Is There Glucose Production Outside of the Liver and Kidney?

Stephen F. Previs, Daniel Z. Brunengraber, and Henri Brunengraber

Department of Nutrition, Case Western Reserve University, Cleveland, Ohio 44106; email: hxb8@case.edu

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gluconeogenesis, intestine, muscle, mass spectrometry, glucose turnover

Abstract

This review analyzes the evidence presented to support the role of organs other than the liver and kidney to release substantial amounts of glucose into the mammalian blood circulation. The evidence includes (*a*) the identification of gluconeogenic enzyme activities in various organs, especially the small intestine, (*b*) levels of mRNA for the same enzymes, and (*c*) measurements of gluconeogenic flux in the small intestine. The latter would be the definite proof of extrahepatic, extrarenal glucose production. We critically evaluate the radioactive and stable isotopic techniques used to measure intestinal gluconeogenesis. We also simulate the impact of unavoidable measurement errors on apparent rates of intestinal gluconeogenesis. We conclude that there is so far no credible evidence to support the concept that glucose can be produced by the intestine or by muscle.

Contents

INTRODUCTION

There is a general consensus that in the postabsorptive state, the liver is the main source of free glucose generated via glycogenolysis and gluconeogenesis. Although the kidney cortex is clearly gluconeogenic in vitro (52, 68), the contribution of the kidneys to whole-body glucose production has been debated for a number of years. Measuring the production of glucose by the kidney using isotopic techniques has been difficult because of the small differences in glucose concentration and labeling across this organ. These small differences result from the high blood flow passing through the kidneys. However, from a number of studies published in the 1990s, one can conclude that glucose production by the kidneys, expressed in percent of whole-body glucose turnover, ranges from close to zero in the control fed state to up to 25% in extended fasting and in diabetes (9–11, 18, 42–44).

The possibility that organs other than liver and kidney could contribute to whole-body glucose production arose from the identification of some gluconeogenic enzymes in tissues not known to be gluconeogenic. This led to a number of reports, most of which concentrated on the capacity of the small intestine to produce glucose. The possibility that the intestine could contribute to glucose production, even under some conditions, is intriguing because it would affect our long-held view that glucose production is restricted to liver and kidney (66). Also, it has been hypothesized (63) that intestinal gluconeogenesis may be involved in the rapid improvement of type II diabetes in obese humans who underwent bariatric surgery (53, 55). Thus, intestinal gluconeogenesis, if it were unequivocally proven to occur, may be relevant to public health.

Studies on intestinal gluconeogenesis that have been conducted so far fall into three categories, i.e., enzymatic activities, mRNA levels, and flux rates. The interpretations of these studies have been debated without a clear consensus. In 2005, Watford reviewed the field and expressed concerns about the validity of some reports, but left the door open to further evaluation of the concept of intestinal gluconeogenesis (66). Although the identification of activities of gluconeogenic enzymes and the measurement of mRNA levels are suggestive of the existence of gluconeogenesis, the proof of the concept requires the unequivocal measurement of a gluconeogenic flux in the intestine. The present review re-evaluates the evidence presented on the existence of intestinal gluconeogenesis, with an emphasis on the critical evaluation of methodologies used to measure tissue-specific flux with isotopic techniques.

ACTIVITIES OF GLUCONEOGENIC ENZYMES IN TISSUES OTHER THAN LIVER AND KIDNEY

The carbon that fuels most de novo glucose production, or gluconeogenesis, is derived from amino acids. Thus, the production of glucose from amino acids (and from lactate derived from Cori cycling) requires the activity of four enzymes: pyruvate carboxylase, PEP carboxykinase, fructose-1,6-diphosphatase, and glucose-6-phosphatase (**Figure 1**). A small fraction of gluconeogenesis, fueled by carbon derived from glycerol, requires only fructose-1,6-diphosphatase and glucose-6-phosphatase. The

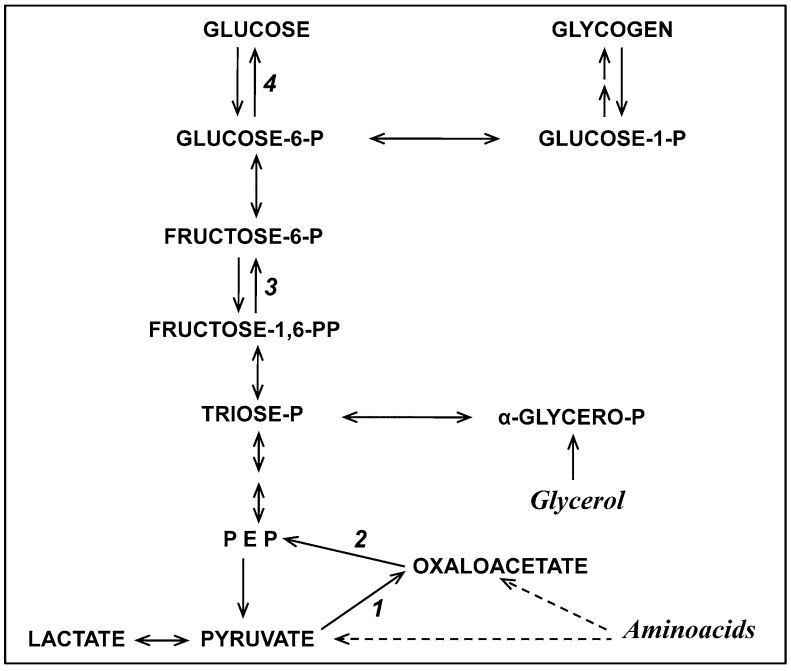

Figure 1

Simplified scheme of gluconeogenesis. The four key enzymes of gluconeogenesis are (*1*) pyruvate carboxylase, (*2*) PEP carboxykinase, (*3*) fructose diphosphatase, and (*4*) glucose-6-phosphatase.

four gluconeogenic enzymes are present in high activity in the liver and the kidney cortex.

Individual gluconeogenic enzymes are present in other organs. For example, PEP carboxykinase, expressed in adipose tissue, is part of the pathway of glyceroneogenesis in this organ (29, 58). In some muscles, fructose-1,6-diphosphatase is a component of a substrate cycle that regulates the rate of glycolysis (12). The four gluconeogenic enzymes (**Figure 1**) are expressed in the small intestine of suckling rats, mice, and rabbits (2, 27, 28, 67, 69), but their activities decrease to very low levels after weaning. However, studies by Mithieux et al. (13, 23, 49, 56, 57) and by others (1, 67) have reported substantial activities of gluconeogenic enzymes in the small intestine of fasted and diabetic rats. Azzout-Marniche et al. (4) have argued that the main glucose-6-phosphatase isoform expressed in the small intestine (G6PC3) is present in many nongluconeogenic organs and is different from the G6PC1 form expressed in gluconeogenic organs. The reports on the presence of gluconeogenic enzymes in the small intestine led to a number of measurements of intestinal gluconeogenic flux (discussed below).

The production of glucose by additional nongluconeogenic organs has been hypothesized by Shieh et al. (60). They reported on a glucose-6-phosphate hydrolase (glucose-6-Pase-β) that can couple with the glucose-6-P transporter to hydrolyze glucose-6-phosphate to glucose. They later showed that astrocytes, which are the main brain cells that accumulate glycogen, express glucose-6-Pase-β and the glucose-6-P transporter (24). These activities can couple to form an active glucose-6-Pase complex. Shieh et al. (60) hypothesized that in hypoglycemia, astrocytes may provide glucose directly to neurons and possibly to plasma. Although this is an attractive hypothesis, they acknowledged that stimulation of glycogenolysis in astrocytes in vitro leads to

the release of lactate, not glucose (17, 54, 70). Also, Shieh et al. (61) hypothesized that muscle glucose-6-phosphatase could catalyze the release of substantial amounts of glucose into the blood.

Eyre et al. (20) reported that in children undergoing cardiopulmonary bypass surgery, the concentration of glucose in the jugular vein transiently increased to levels exceeding arterial concentration by up to 5.3 mM. The authors concluded, "astrocyte glycogen can be exported as glucose, permitting spatial and temporal modulation of glucose delivery to neurons." The glucose concentrations were measured by a standard clinical chemistry technique. The sheer magnitude of the apparent release of glucose by the brain is troubling. It would correspond to the complete degradation of $5.3 \times 0.162\ \text{g} = 0.86\ \text{g}$ of brain glycogen per liter of blood flow through the brain (the molecular weight of glycosyl units in glycogen is 162). In contrast, Wahren et al. (64) reported no release of glucose from the brain of acutely hypoglycemic humans (2.4 mM) but a compensatory increase in glucose extraction by the brain.

MEASUREMENTS OF RATES OF INTESTINAL GLUCONEOGENESIS

In 1978, Windmueller & Spaeth (72) reported data on glucose production by an isolated, vascularly perfused preparation of rat intestine. When [^{14}C]lactate, 3-hydroxy-[^{14}C]butyrate or [^{14}C]glutamine was infused into the arterial blood perfusing a segment of jejunum of an anesthetized rat, a very small amount of [^{14}C]glucose was identified in the venous blood of the jejunal segment. The authors reasoned that (*a*) each of the three tracers would label intermediates of the citric acid cycle, (*b*) reversal of glycolysis would channel label to glucose-6-phosphate, and (*c*) glucose-6-phosphatase would convert glucose-6-phosphate to glucose. This interpretation was in keeping with the report by Anderson (1), who measured substantial activities of PEP carboxykinase, fructose-diphosphatase, and glucose-6-phosphatase in the jejunal mucosa of rats. One concern is that the [^{14}C]tracers used by Windmueller & Spaeth "were checked for radiochemical purity (>95%) by thin layer chromatography." Such a purity criterion leaves room for nonnegligible amounts of radioactive impurities, which could have been carried to the glucose isolated from the venous blood. Also, the amount of radioactivity counted in glucose was a minuscule fraction of the radioactivity infused as [U-^{14}C]glutamine or 3-hydroxy-[^{14}C]butyrate: about 0.07% and 0.2%, respectively (calculated from data presented in tables II and III of Reference 72). Thus, small amounts of radioactive impurities could have ended up in the glucose isolated from the effluent blood. On the other hand, Windmueller & Spaeth were very careful at purifying the labeled glucose by (*a*) chromatography on Dowex-borate, (*b*) oxidation of [^{14}C]glucose to [^{14}C]gluconate by glucose oxidase, and (*c*) chromatography of [^{14}C]gluconate on a Dowex-1-formate column (71). Because the blood perfusing the intestinal segment was not recycled, it appears that a very small amount of labeled glucose was formed in the presence of [^{14}C]lactate, 3-hydroxy-[^{14}C]butyrate or [^{14}C]glutamine.

In 1986, Hahn & Wei-Ning (28) reported data from incubations of fragments of small intestinal mucosa of rats or rabbits in Krebs-Ringer bicarbonate buffer containing 10 mM unlabeled glutamate and 10 mM [^{14}C]lactate. They reported low rates of gluconeogenesis from lactate in the intestinal mucosa of suckling rats and rabbits. The highest rates, measured in intestinal mucosa of 10-day-old rats and rabbits, are equivalent to 0.85 and 3 $\mu\text{mol} \cdot \text{hr}^{-1} \cdot (\text{g wet weight})^{-1}$, respectively. These rates, calculated from the radioactivity incorporated in glucose and the specific activity of lactate, may be underestimated by a factor of about 2 because of the loss of label between lactate and glucose (30, 32). Note that although the fragments of mucosa were incubated with unlabeled glutamate and [^{14}C]lactate, gluconeogenesis from glutamate would generate [^{14}C]glucose because both substrates generate oxaloacetate, an intermediate of gluconeogenesis. Thus, gluconeogenesis

from unlabeled glutamate would incorporate ^{14}C from [^{14}C]oxaloacetate derived from [^{14}C]lactate into glucose. The rates of glucose production would not include any glucose derived from glycogenolysis. However, based on the reported decrease in glycogen content of the mucosa over 2 hr (from 36 to 16.5 mg/100 g wet weight), the maximum rate of glucose production from glycogenolysis (assuming no glycolysis or pentose shunt) would be less than $0.001\ \mu mol \cdot hr^{-1} \cdot (g\ wet\ weight)^{-1}$. The low rates of gluconeogenesis from lactate decreased to almost zero in intestinal mucosa of weaning and adult animals. The data were interpreted as showing that the small intestinal mucosa of suckling rats and rabbits can generate a small amount of glucose, possibly to be used by the muscular cells of the small intestine.

Hahn & Wei-Ning's interpretation of their data is questionable for the following reasons. First, [^{14}C]glucose was identified only as radioactivity that was not retained on Amberlite MB3, a mixed-bed ion exchange resin. The identity and radiochemical purity of [^{14}C]glucose in the resin effluent should have been confirmed by two independent chromatographic systems, such as (*a*) chromatography on a Dowex-1-borate column (40), and (*b*) treatment of the sample with ATP + hexokinase, followed by chromatographic identification of [^{14}C]glucose-6-phosphate on a suitable anion exchange column. Second, glucose was not assayed in the incubation medium by a specific assay (enzymatic or mass spectrometric). Third, the amounts of label from [^{14}C]lactate recovered in glucose and in the glycerol moiety of triglycerides were the same at 1 and 2 hr of incubation. Actually, the authors report that most of the labeled glucose was produced during the first 15 min of incubation. This points to a possible rapid deterioration of the preparation, although the glycogen content of the mucosa decreased by 20% and 50% over the first and second hour of incubation, respectively. Although the report by Hahn and Wei-Ning is often quoted as demonstrating the gluconeogenic capacity of the intestinal mucosa in suckling rats and rabbits (3, 13, 15, 25, 47, 75), we do not view the current evidence as convincing. We hope that this review will stimulate investigators to measure the rates of gluconeogenesis in the intestine of suckling rats, using stable isotopic technology that will allow unequivocal and quantitative assessment of the gluconeogenic capacity of this organ.

Mithieux's group has reported multiple studies in which they measured the production of glucose by the small intestine in anesthetized rats (15, 47, 48, 50, 51) and mice (63). The studies involved (*a*) infusion of [3-^{3}H]glucose in a peripheral vein, (*b*) clamping the circulation of blood in the cecum and colon, (*c*) simultaneous sampling of blood in the carotid artery and the superior mesenteric vein after 90 min of [3-^{3}H]glucose infusion, and (*d*) measuring the concentration and specific activity of glucose in the two plasma samples. The blood flow through the small intestine was measured using radioactive microspheres in other rats, similarly prepared but not infused with [3-^{3}H]glucose (39). [3-^{3}H]Glucose is commonly used to trace glucose metabolism (14, 33) because all its label is released to [^{3}H]water when the hexose skeleton is split to triose phosphates. Thus, a simple way to measure the specific activity of plasma glucose involves (*a*) deproteinization of plasma, (*b*) assay of glucose concentration in the protein-free extract, and (*c*) complete evaporation of the extract to eliminate [^{3}H]water, followed by redissolution in water, and liquid scintillation counting. The uptake and production of glucose by the small intestine was calculated, using standard equations, from the blood flow through the small intestine and from the concentration and specific activity of glucose in the arterial and superior mesenteric vein blood plasma. The authors report that the intestinal glucose release is equivalent to 21%, 16.9%, and 19% of the endogenous glucose production in rats that were 48 hr-fasted, 72 hr-fasted, and streptozotocin-diabetic, respectively (table 1 of Reference 15 and table 1 of Reference 47).

The data from the two studies from Mithieux's group were met with much skepticism in the field. As was pointed out by Watford in a recent review (66), computations

by Mithieux et al. of intestinal glucose release were based on very small differences in glucose-specific activity in mesenteric vein plasma versus arterial plasma. For example, in diabetic rats, the average specific activities of venous versus arterial glucose differed by 1.4%, while the coefficients of variation of the arterial- and venous-specific activities were 21.9% and 11%, respectively [13,646 ± 946 versus 13,464 ± 470 dpm/μmol (SE, n = 10)]. It is not clear how the authors concluded that the two average specific activities were significantly different at the $P < 0.01$ level. Similar questions can be addressed about the data of subsequent studies from Mithieux's group (45, 46, 48, 50, 63).

Martin et al. tested the capacity of the rat small intestine to synthesize glucose from glutamine, the main fuel of intestinal metabolism (73). They incubated segments of small intestine from 72-hr-fasted rats (Wistar and Sprague-Dawley) with L-[3-^{13}C]glutamine, and followed the ^{13}C-labeling of metabolites by NMR. The 72-hr fasting corresponds to the maximal induction of gluconeogenic enzymes in the rat small intestine (47). The viability of the intestinal segments for 30 min was verified by the constancy of ATP concentration, the linearity of [3-^{13}C]glutamine uptake, and the linearity of the accumulation of metabolites (glutamate, alanine, lactate, pyruvate, aspartate). The ^{13}C-labeling of metabolites indicated cycling of the label of [3-^{13}C]glutamine in the citric acid cycle (evidenced by the labeling of multiple carbons of glutamine and glutamate) and its transfer to gluconeogenic intermediates and amino acids (lactate, alanine, ornithine, citrulline, proline, aspartate). The authors report finding a very small amount of glucose in the incubation medium, equivalent to 0.4% of the [3-^{13}C]glutamine uptake, but unlabeled. Because a small amount of unlabeled urea was also found, it is likely that the traces of unlabeled glucose and urea were present in the extracellular fluid of the intestinal segments taken from the rats. Martin et al. (73) concluded that the small intestine of the 72-hr-fasted rat does not synthesize glucose from glutamine, its main substrate. In subsequent studies from the same group, Baverel et al. (5) reported incubations of intestinal cells from adult fed and 48-hr-fasted diabetic ZDF rats with [3-^{13}C]glutamine. The data were similar to those of their previous study on intestinal segments, i.e., labeling of a number of metabolites, but no formation of glucose. In a similar study, Burrin et al. (8) incubated epithelial cells of pig intestine with lactate or glutamine. They could not detect the formation of glucose using a sensitive enzymatic assay.

Burrin et al. (8) also infused [$^{13}C_6$]glucose to 28-day-old piglets fasted for 36 hr. After 4 hr, they measured the concentration and molar percent enrichment (MPE) of glucose in the carotid artery and portal vein. Although the uptake of glucose by the gastrointestinal tract was significantly different from zero, the mean difference in MPE of glucose (measured by gas chromatography–mass spectrometry) across the gut was not significantly different from zero. They concluded that there is "no significant gluconeogenesis by the gastrointestinal tissues in the fasted piglet."

Brunengraber et al. (7) investigated intestinal gluconeogenesis using animals administered [6,6-$^{2}H_2$]glucose. The concentration and isotopic enrichment of glucose were assayed by gas chromatography–mass spectrometry. Spiking the sample with [$^{13}C_6$]glucose internal standard allows measuring both the ^{2}H-enrichment and the concentration of glucose in the same chromatographic peak. The use of a long capillary gas chromatography column, a proper temperature gradient, and selected ion monitoring under chemical ionization insures isotopic and chemical purity of the glucose derivative. The absence of interference from coeluting unknown matrix compounds was assessed by verifying that the natural mass isotopomer distribution of glucose in the baseline sample was identical to the theoretical distribution. The latter was calculated from the atomic composition of the glucose derivative (21).

Anesthetized, 48-hr-fasted rats fitted with carotid, jugular, and portal vein catheters were infused [6,6-$^{2}H_2$]glucose until the arterial enrichment of glucose reached steady state. Then, blood was slowly withdrawn from

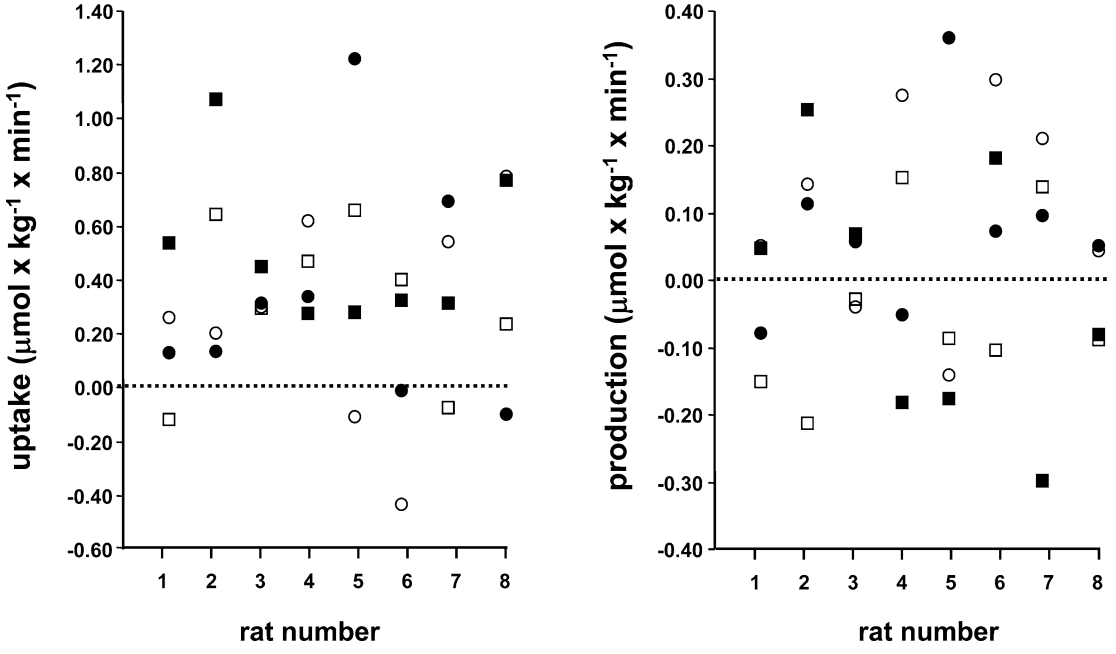

Figure 2

Uptake and production of glucose by the gastrointestinal tract of anesthetized, 48-hr-fasted rats infused with [6,6-$^2H_2$]glucose. Arterial and portal venous blood was sampled simultaneously at 90 min (*solid circles*), 100 min (*solid squares*), 110 min (*open circles*), and 120 min (*open squares*).

the carotid and portal catheters at four time points. Glucose concentration and enrichment were assayed by the technique outlined above with quadruplicate injections into the gas chromatograph-mass spectrometer. Using the equations of Croset et al. (15) adapted to stable isotope technology, Brunengraber et al. (7) calculated rates of uptake and production of glucose by the gastrointestinal tract of 0.356 ± 0.14 and 0.028 ± 0.046 $\mu mol \cdot min^{-1} \cdot kg^{-1}$, respectively (mean ± SD, n = 8 rats). **Figure 2** shows, for each of the eight rats, the calculated rates of glucose uptake and production at four time points. The value for each symbol point represents the mean of four injections of the glucose derivative into the gas chromatography–mass spectrometer. The rates of glucose production amount to only 0.3% of the average whole-body glucose turnover rate, i.e., 52 ± 8.0 $\mu mol \cdot min^{-1} \cdot kg^{-1}$ (mean ± SD, n = 8), which is much less than the value of 21% reported by Croset et al. (15) for 48-hr-fasted rats. Note that the rates of intestinal glucose production shown in **Figure 2** are not significantly different from zero because 14 out of the 32 rates are negative. In contrast, intestinal glucose uptake shows that only 6 out of 32 rates are negative.

Brunengraber et al. (7) also investigated glucose production by the gut, kidney, and leg of acutely hepatectomized dogs and of sham-operated dogs. They reasoned that allowing the deep hypoglycemia that sets in after hepatectomy would maximize the possibility of detecting glucose production by the gut and/or by muscle. Anesthetized dogs were fitted with carotid, jugular, portal vein, renal vein, and femoral vein catheters. They injected a small bolus of [6,6-$^2H_2$]glucose just after completing the hepatectomy (16) and at the end of the sham surgery in control dogs. At various times, blood was slowly sampled through the carotid, portal vein, renal vein, and femoral vein catheters. In hepatectomized dogs, glucose concentration

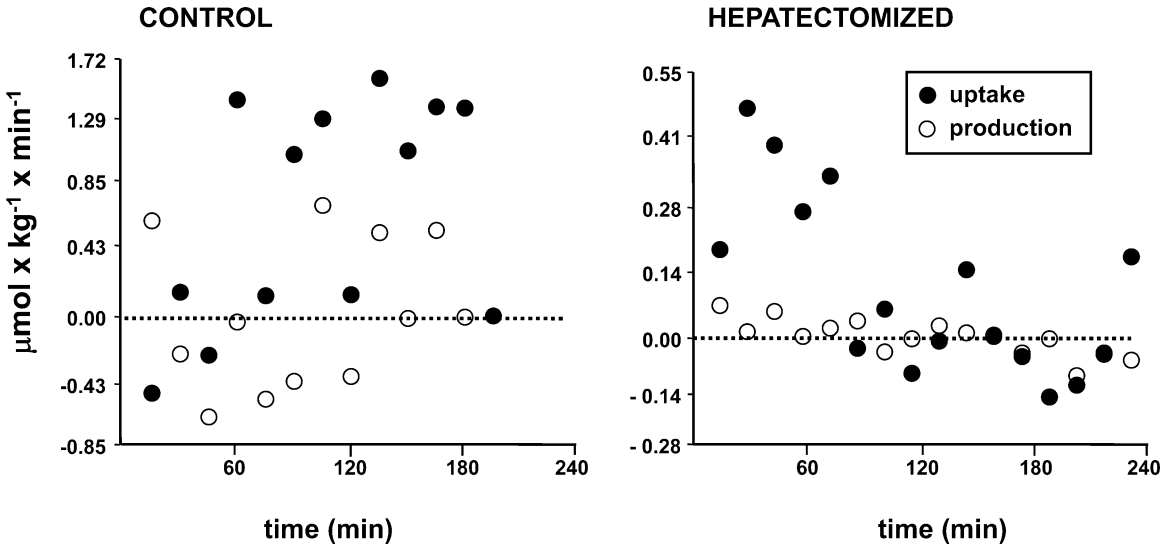

Figure 3

Time profile of the uptake (*solid circles*) and production (*open circles*) of glucose by the gastrointestinal tract of one hepatectomized dog and one sham-operated control dog (both 36-hr-fasted). Zero time corresponds to the completion of hepatectomy and to the bolus injection of [6,6-$^2H_2$]glucose in a peripheral vein. During the course of the experiments, arterial glucose concentration remained at about 5 mM in the control dog and decreased from 5 to 1 mM in the hepatectomized dog.

decreased from 5.0 to 1.0 mM over 240 min, but remained stable at about 5 mM in sham-operated dogs. **Figure 3** shows, for one control and one hepatectomized dog, the time course of glucose uptake and production. In both dogs, the rates of glucose production by the gut show similar numbers of positive and negative values and are thus not significantly different from zero. The average rates of glucose production for the three control and six hepatectomized dogs were 0.000 ± 0.006 and 0.007 ± 0.068 (SD) $\mu mol \cdot min^{-1} \cdot kg^{-1}$, respectively. Thus, even in hepatectomized dogs, where the deep hypoglycemia should have stimulated any gluconeogenic capacity of the intestine, there was no evidence of intestinal gluconeogenesis. Also, no glucose production was detected across the leg of hepatectomized dogs. This argues against the hypothesis that free glucose could be released from muscle glycogen via glucose-6-phosphate hydrolysis (61) or via glycogen debranching enzyme. Finally, glucose production by the kidneys accounted for the remaining glucose production after hepatectomy, i.e., about one-half of glucose production in the sham-operated dogs. However, the stimulation of renal gluconeogenesis by posthepatectomy hypoglycemia did not prevent the decrease of blood glucose concentration to 1 mM. This confirms the central role of the liver in the maintenance of blood glucose concentration.

SIMULATION OF THE IMPACT OF MEASUREMENT ERRORS ON RATES OF GLUCOSE PRODUCTION

The purpose of the following discussion is to illustrate the influence of unavoidable random errors, and possibly systematic errors, on the calculation of the uptake and production of a substrate by an organ in vivo using arteriovenous (A-V) measurements. To determine the rates of glucose uptake and production by an organ, such as the intestine, one needs to measure five parameters: arterial glucose concentration (C_{art}), portal vein glucose concentration (C_{portal}), labeling of arterial glucose as specific activity or molar percent enrichment (SA_{art} or

MPE_{art}), labeling of effluent glucose (SA_{portal} or MPE_{portal}), and blood flow. In cases when there is high flow of blood through an organ (e.g., the kidneys and gastrointestinal tract), differences in glucose concentrations and labeling are very small and difficult to measure with great precision. Relative errors in measurements of glucose concentrations and stable isotopic labeling by gas chromatography–mass spectrometry are of the order of 1%–2%. Relative errors in measurements of specific activity are greater (2%–4%) because of independent measurements of concentration and radioactivity. Also, one can question the measurement of the specific activity of [3-^{3}H]glucose in plasma, without glucose purification, by simply drying the protein-free extract, dissolving the residue, and counting the radioactivity. This technique is adequate for measuring glucose turnover because an error of a few percent in the specific activity of arterial glucose would not affect much the calculated glucose turnover. However, this technique is not adequate when small differences in glucose-specific activity must be measured across an organ, such as the small intestine (15). There, the radioactive glucose should be purified on resins to avoid interferences of compounds present in the matrix on the counting process.

The conversion of counts per minute (cpm) to disintegrations per minute (dpm) is subject to artifacts such as uneven quenching of the scintillation, instrument drift, variability of automatic quench corrections, and chemiluminescence (59, 62, 74). At present, very few investigators use internal standardization that involves (*a*) multiple counting of samples until stabilization of the cpm, (*b*) careful pipetting of an internal standard of [^{14}C]- or [^{3}H]toluene into the scintillation vial, (*c*) multiple recounting of the samples, (*d*) computing the counting efficiency of each sample, and (*e*) using the counting efficiency to convert cpm to dpm (19, 22, 26). Thus, the range of errors of measurements is similar to or greater than the A-V differences in glucose concentration and labeling. If the errors are random, they can cancel or amplify each other. This results in a scattering of calculated values around an average. In some cases, random errors are accompanied by systematic errors resulting from over- or underestimation of dpm because of chemiluminescence or quenching, and/or an error in the concentration of a stock concentration standard. Random errors result in the scattering of computed data, whereas systematic errors move the range of computed data above or below the actual range. Note that although the high blood flow through the kidney or intestine is responsible for the small differences in glucose concentration and labeling across the organ, it does not result in major computation errors unless the flow varies erratically.

In **Table 1**, we simulated the effect of random errors on the computation of glucose uptake and production by the intestine of an adult dog using tracer [6,6-$^2H_2$]glucose. The computations were conducted using the following standard equations, where C is the glucose concentration, IE is the isotopic enrichment of glucose expressed as mol fraction, subscripts a and v refer to arterial and venous blood, respectively, and FE is the fractional extraction of glucose across the organ.

$$FE = [(C_a \times IE_a) - (C_v \times IE_v)]/(C_a \times IE_a)] \quad 1.$$

$$\text{Glucose uptake} = (FE \times C_a)(\text{Blood flow})/(\text{Body wt}) \quad 2.$$

$$\text{Glucose production} = [(C_v \times (1 - IE_v)) - (C_a \times (1 - IE_a)) \times (1 - FE)](\text{Blood flow})/(\text{Body wt}) \quad 3.$$

The simulation was conducted under the following theoretical conditions: (*a*) the glucose concentration decreases by 2% across the intestine (from 1.0 to 0.98 mg/ml), (*b*) the glucose isotopic enrichment does not decrease across the intestine (remains at 0.028), (*c*) the intestinal blood flow is 20 $ml \cdot kg \cdot^{-1} min^{-1}$. These conditions correspond to a glucose uptake of 0.400 $mg \cdot min^{-1} \cdot kg^{-1}$ and to a zero glucose production by the gut. We simulated the effect of random errors that can equally affect each of the four parameters, i.e., the two concentrations and the two isotopic labelings. To simulate

Table 1 Simulation of the effect of random errors on measurements of glucose concentration and isotopic enrichment on computed rate of glucose uptake and production by an organ in vivo. The errors in the four parameters—arterial glucose concentration (Ca), arterial glucose isotopic enrichment (IEa), venous glucose concentration (Cv), and venous glucose isotopic enrichment (IEv)—are +2% (+), −2% (−), or 0% (0). The first combination of errors (0000) corresponds to no error on any parameter. Computations are described in the text

Distributions of four errors				mg • kg^{-1} • min^{-1}		Distributions of four errors				mg • kg^{-1} • min^{-1}		Distributions of four errors				mg • kg^{-1} • min^{-1}	
Ca	IEa	Cv	IEv	Uptake	Production	Ca	IEa	Cv	IEv	Uptake	Production	Ca	IEa	Cv	IEv	Uptake	Production
0	0	0	0	0.400	0.000	+	0	+	+	0.008	−0.400	−	−	+	+	−1.208	−0.816
−	0	0	0	0.000	0.000	0	+	+	+	0.008	0.000	−	0	0	+	−0.392	−0.392
+	0	0	0	0.800	0.000	+	+	+	−	1.192	0.784	−	0	+	0	−0.392	0.000
0	−	0	0	0.000	−0.400	+	+	−	+	1.192	0.000	−	0	−	+	0.008	−0.384
0	+	0	0	0.784	0.384	+	−	+	+	−0.408	−0.816	−	0	+	−	0.008	0.400
0	0	−	0	0.792	0.000	−	+	+	+	−0.392	0.000	−	−	+	0	−0.800	−0.408
0	0	+	0	0.008	0.000	+	+	0	0	1.184	0.384	−	−	0	+	−0.800	−0.800
0	0	0	−	0.792	0.392	+	0	+	0	0.408	0.000	+	0	−	−	1.576	0.384
0	0	0	+	0.008	−0.392	0	+	+	0	0.400	0.392	0	+	−	−	1.545	0.753
−	−	−	−	0.392	0.000	0	0	+	+	−0.392	−0.400	+	−	−	0	0.800	−0.392
+	+	+	+	0.408	0.000	+	0	0	+	0.408	−0.392	+	−	0	−	0.800	0.000
−	−	−	0	0.000	−0.392	0	+	0	+	0.400	0.000	−	+	−	0	0.769	0.377
−	−	0	−	0.000	0.000	0	0	+	−	0.408	0.400	−	+	0	−	0.769	0.769
−	0	−	−	0.776	0.384	0	0	−	+	0.408	−0.384	0	−	−	+	0.008	−0.784
0	−	−	−	0.792	0.000	+	−	0	0	0.400	−0.400	0	−	+	−	0.008	0.000
−	−	−	+	−0.392	−0.784	−	+	0	0	0.384	0.384	+	+	0	−	1.569	0.769
−	−	+	−	−0.392	0.000	+	0	0	−	1.192	0.392	+	+	−	0	1.569	0.377
−	+	−	−	1.145	0.753	+	0	−	0	1.192	0.000	0	−	+	+	−0.808	−0.816
+	−	−	−	1.192	0.000	0	+	−	0	1.169	0.377	−	0	+	+	−0.792	−0.400
−	−	0	0	−0.400	−0.400	0	+	0	−	1.169	0.769	0	+	+	−	0.792	0.784
−	0	−	0	0.392	0.000	0	−	+	0	−0.400	−0.408	0	+	−	+	0.792	0.000
0	−	−	0	0.400	−0.392	0	−	0	+	−0.400	−0.800	+	0	+	−	0.808	0.400
0	0	−	−	1.176	0.384	−	+	−	+	0.392	0.000	+	0	−	+	0.808	−0.384
−	0	0	−	0.392	0.392	−	+	+	−	0.392	0.784	−	+	+	0	0.000	0.392
0	−	0	−	0.400	0.000	+	−	+	−	0.408	0.000	−	+	0	+	0.000	0.000
+	+	+	0	0.800	0.392	+	−	−	+	0.408	−0.784	+	−	0	+	0.000	−0.800
+	+	0	+	0.800	0.000	+	+	−	−	1.945	0.753	+	−	+	0	0.000	−0.408

the randomness of errors, we considered three possible relative errors: positive (+2%), nil (0%), or negative (−2%) for each of the four parameters. This yields $3^4 - 1 = 80$ combinations of errors for each level of error. The values of the four parameters corresponding to each of the 80 combinations were introduced into the equations that yield rates of glucose uptake and production. For a zero relative error on each parameter (listed as 0 0 0 0 at the top of the first column of **Table 1**), the rates of uptake and production of glucose are 0.40 and 0.00 $mg \cdot kg^{-1} \cdot min^{-1}$, respectively. The 80 combinations of three types of error in four parameters result in 80 values of the rates of glucose uptake and 80 values of the rate of glucose production. For truly random 2% errors in all four parameters, the rates of glucose uptake and production are 0.395 ± 0.648, and 0.005 ± 0.456 $\mu mol \cdot min^{-1} \cdot kg^{-1}$, respectively (SD, n = 80). Depending on whether the errors in the four parameters balance or amplify each other, one computes positive, nil, or negative rates of glucose uptake and production.

Figure 4 shows mirror histograms of the distribution of the 80 values of glucose production (top histogram) and glucose uptake (bottom histogram) shown in **Table 1**. The horizontal scale is the deviation from the correct rates of glucose uptake and production. The vertical scale is the number of combinations corresponding to each level of deviation from the correct rates. Out of the 80 combinations of errors in the four parameters, 27 combinations (one third) yield the correct rate of glucose production (at zero deviation from the expected rate). These correspond to conditions where the errors in the enrichment of glucose were the same in the arterial and venous blood (both positive, both nil, or both negative). All other combinations of errors yield incorrect rates of glucose production. The bottom histogram shows that most combinations of errors result in rates of glucose uptake that deviate substantially from the correct value. This occurs even when the difference between C_{art} and C_{ven} is correct because of equal relative errors in the two parameters. This is because the labeling of arterial and venous glucose is part of the calculations of the fractional uptake of glucose. The scattering of the 80 values of each metabolic rate increases with the relative error on the parameters (not shown), but is close to symmetrical because of the randomness of the errors on each of the four parameters. Also, similar simulations (not shown) reveal that the impact of a given percent error in the measurement of glucose concentrations on rates of glucose uptake or production is much greater when the glucose concentration is high (as in diabetic rats) versus normal (as in control rats).

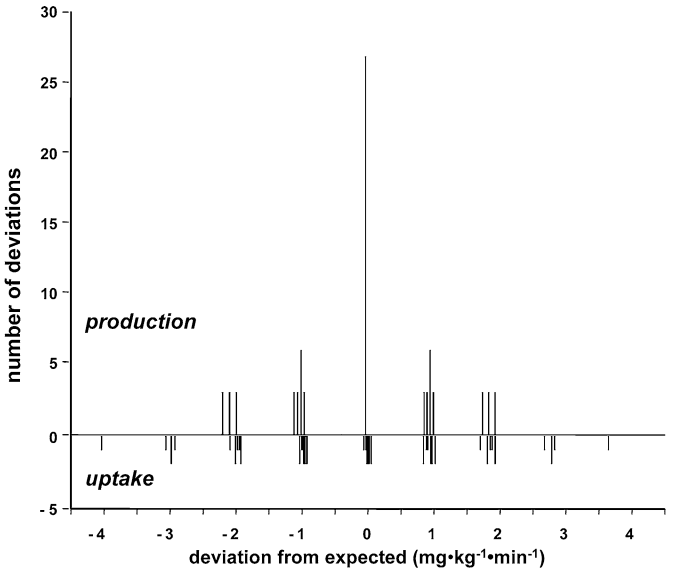

Figure 4

Mirror histograms of the distributions of errors on glucose uptake and production as a result of random 2% errors on the four measured parameters. The data, taken from **Table 1**, are expressed as deviations from the theoretical, zero-error values (*X* axis). The unit of the *Y* axis is the number of simulated rates corresponding to each level of deviation. The total number of combinations of errors is 80 for glucose production and 80 for glucose uptake.

Although the scatter of the simulated data can be very large, the mean of each group of 80 simulated values is close to the simulated error-free value. However, in a given laboratory, it is unlikely that all of the 80 error combinations occur randomly. It is more likely that a small number of error combinations prevails, resulting in systematic overestimation, underestimation, or nearly correct estimation of glucose uptake and production.

In reporting a study on glucose production by the human kidney, Ekberg et al. (18) computed negative as well as positive rates of glucose production in some series of experiments (see figure 1 in Reference 18). They commented on the natural temptation to replace negative rates of glucose production by zero rates. Such censoring of data should never be done because it biases the computations into "demonstrating" a substantial rate of glucose production. As Ekberg et al. (18) emphasized, "Mistakenly setting negative values to zero or repeating the assays giving negative values until zero or positive values are obtained has two consequences: actual glucose production will be overestimated and the variation in the measurements (e.g., expressed as SE) will be erroneously decreased. The larger the experimental error in the measurements, the more such treatment of data will overestimate production by the kidney and its statistical significance."

CONCLUSION

To summarize this discussion, when the arterial and venous concentrations and labeling of glucose differ by only a few percent, computed rates of glucose production and uptake should be viewed critically. One should be wary of using such data to formulate a new biological concept, especially when data from investigations on other models do not support such a concept. As recently pointed out by Troy et al. (63), "One must be conscious that, even when differences in glucose specific activity indicate glucose release, the calculated glucose fluxes are, at best, rough estimates and not accurate values." A major revision of our view of glucose homeostasis should not be based on rough estimates. We therefore conclude that, at the present time, there is no credible evidence that the small intestine is a source of glucose.

DISCLOSURE STATEMENT

In this review, the authors discuss some of their own publications (7, 31). They have made every effort to avoid any biases that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

This review is dedicated to the memory of Dr. Bernard R. Landau, who emphasized throughout his long career the need to critically evaluate data from biological investigations using radioactive or stable isotopes (6, 18, 34–38, 65). The work from the authors' laboratory was supported by Roadmap grant 3R33DK070291 from the National Institutes of Health.

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