhile, whole body glucose disposal can be determined simultaneously as in a classical glucose clamp. Additionally, the PoG-glucose clamp enables the activation of the putative hepatoportal nutrient and hormone-sensing mechanism, extending its use in addressing neurally mediated metabolic effects.

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DISCLOSURES

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