Regulation of postabsorptive glucose production in patients with type 2 diabetes mellitus
Pereira Arias, A.M.

Citation for published version (APA):
Pereira Arias, A. M. (2000). Regulation of postabsorptive glucose production in patients with type 2 diabetes mellitus

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
CHAPTER 6

The Effects of Carbohydrate Variation in Isocaloric Diets on Glycogenolysis and Gluconeogenesis in Healthy Men

P.H. Bisschop\textsuperscript{1,6}, A.M. Pereira Arias\textsuperscript{1,6}, M.T. Ackermans\textsuperscript{2}, E. Endert\textsuperscript{2}, H. Pijl\textsuperscript{3}, F. Kuipers\textsuperscript{4}, A.J. Meijer\textsuperscript{5}, H.P. Sauerwein\textsuperscript{1}, J.A. Romijn\textsuperscript{6}

\textsuperscript{1}Dept. of Endocrinology and Metabolism, \textsuperscript{2}Department of Clinical Chemistry and \textsuperscript{3}Dept. of Biochemistry, Academic Medical Center, University of Amsterdam, Amsterdam, \textsuperscript{4}Center for Liver, Digestive and Metabolic Diseases, Academic Hospital Groningen, Groningen, \textsuperscript{5}Dept. of Internal Medicine and \textsuperscript{6}Dept. of Endocrinology, Leiden University Medical Center, Leiden, the Netherlands

Journal of Clinical Endocrinology & Metabolism, in press
Abstract

To evaluate the effect of dietary carbohydrate content on postabsorptive glucose metabolism, we quantified gluconeogenesis and glycogenolysis after 11 days of a high carbohydrate (85% carbohydrate), control (44% carbohydrate) and very low carbohydrate (2% carbohydrate) diet in six healthy males. Diets were eucaloric and provided 15% of energy as protein. Post-absorptive glucose production was measured by infusion of [6,6-\(^2\)H\(_2\)]glucose and fractional gluconeogenesis by ingestion of \(^2\)H\(_2\)O. Postabsorptive glucose production rates were 13.0 ± 0.7, 11.4 ± 0.4 and 9.7 ± 0.4 μmol·kg\(^{-1}\)·min\(^{-1}\) after high carbohydrate, control and very low carbohydrate diet, respectively (p<0.001 between the three diets). Gluconeogenesis was ≈ 14% higher after the very low carbohydrate diet (6.3 ± 0.2 μmol·kg\(^{-1}\)·min\(^{-1}\); p=0.001) compared to the control diet, but was not different between the high carbohydrate and control diet (5.5 ± 0.3 vs 5.5 ± 0.2 μmol·kg\(^{-1}\)·min\(^{-1}\)). The rates of glycogenolysis were 7.5 ± 0.5 μmol·kg\(^{-1}\)·min\(^{-1}\), 5.9 ± 0.3 μmol·kg\(^{-1}\)·min\(^{-1}\) and 3.4 ± 0.3 μmol·kg\(^{-1}\)·min\(^{-1}\), respectively (p<0.001 between the three diets).

We conclude that, under eucaloric conditions in healthy subjects, dietary carbohydrate content affects the rate of post-absorptive glucose production mainly by modulation of glycogenolysis. In contrast, dietary carbohydrate content affects the postabsorptive rate of gluconeogenesis minimally, evidenced only by a slight increase of gluconeogenesis during severe carbohydrate restriction.

Introduction

Nutritional intake is an important determinant of the rate of postabsorptive glucose production. There is a direct relation between carbohydrate intake and postabsorptive glucose production (23). Carbohydrate overfeeding increases postabsorptive glucose production (4), whereas fasting reduces glucose production (12;22). Changes in post-absorptive glucose production reflect changes in gluconeogenesis and/or glycogenolysis, because endogenous glucose can only be derived from gluconeogenesis and glycogenolysis. Quantification of these two pathways is essential for better understanding of changes in intra-hepatic glucose metabolism induced by variations in carbohydrate intake. Several studies have addressed this issue by measuring incorporation of gluconeogenic precursors into glucose. In perfused livers of rats fed a eucaloric carbohydrate-free diet conversion of alanine and pyruvate to glucose is increased compared to a control diet (6). In humans, conversion of alanine to glucose is decreased after several days of
Effects of carbohydrates on glycogenosis and gluconeogenesis

excessive carbohydrate intake (4). Although alanine is an important precursor of gluconeogenesis, extrapolation to total gluconeogenesis should be interpreted with caution.

Recently, Landau et al. described the use of $^{2}$H$_2$O for the measurement of gluconeogenesis (2;14). This method allows for quantification of total gluconeogenesis irrespective of the contribution of individual gluconeogenic precursors. To study the effect of dietary carbohydrate content on the contribution of gluconeogenesis and glycogenolysis to postabsorptive glucose production, we used $^{2}$H$_2$O and measured the effects of variation of carbohydrate content in isocaloric diets on postabsorptive glucose production and gluconeogenesis in 6 healthy men. Each diet was used for 11 days and contained an identical amount of proteins of similar composition, whereas the remainder of the calories consisted of only carbohydrates (diet 1), only fat (diet 2) or an approximately equal distribution of carbohydrates and fat (diet 3).

Subjects, materials and methods

Subjects

Six healthy males (age 29-55 yr., BMI 21-26 kg/m$^2$) were studied on three separate occasions after an overnight fast. All subjects were in good health and did not use any medication. All participating subjects gave written informed consent. This study was approved by the Medical Ethical Committee of the Academic Medical Center.

Diets

The subjects were studied on three occasions, each time after 11 days on a different diet. The sequence of the three studies was determined by random assignment. The three diets consisted of liquid formulas and contained identical amounts (15 % of the calories) and identical protein composition. In addition to the proteins, the high carbohydrate diet (diet 1) contained 85 % of calories in the form of carbohydrates. The control diet (diet 2) contained 44 % of the calories in the form of carbohydrates and 41 % in the form of lipids. The very low carbohydrate diet (diet 3) contained 2 % of the calories in the form of carbohydrates and 83 % in the form of lipids. Caloric requirements for each subject were assessed by a dietician by means of a 3-day dietary journal. Meals with predetermined amounts of calories were taken at six fixed timepoints each day between 8:00 am and 9:30
pm for eleven days. In addition to the diets, the subjects were only allowed to drink water ad libitum. Subjects were seen daily to receive their diet for the next day. All subjects refrained from alcohol and exercise was limited to normal daily activities during the experimental diets. Compliance with the diet was assessed by measuring the respiratory quotient, which reflects the ratio of carbohydrate/fat intake (17). Respiratory quotients were measured after 10 and 11 days of the experimental diet after an overnight fast of 14 hours with an energy expenditure unit (Sensormedics model 2900, Anaheim, CA, USA) using the ventilated hood technique. For each subject the period between the beginning of two successive experimental diets was 8-10 weeks, during which period the subjects used their habitual diet.

Protocol

The subjects were admitted to the clinical research center and studied in the supine position. At 06:45 a.m., after an overnight fast of 10 hour, a catheter was inserted in an antecubital vein in each arm. One catheter was used for sampling of arterialized blood using a heated handbox (60 °C). The other catheter was used for infusion of \([6,6-^2\text{H}_2]\)-glucose. At 06:55 a.m. urine and blood samples were taken for determination of background enrichments of body water and plasma glucose, respectively. From 07:00 until 9:00 a.m. \(^2\text{H}_2\text{O}\) (>99.8 % enriched, Cambridge Isotopes, Ma) was administered orally every half hour up to a total dose of 5 g/kg body water to achieve a deuterium enrichment of body water of approximately 0.5%. Body water was estimated to be 60 % of total body weight. At 9:00 a.m., after taking a blood sample for background enrichment of plasma glucose, a primed-continuous infusion of [6,6-\(^2\text{H}_2\)]glucose (>99 % enriched, Cambridge Isotopes, Ma) was started at a rate of 0.33 μmol/kg/min (prime 26.4 μmol/kg). The subjects voided urine at 11.00 a.m., which was discarded. Subsequently a urine sample was obtained between 11.00 and 12.00 a.m. for determination of body water enrichment. At 11:30, 11:45 and 12:00 a.m. blood samples were taken for enrichment of [6,6-\(^2\text{H}_2\)]glucose and deuterium at C5 of plasma glucose, glucose concentration and plasma levels of glucoregulatory hormones. During the study, subjects were allowed to drink only water, which was 0.5 % enriched with \(^2\text{H}_2\text{O}\).

Analytical procedures

Plasma samples for glucose enrichments of [6,6-\(^2\text{H}_2\)]glucose were deproteinized with methanol (9). The aldonitril penta-acetate derivative of glucose (10) was injected into a gas chromatograph/mass spectrometer system. Separation
Effects of carbohydrates on glycogenolysis and gluconeogenesis

was achieved on a J&W DB17 column (30 m x 0.25 mm, d_f 0.25 μm). Glucose concentrations were determined by gaschromatography using xylose as an internal standard. Glucose was monitored at m/z 187,188 and 189. The enrichment of [6,6-²H₂]glucose was determined by dividing the peak area of m/z 189 by the total peak area and correcting for natural enrichments.

To measure deuterium enrichment at the C5 position, glucose was converted to hexamethylenetetraamine (HMT) as described by Landau et al. (14). HMT was injected into a gaschromatograph mass spectrometer. Separation was achieved on an AT-Amine column (30 m x 0.25 mm, d_f 0.25 μm). HMT consists of six formaldehyde molecules, originally derived from the C5 of six glucose molecules. The distribution of the different masses in HMT can be used to calculate the original deuterium enrichment at C5 by mass isotopomer distribution analysis (MIDA) (9). This adaptation to the method of Landau et al. (14) was validated in our laboratory and the results from this adapted method were not different from the results obtained by using a calibration curve with [1,2,3,4,5,6,6-²H₇]glucose (98%, CIL, Andover, MA, US) (n=18, p>0.9 paired t-test). Quality control was incorporated at two levels. Within each series, unlabeled glucose (Merck, Darmstadt, Germany) was also converted to HMT and M+1 in this HMT was determined. If the measured M+1 was not within 3% of the theoretical value of natural abundance of M+1 the series was rejected. If the series was accepted a second control was measured, a plasma sample with repeatedly measured deuterium enrichment at C5 (0.31 %, n=15, intra-assay coefficient of variation 8%). The series was also rejected, if the measured enrichment from the second control was not within two standard deviations. Deuterium enrichment in body water was measured by a method adapted from Previs et al (20). All isotopic enrichments were measured on a gaschromatograph mass spectrometer (model 6890 gaschromatograph coupled to a model 5973 mass selective detector, equipped with an electron impact ionization mode, Hewlett-Packard, Palo Alto, CA).

Plasma insulin concentration was determined by RIA (Insulin RIA 100, Pharmacia Diagnostic AB, Uppsala, Sweden), intra-assay coefficient of variation: 3-5 %, inter-assay c.v.: 6-9 %, detection limit: 15 pmol/l. C-peptide was determined RIA (RIA-coat c-peptide, Byk-Sangtec Diagnostica GmbH & Co. KG, Dietzenbach, Germany), intra-assay c.v.: 4-6 %, inter-assay c.v.: 6-8 %, detection limit: 50 pmol/l. Cortisol was measured by enzyme-immunoassay on an Immulite analyser (DPC, Los Angeles, CA), intra-assay c.v.: 2-4 %, inter-assay c.v.: 3-7 %, detection limit: 50 nmol/l. Glucagon was determined by RIA (Linco Research, St.
Charles, MO, USA), intra-assay c.v.: 3-5 %, inter-assay c.v.: 9-13 %, detection limit: 15 ng/l. Norepinephrine and epinephrine were determined by an in-house HPLC method. Norepinephrine: intra-assay c.v.: 6-8 %, inter-assay c.v.: 7-10 %, detection limit: 0.05 nmol/l. Epinephrine: intra-assay c.v.: 6-8 %, inter-assay c.v. 7-12 %, detection limit: 0.05 nmol/l. Serum free fatty acids were measured by an enzymatic method (NEFAC; Wako chemicals GmbH, Neuss, Germany), intra-assay c.v. 2-4 %, inter-assay c.v.: 3-6 %, detection limit: 0.02 mmol/l.

Calculations and statistics

The rate of endogenous glucose production \( (R_a) \) was calculated by dividing the infusion rate of \([6,6^{-2}\text{H}_2]\text{glucose}\) by the resulting M+2 enrichment of plasma aldenotril penta-acetate glucose. The fractional rate of gluconeogenesis was calculated by dividing deuterium enrichment at C5 of plasma glucose by deuterium enrichment in body water. The absolute rates of gluconeogenesis were calculated by multiplying fractional gluconeogenesis with endogenous glucose production. Only absolute rates of gluconeogenesis are reported, unless stated otherwise. Glycogenolysis was calculated by subtracting the absolute rate of gluconeogenesis from endogenous glucose production.

The results of the three diets were analyzed with analysis of variance for randomized block design and Fisher LSD-test when appropriate. A p-value <0.05 was considered to be statistically different. Data are presented as means ± SE.

Results

Dietary compliance was assessed by measuring the postabsorptive respiratory quotient after 10 and 11 days of the experimental diets. The respiratory quotient increased with increasing dietary carbohydrate content from 0.73 ± 0.01 to 0.81 ± 0.01 to 0.86 ± 0.02 (p<0.014 for differences between each diet). The postabsorptive (14 h fast) concentrations of plasma glucose (table 1) were not different between the high carbohydrate and the control diets, but were lower after the very low carbohydrate diet compared to control diet (p<0.05). The rate of postabsorptive glucose production depended on the carbohydrate content of the diets: 13.0 ± 0.7, 11.4 ± 0.4 and 9.7 ± 0.4 μmol·kg⁻¹·min⁻¹ after 11 days of high carbohydrate, control and very low carbohydrate diet respectively (p<0.001 between the three diets).
Effects of carbohydrates on glycogenolysis and gluconeogenesis

Table 1. Postabsorptive concentrations of plasma glucose and glucoregulatory hormones after 11 days on high carbohydrate, control and very low carbohydrate diet.

<table>
<thead>
<tr>
<th></th>
<th>high carbohydrate</th>
<th>control</th>
<th>low carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.11 ± 0.11</td>
<td>5.17 ± 0.17</td>
<td>4.65 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>38 ± 3</td>
<td>37 ± 3</td>
<td>25 ± 4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C-peptide (pmol/l)</td>
<td>362 ± 35</td>
<td>435 ± 73</td>
<td>195 ± 55&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucagon (ng/l)</td>
<td>60 ± 4</td>
<td>57 ± 3</td>
<td>65 ± 7</td>
</tr>
<tr>
<td>Cortisol (nmol/l)</td>
<td>224 ± 14</td>
<td>217 ± 21</td>
<td>265 ± 26</td>
</tr>
<tr>
<td>Epinephrine (nmol/l)</td>
<td>0.31 ± 0.07</td>
<td>0.31 ± 0.05</td>
<td>0.24 ± 0.05</td>
</tr>
<tr>
<td>Norepinephrine (nmol/l)</td>
<td>1.94 ± 0.49</td>
<td>1.88 ± 0.29</td>
<td>1.85 ± 0.08</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SE. <sup>a</sup>p<0.05, <sup>b</sup>p<0.01, <sup>c</sup>p<0.001 vs control diet.

Baseline deuterium enrichments of body water and on the C5 position of glucose at the beginning of each infusion protocol were not different between the diets and equaled natural abundance. Therefore, there was no underestimation of fractional gluconeogenesis due to deuterium label on the C5 position of glucose derived from previous experiments. Deuterium enrichments on the C5 position of glucose between 11.30 and 12.00 were constant within each experiment. Actual enrichments of body water and on the C5 position of glucose are shown in table 2. The postabsorptive rates of gluconeogenesis and glycogenolysis are presented in figure 1. Gluconeogenesis was not affected by high carbohydrate diet compared to the control diet, but was ~14 % higher (p=0.001 versus both other diets) after 11 days of very low carbohydrate diet. The rate of glycogenolysis was related to dietary carbohydrate content with the highest rate after high carbohydrate and the lowest rate after very low carbohydrate intake (p<0.001 between the three diets). After 11 days of eucaloric, very low carbohydrate feeding the rate of
Table 2. Mean deuterium enrichments in body water and on the C5 position of glucose between 11.30 and 12.00 a.m.

<table>
<thead>
<tr>
<th>subject</th>
<th>high carbohydrate</th>
<th>control</th>
<th>low carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C5 body water</td>
<td>C5 body water</td>
<td>C5 body water</td>
</tr>
<tr>
<td>1</td>
<td>0.22 0.55</td>
<td>0.27 0.53</td>
<td>0.34 0.51</td>
</tr>
<tr>
<td>2</td>
<td>0.23 0.48</td>
<td>0.22 0.47</td>
<td>0.29 0.47</td>
</tr>
<tr>
<td>3</td>
<td>0.21 0.53</td>
<td>0.24 0.53</td>
<td>0.32 0.52</td>
</tr>
<tr>
<td>4</td>
<td>0.20 0.44</td>
<td>0.22 0.44</td>
<td>0.31 0.46</td>
</tr>
<tr>
<td>5</td>
<td>0.16 0.39</td>
<td>0.18 0.35</td>
<td>0.28 0.42</td>
</tr>
<tr>
<td>6</td>
<td>0.19 0.43</td>
<td>0.21 0.44</td>
<td>0.34 0.48</td>
</tr>
</tbody>
</table>

Glycogenolysis was $3.4 \pm 0.3 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ or ~35% of post-absorptive glucose production.

Plasma insulin and C-peptide concentrations were lower after the very low carbohydrate diet compared to the other diets. Other glucoregulatory hormones were not different between the diets (table 1). Plasma concentrations of free fatty acids were higher after the very low carbohydrate diet compared to the control diet ($0.78 \pm 0.12$ vs $0.36 \pm 0.05 \text{mmol/l}$, $p=0.001$), but were not different between control and the high carbohydrate diet ($0.36 \pm 0.04 \text{mmol/l}$).
Fig.1. Postabsorptive rates of gluconeogenesis and glycogenolysis after 11 days on high carbohydrate, control and very low carbohydrate diet in 6 healthy men. Values are means ± SE. * Indicates a significant difference (p≤0.001) compared to the control diet.

Discussion

This study describes the effects of modulation of carbohydrate content in isocaloric diets on postabsorptive glucose production. The data indicate that the postabsorptive rate of glucose production is a reflection of dietary carbohydrate content. The main mechanism involved is modulation of the rate of glycogenolysis. High dietary carbohydrate intake results in high postabsorptive rates of glycogenolysis without any change in the rate of gluconeogenesis. After very low carbohydrate intake the rate of glycogenolysis is low compared to control feeding and gluconeogenesis is slightly stimulated.

In the present study $^2$H$_2$O was used to quantify gluconeogenesis. The ratio of deuterium enrichment at the C5 position of glucose over the enrichment in body
water was used to quantify fractional gluconeogenesis. Chandramouli et al. showed that deuterium enrichment in body water equals that at the C2 position of glucose in the same study design of isotope administration that we used in the present study (2). Chandramouli et al. also showed that deuterium enrichment at C2 and in body water was essentially at steady state \( \approx 1 \) h after completion of \(^2\text{H}_2\text{O}\) intake (2). Previously we found that deuterium enrichment in body water was at steady state within 1 h after completion of \(^2\text{H}_2\text{O}\) intake under conditions identical to the present study (unpublished data). Since samples for determination of gluconeogenesis in the present study were taken 2.5 h after completion of \(^2\text{H}_2\text{O}\) it is unlikely that steady state was not achieved. However, under other conditions, for instance in diabetes mellitus, a longer period between \(^2\text{H}_2\text{O}\) administration and sampling might be required.

Another methodological issue may be raised, in that we administered both \(^2\text{H}_2\text{O}\) and [6,6-\(^2\text{H}_2\)]glucose, which might result in analytical interference of the isotopomers. However, administration of [6,6-\(^2\text{H}_2\)]glucose in the absence of \(^2\text{H}_2\text{O}\) did not cause a detectable increase above natural abundance in \(M_i\) at the C5 position of glucose as was observed by others (7) as well as ourselves (unpublished data). The increase due to administration of \(^2\text{H}_2\text{O}\) without [6,6-\(^2\text{H}_2\)]glucose in the enrichment in \(M_2\) in the glucose fragment used to measure enrichment from [6,6-\(^2\text{H}_2\)]glucose was negligible at infusion rates of 0.33 \(\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}\) [6,6-\(^2\text{H}_2\)]glucose (unpublished data). Background enrichments for \(M_2\) were taken 2 hours after the first administration of \(^2\text{H}_2\text{O}\), to reduce possible interference even further. Therefore it is very unlikely that the results in the present study are subject to methodological errors.

To study the effects of varying carbohydrate intake two approaches are possible. Carbohydrates can be simply added to or removed from a standard diet without altering absolute amounts of fat and protein, as has been done before. These studies indicate that post-absorptive glucose production is related to carbohydrate intake (23) and that excessive carbohydrate intake reduces gluconeogenesis (4). However, this approach also affects caloric intake. To our knowledge, studies have not been carried out studying the effect of isocaloric changes in carbohydrate to fat ratio on gluconeogenesis and glycogenolysis. Therefore, in our approach we replaced carbohydrates by fat to maintain a constant caloric intake.

In the present study the amount and composition of proteins between the three diets were identical, precluding any effect of protein intake on the differences
observed in our study in postabsorptive glucose metabolism. Interestingly, post-absorptive glucose production still amounted to 9.7 µmol·kg⁻¹·min⁻¹ after 11 days of carbohydrate but not caloric deprivation, whereas in other studies prolonged fasting resulted in lower rates of glucose production, ranging from 7.9 to 8.7 µmol·kg⁻¹·min⁻¹ (2;10;12;22). These data suggest that the rate of glucose production after isocaloric carbohydrate deprivation is higher than during carbohydrate deprivation in starvation. This might be attributed to an adequate protein intake during the very low carbohydrate diet in contrast to starvation, because modulation of protein intake affects glucose production (15;16).

Our results indicate that the rate of post-absorptive glucose production depends on the amount of carbohydrate intake, as glucose production is reduced by diminishing carbohydrate intake. This reduction in glucose production is caused exclusively by a decrease of the rate of glycogenolysis. Glucoregulatory hormones, such as glucagon, adrenalin, insulin and glucocorticoids, have a distinct modulatory effects on glycogenolysis (1). It seems unlikely that glucoregulatory hormone levels contributed to the differences in glycogenolysis, since most hormone levels were not different between the diets. Plasma insulin levels were even lower after the very low carbohydrate diet, which would favour an increase rather than a decrease of glycogenolysis. Therefore, it is likely that other factors are involved. For instance, the rate of glycogenolysis might, at least in part, be regulated by hepatic glycogen concentrations. In the present study glycogenolysis is defined as the rate of breakdown of glycogen molecules that were already present before administration of ²H₂O, because formation of glycogen from gluconeogenesis and subsequent conversion to glucose during the study would be measured as gluconeogenesis. Surprisingly, glycogenolysis still accounted for ~35 % of post-absorptive glucose production after eleven days of virtually absent carbohydrate intake, which indicates that glycogen stores were not fully depleted. This is supported by the observation that hepatic glycogen concentration in rats after 4 weeks of high-fat feeding was still ≈50 % of that of carbohydrate-fed animals (5). Since glycogen could not have been derived from dietary carbohydrates after 11 days of carbohydrate deprivation, the contribution of glycogen to postabsorptive glucose production must ultimately have been derived from gluconeogenesis, shuttled to glycogen.

The rate of gluconeogenesis is regulated by several factors, including glucoregulatory hormones (19). Insulin suppresses gluconeogenesis, whereas glucagon, glucocorticoids and catecholamines enhance gluconeogenesis (18).
Other factors include free fatty acids, which have been shown to stimulate gluconeogenesis (3). In the present study the rate of gluconeogenesis was not different between high and intermediate carbohydrate feeding, which is compatible with the fact that neither glucoregulatory hormones nor free fatty acid concentrations were different. The fact that eucaloric, high carbohydrate intake had no suppressive effect on postabsorptive gluconeogenesis appears to be in contrast to hypercaloric carbohydrate overfeeding, which reduces gluconeogenesis from alanine (4). This discrepancy may be due to methodological differences as well differences in study design. In the present study total gluconeogenesis was measured, i.e. the sum of all precursors incorporated into glucose, instead of incorporation of a single gluconeogenic precursor. Moreover, hypercaloric carbohydrate overfeeding increased glucose and insulin concentrations (4), both causing inhibition of phosphoenolpyruvate carboxykinase activity (7;11), an enzyme that contributes to the control of gluconeogenesis.

Eucaloric low carbohydrate feeding, i.e. high-fat feeding, stimulated gluconeogenesis, associated with increased plasma free fatty acid and decreased plasma insulin levels. A similar effect of eucaloric low carbohydrate feeding has been observed in rats (6). As has been proposed before (6), mitochondrial acetyl CoA probably plays a pivotal role, because after 11 days of virtually no carbohydrate intake fatty acids are the main substrate for oxidation, which results in production of large amounts acetyl CoA. Acetyl CoA activates pyruvate carboxylase, which might accelerate gluconeogenesis because pyruvate carboxylase has a high flux control coefficient in gluconeogenesis (8). In addition, the decrease in plasma insulin might also stimulate gluconeogenesis by induction of phosphoenolpyruvate carboxykinase. Therefore, a very low carbohydrate diet probably stimulates gluconeogenesis by enhanced fatty acid availability and reduced insulin levels.

In conclusion, carbohydrate intake affects post-absorptive glucose production mainly by modulation of glycogenolysis. The post-absorptive rate of gluconeogenesis is not affected by high carbohydrate intake, but increases after eucaloric very low carbohydrate feeding.

**Acknowledgements**

This study was supported by the Dutch Diabetes Foundation, grant 96.604. We thank An Ruiter from the Dept. of Clinical Chemistry for analytical assistance.
Effects of carbohydrates on glycogenolysis and gluconeogenesis

Reference List


