Effect of resistant starch on breath-hydrogen and methane excretion in healthy volunteers[1–3]

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ABSTRACT Colonic fermentation of dietary carbohydrates and fiber might produce a protective effect against the development of large bowel cancer. Resistant starch, ie, starch that escapes small bowel digestion, is a candidate fermentable substrate that has been hitherto little studied. We supplemented 19 healthy volunteers with 15 g native amylomaize (Hylon-VII) three times a day, containing 28 g type II resistant starch, or with dextrins as a placebo for 7 d in a crossover design. Pre-experimentally, 11 subjects regularly produced breath methane and 8 did not. Resistant starch increased 24-h integrated excretion of breath hydrogen. The mean rise relative to placebo was 35% ($P = 0.03$) for all subjects and 60% for eight subjects not producing methane ($P = 0.02$). The 11 methane producers showed a 93% increase in breath-methane excretion on resistant starch ($P = 0.03$). Continued consumption of 28 g type II resistant starch/d is well tolerated and increases colonic fermentation in healthy volunteers. *Am J Clin Nutr* 1994;59:626–30.

KEY WORDS Resistant starch, fermentation, hydrogen, methane, colon cancer, human diet, experiment

Introduction

Starchy foods such as wheat, potatoes, and cassava are the main source of energy in most human diets. Cooking serves to release starch from the granules in which it is stored, and makes it available for digestion. Dietary starch is largely hydrolyzed and absorbed in the small bowel but a part is resistant to digestion, depending on the processing and type of starch (1–3). This resistant starch fraction enters the cecum and is a potential substrate for fermentation (1, 4). Fiber is another important source of fermentable carbohydrate, but high-fiber diets have proved less than attractive to most Western people, and adherence to such diets is poor. Resistant starch might conceivably provide a useful alternative to fiber; however, very little is known about the actual fate of resistant starch in the colon.

Dietary fiber can offer protection against the development of colonic cancer (5–8). Fermentation of fiber into short-chain fatty acids is one of the proposed mechanisms for this effect. Thornton et al (9) demonstrated that in patients with colonic neoplasms the fraction of dietary starch reaching the colon was smaller than in healthy control subjects (9).

Resistant starch has been classified into three groups: type I represents physically inaccessible starch such as that in intact or partly milled grains; type II consists of starch enclosed in granules, such as in raw potato or unripe bananas; and type III represents retrograded amylose (1).

The proportion of resistant starch in several foods has been calculated by feeding them to colectomized patients and determining the residual starch in the ileostomy output. The proportion of starch that was resistant to small bowel digestion and absorption varied from 1% in potatoes (10) to 89% in unripe bananas (11). Molis et al (12) intubated the cecum of healthy volunteers and found that 49% of ingested retrograded high-amylose maize starch was recovered from the cecum. In common foods only a relatively small proportion of dietary starch appears to be resistant starch (1, 13). Using the intubation technique, Flourie et al (14) demonstrated that 4% of starch in an average meal reaches the colon. Stephen (15) used the same intubation technique and recovered 10% of starch in meals from the terminal ileum; in some subjects the figure was as high as 20%. In general, such studies show 4–10% of dietary starch to be resistant to digestion.

One end product of cecal fermentation of starch is hydrogen, which can be measured in the expired breath. If the colonic bacterial flora contains a sufficient amount of methanogenic bacteria, hydrogen will in turn be used to synthesize methane (16). This can be found in the expired air and flatus of approximately 50% of the Western population (17). Therefore, the excretion of these two gases can be used as a gauge for colonic fermentation (18).

If indeed fermentation is the mechanism through which fiber has a protective effect on colon cancer development, we speculate that resistant starch could have the same effect. As far as we know, only a few single-dose experiments have been done with resistant starch in human subjects, but no data are available about the effect of continued supplementation with resistant starch. Here we report the effects of feeding 45 g native amylomaize/d, containing 28 g resistant starch for 1 wk on colonic fermentation in healthy volunteers.

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TABLE 1
Intakes of energy and carbohydrates from regular foods in volunteers participating in a controlled crossover trial on the fermentation of resistant starch*

Intakes and subjects	Amylomaize	Maltodextrin
Energy (MJ/24 h)		
All (n = 21)	11.4 ± 2.4	12.0 ± 2.7
Methane excreters (n = 12)	11.3 ± 2.4	12.5 ± 3.2
Nonmethane excreters (n = 9)	11.6 ± 2.5	11.2 ± 1.7
Mono- and disaccharides (g/24 h)		
All (n = 21)	153 ± 43	156 ± 30
Methane excreters (n = 12)	153 ± 51	156 ± 33
Nonmethane excreters (n = 9)	153 ± 32	156 ± 29
Starch (g/24 h)		
All (n = 21)	165 ± 41	166 ± 59
Methane excreters (n = 12)	168 ± 43	182 ± 65
Nonmethane excreters (n = 9)	162 ± 40	146 ± 45
Dietary fiber (g/24 h)		
All (n = 21)	29 ± 11	31 ± 14
Methane excreters (n = 12)	32 ± 13	34 ± 18
Nonmethane excreters (n = 9)	26 ± 7	26 ± 7

* $\bar{x}$ ± SD for days 5–7 of the combined supplementation periods and include those for two subjects who provided insufficient breath samples for H_2 and CH_4 analysis. Differences between periods were not significant either biologically or statistically for any nutrient. In addition, subjects received 45 g native amylomaize/d or 45 g rapidly digestible starch (maltodextrin)/d for 7 d each.

Subjects and methods

Hypothesis

The study was set up to test the following hypotheses:

1) Resistant starch supplementation will cause a higher breath-hydrogen excretion in both methane producers and nonmethane producers than will maltodextrin supplementation.

2) Resistant starch supplementation will increase breath-methane excretion in methane producers.

Subjects and design

Twenty-two healthy male volunteers (mean age 46 y, range 21–76 y) entered the study. Women were excluded because of the influence of the menstrual cycle on starch absorption and fermentation (19). Other exclusion criteria were smoking, recent use of laxatives or antibiotics, and previous or current bowel disease. Four breath samples were collected on 2 separate days from each volunteer before the study, to classify him as a methane excreter or nonexcreter. A subject was classified as a methane excreter when the methane concentration exceeded 3 ppm in two or more of the four breath samples (20). Ten volunteers were nonexcreters and 12 were methane excreters. One nonmethane excreter dropped out because of influenza, and one excreter and one nonexcreter provided insufficient breath samples, leaving a total of 19 who completed the trial successfully.

The design was a two-period, placebo-controlled crossover study with periods of 7 d and a 7-d washout period between treatments. During the last 3 d of each supplementation period, volunteers recorded their diet and any symptoms of abdominal discomfort in a special diary. The purpose and design of the study and the possible consequences of resistant starch consumption were thoroughly explained to the subjects, who then gave their informed consent in writing. The study was approved by the Human Experimentation Committee of the University Hospital Nijmegen.

Diets and food intake

Participants consumed their regular diets during the study but were asked to refrain from consuming beans and peas. During the supplementation periods, subjects consumed three times daily 15 g native amylomaize starch (Hylon-VII; National Starch and Chemical Company, Zutphen, The Netherlands) or 15 g maltodextrin (Cerestar SF 01904; Cerestar Benelux BV, Sas van Gent, The Netherlands) with their meals. Native Hylon-VII contains a high proportion of amylose; ≈62% on a gross weight basis is type II resistant starch as determined by the in vitro method of Englyst et al (21). Consequently, 45 g native amylomaize contains 28 g resistant starch. Maltodextrin is a rapidly absorbable, partially hydrolyzed maize-starch. Diet composition was calculated from the dietary records by using the 1987 release of The Netherlands nutrient data bank (22).

Breath hydrogen and methane

The subjects collected breath samples in 60-mL syringes at 2-h intervals from 0800 until 0000 and again at 0800 the next morning during the last 24 h of each supplementation period. Immediately after breath collection another syringe was filled with ambient air, and both syringes were sealed. Hydrogen was measured within 24 h after collection by using a standard electrochemical cell. Calibration was performed with a standard gas (AGA-gas; AGA, Amsterdam) with a hydrogen concentration of 95.7 ± 2.5 ppm. For the determination of methane concentration, 0.4-mL breath samples were injected in triplicate into a Packard gas chromatograph, equipped with a Porapak Q 100-120 mesh column (Chrompack, Middelburg, The Netherlands) at an oven temperature of 50 °C. Calibration was done with a standard gas (AGA-gas; methane concentration 28.4 ± 0.4 ppm). The methane concentration in each breath sample was corrected for the concentration of the matching ambient air sample.

Symptom score

On the final 3 d of each supplementation period, the volunteers scored complaints of bloating, flatulence, abdominal cramps, belching, and diarrhea in a diary on a scale running from 0 (none) to 4 (severe). The mean value of the median symptom score was calculated for each complaint during both periods.

Data analysis and statistics

All values are given as $\bar{x}$ ± SEM. The effect of resistant starch supplementation and maltodextrin supplementation on hydrogen and methane excretion, calculated as the area under the 24-h time-concentration curve, was tested one-tailed by using the method of Pocock (23). With a confidence level of $\alpha = 0.10$, no interaction could be demonstrated between period and supplementation. Under the assumption of equal period effects, differences between methane- and nonmethane-producing subjects were tested with a two-way analysis of variance (ANOVA) without interaction (classifications: type of supplement, methane producer, and methane nonproducer). Calculations were done with the *SAS* analysis system (SAS Institute, Inc., Cary, NC).

TABLE 2
Symptom scores as recorded by 21 subjects during supplementation with 45 g native amylomaize/d or 45 g maltodextrin/d*

Symptoms	Amylomaize	Maltodextrin
Bloating	0.4 (0–2)	0.3 (0–3)
Flatulence	1.4 (0–3)	0.7 (0–2)
Cramps	0.1 (0–1)	0.2 (0–3)
Belching	0.2 (0–1)	0.2 (0–1)
Diarrhea	0.0 (0–1)	0.0 (0–1)

* $\bar{x}$; range in parentheses. Subjects scored symptoms on a scale from 0 (none) to 4 (severe).

Results

As shown in **Table 1,** values for carbohydrate and energy intakes were those typically found in adult Dutch men. No significant differences in food intake were seen between treatments for either methane or nonmethane excreters. Native amylomaize was well tolerated (**Table 2**); only flatulence was somewhat increased.

Hydrogen excretion

The hydrogen concentration in expired air ranged from 1.2 to 91.5 ppm during consumption of resistant starch and from 0 to 57.6 ppm during the maltodextrin period. The mean hydrogen excretion rose during the day and peaked late at night, several hours after the last dose of resistant starch (**Fig 1**). A significant rise in integrated 24-h hydrogen excretion during amylomaize supplementation relative to control treatment was found in both the entire group and in the subjects not excreting methane (**Table 3**). The effect was more modest in methane excreters.

Methane excretion

The mean concentration of methane in ambient air was 3.9 ± 1.2 ppm. During amylomaize supplementation the mean methane concentration in breath, corrected for ambient air concentration, ranged from 17 to 26 ppm in methane excreters (**Fig 2**). The

TABLE 3
Twenty-four–hour integrated breath-hydrogen excretion of healthy subjects after 7 d supplementation with either 45 g native amylomaize/d or 45 g maltodextrin/d*

	Amylomaize	Maltodextrin	Difference†
		ppm/h	
All subjects (n = 19)†	601 ± 61	443 ± 46	155 ± 75§
Methane excreters (n = 11)	592 ± 97	488 ± 62	105 ± 110
Non-methane excreters (n = 8)	612 ± 64	383 ± 65	230 ± 90‖

* $\bar{x}$ ± SEM.
† Corrected for a period effect.
‡ Another two subjects had provided insufficient samples for hydrogen analysis.
§ $P = 0.03$.
‖ $P = 0.02$.

area under the curve of concentration vs time was significantly higher during amylomaize supplementation than during control treatment (506 ± 109 vs 280 ± 73 ppm × h, $P = 0.03$). In subjects initially classified as nonmethane producers, methane excretion was indeed negligible on either treatment (1 ± 1 vs 4 ± 3 ppm × h).

H_2 equivalents in methane-producing subjects

In subjects who produce methane, hydrogen formed by fermentation is converted into methane according to the equation $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$ (24). The primary 24-h integrated hydrogen production in such subjects (H_2 equivalent) was estimated according to the following formula: area under the curve (H_2) + 4 × area under the curve (CH_4). This sum rose significantly in methane-producing subjects from 1607 ± 283 ppm × h during placebo supplementation to 2617 ± 419 ppm × h during resistant starch supplementation. After correction for a period

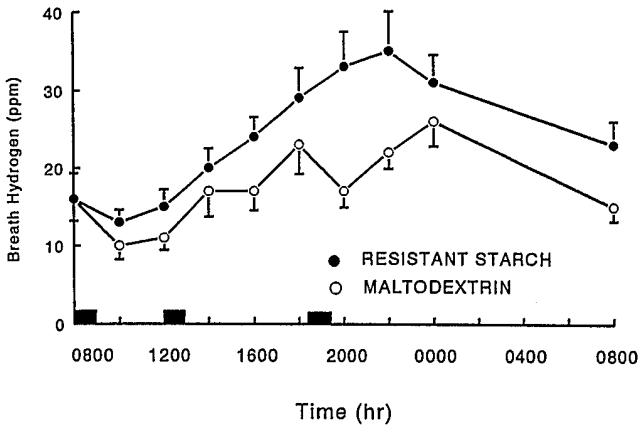

FIG 1. Breath-hydrogen concentration on the seventh day of supplementation of a normal Dutch diet three times daily with 15 g maltodextrin or native amylomaize (Hylon-VII), providing 28 g type II resistant starch/d. The 19 subjects received the two supplements in a 2×7-d crossover design with a 1-wk washout period in between treatments. $\bar{x}$ ± 1 SEM. ■, time of ingestion of supplement.

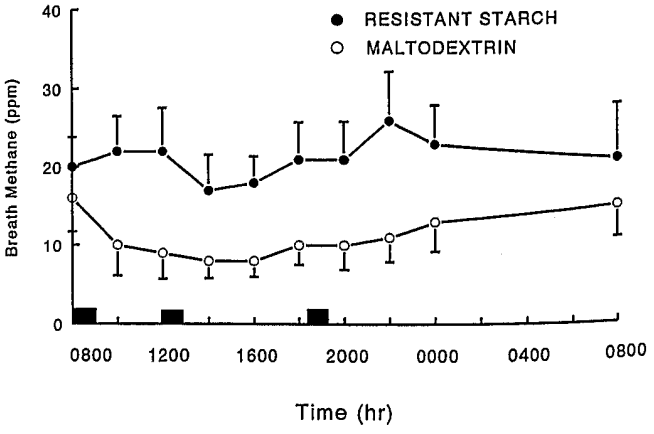

FIG 2. Breath methane concentration of 11 methane-producing subjects on the seventh day of supplementation of a normal Dutch diet three times daily with 15 g maltodextrin or native amylomaize (Hylon-VII), providing 28 g type II resistant starch/d. The 19 subjects received the two supplements in a 2×7-d crossover design with a 1-wk washout period in between treatments. $\bar{x}$ ± SEM. ■, time of ingestion of supplement.

effect, the difference between the two supplementations was 1094 ± 447 ppm ($P = 0.015$).

Discussion

Data about hydrogen excretion after resistant starch ingestion are scarce. A positive response has been demonstrated after single doses of raw potato starch (25) and raw banana starch (26), and consumption of a type III resistant starch as cornflakes resulted in an elevation of breath hydrogen on the first day of consumption (27). However, several studies have demonstrated an adaptation of the colonic flora after chronic consumption of fermentable substrate (28, 29). Therefore, the increased fermentation after continued resistant starch consumption found in this study is more relevant to real-life situations than is the effect of a single dose. Studies concerning hydrogen excretion after fiber ingestion have shown conflicting results (4, 30, 31), which could partly be explained by differences in fermentability of different fibers.

Because hydrogen is produced by bacterial fermentation in the colon, the rise in hydrogen excretion is probably the result of fermentation of amylomaize escaping digestion and absorption in the small bowel. Hydrogen excreted during the maltodextrin period is the result of background fermentation of other food constituents in the normal diet. This integrated hydrogen excretion during the placebo supplementation was similar to that reported by other authors (4). Hydrogen produced in the large bowel may be further metabolized to acetate by acetogenic bacteria, to H_2S by sulphate-reducing bacteria, or to methane by methanogenic bacteria (16). Competition between these metabolic pathways exists, and only half of the Western population shows significant excretion of methane (17). In the present study methane-excreting subjects showed a significant increase of methane excretion after resistant starch supplementation. Flourie et al (14) found that a large increase in the intake of digestible starch also resulted in an increased excretion of methane, but not of hydrogen. Presumably, an overload of digestible starch results in a partial escape from small bowel digestion and to colonic fermentation. Studies about the effect of fiber consumption on methane excretion have shown conflicting results (32–35), although in general the effect is minimal. This finding suggests that resistant starch may more readily increase colonic fermentation than does dietary fiber.

Those of our subjects who did not excrete methane when on their regular diets also did not do so after resistant starch supplementation. A lack of excretion of methane thus appears to be due to a difference in colonic bacterial composition rather than to a lack of fermentable substrate in the habitual diet.

Hydrogen excretion did not increase significantly in the methane-excreting subjects after supplementation with resistant starch. We speculate that this is due to excess hydrogen being channelled into methane production. Formation of one molecule of methane requires four molecules of hydrogen. The equivalent H_2 excretion, calculated as $H_2 + 4CH_4$, rose significantly in methane producers after resistant starch consumption. This suggests that the difference in response of breath hydrogen to resistant starch between methane- and nonmethane-excreting subjects was due to a difference in utilization of the hydrogen produced, rather than to a difference in colonic production of hydrogen from resistant starch. In a previous study using whole-body calorimetry, lactulose consumption caused a lower hydrogen excretion but a higher excretion of total H_2 equivalents in methane producers than in nonmethane-excreting subjects (26). This is in line with our results. The relatively large amount of resistant starch (28 g/d) given by us was well tolerated; the subjects reported an increase in flatulence but no other discomfort or symptoms.

We conclude that supplementation of the diet of healthy volunteers with 28 g type II resistant starch/d in the form of 45 g native amylomaize/d is well tolerated, and results in an increased colonic fermentation. Epidemiological studies are warranted to elucidate a possible protective effect of resistant starch on the development of colon cancer.

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References

1. Cummings JH, Englyst HN. Fermentation in the human large intestine and the available substrates. Am J Clin Nutr 1987;45:1243–55.
2. Anderson IH, Levine AS, Levitt MD. Incomplete absorption of the carbohydrate in all purpose wheat flour. N Engl J Med 1981;304:891–2.
3. Muir JG, O'Dea K. Measurement of resistant starch: factors affecting the amount of starch escaping digestion in vitro. Am J Clin Nutr 1992;56:123–7.
4. Levitt MD, Hirsh P, Fetzer CA, Sheahan M, Levine AS. H_2 excretion after ingestion of complex carbohydrates. Gastroenterology 1987;92:383–9.
5. Vargas PA, Alberts DS, Ritenbauch C, et al. Dietary fiber and colon cancer prevention. Cancer Bull 1991;43:549–61.
6. Willett WC, Stampfer MJ, Colditz GA, Rosner BA, Speizer FE. Relation of meat, fat, and fiber intake to the risk of colon cancer in a prospective study among women. N Engl J Med 1990;323:1664–72.
7. Giovannucci E, Stampfer MJ, Colditz G, Rimm EB, Willett WC. Relationship of diet to risk of colorectal adenoma in men. J Natl Cancer Inst 1992;84:91–8.
8. Alberts DS, Einspahr J, Rees-McGee S, et al. Effects of dietary wheat bran fiber on rectal epithelial cell proliferation in patients with resection for colorectal cancers. J Natl Cancer Inst 1990;82:1280–5.
9. Thornton JR, Dryden A, Kelleher J, Losowsky MS. Super-efficient starch absorption. A risk factor for colonic neoplasia? Dig Dis Sci 1987;32:1088–91.
10. Schweizer TF, Andersson H, Langkilde AM, Reimann S, Torsdottir I. Nutrients excreted in ileostomy effluents after consumption of mixed diets with beans or potatoes. II. Starch, dietary fibre and sugars. Eur J Clin Nutr 1990;44:567–75.
11. Englyst HN, Cummings JH. Non-starch polysaccharides (dietary fiber) and resistant starch. Adv Exp Med Biol 1990;270:205–25.
12. Molis C, Champ M, Flourie B, et al. Small intestine digestibility of processed corn starches in healthy human subjects. Eur J Clin Nutr 1992;46:S131–2.
13. McBurney MI, Thompson LU, Cuff DJ, Jenkins DJ. Comparison of ileal effluents, dietary fibers, and whole foods in predicting the physiological importance of colonic fermentation. Am J Gastroenterol 1988;83:536–40.
14. Flourie B, Leblond A, Florent C, Rautureau M, Bisalli A, Rambaud JC. Starch malabsorption and breath gas excretion in healthy humans consuming low- and high-starch diets. Gastroenterology 1988;95:356–63.

15. Stephen AM. Starch and dietary fibre: their physiological and epidemiological interrelationships. Can J Physiol Pharmacol 1991;69:116–20.
16. Gibson GR, Cummings JH, Macfarlane GT, et al. Alternative pathways for hydrogen disposal during fermentation in the human colon. Gut 1990;31:679–83.
17. McKay LF, Eastwood MA, Brydon WG. Methane excretion in man—a study of breath, flatus, and faeces. Gut 1985;26: 69–74.
18. Olesen M, Rumessen JJ, Gudmand-Hoyer E. The hydrogen breath test in resistant starch research. Eur J Clin Nutr 1992;46:S133–4.
19. McBurney MI. Starch malabsorption and stool excretion are influenzed by the menstrual cycle in women consuming low fibre western diets. Scand J Gastroenterol 1991;26:880–6.
20. Melis O, Oudenhoven I, van Munster IP, Nagengast FM. Variability and prevalence of methane excretion in healthy volunteers. Gastroenterology 1991;100:A834 (abstr).
21. Englyst HN, Kingman SM, Cummings JH. Classification and measurement of nutritionally important starch fractions. Eur J Clin Nutr 1992;46(suppl 2):S33–50.
22. Voorlichtingsbureau voor de voeding. NEVO Table. Meppel, The Netherlands: Krips Repro, 1993.
23. Pocock SJ. Crossover trials. In: Pocock SJ, ed. Clinical trials, a practical approach. New York: John Wiley & Sons, 1983: 110–22.
24. Wolin MJ. Metabolic interactions among intestinal microorganisms. Am J Clin Nutr 1974;27:1320–8.
25. Cummings JH, Englyst HN. Measurement of starch fermentation in the human large intestine. Can J Physiol Pharmacol 1991;69: 121–9.
26. Christl SU, Murgatroyd PR, Gibson GR, Cummings JH. Production, metabolism, and excretion of hydrogen in the large intestine. Gastroenterology 1992;102:1269–77.
27. Tomlin J, Read NW. The effect of resistant starch on colon function in humans. Br J Nutr 1990;64:589–95.
28. Florent C, Flourie B, Leblond A, Rautureau M, Bernier JJ, Rambaud JC. Influence of chronic lactulose ingestion on the colonic metabolism of lactulose in man (an in vivo study). J Clin Invest 1985;75:608–13.
29. Perman JA, Modler S, Olson C. Role of pH in production of hydrogen from carbohydrates by colonic bacterial flora. J Clin Invest 1981;67:643–50.
30. Eastwood MA, Brydon WG, Anderson DM. The effect of the polysaccharide composition and structure of dietary fibers on cecal fermentation and fecal excretion. Am J Clin Nutr 1986; 44:51–5.
31. Hanson CF, Winterfeldt EA. Dietary fiber effects on passage rate and breath hydrogen. Am J Clin Nutr 1985;42:44–8.
32. Wolever TM, Robb PA. Effect of guar, pectin, psyllium, soy polysaccharide, and cellulose on breath hydrogen and methane in healthy subjects. Am J Gastroenterol 1992;87:305–10.
33. Marthinsen D, Flemiong SE. Excretion of breath and flatus gases by humans consuming high-fiber diets. J Nutr 1982;112: 1133–43.
34. Melcher EA, Levitt MD, Slavin JL. Methane production and bowel function parameters in healthy subjects on low- and high-fiber diets. Nutr Cancer 1991;16:85–92.
35. McNamara EA, Levitt MD, Slavin JL. Breath hydrogen and methane: poor indicators of apparent digestion of soy fiber. Am J Clin Nutr 1986;43:898–902.