Glucose appearance in the peripheral circulation and liver glucose output in men after a large $^{13}\text{C}$ starch meal$^{1-3}$

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ABSTRACT

Background: Glucose absorption from starchy food has only been described with small amounts ingested ($\approx 20–75$ g).

Objective: Our aim was to describe total plasma (Ra) and exogenous glucose appearance, glucose release from the liver (HGP), and the metabolic response after ingestion of $5$ g polished or parboiled rice/kg body mass.

Design: Gas exchange and urea excretion were monitored in $8$ healthy subjects before (3.5 h) and after (8 h) ingestion of rice intrinsically labeled with $^{13}\text{C}$; $[6,6-\text{D}_2]$glucose was infused for the measurement of Ra, Raexo, and HGP.

Results: Changes in plasma glucose, insulin, lactate, and free fatty acids and the increase in Raexo and Ra ($\approx 200\%$) and the decrease in HGP ($\approx 90\%$) were not significantly different ($P > 0.05$) after ingestion of either rice. Glucose oxidation was not significantly different (111.6 ± 8.2 compared with 89.0 ± 11.3 g; $P = 0.13$), but fat oxidation was significantly lower (9.9 ± 1.7 compared with 21.3 ± 4.0 g; $P < 0.05$) after parboiled than after polished rice. The percentage of the glucose load that appeared in the circulation over 8 h was not significantly different after ingestion of polished (70.4 ± 4.5%) or parboiled (63.2 ± 2.0%) rice ($P > 0.05$).

Conclusion: Although the starch in parboiled rice is less susceptible to digestion in vitro, exogenous glucose availability was not significantly different after ingestion of large amounts of polished or parboiled rice. Glucose absorption remains incomplete 8 h after ingestion of both types of rice. Am J Clin Nutr 2004;80:881–6.

KEY WORDS Starch, glucose kinetics, stable isotope, indirect respiratory calorimetry, de novo lipogenesis, insulin, healthy men

INTRODUCTION

The consumption of starch, which is encouraged in a healthy diet (1), ranges between 140 and 390 g/d (2). However, exogenous glucose appearance in the peripheral blood (Raexo) from starchy food has only been described with small amounts ingested ($\approx 20–75$ g) (3–5). This is partly because $^{13}\text{C}$-starch is not readily available. The $^{13}\text{C}:^{12}\text{C}$ in starch derived from plants naturally labeled in $^{13}\text{C}$ is only $\approx 1.5\%$ above the background $^{13}\text{C}$ enrichment (6). Higher enrichments can only be obtained by growing starch-producing plants such as wheat (3, 4, 7) or leguminosa (5) in an atmosphere artificially enriched in $^{13}\text{CO}_2$ (intrinsic labeling).

In the present experiment, Raexo, and the metabolic response were described over 8 h after a starch meal in the form of rice (5 g dry mass/kg body mass) intrinsically labeled with $^{13}\text{C}$ in young healthy male subjects, and plasma glucose kinetics and hepatic glucose output (HGP) were measured with use of $[6,6-\text{D}_2]$glucose infusion. Rice can be processed and cooked in various ways, which modify its physicochemical properties and the digestibility of the starch (8–10) and, in turn, could modify the glycemic index (8, 11). For example, consistent data indicate that the susceptibility of starch to digestion in vitro is lower in parboiled than in polished rice (8–10). As for the glycemic index, no significant difference was observed between these 2 types of rice by Larsen et al (12) and Miller et al (13). However, data reported by Casiraghi et al (8) and data compiled by Wolever (11) indicate that the glycemic index (compared with white bread) could be 25–40% lower for parboiled [70 compared with 120 (8)] than for polished rice [51 compared with 68 (11)]. We, thus, compared Raexo, plasma glucose kinetics, and the metabolic response to ingestion of polished or parboiled rice intrinsically labeled with $^{13}\text{C}$. It was hypothesized that Raexo, along with the response of plasma glucose and insulin concentration, plasma glucose turnover, and the reduction in liver glucose production, would be lower after ingestion of the parboiled than polished rice.

SUBJECTS AND METHODS

Subjects

The experiment was conducted in $8$ healthy active male subjects [age: 22.4 ± 0.6 y; weight: 68.0 ± 0.9 kg; height: 175.7 ± 1.3 cm; body mass index (BMI; in kg/m²) = 22.1 ± 0.6; $x$ ± SEM) who gave their informed written consent to participate in the study, which was approved by the institutional board on the use of human subjects in biomedical research at Grenoble University Hospital. All the subjects had a normal fasting plasma

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glucose concentration (5.2 ± 0.1 mmol/L), and none of them had a family history of glucose intolerance or diabetes. None of them were smokers, were heavy drinkers, were taking medications, or had gained or lost weight over the past year (changes of <2 kg).

Experimental protocol

The subjects were studied 3.5 h before and 8 h after ingestion of 5 g (dry mass)/kg body mass of polished or parboiled rice intrinsically enriched in $^{13}$C. The rice was boiled for 14 (parboiled) or 12 min (polished) in tap water (1000 mL/125 g) with 5 g table salt/L water. The protocol followed a crossover design, and the order of presentation of the 2 types of rice was randomized. For 2 d before each experiment, the subjects were asked to rest and were provided with prepackaged meals (125 kJ/g) in the tomatoes were small and negligible compared with those in the rice ingested (Table 1), and there was essentially no fat in the meal (∼2 g). The subjects were then studied for an additional 8 h with measurements of respiratory exchanges and blood sampling every 30 min from 0 to 120 min and every hour thereafter and with urine collection at 60, 180, 300, 420, and 480 min.

Rice intrinsically labeled with $^{13}$C

The rice was prepared by the Centre Français du riz and France riz (Arles, France) according to standard industrial procedures (14) and was made from rice (*Oriza sativa* L. cv Ariète) artificially enriched in $^{13}$C. For this purpose, a small amount of rice was grown hydroponically in a confined atmosphere containing 350 ppm $^{13}$CO$_2$ artificially enriched in $^{13}$C ($^{13}$CO$_2$/CO$_2$ = 11%; Euriso-top, Saint-Aubin, France). The rice artificially labeled with $^{13}$C (108 g; final $^{13}$C/C values measured by mass spectrometry = 10.4%) was mixed with rice grown in the field (6000 g; $^{13}$C/C = 1.0828%) to achieve a final $^{13}$C/C value in the meals of 1.25% (actual value: 1.247%).

Indirect calorimetry

Total protein oxidation and the associated amount of energy provided were computed from urea excretion in urine, and VO$_2$ and VCO$_2$ were corrected for protein oxidation (2.9 g protein oxidized/g urea, and 1.01 L O$_2$ and 0.843 L CO$_2$/g protein oxidized) (15). Glucose and fat oxidation and the amount of energy provided were then computed when the nonprotein respiratory quotient (NPRQ) was <1.0 (15), whereas glucose oxidation, the amount of glucose converted into fat, and the amount of fat synthesized were computed when the NPRQ was >1.0 (16).

Plasma glucose kinetics

The total rate of appearance of glucose in plasma (Ra) at time $t$ was computed with use of Steele’s equation corrected for nonsteady state and modified for stable isotopes, in conjunction with a spline-fitting program for smoothing both tracer and tracee concentrations (17):

$$\text{Ra} = \frac{\text{Fd} - V \times [C/(1 + E_d)] \times (dE_d/dt)/E_d}{1} \quad (I)$$

In this equation, Fd is the rate of [6,6-$^{2}$H$_{2}$] glucose infusion, V is the effective volume of glucose distribution (230 mL/kg) (17), $C_t$ is the plasma glucose concentration, $E_d$ is the plasma glucose enrichment in deuterium (tracer:tracee), and $dE_d/dt$ is its first derivative. The rate of appearance of exogenous glucose arising from ingested labeled starch [Ra$_{exo}$] was computed from the corresponding value of Ra$_{t}$, $C_t$, the $^{13}$C-enrichment of plasma glucose (Ec), and its first derivative (dEc/dt), with use of a reorganization of Steele’s equation applied to $^{13}$C-glucose (17, 18):

$$\text{Ra}_{exo} = [\text{Ra} \times \text{Ec}] + [V \times [C/(1 + E_c)] \times (dE_c/dt)] \quad (II)$$

As suggested by Livesey et al (17), for computing Ra$_{exo}$ when labeled glucose is ingested, the value for V was kept at 230 mL/kg. It should be recognized that the value of Ra$_{exo}$ computed from Ra$_{exo}$ represents the appearance of J) labeled glucose directly absorbed from the gut and which escaped removal in the liver on first pass, 2) labeled glucose removed by the liver on first or subsequent passes early in the observation period and which could be released later, and 3) labeled glucose synthesized in the liver from $^{13}$C-lactate produced from exogenous glucose in the gut or in peripheral tissues. Consequently, the rate of HGP, computed by the difference between Ra and Ra$_{exo}$, actually represents unlabeled glucose released from the liver, from unlabeled glycogen stores, and gluconeogenesis from unlabeled precursors. The total amounts of glucose, glucose released from the liver, and $^{13}$C-glucose, which appeared into the peripheral circulation over the observation period, were computed from the area under the curve of Ra, Ra$_{exo}$, and HGP, respectively.
Analysis

Plasma glucose, lactate (EML105; Radiometer, Copenhagen), free fatty acid [NEFA C assay kit (nonesterified fatty acid assay kit using acyl-CoA synthetase and acyl-CoA oxidase); Wako Chemicals GmbH, Neuss, Germany], and insulin concentrations (CIS Bio International Radioimmunoassay; Biorad, Gif/Yvette, France), and urea concentration in urine (Hitachi 917 analyzer; Roche, Meylan, France) were measured with use of automated assays. Plasma glucose $^{13}$C enrichment was measured by gas chromatography combustion–isotope ratio mass spectrometry as previously described (19). Briefly, the plasma was deproteinized with perchloric acid and neutralized, and glucose was partially purified by sequential anion–cation exchange chromatography (Dowex AG50W*4 and AG1*8; Biorad Laboratory, Richmond, CA). The neutral eluate fraction was lyophilized and derivatized as glucose pentacetate (acetic anhydride; Sigma Chemical, La Verpillière, France). After removing the excess of derivatization products under nitrogen, the sample was resuspended in 50 mL CHCl$_3$, and the glucose was separated by gas chromatography (model 5890; Hewlett-Packard, Evry, France) on a CP SIL19CB column (Chrompack Inc, Bridgewater, NJ) maintained at 260 °C. The glucose peak in the effluent was oxidized at 800 °C in the presence of CuO, and the effluent was driven through a water trap to the isotope-ratio mass spectrometer (SIRA 10; VG Isogas, Middlewich, United Kingdom). For the determination of plasma [6,6-$^2$H$_2$] glucose enrichment, the pentacetate glucose derivative was analyzed by gas chromatography–mass spectrometry (Hewlett-Packard, Palo Alto, CA) in chemical ionization mode with monitoring of $m/z$ 331 and $m/z$ 333.

Statistics

Data are presented as means ± SEMs. The main effects of type of rice (polished or parboiled) and time, as well as type of rice × time interactions were tested by two-factor analysis of variance with repeated measures (Statview; Abacus Concepts, Berkeley, CA) for the kinetics of plasma glucose, NPRQ, and plasma insulin and metabolite concentrations. When no main effect of type of rice or no rice × time interaction was present, the data from the experiments with the 2 types of rice were pooled at each time point for analyzing changes over time, using one-factor analysis of variance with repeated measures. Tukey’s post hoc test was used to identify the location of significant differences when appropriate. The oxidation rate of proteins before (3-h period) and after (8-h period) the meal, as well as the cumulative amounts of glucose and fat oxidized, and the cumulative amounts of total glucose, exogenous glucose, and glucose released from the liver that appeared in the peripheral circulation, over the first and second 4-h periods after ingestion of the polished or parboiled rice, were compared with use of two-factor (type of rice × time) analyses of variance with repeated measures. The level of significance was set at 0.05.

RESULTS

Plasma glucose, lactate, free fatty acid, and insulin concentrations were not significantly different after ingestion of the 2 types of rice (Figure 1). When the data from the experiments with the 2 types of rice were pooled at each time point, the analysis showed that plasma glucose and insulin concentrations significantly increased, whereas plasma free fatty acid concentration

FIGURE 1. Mean (±SEM) plasma glucose, insulin, free fatty acid (FFA), and lactate concentrations before and during the 8 h after ingestion of polished or parboiled rice (the meal was ingested between −60 and 0 min). $n = 8$. No significant difference was seen between the 2 types of rice for any of the variables (two-factor repeated-measures ANOVA: main effect of type of rice, $P > 0.05$). *Line identifies the range of times significantly different from the premeal value (pooled data: one-factor repeated-measures ANOVA and Tukey’s post hoc test, $P < 0.05$).
significantly decreased. A small but significant increase in plasma lactate concentration was also observed. Plasma glucose, insulin, free fatty acid, and lactate concentrations were back to control values, respectively, at 360, 240, 480, and 360 min after the end of the meal.

The oxidation rate of proteins was not significantly different before and after ingestion of polished (4.3 ± 0.4 g/h before the meal, providing 25 ± 2% of the energy yield; 3.6 ± 0.2 g/h after the meal, providing 19.8 ± 2.0% of the energy yield) or parboiled rice (3.2 ± 0.4 g/h before the meal, providing 22 ± 3% of the energy yield; 3.3 ± 0.3 g/h after the meal, providing 20.0 ± 2.1% of the energy yield) (two-factor analysis of variance for repeated measures; \( P > 0.05 \)).

The significant changes in NPRQ after the meal (Figure 2) indicated a marked increase in glucose oxidation and a marked reduction in fat oxidation. However, over the 8 h after the meal, NPRQ only transiently increased above 1.0 (240 min after ingestion of parboiled rice); thus, no net de novo lipogenesis was present. The NPRQ values observed were significantly higher after ingestion of parboiled rice than polished rice (two-factor repeated-measures ANOVA: main effect of type of rice without type of rice \( \times \) time interaction, \( P < 0.05 \)). Line identifies the range of times significantly different from the premeal value (pooled data: one-factor repeated-measures ANOVA and Tukey’s post hoc test, \( P < 0.05 \)).

The changes in tracer:tracee for \([6,6-^2\text{H}_2]\) and \(^{13}\text{C}\)-glucose from −60 to 480 min are shown in Figure 3. The \(^{13}\text{C}\)-glucose:glucose in plasma markedly increased after the meal and leveled off between 120 min and the end of the observation period (Figure 4). No significant difference was observed between the kinetics of Ra, Raexo, and HGP after ingestion of the parboiled or polished rice. As shown in Table 3, the cumulative amounts of glucose, exogenous glucose, and glucose released from the liver appearing in the peripheral circulation were significantly lower between 240–480 min than between 0–240 min. However, no significant difference was observed after ingestion of parboiled or polished rice for any of these 3 variables. At the end of the observation period, the percentages of the glucose load which had appeared in the peripheral blood after ingestion of polished or parboiled rice were not significantly different (70.4 ± 4.5% and 63.8 ± 2.0%; paired \( t \) test, \( P > 0.05 \)). No significant difference was observed between the cumulative amount of glucose that appeared in the circulation and the cumulative amount of glucose released from the liver after ingestion of the 2 types of rice.

**DISCUSSION**

Although consistent data indicate that starch in parboiled rice is less susceptible to digestion than starch in polished rice (8–10), Raexo and the percentages of glucose derived from the ingested meal, providing 25

### Table 2

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<tr>
<th>Time (min)</th>
<th>Polished rice</th>
<th>Parboiled rice</th>
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<tbody>
<tr>
<td>0–240 min</td>
<td>56.6 ± 8.0</td>
<td>65.1 ± 5.9</td>
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<tr>
<td>240–480 min</td>
<td>32.5 ± 4.9(^\dagger)</td>
<td>46.5 ± 2.7(^\dagger)</td>
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<tr>
<td>Fat(^\ddagger)</td>
<td>10.8 ± 2.5</td>
<td>5.9 ± 1.1(^\ddagger)</td>
</tr>
<tr>
<td>240–480 min</td>
<td>10.5 ± 2.0</td>
<td>4.0 ± 0.8(^\ddagger)</td>
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\(^{\dagger}\) All values are \( \bar{x} \pm \text{SEM}; n = 8.\)

\(^{\ddagger}\) Main effect of time (\( P < 0.05 \)), without effect of type of rice and type of rice \( \times \) time interaction.

\(^{\ddagger\ddagger}\) Significantly different from polished rice, \( P < 0.05 \).
starch which appeared into the peripheral circulation over the 8 h after the meal were not significantly different after ingestion of the 2 types of rice. In addition, except for a lower oxidation of fat from a small glucose or starch meal, the absorption of exogenous glucose from a large starch meal is not complete after 8 h, despite the much larger rate of appearance into the peripheral circulation.

As already discussed (21, 22), this could be due to several reasons. First, as indicated by the negative arterioportal gradient of $^{13}$C-glucose reported in pigs by Noah et al (20) 12 h after the end of the meal, glucose could still be absorbed from the gut at the end of the observation period. Second, a portion of exogenous glucose entering the portal vein could be taken up by the liver on first pass. Although this portion remains a matter of debate (21–23), it could be as high as $\approx 30\%$ (22, 23). It is worth mentioning that this percentage is close to the percentage of exogenous glucose that did not appear into the peripheral circulation in the present experiment (30% with polished rice and 36% with parboiled rice). Finally, a portion of exogenous glucose could be converted into 3-carbon products by the gut. For example, Abumrad et al (21) showed that $\approx 11\%$ of a $1.63$ g/kg glucose load actually enters the blood in the form of lactate and alanine.

Starch food, including rice, can be processed and cooked in various ways that could modify their digestibility (8–10, 24), but a paucity of data exists about the actual effect of starch processing on Raexo (20) and on the metabolic response after a meal (25). In the study by Noah et al (20) pregelatinized starch was much more susceptible than native starch to hydrolysis in vitro (71% converted into maltodextrins within 180 min by α-amylase, compared with only 22% for native starch). However, when compared with the native starch, peak Raexo was only 8% higher when the pregelatinized starch was ingested, and the amount of glucose that appeared into the peripheral circulation over the first 4 h after the meal was only 4% higher. Over the subsequent 4 h, the cumulative appearance of exogenous glucose into the peripheral circulation was actually 21% higher from the native than pregelatinized starch. In the present experiment, the susceptibility of the starch in the 2 types of rice was not measured, but consistent data from Casiraghi et al (8), Niba (9), and Rashmi and Urooj (10) indicate that starch from parboiled rice is less digestible than starch from polished rice. However, Raexo and the cumulative amount of exogenous glucose appearing into the peripheral circulation were not significantly different after ingestion of polished or parboiled rice (Figure 4 and Table 3). These observations and those from the study by Noah et al (20) suggest that the susceptibility of starch to hydrolysis might not be the rate-limiting factor for Raexo after ingestion of large starch.

Data from the present study about Raexo after ingestion of a large amount of rice in men ($= 4.0$ g/kg starch) compare well with those reported by Noah et al (20) in pigs with ingestion of 4.3 g/kg corn starch: peak Raexo and the percentage of glucose from the starch load that appeared into the peripheral circulation over 8 h ($= 7–9$ mg·kg$^{-1}$·min$^{-1}$ and 64–70%) were only slightly lower than those reported by Noah et al (20) ($= 11$ mg·kg$^{-1}$·min$^{-1}$ and $= 73\%$). When $= 20–75$ g glucose [reviewed in Livesey et al (17)] or starch (3–5) are ingested in humans, peak Raexo only ranges between $= 3$ and $= 7$ mg·kg$^{-1}$·min$^{-1}$, and 100% of exogenous glucose could appear into the peripheral circulation within 4.5 h after the meal. Thus, unlike the absorption of glucose from a small glucose or starch meal, the absorption of exogenous glucose from a large starch meal is not complete after 8 h, despite the much larger rate of appearance into the peripheral circulation.

All values are $\bar{x} \pm$ SEM; $n = 8$.

Main effect of time ($P < 0.05$), without effect of type of rice and type of rice $\times$ time interaction.

Significantly different from 0–240 min, $P < 0.05$. with parboiled than polished rice, the metabolic responses did not differ markedly: changes in plasma glucose, insulin, lactate, and free fatty acid concentrations and the increase in Ra and the reduction in HGP were not significantly different after ingestion of the 2 types of rice.

<table>
<thead>
<tr>
<th>Glucose released from the liver</th>
<th>Polished rice</th>
<th>Parboiled rice</th>
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<tr>
<td>0–240 min</td>
<td>34.1 ± 4.7</td>
<td>44.0 ± 4.3</td>
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<tr>
<td>240–480 min</td>
<td>11.5 ± 4.6$^d$</td>
<td>14.5 ± 3.4$^d$</td>
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$^d$ Significantly different from 0–240 min, $P < 0.05$.
meals. In this situation, the limiting factor could rather be the rate of glucose absorption by the gut.

This phenomenon could explain that, in the present experiment, changes in plasma glucose and insulin concentrations in response to the meal and changes in HGP were not significantly different after ingestion of polished or parboiled rice. Immediately at the end of the meal, plasma glucose and insulin concentrations were markedly increased but were not significantly different. As for HGP, it progressively decreased over the observation period, which is in line with data from several studies (3, 5, 17, 21–23) after administration of small glucose or starch loads (30–75 g), as well with data from Noah et al (20) after ingestion of a large amount of starch in pigs. However, the reduction was not significantly different after ingestion of polished or parboiled rice. In addition, despite the large value of R_aexo and the elevated concentration of insulin, HGP was not totally suppressed. In fact, changes in HGP did not follow changes in plasma insulin or glucose concentrations. For example, HGP remained high over the first 4 h after the meal, at a time when plasma glucose and insulin concentrations were both high but remained high over the first 4 h after the meal, at a time when insulin concentration was back to premeal values.

In the present experiment, in accordance with consistent data from several studies of disposal of a large carbohydrate load, fat oxidation was markedly reduced, whereas glucose oxidation was stimulated (7, 26–29). However, changes in glucose compared with fat oxidation did not closely follow those in R_aexo, althoughRaexo was not significantly different after ingestion of the 2 types of rice, fat oxidation was significantly lower after ingestion of parboiled than polished rice. A possible explanation for these differences is that a portion of exogenous glucose could be oxidized in the gut, could be absorbed under the form of 3-carbon products, or both, as shown by Abumrad et al (21). This portion, which could be different after ingestion of parboiled or polished rice, escapes detection by the tracer techniques used in the present experiment, which only track the appearance of glucose in the peripheral blood.

We are indebted to Martine Laville and her colleagues from the Centre de Recherche en Nutrition Humaine, Faculté de Médecine RTH Laennec, Lyon, for the measurement of 13C/12C in plasma glucose.

FP, XL, and MP initiated the study and developed the protocol and experimental design. The rice intrinsically labeled with 13C was grown under the supervision of MP. The experiments were performed by MK-A, DB, and HR. Data were compiled and analyzed by MK-A, HR, and FP and discussed with all of the authors. FP wrote the manuscript with contributions from MK-A, HR, and XL. None of the authors had any personal or financial conflicts of interest with regard to the study.

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