Effect of fasting on the intracellular metabolic partition of intravenously infused glucose in humans

F. Fery, L. Plat, and E. O. Balasse

Laboratory of Experimental Medicine and Department of Endocrinology, Erasmus Hospital, University of Brussels, B-1070 Brussels, Belgium

Fery, F. L. Plat, and E. O. Balasse. Effect of fasting on the intracellular metabolic partition of intravenously infused glucose in humans. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E815–E823, 1999.—The effects of fasting on the pathways of insulin-stimulated glucose disposal were explored in three groups of seven normal subjects. Group 1 was submitted to a euglycemic hyperinsulinemic clamp (−100 µU/ml) after both a 12-h and a 4-day fast. The combined use of [3-3H]- and [U-14C]glucose allowed us to demonstrate that fasting inhibits, by ~50%, glucose disposal, glycolysis, glucose oxidation, and glycogen synthesis via the direct path. In group 2, in which the clamp glucose disposal during fasting was restored by hyperglycemia (155 ± 15 mg/dl), fasting stimulated glycogen synthesis (+29 ± 2%) and inhibited glycolysis (−32 ± 3%) but only in its oxidative component (−40 ± 3%). Results were similar in group 3 in which the clamp glucose disposal was restored by a pharmacological elevation of insulin (−2,800 µU/ml), but in this case, both glycogen synthesis and nonoxidative glycolysis participated in the rise in nonoxidative glucose disposal. In all groups, the reduction in total carbohydrate oxidation (indirect calorimetry) induced by fasting markedly exceeded the reduction in circulating glucose oxidation, suggesting that fasting also inhibits intracellular glycogen oxidation. Thus prior fasting favors glycogen retention by three mechanisms: 1) stimulation of glycogen synthesis via the direct pathway; 2) preferential inhibition of oxidative rather than nonoxidative glycolysis, thus allowing carbon conservation for glycogen synthesis via the indirect pathway; and 3) suppression of intracellular glycogen oxidation.

Prior fasting has been shown to inhibit glucose oxidation and to stimulate nonoxidative glucose disposal after glucose refeeding in normal subjects (10, 11) and patients with type 2 diabetes (8, 9), thereby favoring glycogen repletion. To our knowledge, only two studies (22, 34) have examined, in humans, the effects of fasting on the pathways of glucose disposal when glucose is administered intravenously during an euglycemic hyperinsulinemic clamp. In both studies, fasting was seen to reduce insulin sensitivity and the reduction in glucose uptake was entirely due to reduced oxidation, with the nonoxidative disposal remaining unchanged. The difficulty in interpreting such data is that the reduced uptake might in itself affect intracellular glucose partition (oxidation vs. storage; Ref. 5), thus masking the specific effect of fasting on this process. In addition, the aforementioned studies provide only limited information about the metabolic fate of glucose because they rely exclusively on indirect calorimetry as the experimental tool for the exploration of this problem.

In the present work, we attempted to define more precisely the impact of fasting on the pathways of intravenous glucose disposal. For this purpose, we performed euglycemic hyperinsulinemic clamps after 12 h and 4 days of fasting in normal subjects with indirect calorimetry combined with a dual-isotope technique so as to quantify the rates of uptake, oxidation, glycolysis, and nonoxidative glycosylation of circulating glucose, as well as its conversion into glycogen. Because prior fasting inhibited glucose uptake during the clamps, the studies were repeated under conditions in which, despite fasting, glucose uptake was maintained constant through either hyperglycemia or hyperinsulinemia.

Materials and Methods

Protocol

Twenty-one healthy volunteers participated in the study. Their characteristics were the following: 19 males/2 females; age: 27 ± 1 years; body weight: 76.3 ± 1.9 kg; body mass index: 24.7 ± 0.8 kg/m²; and body surface: 1.92 ± 0.02 m². The nature, purpose, and potential risks of the study were explained to the subjects, and their written informed consent was obtained before participation. The protocol was approved by the Ethics Committee of the Faculty of Medicine of the University of Brussels. The subjects were divided into three groups, as follows:

Group 1. Seven subjects were submitted to a euglycemic hyperinsulinemic clamp, both after 12 h and 4 days of total fasting. The two studies were performed in random order and were separated by a 2- to 3-wk interval. On the morning of each study, a short Teflon catheter was inserted into an antecubital vein for infusion of all test substances. Another catheter was placed in a contralateral dorsal hand vein in a retrograde fashion for intermittent blood sampling. This catheter was kept patent with a slow drip of saline, and the hand was placed in a temperature-regulated heating pad to allow arterialization of the venous blood. After a period of ~30 min, four basal blood samples were collected at 10-min intervals. At that time (time 0), three different infusions were started simultaneously and continued for 240 min: 1) a constant infusion of [3-3H]glucose (0.6 µCi/min) and [U-14C]glucose (0.03 µCi/min) diluted in saline, with a priming dose representing 40 times the rate of infusion and prelabeling of the bicarbonate pool with 1.5 µCi of NaH14CO3; 2) a primed (1.6 U/m²) constant infusion of insulin (40 mU·m²·min⁻¹), diluted in saline containing a few milliliters of the subject’s own blood; and 3) a variable infusion of a 20% glucose solution in water, which was frequently adjusted to

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

0193-1849/99 $5.00 Copyright © 1999 the American Physiological Society
maintain plasma glucose at ~85 mg/dl. Blood samples for
determination of $[^3$H$]glucose$, $[^3$H$]H_2O$, insulin, glucagon, and
various substrates were obtained at 120 and 180 min and
every 10 min during the final 60 min of the clamp. Expired
air was collected in rubber bags at 30, 60, 120, 180 min and every
20 min thereafter for immediate measurement of $CO_2$ specific
activity. Respiratory gas exchanges were determined with
computerized open-circuit calorimetry (Deltatrac, Datex, Hel-
sinki, Finland) during the basal period and for 15-min periods
every 30 min until the 180th min. Thereafter, the 15-min
measurements were obtained every 20 min during the inter-
vals between the expired air collections. Before each test, the
Deltatrac monitor was calibrated with a gas of known $CO_2$
and $O_2$ composition and before and after each test, the quality
and stability of the calibration were checked by an ethanol
burning test. Timed urine specimens for determination of
total nitrogen excretion were obtained before and during the
insulin clamp. In the case of starved subjects, the urine was
collected on hydrochloric acid to prevent loss of ammonia.
About 3 wk after the second test, total body water volume was
measured (6). For this purpose, a basal blood sample was
collected to evaluate residual $[^3$H$]H_2O$ content, followed by an
intravenous bolus of 80 $\mu$Ci of $[^3$H$]H_2O$. Blood samples were
collected 2, 2.5, and 3 h after injection of the tracer to
determine the $[^3$H$]H_2O$ content of plasma water. All tracers were
purchased from Du Pont-NEN (Boston, MA).

Group 2. Because, as expected, studies of group 1 showed
that fasting was associated with a marked reduction in
glucose uptake during the euglycemic clamp, a second series of
studies was designed to compare glucose metabolism in
12-h and 4-day fasted subjects at the same rate of glucose
uptake. Accordingly, seven subjects were submitted to the
same protocol as group 1, except for the following differences:
1) the experiments performed after the 12-h fast always came
first; 2) in the second test (4-day fast), glucose was infused at
the same rate as in the first test and no attempt was made to
clamp the glucose concentration, which rose above normal
levels; and 3) somatostatin (UCB Pharma, Brussels, Bel-
gium) was infused at the rate of 10 $\mu$g/min to inhibit
endogenous insulin release and glucagon (Novo Nordisk,
Bagsvaerd, Denmark) was replaced at the rate of 250
pg·kg$^{-1}$·min$^{-1}$.

Five additional subjects belonging to group 1 or 2 gave up
the 4-day fast, but their 12-h fast clamp data were neverthe-
less used for the correlation analysis (see Fig. 2).

Group 3. The protocol used for the seven subjects in this
group was identical to that of group 1, except for the following
differences: 1) the 4-day fast test always came first, and
insulin was delivered at the rate of 400 mU·m$^{-2}$·min$^{-1}$ so as
to attain an insulin concentration with maximal effect on
glucose uptake, with glycaemia clamped at 85 mgidl, and 2) in
the 12-h fast test, glucose was delivered at the same rate as
during the first test and glycaemia was clamped at 85 mgidl by
frequently adjusting the rate of insulin infusion. Thus, during
the two tests, glucose levels and infusion rates were identical,
but insulin infusion rates were different.

Analytical Procedures

Blood samples were collected in heparinized syringes and
transferred to tubes kept on ice. The samples used to measure
the liver glycogen depot and labeled glucose and lactate concentra-
tions contained NaF, and those used to measure the glucose
concentration contained aprotinin. After centrifugation at
4°C, plasma was stored at ~20°C until assay. Plasma glucose was
determined with a glucose oxidase method (test combina-
tion glucose; Boehringer, Mannheim, Germany). Plasma
$[^3$H$]glucose$ and $[^3$H$]H_2O$ were determined after deproteinization
by the Somogyi method. $[^3$H$]glucose$ was counted by dual-
scintillation spectrometry on evaporated filtrates reconsti-
tuted with water. $[^3$H$]H_2O$ was determined as the difference
between the tritium counts obtained with and without evapo-
ratio. $[^3$H$]H_2O$ in plasma water was calculated by dividing its
concentration in total plasma by 0.93. Lactate and 3-hydroxy-
butyrate were determined on a neutralized perchloric filtrate
of plasma with standard enzymatic methods (1). Free fatty
acids (FFAs) were assayed by an enzymatic method (NEFA;
Wako, Neuss, Germany). The levels of plasma insulin (23) and
glucagon (13) were determined by RIA. Total urinary
nitrogen was assayed by the Kjeldahl method, with a Kjeltac
1 apparatus (Tecator, Höganäs, Sweden). To measure $^{14}$CO$_2$
specific activity in expired air, 3 ml of a solution of hyamine
hydroxide (Packard, Groningen, The Netherlands) in metha-
nol (0.33 mol/l) were placed in 10-ml counting vials, and
expired air from the rubber bags was pumped slowly through
the solution until neutralization in the presence of phenolphtha-
lein. The vials were then counted after addition of scintilla-
tion fluid. For each experiment, the hyamine solution was
titrated with HCl before use. All determinations were made
in duplicate.

Calculations

Total body water volume was calculated from the ratio
between the amount of $[^3$H$]H_2O$ injected (dpm) and the steady-
state concentration of $[^3$H$]H_2O$ in plasma water (dpm/ml). Body
water content after the 4-day fast was assumed to represent
the same fraction of body weight as that measured in the
nonfasted state. Because body weight decreased by 3.7 ± 0.3
kg by fasting, this calculation resulted in an estimated
decrease in total body water of 2.1 ± 0.2 liters.

Metabolic fluxes were calculated for the last hour of the clamps
(180-240 min) under near steady-state conditions
(Fig. 1). The coefficients of variation for glucose concentra-
tion, glucose infusion rate and glucose specific activity aver-
aged 3.6 ± 2, 2.9 ± 0.6, and 4.1 ± 0.3%, respectively, for the
studies performed after 12 h of fast and 2.8 ± 0.2, 3.3 ± 0.7,
and 3.5 ± 0.3%, respectively, for those performed after 4 days
of fast.

Total glucose appearance ($R_a$) and disappearance ($R_d$) were
calculated between two consecutive samples from the $[^3$H$]glu-
cose$ infusion rate and the $[^3$H$]glucose$ specific activity in
plasma with the equations of Steele (31), assuming that the
functional volume of distribution represented 13% of body
weight. Endogenous glucose production was calculated as the
difference between total glucose appearance and glucose infu-
sion rate.

Circulating glucose oxidation was calculated as follows: the rate
of production of $^{14}$CO$_2$ (dpm/min) was obtained as the
product between the steady-state $CO_2$ specific activity and
the $V_{CO_2}$ measured by the Deltatrac monitor. The $R_0$ of $^{14}$CO$_2$
was divided by $[^3$H$]glucose$ specific activity to calculate
circulating glucose oxidation. The $[^14$C$]glucose$ specific activity
was not directly measured (owing to the very small
amount of $^{14}$C$^0$ infused) but was calculated as the ratio
between $[U-[^14$C$]glycine$ infused per unit of time and glucose
$R_a$ obtained from the $[^3$H$]glucose$ data. A correcting factor
of 0.81 was applied to account for $CO_2$ retention in the bicarbo-
nate pool (36).

Whole body glycolysis was calculated (28) from the rate of
increment per unit of time of plasma $[^3$H$]H_2O$ as determined by
linear regression, multiplied by total body water volume.
Nonoxidative glycolysis was the difference between glyco-
sis and circulating glucose oxidation. Glycogen synthesis was
calculated as the difference between glucose $R_a$ and glyco-
lisis (28). Note that this glycogen synthesis corresponds only to
the net amount of glycogen formed by the direct pathway
(glucose → glucose 1-phosphate → glycogen) in muscle and
liver. It does not include the glycogen formed in liver through
Carbohydrate and lipid oxidation and energy expenditure were determined from \(\text{VO}_2\), \(\text{VO}_2\), and urinary nitrogen output (12). In the case of starved subjects, the \(\text{VO}_2\) and \(\text{VO}_2\) values used in the calculations were corrected (12) for changes in the ketone body pool occurring between the 180th and 240th min of the studies assuming an operational volume of distribution of ketone bodies of 0.2 L/kg. Because of the small variations in ketonemia that occurred during the 4th h of the clamps, these corrections altered the estimated carbohydrate and lipid oxidation rates by less than 5%.

Glucose storage was calculated in three different ways, leading to different results whose significance will be considered in the DISCUSSION: 1) glycogen synthesis = glucose \(R_d\) – whole body glycolysis (28); 2) nonoxidative glucose disposal (NOGD) = glucose \(R_d\) – circulating glucose oxidation as measured from the \(\text{14CO}_2\) data; 3) nonoxidative glucose disposal (NOGD) = glucose \(R_d\) – glucose oxidation as measured by indirect calorimetry.

Data are means ± SE. The three groups were compared in the overnight-fasted state and in the 4-day fasted state with a one-factor ANOVA, and whenever a significant difference was detected, pairwise comparisons between groups were made with a Scheffé's \(F\) test. Within-group comparisons between the 12-h and 4-day fasts were made with a paired Student's \(t\)-test. Linear regression and covariance analysis were carried out by standard techniques. \(P\) values below 0.05 were considered significant.

**RESULTS**

Effect of Fasting on Basal Parameters

No difference was observed between the groups for any of the basal parameters tested, either in the overnight-fasted or 4-day fasted states (Table 1). As expected, the 4-day fast was associated in each group with a significant decrease in glucose and insulin concentrations and an increase in FFA, 3-hydroxybutyrate, and glucagon levels. Lactate concentrations decreased slightly but not significantly. Carbohydrate oxidation was totally suppressed after 4 days of food deprivation and dropped to slightly negative values, whereas fat oxidation rates were approximately doubled. Protein oxidation was not affected.

Glucose Metabolism During the Euglycemic Hyperinsulinemic Clamp After the 12-h Fast

The main data recorded in group 1 during the last hour of the euglycemic hyperinsulinemic clamp after the 12-h fast are shown in Table 1.

**Table 1. Basal metabolic characteristics of groups 1–3 in the 12-h and 4-day fasted states**

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Group 1 (12-h fast)</th>
<th>Group 1 (4-day fast)</th>
<th>Group 2 (12-h fast)</th>
<th>Group 2 (4-day fast)</th>
<th>Group 3 (12-h fast)</th>
<th>Group 3 (4-day fast)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dl</td>
<td>103 ± 2</td>
<td>68 ± 3*</td>
<td>99 ± 3</td>
<td>65 ± 1*</td>
<td>95 ± 2</td>
<td>66 ± 3*</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>13 ± 1</td>
<td>7 ± 1*</td>
<td>12 ± 1</td>
<td>7 ± 1*</td>
<td>14 ± 1</td>
<td>8 ± 1*</td>
</tr>
<tr>
<td>Glucagon, pg/ml</td>
<td>210 ± 73</td>
<td>326 ± 89*</td>
<td>137 ± 24</td>
<td>278 ± 41*</td>
<td>175 ± 8</td>
<td>244 ± 12*</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td>0.9 ± 0.08</td>
<td>0.93 ± 0.06</td>
<td>0.91 ± 0.10</td>
<td>0.82 ± 0.03</td>
<td>1.13 ± 0.15</td>
<td>0.89 ± 0.03</td>
</tr>
<tr>
<td>FFA, mmol/l</td>
<td>0.47 ± 0.08</td>
<td>1.15 ± 0.09</td>
<td>0.41 ± 0.10</td>
<td>1.18 ± 0.14</td>
<td>0.45 ± 0.05</td>
<td>1.30 ± 0.09</td>
</tr>
<tr>
<td>3-Hydroxybutyrate, mmol/l</td>
<td>0.07 ± 0.02</td>
<td>0.49 ± 0.58</td>
<td>0.11 ± 0.05</td>
<td>0.58 ± 0.72</td>
<td>0.11 ± 0.05</td>
<td>4.87 ± 0.40*</td>
</tr>
<tr>
<td>Substrate oxidation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbohydrate, mg·m⁻²·min⁻¹</td>
<td>61 ± 5</td>
<td>−6 ± 2*</td>
<td>52 ± 7</td>
<td>−18 ± 5*</td>
<td>58 ± 9</td>
<td>−8 ± 4*</td>
</tr>
<tr>
<td>Lipid, mg·m⁻²·min⁻¹</td>
<td>28 ± 4</td>
<td>55 ± 2*</td>
<td>31 ± 4</td>
<td>62 ± 5*</td>
<td>29 ± 4</td>
<td>60 ± 2*</td>
</tr>
<tr>
<td>Protein, mg·m⁻²·min⁻¹</td>
<td>23 ± 3</td>
<td>26 ± 3</td>
<td>27 ± 3</td>
<td>24 ± 2</td>
<td>26 ± 3</td>
<td>27 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE for last 30 min of basal period. FFA, free fatty acids. Significance of difference with the 12-h fast (paired comparison): \(bP < 0.02\); \(cP < 0.01\); \(dP < 0.005\); \(eP < 0.001\).
both the overnight and 4-day fasts (Fig. 1) show that a near-steady state was attained for most parameters. Note, however, that the glucose infusion rate had to be slowly increased to maintain a steady glucose level throughout the clamp, so that glucose specific activity slightly decreased. Calorimetry data remained stable and $^{3}$H$_2$O concentrations rose in a linear fashion. A similar time-course prevailed in all three groups under both nutritional conditions.

Tables 2 and 3 show that during the clamps that followed the 12-h fast, the concentrations of glucose and other substrates, the concentration of glucagon, glucose uptake, and the fluxes in the various pathways of glucose utilization were very similar in the three groups, as confirmed by statistical analysis. However, insulin concentrations tended to be lower in group 3 (77 ± 12 µU/ml) than in group 1 (106 ± 6 µU/ml) and group 2 (109 ± 6 µU/ml). This situation was due to the experimental design itself, because the average amount of insulin, which had to be infused in group 3 in the overnight-fasted state to maintain euglycemia at the rate of glucose infusion used in the fasted state, was significantly lower than the predetermined amount of insulin infused in groups 1 and 2 (26 ± 3 vs. 40 mU·m$^{-2}$·min$^{-1}$; $P < 0.001$). However, despite this difference, insulin concentrations were not significantly different in the three groups.

Taken together, the isotope data for the control clamps in groups 1–3 (n = 21) indicate that at a glucose level of 86 ± 1 mg/dl and an insulin concentration of 97 ± 6 µU/ml, glucose $R_d$ averaged 294 ± 11 mg·m$^{-2}$·min$^{-1}$ with an equal contribution (50 ± 2%) of glycolysis and glycogen synthesis. Within glycolysis, the oxidative and nonoxidative pathways accounted for 69 ± 2 and 31 ± 2%, respectively. Figure 2 demonstrates that within this range of $R_d$, glycolysis, circulating glucose oxidation, glycogen synthesis, and NOGD, correlated positively with $R_d$. Covariance analysis indicates that the slopes of these relationships are much steeper ($P < 0.001$) for the synthetic pathways than for glycolysis and glucose oxidation.

Effect of Fasting on Circulating Glucose Disposal During the Clamps: Isotope Data

In group 1, fasting decreased clamp glucose requirements so that $R_d$ was reduced by ~50% (150 ± 10 vs. 291 ± 18 mg·m$^{-2}$·min$^{-1}$; $P < 0.001$), with an equivalent reduction in glycolysis (76 ± 3 vs. 146 ± 5 mg·m$^{-2}$·min$^{-1}$; $P < 0.001$) and glycogen synthesis (74 ± 7 vs. 145 ± 15 mg·m$^{-2}$·min$^{-1}$; $P < 0.01$; Tables 2 and 3). The fall in glycolysis was almost entirely due to the inhibition of glucose oxidation (44 ± 3 vs. 107 ± 8 mg·m$^{-2}$·min$^{-1}$; $P < 0.001$), as nonoxidative glycolysis remained virtually unchanged (32 ± 3 vs. 39 ± 7 mg·m$^{-2}$·min$^{-1}$; $P > 0.05$).

In group 2, in which identical amounts of glucose were infused during the two clamps, average glucose concentration was higher after the 4-day fast than after the 12-h fast (155 ± 15 vs. 86 ± 2 mg/dl; $P < 0.001$), whereas insulin levels were similar under both conditions (106 ± 5 vs. 109 ± 6 µU/ml; $P > 0.05$). Despite a virtually identical $R_d$ (305 vs. 307 mg·m$^{-2}$·min$^{-1}$), the pathways of glucose disposal were markedly altered by fasting; thus glycolysis fell by 32 ± 3% and glycogen synthesis rose by 29 ± 2% ($P < 0.001$ for both). The reduction in glycolysis was mainly due to a fall in its oxidative component (~40 ± 3%; $P < 0.001$), as nonoxidative glycolysis remained unchanged (~11 ± 10%; $P > 0.05$).

In group 3, in which the $R_d$ similar to that observed after the 12-h fast was achieved after the 4-day fast (281 ± 24 vs. 283 ± 22 mg·m$^{-2}$·min$^{-1}$; $P > 0.05$) by means of very high insulin levels (2,788 ± 99 vs. 77 ± 12 µU/ml), the alterations in glucose metabolism due to fasting differed slightly from those observed in group 2. Thus glycolysis decreased only modestly (~15 ± 3%; $P < 0.005$) and glycogen synthesis rose slightly (~19 ± 10%) and not significantly. Note that the two components of glycolysis changed in opposite directions with a decrease in oxidation (~37 ± 2%; $P < 0.001$) and an increase in nonoxidative glycolysis, which did not, however, reach statistical significance (~25 ± 12%; $P > 0.05$).

Effect of Fasting on Carbohydrate and Fat Metabolism During the Clamps: Calorimetry Data

In the three groups, prior fasting had a much greater inhibitory effect on total carbohydrate oxidation than on circulating glucose oxidation during the clamps (Tables 2 and 3). Consequently, in group I, NOGD, was not significantly reduced by fasting (~35 ± 15 mg·m$^{-2}$·min$^{-1}$) despite a highly significant ($P < 0.001$) inhibition of NOGD, (~77 ± 15 mg·m$^{-2}$·min$^{-1}$). In the other two groups, the rise in NOGD, induced by fasting significantly exceeded the rise in NOGD, (+67 ± 11 vs. +41 ± 6 mg·m$^{-2}$·min$^{-1}$ in group 2, $P < 0.02$, and

Table 2. Plasma concentrations of substrates and hormones during clamp studies in groups 1–3

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Group 1</th>
<th></th>
<th>Group 2</th>
<th></th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12-h fast</td>
<td>4-day fast</td>
<td>12-h fast</td>
<td>4-day fast</td>
<td>12-h fast</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>86 ± 3</td>
<td>85 ± 3</td>
<td>86 ± 2</td>
<td>155 ± 15$^{a}$</td>
<td>87 ± 2</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>106 ± 6</td>
<td>103 ± 6</td>
<td>109 ± 6</td>
<td>106 ± 5</td>
<td>77 ± 12</td>
</tr>
<tr>
<td>Glucagon, pg/ml</td>
<td>123 ± 19</td>
<td>132 ± 25</td>
<td>109 ± 30</td>
<td>127 ± 33</td>
<td>133 ± 16</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td>1.34 ± 0.11</td>
<td>1.01 ± 0.04$^{a}$</td>
<td>1.21 ± 0.05</td>
<td>1.32 ± 0.06$^{a}$</td>
<td>1.31 ± 0.10</td>
</tr>
<tr>
<td>FFA, mmol/l</td>
<td>0.06 ± 0.01</td>
<td>0.37 ± 0.05$^{a}$</td>
<td>0.04 ± 0.02</td>
<td>0.20 ± 0.05$^{a}$</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>3-Hydroxybutyrate, mmol/l</td>
<td>0.01 ± 0.00</td>
<td>0.61 ± 0.21$^{a}$</td>
<td>0.01 ± 0.00</td>
<td>0.31 ± 0.15$^{a}$</td>
<td>0.01 ± 0.00</td>
</tr>
</tbody>
</table>

Values are means ± SE for last hour of clamp. Significance of difference with 12-h fast (paired comparison): $^{a}$P < 0.05; $^{b}$P < 0.02; $^{c}$P < 0.01; $^{d}$P < 0.005; $^{e}$P < 0.001.
remained, clamps (Table 3). These high fat oxidation rates diminished from baseline (Table 1) by only 20%, groups, despite the 4-h insulin infusions, fat oxidation 0.001).

As regards fat oxidation after fasting, the three groups behaved similarly. On an average for the three groups, despite the 4-h insulin infusions, fat oxidation diminished from baseline (Table 1) by only 20 ± 2% and remained ~4 times higher than during the control clamps (Table 3). These high fat oxidation rates persisted despite a reduction in FFA levels from baseline averaging 75 ± 3% (Tables 1–3).

Energy expenditure during the clamps was only affected by prior fasting in group 3 in which the high insulin concentrations prevailing during the tests performed after fasting were associated with a significant increase in caloric expenditure (958 ± 23 vs. 858 ± 22 Kcal·m⁻²·24 h⁻¹; P < 0.005).

**DISCUSSION**

Significance of the Components of Oxidative and Nonoxidative Glucose Disposal

Glucose oxidation, as estimated from ¹⁴CO₂ data, includes the oxidation of all circulating glucose, including exogenous glucose and endogenous glucose derived from both glycojenolysis and gluconeogenesis. It is admitted that the reliability of the correcting factor 0.81 used to account for ¹⁴CO₂ retention in the bicarbonate pool is questionable (21, 32). However, it is important to note that recent [¹⁴C]bicarbonate-infusion studies from our laboratory showed that recovery is not affected by previous fasting (11). Therefore, any inaccuracy in the recovery factor used should have the same impact on the calculation of glucose oxidation in individuals fasted overnight and those fasted for 4 days and should not invalidate their comparison.

Nonoxidative glycolysis corresponds to glucose metabolized to C₃ fragments (mainly lactate) that escaped oxidation within the time limits of the experiments. Theoretically, this lactate could either recirculate in the residual hepatic glucose output, which under the present clamp conditions was minimal (Table 3) or be converted into glycogen in the liver. Assuming that the volume of lactate distribution constitutes 20% of body weight (26), it can be calculated from the slow increase in the lactate levels that the rate of accumulation of unmetabolized lactate represented at most 10% of nonoxidative glycolysis. Although nonoxidative glycolysis might also include glucose conversion to fat, de novo
lipogenesis was probably minimal, because under our experimental conditions, the nonprotein respiratory quotient never exceeded 1.0. It therefore seems reasonable to assume that most of the flux of carbon in nonoxidative glycolysis was eventually incorporated into liver glycogen by the indirect pathway. Therefore, NOGD\(_i\) (R\(_d\) – circulating glucose oxidation) should approximate whole body glycogen synthesis in muscle and liver, both pathways included.

Indirect calorimetry constitutes an entirely different approach to measuring glucose oxidation and storage. When estimated by this technique, carbohydrate oxidation corresponds to whole body net carbohydrate loss and includes (32): 1) oxidation of exogenous glucose; 2) oxidation of the portion of endogenous glucose derived from hepatic glycogenolysis; and 3) oxidation of hepatic and muscle glycogen without passage through extracellular glucose. However, it does not include the fraction of circulating glucose derived from gluconeogenesis.

The NOGD\(_c\) obtained as the difference between glucose R\(_d\) and carbohydrate oxidation estimated by indirect calorimetry corresponds to a net carbohydrate balance that includes net glycogen accumulation (synthesis – oxidation), lactate accumulation, and net de novo lipogenesis (32). Because, as discussed above, these two latter components are quantitatively small, NOGD\(_c\) should correspond essentially to net glycogen accumulation in muscle and liver, both pathways included.

The fact that during the clamps performed after an overnight fast total carbohydrate and circulating glucose oxidation were similar (mean for the 3 groups: 109 ± 4 vs. 99 ± 4 mg·m\(^{-2}\)·min\(^{-1}\); P > 0.05) suggests that on an average, glycogen oxidation was of the same order of magnitude as the oxidation of glucose derived from gluconeogenesis. On the other hand, the fact that during the clamps after fasting carbohydrate oxidation in all three groups was always lower than glucose oxidation (21 ± 3 vs. 53 ± 2 mg·m\(^{-2}\)·min\(^{-1}\); P < 0.001) can probably be explained by the persistence of significant gluconeogenesis concomitantly with total suppression of glycogen oxidation. In this connection, it is noteworthy that basal carbohydrate oxidation, which corresponds solely to glycogen oxidation was totally suppressed in the 4-day fasted state (Table 1). It should be kept in mind that both the isotope and calorimetry techniques include several sources of potential inaccuracies, so that the data obtained from their combined use should be regarded as semiquantitative.

Effect of Fasting on the Pathways of Glucose Utilization During the Euglycemic Hyperinsulinemic Clamps

During the euglycemic hyperinsulinemic glucose clamps performed after the overnight fast (groups 1–3 combined), glycogen synthesis, circulating glucose oxidation, and nonoxidative glycolysis accounted for 50, 35, and 15%, respectively, of R\(_d\), in agreement with previously published data (4, 28). These are average proportions for a mean R\(_d\) of ~300 mg·m\(^{-2}\)·min\(^{-1}\), but, as shown in Fig. 2, they depend on R\(_d\), as any change in R\(_d\) has a greater impact on synthetic pathways than on glycolysis or oxidation.

The results for group 1 indicate that several days of fast induced a state of insulin resistance, as shown by the ~50% reduction in R\(_d\) at an insulin concentration of ~100 μU/ml during euglycemia (Table 3), thus confirming the results of previous studies (2, 22, 24). Whether the antilipolytic effect of insulin is inhibited by fasting remains a controversial issue (16, 24). Our results indicate that this process participates in insulin resistance, because even maximal insulin concentrations for 4 h (group 3) failed to bring FFAs back to the levels observed during the control clamps. Fat oxidation was even less reduced by hyperinsulinemia than FFA levels (approximately –22% vs. approximately –75%), suggesting that fasting is associated with a high rate of intracellular triglyceride oxidation, a process known to be less sensitive to insulin than FFA concentration and oxidation (38).

The fact that in group 1, the decrease in R\(_d\) induced by fasting was associated with an equivalent decrease in glycogen and glycogen synthesis that suggests besides inhibiting glucose transport-phosphorylation, fasting fails to alter the partition between these two pathways. However, this interpretation is probably not correct, because according to Fig. 2, one would expect any decrease in R\(_d\) in the absence of fasting to induce a greater absolute decrease in glycogen synthesis than in glycolysis. Therefore, fasting probably exerts a selective stimulation of glycogen synthesis that is masked by the 50% decrease in glucose R\(_d\). This interpretation is corroborated by the results obtained at a constant R\(_d\) in groups 2 and 3, as discussed later.

The observation that ~90% of the decrease in glycogenesis was due to a reduction in oxidation, with nonoxidative glycolysis remaining virtually unchanged, is compatible with reduced activity of the pyruvate dehydrogenase complex. Such a decrease has been documented in several tissues of fasted animals, including skeletal muscle (25). Because, as stated above, the products of nonoxidative glycolysis probably serve as precursors for glycogen synthesis in the liver, the preservation of nonoxidative glycolysis, despite the ~50% decrease in R\(_d\), represents an indirect mechanism of carbohydrate retention. An additional mechanism of carbohydrate conservation that operates in fasting individuals during a euglycemic hyperinsulinemic clamp is the apparent cessation of intracellular glycogen oxidation, as suggested by the near maintenance of the net carbohydrate balance (NOGD\(_i\)) despite the ~42% decrease in total glycogen synthesis (NOGD\(_i\); Table 3).

Effect of Fasting on the Pathways of Intravenous Glucose Utilization at Constant R\(_d\)

The stimulatory effect of prior fasting on glucose storage was fully expressed in our experiments at constant R\(_d\) obtained through either hyperglycemia or hyperinsulinemia. For instance, the isotope data summarized in Fig. 3 indicate that under both conditions, fasting induced an equivalent decrease in glucose oxidation and therefore an equivalent increase in NOGD\(_i\),
i.e., in total glycogen synthesis by the direct and indirect pathways combined. However, the mechanisms of action of hyperglycemia and hyperinsulinemia are not identical. In the case of hyperglycemia (B compared with A in Fig. 3), the increase in glycogen synthesis occurred entirely by the direct route without modification in nonoxidative glycolysis, whereas hyperinsulinemia (C compared with A) stimulated both pathways. The prediction that the indirect pathway has a higher energy cost was confirmed by the greater energy expenditure observed in group 3 subjects whose glucose disposal was stimulated by pharmacological hyperinsulinemia (Table 3).

It could be argued that the observed stimulation of glycogen synthesis is related to the hyperglycemia and/or hyperinsulinemia rather than to the fast itself. Data obtained by Youn and Buchanan (39) in rats make this hypothesis unlikely. Because rats do not develop insulin resistance after fasting, these authors could compare fed and 48-h fasted animals at an identical glucose Rd in the presence of the same insulin and glucose concentrations. Under these conditions, fasting inhibited whole body glycolysis by 16% and stimulated glycogen synthesis by 44%.

It is important to stress that the present study describes the changes occurring at the whole body level with no indication of the individual tissues involved. It has been established that after an overnight fast, at least 85% of glucose uptake during a hyperinsulinemic euglycemic clamp is taken up by peripheral tissues, with splanchnic bed being a relatively minor site of glucose disposal (5). Whether this also occurs after prolonged fasting is not known. One might expect that as liver glycogen is totally depleted after 4 days of fast, a greater proportion of infused glucose would ultimately end up in the liver to replenish the glycogen stores whichever pathway is involved. Our data do not prove, but are compatible with, the idea that during fasting, hyperglycemia combined with physiological hyperinsulinemia favors the direct pathway, whereas pharmacological hyperinsulinemia during euglycemia preferentially enhances the indirect pathway (Fig. 3). This interpretation is in line with the results of earlier studies on the pathways of hepatic glycogen repletion in rats (19, 30).

Our observation that during hyperinsulinemia, the fraction of $R_d$ accounted for by nonoxidative glycolysis is greater than after hyperglycemia (Fig. 3) is in agreement with the higher lactate concentrations observed during hyperinsulinemia. Note that when the results for our three groups are combined, lactate concentration during the clamps correlates strongly ($P < 0.001$) with glycolysis ($r = 0.87$) and with nonoxidative glycolysis ($r = 0.83$) under fasting conditions but not in the overnight-fasted state ($P > 0.05$ for both correlations; Fig. 4). One possible explanation is that in the 12-h fasted subjects the expected relationship between glycolysis and the lactate concentration is masked, because part of lactate production is derived from intracellular glycogen breakdown, a process that is not detected by the tracers and which is probably switched off after fasting as mentioned earlier. Total

**Fig. 3. Intracellular metabolic partition of intravenous glucose at a similar Rd under 3 different conditions.** A: euglycemic hyperinsulinemic clamp after a 12-h fast (groups 1 and 2 combined). B: 4-day fasted subjects in whom insulin resistance was compensated by hyperglycemia (group 2). C: 4-day fasted subjects in whom insulin resistance was compensated by pharmacological hyperinsulinemia (group 3). Figures and symbols in italics correspond to statistical significance ($P$ value) between adjacent columns. NS, not significant ($P > 0.05$).

**Fig. 4. Correlation between plasma lactate and nonoxidative glycolysis during last hour of clamps performed after a 12-h fast (● and solid line, $r = 0.10, P > 0.05$) and a 4-day fast (○ and dotted line, $r = 0.85, P < 0.001$) in subjects in groups 1-3.**
carbohydrate oxidation was much more reduced by fasting than circulating glucose oxidation in all three groups, thus corroborating the concept that the suppression of glycogen oxidation helps to enhance net carbohydrate storage induced by fasting. Similar findings were recently reported for normal 4-day fasted volunteers refed with oral glucose (11).

Potential Mechanisms by Which Fasting Alters the Pathways of Intravenous Glucose Metabolism

According to the glucose-fatty acid cycle concept (24), the effects of fasting on glucose metabolism might be mediated by the elevated rates of fat oxidation. Thus, during euglycemic hyperinsulinemic clamping, fat infusion was observed to inhibit glucose uptake and carbohydrate oxidation and NOGDc was either unchanged (17, 29, 33, 37) or reduced (3, 18, 20). Although the results obtained in group 1 fit these observations, there seem to be quantitative differences between the effects of fat infusion and fasting. Comparison of the data in the literature (3, 17, 18, 20, 27, 29, 33, 37) with the present results indicates that at similar rates of fat oxidation (~45 mg·m⁻²·min⁻¹), fat infusion has a smaller inhibitory effect on carbohydrate oxidation than fasting. As a result, NOGDc constituted on an average 69% of R₉ during lipid administration against 92% during the clamps performed after fasting. In the studies; and the present 4-day fast than during the fat infusion. In addition to the triglyceride content of liver, the relative contribution to fat oxidation than during fat infusion. That after fasting, intracellular fat makes a larger relative contribution to fat oxidation than reducing FFA to higher levels (usually over 1 mmol/l) than those observed after fasting (~0.30 mmol/l). This suggests that after fasting, intracellular fat makes a larger relative contribution to fat oxidation than during fat infusion. In addition to the triglyceride content of liver, that of muscle is known to increase after fasting (7) and might participate more efficiently in muscle fat oxidation than circulating FFA and therefore have a greater impact on muscle glucose metabolism (20).

In conclusion, our results strongly suggest that at a given rate of whole body glucose disposal induced by glucose and insulin infusion, prior fasting favors glycogen retention. Three mechanisms are involved: 1) stimulation of glycogen synthesis by the direct pathway combined with inhibition of glycogenolysis; 2) within glycogenolysis, preferential inhibition of glucose oxidation with the relative preservation or even slight stimulation of nonoxidative glycogenolysis, which represents an indirect mechanism for conserving carbon for glycogen synthesis by the indirect pathway in the liver; and 3) suppression of intracellular glycogen oxidation. The overall effect of hyperglycemia and hyperinsulinemia on total glycogen synthesis (direct + indirect pathways) is quantitatively similar (Fig. 3), but hyperglycemia only stimulates the direct pathway, whereas insulin also enhances the indirect pathway. In addition to the elevated rates of fat oxidation, other factors such as glycogen depletion are probably involved in the overall stimulatory effect of prior fasting on the storage of exogenous glucose.

We thank M. A. Neef for expert technical help, C. Demesmaeker for excellent secretarial assistance, and M. Dreyfus for the English correction. We are indebted to UCB-Pharma Belgium for the generous gift of somatostatin.

This work was supported by Grant No. 3.4542.96 from the Fonds de la Recherche Scientifique Médicale Bélgique.

Address for reprint requests and other correspondence: E. O. Balasse, Laboratory of Experimental Medicine, Univ. of Brussels, 808 Route de Lennik, B-1070 Brussels, Belgium.

Received 1 March 1999; accepted in final form 23 June 1999.

REFERENCES


