Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism¹–³

M Denise Robertson, Alex S Bickerton, A Louise Dennis, Hubert Vidal, and Keith N Frayn

ABSTRACT

Background: Resistant starch may modulate insulin sensitivity, although the precise mechanism of this action is unknown.

Objective: We studied the effects of resistant starch on insulin sensitivity and tissue metabolism.

Design: We used a 4-wk supplementation period with 30 g resistant starch/d, compared with placebo, in 10 healthy subjects and assessed the results by using arteriovenous difference methods.

Results: When assessed by euglycemic-hyperinsulinemic clamp, insulin sensitivity was higher after resistant starch supplementation than after placebo treatment (9.7 and 8.5 × 10⁻² mg glucose · kg⁻¹ · min⁻¹ · (mU insulin/L)⁻¹, respectively; P = 0.03); insulin sensitivity during the meal tolerance test (MTT) was 33% higher (P = 0.05). Forearm muscle glucose clearance during the MTT was also higher after resistant starch supplementation (P = 0.03) despite lower insulin concentrations (P = 0.02); glucose clearance adjusted for insulin was 44% higher. Subcutaneous abdominal adipose tissue non-esterified fatty acid (NEFA; P = 0.02) and glycerol (P = 0.05) release were lower with resistant starch supplementation, although systemic NEFA concentrations were not significantly altered. Short-chain fatty acid concentrations (acetate and propionate) were higher during the MTT (P = 0.05 and 0.01, respectively), as was acetate uptake by adipose tissue (P = 0.03). Fasting plasma ghrelin concentrations were higher with resistant starch supplementation (2769 compared with 2062 pg/mL; P = 0.03), although postprandial suppression (40–44%) did not differ significantly. Measurements of gene expression in adipose tissue and muscle were uninformative, which suggests effects at a metabolic level. The resistant starch supplement was well tolerated.

Conclusion: These results suggest that dietary supplementation with resistant starch has the potential to improve insulin sensitivity. Further studies in insulin-resistant persons are needed.

Key Words: Ghrelin, lipolysis, short-chain fatty acids, skeletal muscle, insulin sensitivity

INTRODUCTION

Type 2 diabetes affects ≈8% of adults in the United States (1), and this figure is likely to increase with the growing incidence of childhood obesity. Although treatments exist to alleviate some of the complications of diabetes, the preferable long-term strategy is still to increase tissue insulin sensitivity and thus prevent the development of overt type 2 diabetes. Recent large-scale studies clearly showed that lifestyle intervention (diet and exercise) can be highly effective in delaying the onset of type 2 diabetes (2), even when compared with pharmacologic agents (3). The results of controlled intervention studies suggest that certain nutritional factors are consistently linked to a reduction in insulin sensitivity: low dietary fiber intake, high trans fatty acid intake; and high saturated fat intake (4). The potential metabolic effects of dietary fatty acids on insulin sensitivity have been extensively studied both from observational (5) and mechanistic viewpoints (6). The effects of dietary fiber, however, remain underdocumented.

Dietary fibers can be classified chemically as either soluble or insoluble. Soluble fibers have viscous properties within the gastrointestinal tract and cause a well-documented reduction in the rate of glucose absorption (7) and thus reduce the glycemic excursion after carbohydrate intake. Insoluble fibers, such as resistant starch (RS), are nonviscous and thus have no effect on glucose absorption, yet they have been shown in short-term human studies (8) to increase insulin sensitivity. Despite epidemiologic evidence linking insoluble fiber intake to a reduced incidence of type 2 diabetes (9–11), the metabolic link between chronic RS ingestion and insulin sensitivity has yet to be proven in humans.

One possible mechanism by which dietary RS intake might modulate insulin sensitivity is through alterations in fatty acid flux. Fatty acid metabolism is a key feature in determining tissue insulin sensitivity. Abnormalities in fatty acid storage and lipolysis in insulin-sensitive tissues with increased flux from adipose to nonadipose tissues such as skeletal muscle may be a critical event in the development of insulin resistance (12). The direct effect of RS consumption on fatty acid flux is unknown beyond studies that have measured fasting triacylglycerol and cholesterol concentrations after RS intervention (7). In isolation, short-chain fatty acids (SCFAs), which are produced during colonic fermentation of RS, inhibit adipose tissue lipolysis (13), but an in vivo effect of dietary RS intake has yet to be shown.

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We showed previously that short-term (24 h) high doses of RS (60 g/d) significantly elevate postprandial insulin sensitivity, with lower circulating concentrations of both NEFAs and SCFAs (8). In the present study, we assessed the effect of a more sustainable dose of RS of 30 g/d for 4 wk to allow the assessment of longer-term metabolic adaptation to RS. By using an integrative approach to this nutritional question, we have assessed adaptation to RS intake at the gene-tissue and whole-body levels in humans.

SUBJECTS AND METHODS

Ten healthy subjects were recruited (6 female). Their ages ranged from 24 to 61 y (x: 48.5 ± 3.4 y), and their body mass indexes (BMIs; in kg/m²) ranged from 18.4 to 32.3 (x: 23.4 ± 1.4). The subjects had no history of gastrointestinal, endocrine, or cardiovascular disease and were currently not taking any prescribed medication (with the exception of hormone replacement therapy). Habitual diet was assessed by use of 7-d food records and the US Department of Agriculture database.

General protocol

This was a single-blind, crossover dietary intervention study in which the subjects visited the metabolic unit for 4 experimental studies over a period of 12 wk, twice for hyperinsulinemic-euglycemic clamps and twice for meal tolerance tests (MTTs; Figure 1).

The subjects were initially randomly assigned to receive either Hi-Maize 260 (National Starch and Chemical, Manchester, United Kingdom) at 50 g/d (30 g type II RS and 20 g rapidly digestible starch) or Amioca (National Starch and Chemical) at 20 g/d (0 g type II RS and 20 g rapidly digestible starch) for 4 wk added to their habitual diet, separated by a 4-wk washout period. The starch was supplied in ready-to-use sachets, and the subjects were instructed how to incorporate these into everyday foods. The subjects were then asked to follow their 7-d food record and to complete new diet diaries (completed during the run-in week) during each 4-wk intervention to standardize their background diet. Bowel habit diaries were also completed during each intervention for assessment of tolerance.

At the end of the third week of each 4-wk dietary intervention, the subjects attended the metabolic unit for a hyperinsulinemic-euglycemic clamp in the fasting state. At the end of the fourth week, the subjects attended for a full-day MTT with arteriovenous blood sampling across both skeletal muscle and subcutaneous abdominal adipose tissue. The subjects avoided vigorous exercise and alcohol for 48 h before each test and were provided with a low-fat, low-fiber evening meal (pasta and tomato sauce) before each study day to reduce variability (14). This study was approved by the Oxfordshire Clinical Research Ethics Committee, and all subjects gave written informed consent. For clarity of presentation in the results and discussion sections, the high-RS supplement (Hi-Maize 260) will be referred to as RS and the RS-free supplement (Amioca) as placebo.

Metabolic investigations

Euglycemic-hyperinsulinemic clamp

This was performed as described by DeFronzo et al (15). Beginning at 0700 after the subjects had fasted for 12 h overnight, an antecubital vein was cannulated for the infusion of both a 20% glucose solution and insulin [Actrapid (Novo Nordisk, Bagsvaerd, Denmark) in a solution of 0.9% saline containing 2 mL of the subject’s blood to prevent adhesion of insulin to plastics]. A hand vein was cannulated retrogradely, and the hand was placed in a heated box (55 °C) for sampling of arterialized blood. After fasting measurements were made, the insulin infusion was started at a rate of 35 mU insulin · m² · min⁻¹. The glucose concentration of whole blood was measured every 5 min by use of a glucose analyzer (Hemocue B-glucose analyzer; Hemocue Ltd, Sheffield, United Kingdom), and the infusion of 20% glucose was adjusted as needed to maintain blood glucose concentrations at 5 mmol/L. Blood was drawn every 10 min during the clamp for the measurement of plasma insulin, nonesterified fatty acids (NEFAs), C-peptide, and ghrelin. The insulin infusion was continued for 120 min to obtain a measure of the rate of insulin-stimulated glucose disposal during the last 30 min of the clamp.

Meal tolerance test with tissue-specific arteriovenous sampling

To assess the metabolism of adipose tissue and skeletal muscle in vivo in humans, we measured arteriovenous differences across these tissues. Serial blood samples were taken with the subjects in the fasting state and for 5 h after a liquid MTT (60 g carbohydrate, 21 g fat, 500 kcal).

Arterialized blood was obtained from a vein draining a heated hand. Venous blood from muscle was taken from a vein draining...
the deep tissues of the contralateral forearm. To prevent contamination of the blood from the forearm vein with blood from the hand, a wrist cuff was inflated to 200 mm Hg for 3 min before the samples were taken. Venous blood from adipose tissue was obtained from the superficial epigastric vein (16). This vein drains subcutaneous abdominal adipose tissue with negligible contribution from other tissues (17). Oxygen saturation and ultrasound were used to assess correct positioning of the cannulae. Simultaneous sampling from 3 sites began at 0700 after the subjects had fasted for 12 h overnight. Two sets of baseline samples were taken 30 min apart. Subjects then ingested the test meal as described previously (8), and further blood samples were taken for 5 h after the meal.

Subcutaneous abdominal adipose tissue blood flow was measured by $^{133}$Xe washout (18). Forearm muscle blood flow was assessed by occlusion strain-gauge plethysmography (19).

Skeletal muscle and adipose tissue biopsies were performed under local anesthesia (1% lignocaine) 5.5 h after the meal. Subcutaneous abdominal adipose tissue was biopsied with a 12-gauge needle, and muscle biopsy samples were taken from the vastus lateralis muscle by using a percutaneous needle technique. Samples were snap frozen in liquid nitrogen and stored at $-80^\circ$C for later RNA quantification. Total RNA was prepared from the frozen tissue according to an established procedure (20), with total RNA solutions stored at $-70^\circ$C.

Biochemistry

Whole blood for metabolite and insulin measurement was collected into heparin-containing syringes (Sarstedt, Leicester, United Kingdom). Plasma glucose, triacylglycerol (Instrumentation Laboratory, Warrington, United Kingdom), and NEFA concentrations (Wako Chemicals, Neuss, Germany) were measured enzymatically with an Instrumentation Laboratory Monarch automated analyzer. Whole blood for 3-hydroxybutyrate and glycerol measurement was deproteinized with 7% (w/vol) perchloric acid, and concentrations were measured enzymatically. Metabolites from the placebo and the RS study arms were analyzed together, and the intra-assay variation was <2.5%. Concentrations of insulin, C-peptide, and leptin were measured by radioimmunoassay with commercially available kits (Linco, St Louis, MO). Blood for total ghrelin analysis was collected into potassium-EDTA containing 200 Kallikrein inhibiting units aprotinin/mL (Bayer, Newbury, United Kingdom). For the ghrelin radioimmunoassay (Linco), antibodies were raised against the C-terminal region (acylated and des-acylated ghrelin) and showed no detectable cross-reactivity with motilin-related peptide. The sensitivity of this assay was 10 pg/mL. Glucagon-like peptide 1 (GLP-1) was measured by radioimmunoassay as described previously (21). The GLP-1 radioimmunoassay detected changes of 7.5 pmol/L, with an intraassay CV of 6.1%. The GLP-1 antibody was specific for the N-terminal amidated GLP-1.

SCFAs were analyzed by gas-liquid chromatography. Heparin-treated plasma was deproteinized with 16% metaphosphoric acid and denatured for 30 min at 60 °C before splitless injection of 1 μL of the supernatant portion onto an FFAP column (Agilent Technologies, Palo Alto, CA). Measurements were made with a flame ionization detector, and isovaleric acid was used as the internal standard.

Quantitation of messenger RNAs

Concentrations of the messenger RNAs (mRNAs) corresponding to the genes of interest were measured by reverse transcription (RT) followed by real-time polymerase chain reaction (PCR) with a light cycler (Roche Diagnostics, Meylan, France). First-strand complementary DNAs (cDNAs) were first synthesized from 500 ng of total RNA in the presence of 100 units of Superscript II (Invitrogen, Eryang, France) using both random hexamers and oligo (dT) primers (Promega, Charbonnières, France). The real-time PCR was performed in a final volume of 20 μL containing 5 μL of a 60-fold dilution of the RT reaction medium, 15 μL of reaction buffer from the FastStart DNA master SYBR Green kit (Roche Diagnostics), and 10.5 pmol of the specific forward and reverse primers (Eurobio, Let Ulis, France). Primers were selected to amplify small fragments (80 to 200 bp) and to hybridize in different exons of the target sequences. For quantification, a standard curve was systematically generated with 6 different amounts (150 to 30 000 molecules/tube) of purified target cDNA cloned in the pGEM plasmid (Promega).

Each assay was performed in duplicate. The results are presented in absolute concentrations, in amol/μg of total RNA. Cyclophilin mRNA levels were measured as the internal standard.

Calculations and statistical analysis

Insulin sensitivity and β-cell function were assessed by homeostatic model assessment (HOMA %S and HOMA %B, respectively; 23), hyperinsulinemic-euglycemic clamp [equal to the glucose infusion rate during steady state (M)] divided by the prevailing plasma insulin concentration (I)], and in the postprandial state during the MTT with the use of a minimal model approach (24). This approach uses cumulative integrated area under the curve (AUC) measures of both insulin and glucose concentrations assuming that the total glucose disposal from the system after 120 min (or when basal values have been reached) equals the glucose entering the peripheral circulation, allowing for first-pass extraction by the liver. The incremental ratio of C-peptide to insulin over the first 2 h of the study, which is an index of hepatic insulin extraction, was calculated with the trapezoid method.

During the MTT, adipose tissue blood flow was calculated as described previously (18). Arteriovenous and venoarterial differences in metabolite concentrations were calculated. Absolute flux was calculated as the product of the arteriovenous or venoarterial difference and tissue blood or plasma flow as appropriate. The rate of action of lipoprotein lipase in adipose tissue in vivo was calculated from triacylglycerol extraction. The rate of action of hormone-sensitive lipase in adipose tissue in vivo was calculated from the total adipose tissue glycerol release after subtraction of the lipoprotein lipase rate of action (16). Total fatty acid uptake into muscle was calculated from the rate of triacylglycerol and NEFA removal across the tissue (16).

All statistical analyses were performed with SPSS version 12.0.1 for WINDOWS (SPSS Inc, Chicago, IL). Time-course data were analyzed by repeated-measures analysis of variance when normally distributed. Postprandial data are also presented in the tables, text, and figures in summary form, i.e., fasting and AUC [AUC values were calculated by using the trapezoid rule (25)]. Summary data were analyzed by paired Student’s t tests. Partial correlations (controlled for subject) were also performed between metabolites by using Pearson’s correlation. Values of
TABLE 1
Anthropometric measurements taken after 4 wk of supplementation with either placebo or resistant starch

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<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Resistant starch</th>
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<tbody>
<tr>
<td>Body weight (kg)</td>
<td>70.6 ± 3.74</td>
<td>71.0 ± 3.88</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.7 ± 1.33</td>
<td>23.8 ± 1.40</td>
<td>NS</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>19.2 ± 2.05</td>
<td>18.5 ± 2.36</td>
<td>NS</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>51.4 ± 3.03</td>
<td>52.5 ± 3.08</td>
<td>0.003</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>118 ± 3.07</td>
<td>117 ± 4.08</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>74.4 ± 1.78</td>
<td>74.7 ± 3.39</td>
<td>NS</td>
</tr>
</tbody>
</table>

1 All values are x ± SEM; n = 10. All measurements were taken in the morning after the subjects had fasted for 12 h. The placebo contained 0 g resistant starch and 20 g rapidly digestible starch; the resistant starch supplement contained 30 g resistant starch and 20 g rapidly digestible starch. Comparisons were made with the paired t test.

2 Measured by using foot-to-hand bioimpedance (Bodystat 1500; Bodystat, Isle of Man, United Kingdom).

3 Mean of 3 readings taken with the subject in a sitting position.

P < 0.05 were taken as significant. Values in the text are displayed as means with ranges or SEMs. Gene expression data were analyzed by using the nonparametric Wilcoxon’s test to compare mRNA levels between the placebo and RS diets.

RESULTS
The inclusion of an additional 30 g RS/d in the diet was well tolerated [mean fiber intake increased from 17.5 g/d (range: 14.6–21.3 g/d) to 46.5 g/d]. There was no significant effect of the RS supplement on stool frequency; adverse gastrointestinal symptoms (abdominal pain, flatulence, bloating, and constipation) were reported with a mean frequency of 1.0 (range: 0–1) incident per month on the placebo treatment compared with 3.0 (range: 0–5) incidents per month with the RS supplement (P > 0.05). Mean daily macronutrient intake as assessed by 7-d food records was 17.5 g dietary fiber (Association of Official Analytical Chemists classification), 256 g carbohydrate, 57.8 g fat, and 7.76 MJ/d during the placebo intervention and 47.9 g dietary fiber, 266 g carbohydrate, 62 g fat, and 7.9 MJ/d during the RS intervention. There was no significant difference in reported food intake between the 2 supplementation periods and no subsequent change in either body weight or BMI (Table 1). There was a small but significant increase in lean body mass (P = 0.003) averaging 1.1 kg (0.6–1.6 kg) over the 4-wk period of RS supplementation (Table 1).

Glucose metabolism and insulin sensitivity
There was no significant effect of RS supplementation on either β-cell function or fasting insulin sensitivity (23; Table 2) when measured by HOMA. During the hyperinsulinemic-euglycemic clamp study, insulin sensitivity was significantly higher (P = 0.027) after RS intake than with placebo. Oral insulin sensitivity (incorporating the effects of the gastrointestinal tract) was also significantly higher after RS supplementation (P = 0.05). The postprandial insulin concentration was significantly lower after RS supplementation (Figure 2) and the molar ratio of C-peptide to insulin was significantly higher, which indicated increased hepatic insulin extraction.

During the MTT, insulin sensitization was noted in both skeletal muscle and adipose tissue after the RS supplementation. Muscle glucose clearance was significantly higher despite lower prevailing insulin concentrations (Figure 2C). The mean glucose clearance per pmol/L insulin, averaged across the study period, was 43.9% higher after RS intake than after the placebo (P = 0.013). A similar trend was observed for adipose tissue, with glucose uptake being higher by almost 3-fold (Table 2; P = 0.007). There

TABLE 2
Indexes of insulin sensitivity after 3 or 4 wk of a high–resistant starch (RS) supplement (30 g RS/d) or placebo (0 g RS/d)

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Resistant starch</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperinsulinemic-euglycemic clamp (week 3)</td>
<td>8.5 × 10⁻² ± 8.7 × 10⁻³</td>
<td>9.7 × 10⁻² ± 1.09 × 10⁻²</td>
<td>0.027</td>
</tr>
<tr>
<td>M/I</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>MTT (week 4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA %S</td>
<td>77.4 ± 5.55</td>
<td>76.75 ± 6.72</td>
<td>NS</td>
</tr>
<tr>
<td>HOMA %B</td>
<td>128 ± 9.21</td>
<td>138 ± 8.83</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>5.04 ± 0.118</td>
<td>5.06 ± 0.139</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma glucose AUC (mmol · 300 min/L)</td>
<td>1890 ± 27.7</td>
<td>1830 ± 28.9</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting plasma insulin (pmol/L)</td>
<td>79.8 ± 16.0</td>
<td>84 ± 17.4</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma insulin AUC (pmol · 300 min/L)</td>
<td>63 000 ± 10 600</td>
<td>55 200 ± 10 500</td>
<td>0.024</td>
</tr>
<tr>
<td>C-peptide/insulin AUC</td>
<td>6.08 ± 0.523</td>
<td>7.48 ± 0.734</td>
<td>0.034</td>
</tr>
<tr>
<td>Oral G1</td>
<td>1.36 × 10⁻³ ± 1.9 × 10⁻⁴</td>
<td>1.82 × 10⁻³ ± 3.6 × 10⁻⁴</td>
<td>0.050</td>
</tr>
<tr>
<td>Total glucose uptake by AT (μmol/100 mL tissue)</td>
<td>54.4 ± 62.5</td>
<td>141 ± 59.3</td>
<td>0.007</td>
</tr>
</tbody>
</table>

1 All values are x ± SEM; n = 10. The total uptake of glucose across adipose tissue (AT) is the arteriovenous difference times the AT plasma flow calculated as an area under the curve (AUC) between 0 and 300 min. M/I, glucose infusion rate during steady state (mg · kg⁻¹ · min⁻¹) divided by the prevailing plasma insulin concentration (mU insulin/L); MTT, meal tolerance test; HOMA %S and HOMA %B, insulin sensitivity and β cell function, respectively, assessed by homeostatic model assessment; G1, insulin sensitivity assessed during the meal tolerance test [(dL · kg⁻¹ · min⁻¹)] · (μU/mL)]. Comparisons were made with the paired t test.
was no significant relation between insulin sensitization with the RS supplement and BMI.

**Fatty acid metabolism**

There was no significant effect of RS intake on either fasting triacylglycerol or NEFA concentrations. During the MTT, however, the postprandial output of both NEFAs and glycerol from adipose tissue was significantly lower with RS supplementation (Figure 3). The decreased NEFA output from adipose tissue reflected a significant postprandial reduction in the rate of action of hormone-sensitive lipase, the enzyme responsible for the mobilization of stored triacylglycerol (from 128 ± 48 to 55 ± 36 nmol · 100 mL tissue⁻¹ · min⁻¹; P = 0.046). There was no significant effect of RS intake on plasma triacylglycerol (data not shown) despite a significant reduction in the calculated rate of action of lipoprotein lipase (from 188 ± 39 to 114 ± 29 nmol · 100 mL tissue⁻¹ · min⁻¹; P = 0.026). Although the release of NEFAs from adipose tissue was lower, the arterialized NEFA concentration and the total uptake of fatty acids by muscle were unchanged by supplementation (data not shown).

**Short-chain fatty acids**

Systemic concentrations of both acetate and propionate were higher after RS supplementation than with placebo (Table 3 and Figure 4), although no significant effect of supplementation was seen on plasma butyrate. During the MTT, there was significant net uptake of SCFAs across both muscle and adipose tissue after both the placebo and the RS-supplemented diets. The uptake of acetate was significant after both the placebo (P = 0.009 across muscle; P = 0.037 across fat) and the high-RS diet (P = 0.002 across muscle; P = 0.004 across fat). (The P values refer to whether we were able to measure significant uptake by the tissue; comparisons of uptake between the dietary periods are given in Table 3.) However, propionate uptake was significant only after the high-RS diet (P = 0.006 across muscle; P = 0.027 across fat). Butyrate uptake was not significant. Acetate uptake increased after RS supplementation across both tissues: as a function of increased plasma concentration (adipose tissue) and as increased fractional extraction (skeletal muscle) (Table 3).
Propionate was present at much lower concentrations and had a higher fractional extraction across adipose tissue after the RS supplement (Table 3).

Hormones
RS supplementation resulted in a significant increase in fasting plasma ghrelin concentrations, although the degree of postprandial suppression during the MTT (40–44%) remained unchanged (Figure 5). The postprandial AUC for ghrelin was significantly correlated with both BMI ($r = 0.523, P = 0.021$) and $MI$ ($r = 0.496, P = 0.03$). There was no significant effect of RS supplementation on plasma leptin or GLP-1 concentrations (data not shown).

### TABLE 3

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Resistant starch</th>
<th>$P$</th>
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<tbody>
<tr>
<td><strong>Plasma acetate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting (μmol/L)</td>
<td>212 ± 23.7</td>
<td>217 ± 18.2</td>
<td>NS</td>
</tr>
<tr>
<td>AUC (μmol · 300 min/L)</td>
<td>53 800 ± 4260</td>
<td>69 200 ± 5970</td>
<td>0.037</td>
</tr>
<tr>
<td>Fractional extraction across muscle (%)</td>
<td>15.7 ± 4.33</td>
<td>24.2 ± 3.68</td>
<td>0.05</td>
</tr>
<tr>
<td>Net uptake by muscle (nmol/100 g tissue)</td>
<td>14 600 ± 4400</td>
<td>25 900 ± 5900</td>
<td>0.064</td>
</tr>
<tr>
<td>Fractional extraction across AT (%)</td>
<td>8.62 ± 2.88</td>
<td>14.6 ± 2.34</td>
<td>NS</td>
</tr>
<tr>
<td>Net uptake by AT (nmol/100 mL tissue)</td>
<td>8200 ± 2890</td>
<td>18 200 ± 3640</td>
<td>0.034</td>
</tr>
<tr>
<td><strong>Plasma propionate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting (μmol/L)</td>
<td>5.74 ± 1.26</td>
<td>9.09 ± 2.48</td>
<td>NS</td>
</tr>
<tr>
<td>AUC (μmol · 300 min/L)</td>
<td>1490 ± 155</td>
<td>2690 ± 320</td>
<td>0.012</td>
</tr>
<tr>
<td>Fractional extraction across muscle (%)</td>
<td>25.2 ± 12.8</td>
<td>39.3 ± 7.98</td>
<td>NS</td>
</tr>
<tr>
<td>Net uptake by muscle (nmol/100 g tissue)</td>
<td>846 ± 453</td>
<td>1100 ± 310</td>
<td>NS</td>
</tr>
<tr>
<td>Fractional extraction across AT (%)</td>
<td>14.1 ± 9.05</td>
<td>35.3 ± 13.5</td>
<td>0.048</td>
</tr>
<tr>
<td>Net uptake by AT (nmol/100 mL tissue)</td>
<td>791 ± 424</td>
<td>1530 ± 495</td>
<td>NS</td>
</tr>
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</table>

1 All values are $\bar{x} ±$ SEM; $n = 10$ for muscle and $n = 8$ for adipose tissue (AT). The fractional extraction across both muscle and AT is calculated as the arteriovenous difference across the individual tissue divided by the arterIALIZED concentration and is expressed as a percentage. The total uptake of fatty acid across tissue is the arteriovenous difference across the individual tissue times the tissue plasma flow calculated as an area under the curve (AUC) between 0 and 300 min. Comparisons were made with the paired $t$ test.

**FIGURE 4.** Mean ($±$ SEM) arterIALIZED short-chain fatty acid concentrations after a meal tolerance test (at time 0) in healthy subjects after a 4-wk intervention of 30 g resistant starch (RS)/d (●) compared with placebo (○). $n = 10$. Repeated-measures ANOVA showed significant effects of the RS intervention for both acetate ($P = 0.048$) and propionate ($P = 0.009$), with a significant effect of time after meal for acetate ($P = 0.036$) but not for propionate ($P = 0.069$).

**FIGURE 5.** Mean ($±$ SEM) plasma ghrelin concentrations after a meal tolerance test (dashed line) in healthy subjects after a 4-wk intervention of 30 g resistant starch (RS)/d (●) compared with placebo (○). $n = 10$. Repeated-measures ANOVA showed a significant effect of the RS intervention ($P = 0.027$).
Gene expression

In adipose tissue, there was no significant change in the expression of the following genes after RS supplementation: hexokinase II, CD36, lipoprotein lipase, peroxisome proliferator activated receptor γ, phosphatidyl-inositol-3 kinase, glucose transporter 4, or leptin. There was, however, significantly greater expression of hormone-sensitive lipase (35.7 ± 5.5 to 45.8 ± 7.7 amol/μg total RNA; P = 0.036) and lower expression of insulin receptor substrate 1 (11.2 ± 1.5 to 8.8 ± 1.4 amol/μg total RNA; P = 0.016) after RS supplementation than with placebo. In skeletal muscle samples, however, there was no significant effect of dietary period on the expression of any of the genes measured (hexokinase II, CD36, lipoprotein lipase, insulin receptor substrate 1, phosphatidyl-inositol-3 kinase, or glucose transporter 4; data not shown).

DISCUSSION

We used an integrative approach to study the metabolic effects of RS intake and obtained novel data concerning the function of individual tissues (adipose tissue and skeletal muscle) in vivo in humans after a period of dietary supplementation. In an earlier short-term study (60 g RS for 24 h) (8), we found RS intake to increase insulin sensitivity at the whole-body level and to increase hepatic insulin clearance, a finding that was corroborated in the present study when RS was included in the diet at more physiologic levels (30 g RS/d for 4 wk). We have now shown insulin sensitization at the whole-body level assessed by both the hyperinsulinemic-euglycemic clamp and the MTT methods. In addition, we showed a reduction in adipose tissue lipolysis and an increase in the insulin sensitivity of skeletal muscle glucose clearance. These effects of RS may be due to changes in the peripheral metabolism of SCFAs or in the secretion of ghrelin.

The concept that dietary fibers induce insulin sensitization by reducing the plasma NEFA concentration (26–28) and thus that they serve as a signal between adipose tissue and muscle has not been supported by conclusive in vivo data. Through direct measurement of tissue metabolic flux rates, we have been able to show the metabolic changes induced by fermentable fiber at both the whole-body and the tissue level.

Adipose tissue lipolysis, as assessed by the calculated rate of action of hormone-sensitive lipase, was significantly lower after RS supplementation than with placebo. Hormone-sensitive lipase is suppressed by insulin after meals, yet we observed lower insulin during the RS study, and thus the results are not consistent with adipose tissue simply responding to concentrations of insulin. To suppress lipolysis, only small excursions in the plasma insulin concentration are required, and this may explain why the maximal effects of RS supplementation were not evident until the late postprandial period when insulin concentrations were low. Insulin-mediated skeletal muscle glucose clearance (glucose clearance/plasma insulin concentration) was significantly higher after RS supplementation. However, we found no direct link between a reduced rate of fatty acid release from adipose tissue and the increased muscle insulin sensitivity, because the skeletal muscle uptake of total fatty acids was unchanged. We cannot, however, exclude the possibility that changes in NEFA flux may influence insulin sensitization in other organs, such as the liver (29). Adipose tissue itself is also an important site for maintaining glucose homeostasis, with total glucose removal increasing by 200% after RS supplementation. The metabolic fate of this additional glucose is at present unclear.

A novel aspect of the present study was the direct measurement of SCFA uptake into both skeletal muscle and adipose tissue. After RS supplementation, the peripheral concentrations of both acetate and propionate were increased, as was the rate of uptake into the specific tissues. SCFAs were recently shown to bind to the G protein–coupled receptors GPR41 and GPR43, which have been isolated from both adipose tissue (30) and skeletal muscle (31). The function of these receptors is at present ill defined, but they have been proposed to trigger leptin release from adipocytes. Despite significantly higher SCFA concentrations, no significant change in plasma leptin or leptin mRNA was found after RS supplementation. SCFAs have been shown to inhibit adipose tissue lipolysis in vivo (13, 32) and thus may contribute to the observed reduction in lipolysis. However, this has never been reported physiologically after fiber intake. We found no relation between NEFA release from adipose tissue and SCFA concentrations, so additional mechanisms may be involved. The fate of the SCFAs taken up into skeletal muscle is likely to be rapid oxidation (33). The accumulation of acetyl CoA from acetate may result in acetylation of the carnitine pool, thus limiting the availability of free carnitine (34) and potentially reducing fatty acid transport into the mitochondria for oxidation. Increased muscle insulin sensitivity could therefore be partly explained by the increased muscle uptake of SCFAs.

An interesting observation was the increase in the circulating concentration of total ghrelin after RS supplementation. This result is counterintuitive from what we know about the satiating effects of RS (35, 36) and the appetite-stimulating effects of ghrelin (37). Perhaps the systemic concentration of ghrelin is more relevant in determining its peripheral actions, independent of those induced within the hypothalamus. Elevations in plasma ghrelin have been linked to increased insulin sensitivity in numerous studies (38–40), although debate still exists as to the mechanism. It is hypothesized that the hyperinsulinemia of insulin resistance down-regulates ghrelin release and thus that elevated ghrelin is merely a consequence of the low insulin concentrations. In the present study, fasting ghrelin concentrations were significantly elevated with no significant change in fasting insulin concentrations, and thus it is doubtful that this mechanism is in place. Ghrelin has been shown to inhibit lipolysis, stimulate lipogenesis, and stimulate the expression of peroxisome proliferator activated receptor γ in vitro (41), which potentially influence insulin sensitivity in vivo. The lack of a change in fatty acid uptake into muscle in our study implies that any insulin-sensitizing effects of ghrelin are independent of those induced in adipose tissue. Ghrelin may have direct insulin-sensitizing effects on skeletal muscle. Muscle expresses the putative ghrelin receptor GHS-R1b (42), although a function for this receptor remains to be described. It is perhaps more likely that the skeletal muscle ghrelin receptor is a protein independent of either GHS-R1a or 1b (43), such as the fatty acid translocase protein CD36/FAT, as has been shown in cardiac muscle (44). If elevated plasma ghrelin concentrations are partly responsible for the insulin-sensitizing effects of RS, then the link between fermentation in the colon and ghrelin production from the stomach warrants further investigation.

Increases in systematic SCFA concentrations have been shown to augment the expression of functional proteins within the intestine (45), potentially because of the ability of SCFAs to...
mediate the release of glucagon-like peptide 2 (GLP-2) (46) or gastrin (47). We suggest that increased peripheral concentrations of SCFA [not colonic SCFAs (48)], either directly or indirectly via GLP-2 or gastrin, are linked to ghrelin release. However, more work is needed to clarify the link between colonic fermentation and gastric ghrelin. In contrast with the results of animal studies, the present RS intervention did not increase plasma concentrations of GLP-1. In terms of systemic effects, GLP-1 is an important incretin; however, tissue-specific studies in humans have clearly shown that GLP-1 does not directly inhibit adipose tissue lipolysis when measured by microdialysis (49) and so the change observed in adipose tissue function would not be predicted to be dependent on GLP-1. The measurement of gene regulation after RS intake is novel, yet, unlike pharmacologic interventions (50), the increased uptake of glucose into skeletal muscle could not be explained by changes in expression of the key genes involved in either insulin signaling or glucose uptake. This does not, however, exclude the possibility that gene expression in other tissues such as the gastrointestinal tract contributes to the metabolic changes observed.

In conclusion, RS intake increases insulin sensitivity in non-insulin-resistant subjects by changing both adipose tissue and skeletal muscle metabolism. This is potentially due to elevations in the systemic concentrations of both ghrelin and SCFAs. RS intake at this dose (30 g/d) was well tolerated and thus could have beneficial effects for the treatment of insulin-resistant persons or those with type 2 diabetes. This would require further investigation.

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