SHORT COMMUNICATION

Urea, glucose and alanine kinetics in man: effects of glucose infusion

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Summary
1. The simultaneous effects of an intravenous glucose infusion on plasma urea, glucose and alanine kinetics were investigated in normal post-absorptive man.
2. The primed constant intravenous infusion of compounds labelled with stable isotopes, [15N2]urea, [6-2H]glucose and [3-13C]alanine, was used.
3. The rate of appearance of glucose and urea in the plasma was rapidly reduced by the 17.7 pmol min⁻¹ kg⁻¹ glucose infusion.
4. In contrast, during the glucose infusion there was an increased rate of appearance of alanine in the plasma, and an increased percentage of glucose carbon atoms derived from alanine.
5. Reduced production of glucose and urea during the glucose infusion was not due to decreased gluconeogenesis from alanine.

Key words: alanine, glucose, kinetics, stable isotope, urea.

Abbreviations: APE, atoms % excess.

Introduction
The metabolism of glucose, alanine and urea is closely related. Alanine is the major gluconeogenic amino acid in man [1] and glucose intake limits urinary nitrogen excretion in both fasting [2] and injury [3]. We have recently shown [4] that an intravenous infusion of glucose given at approximately twice the endogenous production rate will reduce endogenous glucose production by about 90% in man. This reduction in the rate of appearance of glucose may be due to a reduction in glycogenolysis or gluconeogenesis.

The purpose of the present study was to investigate simultaneously plasma glucose, alanine and urea kinetics in man before and during glucose infusion by using the primed constant intravenous infusion of the stable isotopes [6-2H]glucose, [3-13C]alanine and [15N2]urea. Infusions of [6-2H]glucose and [15N2]urea in tracer quantities have been previously validated [5, 6] as accurate methods for quantitatively measuring the rate of appearance of glucose and urea in the plasma in dogs.

Methods
Subjects
Four healthy male volunteers (age 20–25 years; weight 58–89 kg) were studied at rest after a 10 h overnight fast. The study was first approved by the Human Studies Committee of the Massachusetts General Hospital and informed consent was obtained.

Infusions of labelled compounds
A primed, constant infusion of [15N2]urea (0.58 μmol min⁻¹ kg⁻¹; prime:infusion ratio 300:1, 99% enrichment), [6-2H]glucose (0.28 μmol min⁻¹ kg⁻¹; prime:infusion ratio 80:1, 98%
enrichment) and \[^{3-13}C\text{alanine} \ (0.07\mu\text{mol min}^{-1} \ \text{kg}^{-1}, \ \text{prime:infusion ratio 100:1, 99\% enrichment)}\] was made into an arm vein with a Harvard constant infusion pump at a rate of 0.191 ml/min for 210 min. All labelled compounds were obtained from KOR isotopes, Cambridge, MA, U.S.A.

**Glucose infusion**

After 90 min of infusion of labelled compound (period 1), unlabelled glucose was also infused intravenously at 17.7 \(\mu\text{mol min}^{-1} \ \text{kg}^{-1}\) via a volumetric pump for 120 min (period 2) in three subjects (subjects 1, 2 and 3). Subject 4 received sodium chloride solution (154 mmol/l: saline) in period 2. The infusion of labelled compound was not interrupted in period 2.

**Blood samples**

Venous blood (9 ml) was withdrawn from the non-infusion arm at -10, -5, +75, +80 and +90 min during period 1 and at 20, 40, 60, 80, 100 and 120 min during period 2 into heparinized tubes.

**Blood analysis**

\([6-^{3}H\text{glucose}, \ [3-^{13}C\text{alanine and } [^{15}N\text{]urea} \text{ enrichments were determined by gas chromatography–mass spectrometry as the following derivatives: butylboronate glucose, n-acetyl-propylalanine, and bis-(trimethylsilyl)urea. A Hewlett-Packard 5985 B GCMS quadrupole mass spectrometer –21 MY computer system was used for analysis. Electron impact ionization was used for the glucose and urea derivatives; ions at } m/e 197 and 199 were selectively monitored for the butylboronate glucose, and ions at } m/e 189, 190 and 191 were selectively monitored for the bis-(trimethylsilyl)urea. Chemical ionization was used for the alanine derivative; ions at } m/e 174 and 175 were selectively monitored. The \(^{13}C\) enrichment of plasma glucose was determined by isolation of the glucose, combustion in a vacuum oven at 1000°C, and analysis of the CO\(_2\) enrichment with a Nuclide-3-60 isotope ratio mass spectrometer as we have described before [4].

**Calculations**

In all experiments the enrichment of plasma urea, glucose and alanine were at plateau. Therefore, the rates of appearance of urea, glucose and alanine in periods 1 and 2 were calculated from the previously described [7] equation for stable isotope infusions.

Rate of appearance = \[
\frac{\text{APE infusion} - 1}{\text{APE plasma}} \times F \ (\mu\text{mol min}^{-1} \ \text{kg}^{-1})
\]

Where APE = atoms % excess, \(F = \) rate of isotope infusion (\(\mu\text{mol min}^{-1} \ \text{kg}^{-1}\))

% glucose from alanine = \[
\frac{[^{13}C\text{glucose APE} \times 3]}{[^{13}C\text{alanine APE}]}
\]

because each \(^{13}C\)glucose atom represents the donation of three carbon atoms from alanine.

The APE of the infused glucose solution (Travenol Ltd) was not different from that of the plasma glucose before infusion of labelled glucose. Consequently no allowance was made in the calculations for enrichment of plasma glucose resulting from the exogenous glucose infusion.

Urea synthesis \textit{de novo} was calculated by measuring [6] the plasma APE of the 191 (M + 2) ion. Urea recycling was calculated [6] by measuring the APE of the 190 (M + 1) ion. A paired \(t\)-test was used to compare results.

**Results**

The glucose infusion increased mean plasma glucose concentration from 5.1 \(\pm\) SEM 1.5 to 7.9 \(\pm\) 3.9 \(\mu\text{mol/ml}\) \((P < 0.01)\). Mean plasma urea concentration, however, did not significantly change (6.0 \(\pm\) 0.4 and 5.8 \(\pm\) 0.5 \(\mu\text{mol/ml}\)). The results for labelled compounds show that the glucose infusion resulted in a marked (84–93\%) decrease in the rate of endogenous glucose production together with a 10–13\% reduction in the rate of appearance of urea into the plasma \((P < 0.01)\) (Table 1).

In contrast, the percentage of endogenous glucose production from alanine increased from only 2\% in period 1 to approximately 20\% during the glucose infusion. The actual rate of glucose production derived from alanine was not significantly altered by glucose infusion (Table 1).

Urea recycling in both period 1 and period 2 was less than 1\% of the rate of appearance as calculated from the M + 1 ion.

**Discussion**

We have shown that an intravenous glucose infusion given at a rate of less than twice the endogenous rate of glucose production in post-absorptive man rapidly reduced both endogenous glucose production and urea production. In contrast, alanine release tended to increase,
Glucose and alanine kinetics before (period 1) and after (period 2) glucose infusion in three subjects

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Period 1</th>
<th>Period 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea appearance (mmol kg⁻¹)</td>
<td>11.3</td>
<td>9.8</td>
</tr>
<tr>
<td>Endogenous glucose appearance (mmol kg⁻¹)</td>
<td>15.3</td>
<td>1.9</td>
</tr>
<tr>
<td>Alanine appearance (mmol kg⁻¹)</td>
<td>6.7</td>
<td>13.6</td>
</tr>
<tr>
<td>Glucose production from alanine (%)</td>
<td>2.0</td>
<td>33.6</td>
</tr>
</tbody>
</table>

This table shows the data for glucose and alanine kinetics before and after glucose infusion in three subjects. The table includes columns for subject number, period 1, and period 2, with measurements for Urea appearance, Endogenous glucose appearance, Alanine appearance, and Glucose production from alanine.

and the rate of glucose production from alanine corresponded to the rate of alanine release. Consequently, the percentage of endogenous glucose production derived from alanine rose from the postprandial level of 2–3% to 27–33% during the glucose infusion.

Since alanine is the major gluconeogenic amino acid in man [11], our estimate that in the post-absorptive state only 2–3% of glucose production was derived from alanine appears low. Others, however, using labelling techniques in both animals [8] and men [9] have also shown similar low rates of gluconeogenesis from alanine. Any underestimation is probably due to the fact that the true enrichment of the alanine precursor for gluconeogenesis is lower than that of the venous blood we sampled, though loss of labelled isotope in the tricarboxylic acid cycle is also possible [10].

Alanine assumed an increased importance, in a relative sense, as a glucogenic precursor during the glucose infusion. Despite an 88% reduction in endogenous glucose production, the rate of glucose production from alanine did not fall. Consequently, there was a marked increase in the percentage of glucose production from alanine. This is consistent with our earlier observation that all residual glucose production during glucose infusion was attributable to recycling of glucose carbon atoms [4]. It would thus seem that in the present work the rate of appearance of alanine in the plasma was related to the rate of peripheral glucose uptake and subsequent production of pyruvate. Similarly, gluconeogenesis from alanine was related to the delivery of alanine. Gluconeogenesis from alanine in postabsorptive man, however, appears to be of minor importance in terms of total endogenous glucose production. Glycogenolysis or possibly gluconeogenesis from other substrates appears to be of greater importance.

The rapidity with which urea production fell during the glucose infusion was quite striking, half of the fall in urea production occurring within 60 min of the start of the glucose infusion. This appears to have been due to changes in the delivery to the liver of amino acids other than alanine. The insulin response to infused glucose may be involved, since insulin has been shown to decrease the release of branched-chain amino acids, but not alanine, from muscle [11].

In conclusion, the primed constant infusion of stable isotopes appears to be a useful method for studying simultaneous changes in the metabolism of different substrates in man. A rapid reduction in both urea and glucose production has been demonstrated with a low-dose glucose infusion in postabsorptive man. Changes in alanine metabolism appear quantitatively to be of minor importance in causing the observed changes in glucose and urea metabolism.

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References


