Role of glucose in the regulation of glutamine metabolism in health and in type 1 insulin-dependent diabetes

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Hankard, Régis G., Morey W. Haymond, and Dominique Darmaun. Role of glucose in the regulation of glutamine metabolism in health and in type 1 insulin-dependent diabetes. Am J Physiol Endocrinol Metab 279: E608–E613, 2000.—To determine the effect of glucose availability on glutamine metabolism, glutamine kinetics were assessed under conditions of hyperglycemia resulting from 1) intravenous infusion of 7.5% dextrose in healthy adults and 2) insulin deficiency in young adults with insulin-dependent diabetes mellitus (IDDM). Eight healthy adults and five young adults with IDDM were studied in the postabsorptive state by use of a primed continuous infusion of D-[U-14C]glucose, L-[5,5,5-2H3]leucine, and L-[3,4-13C2]glutamine. Whether resulting from insulin deficiency or dextrose infusion, the rise in plasma glucose was associated with increased glucose turnover (23.5 ± 0.7 vs. 12.9 ± 0.3 μmol·kg⁻¹·min⁻¹, P < 0.01 and 20.9 ± 2.5 vs. 12.8 ± 0.4 μmol·kg⁻¹·min⁻¹, P = 0.03, in health and IDDM, respectively). In both cases, high blood glucose failed to alter glutamine appearance rate (Rα) into plasma (298 ± 9 vs. 312 ± 14 μmol·kg⁻¹·h⁻¹, not significant [NS] and 309 ± 23 vs 296 ± 26 μmol·kg⁻¹·h⁻¹, NS, in health and IDDM, respectively) and the estimated fraction of glutamine Rα arising from de novo synthesis (210 ± 7 vs. 217 ± 10 μmol·kg⁻¹·h⁻¹, NS and 210 ± 16 vs. 207 ± 21 μmol·kg⁻¹·h⁻¹, NS in health and IDDM, respectively). When compared with the euglycemic day, the apparent contribution of glucose to glutamine carbon skeleton increased when high plasma glucose resulted from intravenous dextrose infusion in healthy volunteers (10 ± 0.8 vs. 4.8 ± 0.3%, P < 0.01) but failed to do so when hyperglycemia resulted from insulin deficiency in IDDM. We conclude that 1) the contribution of glucose to the estimated rate of glutamine de novo synthesis does not increase when elevation of plasma glucose results from insulin deficiency, and 2) the transfer of carbon from glucose to glutamine may depend on insulin availability.

SUBJECTS AND METHODS

Materials

D-[U-14C]glucose was purchased from New England Nuclear (Boston, MA). L-[5,5,5-2H3]leucine (98% 2H3) and L-[3,4-13C2]glutamine (98% 13C2) were obtained from Cambridge Isotopes Laboratories (Andover, MA), as were the internal standards utilized in analytical procedures (D-,ketosooaric acid (D-, KIC), [3H]glucose, [2H3]glutamine). Solutions of tracers were prepared in sterile 0.45 g/dl NaCl, passed through 0.22-μm filters, and tested for sterility (plate culture) and absence of pyrogen (limulus lysate assay). Infusates were prepared <24 h before infusion and kept at 4°C until used.

Subjects

The present study was reviewed and approved by the Nemours Children’s Clinic Research Committee and the Institutional Review and Radiation Safety Committees of the Baptist Medical Center in Jacksonville, FL.

Eight healthy adults [weight, 64 ± 3 kg, age, 28 ± 3 yr, body mass index (BMI), 22 ± 1 kg/m², means ± SE] and five

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patients with IDDM (weight, 60 ± 4 kg, age, 19 ± 1 yr, BMI, 22 ± 1 kg/m²) gave their written consent after the aims, study design, and potential risks of the study were explained.

Protocol Design

Glucose, glutamine, and leucine kinetics were studied using two 4-h tracer infusion studies performed on two consecutive days in healthy volunteers (protocol 1) and in IDDM patients (protocol 2). In both protocols, volunteers were studied under conditions of mild hyperglycemia on one day, whereas normal postabsorptive plasma glucose levels were maintained on the other day.

Protocol 1. Volunteers received in random order an intravenous infusion of 7.5% dextrose (19.6 ± 0.6 μmol·kg⁻¹·min⁻¹) on one day or 0.9% saline providing the same fluid load (3.2 ml·kg⁻¹·h⁻¹) on the other day. On each study day, subjects ate dinner at 2000 and remained fasting until 1300 the next day. The tracer infusion procedure took place from 0900 to 1300.

Protocol 2. The night before the first study day, subjects ate dinner at 2000 with their normal subcutaneous injection of regular insulin and then remained fasted until 1200 the next day. Their blood glucose was kept between 200 and 250 mg/dl (10–14 mmol/l) overnight and throughout the next morning. Insulin was administered for 5.0–6.7 mmol/l to maintain plasma glucose between 90 and 120 mg/dl. Algorithms for the choice of insulin infusion rates were already determined during two 4-h tracer infusion studies performed on two consecutive days in healthy volunteers and in IDDM patients (protocol 2).

Tracer Infusion Procedure

The first study day, two short catheters were placed, one in a forearm vein for isotope infusion and the other in a contralateral hand vein for arterialized venous blood sampling. Before the start of tracer infusion, two blood and air samples were obtained for determination of baseline isotopic enrichment and specific activity (SA) in plasma and expired air. For [13C₂]glucose enrichment and CO₂ SA determination, expired air was bubbled into Vacutainer evacuated glass tubes with a 50-ml syringe. 15-ml aliquots were transferred from the Douglas rubber bag into Vacutainer evacuated glass tubes with a 50-ml syringe. E609EFFECT OF GLUCOSE ON GLUTAMINE METABOLISM

Analytical Procedures

Glucose assay. Plasma glucose concentration was determined by means of automated glucose oxidase reaction (Beckman II glucose analyzer).

For determination of plasma [3H₆]glucose enrichment, 50 μl of plasma were deproteinized using 300 μl ice-cold acetone, and the deproteinized sample was derivatized with acetic anhydride to form the glucose pentacetate derivative. Glucose pentacetate was analyzed for mass-to-charge (m/z) fragments 98 and 100 using gas chromatography-mass spectrometry (GC-MS) (24).

For determination of plasma [14C]SA (Glc SA, dpm/μmol), a known aliquot of a [3H]glucose internal standard was first added to plasma. The plasma was deproteinized with barium hydroxide and zinc sulfate, and the sample was submitted to cation and anion exchange chromatography. The neutral eluate was counted for [3H] and [14C] radioactivity with a liquid scintillation counter (LS9800 series, Beckman Instruments, Palo Alto, CA) with correction for quenching and 14C contribution into the [3H] spectrum. Plasma glucose SA was calculated as the ratio of glucose dpm to plasma glucose concentration, with a correction for glucose recovery, by use of the [3H]glucose as an internal standard.

Plasma α-KIC assay. Plasma α-KIC was isolated from plasma by ion exchange chromatography and derivatized to its t-butyldimethylsilylexiloxime derivative. Plasma α-KIC enrichment was determined by GC-MS monitoring ions with m/z 316 and 319 (16). Plasma α-KIC concentration was determined by the reverse dilution method with d3-KIC as an internal standard (16).

Glutamine assay. Plasma [14C]glutamine enrichment: glutamine was first extracted from plasma by ion exchange chromatography and then derivatized to N-acetyl-n-propyl-NAP-glutamate, as described previously (4). Injections were made into a 0.25 x 25 mm HP1, capillary column by use of an isothermal program and the split mode. Ions m/z ratios of 186 and 188, corresponding to natural glutamine and [14C]glutamine, respectively, were selectively monitored with GC-MS (HP5971, Hewlett-Packard, Palo Alto, CA). Plasma glutamine concentration was determined by the reverse dilution method with [3H]glutamine as internal standard and GC-MS (4).

Plasma glutamine [14C]SA: glutamine was extracted from plasma by ion exchange chromatography, and the glutamine-containing eluate was specifically converted to glutamate using glutaminase (EC 3.5.1.2, Sigma, St. Louis, MO) to remove labeled compounds other than glutamine. The glutamate was extracted by ion exchange chromatography and then counted for disintegrations per minute (dpm) with a scintillation counter with quenching correction.

Breath CO₂. CO₂ SA (dpm/mmol) was determined by counting the dpm in hyamine and ethanolamine CO₂ trapping solutions (24). Breath [13C]CO₂ was measured by gas chromatography-isotope ratio mass spectrometry (GC-IRMS; VG-Isocrom-III, VG Isogas, Ipswich, UK).

Insulin assay. Plasma insulin concentration was determined by radioimmunoassay (Endocrine Science, Calabasas Hills, CA).

Calculations

Stable isotopes. Rates of appearance (Ra) of glutamine (Ra,Gln), leucine (Ra,Leu), and glucose (Ra,Glc) in plasma were calculated, in micromoles per kilogram per minute, as

\[ Ra_i = \frac{i \times (E_i / E_p)}{1} - 1, \]

where i is the tracer infusion rate, Ei is the tracer enrichment in the intravenous infusate (mol % excess), and Ep is the
tracer enrichment in the relevant plasma substrate at steady state (mol % excess) (3, 4, 5, 15).

The apparent rate of glutamine release from whole body protein breakdown (Bgin, μmol·kg⁻¹·h⁻¹) was roughly estimated from Ra,Leu, an index of whole body protein degradation, as

\[ B_{\text{gin}} = R_{a,\text{Leu}} \times 0.07 \times 131/(146 \times 0.08) \]

where 0.07 and 0.08 are grams of glutamine and leucine contents per gram of protein, respectively (10), and 146 and 131 are, respectively, glutamine and leucine molecular weights.

The fraction of glutamine Ra arising from de novo synthesis (D\(_{\text{Gln}}\); μmol·kg⁻¹·h⁻¹) was estimated as

\[ D_{\text{Gln}} = R_{a,\text{Gln}} - B_{\text{Gln}} \]

Endogenous glucose Ra in plasma (Endo Ra,Glc) was calculated as

\[ \text{Endo } R_{a,\text{Glc}} = R_{a,\text{Glc}} - \text{Exo } R_{a,\text{Glc}} \]

where Exo Ra,Glc is the intravenous unlabeled glucose infusion rate (μmol·kg⁻¹·min⁻¹).

Radioactive isotopes. Glucose oxidation rate (OxGlc, μmol·kg⁻¹·h⁻¹) was estimated from the \(^{14}\)CO\(_2\) excretion rate in expired air (F\(^{14}\)CO\(_2\), μmol·kg⁻¹·min⁻¹) by two methods (hyamine and ethanolamine) and averaged.

F\(^{14}\)CO\(_2\) was calculated from ethanolamine CO\(_2\) trapping solution as

\[ F^{14}\text{CO}_2 = \text{CO}_2A(E) \times 250/(W \times 2) \]

where CO\(_2\) A (E) is CO\(_2\) activity in a 1-ml ethanolamine aliquot (dpm/ml), 250 corrects 1 ml to 250 ml ethanolamine total volume of trapping solution, 2 is the 2-min collecting period, and W is the weight (kg).

F\(^{14}\)CO\(_2\) was calculated from hyamine CO\(_2\) trapping solution as

\[ F^{14}\text{CO}_2 = \text{CO}_2SA(H) \times \text{VCO}_2/(W \times 22.4) \]

where CO\(_2\) SA (H) is CO\(_2\) SA in 1 mmol hyamine (dpm/mmol).

OxGlc was then calculated from mean F\(^{14}\)CO\(_2\) as

\[ \text{OxGlc} = F^{14}\text{CO}_2/(\text{Glc SA} \times R \times 10^{-2}) \]

where R is labeled carbon fractional recovery in expired CO\(_2\). R was set at 70% in basal conditions and at 82% during glucose intravenous infusion in protocol 1 (13) and at 70% on both days in protocol 2 (3).

The fraction of glutamine arising from glucose was estimated from the glucose-to-glucose SA ratio.

Indirect calorimetry. V\(_{\text{CO}_2}\) and V\(_{\text{O}_2}\) were calculated during the two 20- to 30-min sets from CO\(_2\) and O\(_2\) concentrations in inspired and expired air and dilution airflow using Haldane’s transformation (23).

Statistical Analysis

Data are expressed as means ± SE. Comparisons within groups were performed using paired t-tests. Significance was established at P < 0.05.

RESULTS

Glucose Metabolism

In healthy volunteers, plasma glucose concentration was higher (~34%) during dextrose than during saline infusion (6.3 ± 0.2 vs. 4.7 ± 0.6 mmol/l, P < 0.01). During saline infusion, plasma insulin concentration decreased from 11 ± 1 to 7 ± 1 mU/l (P < 0.01) over the course of the infusion, whereas insulin increased from 12 ± 2 to 18 ± 3 mU/l (P < 0.01) during dextrose infusion.

In IDDM patients, plasma glucose concentration was significantly higher on the first study day (13.5 ± 3.8 vs. 7.2 ± 1.3 mmol/l, P < 0.01) resulting from the lower insulin infusion rate (0.6 ± 0.7 vs. 1.6 ± 0.6 IU/h, P < 0.01; Fig. 1). On both study days, plasma glucose concentration remained stable throughout the tracer infusion period (Fig. 1).

Glucose SA and enrichments were at isotopic steady state on both study days (Table 1). In healthy volunteers, intravenous dextrose infusion resulted in increased glucose turnover (23.5 ± 0.7 vs. 12.9 ± 0.3 μmol·kg⁻¹·h⁻¹, P < 0.01) and oxidation rate (6.8 ± 0.2 vs. 4.8 ± 0.3 μmol·kg⁻¹·min⁻¹, P < 0.01; Fig. 2), and decreased endogenous glucose production rate (3.9 ± 0.7 vs. 12.9 ± 0.3 μmol·kg⁻¹·h⁻¹, P < 0.01). Similarly, in IDDM patients, glucose turnover rate was
higher when blood glucose level was maintained high, compared with the euglycemic day (20.9 ± 2.5 vs. 12.8 ± 0.4 \( \mu\)mol·kg\(^{-1}\)·min\(^{-1}\), \(P = 0.03\)). However, elevation of blood glucose resulting from insulin deficiency was not associated with an increase in glucose oxidation rate (3.7 ± 0.4 vs. 3.9 ± 0.5 \( \mu\)mol·kg\(^{-1}\)·min\(^{-1}\), NS; Fig. 2).

The minimum fraction of plasma glutamine carbon arising from glucose (estimated from the ratio of glutamine SA to glucose SA) doubled between the saline and the dextrose infusion days in healthy subjects: 10.4 ± 0.8 vs. 4.8 ± 0.3\% (\(P < 0.01\)).

In contrast, the apparent glucose-to-glutamine carbon transfer did not increase when high blood glucose resulted from a lower insulin infusion rate (4.4 ± 0.5 vs. 4.6 ± 0.7\%, NS) in IDDM subjects.

### Leucine Metabolism

On both days, plasma \(\alpha\)-KIC enrichments were at isotopic steady state over the last 2 h of isotope infusion (Table 1). In healthy volunteers, leucine turnover rate, an index of protein degradation, and plasma \(\alpha\)-KIC concentration were lower during intravenous dextrose compared with the saline infusion (112 ± 5 vs. 120 ± 7 \(\mu\)mol·kg\(^{-1}\)·h\(^{-1}\), \(P = 0.03\), and 18 ± 1 vs. 24 ± 2 \(\mu\)mol/l, \(P = 0.006\), respectively). In contrast, in IDDM patients, high blood glucose resulting from a reduction in the insulin infusion rate was associated with a higher leucine turnover rate (126 ± 11 vs. 112 ± 7 \(\mu\)mol·kg\(^{-1}\)·h\(^{-1}\), \(P = 0.03\)) and plasma concentrations of \(\alpha\)-KIC, the intracellular metabolite of leucine (48 ± 2 vs. 43 ± 2 \(\mu\)mol/l, \(P = 0.03\)).

### Glutamine Metabolism

Regardless of the group studied (healthy volunteers or IDDM patients), elevation of plasma glucose failed to alter plasma glutamine concentration, glutamine R\(_a\) into plasma, and the estimated fraction of glutamine R\(_a\) arising from de novo synthesis (Table 2). A decrease in glutamine arising from whole body protein breakdown resulting from lower rate of protein degradation was observed in healthy volunteers receiving 7.5% dextrose (88 ± 4 vs. 94 ± 6 \(\mu\)mol·kg\(^{-1}\)·h\(^{-1}\), \(P = 0.03\)) and in normoglycemic IDDM patients (88 ± 6 vs. 99 ± 9 \(\mu\)mol·kg\(^{-1}\)·h\(^{-1}\), \(P = 0.03\)).

### DISCUSSION

We determined the effect of elevation of plasma glucose on glutamine metabolism in two different “models”: hyperglycemia resulting from intravenous dextrose infusion in healthy adults on one hand and hyperglycemia resulting from insulinopenia in type 1 diabetic subjects on the other hand. We observed that 1) high plasma glucose level, whether resulting from either insulin deficiency or exogenous glucose infusion, failed to increase the estimated rate of glutamine de novo synthesis, and 2) the apparent contribution of glucose to glutamine carbon skeleton increased when exogenous glucose was infused but failed to do so when high plasma glucose resulted from insulinopenia.

In the present study, as in previous ones (8), leucine turnover rate, an index of whole body proteolysis, in-

### Table 1. Plasma substrates specific activity and enrichments in the 2 experiments

<table>
<thead>
<tr>
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<th>Normal Plasma Glucose</th>
<th>High Plasma Glucose</th>
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<tbody>
<tr>
<td></td>
<td>H D</td>
<td>H D</td>
</tr>
<tr>
<td>Ep Gln, APE</td>
<td>1.8 ± 0.1 1.9 ± 0.2</td>
<td>1.9 ± 0.1 1.8 ± 0.2</td>
</tr>
<tr>
<td>Ep (\alpha)-KIC, APE</td>
<td>2.6 ± 0.2 2.8 ± 0.2</td>
<td>2.8 ± 0.2 2.5 ± 0.3</td>
</tr>
<tr>
<td>Gln SA, dpm/(\mu)mol</td>
<td>2,847 ± 168 2,838 ± 279</td>
<td>1,550 ± 98 1,915 ± 359</td>
</tr>
<tr>
<td>Glucose SA, dpm/(\mu)mol</td>
<td>137 ± 13 131 ± 33</td>
<td>162 ± 17 86 ± 22</td>
</tr>
</tbody>
</table>

Values are means ± SE. H, healthy volunteers; D, insulin-dependent diabetic volunteers; Ep, plasma enrichment; APE, atom percent excess; SA, specific activity; Gln, glutamine; \(\alpha\)-KIC, \(\alpha\)-ketoisocaproic acid.
creased by 12% when high blood glucose resulted from insulin deficiency. Conversely, the enhanced insulin secretion induced by glucose most likely accounts for the decrease in protein degradation observed during dextrose infusion in healthy subjects. These data highlight the anticoncatabolic effect of insulin on protein metabolism (2).

The methodology used in the present study yields estimates of the fraction of overall glutamine appearance into plasma that arises from de novo synthesis. These estimates are, however, based on the assumptions that 1) in the postabsorptive state, leucine appearance rate reflects whole body protein breakdown, and 2) the release of amino acids from proteolysis is proportional to their relative abundance as bound residues in body protein. The glutamine content of muscle protein was recently reevaluated and found to be lower than the value used in the present study (14). This reevaluation emphasizes the fact that estimates of $D_{\text{Gln}}$ are imperfect and do not measure the true rates of glutamine synthesis. Although lowering the glutamine content of body protein in calculations would result in higher estimates of $D_{\text{Gln}}$, it would not alter the conclusions reached in the current study.

Whether hyperglycemia resulted from insulin deficiency or 7.5% dextrose intravenous infusion, it failed to stimulate the estimated fraction of glutamine $R_a$ arising from de novo synthesis. These results support those obtained in a previous study using $^{13}\text{N} \text{N}$ glutamine as a tracer (8) in IDDM. Lack of carbon and nitrogen availability cannot account for the lack of stimulation of $D_{\text{Gln}}$ observed in the current study. Indeed, abundant carbon was available because of hyperglycemia. Nitrogen availability may have been a limiting factor in healthy subjects, in whom the release of precursor amino acids from proteolysis was depressed by insulin. However, in IDDM subjects, nitrogen was not limiting, because precursor amino acid availability was enhanced as a result of accelerated protein breakdown on the hyperglycemic day.

Taken together with findings from earlier studies, the results of the current study are consistent with the view that glutamine appearance into plasma and the estimated release of glutamine from de novo synthesis are more responsive to increased demand than they are to increased precursor availability. Glutamine is indeed released mainly by skeletal muscle and is utilized in tissues with a high cell turnover rate such as gut and bone marrow. Estimated rates of glutamine synthesis clearly increased during hypercortisolemia (6), a condition that enhances glutamine utilization in the gut (22), as well as in children suffering from sickle cell disease (21), a condition associated with increased cell replication in the bone marrow. In contrast, glutamine $R_a$ and estimated glutamine synthesis were found to be depressed in patients with short bowel syndrome (7, 11), a condition associated with a reduced intestinal cell mass.

The current findings also suggest that glutamine synthesis may not be insulin dependent in vivo. They are consistent with in vitro studies showing that glutamine synthetase is not dependent on the presence of insulin (9). Further studies using hyperinsulinemic euglycemic clamp and/or somatostatin infusion would be warranted to further delineate the role of substrate (i.e., glucose) availability on glutamine synthesis in vivo. Interestingly, the synthetic rate of alanine, the other major amino acid precursor for gluconeogenesis, failed to increase in IDDM patients under conditions of insulinopenia (20), whereas hyperglycemia was able to stimulate alanine synthesis in the presence of insulin (19). Taken together, these data suggest that, although both glutamine and alanine are nonessential gluconeogenic amino acids, alanine metabolism is dependent on insulin, whereas glutamine synthesis is not.

Table 2. Effect of “high blood glucose” on glutamine metabolism in H and D subjects

<table>
<thead>
<tr>
<th>[Glc], mmol/l</th>
<th>H</th>
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<tbody>
<tr>
<td>Gln, μmol/l</td>
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<tr>
<td>High</td>
<td>482 ± 31</td>
<td>480 ± 20</td>
<td>298 ± 9</td>
<td>309 ± 23</td>
<td>88 ± 4</td>
<td>99 ± 9</td>
</tr>
<tr>
<td>Normal</td>
<td>513 ± 29</td>
<td>485 ± 25</td>
<td>312 ± 14</td>
<td>296 ± 26</td>
<td>94 ± 6</td>
<td>88 ± 6</td>
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<tr>
<td>$P$</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.03</td>
<td>0.03</td>
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<tr>
<td>$R_{an,Gln}$, μmol·kg⁻¹·h⁻¹</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>High</td>
<td>23 88</td>
<td>26 94</td>
<td>9 210</td>
<td>210 ± 16</td>
<td>217 ± 10</td>
<td>207 ± 21</td>
</tr>
<tr>
<td>Normal</td>
<td>29 485</td>
<td>26 252</td>
<td>16 211</td>
<td>217 ± 10</td>
<td>207 ± 21</td>
<td>207 ± 21</td>
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<tr>
<td>$B_{Gln}$, μmol·kg⁻¹·h⁻¹</td>
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<td>207 ± 21</td>
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</table>

Values are means ± SE. [Glc], blood glucose; [Gln], plasma glutamine concentration; $R_{an,Gln}$, whole body glutamine turnover; $B_{Gln}$, glutamine arising from protein breakdown; $D_{Gln}$, glutamine de novo synthesis; $P$, level of significance (paired t-test); NS, not significant.

Even though glucose infusion failed to alter the overall rate of glutamine de novo synthesis, glucose infusion enhanced the apparent contribution of glucose carbon to glutamine synthesis in healthy subjects. Carbons arising from glucose oxidation can appear into glutamine because they enter the tricarboxylic acid cycle and end up in α-ketoglutarate, which is a precursor of glutamate and glutamine. A rise in glucose oxidation may therefore enhance the incorporation of glucose-derived carbons into the glutamine molecule. This should, however, be interpreted with caution, because glutamine labeling mostly reflects $^{14}$C loading of metabolic intermediates rather than a true glucose-to-glutamine conversion. This increased carbon availability may nevertheless help spare other precursors for glutamine synthesis. In fact, we observed an apparent increase in the contribution of glucose-to-glutamine’s carbon skeleton when high blood glucose resulted from 7.5% dextrose intravenous infusion. Interestingly, the rise in the apparent carbon transfer from glucose to glutamine was not observed in IDDM when high blood glucose resulted from insulin deficiency. In the latter situation, high blood glucose failed
to elicit an increase in glucose oxidation. Insulin might therefore play a key, albeit indirect, role in regulating the carbon transfer from glucose to glutamine through its effect on glucose utilization and/or glucose oxidation to CO₂.

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