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Diets containing soluble oat extracts reduce urinary malondialdehyde in moderately hypercholesterolemic men and women

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Soluble oat extracts have been used successfully in a number of diet studies to lower plasma blood lipids. One of the mechanisms proposed for this reduction in lipids is the production of short-chain fatty acids in the large intestine, which, in turn, may inhibit low-density lipoprotein cholesterol synthesis, thereby limiting oxidation. Urinary excretion of malondialdehyde (MDA) is proportional to endogenous lipid peroxidation. The purpose of this report is to examine the effects of consuming oat extracts with low (1%) or high (10%) levels of soluble β-glucans on lipid peroxidation as measured by urinary malondialdehyde excretion. Twenty-four subjects with moderately elevated cholesterol levels (6.0 mmol/L) were selected for the study. A 1-week equilibration period was followed by two 5-week experimental periods during which defined diets contained the 1% or 10% oat extracts in a crossover design. Seventy-two-hr urine samples (3 × 24-hr) were collected at the end of each period. Urinary levels of MDA excreted were 10–18% of dietary MDA consumed. Consumption of either of the oat extracts resulted in highly significant reductions in urinary excretion of malondialdehyde ($P < 0.0001$*). These data would support the hypothesis that oat extracts inhibit endogenous lipid peroxidation by reduction of MDA (a marker for fatty acid oxidation); however, because no significant differences were found in MDA excretion after subjects consumed the two levels of β-glucans, this is apparently not the responsible component. Further examination of this mechanism in the control of lipid metabolism is warranted.* (J. Nutr. Biochem. 8:497–501, 1997) © *Elsevier Science Inc. 1997*

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Introduction

Soluble fibers have been successful in lowering blood cholesterol in a number of human feeding studies.[1] Oat fibers have been the most successful at lowering this risk for heart disease.[2] Some reports suggest that it is the amount of β-glucan that is responsible for lowering plasma cholesterol levels.[3] A number of possible mechanisms have been proposed as responsible for this beneficial effect. They include reduced rate of absorption because of increased viscosity of the gut contents;[4] binding of bile salts to increase excretion;[5] and production of short-chain fatty acids in the large intestine from fermentation of undigested carbohydrate that then inhibit cholesterol synthesis.[6–8] This study was conducted to test the hypothesis that it is the level of soluble β-glucan that is responsible for the beneficial effects associated with consumption of oats and oat extracts. Although consumption of menus containing oat extracts with both 1% and 10% β-glucan was found previously to be successful in lowering cholesterol,[9] glucose, and insulin responses[10] in moderately hypercholesterolemic men and women, and systolic blood pressure[11] in men; the menus containing 10% β-glucan were generally more effective. Malondialdehyde (MDA) is one end product of lipid per-

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Table 1 Characteristics of men and women completing 11-week oatrim study

Sex	*n*	Age (yr)	Weight (kg)	BMI[1]
Women	17	52 ± 2	70 ± 4	26 ± 1
Men	7	50 ± 2	90 ± 6	28 ± 2

[1]Kg/M^2.

oxidation. Urinary excretion of MDA can be used as a measure of in vivo oxidative stress.[12] In the present report the effect of sex and diet on urinary excretion of MDA equivalents of moderately hypercholesterolemic men and women are discussed.

Methods and materials

Subjects

Twenty-four subjects completed the study. They were selected on the basis of moderately elevated plasma cholesterol levels (6.0 mmol/L), but were otherwise healthy as determined by a medical evaluation and clinical blood and urine screening. Details of the methods and selection have been reported.[10] The study was approved by the Institutional Review Board of the Johns Hopkins School of Public Health which provided medical supervision and the USDA Human Studies Committee. Subjects were informed both orally and in writing of the procedures to be conducted during the study. All subjects gave written consent. Data from 17 women and 7 men are included in this report (*Table 1*).

Diets

Diets consisted of a 7-day rotating menu composed of commonly consumed foods. During the equilibration week the diet was 35% fat, 50% carbohydrate, and 15% protein. During the two experimental periods (each 5-week) a number of the recipes used in the equilibration diet were modified to reduce fat and increase carbohydrate by adding a soluble oat extract containing either 1% or 10% β-glucans. The oat extracts used in this study were produced by ConAgra and Quaker Oats using a method patented by Agricultural Research Service scientist George Inglett, Northern Research Laboratory, Peoria, IL.[13] The oat extracts (Oatrims 1 and 10) are soluble tasteless powders that can be added to a variety of foods in modest amounts without lowering their palatability. Resultant menus contained 30% fat, 55% carbohydrate, and 15% protein, but seemed identical to the equilibration menus (*Table 2*). All foods were weighed to 0.5 g. Subjects consumed breakfast and dinner Monday through Friday under supervision in the Human Study Facility of the Beltsville Human Nutrition Research Center. Lunches and weekend meals were packed with instructions for preparation and consumption. Subjects were required to consume all foods presented to them and no others except those approved by the investigators (water, diet sodas, tea, coffee). The three menus consumed during the urine collections are listed in *Table 3*. Gram amounts listed represent the amount consumed for a 8.36 MJ (2000 Kcal) menu, which was the level composited for analyses.

Analyses

Daily food composites of all three menus (equilibration, 1% and 10% soluble oat extracts) were collected as served during the study. These composites were frozen until they could be analyzed. Daily composites were thawed and blended until homogeneous.

Table 2 Macronutrient composition of menus during equilibration week and 5-week periods during which oat extract either with 1% or 10% β-glucan was added to selected foods[1]

Nutrient	Equilibration	1%	10%
Protein (%)	15%	15%	15%
Carbohydrate (%)	50%	55%	55%
Fat (%)	35%	30%	30%
P:S[2]	0.7	0.6	0.6
Cholesterol (mg/MJ)	33	33	33
Fiber, insoluble g/MJ	1.6	1.5	2.1
Soluble glucans (g/MJ)	0.17	0.20	1.03

[1]In the 1% and 10% diets, 5% of energy from fat was replaced and 5% of energy from carbohydrate was added as soluble oat extract (oatrim).
[2]Polyunsaturated to saturated fatty acid ratio.

Duplicate aliquots were taken from 3-day composites and extracted with 10% trichloroacetic acid before analysis. All urine was collected the last 3 days of each of the three periods in 24-hr samples. Each 24-hr urine sample began after the first void of the day and continued to include the first void of the following day. Urine was collected in plastic containers and kept in coolers with ice packs until daily delivery before breakfast. Urine volume was measured daily and then refrigerated until the end of the 3 days when composites were made and frozen at −20°C until analyzed. Food and urine were analyzed for MDA using a modified high-pressure liquid chromatography (HPLC) thiobarbituric acid technique originally reported by Halliwell and Chirico.[14] The concentration of MDA in samples was calculated using peak areas at identical elution times of varying amounts of thiobarbituric acid. A blank, three standards, and an internal urine control sample were included with each set of 12 unknowns. Food composition and urinary excretion of MDA were expressed as average daily number of μmol/day of thiobarbituric acid reactive substances (TBARS). The acronym MDA will be used throughout the paper, referring to malondialdehyde equivalents or malondialdehyde adducts as determined by this HPLC technique.

Statistical Analyses

Repeated measures analysis of variance[15] (ANOVA) was used to determine diet and sex differences in MDA. Excretion was expressed as average daily level, percent of dietary intake, excretion/g fat intake; $P < 0.05$ was considered significant. Differences between means were determined by least squares means. Pearson correlation coefficients were calculated among MDA and plasma lipid values.

Results and discussion

MDA intakes of the three diets show that intake was actually higher when subjects consumed the menus containing Oatrim (*Table 4*). This is partly because the energy level was increased to attempt to maintain body weight so that more energy was consumed during the oatrim periods. Men were consuming more energy, therefore, they consumed more MDA than women for all three diet periods. These calculations are based on analyses of duplicate composites for the three periods that were then adjusted for individual variation in energy intakes. The variation is determined by the variation in energy intake and not by the variation in food composite values or day-to-day variation in menus. A number of the foods in the menus have been analyzed for

Table 3 Menus consumed during the equilibration week and the two 5-wk periods[1]

Day 1		Day 2		Day 3	
Food	g	Food	g	Food	g
*Orange juice	187	*Waffles	71	40% bran	46
Egg substitute	30	Strawberries	125	*Orange juice	125
Milk	184	*Gelatin	32	*Muffin	40
Margarine	10	Butter	15	Margarine	10
*Muffin	40	Milk	122	Milk	186
		*Grapefruit juice	124		
Lunch					
*Tomato soup	201	Tuna	56	Cheese	10
Peanut butter	27	*Cookies	42	Turkey	56
*Pudding	156	Ranch dressing	15	Cucumber	30
Celery	60	Lettuce	20	Lettuce	46
Cucumber	52	Celery	43	Tomato	30
Fruit cocktail	88	*Muffin	40	Salad dressing	30
*Gelatin	37	*Applesauce	134	*Muffins	40
*Muffin	62			*Cookies	42
Ranch dressing	15				
Dinner					
Roast beef	85	Turkey	85	Flounder	90
*Gravy	105	Potatoes	100	Rice	100
Sweet potatoes	33	Asparagus	60	Spinach	95
Lettuce	42	Celery	40	Lemon juice	8
Radishes	30	Tomatoes	60	Lettuce	46
Salad dressing	10	Lettuce	42	Tomatoes	40
Margarine	5	French dressing	15	Salad dressing	15
*Yogurt	113	*Muffin	40	Margarine	5
Cherries	88	Blueberries	125	*Pudding	156
*Gelatin	37	*Gelatin	32	Pears	77
*Muffin	40	*Cupcake	62	*Gelatin	37
		*Gravy	33	*Brownie	43

[1]Foods with asterisks (*) had either 1% or 10% oatrim added during the experimental periods. Amounts are for menus containing approximately 8.36 mj (2000 Kcal).

MDA.[16–18] Most of the MDA in the diets would be expected to come from these meat sources: flounder, tuna, beef, turkey; but other foods, including cheese, nuts, green beans, and vegetable oils have been reported to have detectable amounts.[16] Cooking methods and storage time affect the MDA content of foods.[17–19] Grinding the samples to make them homogeneous was essential for analyses of other components of the diet, but it releases MDA.[20] The method of extraction may also alter MDA as determined by thiobarbituric acid.[21] Use of the HPLC method eliminates some of the interfering substances detected by the colorimetric method.[22] Because we analyzed only duplicate samples of each menu, our values should not be considered definitive, but only a rough estimate of intakes. Subjects took lunches with them and may have microwaved them before consumption or reheated them, which could have altered the content. The length of time some prepared foods sat in hot and cold storage could have varied as much as 1.5 hr/day and could have affected intake amounts.

Table 4 Dietary intakes of malondialdehyde equivalents of men and women consuming equilibration diets and diets containing 1% or 10% β-glucan oat extracts[1] (μmol/day)

	Men (n = 7)	Women (n = 17)
Equilibration	36.0 ± 1.0[c]	26.1 ± 0.6[a]
1% Diet	48.1 ± 1.0[e]	33.9 ± 0.6[c]
10% Diet	42.5 ± 1.0[d]	30.4 ± 0.6[b]

[1]*ANOVA*: diet*sex, $P < 0.05$. Values not sharing a common superscript are different, $P < 0.05$ according to least squares means.

Urinary excretion of MDA was dramatically reduced after subjects consumed the diets containing Oatrim (*Table 5*). Men consumed more energy and excreted more MDA. Fat intake has been reported to influence urinary excretion of MDA.[12] Draper et al.[12] conducted a number of animal experiments in which the level of polyunsaturated fatty acids (PUFA) was changed. Rats consuming higher levels of PUFA excreted more MDA in the urine than those consuming either lower levels or no PUFA. Although the p/s ratio of the three menus in the present study were similar (0.6–0.7), the actual amount of fat consumed changed, because the percent of energy from fat was reduced from 35% in the equilibration diet to 30% in the oat extract diets. Therefore, MDA excretion expressed as nmol/g fat intake was also analyzed for diet and sex differences (*Table 5*). This adjustment eliminates the significant difference in MDA excretion between men and women, because it accounts for the differences in energy intake between the sexes. The reduction in MDA excretion when consuming the oat extracts is still highly significant ($P < 0.0001$).

Although MDA excretion was slightly lower during the high soluble β-glucan consumption period, the hypothesis

Table 5 Urinary excretion of malondialdehyde of men and women consuming equilibration diet and diets containing 1% or 10% β-glucan oat extracts[1]

	Men			Women		
	μmol/day	nmol/g fat	intake (%)	μmol/day	nmol/g fat	intake (%)
Equilibration	5.8 ± 0.4[a]	54.8 ± 6.1[ab]	16.2 ± 1.6[a]	5.1 ± 0.3[ab]	67.7 ± 3.9[a]	19.9 ± 1.0[a]
1%	5.0 ± 0.4[a]	50.5 ± 6.1[b]	10.3 ± 1.6[b]	3.3 ± 0.3[c]	48.8 ± 3.9[b]	10.0 ± 1.0[b]
10%	4.4 ± 0.4[b]	44.9 ± 6.1[b]	10.4 ± 1.6[b]	2.9 ± 0.3[c]	42.1 ± 3.9[b]	9.7 ± 1.0[b]

[1]ANOVA: for μmol/day: sex $P < 0.05$ (men > women), diet $P < 0.0001$ (equilibration > 1% and 10%); for /g fat: diet $P < 0.005$ (equilibration > 1% and 10%); for percent intake MDA: diet $P < 0.0001$ (equilibration > 1% and 10%). Values within a parameter are different if superscripts are different ($P < 0.05$).

that β-glucan is responsible for reduction in MDA excretion was not supported by the data. Other compounds such as avenanthramides, which are present in oats, have been reported to have antioxidant properties.[23] Fiber-containing sources including coconut and blackgram have been reported to reduce MDA and conjugated dienes in rats indicating the antioxidant properties of these foods.[24]

There is controversy over the proportion of ingested MDA excreted in the urine. Jacobson et al.[19] fed five volunteers 200 g of beef, pork, or chicken and reported that 50% to 60% of ingested MDA was recovered in the urine within 24 hr. The majority was recovered during the first 12 hr. Siu and Draper[25] reported that only 9% to 17% of [1,3-^{14}C] malonaldehyde administered to rats was recovered in urine in 12 hr. Nelson et al.[26] fed nine men diets containing stabilization diets for 20 days and then switched six of the men to a diet containing salmon as the only source of n-3 long-chain PUFA for 40 days. Urinary output of MDA was elevated after consumption of the salmon, but the authors state that they believe that the source of these compounds is in vivo production from the fatty acids, not MDA in the fish. Unfortunately, the fish was not analyzed and the amounts consumed not reported. Siu and Draper[11] report fresh salmon to contain 0.88 μg/g. Brown recovered 84% to 99% of MDA equivalents from beef in 24-hr urine samples of 10 men and 9 women consuming meat cooked at low and high temperatures;[27] however, the rest of the menus were not analyzed for MDA. For the present study, when MDA excretion is expressed as percent of intake, values are comparable to those reported by Draper et al.[23] with mean values ranging from 10% to 18% of intake. There was a highly significant diet difference in MDA excretion between the equilibration diet and the oat extract diets ($P < 0.0001$).

Previously reported lipid data[9] show a significant reduction after consumption of either Oatrim in total and LDL-cholesterol with no significant decrease in HDL-cholesterol. Pearson correlation coefficients within diet among blood lipids and MDA intake and excretion were nonsignificant with few exceptions. MDA excretion was positively correlated with baseline VLDL ($r = 0.54$) and triglyceride ($r = 0.52$) levels and inversely related to final HDL ($r = -0.49$) levels. Total cholesterol that did decline was not related to MDA excretion.

In summary, regardless of how urinary excretion of MDA is expressed, there was a highly significant decrease when subjects consumed oat extracts with high or low levels of β-glucans. Although the diet higher in β-glucan was slightly more effective in lowering MDA excretion, differences between the two oat extracts were not significant. Because the 1% diet had amounts of soluble fiber comparable to the amount in the equilibration diet, it is doubtful that both Oatrim diets were above the threshold for effectiveness in lowering MDA excretion. Soluble β-glucan therefore, does not seem to be the effective component of oats. If excretion of malondialdehyde equivalents can be viewed as a quantitative measure of in vivo lipid oxidation or peroxidation as determined by this HPLC method, then this decrease should be considered desirable. Further research is needed to determine the mechanism controlling this beneficial effect.

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