Neither raw nor retrograded resistant starch lowers fasting serum cholesterol concentrations in healthy normolipidemic subjects\textsuperscript{1–3} 

Marie-Louise A Heijnen, Johan MM van Amelsvoort, Paul Deurenberg, and Anton C Beynen

ABSTRACT The question addressed was whether dietary resistant starch would lower serum cholesterol and triacylglycerol concentrations in healthy normolipidemic subjects. In a randomized single-blind 3 × 3 Latin-square study with corrections for any carryover effects, 27 males and 30 females consumed supplements containing glucose or resistant starch (RS) from raw high-amylose cornstarch (RS\textsubscript{2}) or from retrograded high-amylose cornstarch (RS\textsubscript{3}). The RS\textsubscript{2} and RS\textsubscript{3} supplements provided 30 g RS/d. Each type of supplement was consumed in addition to the habitual diet for 3 wk. At the end of each 3-wk period, fasting blood samples and a 24-h food-consumption recall were obtained from each subject. The subjects collected 24-h urine samples for lithium determination, which was added to the supplements to check compliance. Mean lithium recovery was 97% and did not differ between supplements. The mean composition of the background diet was similar when the three supplements were taken. Body weight remained constant throughout the study. There were no significant differences in the fasting concentrations of serum total, high-density-lipoprotein (HDL), and low-density-lipoprotein (LDL) cholesterol, and triacylglycerols, or 3α-hydroxy bile acids after consumption of glucose, RS\textsubscript{2}, or RS\textsubscript{3}. Evidence is presented that the lack of effect of RS\textsubscript{2} and RS\textsubscript{3} on serum lipid concentrations cannot be explained by insufficient statistical power, a low dose, or a short duration of treatment. The subjects reported softer stools and more gastrointestinal symptoms after supplementation with RS than after glucose. Neither the RS\textsubscript{2} nor the RS\textsubscript{3} supplements lowered serum lipid concentrations in healthy, normolipidemic men and women. Am J Clin Nutr 1996;64:312–8.

KEY WORDS Resistant starch, raw starch, retrograded starch, serum cholesterol, serum triacylglycerols, serum bile acids, low-density lipoproteins, high-density lipoproteins, normolipidemia, humans

INTRODUCTION

Resistant starch (RS) is defined as the sum of starch and products of starch degradation not absorbed in the small intestine of healthy subjects (1). After ingestion, RS will enter the colon, where it may be fermented by the bacterial flora. Three types of RS have been identified (2): RS\textsubscript{1}, starch physically inaccessible to α-amylase because of partitioning by intact plant cell walls (eg, starch in lentils or in partly milled grains); RS\textsubscript{2}, native granular starch (B-type starch such as that in unripe bananas or in raw potato); and RS\textsubscript{3}, retrograded starch consisting mainly of retrograded amylose (eg, part of the starch in cooled, cooked potato and in stale bread).

The effect of high-amylose cornstarch on serum lipid concentrations in healthy men was determined in three crossover experiments. Reiser et al (3) found in 11 men that the concentrations of total cholesterol, low-density-lipoprotein (LDL) cholesterol, and triacylglycerols were 7%, 11%, and 17% lower after consumption of a diet with 20% of energy from high-amylose cornstarch instead of fructose for 5 wk. Behall et al (4) reported 6% lower total cholesterol and 19% lower triacylglycerol concentrations in 12 men after consumption for 5 wk of 35% of total energy as high- compared with low-amylose cornstarch. In contrast, in 10 men who had consumed diets with 55% of carbohydrates provided by test products containing either high- or low-amylose cornstarch, the total cholesterol concentration was 11% higher and the triacylglycerol concentration 28% lower after 4 wk of the high-amylose diet; no significant differences were observed after 8 and 13 wk (5). It is unclear whether the lipidemic effects found in these three studies are rightly ascribed to amylose or whether they should be ascribed to amylose plus RS or to RS alone (6, 7).

Direct evidence for the notion that consumption of RS may affect serum lipid concentrations comes from studies with rats. Dietary high-amylose cornstarch, when compared with low-amylose cornstarch, lowered the blood cholesterol concentration in rats by 30–36% (8) and the triacylglycerol concentration by 43% (9). Several studies showed that feeding RS\textsubscript{2} lowered serum cholesterol in rats, the decrease ranging from 8% to 23%, whereas serum triacylglycerol concentrations dropped from 0% to 42% (10, 11). Verbeek et al (12) found that RS\textsubscript{3}...
reduced the increase in serum cholesterol concentrations during the course of the experiment by 50% and reduced the increase in serum triacylglycerol concentration by 77%. In a recent study using feeds with different types and amounts of RS, de Deckere et al (13) found no effect on serum total cholesterol but a decrease in serum triacylglycerol. RS2 fed to rats in the form of various raw starches was shown to lower serum cholesterol by 22–32% and triacylglycerols by 30–34% (14–16).

Thus, feeding RS2 or RS3 may lower serum cholesterol and triacylglycerol concentrations in rats, but it is not clear whether the same is true in humans. This prompted us to investigate whether RS2 and RS3 would decrease serum total cholesterol and triacylglycerol concentrations in healthy, normolipidemic subjects. The study had a 3 × 3 Latin-square design with dietary supplements providing either glucose, RS2, or RS3 as treatments. Apart from serum total cholesterol concentrations we also measured cholesterol in the LDL and HDL fractions. We decided to study normolipidemic subjects because several investigators (3–5, 17–19) found both statistically and physiologically significant changes in serum cholesterol concentrations (ranging from 3% to 13%) in normolipidemic subjects (prestudy mean total cholesterol concentration ranging from 4.7 to 5.4 mmol/L). In normolipidemic rats, large effects (ranging from 8% to 32%) of RS on serum cholesterol concentration were found (10–12, 14–16). An additional practical advantage was that the group of normolipidemic subjects was most accessible to us.

SUBJECTS AND METHODS

Subjects

Sixty apparently healthy subjects (27 males and 33 females) were recruited by advertisements in local newspapers and posters mounted in public buildings in Wageningen. The inclusion criteria were as follows: age between 18 and 65 y; serum cholesterol concentration < 7 mmol/L; serum triacylglycerol concentration < 2 mmol/L; weight fluctuation during the previous 3 mo not more than 2.5 kg; no anemia; no diseases of the kidneys or gastrointestinal tract; no stomach or bowel surgeries other than removal of the appendix; no complaints of diarrhea, obstipation or abdominal pain; normal values for glucose, protein, pH, nitrate, blood, ketones, bilirubin, and urobilinogen in urine (Combur™ Test; Boehringer Mannheim GmbH, Mannheim, Germany); no use of medication known to affect blood lipids; and no use of antibiotics or laxatives during the previous 3 mo.

Characteristics of the subjects were as follows (x ± SD): age, 24 ± 7 y; height, 1.78 ± 0.10 m; body weight, 70.8 ± 10.9 kg; body mass index (BMI; in kg/m²), 22.3 ± 2.3; hemoglobin, 9.1 ± 0.8 mmol/L; fasting serum cholesterol concentration, 4.78 ± 1.07 mmol/L; and fasting serum triacylglycerol concentration, 1.09 ± 0.59 mmol/L. Five men smoked cigarettes, four of them regularly. Two women were postmenopausal and 14 women used oral contraceptives.

The experimental design of the study and possible discomforts were explained to the subjects before they gave their written informed consent. The study protocol was approved by the Medical-Ethical Committee of the Department of Human Nutrition of the Wageningen Agricultural University. Subjects were paid $125 for their participation after they had completed the experiment.

Study design

The study ran from March 13 until May 15, 1995, and had a single-blind randomized 3 × 3 Latin-square design. There were three consecutive treatment periods of 3 wk each, during which the subjects consumed a daily supplement in addition to their habitual diet. The supplements provided glucose, RS2, or RS3 and each subject consumed each type of supplement for 3 wk. The 60 subjects were randomly divided into six groups with equal numbers of men and women before the experiment began. Each group consumed the supplements in one of the six possible sequences to eliminate variation due to residual effects of the previous diet or to drift of variables over time (20). The groups were comparable with respect to age, height, body weight, and BMI (data not shown).

Blood samples were taken after a 12-h fast in the morning on days 18 and 22 of each 3-wk period. All venipunctures were performed by the same technicians, at one location, and at identical times and days of the week. All participants were assigned a random number that was used for labeling blood and serum tubes. In this way the laboratory technicians were unaware of the subjects’ supplement sequence. In the last week of each 3-wk period, a 24-h food-consumption recall was obtained from each subject to check whether the amount and composition of the habitual diet had remained constant. On day 10 of each 3-wk period, 24-h urine samples were collected for lithium determination (see below). Subjects were weighed twice a week while wearing light indoor clothes with empty pockets and no shoes.

Supplements

The ingredients in the dietary supplements, which consisted of a mixture of skimmed yogurt, skimmed milk, fruit syrup, and either glucose (control) or raw RS (RS2) or retrograded RS (RS3), are shown in Table 1. The supplements had identical

### TABLE 1

<table>
<thead>
<tr>
<th>Dietary supplement</th>
<th>Glucose</th>
<th>RS2</th>
<th>RS3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (g/d)²</td>
<td>77</td>
<td>26.4</td>
<td>0</td>
</tr>
<tr>
<td>Raw high-amylose cornstarch (g/d)³</td>
<td>0</td>
<td>54.2</td>
<td>0</td>
</tr>
<tr>
<td>Retrograded high-amylose cornstarch (g/d)⁴</td>
<td>0</td>
<td>0</td>
<td>73.0</td>
</tr>
<tr>
<td>Nonfat yogurt (g/d)</td>
<td>120</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Skim milk (g/d)</td>
<td>120</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Fruit syrup (g/d)</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Lithium chloride (μmol/L)⁵</td>
<td>240</td>
<td>240</td>
<td>240</td>
</tr>
</tbody>
</table>

¹ RS₂, resistant starch from raw high-amylose cornstarch; RS₃, resistant starch from retrograded high-amylose cornstarch.
² Glucose monohydrate (CL2007); Cerestar, Vilvoorde, Belgium.
³ Hylon VII; National Starch and Chemical Company, Bridgewater, NJ; contains 55.4% RS by wt as measured in vitro according to the procedure of Englyst et al (2).
⁴ Novelose; National Starch and Chemical Company, Bridgewater, NJ; contains 41.1% RS by wt as measured in vitro according to the procedure of Englyst et al (2).
⁵ Only added to the supplements consumed in the week before the 24-h urine collection (on day 10 of each 3-wk period).
nutrient compositions except that the control supplement contained digestible carbohydrate only, whereas the RS2 and the RS3 supplements contained 30 g RS/d (Table 2). We did not try to equalize the energy content of the supplements because there is no accurate estimate of the amount of energy that RS supplies and because the glucose supplement contained at most 500 kJ (~4% of total energy intake in this group of subjects) more than the RS supplements if RS supplies no energy at all.

The supplements were prepared in the kitchen of the Department of Human Nutrition three times a week and the subjects took them home for consumption. Supplements were stored at 4 °C until consumed. Supplements were consumed in three equal portions of ~115 g/d, essentially with breakfast, lunch, and dinner. Supplements had to be consumed as provided and after stirring.

Compliance

To check whether the subjects really consumed the supplements, 80 μmol lithium chloride was added per supplement portion (22, 23). This amount is 100 times the amount found in food and 100 times less than the dose used in antidepressant drugs. About 95% of the ingested lithium is recovered in urine (22, 24). After a continuous intake of lithium, it takes 4–5 d for its excretion in urine to reach a steady concentration (24). Lithium was only added to the supplement portions that were to be consumed in the week before 24-h urine samples were collected (day 10 of each 3-wk period) to limit as much as possible the amount of lithium consumed. Subjects were told that a safe substance was added to the supplements to check their compliance. They were given the impression that the substance was added to every supplement portion and that measurements in urine and blood could show their compliance over the last days before collection of urine or blood. When the urine was turned in, subjects were asked whether the collection had been successful. The urinary lithium concentration was measured by atomic-absorption spectrophotometry (model 2380; Perkin-Elmer, Norwalk, CT), with a precision of 2.5%. Furthermore, the subjects were asked to report daily in a diary the times of consumption of the supplement portions.

Food consumption

The subjects were free to eat and drink what they wanted in addition to the supplements, but they were encouraged to maintain their habitual diet as much as possible. Subjects were instructed to minimize the consumption of products presumed to contribute significantly to their RS intake, such as muesli; unripe bananas; lentils; beans; fried or baked potatoes; cooked and cooled potatoes, rice, and pasta; and potato chips. Subjects were asked to report the consumption of these foods and also deviations from their habitual diet or activity pattern in a diary. In the last week of every 3-wk period, a 24-h recall was obtained from each subject by one of the four dietitians involved. Each subject was interviewed by the same dietitian throughout the study. The way of interviewing and coding the foods was standardized. Energy and nutrient intakes were calculated by using a computerized food-consumption table (HUVO-95) that was developed at the Department of Human Nutrition and based on the NEVO-93 table (21).

Because a change in coffee (25) or alcohol consumption (26) can influence serum cholesterol concentrations, subjects were told to maintain their usual coffee and alcohol consumption patterns. Subjects were asked to report daily in their diaries what type and the amount of coffee they drank and whether they had deviated from their habitual alcohol consumption.

Gastrointestinal complaints

In the same diary the subjects were asked to report daily whether they suffered from flatulence, a bloated feeling, belching, stomachache, bellyache, nausea, vomiting, appetite disturbance, diarrhea, or constipation. The severity of the complaints was rated as 0 (absent), 1 (minor), 2 (moderate), or 3 (severe). For each subject a mean score for each type of complaint was calculated per supplement period. Furthermore, the subjects were asked to record in their diaries illness, medicine use, start of menstruation, and time of defecation. They also rated the consistency of their feces from 1 (watery) to 5 (like pellets). A mean consistency score was calculated per subject for each supplement period.

Blood analysis

Blood was obtained by venipuncture and within 1 h serum was obtained by low-speed centrifugation for 10 min at 1500 × g and 4 °C (Sigma 4K10; Salm en Kipp BV, Breukelen, Netherlands) and analyzed enzymatically for total cholesterol (27), HDL cholesterol (28), triacylglycerols (29), and 3α-hydroxy bile acids. Triacylglycerols and total and HDL cholesterol were analyzed with a Spectrum Analyzer (Abbott Laboratories, Chicago). 3α-Hydroxy bile acids were measured with a commercial test kit (Enzabile; Nycomed Pharma AS, Oslo) and a Cobas-Bio Analyzer (Roche Diagnostica, Basel, Switzerland). All samples from a particular subject were analyzed in one run. The CV within runs was 1.0% for total cholesterol, 0.8% for HDL cholesterol, 1.4% for triacylglycerols, and 5% for 3α-hydroxy bile acids. Mean bias with regard to the target values from serum pools provided by the Centers for Disease Control and Prevention, Atlanta, was 0.05 mmol/L for total cholesterol, 0.01 mmol/L for HDL cholesterol, and 0.05 mmol/L for triacylglycerols. LDL cholesterol was calculated by using the equation of Friedewald et al (30). The mean of the values of the two blood samples per period was used in the statistical analysis to exclude as much as possible the within-subject fluctuations in total and HDL-cholesterol concentrations (31).

<table>
<thead>
<tr>
<th>Dietary supplement</th>
<th>Glucose</th>
<th>RS2</th>
<th>RS3</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS (in glucose equivalents, g/d)</td>
<td>0</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Digestible glucose equivalents (g/d)</td>
<td>87</td>
<td>57</td>
<td>57</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Cholesterol (mg/d)</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Energy (kJ/d)</td>
<td>1824</td>
<td>1314</td>
<td>1314</td>
</tr>
</tbody>
</table>

* Calculated by using a computerized food-composition table (HUVO-95) that was developed at the Department of Human Nutrition and is based on the NEVO-93 table (21). RS2, resistant starch from raw high-amylase cornstarch; RS3, resistant starch from retrograded high-amylase cornstarch.

* As measured in vitro according to the procedure of Englyst et al (2).

* Does not include the energy that RS provides when it is fermented in the colon.
Statistical analysis

Differences between the variables as induced by the three supplements were tested by analysis of variance with the GLM (general linear model) procedure of SAS (release 6.09; Statistical Analysis Systems Institute Inc, Cary, NC). The model contained “subject” as a random factor, thus taking the intrinsic individual values into account, and “supplement” as a fixed factor. When the analysis of variance indicated a significant ($P \leq 0.05$) effect of supplement, Tukey’s Studentized range test was used for pair-wise comparison of the supplements and for calculation of the 95% CIs for the differences between the two supplements. This method encompasses a downward adjustment of the significance limit for multiple testing. With 60 subjects the a priori power was calculated to be $\geq 90\%$ for detecting a significant effect ($P \leq 0.05$) of RS compared with glucose on the serum total cholesterol concentration when testing one-sided, if the real population effect was $\pm 0.15$ mmol/L and assuming the same within-subject variation as in previous studies at our department.

RESULTS

Within 3 wk after the study began, three women dropped out for personal reasons. A fourth female participant had a traffic accident that lead to hospitalization; therefore, she could not finish the experiment. However, she had completed the RS$_2$ and RS$_3$ supplementation periods. One woman developed a bladder infection in the beginning of her RS$_3$ period; she took antibiotics for 1 wk, which might have affected her colonic flora. The results analyzed with and without the data from the subject who took antibiotics were similar unless stated otherwise.

Food consumption and body weight

No significant differences were found in energy and nutrient intakes when the various supplements were given (Table 3). Between treatment periods, no changes in coffee and alcohol consumption were reported. Body weight remained constant throughout the study. The mean (± SD) change in body weight was $-0.2 \pm 0.8$ kg (range: $-2.0$-1.7 kg) over the glucose periods, 0.2 ± 1.1 kg (range: $-2.1$-4.0 kg) over the RS$_2$ periods, and 0.0 ± 1.0 kg (range: $-2.6$-1.9 kg) over the RS$_3$ periods.

Compliance

According to the diaries, 99% of the supplements provided were consumed. It was reported that 1% of the glucose supplements, 1.1% of the RS$_2$ supplements, and 1.3% of the RS$_3$ supplements were not consumed. Mean lithium recovery was > 95% and did not differ significantly among the three supplementation periods (Table 4). Mean lithium recovery increased by 1-2% when the data from urine collections that were reported to be incomplete were excluded. The three lowest lithium recoveries found in individual subjects (36%, 39%, and 45%) corresponded with diaries reporting failure to consume all supplement portions.

Serum concentrations of lipids and 3α-hydroxy bile acids

No treatment effects were found with regard to fasting concentrations of serum total cholesterol, HDL and LDL choles-

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between RS2 and RS3 was 0.03 mmol/L (95% CI: -0.12, 0.07; P > 0.05). Because the distribution of the serum triacylglycerol concentration was rather skewed, the statistical analysis was repeated on the logarithm of the triacylglycerol concentration. No significant differences were found then, neither when all data were analyzed (P = 0.09) nor when the data of the above-mentioned subjects were excluded (P = 0.11).

**Gastrointestinal complaints**

During supplementation with RS (n = 57), more flatulence, bloated feelings, belching, and bellyaches were reported than during glucose supplementation (n = 56). The scores (x ± SEM) for flatulence were 0.27 ± 0.04 for glucose, 0.73 ± 0.06 for RS2, and 1.09 ± 0.07 for RS3 (P < 0.0001 for all comparisons). RS3 ingestion caused more bloating (0.33 ± 0.05) than did either the consumption of glucose (0.08 ± 0.02, P < 0.0001) or RS2 (0.19 ± 0.05, P < 0.05). More belching (P < 0.05) was reported during supplementation with RS3 (0.13 ± 0.03) than during supplementation with either RS2 (0.07 ± 0.02) or glucose (0.06 ± 0.02). Bellyache was reported more during RS2 (0.09 ± 0.03) and RS3 (0.10 ± 0.02) supplementation than during glucose consumption (0.05 ± 0.01, P < 0.05).

**Frequency and consistency of feces**

During supplementation with RS3, a slightly higher number of bowel movements per day (x ± SEM: 1.4 ± 0.05; n = 57) was reported than during supplementation with either RS2 (1.3 ± 0.05; n = 57) or glucose (1.3 ± 0.06, P < 0.05; n = 56). During glucose supplementation, somewhat harder stools were reported (rated consistency/stool, x ± SEM: 3.4 ± 0.08; n = 56) than during either the RS2 (3.2 ± 0.07, P < 0.05; n = 57) or RS3 (3.1 ± 0.07, P < 0.01; n = 57) supplementation periods.

**Awareness of the nature of the supplements**

The subjects were not told the sequence in which they would receive their supplements until they completed the experiment. At the end of the study the participants were asked to guess their supplement sequence. The sequence was guessed right by 78% of the participants. Eighteen percent of the volunteers were able to discern the glucose from the RS supplements but could not discern between the RS2 and RS3 supplements. Two subjects (4%) guessed totally wrong.

**DISCUSSION**

This study shows that in healthy normolipidemic men and women, supplementation of their habitual diet for 3 wk with 30 g RS/d from either raw (RS2) or retrograded (RS3) starch did not lower fasting concentrations of serum lipids when compared with supplementation of the diet with glucose.

With 57 subjects and one-sided testing at P ≤ 0.05, this study had a statistical power of 56% to detect a significant treatment effect on serum total cholesterol between RS and glucose = 0.10 mmol/L. The power was 85% for a treatment-induced difference ≥ 0.15 mmol/L and 97% for a difference ≥ 0.20 mmol/L. A serum cholesterol lowering of ≥ 0.20 mmol/L, or ≥ 4% for a baseline value of 5.0 mmol/L, is considered meaningful with regard to the risk of coronary heart disease.

Both reported compliance and compliance as assessed by lithium recovery in urine were high and did not differ between treatment periods. The variation in lithium recoveries was relatively large, but it was symmetrical around the mean and similar for all three supplement periods. The large variation most likely was due to day-to-day variation in urine production and composition, which was not accounted for by the single 24-h urine collection per treatment period. The sequences in which the supplements were consumed were guessed at least partly correctly by 96% of the subjects. However, it is unlikely that awareness of the nature of the supplements could have affected the study outcome with regard to serum lipid concentrations. The consumption of the different supplements was confirmed by the reported severity of gastrointestinal complaints and stool consistencies. As anticipated, consumption of RS2 and RS3 elicited more gastrointestinal complaints and softer stools than did consumption of glucose.

Serum lipid concentrations have been found to stabilize within 2 wk after a dietary change (17, 32–36), so that the 3-wk treatment period used can be considered sufficiently long to detect any changes in serum lipid concentrations. The subjects consumed 30 g RS/d in addition to their habitual diet. This daily dose of RS is estimated to be about six times the average intake of RS in the Netherlands (37). Assuming that RS may be regarded as a kind of dietary fiber, the 30 g RS applied in the supplements was a significant increase in dietary fiber intake compared with the habitual mean intake of 15 g/d in the Netherlands (38), or with the 20 g/d in our group of volunteers (Table 3). The glucose supplement provided at most 500 kJ (4% of total energy intake) more than did the RS supplements on the basis that RS supplies no energy at all. Probably, the subjects compensated for the difference in energy intake because no treatment effects on body weight were found.

It is often believed that women are less suitable subjects for studying dietary effects on serum lipids because of confounding effects of the menstrual cycle (39, 40) or the use of oral contraceptives (41). With a proper study design, however, such confounding effects are eliminated. In our randomized study the women entered the trial at different stages of their menstrual cycle so that the start of menstruation in the premeno-
pausal women was equally spread over the three periods. The number of women starting menstruation was 17 during the glucose period, 22 during the RS\textsubscript{2} period, and 18 during the RS\textsubscript{1} period. Moreover, the supplements were given in random sequence, which provided that any effects of menstrual cycle would be averaged out and thus could not have systematically biased the comparisons of the supplements.

Our results do not agree with those of several studies in rats (10–12, 14–16) in which RS was found to lower blood cholesterol and triacylglycerol concentrations. This discrepancy might be due to either a species effect or to comparable doses. The rats in the study of de Deckere et al (11) were fed daily 4.6 g RS/kg metabolic wt (body weight\textsuperscript{0.72}), in the study of Verbeek et al (12) rats were fed daily 5.6 g RS/kg metabolic wt, and in the study of Younes et al (16) rats were fed daily 3 g RS/kg metabolic wt. Other studies with rats reported insufficient information to calculate the intake of RS on the basis of metabolic weight. The subjects in our study consumed daily 1.2 g RS/kg metabolic wt. It is not feasible for humans to consume more RS per day because with 30 g/d, flatulence, bloating, and belching were frequently reported in this study and also in other studies (42, 43). Thus, it appears that the lack of effect of RS consumption on serum lipid concentrations in humans, as seen in this study, and the lowering effect found earlier in rats relates to the 4- to 10-fold higher RS doses administered to the rats.

To our knowledge a study on the intake of foods containing well-defined RS in relation to blood lipid concentrations in humans has not been reported before. In three crossover studies the effect of high-amylose starch on blood lipid concentrations in humans was investigated (3–5). In these studies 10–12 healthy men consumed high-amylose foods that were incorporated into their diet for \( \geq 5 \) wk. Reiser et al (3) and Behall et al (4) found a 7% decrease in the total cholesterol concentration and an 18% decrease in the triacylglycerol concentration. Behall and Howe (5) reported that the total cholesterol concentration was elevated by 11% and the triacylglycerol concentration was lowered by 28%. Thus, a high-amylose starch diet did not consistently affect the blood cholesterol concentration, whereas it lowered the triacylglycerol concentration. It is difficult to compare results from the reported studies on high amylose intakes and those from the present study because the extent to which amylose intake was associated with either RS\textsubscript{2} or RS\textsubscript{3} is unknown. Furthermore, Reiser et al (3) used fructose administration as a control. Fructose compared with regular starches has been found to increase blood cholesterol and triacylglycerol concentrations in some studies (44). Thus, when a high amylose intake is compared with a high fructose intake, as in the study of Reiser et al (3), the observed lipidemic effects may be enhanced.

The major determinant of the serum bile acid concentration in healthy subjects is the rate of intestinal absorption of bile acids (45). In the present study neither RS\textsubscript{2} nor RS\textsubscript{3} affected the serum concentration of 3α-hydroxy bile acids. This finding is in line with the unlabeled serum cholesterol concentrations and also points to an unaltered enterohepatic cycle and cholesterol absorption and synthesis. In contrast, Verbeek et al (12) found in rats that RS\textsubscript{3} (compared with digestible starch) significantly increased the serum 3α-hydroxy bile acid concentration by 72%. Again, the large RS dose used in the rat study could explain why an effect was found.

In conclusion, this study showed that daily supplementation of the habitual diet with 30 g RS from either raw or retrograded starch for 3 wk did not lower serum lipid concentrations in healthy normolipidemic men and women. It is possible that RS supplementation lowers serum cholesterol concentrations in hyperlipidemic subjects. In any event, the lack of effect found in this study cannot be explained by insufficient statistical power, low RS doses, the short duration of the trial, or inferior compliance by the subjects.

We are indebted to the volunteers for their cooperation. We thank Helmie Arendsen, Ilse van Gils, Marian van Opzeeland, Marliene van Wijk, and Misja van Zadel for their help in conducting the experiment; Joke Barendse, Jan Harryvan, Robert Hovenier, and Marga van der Steen for collection of the blood samples; Paul Huishof, Inez Lemmens, and Marga van der Steen for laboratory analyses; Lidwien van der Heijden and Saskia Meyboom for dietary advice; and Jan Burema for statistical advice.

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