Effects of hyperglycemia on glucose metabolism before and after oral glucose ingestion in normal men

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1Service de Nutrition-Diabétologie, Hôpital Haut-Lévêque, Pessac, and Université de Bordeaux 2 - Victor Segalen, Bordeaux, France; and 2Institut de Physiologie, Université de Lausanne, Lausanne, Switzerland

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Rigalleau, Vincent, Marie-Christine Beauvieux, Jean-Louis Gallis, Henri Gin, Philippe Schneiter, and Luc Tappy. Effects of hyperglycemia on glucose metabolism before and after oral glucose ingestion in normal men. Am J Physiol Endocrinol Metab 290: E1198–E1204, 2006.—The plasma glucose excursion may influence the metabolic responses after oral glucose ingestion. Although previous studies addressed the effects of hyperglycemia in conditions of hyperinsulinemia, it has not been evaluated whether the route of glucose administration (oral vs. intravenous) plays a role. Our aim was to determine the effects of moderately controlled hyperglycemia on glucose metabolism before and after oral glucose ingestion. Eight normal men underwent two oral glucose clamps at 6 and 10 mmol/l plasma glucose. Glucose turnover and cycling rates were measured by infusion of [2H7]glucose. The oral glucose load was labeled by i-6,6-3H2glucose to monitor exogenous glucose appearance, and respiratory exchanges were measured by indirect calorimetry. Sixty percent of the oral glucose load appeared in the systemic circulation during both the 6 and 10 mmol/l plasma glucose tests, although less endogenous glucose appeared during the 10 mmol/l tests before glucose ingestion (∆P < 0.05). This inhibitory effect of hyperglycemia was not detectable after oral glucose ingestion, although glucose utilization was increased (+28%, ∆P < 0.05) due to increased nonoxidative glucose disposal [10 vs. 6 mmol/l: +20%, not significant (NS) before oral glucose ingestion; +40%, ∆P < 0.05 after oral glucose ingestion]. Glucose cycling rates were increased by hyperglycemia (+13% before oral glucose ingestion, ∆P < 0.001; +31% after oral glucose ingestion, ∆P < 0.05) and oral glucose ingestion during the 6 (+10%, ∆P < 0.05) and 10 mmol/l (+26%, ∆P < 0.005) tests. A moderate hyperglycemia inhibits endogenous glucose production and contributes to glucose tolerance by enhancing nonoxidative glucose disposal. Hyperglycemia and oral glucose ingestion both stimulate glucose cycling.

Hormonal responses play an important role in these changes. Euglycemic clamp studies have shown that insulin stimulates glucose utilization (8) and inhibits endogenous glucose production (22). Low plasma glucagon levels also play a role in the reduction of endogenous glucose production (6, 25).

The glycemic response after glucose ingestion may also contribute to these changes. Hyperglycemic clamp studies have shown that hyperglycemia can specifically reduce endogenous glucose production (7, 23) and stimulate oxidative and nonoxidative glucose disposal (21, 28). However, the fate of glucose may differ according to the route of glucose administration (i.e., oral vs. intravenous). Therefore, we used an oral glucose-clamp technique (OG-clamp) (27), which specifically enables the study of the route of glucose administration at constant glyceremia.

We measured endogenous glucose production and oxidative and nonoxidative glucose disposal in eight normal middle-aged men before and after ingestion of a 0.7 g/kg glucose load. The oral load was labeled by deuterated glucose to measure the rate of exogenous oral glucose appearance. Glucose turnover and cycling rates were measured by the infusion of [3H2]glucose (41). Plasma glucose, insulin, and glucagon levels were controlled by the pancreatic clamp technique (infusion of somatostatin plus replacement dose of glucagon plus insulin infusion). To determine the effect of a moderate hyperglycemia, all of the subjects were studied twice at plasma glucose levels of 6 and 10 mmol/l.

MATERIALS AND METHODS

Subjects. Eight subjects were studied and underwent the two tests in a random order. Subjects were healthy, middle-aged men (age: 51.6 ± 2.1 yr, body mass index: 24.5 ± 1.1 kg/m2). None of them had a personal or family history of diabetes or obesity, nor were they receiving any medication. Subjects gave their written consent to the study after being informed of its nature, purpose, and potential risks. The protocol was approved by the ethical committee of the Centre Hospitalier Universitaire de Bordeaux (Bordeaux University Hospital).

Experimental protocol. All subjects were studied in the postabsorptive state after a 12-h overnight fast. To collect arterialized venous blood, a retrograde catheter was inserted in a dorsal hand vein, with the hand kept in a warming blanket. A forearm vein of the contralateral arm was catheterized for the infusions, and respiratory exchanges were measured with a Deltatrac metabolic monitor (Datex).

One hour after the beginning of respiratory gas exchange monitoring, a hyperinsulinemic hyperglycemic clamp was started, with con-

IN NORMAL INDIVIDUALS, oral glucose ingestion is followed by a number of metabolic events that condition glucose tolerance. The major portion of ingested glucose appears in the peripheral circulation, although some is retained in the splanchnic bed, and endogenous glucose production is slightly reduced (25, 35). Ten to fifteen percent of the ingested glucose is cycled in the liver participating in the total glucose output after a first-pass splanchnic uptake (6). There is an increase in both oxidative (energy production) and nonoxidative (energy storage essentially as glycogen in muscles and liver, but also possibly as lipids) glucose disposal (15, 25, 35).

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comitant infusions of somatostatin (50 nmol·kg⁻¹·min⁻¹), insulin (2.44 pmol·kg⁻¹·min⁻¹), and glucose (0.5 ng·kg⁻¹·min⁻¹) aimed at reproducing plasma insulin and glucagon concentrations usually observed 2 h after a 75-g oral glucose tolerance test (OGTT) (30). These infusions were continuously administered during the next 420-min period, whereas plasma glucose levels were held constant at 6 or 10 mmol/l by means of a variable infusion of glucose (15 g/100 ml). A primed infusion of [³H]glucose (prime 12 μmol/kg; Cambridge Isotope Laboratories, Andover, MA) was started at the same time. The infusion rate was proportional (0.6%) to the “cold” glucose infusion rate according to the hot-clamp technique (17). An oral glucose load (700 mg/kg glucose, enriched with 5% d-[6,6-³H₂]glucose) was ingested at 240 min.

Blood samples were drawn at 0, 45, and 120 min and every 30 min thereafter for determination of plasma metabolites, hormones, and isotopic enrichments.

**Analytical procedures.** Plasma glucose was measured with a Hexose glucose analyzer (Angelholm, Sweden). Plasma insulin, C-peptide, and glucagon were measured using radioimmunoassays.

For isotopic analysis, plasma was deproteinized with 6% perchloric acid, neutralized with 3.2 M K₂CO₃, and partially purified over sequential cation exchange anion exchange resins (AG 50W-X8 and AG1X8; Bio-Rad, Richmond, CA). Pentacyclyl tertbutyl derivatives were analyzed by gas chromatography-mass spectrometry (GC 5890-MS5971; Hewlett Packard, Palo Alto, CA) in chemical ionization mode, with isotopic enrichments being measured using mass spectrometry (GC 5890-MS5971; Hewlett Packard, Palo Alto, CA) in chemical ionization mode, with selective monitoring of mass-to-charge ratios (m/z) 331, 333, 337, and 338 to determine concentrations of d-[6,6-²H₂]-, [³H₆]-, and [³H₂]glucose, respectively. With the use of [³H]glucose and cold glucose, two calibration curves were established by measuring abundances of m/z 331, 337, and 338 on samples with increasing [³H] glucose isotopic enrichments. The first calibration curve

\[
\frac{338}{331} = a \cdot \left(\frac{[²H₂] \text{glucose}}{\text{cold glucose}}\right) - b
\]

was used to calculate [²H₂]glucose isotopic enrichments from 338/331 abundance ratios. The second calibration curve

\[
\frac{337}{331} = a' \cdot \left(\frac{338}{331}\right) - b'
\]

was used to correct 337 abundances for the contribution of [³H₂]glucose to mass 337 (36).

**Calculations.** Rates of total glucose appearance (RaT) and disappearance (RaD) were calculated from [²H₂]glucose isotopic enrichments expressed as molar ratios (tracer-to-tracee; MRD') and plasma glucose concentrations (C) by the use of non-steady-state equations, with a pool fraction (p) of 0.75 and a distribution volume (V) of 0.2 l/kg (40):

\[
RaT = \frac{F - pVC(dMRD'/dt)}{MRD'}
\]

\[
RaD = RaT - pV\frac{dC}{dt}
\]

By using d-[6,6-²H₂]glucose enrichments expressed as molar ratios MRD2, non-steady-state calculations were also performed for determination of rates of exogenous glucose appearance (Ra ineff.), as proposed by Proietto et al. (33):

\[
Ra ineff = \frac{[RaT \times MRD2] + [pV \times (dMRD2/dt)]}{MRD2 \text{ of ingested glucose}}
\]

Total glucose output (TGO), representing the total flux through glucose-6-phosphatase, was calculated from RaT, Ra ineff, and glucose infusion rate (M) before oral glucose ingestion

\[
TGO = RaT - (M + Ra ineff)
\]

The same calculations were also performed using ([³H₂] + [³H₆]-)glucose isotopic enrichments to obtain net endogenous glucose production (EGP). The rate of glucose cycling (GC) was calculated as

\[
GC = TGO - EGP
\]

To be detected as [³H₆]glucose, the infused [³H₂]glucose must (1) penetrate in glucose-producing cells and be phosphorylated by glucokinase, (2) release one deuterium in the reversible hexose-isomerase reaction, (3) be dephosphorylated by glucose-6-phosphatase, and (4) finally be released in the circulation. GC, therefore, represents the balance between the simultaneously occurring glucose phosphorylation catalyzed by glucokinase and dephosphorylation catalyzed by glucose-6-phosphatase.

Total glucose oxidation (GOX) and lipid oxidation rates were estimated from gaseous exchanges measurements, using the specific equation for GOX when negative lipid oxidation rates indicative of net lipogenesis from glucose were found (12). Nonoxidative glucose disposal rates, representing the portion of plasma glucose stored as muscular and hepatic glycogen, and lipids, or recycled through the Cori cycle, were calculated (14) as

\[
\text{GnomOX} = RaT - \text{GOX}
\]

**Statistical analysis.** Results are shown as means ± SE. The changes in hormones, substrate concentrations, and turnover rates over time, the comparisons between parameters measured with intravenous glucose alone (180–240 min) and oral glucose (240–480 min), and the results from tests at 6 and 10 mmol/l plasma glucose levels were analyzed by paired t-tests corrected for multiple comparisons. P < 0.05 was considered as significant.

**RESULTS**

Clamp conditions. In the postabsorptive state, plasma glucose and insulin levels did not differ before the 6 and 10 mmol/l tests. As shown on Fig. 1, insulin levels were similarly increased during the 6 and 10 mmol/l tests, and a 4 mmol/l plasma glucose difference (P < 0.0001) was maintained throughout the clamp period. C-peptide levels were promptly suppressed after the onset of the somatostatin-insulin infusions and remained suppressed until the end of the tests. Glucagon levels were lower during the clamps than before, with no difference between the 6 and 10 mmol/l tests. Table 1 provides the hormonal levels at 0, 240 (before oral glucose ingestion), and 480 min (end of the tests). The glucose infusion rates before oral glucose ingestion were higher during the 10 mmol/l tests (10 mmol/l: 39.6 ± 5.4 μmol·kg⁻¹·min⁻¹, 6 mmol/l: 31.3 ± 5.3 μmol·kg⁻¹·min⁻¹, P < 0.05). They similarly declined to a nadir during the second hour after glucose ingestion (10 mmol/l: 28.5 ± 5.9 μmol·kg⁻¹·min⁻¹, 6 mmol/l: 22.2 ± 6.0 μmol·kg⁻¹·min⁻¹) and progressively increased thereafter (last hour: 10 mmol/l, 36.0 ± 6.1 μmol·kg⁻¹·min⁻¹; 6 mmol/l, 27.6 ± 6.4 μmol·kg⁻¹·min⁻¹).

**Isotopic enrichments.** The time course of the labeling of plasma glucose as [³H₂]-, [³H₆]-, and d-[6,6-²H₂]glucose is depicted in Fig. 2. Conversion of fructose 6-phosphate to mannose 6-phosphate, may theoretically, generate 2H₅; however, because we did not detect any significant m + 5 enrichment of plasma glucose during the experiments, this pathway was considered to be very low and, hence, was neglected.

According to the hot-clamp technique, [²H₂]glucose enrichment changes were minimal throughout the experiments. The time courses of d-[6,6-²H₂]glucose enrichments were similar...
During the 6 and 10 mmol/l tests, with slightly lower levels during 10 mmol/l tests that required higher glucose infusion rates.

Rates of glucose disappearance. Before oral glucose ingestion, total $R_{dT}$ were slightly (+14%), although not significantly, increased during the 10 mmol/l tests (Fig. 3). This increase was amplified and became significant (+28%, $P < 0.05$) after oral glucose ingestion. This was entirely due to an increased nonoxidative glucose disposal rate (10 vs. 6 mmol/l: +20%, NS before oral glucose ingestion; +40%, $P < 0.05$ after oral glucose ingestion), whereas glucose oxidation was similarly increased after oral glucose ingestion during the 6- (+20%, $P < 0.05$) and 10 mmol/l tests (+21%, $P < 0.01$). Lipid oxidation rates declined after oral glucose ingestion during both the 6 (−57%, $P < 0.01$) and 10 mmol/l tests (−21%, $P < 0.01$). They did not differ between 6 and 10 mmol/l tests, and net de novo lipogenesis, as reflected by negative lipid oxidation rates, occurred only marginally in two of the 6 mmol/l tests after oral glucose ingestion and one of the 10 mmol/l tests before oral glucose ingestion.

Rates of glucose appearance. $R_{aE}$ did not differ between the 6 and 10 mmol/l tests (Fig. 4). They represented 55 and 63% of the 700 mg/kg oral glucose load, respectively. TGO also did not differ, although it tended to be lower before oral glucose ingestion during the 10 mmol/l tests. EGP was more inhibited during the 10 mmol/l tests ($P < 0.05$) before oral glucose ingestion but became similar in both tests after oral glucose ingestion. GC rates were higher throughout the 10 mmol/l tests (+13% before oral glucose ingestion, $P < 0.001$; +31% after oral glucose ingestion, $P < 0.05$). They were also higher after than before they were before the oral glucose ingestion, both during the 6 (+10%, $P < 0.05$) and 10 mmol/l (+26%, $P < 0.005$) tests.

DISCUSSION

The major aim of this study was to find out whether and how hyperglycemia contributes to glucose tolerance in normal men. We examined the influence of a moderate and controlled hyperglycemia on glucose turnover rates both before and after oral glucose ingestion.

About 60% of the oral glucose load appeared in the systemic circulation during the 4 h after ingestion. This proportion is similar to those of a double-labeled OGTT study (25) or our previous double-labeled OG-clamp study (36) but lower than the 100% appearance of labeled glucose administered by duodenal infusions (16, 26). The remaining 40% may be exogenous glucose metabolized in splanchic tissues, which did not, therefore, reach the systemic circulation. Alternatively, it is possible that substantial portions of oral glucose remained unabsorbed in the intestinal lumen during the study period. Fery et al. (16) have indeed shown that somatostatin infusion during the 6 and 10 mmol/l tests, with slightly lower levels during 10 mmol/l tests that required higher glucose infusion rates.

Table 1. Insulin, C-peptide, and glucagon levels during 6 and 10 mmol/l plasma glucose tests

<table>
<thead>
<tr>
<th>Tests, mmol/l</th>
<th>Time 0 min</th>
<th>Before Oral Glucose Ingestion</th>
<th>After Oral Glucose Ingestion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Plasma insulin, pmol/l</td>
<td>46±8</td>
<td>41±9</td>
<td>164±12</td>
</tr>
<tr>
<td>Plasma C-peptide, pmol/l</td>
<td>187±26</td>
<td>204±25</td>
<td>&lt;40</td>
</tr>
<tr>
<td>Plasma glucagon, ng/l</td>
<td>93±14</td>
<td>100±9</td>
<td>61±16</td>
</tr>
</tbody>
</table>

Values are means ± SE.
substantially delays the rate of glucose absorption during constant duodenal glucose infusion and that about 36% of duodenally administered glucose remained in the intestinal lumen during the initial 3 h of infusion. Unfortunately, due to the known effect of somatostatin infusion, our present protocol cannot reliably assess first-pass splanchnic glucose uptake.

Hyperglycemia alone, i.e., before glucose ingestion, increased glucose $R_{dT}$, as reported during hyperglycemic clamps (1, 2, 5, 18, 28, 29, 43). The increase of glucose $R_{dT}$ during 10 mmol/l tests did not reach significance, probably because of the moderate level of hyperglycemia chosen in these experiments. Glucose $R_{dT}$ further increased significantly after oral glucose ingestion. This was probably not a time effect because the tests conducted at 6 and 10 mmol/l had the same duration. The increase in glucose infusion rates during prolonged euglycemic clamps reached a plateau by 5–6 h (39), whereas the difference between 6 and 10 mmol/l tests was maintained during the 7th hour of our tests. It may reflect the synergistic effect of hyperglycemia and hyperinsulinemia on major glucose disposal pathways (13). In contrast, a time effect could explain the slight increase in glucose oxidation that was observed after oral glucose ingestion: a +10% increment has indeed been reported by Soop et al. (39) between the 4th and 7th hours of prolonged euglycemic clamps. Glucose oxidation did not differ between

![Fig. 2. Time course of plasma glucose enrichments (expressed as molar ratios × 100) by [2H7]- (A), [2H6]- (B), and D-[6,6-2H2]glucose (C). ○, 6 mmol/l plasma glucose tests; ●, 10 mmol/l plasma glucose tests.](image-url)
our 6 and 10 mmol/l tests; the higher glucose RdT during the 10 mmol/l tests were due to a significant increase of nonoxidative glucose disposal, which tended to rise further after oral glucose ingestion. Nonoxidative glucose disposal has been shown to plateau after the 6th hour of hyperinsulinemic euglycemic clamps (39). Although hyperglycemia increases both glucose oxidative and nonoxidative disposal at basal insulin levels (44), the effect on nonoxidative glucose disposal is more pronounced during hyperinsulinemia (11, 28). Our results do not differ from those of hyperglycemic clamp experiments on this point, but they extend these observations by showing that hyperglycemia induced by oral glucose further stimulates nonoxidative glucose disposal. Other investigators compared hepatic glucose storage (15) or direct vs. indirect hepatic glycogen synthesis (34) during intraduodenal (15) or oral vs. intravenous glucose administrations (34), but they failed to notice a specific effect of enteral glucose administration on hepatic glucose storage and glycogen synthesis. However, in these experiments, similar effects on hepatic and peripheral glucose handling were obtained at higher plasma glucose levels when glucose was administered intravenously. Our results show that such high levels of glycemia stimulate nonoxidative glucose disposal during oral glucose administration. This stimulation seems more effective after oral glucose ingestion, which accords with the hypothesis that a hepatic insulin sensitizing substance (32) may be secreted in that occurrence. The fate of the nonoxidized glucose could not be determined from our results. Similar (and marginally negative) rates of total lipid oxidation during the 6 and 10 mmol/l tests suggest that de novo lipogenesis can be neglected and that glycogen storage accounts for the major portion of nonoxidative glucose disposal. Lactate production and glucose lactate cycling may have been stimulated as well and may have contributed to maintain a significant glucose production after oral glucose.

EGP was more suppressed during the 10 mmol/l tests than it was during the 6 mmol/l tests before oral glucose ingestion, as reported in normal (4, 7) or type 1 diabetic subjects (23). We did not observe this suppressive effect of hyperglycemia after oral glucose ingestion; one-half of the suppression of EGP during OGTTs, therefore, seemed attributable to insulin. It is not clear why hyperglycemia did not further suppress it after oral glucose ingestion. Although systemic glycemia was maintained at 6 and 10 mmol/l throughout the tests, oral glucose administration would be expected to lead to a much larger increase in portal glycemia. Therefore, it is possible that the difference in systemic glycemia did not accurately reflect differences in portal glycemia during this part of the protocol. Alternatively, plasma glucagon concentrations are normally suppressed after oral glucose (30), and the decline in glucagonemia contributes to inhibit glucose production (9). This regulatory mechanism was disrupted in our experiments due to the infusion of somatostatin and maintenance of constant systemic and portal glucagon concentrations.

Net hepatic glucose output is determined by the balance of flux through glucokinase and glucose-6-phosphatase, the im-

![Fig. 3. Rates of total glucose disappearance (RdT) (μmol·kg⁻¹·min⁻¹) during the 6 and 10 mmol/l plasma glucose tests before (180–240 min) and after (240–480 min) oral glucose ingestion. White bars represent nonoxidative glucose disposal (GnonOx), and gray bars represent total glucose oxidation (GOx). *P < 0.05; **P < 0.01 between, before, and after oral glucose ingestion for the same plasma glucose level; §P < 0.05 between the 6 and 10 mmol/l plasma glucose tests at the same time.](http://ajpendo.physiology.org/)

![Fig. 4. Rates of total glucose appearance (RaT; μmol·kg⁻¹·min⁻¹) during the 6 and 10 mmol/l plasma glucose tests before (180–240 min) and after (240–480 min) oral glucose ingestion. White bars represent glucose cycling (GC), hatched bars represent endogenous glucose production (EGP), gray bars represent glucose infusion rates (M), and black bars represent rates of exogenous (oral) glucose appearance (RaE). *P < 0.05; ***P < 0.001 between before and after oral glucose ingestion for the same plasma glucose level; §P < 0.05; §§P < 0.01; §§§P < 0.001 between 6 and 10 mmol/l plasma glucose tests at the same time.](http://ajpendo.physiology.org/)
portance of which is reflected in GC (10). Hyperglycemia per se may inhibit net glucose output by increasing the flux via glucokinase (38). For example, it has been shown that suppression of glucose production by hyperglycemia is blunted in maturity onset diabetes of the young patients with glucokinase mutations (42). In addition, hyperglycemia has been reported to reduce the glucose-6-phosphatase activity, whereas the reduction of hepatic glucose production by insulin in euglycemic conditions is mediated by a decrease of intrahepatic glucose 6-phosphate concentrations (19, 38). Our observation of a decreased net glucose production together with increased GC suggests that hyperglycemia increased the absolute fluxes through both glucokinase and glucose-6-phosphatase, but predominantly on glucokinase.

A GC rate increased by hyperglycemia during somatostatin-insulin infusions has been reported in rats (20, 38) and mice (37). This effect was not detected in humans when glucose cycle was determined by coinfections of [2H3]- and [6H]glucose (24) or [6,14C]glucose (4). Methodological differences may account for this discrepancy. The monitoring of m + 6 and m + 7 during the infusion of only one isotope as permitted by the use of [2H3]glucose is probably more sensitive. In the experiments done by Bell et al., (4) the difference between the turnover rates tended to increase, although not significantly, with the plasma glucose levels (+0.5, +0.6, and +0.8 mg·kg−1·min−1 at 95, 135, and 175 mg/dl, respectively). The slight hyperglycemia and lack of glucagon infusion may explain why the GC rate did not increase in the experiments done by Karlander et al. (24); glucagon is known to stimulate GC (31). Similar isotope infusion rates during variable “cold” M, as employed in these previous studies, are also known to erroneously underestimate tracer-derived turnover rates, which may underestimate the difference between two calculated rates.

In one study (42) with [2-2H]- and [6-6,2H2]glucose administered together with the nonlabeled glucose, glucose cycle was higher at 10 than at 5 mmol/l plasma glucose (2.1 vs. 1.5 μmol·kg−1·min−1) and even higher with oral glucose ingestion (3.4 μmol·kg−1·min−1). The difference between 2-3H- and 3-3H-derived turnover rates was also higher in the postprandial than in the postabsorptive state in normal subjects (3).

In summary, we show that a moderately controlled hyperglycemia, such as type 2 diabetes or impaired glucose tolerance, such as type 2 diabetes or impaired glucose tolerance, remain to be determined.

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