Regulators of hepatic glucose and glycogen metabolism

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CHAPTER 8

Glycogenolysis and gluconeogenesis in an extended overnight fast in type 2 diabetes mellitus

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\textit{Diabetes}, submitted
Abstract

In healthy subjects, endogenous glucose production adapts to short term starvation (< 24 h) by a decrease in glycolysis, whereas gluconeogenesis does not change. In type 2 diabetes mellitus plasma glucose concentration decreases faster during short term starvation. To evaluate the adaptation of glycolysis and gluconeogenesis to a short extension of the postabsorptive state, we compared in six patients with type 2 diabetes mellitus plasma glucose concentration, endogenous glucose production and gluconeogenesis between 16 to 20 hours of fasting versus between 20 to 24 hours of fasting. Endogenous glucose production was measured by infusion of [6,6-²H₂]glucose, and gluconeogenesis by administration of ²H₂O. Between 16 to 20 h of fasting, plasma glucose concentration as well as endogenous glucose production decreased by 16% due to a decrease in glycogenolysis by 43%. These changes occurred without changes in glucoregulatory hormones or FFA. Between 20 to 24 h of fasting plasma glucose nor endogenous glucose production changed. Glycogenolysis decreased by only 8% (p < .05), whereas gluconeogenesis increased by 10% (p < .05). Plasma concentrations of FFA increased by 31%. These changes were associated with a decrease in C-peptide levels. These data demonstrate that in type 2 diabetes mellitus, the adaptation of glucose concentration to the postabsorptive state is at least in part caused by a fall in glycogenolysis. Subsequently, glycogenolysis decreases only minimally and a further decrease in endogenous glucose production is prevented by an increase in gluconeogenesis. Therefore, the pattern of changes in glycogenolysis and gluconeogenesis induced by short term starvation differs between patients with 2 diabetes mellitus and those previously published in healthy controls.
**Introduction**

The change in plasma glucose concentration during fasting is the result of the changes in postabsorptive endogenous glucose production and peripheral glucose uptake. In healthy individuals, the decrease in plasma glucose concentration between 16 and 22 hours is minimal (less than 10% from basal) (1-3), despite a linear decrease in endogenous glucose production in the same period by ~20% (1,2,4). Apparently, a major decrease in plasma glucose is prevented by a decrease in peripheral uptake. The decrease in endogenous glucose production is due to a decrease in the rate of glycogenolysis, whereas the absolute rate of gluconeogenesis remains unchanged (2,4).

In type 2 diabetes mellitus a different pattern is seen. In several studies plasma glucose concentration was measured at regular intervals during a 24 hour fast (5,6,7,8,9). These studies show two differences with the data obtained in healthy subjects: a) plasma glucose concentration decreased substantially (~30%) between 16 h and 24 hours of fasting, and b) this decrease was non-linear due to a levelling of the rate of decrease after 20 hrs of fasting. Whether this non-linear decrease in plasma glucose concentration in type 2 diabetes mellitus is due to changes in gluconeogenesis and/or glycogenolysis is currently unknown. Therefore, we compared in six patients with type 2 diabetes mellitus the changes in plasma glucose concentration, the rates of endogenous glucose production and gluconeogenesis after 16 to 20 hours of fasting versus 20 to 24 hours of fasting. Endogenous glucose production was measured by infusion of [6,6-2H₂]glucose, and gluconeogenesis by the deuterated water method (10).

**Study design and methods**

*Subjects:* six patients with type 2 diabetes mellitus were studied. They had no complications from their diabetes. Their clinical characteristics are shown in table 1. Their mean glycosylated hemoglobin level was 7.5 ± 0.5 % and their BMI 28.0 ± 1.5. Except for the presence of type 2 diabetes, they were healthy and were taking no other medication known to affect glucose metabolism. None had been treated with insulin. Oral antidiabetic drugs were discontinued 72 hours before the start of the study. They consumed a weight-maintaining diet of at least 250 g carbohydrates for 3 days before the study. Written informed consent was obtained.
from all subjects. The studies were approved by the Institutional Ethics and Research Committees.

**Table 1: clinical characteristics.**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex (m/f)</th>
<th>Age (yrs)</th>
<th>BMI (kg/m²)</th>
<th>Glyc Hb (%)</th>
<th>FPG (mmol L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>m</td>
<td>54</td>
<td>27.7</td>
<td>6.7</td>
<td>7.5</td>
</tr>
<tr>
<td>2</td>
<td>m</td>
<td>52</td>
<td>32.5</td>
<td>8.5</td>
<td>11.5</td>
</tr>
<tr>
<td>3</td>
<td>m</td>
<td>73</td>
<td>22.0</td>
<td>5.8</td>
<td>7.2</td>
</tr>
<tr>
<td>4</td>
<td>m</td>
<td>65</td>
<td>26.9</td>
<td>8.3</td>
<td>11.9</td>
</tr>
<tr>
<td>5</td>
<td>f</td>
<td>57</td>
<td>31.0</td>
<td>8.9</td>
<td>9.5</td>
</tr>
<tr>
<td>6</td>
<td>m</td>
<td>54</td>
<td>28.0</td>
<td>7.0</td>
<td>8.7</td>
</tr>
</tbody>
</table>

Mean ± SE 5 / 1 59.1 ± 3.3 28.0 ± 1.5 7.5 ± 0.5 9.4 ± 0.81

BMI: body mass index; Glyc Hb: glycosylated hemoglobin; FPG: fasting plasma glucose concentration after a 16 hour fast

**Study design:** the study was designed to compare the adaptation to two different lengths of fast in type 2 diabetes. Since the nadir in plasma glucose concentration is expected around 20 hours of fasting (7,8,9), an extended overnight fast (16-24 hours) was divided into two periods of equal length: one in which the decrease in glucose concentration per hour is supposed to be constant (period A: 16-20 hours of fasting), and one in which the decline in glucose concentration is supposed to be minimal (period B: 20-24 hours of fasting). Each subject served as his or her own control and was studied twice on the same day, to exclude confounding effects that might occur during studies on separate days.

The study started at 8.00 a.m. after a fasting period of 10 hours. A 19-gauge catheter was inserted in a forearm vein for infusion of [6,6-²H₂]glucose. Another 19-gauge catheter was inserted retrogradely into a wrist vein of the contralateral arm and maintained at 60 °C in a thermoregulated plexiglass box for sampling of arterialized venous blood. After obtaining a baseline sample for determination of
background isotopic enrichment and plasma glucose concentration, the subjects ingested 1g/kg body water $^2$H$_2$O (99.7 % enriched, Cambridge Isotopes, Cambridge, MA) with intervals of 30 min until a total dose of 5 g/kg body water was reached. Body water was estimated to be 60% of body weight in men and 50% in women. At the same time a primed, continuous (0.22 μmol/kg/min) infusion of [6,6-$^2$H$_2$]glucose (99 % enriched, Isotech, Miamisburg, OH) dissolved in sterile isotonic saline and sterilized by passage of the solution through a millipore filter (0.2 μm, Minisart; Sartorius, Gottingen, Germany) was started, and continued throughout the study. The priming dose was adapted to plasma concentrations according to the formula derived by Hother-Nielsen et al. (11):

$$\text{adjusted prime} = \text{normal prime} (17.6 \ \mu\text{mol/kg}) \times \left[ \frac{\text{actual plasma glucose concentration (mmol/l)}}{5} \right]$$

Fasting plasma glucose concentration at 8 a.m. was measured at the bedside using a Precision Q.I.D.$^*$TM glucometer (Medisense®, Abbott Laboratories Company, Chicago, Ill).

At t = 0 (i.e. 16 h of starvation), after a six hour equilibration period of [6,6-$^2$H$_2$]glucose infusion, blood samples for measurement of plasma glucose concentration, [6,6-$^2$H$_2$]glucose enrichment, glucoregulatory hormones and free fatty acids (FFA) were obtained every hour until the end of the study (i.e. 24 h of starvation). Blood samples for measurement of enrichment of deuterium at carbon 5 of glucose and of plasma water were obtained every two hours until the end of the study. During the study water for drinking was allowed which was 0.5% enriched with $^2$H$_2$O.

Assays: all measurements were performed in duplicate, except of the deuterium enrichment at carbon 5 of glucose, and all samples from each individual subject were analyzed in the same run. Glucose concentrations and [6,6-$^2$H$_2$]glucose enrichment in plasma were measured using a method adapted from Reinauer et al (12). The aldonitril penta-acetate derivative of glucose was dissolved in ethylacetate. A calibration graph using xylose as an internal standard was used for the determination of glucose concentration. The enrichment of [6,6-$^2$H$_2$]glucose was determined by dividing the peak area at M+2 by the total peak area of the glucose aldonitril penta-acetate peak and correction for the natural abundance by substracting the natural abundance of the M+2 enrichment from the measured M+2 enrichment. The deuterium enrichment at carbon 5 of glucose was measured as
described by Landau et al. (10). Instead of measuring deuterium enrichment at carbon 2 of glucose, we measured body water enrichment as described by van Kreeel et al. (13), which yields similar enrichments as hydrogen enrichment at carbon 2 of plasma glucose in the study design used (4). All isotopic enrichments were measured on a gas chromatograph mass spectrometer (model 6890 gas chromatograph coupled to a model 5973 mass selective detector, equipped with an electron impact ionization mode, Hewlett-Packard, Palo Alto, CA).

Plasma insulin concentration was measured by commercial RIA (Pharmacia Diagnostics AB, Uppsala, Sweden), plasma cortisol levels by enzyme-immunoassay on an Immulite analyser (DPC, Los Angeles, CA), glucagon by RIA (Linco Research Inc., St. Charles, MO); glucagon-antiserum elicited in guinea pigs against pancreatic specific glucagon; cross reactivity with glucagon-like substances of intestinal origin less than 0.1%), and plasma epinephrine and norepinephrine by high performance liquid chromatography with fluorescence detection, using α-methyl norepinephrine as internal standard. Free fatty acids were determined by using the NEFA C kit (code no. 994-75409) from Wako Chemicals (Neuss, Germany).

Calculations and Statistics: endogenous glucose production was calculated by the non-steady state equations of Steele (14) in their derivative form. The effective distribution volume for glucose was assumed to be 165 ml/kg. Gluconeogenesis was calculated by multiplying the fractional contribution of gluconeogenesis (measured deuterium enrichment at carbon 5 of glucose divided by the measured enrichment in body water) with EGP. Glycogenolysis was calculated as the difference of EGP and gluconeogenesis.

The results are reported as mean ± SEM. The data of both periods of starvation were compared by a two-sided non-parametric test for paired samples (Wilcoxon Signed Rank test) and by Spearman's rank test for calculation of correlation coefficients. A p-value of less than 0.05 was considered to represent a statistical significant difference.
Results

Plasma concentrations of glucose and free fatty acids
Between 16 to 20 h of fasting plasma glucose concentration decreased by 16% (p=0.027), whereas between 20 to 24 h of fasting plasma glucose concentration did not change significantly (Fig. 1 and Table 2). Between 16 to 20 h of fasting plasma concentrations of FFA did not change (0.49±0.04 vs 0.51±0.02 mmol/l), whereas from 20 to 24 h of fasting the plasma concentrations of FFA increased by 31% (0.51±0.02 vs 0.67±0.06 mmol/l; p=0.027).

Glucose kinetics
Between 16 to 20 h of fasting endogenous glucose production decreased by 16% (p=0.028), whereas prolongation of the postabsorptive state by another 4 hours did not significantly affect endogenous glucose production (Table 2).

![Graphs showing changes in plasma glucose concentration and endogenous glucose production](image)

Fig 1. Changes in plasma glucose concentration (upper panel) and endogenous glucose production and gluconeogenesis (lower panel) from 16 to 20 hours of fasting and from 20 to 24 hours of fasting.
Metabolic clearance rate did not change between 16 to 20 h of fasting (1.11±0.11 vs 0.13±0.1 ml/kg/min) nor during the subsequent 4 hours of fasting (0.13±0.1 vs 0.12±0.1 ml/kg/min).

The deuterium enrichment at carbon 5 of glucose increased gradually from 26% after an overnight fast of 16 h to 37% at 24 h of starvation (p=0.003). This was not associated with changes in the deuterium enrichment of plasma water (Table 3). Between 16 and 20 h of starvation glycogenolysis decreased by 43% (p=0.028), whereas there was no change in the rate of gluconeogenesis (Table 2). Between 20 to 24 h of fasting glycogenolysis decreased by only 8% (p=0.028), whereas gluconeogenesis increased by 10% (p=0.028). Although glycogenolysis decreased in both periods of fasting, the rate of change was significantly faster between 16-20 h versus 20-24 h of fasting (p=0.028).

**Table 2: The changes in glucose kinetics within the two different fasting periods.**

<table>
<thead>
<tr>
<th>postabsorptive state</th>
<th>16–20 h</th>
<th>p value</th>
<th>20–24 h</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>9.4 ±0.81 - 7.9 ±0.45</td>
<td>0.027</td>
<td>7.9 ±0.45 - 7.5 ±0.37</td>
<td>NS</td>
</tr>
<tr>
<td>Endogenous glucose production (μmol kg/min)</td>
<td>9.5 ±0.42 - 8.0 ±0.62</td>
<td>0.028</td>
<td>8.0 ±0.62 - 8.4 ±0.58</td>
<td>NS</td>
</tr>
<tr>
<td>Gluconeogenesis (μmol kg/min)</td>
<td>4.8 ±0.40 - 5.3 ±0.33</td>
<td>NS</td>
<td>5.3 ±0.33 - 5.9 ±0.30</td>
<td>0.028</td>
</tr>
<tr>
<td>Glycogenolysis (μmol kg/min)</td>
<td>4.7 ±0.43 - 2.7 ±0.43</td>
<td>0.028</td>
<td>2.7 ±0.43 - 2.5 ±0.44</td>
<td>0.028</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; NS: not significant. p < .05 represents a statistical significant difference.
Glycogenolysis and gluconeogenesis in type 2 diabetes

Table 3: Deuterium enrichment (in %) at carbon 5 of glucose and in plasma water in relation to the extension of an overnight fast.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Enrichment at carbon 5 of glucose</th>
<th>p value</th>
<th>Deuterium enrichment of plasma water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16 h</td>
<td>18 h</td>
<td>20 h</td>
</tr>
<tr>
<td>1</td>
<td>0.18</td>
<td>0.21</td>
<td>0.25</td>
</tr>
<tr>
<td>2</td>
<td>0.27</td>
<td>0.32</td>
<td>0.37</td>
</tr>
<tr>
<td>3</td>
<td>0.29</td>
<td>0.31</td>
<td>0.31</td>
</tr>
<tr>
<td>4</td>
<td>0.29</td>
<td>0.34</td>
<td>0.37</td>
</tr>
<tr>
<td>5</td>
<td>0.26</td>
<td>0.31</td>
<td>0.35</td>
</tr>
<tr>
<td>6</td>
<td>0.27</td>
<td>0.32</td>
<td>0.35</td>
</tr>
<tr>
<td>Mean</td>
<td>0.26</td>
<td>0.30</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Hormone concentrations
Between 16 to 20 h of fasting plasma concentrations of insulin, C-peptide, glucagon, cortisol, epinephrine and norepinephrine did not change significantly. Between 20 to 24 of fasting plasma only plasma C-peptide concentrations decreased significantly (Table 4).

Table 4: The changes in hormone concentrations within the two different fasting periods.

<table>
<thead>
<tr>
<th>Postabsorptive state</th>
<th>16 - 20 h</th>
<th>p value</th>
<th>20 - 24 h</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>insulin (pmol/l)</td>
<td>100±17 - 91±17</td>
<td>NS</td>
<td>91±17 - 73±13</td>
<td>NS</td>
</tr>
<tr>
<td>C-peptide (pmol/l)</td>
<td>1040±200 - 1000±252</td>
<td>NS</td>
<td>1000±252 - 792±171</td>
<td>0.028</td>
</tr>
<tr>
<td>cortisol (nmol/l)</td>
<td>254±32 - 243±49</td>
<td>NS</td>
<td>243±49 - 167±44</td>
<td>NS</td>
</tr>
<tr>
<td>glucagon (ng/l)</td>
<td>58±4 - 59±4</td>
<td>NS</td>
<td>59±4 - 60±4</td>
<td>NS</td>
</tr>
<tr>
<td>norepinephrine (nmol/l)</td>
<td>2,01±0,36 - 2,03±0,37</td>
<td>NS</td>
<td>2,03±0,37 - 2,08±0,38</td>
<td>NS</td>
</tr>
<tr>
<td>epinephrine (nmol/l)</td>
<td>0,14±0,07 - 0,18±0,06</td>
<td>NS</td>
<td>0,18±0,06 - 0,21±0,05</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE, NS: not significant. p<0.05: statistical significant difference
Chapter 8

Discussion

This study describes the adaptation of glycogenolysis and gluconeogenesis during a short prolongation of the postabsorptive state in patients with type 2 diabetes mellitus. In line with previous observations plasma glucose concentrations decrease non-linearly during the first 24 hours of fasting in these patients, unlike the linear changes in healthy subjects (2,4). Moreover, our data indicate that the initial decrease in postabsorptive glucose concentrations are at least in part the result of a decrease in glycogenolysis. whereas during a slight prolongation of this overnight fast plasma glucose concentrations do not decline more because an increase in the rate of gluconeogenesis compensates for the further decrease in glycogenolysis.

The rate of change in plasma glucose concentrations was significantly different between the two periods of fasting. This is in accordance with previous studies (5, 6, 8, 9). For instance, Faiman and Moorhouse (5) fasted five patients with type 2 diabetes for 72 h and observed that glucose concentrations did not further decrease after approximately 24 h of fasting. Thus, stabilization of the adaptation of plasma glucose concentration occurs in type 2 diabetes after approximately 20 h of fasting.

A non-linear decrease in endogenous glucose production apparently precedes the non-linear decrease in plasma glucose. Only one of the abovementioned studies measured endogenous glucose production during the initial 24 h of starvation in patients with type 2 diabetes (6). Our data are in line with their observation that endogenous glucose production decreases non-linearly during a prolongation of the postabsorptive state. However, their data obtained between 16 and 19 h of fasting might be subject to error, because the priming dose of the glucose tracer was not adjusted for hyperglycemia and isotopic equilibration was not achieved within the initial 19 h of starvation (6). Nonetheless, in type 2 diabetes the decrease in plasma glucose concentration is non-linear, at least in part as a result of a non-linear fall in endogenous glucose production.

The change in plasma glucose concentration from 16 to 24 h of fasting in healthy subjects is different from type 2 diabetic subjects. In healthy subjects the decrease in glucose concentration is linear (1,2,5). This is associated with a linear decrease in endogenous glucose production between 16 and 24 h of fasting (1,2), due to a decrease in glycogenolysis whereas gluconeogenesis does not change, as has been convincingly shown by Landau et al and Boden et al (2,4). The decrease in the rate
of glycogenolysis seems to be larger in our patients with type 2 diabetes mellitus, than described in healthy controls (~47 vs ~35%). Finally, the regulation of gluconeogenesis during the adaptation to the postabsorptive state is altered in patients with type 2 diabetes, because gluconeogenesis does not change in healthy volunteers between 16 and 24 h of fasting (2,4), whereas gluconeogenesis slightly increases during the same period in our type 2 diabetic patients.

The changes in endogenous glucose production, and thus in gluconeogenesis and glycogenolysis within the first period of fasting occurred without significant changes in glucocounterregulatory hormone concentrations. They can therefore not explain these changes. Between 20 to 24 h of fasting, insulin secretion diminished, as is reflected by the decrease in C-peptide concentrations. Portal insulin concentrations could thus be decreased between 20 to 24 h of fasting, possibly explaining the stabilisation of endogenous glucose production between 20 to 24 h of fasting. Recently the role of FFA as a potential regulator of glucose production has got attention. In healthy volunteers Chen et al recently (2) demonstrated that lowering of plasma FFA by oral administration of nicotinic acid led to a decrease in gluconeogenesis. After stopping the nicotinic acid a FFA rebound led to an acute increase in gluconeogenesis without influencing endogenous glucose production suggesting a decrease in glycogenolysis. In our observational study in patients with type 2 diabetes similar correlations were found between plasma concentrations of FFA and gluconeogenesis. In the first period of starvation plasma FFA nor gluconeogenesis changed. In the second period of fasting plasma FFA increased associated with an increase in gluconeogenesis, as could be expected from the data obtained by Boden and others. They proposed that FFA promote gluconeogenesis by increasing the production of ATP and NADH, as well as by increasing pyruvate carboxylase activity via acetyl-CoA or long-chain fatty acyl-CoA, generated during FFA oxidation (2,15). However, the data from our study do not permit a conclusion with respect to a causal relationship between the changes in FFA and gluconeogenesis.

We conclude that in type 2 diabetes mellitus, the decrease in glucose concentrations during an extension of an overnight fast is, at least in part, caused by a fall in glycogenolysis during the initial period of the fast. Subsequently, the decrease in the rate of glycogenolysis is minimal and a further decrease in endogenous glucose production is prevented by an increase in gluconeogenesis, associated with an increase in plasma FFA concentrations. Therefore, this pattern
of adaptation of glycogenolysis and gluconeogenesis to short term starvation of 24 h differs between patients with type 2 diabetes mellitus and those previously published in healthy volunteers.

Acknowledgements

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References
