Assessment of Postabsorptive Renal Glucose Metabolism in Humans With Multiple Glucose Tracers

Niels Moller, Robert A. Rizza, G. Charles Ford, and K. Sreekumaran Nair

The contribution of the kidneys to postabsorptive endogenous glucose production is a matter of controversy. To assess whether this could relate to the use of various isotopic methods with different analytical performance capabilities, we measured glucose kinetics in 12 healthy subjects. Blood samples were taken from the femoral artery and the renal vein after 4 h of \([\text{6,6-}^2\text{H}_2]\)glucose infusion (for gas chromatography [GC]/mass spectrometry [MS] analysis), and renal plasma flow was determined with paraaminohippurate. In addition, six subjects received uniformly labeled \([\text{13C}]\)glucose (for GC/combustion/isotope ratio MS [IRMS]) and \([3-3\text{H}]\)glucose (for counting of radioactive disintegrations). Arterial glucose concentrations (means ± SD) were 4.2 ± 0.1 mmol/l, and endogenous glucose production rates using \([2\text{H}_2]\)glucose were 2.2 ± 0.1 mg · kg⁻¹ · min⁻¹ or 818 ± 50 μmol/min. Dilution of \([2\text{H}_2]\)glucose across the kidney was 0.79 ± 1.32%, and renal glucose production (RGP) rates were 27 ± 72 μmol/min. In the six subjects receiving additional tracers, dilutions across the kidney were 2.83 ± 0.72 and 0.54 ± 1.20 (for \([\text{U-13C}]\)glucose and \([3-3\text{H}]\)glucose, respectively, the dilution with \([\text{U-13C}]\) being higher than that with \([\text{2H}_2]\) \((P = 0.007)\). Corresponding RGP values were 144 ± 39 and 43 ± 76 μmol/min for \([\text{U-13C}]\) and \([3-3\text{H}]\), respectively. In conclusion, we found that the highly sensitive \([\text{U-13C}]\) GC/Combustion/IRMS technique showed consistent dilution of label across the kidney, whereas the less sensitive techniques gave some negative values and smaller RGP rates. Thus, depending on which technique is being used, a fivefold difference in calculated RGP values may be encountered. The methodological variability of our data suggests that extrapolation from regional renal measurements to the whole-body level should be perfumed with caution. Diabetes 50:747–751, 2001

Interests in the potential role of the kidney in the regulation of glucose metabolism in humans has been revived within the past decade (1). The capacity of the renal cortex to produce glucose de novo was first recognized in 1937 (2). Later studies failed to show any significant net renal glucose output in the postabsorptive state in humans (3–6), although it was observed that with prolonged fasting the kidneys contribute ≥50% of endogenous glucose production (3). However, the perception of the kidney as playing a minor role in overall glucose homeostasis postabsorptively was recently challenged by a study using isotope dilution and renal vein catheterization concomitantly; the results showed that in postabsorptive dogs, renal glucose production (RGP) and renal glucose uptake each account for 30% of total whole-body glucose turnover in the presence of net balances close to zero (7). Subsequent human studies from two groups using similar techniques showed that the kidney may be responsible for 15–30% of endogenous glucose production in the basal postabsorptive state, and they also demonstrated that RGP increased in response to hypoglycemia and epinephrine (8–14). However, these results were not confirmed by Ekberg et al. (15), who reported that there was no significant renal dilution of labeled glucose and that postabsorptive RGP at most would be ~5%. Although currently unclear, some of the controversy could relate to different methodological approaches.

When using the combined glucose label–dilution and arteriovenous balance technique across the kidney, calculations of glucose exchange depend on small across-organ differences of both labeled and total glucose concentrations. These difficulties are further complicated by the high renal blood flow, which will magnify any imprecision in the determination of glucose concentrations and dilution. The gas chromatography (GC)-to-combustion-to-isotope ratio mass spectrometry (IRMS) technique is very precise, because it exploits the high sensitivity of the isotope ratio mass spectrometer (16). To our knowledge, this powerful technique has not been used previously to describe regional renal glucose kinetics.

This study was designed in an attempt to resolve the discrepancies pertaining to assessment of the role of the kidney in glucose homeostasis. We proposed to assess whether the GC/IRMS technique, because of its higher sensitivity, offers advantages compared with more conventional dilution methods. We studied 12 healthy subjects receiving \([\text{6,6-}^2\text{H}_2]\)glucose (GC/mass spectrometry [MS] analysis), \([3-3\text{H}]\)glucose (for counting of radioactive disintetusions of glucose exchange depend on small across-organ differences of both labeled and total glucose concentrations. These difficulties are further complicated by the high renal blood flow, which will magnify any imprecision in the determination of glucose concentrations and dilution. The gas chromatography (GC)-to-combustion-to-isotope ratio mass spectrometry (IRMS) technique is very precise, because it exploits the high sensitivity of the isotope ratio mass spectrometer (16). To our knowledge, this powerful technique has not been used previously to describe regional renal glucose kinetics.

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Table 1
Arterial blood glucose concentrations, whole-body glucose rate of appearance by [3-3H]glucose, and circulating hormones in 12 subjects undergoing renal vein catheterization

<table>
<thead>
<tr>
<th>Glucose (mmol/l)</th>
<th>Rg (µmol/min)</th>
<th>Glucagon (pg/ml)</th>
<th>Growth hormone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rg</td>
<td>Insulin (µmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.2 ± 0.1</td>
<td>2.2 ± 0.1</td>
<td>33.1 ± 4.8</td>
<td>63.2 ± 3.1</td>
</tr>
</tbody>
</table>

Data are means ± SE.

Glucone production by kidney

Plasma enrichment of [U-13C]glucose was determined by IRMS using a Finnigan MAT Delta+ system (Bremen, Germany) fitted with an on-line GC/combustion inlet. The proteins from 40 µl plasma were precipitated with 1 ml of ice-cold ethanol, and the supernatant, after centrifugation, was evaporated to dryness using a centrifugal evaporator (Savant, Farmingdale, NY). The methyl boronate derivative of glucose was prepared (21) and separated using a 30 mm × 0.25 mm id × 0.25 µm film fused-silica capillary column (DB-1701; J&W, Folsom, CA). The coefficient of variation of this analysis was 1% (i.e., 0.02 ± 0.0002 atom percent excess).

The specific activity of [3-3H]glucose was determined as described previously (22). The coefficient of variation of this measurement was 2 ± 0.24%

Calculations. Plasma flows (PFs) from the kidney were calculated as described previously (5) and converted to blood flows (BFs) using the following equation: BF = PF/(1-hematocrit). Measured hematocrit values were 40 ± 1%

Isotopic plateau was observed from 180 to 240 min. This was assessed based on observation that when isotopic enrichment values or specific activities of the glucose tracers used in different sites were plotted against time, the ensuing slopes were not different from zero. The mean values of five measurements for any isotope at each plateau were used for all calculations of glucose kinetics.

Whole-body rate of appearance (Rg) for glucose was calculated by dividing the rate of infusion of labeled glucose by enrichment or specific activity.

Net renal glucose exchange was calculated as the product of arteriovenous plasma glucose concentration differences and renal blood flow.

Regional renal production of glucose was calculated in all circumstances using the following equation (15,21):

Rg(renal) = BF × [glucose] arter. × (Eart/Even - 1)

in which Rg(renal) is Rg, BF is total renal blood flow, [glucose] arter. is the arterial glucose blood concentration, and Eart and Eeven represent enrichment (or specific activity) in venous or arterial blood. As pointed out by Eldberg et al. (15), the low fractional extractions (<2%) of glucose across the renal bed means that their contributions to the calculation become negligible. For that reason, the parameter was omitted. Negative values for regional glucose production were included in the mean calculations.

Regional renal glucose uptake was determined as the sum of RGP and net renal glucose exchange.

Statistical analysis. The mean of all five measurements during the 4h infusion was used for calculations. All values given are means ± SE. Differences among tracers were assessed by Student’s t test.

Results
Parameters of whole-body glucose metabolism and circulating hormones are given in Table 1. Circulating arterial blood glucose concentrations were 4.2 ± 0.1 mmol/l, and the total glucose Rg determined with [3H]glucose was 2.2 ± 0.1 mg·kg⁻¹·min⁻¹ (or 818 ± 50 µmol/min) in the 12 subjects (Table 1).

Table 2 shows isotopic enrichment and specific activity obtained with the three tracers in the femoral artery and in the renal vein. In all cases, a small overall dilution of tracer was recorded across the renal bed, ranging from 0.54% with [3H]glucose to 2.83% with [U-13C]glucose. The percent dilution was higher with [U-13C]glucose compared with [3H]glucose (F = 0.007), but not different between [U-13C]glucose and [3H]glucose (P = 0.22). As shown in Fig. 1, individual values of dilution for each subject were quite scattered, and a considerable number of subjects
exhibited negative values. However, for [U-13C]glucose (followed by GC/IRMS), only positive values were recorded for all six subjects.

Calculated values for renal glucose dynamics are given in Table 3. Arterial and venous glucose concentrations were very similar; some were positive, whereas others were negative, as shown in Fig. 2. This gave rise to a small average net glucose release in the [6,6-2H2]glucose, [3-3H]glucose, and [U-13C]glucose experiments. RGP rates were 27 ± 72, 144 ± 39, and 43 ± 76 μmol/min (for [6H2], [U-13C], and [3-3H], respectively). Only utilization of [U-13C] gave consistently positive values for RGP. Calculated values for the renal contribution to the entire endogenous glucose production varied from 4.9 ± 9.0% ([6,6-2H2]) and 4.3 ± 8.3% ([3-3H]) to 18.3 ± 4.3% [U-13C].

**DISCUSSION**

This study was undertaken to assess whether methodological differences among the use of various glucose tracers may be 15–20% lower than calculated. For this reason, previous studies examining RGP in the basal state in humans have reported values that range from ~0% (15) to ~30% (8) of endogenous glucose production. A summary of reported parameters of renal glucose metabolism in humans is given in Table 4. It may be noted that both renal net exchange of glucose (ranging from 0 to 70 μmol/min) and tracer dilution across the renal bed (ranging from 0 to 4.2%) vary considerably. Our findings of a renal net release of glucose between 60 and 90 mmol/min and isotope dilutions ranging from 0.5 to 2.8% are thus comparable to those of the literature (24,25). Conceivably, the excessive variability of results relates to the fact that measured arteriovenous differences are very close to the detection limits of the assays used. The resulting imprecision is amplified manifold by the large renal blood flow of >1 l/min.

In the studies above, extrapolation from data on renal blood flow, arterial and venous glucose concentrations, and dilution of labeled glucose molecules across the renal bed to whole-body glucose kinetics rests on a number of assumptions. Total renal blood flow is generally used to convert plasma measurements in one kidney to an estimate of the entire renal contribution. This assumes that the two kidneys are metabolically identical and that conversion from plasma determinations to whole blood by using hematocrit values is appropriate. However, the use of whole-blood flow rates may overestimate actual glucose kinetic events to the extent that blood is not composed of water alone, but also inert dry matter (e.g., plasma membranes, hemoglobin, and plasma proteins). The concentration of water is ~93% in plasma and ~70% in blood cells (26,27), meaning that across-organ flux rates in free water alone, but also inert dry matter may lead to an overestimation of regional glucose production. Although the role of red cell glycolysis during passage through the renal bed remains to be defined, equilibration of glucose across red cell membranes occurs rapidly, and it seems

**FIG. 1.** Individual values (percent-wise) of dilution of isotopic enrichment across kidney of [6H2], [U-13C]glucose and [3-3H]glucose as well as the specific activity of [3-3H]glucose, demonstrating that there was a dilution of label across kidney in all subjects when [U-13C]glucose was used as a tracer.

**FIG. 2.** Individual values of arteriovenous differences in glucose (mmol/l) concentrations across kidney, showing that the differences are small and vary from subject to subject.
fair to assume that intracellular water concentrations of labeled and unlabeled glucose are identical to plasma concentrations (28). Alternatively, if it is assumed that no equilibration occurs between red cells and plasma, the calculated values of RGP will be increased by 30%. Finally, it is possible that hemoconcentration due to urine and lymph production may elevate effluent renal vein glucose concentrations, thus spuriously increasing calculated rates for net RGP. Most of these potential limitations will tend to induce an overestimate of the renal contribution to whole-body glucose production.

On the other hand, underestimation of renal glucose output may occur to the extent that there is recycling of labels and that contamination of renal venous blood with caval or gonadal blood occurs. Recycling of deuterium and tritium from labeled water may take place, and synthesis of glucose from \(^{13}\)C-labeled 3-carbon precursors (lactate, pyruvate, alanine, and glycerol) may also contribute. Any possible role of these processes remains uncertain. Recycling of labeled glycogen is probably negligible, because normal human kidney does not contain appreciable amounts of glycogen (29). In this study, renal vein concentrations of PAH ranged from 7 to 16% of the arterial concentrations, virtually excluding the possibility that any significant contamination from caval or gonadal blood occurred.

The above considerations, however, do not explain the variability of results in the literature concerning the contribution of kidney to endogenous glucose production. As pointed out by Ekberg et al. (15), the existing discrepancies could relate to omission or reanalysis of negative values for renal dilution of tracers in some cases. When using the GC/combustion/IRMS technique with high accuracy and precision for the analysis of \([^{13}C]glucose\) kinetics across the kidney, we found clear evidence for a small but consistent dilution of labeled glucose. These results could not be reproduced with less sensitive tracer techniques, probably due to lack of analytical precision to some extent. It is intriguing that calculated mean values for RGP were higher with \([^{13}C]glucose\) than with \([^{2}H_{2}]\)glucose and \([^{3}H]glucose\). One would expect intrarenal dilution of deuterium and tritium to be increased due to potential loss of label in glycolysis. Another potential reason for the variable results in the existing literature is that the degree of stress to which the participants had been exposed may have varied. It has been shown that RGP is substantially increased by epinephrine (8).

In conclusion, the data show that tracer dilution in the kidney may vary from 0.5 to 2.8%, depending on the method used, and that the use of the highly sensitive GC/combustion/IRMS technique gives consistently positive results for RGP. This implies that analysis of renal glucose metabolism is very susceptible to methodological noise and that renal contribution to endogenous glucose production is between 4 and 18% in postabsorptive humans. In view of the small arteriovenous differences in isotopic enrichment (or specific activity), future studies should consider larger numbers of subjects to minimize the effect of analytical errors.

ACKNOWLEDGMENTS

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TABLE 4
Reported values for postabsorptive renal glucose metabolism in humans

<table>
<thead>
<tr>
<th>Reference</th>
<th>n</th>
<th>Tracer used</th>
<th>Net renal glucose exchange (μmol/min)</th>
<th>Tracer dilution (%)</th>
<th>RGP (μmol/min)</th>
<th>% Renal contribution to endogenous glucose production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nieth et al., 1966 (22)</td>
<td>58</td>
<td>—</td>
<td>~0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Owen et al., 1969 (3)</td>
<td>5 (5–6 weeks starvation)</td>
<td>—</td>
<td>~122</td>
<td>—</td>
<td>~40</td>
<td>—</td>
</tr>
<tr>
<td>Bjorkman et al., 1980 (23)</td>
<td>17</td>
<td>—</td>
<td>~40</td>
<td>—</td>
<td>~10</td>
<td>—</td>
</tr>
<tr>
<td>Stumvoll et al., 1995 (8)</td>
<td>10</td>
<td>[6-2H]glucose</td>
<td>~69</td>
<td>3.9</td>
<td>256</td>
<td>28</td>
</tr>
<tr>
<td>Stumvoll et al., 1998 (9)</td>
<td>9</td>
<td>[6-3H]glucose</td>
<td>~60</td>
<td>3.6</td>
<td>199</td>
<td>24</td>
</tr>
<tr>
<td>Meyer et al., 1998 (12)</td>
<td>15</td>
<td>[6-3H]glucose</td>
<td>~21</td>
<td>2.0</td>
<td>125</td>
<td>~15</td>
</tr>
<tr>
<td>Stumvoll et al., 1998 (11)</td>
<td>6</td>
<td>[6-3H]glucose</td>
<td>~29</td>
<td>ND</td>
<td>208</td>
<td>22</td>
</tr>
<tr>
<td>Cersosimo et al., 1999 (13)*</td>
<td>8</td>
<td>[6-3H]glucose</td>
<td>~55</td>
<td>4.2</td>
<td>168</td>
<td>24</td>
</tr>
<tr>
<td>Cersosimo et al., 1999 (14)*</td>
<td>18</td>
<td>[6-3H]glucose</td>
<td>~51</td>
<td>3.1</td>
<td>159</td>
<td>21</td>
</tr>
<tr>
<td>Ekberg et al., 1999 (15)</td>
<td>7</td>
<td>7-[U-13C]</td>
<td>14</td>
<td>~0</td>
<td>ND</td>
<td>≤5</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10-[6-2H]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>6-[3-3H]</td>
<td></td>
<td></td>
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</tbody>
</table>

*Based on plasma flow determinations. ND, no raw data.
REFERENCES


