Effect of Resistant Starch on Blood and Liver Lipids in Hamsters

G. S. RANHOTRA,[1] J. A. GELROTH, and B. K. GLASER

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ABSTRACT

Serum and liver lipid responses were studied in young hamsters fed diets containing cholesterol (CH) and either no fiber or 20.5% fiber. Fiber was provided as resistant starch (RS) or as cellulose (CL); the no-fiber diet was based on normal starch (NS). After four weeks, serum total CH in hamsters fed RS was 16.2% lower, and in those fed CL 13.1% lower, than in hamsters fed NS. This effect may be specific to RS and CL, may be the result of lower weight gains in hamsters fed RS and CL as compared to those fed NS, or both. The lowering of CH resulted from a reduction of both high-density lipoprotein (HDL) CH and non-HDL CH. Like serum CH, serum triglyceride levels were also lower in hamsters fed RS and CL—nearly 50% lower—compared with those fed NS. Liver CH and lipid levels defied this trend in that CH levels were higher in hamsters fed RS than in those fed NS or CL.

Resistant starch (RS) is defined as the sum of starch and starch degradation products that resist digestion in the small intestine (Asp 1992). RS occurs naturally in some prepared foods in small amounts (Englyst and Cummings 1985); however, high-amylose corn starch can be modified to contain significant levels of RS (Kevin 1995). Although RS is starch, it analyzes as fiber (Berry 1986) and functions as fiber (Ranhotra et al 1991, Van Munster et al 1994, Phillips et al 1995).

RS is becoming a food ingredient of increasing interest. It does not hold much water and, thus, may be a preferred fiber source for use in low-moisture products such as cookies and crackers. RS is also free of gritty mouthfeel and, unlike traditional fiber sources, is reported not to alter flavor and textural properties of foods. These characteristics can improve the processing and quality of foods such as baked and extruded products where RS may be added.

If RS can be shown to also favorably affect health parameters, this may further underscore the importance of this ingredient. A study with healthy young men recently showed RS caused a significant reduction in postprandial glycemia and insulinemia (Raben et al 1994), as compared to normal starch (NS). RS may also affect blood cholesterol (CH) and triglyceride (TG) levels favorably. This study was undertaken to examine this possibility using hamsters, a more suitable model for lipid studies than rats (Beher et al 1992), as the test model.

MATERIALS AND METHODS

Test Materials

RS (CrystaLean) was obtained from Opta Food Ingredients, (Bedford, MA). It is prepared from high-amylose corn starch by subjecting corn starch to repeated heating and cooling cycles and other processing steps. NS, a pregelatinized corn starch, was obtained from American Maize-Products Co., Hammond, IN. Cellulose (CL, Solka-Floc) was obtained from FS & D Corp. (Urbana, OH). These materials were analyzed (Table I) and used to formulate test diets (Table II).

Test Diets

Diet NS (NS-based) contained virtually no fiber, while diet RS (RS-based) and diet CL (CL-based) contained 20.5% fiber each (Table II). All diets contained the same level of protein (15%) and fat (10%) and the sources differed minimally. All diets also contained 0.5% CH and were complete in nutrients required by weanling hamsters (NRC 1987).

Animals

Thirty three-week-old male Syrian golden hamsters (Harlan Sprague-Dawley, Indianapolis, IN) were housed in suspended mesh-bottomed cages in a controlled environment (23.9°C, 60% rh, 12-hr light and dark cycle). After one week on a fiber-free diet, these animals were weighed, assigned by selective randomization to three groups (nine animals per group; mean weight of 50 ± 6 g), and fed test diets (Table II); three hamsters were sacrificed on day zero to obtain baseline serum lipid values.

Feeding

All animals were fed daily. Their food intake, which gradually increased as the study progressed, was, however, equalized among groups. Deionized water was offered ad libitum. Hamsters were weighed weekly.

Blood and Liver Sampling

After four weeks on test diets, animals were fasted overnight and then lightly anesthetized under ether. About 2 ml of blood was withdrawn by cardiac puncture. The blood was allowed to clot and then centrifuged (8 min) to obtain serum. Lipid analyses were run on the refrigerated serum the next day. Livers were excised, rinsed, blotted dry, weighed, and homogenized. The homogenate volume was recorded, and the samples were frozen until needed for CH and total lipid determinations.

Analytical

Test materials were analyzed for moisture, protein (N × 5.7), fat (ether extract), and ash using standard AACC methods (1995). Fiber (total, soluble, and insoluble) in CL was determined by the AACC (1995) enzymatic-gravimetric method; the same method (fiber method) was used to measure RS. Serum total CH and TG

TABLE I
Percent Composition of Test Materials

	Normal Starch	Resistant Starch	Cellulose
Moisture	3.8	6.8	4.2
Protein (N × 5.7)	0.3	0.7	0.0
Fat	0.3	1.1	0.0
Ash	0.7	0.3	0.2
Total dietary fiber[a]	0.3	30.2	95.6
Total carbohydrates	94.9	91.1	95.6

[a] No measurable amount of soluble fiber was detected in any of the test materials.

[1]Corresponding author: American Institute of Baking, 1213 Bakers Way, Manhattan, KS 66502. Phone: 913/537-4750. Fax: 913/537-1493.

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levels were determined enzymatically using kits 352 and 336, respectively, from Sigma Chemical Co. (St. Louis, MO). High-density lipoprotein (HDL) CH was determined (using kit 352) after phosphotungstic acid precipitation of very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL). Total CH in liver was determined by the method of Abell et al (1952). Liver lipids were determined by ether-extracting the fat using a freeze-dried sample of liver homogenate. The data were subjected to analysis of variance using the Statistical Analysis System (SAS 1982).

RESULTS AND DISCUSSION

Test Diets

The two fiber-containing diets contained the same level of fiber (Table II). Fiber was included in these diets—diets RS and CL—at a high level to enable observing a possible hypolipidemic effect; a follow-up study would test lower levels of RS and CL. All three diets contained 0.5% added CH. With isolated exceptions, investigators have routinely added CH (range: 0.1–1.0%) to hamster diets to induce hypercholesterolemia (Beher et al 1979, Kahlon et al 1990, Jonnalagadda et al 1993).

All diets contained identical levels of protein and fat (Table II). Carbohydrate levels in the diets were also nearly identical because the test materials used contained nearly identical levels, represented either as all digestible starch (NS), digestible and indigestible starch combined (RS), or as indigestible fiber (CL). Only the type of carbohydrate (NS, RS, or CL) differed, the basis of this study.

Diet Intake and Weight Gains

Caloric density of the diet as well as diet components such as cholesterol, fat, and protein affect blood lipid levels differently. To at least rule out the effect of diet components (different intake), all groups of hamsters were fed identical amounts of diets (Table III). This approach did not allow compensating for differences in caloric density of the diets. Consequently, weight gains of hamsters differed significantly ($P < 0.05$) between groups (Table III). This may have contributed to differences observed in blood and liver lipid responses.

Serum Total Cholesterol

Compared to the initial level (136 ± 2 mg/dl), serum CH levels were quite elevated in all groups of hamsters after four weeks on test diets (Table III). Still, differences between the three groups became apparent. Serum total CH levels were 16.2% lower in hamsters fed RS, and 13.1% lower in those fed CL, as compared to the group fed NS (Table III). At modest levels of intake, CL has not been shown to lower CH (Hillman et al 1985, Anderson et al 1994). However, when intake levels are high, CL may show a lowering effect as we observed in this study. This may hold true for RS also.

The CH-lowering effect may be specific to CL and RS, may be the consequence of differences in weight gains of hamsters (diet NS vs. diets RS and CL), or both. Alternatively, it could be that CL and RS will show a CH-lowering effect when compared to NS but perhaps not when compared to soluble fibers, which have repeatedly been shown as potent CH-lowering agents (Pilch 1987, Ranhotra et al 1993, Anderson et al 1994).

Serum HDL and VLDL + LDL Cholesterol

Dietary measures taken to lower serum CH rarely affect only VLDL and LDL cholesterol; HDL, the "good" cholesterol, is often also affected, sometimes adversely. This adverse effect was observed in this study also. The lowering of total CH due to RS and CL resulted from a decrease in both HDL and VLDL and LDL CH (Table III). The lowering of VLDL and LDL CH in hamsters fed CL has also been reported by Jonnalagadda et al (1993).

As compared to hamsters fed NS, those fed RS showed little change in HDL CH to VLDL and LDL CH ratios, even when total CH levels dropped significantly (321 ± 57 vs. 269 ± 35 mg/dl). In contrast, CL-based diet favored this ratio, i.e., the drop in total CH (42 ± 17 mg/dl) resulted from a drop more in VLDL and LDL CH than in HDL CH (Table III). In spite of these differences, both fiber components lowered serum total CH effectively. A lowering of serum total CH alone is linked to lowering of the risk of heart disease.

Serum Triglycerides

Elevated serum TG levels are viewed by some as an independent risk factor in heart disease (Austin 1991, McNamara 1992). TG levels usually increase on diets high in available carbohydrates—starches and sugars. This occurred in hamsters fed NS but not in those fed RS or CL (Table III). In fact, TG levels in hamsters fed RS or CL were no higher after four weeks than ob-

TABLE II
Composition of Test Diets[a]

	Diet		
	NS	RS	CL
Component, %			
NS	66.92	0.0	45.5
RS	0.0	67.78	0.0
CL	0.0	0.0	21.36
Casein	17.8	17.5	17.82
Shortening[b]	7.82	7.26	7.86
Constants[c]	7.46	7.46	7.46
Composition, %			
Protein	15	15	15
Fat	10	10	10
Total dietary fiber[d]	0.2	20.5	20.5

[a] Using test materials identified in Table I. NS = normal starch, RS = resistant starch, CL = cellulose.
[b] Partially hydrogenated soybean oil.
[c] Includes (%): soybean oil, 2; mineral mix, 3.5; vitamin mix, 1; dl-methionine, 0.3; choline chloride, 0.16; and cholesterol, 0.5.
[d] As resistant starch in diet RS and as cellulose in diet CL.

TABLE III
Physiological Responses in Hamsters Fed Test Diets (4 week)[a]

	Diet		
	NS	RS	CL
Diet intake, g	200 ± 1	202 ± 1	202 ± 2
Body weight gain[b], g	44 ± 3 a	37 ± 5 b	31 ± 3 c
Liver weight, g	5.4 ± 0.3 a	4.8±0.3 b	4.7±0.3 b
Serum cholesterol, mg/dl[c]			
Total	321 ± 57 a	269 ± 35 b	279 ± 17 b
HDL	180 ± 17 a	145 ± 17 b	170 ± 19 a
VLDL+LDL[d]	141 ± 44 a	124 ± 23 a,b	109 ± 18 b
Decrease in serum cholesterol, mg/dl[e]			
Total	–	52 ± 35 a	42 ± 17 a
HDL	–	35 ± 17 a	10 ± 19 b
VLDL+LDL	–	17 ± 23 a	32 ± 18 a
Serum triglycerides, mg/dl	276 ± 96 a	149 ± 31 b	147 ± 27 b
Liver cholesterol, mg/g	56 ± 7 b	74 ± 10 a	57 ± 7 b
Liver lipid, mg/g	97 ± 11 b	135 ± 20 a	86 ± 11 b

[a] NS = normal starch, RS = resistant starch, CL = cellulose. Values are averages ± SD for nine hamsters per diet. Within a row, values not sharing common letter are significantly different ($P < 0.05$).
[b] Initial body weight: 50 ± 6 g.
[c] Initial (0-day) levels (mg/dl): serum total CH, 136 ± 2; serum HDL-CH, 82 ± 7; serum VLDL+LDL-CH, 54 ± 9; serum triglycerides, 158 ± 66.
[d] Very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) cholesterol.
[e] In comparison to diet NS.

served initially. Both fiber diets (diets RS and CL) contained significant levels of NS, but it appears that the presence of fiber (RS or CL) in these diets negated the TG-raising potential of NS.

Liver Cholesterol and Lipids

While hamsters fed RS or CL showed near similar patterns of serum total CH and TG responses, this similarity did not hold true for liver CH and lipid responses. Liver CH and lipid levels were similar in hamsters fed NS or CL but were significantly ($P < 0.05$) higher in those fed RS (Table III). Physiological significance of this is difficult to assess, but may be related, as far as CH levels are concerned, to the mechanism(s) that regulates hepatic CH storage and excretion.

CONCLUSIONS

At a high level of intake and as compared to normal starch, resistant starch lowered serum total cholesterol and triglyceride levels in hamsters profoundly, an effect also observed with cellulose. If these observations can be affirmed under more modest intakes of resistant starch, it may mean that resistant starch, a highly functional ingredient in food systems, may not only add fiber to our diet, it may also favorably affect blood lipid levels.

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