

# Physicochemical properties of oat $\beta$ -glucan influence its ability to reduce serum LDL cholesterol in humans: a randomized clinical trial<sup>1-3</sup>

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## ABSTRACT

**Background:** Consumption of 3 g oat  $\beta$ -glucan/d is considered sufficient to lower serum LDL cholesterol, but some studies have shown no effect. LDL cholesterol lowering by oat  $\beta$ -glucan may depend on viscosity, which is controlled by the molecular weight (MW) and amount of oat  $\beta$ -glucan solubilized in the intestine (C).

**Objectives:** Our 2 primary objectives were to determine whether consumption of 3 g high-MW oat  $\beta$ -glucan/d would reduce LDL cholesterol and whether LDL cholesterol lowering was related to the  $\log(\text{MW} \times \text{C})$  of oat  $\beta$ -glucan.

**Design:** In a double-blind, parallel-design, multicenter clinical trial, subjects with LDL cholesterol  $\geq 3.0$  and  $\leq 5.0$  mmol/L ( $n = 786$  screened,  $n = 400$  ineligible,  $n = 19$  refused,  $n = 367$  enrolled, and  $n = 345$  completed) were randomly assigned to receive cereal containing wheat fiber ( $n = 87$ ) or 3 g high-MW (2,210,000 g/mol,  $n = 86$ ), 4 g medium-MW (850,000 g/mol,  $n = 67$ ), 3 g medium-MW (530,000 g/mol,  $n = 64$ ), or 4 g low-MW (210,000 g/mol,  $n = 63$ ) oat  $\beta$ -glucan/d (divided doses, twice daily) for 4 wk.

**Results:** LDL cholesterol was significantly less with 3 g high-MW, 4 g medium-MW, and 3 g medium-MW oat  $\beta$ -glucan cereals than with the wheat-fiber cereal by 0.21 (5.5%; 95% CI:  $-0.11$ ,  $-0.30$ ;  $P = 0.002$ ), 0.26 (6.5%; 95% CI:  $-0.14$ ,  $-0.37$ ;  $P = 0.0007$ ), and 0.19 (4.7%; 95% CI:  $-0.08$ ,  $-0.30$ ;  $P = 0.01$ ) mmol/L, respectively. However, the effect of 4 g low-MW oat  $\beta$ -glucan/d (0.10 mmol/L) was not significant (2.3%; 95% CI: 0.02,  $-0.20$ ). By analysis of covariance,  $\log(\text{MW} \times \text{C})$  was a significant determinant of LDL cholesterol ( $P = 0.003$ ). Treatment effects were not significantly influenced by age, sex, study center, or baseline LDL cholesterol.

**Conclusions:** The physicochemical properties of oat  $\beta$ -glucan should be considered when assessing the cholesterol-lowering ability of oat-containing products: an extruded breakfast cereal containing 3 g oat  $\beta$ -glucan/d with a high-MW (2,210,000 g/mol) or a medium-MW (530,000 g/mol) lowered LDL cholesterol similarly by  $\approx 0.2$  mmol/L (5%), but efficacy was reduced by 50% when MW was reduced to 210,000 g/mol. This trial was registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) as NCT00981981. *Am J Clin Nutr* 2010;92:723-32.

## INTRODUCTION

Coronary artery disease (CAD) is a leading cause of morbidity and mortality in North America (1), Europe (2), and Australia. It is well established that reducing the serum LDL-cholesterol concentration reduces the risk of CAD (3). Lifestyle mod-

ifications to reduce CAD risk include the recommendation to increase consumption of viscous soluble dietary fiber from the current level of 3-4 g/d (4) to 5-10g/d (1) to lower LDL cholesterol. Oats are a source of soluble fiber in the Western diet. The main component of oat soluble fiber is (1 $\rightarrow$ 3)(1 $\rightarrow$ 4)- $\beta$ -D-glucan, commonly known as  $\beta$ -glucan. Numerous studies (5-13) have shown that oat-containing products reduce LDL cholesterol. The US Food and Drug Administration (14) allows a claim that food products containing oats and that can deliver 3 g  $\beta$ -glucan/d can reduce risk of heart disease. The European Food Safety Authority recently issued an opinion that a cause and effect relation has been established between the consumption of oat  $\beta$ -glucan and a reduction in blood cholesterol (15). In addition, the French Food Safety Agency (16) and the Joint Health Claims Initiative in the United Kingdom (17) allow a claim that the inclusion of 3 g oat  $\beta$ -glucan in foods products can help reduce blood cholesterol.

However, not all studies have shown a cholesterol-lowering effect of oat products (8, 18, 19), partly because the ability of oat  $\beta$ -glucan to reduce serum cholesterol depends on its ability to increase the viscosity of intestinal contents. The viscosity of  $\beta$ -glucan solutions is determined by its molecular weight (MW) and solubility (20), both of which can be altered by normal methods of processing and storage (21). The role of viscosity in determining the cholesterol-lowering effect of soluble fiber has been shown in hamsters (22) and recently in humans (23) with the use of high-dose (15 g/d) hydroxypropylmethylcellulose.

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However, although the assumption that viscosity determines the cholesterol-lowering effect of  $\beta$ -glucan is often evident in discussion of the literature (24), it has yet to be established in clinical studies. Indeed, there remains some doubt about the value of soluble fiber and oats for lowering serum cholesterol (8).

Thus, we hypothesized that consuming 1.5 g high-MW oat  $\beta$ -glucan incorporated into a ready-to-eat cereal twice daily would reduce serum LDL cholesterol compared with a control wheat-bran cereal. We showed a role for viscosity in determining the blood glucose-lowering effect of soluble fibers in humans (20, 25, 26) and that food-processing methods that reduce the MW and solubility of oat  $\beta$ -glucan reduce its ability to lower blood glucose (21, 27, 28). Therefore, we also hypothesized that the LDL-lowering effect of oat  $\beta$ -glucan is related to its ability to increase the viscosity of the contents of the small intestine, which, in turn, is related to the MW and amount of  $\beta$ -glucan solubilized in the small intestine.

## SUBJECTS AND METHODS

### Subjects

We conducted a double-blind, randomized, multicenter, parallel design, controlled clinical trial at 2 contract research organizations and 3 university nutrition research centers. Males and nonpregnant females aged 35–70 y with a body mass index (BMI; in  $\text{kg}/\text{m}^2$ )  $\geq 18.5$  and  $\leq 40.0$ , fasting serum total cholesterol  $\geq 5.0$  and  $\leq 8.0$  mmol/L, and fasting serum LDL cholesterol  $\geq 3.0$  and  $\leq 5.0$  mmol/L were invited to participate. Subjects were excluded for any of the following: fasting serum triglycerides  $\geq 4.0$  mmol/L, serum aspartate transaminase (AST)  $\geq 1.5$  times the upper limit of normal (ULN), serum urea and creatinine  $\geq 1.8$  times the ULN, unstable body weight or intention to lose or gain weight, presence of diabetes mellitus (fasting plasma glucose  $\geq 7.0$  mmol/L or use of insulin or any hypoglycemic or antihyperglycemic medication), presence of any prescription or nonprescription drug, herbal or nutritional supplement known to affect blood lipids (except for stable doses of thyroxine, oral contraceptive agents, hormone replacement therapy, and medications for controlling blood pressure), recent major surgical or medical events, presence of a gastrointestinal disorder or medication that alters the digestion and absorption of nutrients, consumption of a diet containing  $\geq 15\%$  of energy from saturated fat, allergy to wheat or oats, or consumption of  $\geq 5$

servings of oatmeal, oat bran, or psyllium-containing cereals weekly.

Written informed consent was obtained from all subjects. The procedures followed were in accordance with the ethical standards of each institution involved, and approval was obtained from the relevant ethics review committee on human subjects.

### Baseline period

After eligibility had been determined on the basis of medical history and analysis of a screening blood sample, the subjects were instructed how to record all foods and drinks consumed during 2 typical weekdays and 1 weekend day (3-d food record). These baseline food records were analyzed for nutrient content at each participating center (Toronto, Guelph, Quebec City, Sydney, and Reading) by using local diet analysis software. Subjects were excluded if their intake of saturated fat was  $\geq 15\%$  of energy. Eligible subjects were seen 1–2 wk later for collection of a baseline fasting blood sample and were then randomly assigned to receive 2 servings per day of a ready-to-eat wheat-bran cereal (W) or oat bran cereal providing a total of 3 g high-MW (3H), 4 g medium-MW (4M), 3 g medium-MW (3M), or 4 g low-MW (4L) oat  $\beta$ -glucan/d for 4 wk.

### Randomization and concealment

Subjects, stratified by center and by LDL cholesterol (stratum: low,  $3.0 \leq \text{LDL cholesterol} \leq 3.8$  mmol/L; or high,  $3.8 < \text{LDL cholesterol} \leq 5.0$  mmol/L), were randomly assigned to 1 of the 5 treatments with the use of blocks of various sizes to maintain balance among centers while treatment allocations were concealed from the study practitioners (29, 30). Treatment assignments were sealed in sequentially numbered opaque envelopes kept by a person not involved with the study and assigned to subjects in order on the day that the baseline blood samples were collected. Randomization (generated by computer with random seed chosen from a table of random numbers) was done by one of us (BAT), and the sealed envelopes were created by a research assistant not involved in the study.

### Interventions

The interventions consisted of ready-to-eat breakfast cereals manufactured by extrusion. The cereals were packed into air-tight foil sachets, each containing half the daily dose (Table 1) and

**TABLE 1**

Nutritional composition and physicochemical properties of the test and control cereals<sup>1</sup>

Cereal	Weight	Energy	Protein	Fat	CHO	Fiber <sup>2</sup>	$\beta$ -Glucan <sup>3</sup>			
							Total	Sol	Peak MW	Viscosity
	<i>g/d</i>	<i>kcal</i>	<i>g</i>	<i>g</i>	<i>g</i>	<i>g</i>	<i>g</i>	<i>g</i>	<i>g/mol</i>	<i>Pa/s at 30 s<sup>-1</sup></i>
3H	20.2	56	3.1	0.7	8.9	5.6	3.0	2.0	$22.1 \times 10^5$	2.93
4M	28.5	81	4.4	1.1	12.7	7.9	4.0	3.2	$8.5 \times 10^5$	1.67
3M	21.1	59	3.4	0.7	9.2	6.0	3.0	2.8	$5.3 \times 10^5$	0.80
4L	28.7	81	4.3	1.0	13.0	8.1	4.0	4.0	$2.1 \times 10^5$	0.13
W	21.0	59	3.3	0.8	9.3	5.9	0.5	0.04	$0.4 \times 10^5$	0.003

<sup>1</sup> CHO, available carbohydrate (starch plus sugars); Sol, soluble amount; MW, molecular weight; 3H, 3 g high-MW oat  $\beta$ -glucan/d; 4M, 4 g medium-MW oat  $\beta$ -glucan/d; 3M, 3 g medium-MW oat  $\beta$ -glucan/d; 4L, 4 g low-MW oat  $\beta$ -glucan/d; W, wheat-bran control; Pa, pascal.

<sup>2</sup> Total dietary fiber.

<sup>3</sup> The source of the  $\beta$ -glucan present in the cereals (3H, 4M, 3M, and 4L) was OatWell oat bran (CreaNutrition AG, Zug, Switzerland).

labeled with a code number. The code was not revealed to those involved in conducting the study, entering the data, or conducting the statistical analysis until the study had been completed, the data entered, and the database locked. After the baseline blood samples were collected, the subjects were provided with a 1-wk supply (14 sachets) of their randomly assigned cereal and instructed to consume 2 sachets daily: 1 with breakfast and 1 with another meal or snack. Otherwise, the subjects were asked to maintain their usual diets and other lifestyle habits throughout the study.

The oat-containing cereals consisted of crisp nuggets ( $\approx 5$  mm in diameter) containing oat bran (CreaNutrition AG, Zug, Switzerland) as the source of  $\beta$ -glucan (Table 1). The MW of the  $\beta$ -glucan in the 3H cereal was similar to that of the  $\beta$ -glucan contained in the oat bran used as an ingredient (21). The MW of  $\beta$ -glucan was reduced in the 4M, 3M, and 4L cereals by graded increases in the temperature and pressure used in the extrusion process. The control cereal was in the form of sticks ( $\approx 1$  mm diameter and  $\approx 5$  mm in length) made with wheat bran as the source of fiber. There was much less  $\beta$ -glucan in the wheat cereal than in the oat cereals, and it was low in MW and solubility such that it had low viscosity. After preliminary runs to establish the manufacturing conditions necessary to obtain the desired MW, each cereal used in the study was produced in a single large batch, samples of which were analyzed for nutrient and  $\beta$ -glucan contents and physicochemical properties as described below and packed as described above. The cereals were re-analyzed several times throughout the course of the study to confirm that the properties of the  $\beta$ -glucan did not change with time ( $\approx 18$  mo from manufacture to the end of the study). Proximate analysis was conducted by using standard methods (31) to determine contents of fat (AOAC 933.05), protein ( $N \times 5.7$ , AOAC 990.03), starch (AOAC 996.11), sugars (extracted with water at  $85^\circ\text{C}$  for 15 min and then analyzed with high-performance anion-exchange chromatography with an electrochemical detector), and dietary fiber (AOAC 45.4.07). Total  $\beta$ -glucan was measured enzymatically (AOAC 995.16). The physicochemical properties of  $\beta$ -glucan were measured as previously described (27, 32). Briefly, cereals were subjected to in vitro digestion with known volumes of enzymes and buffer at  $37^\circ\text{C}$ , and the resulting digesta were centrifuged to obtain the supernatant fluid.  $\beta$ -Glucan solubility was considered to be the proportion of the total  $\beta$ -glucan recovered in the supernatant fluid as measured by using a dye binding method (27). The value for solubility was multiplied by the dose of  $\beta$ -glucan consumed to provide an estimate of the amount of  $\beta$ -glucan solubilized in the small intestine, referred to as C. MW was considered to be the peak MW of the  $\beta$ -glucan in the supernatant fluid as measured by size-exclusion chromatography (21). The viscosity of the supernatant fluid was measured by using a controlled strain rheometer fitted with a cone and plate geometry (21). A more detailed analysis of the cereals and the physicochemical properties of their  $\beta$ -glucan is reported elsewhere (33).

### Procedures

The subjects were seen at weekly intervals for 4 wk. On each occasion, the subjects were weighed, blood pressure was measured, changes in medication use and adverse events (if any) were recorded, a fasting blood sample was obtained, the number of

unused cereal sachets returned from the previous week were counted, and a new supply of cereal was provided for the following week. During the last week of the study, subjects completed a 3-d diet record that was handed in at the last visit. A symptom questionnaire was administered at baseline and week 4, with the severity of symptoms over the past 4 wk being rated as none, mild, moderate, or severe (34).

Blood samples were analyzed for total and HDL cholesterol, triglycerides, glucose, AST, CRP, urea, and creatinine locally at each center by using whatever methods were normally used (typically at a hospital or commercial medical biochemistry laboratory). LDL cholesterol (in mmol/L) was calculated as total cholesterol minus HDL cholesterol minus triglycerides divided by 2.2 (LDL cholesterol was not calculated if triglycerides were  $>4.50$  mmol/L). All results were entered onto case report forms that were sent by FAX to the coordinating center (Glycemic Index Laboratories, Toronto, Canada) for verification and entry. Data entry and statistical analysis were performed by individuals who were unaware of the treatment assignments.

### Sample size

Sample size was estimated by using measures of LDL cholesterol at 0 and 4 wk from 12 normal subjects (TMS Wolever, unpublished observations, 2005) and 52 subjects with diet-treated diabetes (35) who met the lipid inclusion criteria for this study. The SDs of LDL cholesterol at 0 and 4 wk were 0.50 and 0.64 mmol/L, respectively, with a correlation between the 0- and 4-wk values of 0.66. Because there were 2 primary objectives, the criterion for significance was set at 2-tailed  $<0.025$  for each objective. For the comparison of W with 3H, the expected effect size was a 7% difference; there was 90% power to detect a 7% difference in serum LDL cholesterol at 4 wk between the W and 3H treatments ( $P < 0.025$ ,  $n = 73$  subjects in each treatment group). For the correlation analysis, the expected correlation was determined for values of  $C \times MW$  for the 5 treatments of 40, 20, 8, 2, and  $0 \times 10^5$  g/mol and values for LDL cholesterol at 4 wk of 3.38, 3.43, 3.52, 3.61, and 3.65 mmol/L, respectively. The power to detect that correlation between  $\log(MW \times C)$  and LDL cholesterol ( $P < 0.025$ ) was 90% ( $n = 73$  subjects in the control and 3H groups and  $n = 56$  subjects in the other 3 treatment groups). To allow for 15% dropout, we aimed to recruit a total of 354 subjects, 81 for the W and 3H treatments and 64 for the other 3 treatments.

### Statistical analysis

Statistical analyses were performed by using SAS (SAS 9.2, XP-Pro, 2002–2008; SAS Institute Inc, Cary, NC) on an intent-to-treat basis by using all available data from the 367 subjects who were randomly assigned to treatments. Log transformations were used for data that were not normally distributed. Because there were 2 primary objectives, the criterion for significance was set at  $P < 0.025$ . For secondary analyses, we used a criterion of 0.05.

The primary outcome measurement was week 4 LDL cholesterol for 2 different analyses; each primary analysis had related secondary analyses. For one primary analysis, the significance of the difference in week 4 LDL cholesterol between the W and 3H groups was examined by using analysis of covariance (ANCOVA), with control for baseline LDL cholesterol and testing for



confounding factors (stratum, sex, and center) and potential covariates (age, waist circumference, and BMI). Only factors and covariates significant at  $P < 0.1$  were included in the final model. Related analyses tested for differences in week 4 LDL cholesterol between all 5 diets, by using the same procedure as described above, and a repeated-measures analysis that included week (1–4) and the interaction between treatment and week. For the second primary analysis, we regressed week 4 LDL cholesterol on  $\log(\text{MW} \times \text{C})$ , with control for baseline LDL cholesterol and other potential factors and covariates as described above. In a related analysis we also regressed week 4 LDL cholesterol on  $\log(\text{viscosity})$ . Post hoc testing was performed after demonstration of significant heterogeneity by ANCOVA with Fisher's least significant difference test and adjusted for multiple comparisons by using the Bonferroni method. Control for multiple comparisons reduces the number of significant differences detected by chance (type I error)—a particularly important consideration for primary comparisons. Thus, the conclusions relating to our primary objectives were based on Bonferroni-adjusted criteria. However, control for multiple comparisons reduces the chance of detecting differences that really exist (type II error), a consideration relevant for secondary comparisons. Because there is controversy about the use of the Bonferroni method (36), we present  $P$  values for the secondary comparisons both before and after Bonferroni correction. A comparison of nonlinear relations with linear relations used Akaike's information criterion.

We also tested the effect of diet on several secondary outcomes, including body weight, blood pressure, glucose, CRP, lipids, and other variables measured in the fasting blood samples. Nutrient intakes for each subject were calculated, and the differences between baseline and 4 wk were compared by paired  $t$  tests; the differences between treatments were assessed by one-factor analysis of variance. Symptom ratings at 4 wk were categorized as less severe, the same, or more severe than at baseline, and the counts of subjects who experienced changes were analyzed by using McNemar's test for significance of changes. Differences between treatments in the numbers of subjects with symptoms of greater, lesser, or the same severity were assessed by using chi-square tests.

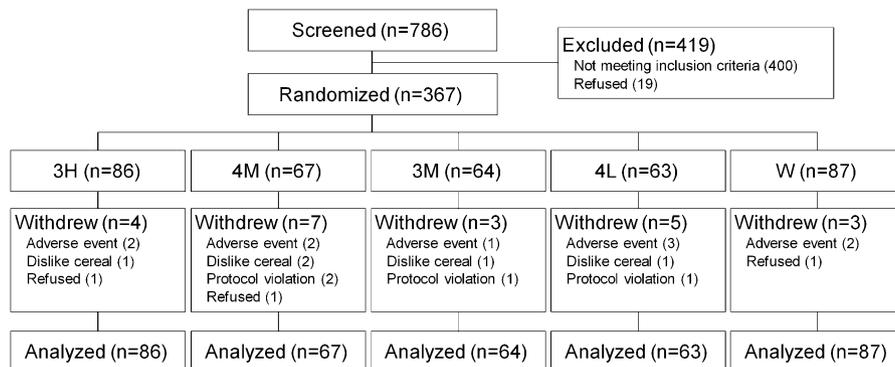
## RESULTS

Seven-hundred and eighty-six subjects were screened; 400 did not meet the inclusion criteria and 19 declined to participate,

which left 367 who were randomly assigned to 1 of the 5 treatments (**Figure 1**). Enrollment began in November 2008, and the last subject finished the study in July 2009. Data were incomplete for the 22 subjects (6%) who discontinued treatment before the end of the trial because of an adverse event (persistent elevation of glucose or triglycerides,  $n = 7$ ; flu-like symptoms,  $n = 2$ ; root canal,  $n = 1$ ), dislike of the study cereal ( $n = 5$ ), protocol violation (left for extended trips,  $n = 4$ ), and refusal to continue the study ( $n = 3$ ). Fifty-five percent of subjects were taking medications, which continued at the same dose throughout the trial (herbals,  $n = 5$ ; nutritionals,  $n = 109$ ; over-the-counter,  $n = 26$ ; prescription,  $n = 140$ ; some subjects took more than one type of medication). Demographic characteristics of the subjects assigned to the different treatments were similar at baseline (**Table 2**). Use of vitamin-mineral supplements (65% of nonprescription medications) was similar in the different groups at baseline, as was the use of antihypertensive (18% of prescription drugs) and endocrine (29% of prescription drugs) agents (**Table 2**). The subjects' body weight and LDL cholesterol did not differ significantly between treatment groups at baseline (not shown).

On the basis of the number of sachets of cereal returned, the percentage of prescribed sachets of cereal consumed during the 5 treatments were as follows: 3H, 98.6%; 4M, 98.0%; 3M, 97.2%; 4L, 97.1%; and control, 97.8%. Energy, total and saturated fat, cholesterol, carbohydrate, and alcohol intakes did not change significantly during the trial, and there were no significant differences between treatment groups for these measurements (**Table 3**). During the trial, polyunsaturated fat intake decreased by  $0.4 \pm 0.1\%$  of energy ( $P = 0.004$ ), protein intake increased by  $1.2 \pm 0.2\%$  of energy ( $P < 0.001$ ), and dietary fiber intake increased by  $3.9 \pm 0.7$  g/d ( $P < 0.001$ ), but the changes were similar between treatment groups (**Table 3**). Body weight did not change throughout the trial and was not significantly different between treatment groups (**Table 4**).

No significant difference in baseline LDL cholesterol was observed between the 5 treatment groups:  $3.88 \pm 0.07$ ,  $3.74 \pm 0.07$ ,  $3.75 \pm 0.08$ ,  $3.81 \pm 0.07$ , and  $3.74 \pm 0.08$  mmol/L for W, 3H, 4M, 3M, and 4L, respectively. Crude changes in LDL cholesterol from baseline to 4 wk on each treatment are as follows (paired  $t$  test): W,  $0.02 \pm 0.05$  mmol/L (NS); 3H,  $-0.15 \pm 0.05$  ( $P = 0.004$ ); 4M,  $-0.17 \pm 0.07$  ( $P = 0.015$ ); 3M,  $-0.14 \pm 0.07$  ( $P = 0.048$ ); and 4L,  $0.00 \pm 0.06$  (NS). The results of the ANCOVA are provided below.



**FIGURE 1.** Study flowchart. 3H, 3 g high-molecular-weight (MW) oat  $\beta$ -glucan/d; 4M, 4 g medium-MW oat  $\beta$ -glucan/d; 3M, 3 g medium-MW oat  $\beta$ -glucan/d; 4L, 4 g low-MW oat  $\beta$ -glucan/d; W, wheat-bran control.



**TABLE 2**  
Subject characteristics at baseline<sup>1</sup>

	Cereal				
	3H (n = 86)	4M (n = 67)	3M (n = 64)	4L (n = 63)	W (n = 87)
Sex (M:F)	43:43	33:34	27:37	22:41	36:51
Male sex (%)	50	49	42	35	41
Stratum (%)					
Low LDL cholesterol <sup>2</sup>	44	31	29	29	41
High LDL cholesterol <sup>3</sup>	42	36	35	34	46
Low stratum (%)	51	46	45	46	47
Ethnicity (n)					
White	67	50	54	51	74
Asian	10	8	4	7	8
African	1	6	2	2	2
Hispanic	4	0	0	1	3
Other	4	3	3	2	0
White (%)	78	75	86	81	85
Age (y)	52 $\pm$ 10 <sup>4</sup>	52 $\pm$ 9	52 $\pm$ 9	53 $\pm$ 9	52 $\pm$ 9
BMI (kg/m <sup>2</sup> )	27.3 $\pm$ 4.2	27.9 $\pm$ 4.0	26.9 $\pm$ 4.2	27.5 $\pm$ 4.3	28.0 $\pm$ 4.3
Waist circumference (cm)	93 $\pm$ 11	94 $\pm$ 12	92 $\pm$ 11	92 $\pm$ 13	93 $\pm$ 14
Nonsmokers (%)	80 $\pm$ 93	62 $\pm$ 93	59 $\pm$ 92	60 $\pm$ 95	73 $\pm$ 84
Vitamin-mineral supplement (%)	20 $\pm$ 23	20 $\pm$ 30	16 $\pm$ 25	20 $\pm$ 32	21 $\pm$ 24
Antihypertensive agents (n) <sup>5</sup>					
Angiotensin-converting enzyme inhibitors	4	3	0	1	3
Angiotensin receptor blockers	1	1	1	2	3
$\beta$ -Blockers	3	2	2	2	2
Calcium-channel blockers	3	0	0	0	3
Diuretic	2	0	2	1	2
Subjects taking antihypertensives (%)	13	9	6	8	10
Endocrine agents (n) <sup>6</sup>					
Estrogen	4	5	3	5	4
Bisphosphonate	3	3	1	5	3
Thyroxine	6	1	4	8	6
Subjects taking endocrine agents (%)	15	12	9	25	14

<sup>1</sup> 3H, 3 g high-molecular-weight (MW) oat  $\beta$ -glucan/d; 4M, 4 g medium-MW oat  $\beta$ -glucan/d; 3M, 3 g medium-MW oat  $\beta$ -glucan/d; 4L, 4 g low-MW oat  $\beta$ -glucan/d; W, wheat-bran control.

<sup>2</sup> 3.0 mmol/L < LDL cholesterol  $\leq$  3.8 mmol/L.

<sup>3</sup> 3.8 mmol/L < LDL cholesterol  $\leq$  5.0 mmol/L.

<sup>4</sup> Mean  $\pm$  SD (all such values).

<sup>5</sup> 43 drugs taken by 35 subjects.

<sup>6</sup> 61 drugs taken by 55 subjects.

When only W and 3H were included in the analysis (first primary objective), the mean week 4 LDL cholesterol for treatment 3H (3.63 mmol/L) was significantly lower than the week 4 LDL cholesterol for W (3.84 mmol/L) ( $P = 0.002$ ) after adjustment for baseline LDL cholesterol and stratum; there were no significant effects of age, sex, BMI, waist circumference, or center, and there were no significant interaction effects. Serum LDL cholesterol at 4 wk was significantly related to baseline LDL cholesterol for both treatments, and the regression line for 3H was significantly lower than that for W ( $P = 0.006$ ), which reflected the ability of 3H to reduce LDL cholesterol relative to W (**Figure 2**). However, the slopes of the regression lines did not differ significantly ( $P = 0.67$ ), which indicates that the difference in week 4 LDL cholesterol between the 3H and W groups did not depend on baseline LDL cholesterol.

When all 5 diets were included in the analysis, there was a significant effect of diet ( $P = 0.0033$ ) on week 4 LDL cholesterol, after adjustment for baseline LDL cholesterol and

stratum; again, there were no significant effects of age, sex, BMI, waist circumference, or center, and there were no significant interaction effects. Compared with W, 3H lowered LDL cholesterol by 5.5% (0.21 mmol/L; 95% CI: -0.11, -0.30; Fisher's test,  $P = 0.002$ ; Bonferroni,  $P = 0.02$ ), 4M lowered LDL cholesterol by 6.5% (0.26 mmol/L; 95% CI: -0.14, -0.37; Fisher's test,  $P = 0.0007$ ; Bonferroni,  $P = 0.007$ ), and 3M lowered LDL cholesterol by 4.7% (0.19 mmol/L; 95% CI: -0.08, -0.30; Fisher's test,  $P = 0.012$ ; Bonferroni,  $P = 0.12$ ); however, the 2.3% reduction with 4L (0.10 mmol/L; 95% CI: 0.02, -0.20) was not significant (Fisher's test,  $P = 0.205$ ). Compared with 4L, 4M lowered LDL cholesterol by 0.16 mmol/L (4.3%, Fisher's test,  $P = 0.047$ ; Bonferroni  $P = 0.47$ ) (Table 4).

In the repeated-measures analysis including all 5 diets, LDL cholesterol was considered at each week (1-4). The effect of treatment was significant ( $P = 0.0081$ ) after control for baseline, stratum, week, and the interaction between diet and week. LDL cholesterol remained relatively constant over time for W, but



**TABLE 3**  
Composition of the subjects' diets<sup>1</sup>

	Cereal				
	3H (n = 86)	4M (n = 67)	3M (n = 64)	4L (n = 63)	W (n = 87)
Energy (kcal)					
Baseline	2133 ± 70	2190 ± 94	2121 ± 87	1993 ± 68	2143 ± 88
End	2153 ± 67	2159 ± 71	2060 ± 73	2112 ± 93	2122 ± 76
Total fat (% of energy)					
Baseline	30.6 ± 0.7	32.6 ± 0.8	31.5 ± 0.7	33.7 ± 0.8	32.9 ± 0.7
End	30.3 ± 0.6	31.9 ± 0.8	32.1 ± 0.7	31.4 ± 0.8	32.0 ± 0.7
Saturated fat (% of energy)					
Baseline	9.9 ± 0.3	10.5 ± 0.4	10.4 ± 0.4	10.8 ± 0.3	10.8 ± 0.3
End	10.0 ± 0.3	10.3 ± 0.3	10.8 ± 0.4	10.2 ± 0.5	10.5 ± 0.3
Polyunsaturated fat (% of energy) <sup>2</sup>					
Baseline	5.0 ± 0.3	4.8 ± 0.3	5.0 ± 0.3	5.2 ± 0.4	5.0 ± 0.2
End	4.4 ± 0.3	4.5 ± 0.2	4.8 ± 0.3	4.5 ± 0.3	4.4 ± 0.2
Cholesterol (mg)					
Baseline	261 ± 13	302 ± 24	248 ± 15	262 ± 35	285 ± 19
End	263 ± 14	291 ± 17	268 ± 16	245 ± 12	307 ± 20
Protein (% of energy) <sup>2</sup>					
Baseline	16.3 ± 0.3	17.1 ± 0.4	17.0 ± 0.5	16.4 ± 0.4	17.7 ± 0.5
End	17.6 ± 0.3	17.9 ± 0.5	18.2 ± 0.5	18.1 ± 0.4	18.6 ± 0.4
Carbohydrate (% of energy)					
Baseline	52.4 ± 0.9	49.6 ± 1.1	50.6 ± 1.1	49.5 ± 1.0	48.4 ± 0.8
End	52.2 ± 0.9	50.5 ± 1.1	49.7 ± 0.9	51.6 ± 0.8	49.9 ± 0.8
Dietary fiber (g) <sup>2</sup>					
Baseline	22.9 ± 1.1	22.8 ± 1.5	22.9 ± 1.5	22.3 ± 1.2	22.0 ± 1.2
End	25.9 ± 0.9	27.6 ± 1.1	26.2 ± 1.3	28.5 ± 1.7	25.4 ± 0.9
Alcohol (% of energy)					
Baseline	2.8 ± 0.5	2.4 ± 0.4	3.0 ± 0.6	2.3 ± 0.4	2.5 ± 0.4
End	2.8 ± 0.4	2.7 ± 0.5	3.1 ± 0.6	2.2 ± 0.4	2.1 ± 0.4

<sup>1</sup> All values are means ± SEMs. 3H, 3 g high-molecular-weight (MW) oat  $\beta$ -glucan/d; 4M, 4 g medium-MW oat  $\beta$ -glucan/d; 3M, 3 g medium-MW oat  $\beta$ -glucan/d; 4L, 4 g low-MW oat  $\beta$ -glucan/d; W, wheat-bran control.

<sup>2</sup> Main effect of time by ANOVA (ie, Baseline compared with End), but no significant difference between treatments.

LDL cholesterol fell over the first 2 weeks for 3H to approximately its final week 4 value. There was a similar pattern for total cholesterol (**Figure 3**).

The regression of week 4 LDL cholesterol on  $\log(\text{MW} \times \text{C})$ , after adjustment for baseline LDL cholesterol and stratum, was significant ( $P = 0.0027$ ; **Figure 4**). Similarly, the regression of week 4 LDL cholesterol on  $\log(\text{viscosity})$ , after adjustment for baseline LDL cholesterol and stratum, was significant ( $P = 0.001$ ; **Figure 4**). No other factors or covariates were significant in either model. There was no evidence from our data that the relation between LDL cholesterol and  $\log(\text{MW} \times \text{C})$  or that between LDL cholesterol and  $\log(\text{viscosity})$  is described significantly better by a nonlinear function.  $\log(\text{MW} \times \text{C})$  was positively related to the  $\log(\text{viscosity})$  of the solutions of  $\beta$ -glucan obtained from in vitro digestion of the 5 treatment cereals (**Figure 4**).

The results of the analyses of secondary outcomes are shown in **Table 4**. There was a significant effect of treatment on week 4 total cholesterol ( $P = 0.031$ ). After adjustment for sex, baseline total cholesterol, and stratum, the 3H and 4M treatments tended to reduce total cholesterol more than did 3M and 4L, but the only significant difference between treatments was that total cholesterol in the 3H group was 0.23 mmol/L (3.9%) lower than that in the W group (Fisher's test,  $P = 0.0038$ ; Bonferroni,  $P = 0.038$ ; **Table 4**). No significant effect of treatment on triglycerides, HDL cholesterol, total:HDL cholesterol ratio, fasting glucose, creatinine, CRP, AST or blood pressure was observed.

Serum urea was significantly lower in the 4M than in the 4L group (**Table 4**).

The severity of the 9 symptoms differed significantly between baseline and the study, but the changes were not significantly different between treatment groups (*see* Supplemental Material under "Supplemental data" in the online issue).

## DISCUSSION

The results supported both of our hypotheses: consumption of 3 g of high-MW oat  $\beta$ -glucan (3H) daily in a ready-to-eat cereal reduced LDL cholesterol by 0.21 mmol/L (5.5%; 0.07 mmol/L per gram  $\beta$ -glucan). Also, the effect of oat  $\beta$ -glucan on LDL cholesterol was significantly related to its viscosity, which, in turn, was determined by MW and C (dose  $\times$  solubility). As secondary endpoints, the results also showed that consumption of 4M and 3M reduced LDL cholesterol significantly and to an extent similar to that observed with 3H. The significant LDL cholesterol reductions elicited by 3H, 4M, and 3M are less than the 30–50% reductions achieved with statins (3); however, unlike statins, foods containing oat  $\beta$ -glucan are appropriate for almost everyone. It is recognized that, "...at the population level, a few percentage change [in LDL cholesterol] has large implications for the public health burden of coronary heart disease." (37). Because each 1% reduction in LDL cholesterol reduces CAD risk by 1–2% (1), the 5–6% LDL cholesterol

TABLE 4

Least-squares means of endpoints after 4 wk of treatment<sup>1</sup>

	Cereal <sup>2</sup>				
	3H (n = 86)	4M (n = 67)	3M (n = 64)	4L (n = 63)	W (n = 87)
Body weight (kg) <sup>2,3</sup>	77.0 $\pm$ 0.1 <sup>4</sup>	77.2 $\pm$ 0.1	77.3 $\pm$ 0.1	77.1 $\pm$ 0.1	77.1 $\pm$ 0.1
LDL cholesterol (mmol/L) <sup>2,5</sup>	3.63 $\pm$ 0.05 <sup>b,c,6</sup>	3.59 $\pm$ 0.06 <sup>c,6</sup>	3.66 $\pm$ 0.06 <sup>b,c</sup>	3.75 $\pm$ 0.06 <sup>a,b</sup>	3.84 $\pm$ 0.05 <sup>a</sup>
Cholesterol (mmol/L) <sup>2,5,7</sup>	5.73 $\pm$ 0.06 <sup>b,6</sup>	5.73 $\pm$ 0.07 <sup>b</sup>	5.83 $\pm$ 0.07 <sup>a,b</sup>	5.84 $\pm$ 0.07 <sup>a,b</sup>	5.96 $\pm$ 0.06 <sup>a</sup>
TG (mmol/L) <sup>2,3</sup>	1.29 (1.10, 1.38) <sup>8</sup>	1.38 (1.28, 1.50)	1.37 (1.26, 1.48)	1.27 (1.17, 1.38)	1.33 (1.24, 1.42)
HDL (mmol/L) <sup>2,9</sup>	1.39 (1.36, 1.42)	1.38 (1.34, 1.42)	1.39 (1.35, 1.43)	1.43 (1.40, 1.47)	1.43 (1.40, 1.46)
Total:HDL ratio <sup>2,3,7,9,10</sup>	4.10 (4.07, 4.20)	4.13 (4.02, 4.24)	4.15 (4.04, 4.27)	4.09 (3.98, 4.21)	4.12 (4.02, 4.21)
Glucose (mmol/L) <sup>2,3,9</sup>	4.92 $\pm$ 0.05	4.95 $\pm$ 0.06	4.84 $\pm$ 0.06	5.01 $\pm$ 0.06	4.87 $\pm$ 0.05
CRP (mg/L) <sup>2,3,7,9</sup>	1.54 (1.31, 1.79)	1.51 (1.25, 1.81)	1.53 (1.26, 1.83)	1.32 (1.07, 1.61)	1.74 (1.49, 2.01)
Urea (mmol/L) <sup>2,5</sup>	5.42 $\pm$ 0.11 <sup>a,b</sup>	5.07 $\pm$ 0.12 <sup>b</sup>	5.38 $\pm$ 0.12 <sup>a,b</sup>	5.66 $\pm$ 0.13 <sup>a</sup>	5.42 $\pm$ 0.11 <sup>a,b</sup>
Creatinine ( $\mu$ mol/L) <sup>2,3,7</sup>	73.4 $\pm$ 1.0	73.0 $\pm$ 1.2	71.7 $\pm$ 1.2	74.1 $\pm$ 1.2	73.6 $\pm$ 1.0
AST (U/L) <sup>2,3,7,10</sup>	22.3 (21.3, 23.3)	22.2 (21.1, 23.3)	22.6 (21.5, 23.8)	22.0 (20.9, 23.2)	22.9 (21.9, 24.0)
Systolic BP (mm Hg) <sup>2,3,10</sup>	123 $\pm$ 1	125 $\pm$ 1	124 $\pm$ 1	123 $\pm$ 1	127 $\pm$ 1
Diastolic BP (mm Hg) <sup>2,9</sup>	78 $\pm$ 0.8	78 $\pm$ 0.9	77 $\pm$ 0.9	79 $\pm$ 0.9	77 $\pm$ 0.8

<sup>1</sup> TG, triacylglycerols; CRP, C-reactive protein; AST, aspartate transaminase; BP, blood pressure; 3H, 3 g high-molecular-weight (MW) oat  $\beta$ -glucan/d; 4M, 4 g medium-MW oat  $\beta$ -glucan/d; 3M, 3 g medium-MW oat  $\beta$ -glucan/d; 4L, 4 g low-MW oat  $\beta$ -glucan/d; W, wheat-bran control. Values with different superscript letters are significantly different,  $P < 0.05$  (Fisher's test).

<sup>2,3,5,7,9,10</sup> Covariates included in the model (correlated with 4-wk value,  $P < 0.1$ ): <sup>2</sup>baseline value, <sup>3</sup>center, <sup>5</sup>stratum, <sup>7</sup>sex, <sup>9</sup>BMI, <sup>10</sup>waist circumference.

<sup>4</sup> Mean  $\pm$  SEM (all such values).

<sup>6</sup> Significantly different from W,  $P < 0.05$  (after Bonferroni adjustment for 10 comparisons).

<sup>8</sup> 95% CI for log-transformed values (all such values).

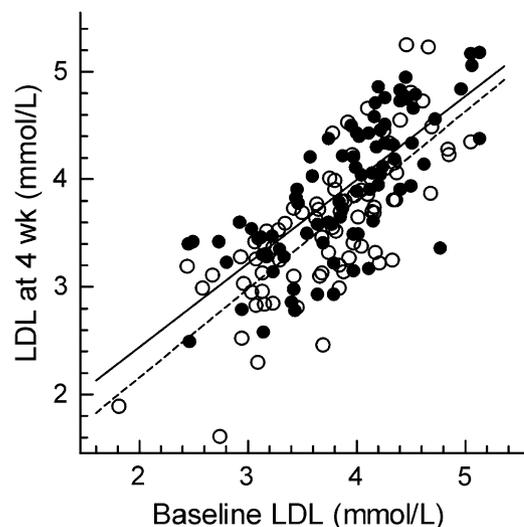
lowering elicited by 3–4 g oat  $\beta$ -glucan from 3H, 4M, or 3M could reduce the risk of CAD by 5–12%. Meta-analyses of the statin trials (3, 38) suggest that lowering LDL cholesterol by 0.2 mmol/L would reduce the risk of CAD by  $\approx$ 5%. Our results apply directly to the  $\approx$ 60–65% of the US population aged 35–70 y who have LDL cholesterol between 3 and 5 mmol/L and triglycerides  $<$ 4 mmol/L (1).

Meta-analyses have shown that oat products lower LDL cholesterol by 0.032–0.037 mmol/L/g soluble fiber; however, there was significant heterogeneity between the 22–25 studies analyzed (7, 8). This could have been due to differences in the responses between subjects in different trials or to variable efficacy of the food products used. In one study, oat  $\beta$ -glucan lowered LDL cholesterol more in subjects with a baseline LDL cholesterol  $>$ 4.3 mmol/L (0.034 mmol/L per g;  $P = 0.02$ ) than in those with LDL cholesterol  $<$ 4.3 mmol/L (0.015 mmol/L per g; NS) (8), but the difference was only marginally significant. We showed that the LDL cholesterol-lowering effect of oat  $\beta$ -glucan did not differ significantly across a range of LDL-cholesterol concentrations from 3 to 5 mmol/L. The mechanism by which oat  $\beta$ -glucan lowers LDL cholesterol is not entirely clear, but it is thought to be related to reduced intestinal bile acid reabsorption (39, 40). This stimulates bile acid synthesis from cholesterol, which is supplied by a combination of increased cholesterol synthesis, as indicated by increased serum lathosterol and 7 $\alpha$ -hydroxy-4-cholesteron-3-one (11, 13) and, presumably, an increased uptake of LDL cholesterol from the plasma.

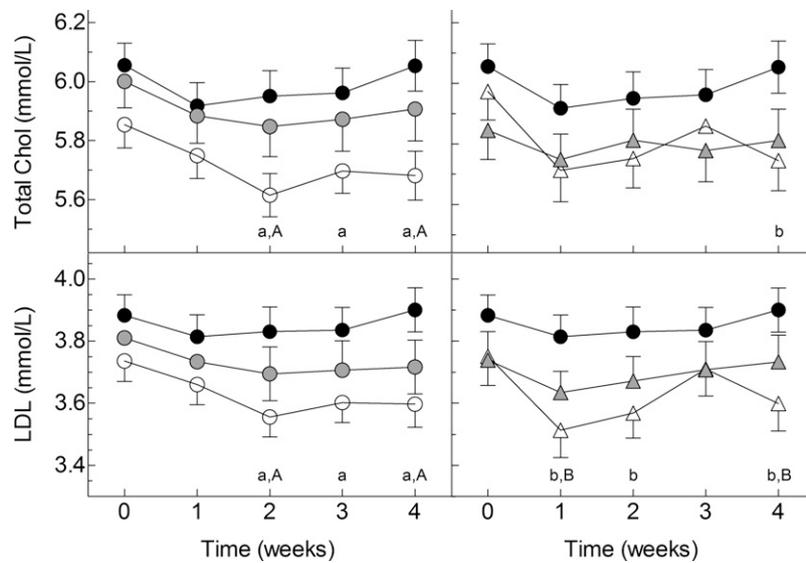
The lack of effect of oat products on LDL cholesterol in some studies has been ascribed, often without supporting data, to an adverse effect of food processing or the food matrix on the MW and/or the solubility of oat  $\beta$ -glucan, resulting in reduced viscosity and bioactivity (24). Many studies compared the cholesterol-lowering effects of cereal  $\beta$ -glucans at different doses and/or MWs (41–43), but they provide contradictory or equivocal

evidence for an effect of dose and MW. Possible reasons for this are that the  $\beta$ -glucan ingested was not completely solubilized (or dispersed) within the intestine, that the range of dose/MW tested was not large enough to affect serum cholesterol, or that the MW of the  $\beta$ -glucan was reduced by food processing or storage.

We modified the MW and solubility of oat  $\beta$ -glucan in extruded cereal products to provide a range of values consumed by subjects that we knew was large enough to elicit differences in



**FIGURE 2.** Regression of LDL cholesterol at baseline on LDL cholesterol for individual subjects after 4 wk of consuming wheat-bran cereal (W; ●, solid line,  $n = 87$ ) or cereal containing 3 g high-molecular-weight  $\beta$ -glucan (3H; ○, dashed line,  $n = 86$ ). The regression equation for W is as follows: 4-wk LDL = 0.776  $\times$  LDL<sub>baseline</sub> + 0.889 ( $P < 0.0001$ ). The regression equation for 3H is as follows: 4-wk LDL = 0.824  $\times$  LDL<sub>baseline</sub> + 0.511 ( $P < 0.0001$ ). The slopes of the regression lines do not differ significantly ( $P = 0.67$ ), but the line for 3H is significantly below that for W ( $P = 0.006$ ).



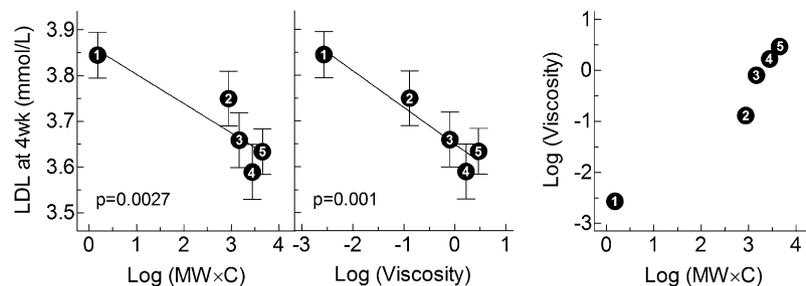
**FIGURE 3.** Unadjusted mean ( $\pm$ SEM) serum total and LDL cholesterol (Chol) over the study period for subjects who consumed wheat bran (W; ●,  $n = 87$ ), 3 g medium-molecular-weight (MW)  $\beta$ -glucan (3M; ●,  $n = 64$ ), 3 g high-MW  $\beta$ -glucan (3H; ○,  $n = 86$ ), 4 g low-MW  $\beta$ -glucan (4L; ▲,  $n = 63$ ), and 4 g medium-MW  $\beta$ -glucan (4M; △,  $n = 67$ ). <sup>a,A</sup> Values for 3H are significantly different from W by unpaired  $t$  test before (a) and after (A) Bonferroni adjustment. <sup>b,B</sup> Values for 4M are significantly different from W by unpaired  $t$  test before (b) and after (B) Bonferroni adjustment.

glycemic response (21, 27, 28), and, thus, we believed would differentially affect serum cholesterol. We measured the viscosity of the  $\beta$ -glucan extracted from the test foods, but because viscosity is shear thinning (ie, the value decreases as the shear rate of measurement increases), measurement of the proportion of  $\beta$ -glucan solubilized (C) and its MW may be a more robust way to estimate bioactivity because MW and C are relatively insensitive to extrinsic factors. Except in dilute solutions (as for the W extract), there is a linear relation between  $\log(\text{viscosity})$  and  $\log(\text{MW} \times \text{C})$  (20, 33) (Figure 4). We included 5 levels of  $\text{MW} \times \text{C}$  so that the primary test of its effect on LDL cholesterol was based on regression analysis rather than on a comparison of individual means. The MW and C of the  $\beta$ -glucan in the test foods were assessed before and during the study to ensure that the expected values were obtained and maintained over the 18 mo from production to the end of the trial. We measured the MW and C of  $\beta$ -glucan in *in vitro* (37°C) digests, which may reflect more closely the physiologic effect of  $\beta$ -glucan than does the 100°C extractions used to measure soluble fiber. Finally, we varied MW over a greater range (500-fold) and included higher

values than used in other studies; indeed the highest MW used in some studies (40, 42) was less than the MW of our low-MW treatment (33).

The LDL cholesterol-lowering effects of 3H (0.070 mmol/L per g  $\beta$ -glucan), 4M (0.063 mmol/L per g), and 3M (0.060 mmol/L per g) were about twice the mean (0.032 mmol/L per g), and over the upper limit of the 95% CI (0.017–0.047 mmol/L per g) of the effect of oat  $\beta$ -glucan estimated from meta-analyses (7, 8); however, the effect of 4L, 0.023 mmol/L per g, was not significant. This suggests that 3 g oat  $\beta$ -glucan/d will lower LDL cholesterol by  $\approx 0.2$  mmol/L if its MW is  $\geq 530,000$  g/mol but will have  $<50\%$  of this effect if its MW is  $\leq 210,000$  g/mol. However, because the regression represents a continuous range of response of LDL cholesterol against  $\log(\text{C} \times \text{MW})$ , a low MW may be effective if C could be increased enough to compensate. Unfortunately, we could not determine the independent effects of MW and C, because C and MW did not vary independently in the cereals used.

The demonstration that the physicochemical properties of oat  $\beta$ -glucan influence its cholesterol-lowering effect has major



**FIGURE 4.** Relations between  $\log(\text{MW} \times \text{C})$  and serum LDL cholesterol,  $\log(\text{viscosity})$  and serum LDL cholesterol, and  $\log(\text{MW} \times \text{C})$  and  $\log(\text{viscosity})$ . Values are means  $\pm$  SEMs for the 5 treatments: 1 = wheat-bran cereal ( $n = 87$ ), 2 = 4 g low-MW;  $\beta$ -glucan ( $n = 63$ ), 3 = 3 g medium-MW  $\beta$ -glucan ( $n = 64$ ), 4 = 4 g medium-MW  $\beta$ -glucan ( $n = 67$ ), 5 = 3 g high-MW  $\beta$ -glucan ( $n = 86$ ). Solid lines are regression equations.  $P$  values for the correlations with LDL cholesterol are from the ANCOVA. No regression line or  $P$  value for  $\log(\text{MW} \times \text{C})$  and  $\log(\text{viscosity})$  is shown because the relation is not linear across the observed range of viscosity (44). MW, molecular weight; C, amount of oat  $\beta$ -glucan solubilized in the intestine.



implications for health claims. Currently, the only criteria that must be fulfilled to make a health claim are that the food product contains  $\beta$ -glucan from an eligible source and in a sufficient amount to provide a daily intake of 3 g. However, our results show that not only the amount, but also the MW and bioavailability (C) of oat  $\beta$ -glucan, should be considered. The MW and C of the  $\beta$ -glucan in a food product depends on the source of the  $\beta$ -glucan used as an ingredient and may be affected by food processing. The MW of  $\beta$ -glucan in raw oats or oat bran is  $\approx$ 2 million, but when extracted to create a purified food ingredient, the MW of  $\beta$ -glucan may be reduced unintentionally during the extraction and purification process (45, 46) or intentionally to improve the functional properties and acceptability of food products into which it is incorporated (47). The MW of commercial preparations of  $\beta$ -glucan whose effects on LDL cholesterol have been tested ranged from 40,000–80,000 g/mol. In 2 cases these low-MW  $\beta$ -glucans significantly reduced LDL cholesterol [5 g oat  $\beta$ -glucan/d with an MW of 70,000 (41) or 80,000 (48) given in beverages], but in 6 cases they did not [10 g oat  $\beta$ -glucan with an MW of 70,000 and 5 or 10 g barley  $\beta$ -glucan with an MW of 40,000 given in beverages (41); 8–12 g barley  $\beta$ -glucan with an MW <80,000 (45, 46) given in meals and snacks (49); and 4 g/d (50) or 3.5 g/d (51) oat  $\beta$ -glucan with an MW of 80,000 given in soup]. Food processing methods can reduce the MW of  $\beta$ -glucan, but these effects can be controlled. Indeed, our results indicate not only that the MW of the  $\beta$ -glucan in oat-bran cereal produced under typical industrial conditions can be controlled by varying the water content, extrusion temperature, and shear rate but also that it is possible to make a palatable processed (extruded) breakfast cereal containing oat  $\beta$ -glucan with an MW similar to that of unprocessed oat bran (33). The  $\beta$ -glucanase enzymes in wheat flour can depolymerize  $\beta$ -glucan and markedly reduce its MW (52); this effect can also be controlled, and baked products may be a good vehicle for oat  $\beta$ -glucan. Indeed, some studies showed that oat  $\beta$ -glucan given in bread lowered serum cholesterol (53, 54). However, no amount of processing can increase the MW of  $\beta$ -glucan in a food if its ingredients contain low-MW  $\beta$ -glucan to start with.

We conclude that the physicochemical properties of oat  $\beta$ -glucan should be considered when assessing the ability of oat-containing products to lower serum cholesterol. Our results show that an extruded breakfast cereal providing 3 g oat  $\beta$ -glucan/d with a high-MW (2,210,000) or medium-MW (530,000) lowered LDL cholesterol similarly by  $\approx$ 0.2 mmol/L (5%), but the efficacy was reduced by 50% when the MW was reduced to 210,000.

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The authors' responsibilities were as follows—TMSW: had access to all of the data in the study and takes responsibility for the integrity of the data and accuracy of the data analysis; TMSW, SMT, ALG, and PJW: primarily responsible for the conception and design; JB-M, AMD, VH, BL, BAT, and RD: contributed to the conception and design; JB-M, AMD, VH, and BL: data acquisition; BAT, ALG, TMSW, SMT, and PJW: data analysis and interpretation; TMSW: drafting of the manuscript; BAT, ALG, and TMSW: statistical analysis; RD and PJW: obtaining funding; TMSW, SMT, PJW, and RD: administrative, technical, or material support; and TMSW, JB-M, AMD, VH, and BL: supervision. All authors were responsible for the critical

revision of the manuscript for important intellectual content. TMSW is president of Glycemic Index Laboratories Inc; VH is employed by Reading Scientific Services, Ltd; RD is employed by CreaNutrition AG. None of the other authors had a conflict of interest to disclose. The role CreaNutrition played in designing the study was restricted to designing and creating the wheat and oat cereals used. CreaNutrition played no role in the conduct of the study, collection, management, analysis, and interpretation of the data or in the initial preparation of the manuscript. The other sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

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