Hepatic autoregulation: response of glucose production and gluconeogenesis to increased glycogenolysis

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Hepatic autoregulation: response of glucose production and gluconeogenesis to increased glycogenolysis. Am J Physiol Endocrinol Metab 292: E1265–E1269, 2007. First published January 9, 2007; doi:10.1152/ajpendo.00411.2006.—The effect of increased glycogenolysis, simulated by galactose’s conversion to glucose, on the contribution of gluconeogenesis (GNG) to hepatic glucose production (GP) was determined. The conversion of galactose to glucose is by the same pathway as glycogen’s conversion to glucose, i.e., glucose 1-phosphate → glucose 6-phosphate → glucose. Healthy men (n = 7) were fasted for 44 h. At 40 h, hepatic glycogen stores were depleted. GNG then contributed ~90% to a GP of ~8 μmol·kg⁻¹·min⁻¹. Galactose, 9 g/h, was infused over the next 4 h. The contribution of GNG to GP declined from ~90% to 65%, i.e., by ~2 μmol·kg⁻¹·min⁻¹. The rate of galactose conversion to blood glucose, measured by labeling the infused galactose with [1-2H]galactose (n = 4), was also ~2 μmol·kg⁻¹·min⁻¹. The 41st h GP rose by ~1.5 μmol·kg⁻¹·min⁻¹ and then returned to ~9 μmol·kg⁻¹·min⁻¹, while plasma glucose concentration increased from ~4.5 to 5.3 mM, accompanied by a rise in plasma insulin concentration. Over 50% of the galactose infused was accounted for in blood glucose and hepatic glycogen formation. Thus an increase in the rate of GP via the glycogenolytic pathway resulted in a concomitant decrease in the rate of GP via GNG. While the compensatory response to the galactose administration was not complete, since GP increased, hepatic autoregulation is operative in healthy humans during prolonged fasting.

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GLUCOSE IS PRODUCED BY LIVER, also to some extent by kidney, and perhaps intestine. The amount produced is normally regulated to the needs of the body, with the brain being a major user. The glucose is produced by two processes: glycogenolysis and gluconeogenesis (GNG). An interaction between these processes in liver, termed hepatic autoregulation or interregulation (8), has been postulated. Thus production in the fasted state has been found unchanged, whether GNG is increased in normal subjects by infusing gluconeogenic precursors, or inhibited by ethanol administration in Type 2 diabetic subjects (12, 16–18, 27, 36, 37). Also, in dogs, an inhibitor of glycogenolysis has been reported to enhance glucagon-stimulated hepatic uptake of gluconeogenic precursors (31). However, using another inhibitor, in a similar study, glycogenolysis declined without a compensatory increase in GNG (8).

The biochemical reactions in glycogenolysis are glycogen → glucose 1-phosphate (G-1-P) → glucose 6-phosphate (G-6-P) → glucose (Fig. 1). Initial reactions in the hepatic utilization of galactose are galactose → galactose 1-phosphate → UDP-galactose → UDP-glucose. The UDP-glucose can be converted to glycogen, and hence to glucose via glycogenolysis, or be directly converted to G-1-P, and hence glucose without glycogen, as an intermediate (6, 22).

We asked, if glycogenolysis were increased in humans, would there be a compensatory decrease in GNG, and, if so, to what extent? Galactose was infused into subjects fasted 40 h. Hepatic glycogen stores are then depleted, and most production is by GNG. Glucose was formed from the galactose. Since conversion of galactose to glucose is via the reactions of glycogenolysis, i.e., G-1-P → G-6-P → glucose (Fig. 1), that conversion served as a surrogate for increasing glucose formation by glycogenolysis.

MATERIALS AND METHODS

Subjects. Written, informed consent was obtained from 10 healthy, normal male volunteers, ages 33–45 yr, with body mass indexes of 23.0–27.1 kg/m². Our Institutional Research Boards reviewed and approved the study.

Procedure. In seven of the subjects, glucose production (GP) was measured using [6,6-2H₂]glucose, and the contribution of GNG to GP using 2H₂O (25). The subjects were fasted for 44 h, beginning after dinner on the first day of study. They had been on their regular diets and were allowed to drink noncaloric fluids ad libitum during the fast. At 5 PM on the second day, i.e., 21 h into the fast, they were admitted to the Diabetes Research Centre of the Odense University Hospital. Drinking of 2H₂O was then begun. The dose, 5 ml/kg body water, was intended to achieve a body water enrichment of ~0.5%. Body water weight was calculated at 60% of body weight (25). The dose was drunk in four equal portions spaced 2 h apart, so that the last portion was ingested at 11 PM. Neither dizziness, nausea, nor any other side effect occurred. Fluids ingested after 11 PM were enriched to 0.5% with 2H₂O.

At 8 AM on the 3rd day, 36 h into the fast, a catheter was inserted into a superficial vein of one hand for blood collection. Another catheter was placed in a vein of the other hand, and through it a prime of 750 mg of glucose composed of 166 mg of [6,6-2H₂]glucose, 98% 2H enriched (Isotec, Miamisburg, OH), and 584 mg of unlabeled glucose in 15 ml of water, sterile and negative for pyrogen, was
infused rapidly. Then 500 mg of glucose composed of 100 mg of the [6,6-2H]glucose and 400 mg of unlabeled glucose, also at a concentration of 5% in water, were infused hourly at a constant rate for the next 8 h. A 5% water solution of 6-galactose was infused at a rate of 9 g/h for the last 4 h. The study ended after the 44th h of fasting. During those last 8 h, blood was collected at 0.5- to 1-h intervals for measurements of plasma glucose, insulin, C-peptide, and glucagon concentrations and 2H enrichments at carbons 2, 5, and 6 of the glucose. Plasma was frozen until analyzed.

To measure the fraction of the infused galactose converted to blood glucose, three other subjects and one of the seven subjects given 2H2O and [6,6-2H]glucose were treated in the same way, except 2H2O and [6,6-2H]glucose were not given. Instead, the 6-galactose infused was 2H enriched to ~3% at its carbon 1 by adding 6-[1-2H]galactose, 98% 2H enriched (Omicron Biochemicals, South Bend, IN). Blood was collected at hourly intervals from the 41st through the 44th h, for measurement of the 2H enrichment at carbon 1 of the blood glucose.

**Analyses.** Plasma glucose concentrations were determined using a glucose oxidase method (Beckman Glucose Analyzer I, Fullerton, CA). Enrichments of the hydrogens bound to carbons 2, 5, and 6 of glucose were determined as previously detailed (3, 25, 30). Briefly, the supernatant, obtained after deproteinizing a blood sample by ZnSO4 and Ba(OH)2 addition, was deionized by passage through a column of AG1-X8 in the formate form over AG50 W-X8 in the hydrogen form (Bio-Rad, Hercules, CA). The column was washed with water, and the effluent evaporated to dryness. The residue was applied to a Bio-Rad HPX-87P column in an HPLC system with water at 80°C as solvent and a flow rate of 0.5 ml/min. Glucose eluted between 15 and 17 min, and galactose between 18 and 20 min. The quantity of galactose was one-twentieth or less than that of glucose. The fraction containing the glucose peak was collected.

To determine 2H enrichments at carbon 6, an aliquot of the glucose collected at the end of the last hour of the infusion, divided by the enrichment of the hydrogen bound to carbon 1 was calculated, taking into account that the formaldehyde formed from the –CH2OH group containing carbon 6 was unlabeled.

Plasma insulin and C-peptide concentrations were measured by a two-site time-resolved immunofluorometric assay (Wallac Dy, Tuku, Finland). Plasma glucagon concentration was measured by radioimmunoassay (13).

**Calculations.** The percent contribution of GNG to GP (%GNG) was set equal to 100 times the enrichment of the hydrogen bound to carbon 5 of blood glucose, divided by the enrichment of the hydrogen bound to carbon 2 (25). The percent contribution via the glycolytic pathway (GLY) then equals 100 – %GNG.

The rate of appearance (Ra) of glucose in blood in micromoles per kilogram per minute was calculated using the equation (38):

\[
Ra = \frac{F - pV(C_2 + C_1)/2[(E_2 - E_1)/(t_2 - t_1)]}{(E_2 + E_1)/2}
\]

where F is the 2H enrichment of the [6,6-2H2]glucose infused, times the quantity of glucose infused in mmol·kg⁻¹·min⁻¹, pV is the extracellular space occupied by glucose, taken to be 200 ml times kg⁻¹, and C2 and C1 are the concentrations in mM of glucose in the plasma for the time interval t2 – t1 in minutes over which the Ra is calculated; and E2 and E1 are the corresponding 2H enrichments of the hydrogens at carbon 6 of plasma glucose at those times. GP in micromoles per kilogram per minute was calculated by subtracting the rate of infusion of the glucose from the Ra. The quantities GNG and GLY contributed to GP were calculated for each time interval, t2 – t1, over which the GP was determined by multiplying the GP by the average of the percent contributions of GNG and GLY to GP at times t2 and t1.

The fraction of GP derived from galactose, when [1-2H]galactose was infused from the 41st through the 44th h of fasting, was set equal to the 2H enrichment in the hydrogen bound to carbon 1 of blood glucose collected at the end of the last hour of the infusion, divided by the enrichment in the [1-2H]galactose infused (35).

**Statistics.** All results are given as means ± SDs. Changes in rates of GP with time were examined using a randomized block design in which the subject was the block. Comparison of GP in specified time intervals was accomplished using t-tests with contrasts, controlling for the mean GP of the subject (20).

**RESULTS**

Glucose concentration did not change from the 36th to 40th h (Table 1). Following the initiation of the galactose infusion, glucose concentration increased from ~4.5 to ~5.3 mM and then remained at that concentration. GP (Table 2) was ~8

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**Table 1. Plasma glucose concentrations**

<table>
<thead>
<tr>
<th>Hour</th>
<th>Plasma Glucose Concentration, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>4.8 ± 0.4</td>
</tr>
<tr>
<td>37</td>
<td>4.8 ± 0.5</td>
</tr>
<tr>
<td>38</td>
<td>4.7 ± 0.5</td>
</tr>
<tr>
<td>39</td>
<td>4.6 ± 0.5</td>
</tr>
<tr>
<td>39½</td>
<td>4.6 ± 0.6</td>
</tr>
<tr>
<td>40</td>
<td>4.4 ± 0.5</td>
</tr>
<tr>
<td>41</td>
<td>5.2 ± 0.4</td>
</tr>
<tr>
<td>42</td>
<td>5.4 ± 0.5</td>
</tr>
<tr>
<td>43</td>
<td>5.3 ± 0.2</td>
</tr>
<tr>
<td>43½</td>
<td>5.3 ± 0.2</td>
</tr>
<tr>
<td>44</td>
<td>5.2 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 7.
Galactose infusion, decreasing to 0.0001, and an F-test for departure from linearity was not significant.

The absolute amount GNG contributed to GP (Table 4) was about 7 μmol·kg⁻¹·min⁻¹ before galactose infusion, rose transiently in the first hour of galactose infusion, and then declined to 6 μmol·kg⁻¹·min⁻¹. The decline in absolute GNG in the first 2 h of galactose infusion was statistically significant (t = -5.96, df = 36, P < 0.0001). The absolute contribution of GLY to GP was about 1 μmol·kg⁻¹·min⁻¹ before infusion and rose to about 3 μmol·kg⁻¹·min⁻¹ on galactose infusion. The test for linear trend from before infusion to the 44th h was statistically significant (t = 19.49, df = 36, P < 0.0001), and an F-test for departure from linearity was not significant.

The ²H enrichment at carbon 1 of blood glucose increased with time during the infusion of the [1-²H]galactose (Table 5). At the end of the infusion, the 44th h of fasting, the enrichment in the glucose was 24.6 ± 1.5% of the enrichment in the infused galactose.

Insulin and C-peptide concentrations increased about two-fold upon galactose infusion (Table 6). A small decline in glucagon concentration is suggested.

DISCUSSION

Only a small amount of glycogen remains in the liver at 40 h of fasting (19, 29). At that time in our subjects, GNG contrib-

Table 2. Glucose production

<table>
<thead>
<tr>
<th>Hour</th>
<th>Glucose Production, μmol·kg⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>38–39½</td>
<td>8.44 ± 1.60</td>
</tr>
<tr>
<td>39½–40</td>
<td>7.93 ± 1.45</td>
</tr>
<tr>
<td>40–41</td>
<td>9.85 ± 1.61</td>
</tr>
<tr>
<td>41–42</td>
<td>9.44 ± 1.02</td>
</tr>
<tr>
<td>42–43</td>
<td>8.61 ± 1.46</td>
</tr>
<tr>
<td>43–43½</td>
<td>9.16 ± 1.47</td>
</tr>
<tr>
<td>43½–44</td>
<td>9.01 ± 1.10</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 7.

μmol·kg⁻¹·min⁻¹ before galactose infusion, increased by about 1.5 μmol·kg⁻¹·min⁻¹ in the first 2 h of infusion, and then declined to about 0.8 μmol·kg⁻¹·min⁻¹. The increase in GP in the first 2 h of infusion was statistically significant (t = 5.12, df = 36, P < 0.0001), and GP during the next 2 h remained higher than that before infusion (t = 2.86, df = 36, P < 0.01).

The percent contribution of GNG to GP was about 90% before galactose infusion, decreasing to about 10% with infusion (Table 3). The test for linear trend from before infusion to the 44th h was statistically significant (t = -21.54, df = 36, P < 0.0001), and an F-test for departure from linearity was not significant.

The absolute amount GNG contributed to GP (Table 4) was about 7 μmol·kg⁻¹·min⁻¹ before galactose infusion, rose transiently in the first hour of galactose infusion, and then declined to about 6 μmol·kg⁻¹·min⁻¹. The decline in absolute GNG in the first 2 h of galactose infusion was not statistically significant, but the decline in the next 2 h was statistically significant (t = -5.96, df = 36, P < 0.0001). The absolute contribution of GLY to GP was about 1 μmol·kg⁻¹·min⁻¹ before infusion and rose to about 3 μmol·kg⁻¹·min⁻¹ on galactose infusion. The test for linear trend from before infusion to the 44th h was statistically significant (t = 19.49, df = 36, P < 0.0001), and an F-test for departure from linearity was not significant.

The ²H enrichment at carbon 1 of blood glucose increased with time during the infusion of the [1-²H]galactose (Table 5). At the end of the infusion, the 44th h of fasting, the enrichment in the glucose was 24.6 ± 1.5% of the enrichment in the infused galactose.

Insulin and C-peptide concentrations increased about two-fold upon galactose infusion (Table 6). A small decline in glucagon concentration is suggested.
\( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) might have been expected. The decline of only a little more than 1 \( \mu \text{mol} \) may be explained by the increase in GP of \( \sim 1 \mu \text{mol} \) on galactose infusion. Glucose from galactose contributed \( \sim 25\% \) to GP, since the \( ^2\text{H} \) enrichment in blood glucose was \( \sim 25\% \) of that in the \( [1-^2\text{H}] \)galactose infused (Table 5). Since GP was \( \sim 9 \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) during galactose infusion, galactose then contributed \( \sim 2 \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \). In accord with that, the contribution of GLY to GP rose \( \sim 2 \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) above that before galactose infusion. Not only did GNG decline on galactose infusion, but the small contribution glycolysis made from stored glycogen to GP before infusion, \( \sim 1 \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \), could also have declined.

Estimates of GNG and GLY contributions can also be affected by glycolysis cycling (15, 23, 34) and transaldolase exchange (23, 24). The contribution of glycolysis cycling is likely minimal, while the contribution of transaldolase may be significant. Also, to the extent, if any, that \( [2.5-^2\text{H}] \)G-6-P formed via the gluconeogenic pathway was converted to glycogen, GNG could be underestimated. Galactose conversion to glucose via G-1-P and G-6-P could have inhibited that conversion, perhaps resulting in its infusion in the apparent transient increase in GNG. The transaldolase catalyzed exchange between F-6-P formed from the galactose with GAP, i.e., F-6-P + \( [2.5-^2\text{H}] \)GAP \( \leftrightarrow [5.2-^2\text{H}] \)G-6-P + GAP, followed by its isomerization to G-6-P and hence to \( [2.5-^2\text{H}] \)glucose, could result in some of the galactose converted to glucose appearing to originate via GNG.

The contribution to GP of glucose formed from the galactose could have been more than \( \sim 25\% \), since the \( ^2\text{H} \) enrichment in blood glucose appears to be still rising (Table 4). Also \( [1-^2\text{H}] \)G-6-P formed from the \( [1-^2\text{H}] \)galactose, before its conversion to glucose, could have lost \( ^2\text{H} \) in the pentose phosphate cycle, i.e., \( 3[1-^2\text{H}] \)G-6-P + 3NADP \( \rightarrow 3\text{CO}_2 + 2 \text{G}-6-P + \text{NADP}^+ \) (26), and in its equilibration with mannose 6-phosphate, i.e., \( [1-^2\text{H}] \)G-6-P \( \rightarrow [1-^2\text{H}] \)F-6-P \( \rightarrow \) mannose 6-phosphate \( \rightarrow \) F-6-P \( \rightarrow \) G-6-P (4).

That amount of glucose released into the circulation to fulfill the body’s needs results from the hydrolysis of G-6-P to glucose, catalyzed by glucose 6-phosphatase (21, 28, 36). The quantity each source of the G-6-P contributes to forming that amount depends only on the relative contribution of each source to the G-6-P. Contributions of the sources to the glucose produced will then be in the same proportion as their contributions to the formation of the G-6-P. As those contributions change, so must the fluxes through the pathways by which the G-6-P is generated change. Thus, in response to the increased formation of G-6-P from galactose via GLY, there must have been a compensatory reduction in the flux of gluconeogenic substrates to G-6-P.

The mechanism by which the so-called hepatic autoregulation is achieved is not well understood (8, 18, 21, 28). The increase in insulin concentration upon galactose infusion was probably due to the small rise in glucose concentration, since galactose is reported not to stimulate insulin release from islets (11). A decline in free fatty acid concentration could have contributed to the decreased GNG (2, 5, 21), but under the study conditions the decrease in free fatty acid concentration was likely small (19). Also, while insulin can inhibit GNG, as well as glycolysis, its increase seems unlikely to explain the decrease in GNG’s contribution because of the relative insensitivity of the gluconeogenic pathway to insulin (1, 7). Conceivably, an intermediate in galactose’s metabolism could have inhibited GNG, but no such inhibition has been reported. Galactose 1-phosphate was reported to inhibit phosphoglucomutase in vitro, but that was in the absence of glucose 1,6-bisphosphate, and no inhibition was demonstrated in vivo (32). Furthermore, inhibition of that enzyme would be expected to decrease glycolysis and not GNG. An initial period of hepatic glycolysis was reported on intravenous injection of a bolus of galactose into men fasted overnight, perhaps due to an inhibition of UDP-glucose pyrophosphorylase by UDP-galactose (9).

Recently, our laboratory reported a \( ^2\text{H}-\text{NMR} \) procedure for measuring enrichments of \( ^2\text{H} \) from \( ^2\text{H}_2 \)O at carbons 5 and 2 of glucose (19). Measurements, made in the postabsorptive state and after long-term fasting, were compared with measurements made by the chemical procedure used in this study (3, 30). Three men were treated the same way as in this study, i.e., galactose infused for 4 h beginning after 40 h of fasting and GP estimated using \( [6,6-^2\text{H}_2] \)glucose, except the \( ^2\text{H}_2 \)O was given 12 h after fasting was begun. GNG again declined from 94 \( \pm 5\% \) to 69 \( \pm 6\% \), following galactose infusion. GP was about the same at 40 h, 9.2 \( \pm 0.6 \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \), as 44 h of fasting, 8.8 \( \pm 0.2 \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \). The rate of glycogen synthesis, measured using nuclear mass resonance spectrometry, was 3.1 \( \pm 0.6 \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \). Thus, of the \( \sim 10 \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) of galactose infused, over 50\% can then be accounted for in glucose and glycogen formation. Most of the galactose infused would be expected to be taken up by liver (6).

Sunehag and Haymond reported giving galactose to healthy women fasted overnight (35). Doses of \( \sim 7.5 \) and 22.5 g/h were ingested over 2-h periods. At the end of that time, at both doses, GP was about \( \sim 12 \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \). At the higher dose, galactose contributed \( \sim 75\% \) to GP. After an overnight fast, before galactose infusion, GNG and glycolysis would be expected to have each contributed \( \sim 50\% \) to GP (e.g., Refs. 1, 3, 5, 15). Therefore, the results at the higher dose provide further support for a compensatory decline in contribution of GNG when glucose is produced from galactose. In that study, similar to the present study, plasma glucose concentration was 4.7 mM before and 5.1 mM after galactose ingestion, and there was a small increase, about a doubling, in insulin concentration. When men, fasted overnight, ingested 50 g of galactose, \( \sim 20\% \) appeared in circulating glucose over the next 8 h, in accord with our estimate of 25\%, although under other condi-

| Table 6. Insulin, C-peptide, and glucagon concentrations |
|----------------|----------------|----------------|
| Hour | Insulin | C-Peptide | Glucagon |
| 36 | 26±18 | 362±113 | 18.6±9.6 |
| 37 | 10±5 | 301±109 | 12.8±6.1 |
| 38 | 11±4 | 249±86 | 13.0±4.2 |
| 39 | 8±3 | 229±90 | 12.2±7.3 |
| 40 | 10±7 | 226±114 | 14.9±5.8 |
| 41 | 18±9 | 306±129 | 10.2±5.0 |
| 42 | 28±18 | 442±142 | 10.6±5.5 |
| 43 | 26±15 | 458±138 | 12.6±5.4 |
| 44 | 21±10 | 424±101 | 11.4±5.1 |

Values are means ± SD in pm; \( n = 7 \).
tions. There was only a transient increase in plasma glucose and insulin concentrations upon the galactose ingestion (10).

Inhibitors of hepatic phosphorylase have been considered for possible use in the treatment of diabetics. To be effective, GP could have to decline without a compensatory increase in GNG. The report of Fosgerau et al. (8) suggests that that would be the case. The present study suggests that there could be a compensatory increase. That assumes the mechanism resulting in a decrease in GNG when glycogenolysis is increased in normal subjects, as simulated by galactose’s conversion to glucose, operates in diabetic patients to increase GNG when glycogenolysis is decreased.

In conclusion, galactose has been used as a surrogate for glycogen as a source of hepatic GP, since both glycogen and galactose are converted to glucose by the same reaction steps. Glycogen is a more important source of hepatic GP, since both glycogen and glycogenolysis is decreased.

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