Do colonic short-chain fatty acids contribute to the long-term adaptation of blood lipids in subjects with type 2 diabetes consuming a high-fiber diet?[1–3]

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ABSTRACT

Background: We recently obtained evidence of long-term adaptation of blood lipids to changes in intakes of carbohydrate and fiber in subjects with type 2 diabetes.

Objective: We determined the effect of increased carbohydrate and fiber intakes on serum short-chain fatty acids (SCFAs) and the relation between changes in serum acetate and changes in blood lipids.

Design: Subjects with type 2 diabetes (n = 62) were randomly assigned to receive ≈10% of energy from low-fiber breakfast cereal (LF diet), high-fiber breakfast cereal (HF diet), or monounsaturated fatty acids (MUFA diet) for 6 mo.

Results: Carbohydrate intakes were higher in the LF and HF groups than in the MUFA group (54% compared with 43%), and more fiber was consumed by the HF group (≈50 g/d) than by the LF or MUFA group (≈23 g/d). Fasting serum SCFAs did not change significantly over the first 3 mo. Between 3 and 6 mo, serum acetate tended (NS) to decrease in the LF group (from 69 ± 4 to 59 ± 5 μmol/L) and increase in the HF group (from 100 ± 18 to 107 ± 17 μmol/L), with no significant change in the MUFA group. Serum butyrate did not change significantly in the LF or MUFA group but increased in the HF group (from 2.5 ± 0.5 to 3.1 ± 0.6 μmol/L; $P < 0.001$). Changes in serum acetate from 0 to 3 mo were not related to changes in lipids. However, changes in serum acetate from 3 to 6 mo were positively related to changes in the ratio of total to HDL cholesterol ($P = 0.041$) and in fasting ($P = 0.013$) and postprandial ($P = 0.016$) triacylglycerols.

Conclusions: In subjects with type 2 diabetes, changes in serum SCFAs in response to changes in carbohydrate and fiber intakes took many months to occur, and the changes in serum acetate were significantly related to the long-term adaptive changes in blood lipids. *Am J Clin Nutr* 2002;75:1023–30.

KEY WORDS Type 2 diabetes, short-chain fatty acids, high-fiber diet, acetate, carbohydrate intake, fiber intake

INTRODUCTION

High-carbohydrate diets may increase cardiovascular risk in diabetic subjects by raising serum triacylglycerol and reducing HDL-cholesterol concentrations compared with diets rich in monounsaturated fatty acids (MUFAs) (1). However, there is concern that these deleterious effects may not persist in the long term (2) and that the effects of different sources of carbohydrate have not been considered (3). To address these issues, we previously randomly assigned 91 subjects with diabetes to a high-carbohydrate, low-fiber (LF) diet; a high-carbohydrate, high-fiber (HF) diet; or a low-carbohydrate, high-MUFA diet for 6 mo (4). Over the first 3 mo, the ratio of total to HDL cholesterol increased significantly more with the LF diet than with the MUFA diet, but this difference disappeared by 6 mo. Unexpectedly, fasting triacylglycerol and cholesterol concentrations tended to rise between 3 and 6 mo with the HF diet, and postprandial triacylglycerols were significantly higher after 6 mo of the HF diet than after 6 mo of the LF diet.

We hypothesized that the adaptations in blood lipids we observed were due to changes in acetate production from colonic bacterial fermentation of unabsorbed carbohydrate. Acetate is the primary substrate for sterol and fatty acid synthesis (5), and colonic acetate rapidly appears in blood lipids (6). Increasing colonic acetate by rectal infusion (7) or by feeding the unabsorbed sugar lactulose (8) raises blood lipids (9, 10). However, changes in colonic acetate production in response to changes in diet may take months to develop, as suggested by the finding that cecal concentrations of short-chain fatty acids (SCFAs) in rats fed resistant starch continue to increase for 6 mo (11).

To test this, we measured fasting serum SCFA concentrations before and after 3 and 6 mo of study diets. If our hypothesis were true, we would expect to see progressive changes in serum SCFAs with time. Also, we would expect there to be no correlation between changes in serum acetate and changes in blood lipids from 0 to 3 mo, but a significant correlation between changes in serum acetate and changes in blood lipids from 3 to 6 mo.

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TABLE 1
Daily fiber intake from high-fiber cereals[1]

	Bran Buds[2]	Oat loops	Total
		g/d	
Total dietary fiber	22.9 ± 1.5	6.1 ± 0.6	29.0 ± 1.8
Insoluble fiber	17.5 ± 1.2	2.0 ± 0.2	19.5 ± 1.2
Soluble fiber	5.4 ± 0.4	4.2 ± 0.4	9.6 ± 0.6
Psyllium fiber	5.3 ± 0.4	3.1 ± 0.3	8.5 ± 0.5

[1] $\bar{x}$ ± SEM.
[2] Bran Buds with Psyllium; Kellogg Co, Battle Creek, MI.

SUBJECTS AND METHODS

The detailed methods and main results of the study were published previously (4). The study protocol was approved by the St Michael's Hospital Research Ethics Board. Subjects gave their written, informed consent and obtained their physicians' written consent to participate. Briefly, 91 subjects with type 2 diabetes, treated by diet alone or by diet plus metformin or a sulfonylurea or both were recruited to take part in a randomized, parallel-design study with treatments lasting 6 mo. Subjects receiving insulin or acarbose were excluded. To be eligible, subjects had to have a body mass index (BMI; in kg/m^2) <36, glycated hemoglobin >0.065 (6.5%) and <0.101 (10.1%), and serum triacylglycerol <10.0 mmol/L. Of the 62 subjects whose results are reported here, 9 were receiving stable doses of lipid-lowering drugs (6 were receiving a statin and 3 a fibrate) throughout the study. Subjects whose dose of lipid drug changed were excluded.

Subjects were randomly assigned to 1 of 3 treatments: low-fiber, high–glycemic index breakfast cereal (LF diet); high-fiber, low–glycemic index breakfast cereal (HF diet); or high–monounsaturated fat oil and margarine, with use of breakfast cereals forbidden (MUFA diet). The aim of the dietary intervention was to achieve a 10% difference in the intake of carbohydrate and a difference in overall dietary glycemic index of ≈10. Treatment foods were provided to subjects and were prescribed to meet 10–15% of energy requirements according to data from the Lipid Research Clinics Prevalence Study (12) plus 1254 kJ (300 kcal) for weight maintenance or minus 836 kJ (200 kcal) for weight loss (subjects with BMIs >27). Subjects were free-living and attended clinic visits approximately once per month. At each visit, body weight was measured and compliance assessed by the difference between the amount of treatment food provided at the previous visit and the amount returned at the present visit.

Fasting blood samples were drawn for analysis of total and HDL cholesterol and triacylglycerol at baseline, 3 mo, and 6 mo. Aliquots of serum were saved at –70°C for later analysis of SCFAs. Serum samples at 2 or 3 time points were available for SCFA analysis from 59 of the 72 subjects who finished the study plus 3 who dropped out after 3 mo. Of these 62 subjects, 8 had missing baseline samples and 22 had missing 6-mo samples.

At baseline and at the end of the study, the subjects came to the nutrition center after 10–14-h overnight fasts for completion of an 8-h metabolic profile. After we obtained a fasting blood sample and the subjects took their medications, if any, the subjects ate a breakfast meal and gave further blood samples at hourly intervals for 8 h. Immediately after the 4-h blood sample, a lunch meal was consumed. At baseline, the same standard breakfast and lunch meals, representing a high-carbohydrate, low-fiber diet, were provided to all subjects. At the end of the study, the breakfast meals contained the same energy as at baseline, but were altered to include the amount of treatment foods used at home during the study. The lunch meals were the same at the beginning and at the end of the study.

The low-fiber cereals were corn flakes (Nature's Path, Delta, Canada), Crispy Rice (Our Compliments; The Oshawa Group Ltd, Toronto), and puffed rice (Arrowhead Mills, Hereford, TX). The high-fiber cereals were Bran Buds with Psyllium (Kellogg Co, Battle Creek, MI) and a prototype oat-loop cereal enriched with psyllium. Subjects were allowed to consume only the breakfast cereals provided. Subjects in the MUFA group were not allowed to eat breakfast cereals at all and were given unhydrogenated salted or unsalted soft tub margarine (Fleischmann's 20% Corn Oil Margarine; Lipton, Toronto), olive oil (President's Choice Extra Virgin Olive Oil; Sunfresh Ltd, Toronto), or both. Treatment foods were incorporated into individualized diabetes meal plans by the study dietitian. Either a portion or all of the prescribed food was consumed at the breakfast meal, with the rest consumed later in the day according to individual preference. Subjects were advised to increase their consumption of treatment foods slowly during the first month to avoid abdominal discomfort.

Subjects were instructed on completing 3-d food records, 2 of which were collected during the baseline period and 4 during the study. The food records were reviewed with the subjects for accuracy by a dietitian. Nutrient intakes were analyzed by using NUTRIPUT (version 2.02; University of Toronto), incorporating the US Department of Agriculture *Handbook No. 8* (13) database; missing values for dietary fiber were added and values for carbohydrate were modified to reflect glycemic carbohydrate (ie, total carbohydrate minus dietary fiber). Soluble and insoluble fiber intakes were not available because the nutrient database does not contain this information. However, the fiber content of the high-fiber test cereals was available from the manufacturer and intake from these sources, based on recorded average daily cereal intakes over the last 3 mo of the study, is given in **Table 1**.

Fasting serum total cholesterol and triacylglycerol were measured enzymatically by using a Vitros Analyzer 950 (Johnson & Johnson Clinical Diagnostics, Rochester, NY). HDL cholesterol was measured after precipitation of other lipoproteins with dextran sulfate and magnesium chloride. Serum SCFAs were measured by gas chromatography after sample preparation by ultrafiltration to remove plasma proteins followed by vacuum distillation (14).

Data were subjected to repeated-measures analysis of variance (ANOVA), examining for differences by diet and time and diet × time interactions. Blood lipid results were presented before but are included here for completeness. Missing values of SCFAs in the ANOVA were imputed by using Rubin's nonitterative method (15). Pairwise comparisons of individual means for effects found to be significant in the ANOVA were carried out by using Tukey's procedure to control for multiple comparisons. Changes across time (3 mo minus baseline, 6 mo minus 3 mo, and, for metabolic profile comparisons, 6 mo minus baseline) in blood SCFAs were correlated with changes in blood lipids over the corresponding time period by using least-squares linear regression analysis (GRAPHPAD PRISM; GraphPad Software, San Diego), with all missing values excluded from the analysis. For nonnormally distributed data, Spearman's rank correlation was used. The criterion for significance was taken to be 2-tailed $P < 0.05$.

TABLE 2
Details of included subjects at baseline[1]

	LF group (n = 13 M, 8 F)	MUFA group (n = 9 M, 11 F)	HF group (n = 12 M, 9 F)
Age (y)	62.9 ± 2.1[2]	63.2 ± 1.8	64.4 ± 2.1
BMI (kg/m^2)	28.0 ± 0.9	28.4 ± 1.0	27.9 ± 0.9
Glycated hemoglobin (%)	7.9 ± 0.3	7.8 ± 0.2	7.8 ± 0.2
Diabetes therapy (n)			
Diet alone	3	8	5
Sulfonylurea	7	5	4
Metformin	2	0	2
Metformin plus sulfonylurea	9	7	10
Lipid-lowering agent (n)	4	1	4

[1] LF group, received a low-fiber breakfast cereal; HF group, received a high-fiber breakfast cereal; MUFA group, received monounsaturated fatty acids. There were no significant differences between groups.
[2] $\bar{x}$ ± SEM.

RESULTS

At baseline, there were no significant differences between the subjects assigned to the 3 diet groups with respect to sex distribution, age, BMI, glycated hemoglobin, or use of drugs to treat diabetes, blood pressure, or blood lipids (**Table 2**). The subjects' nutrient intakes were presented in full elsewhere (4) and are summarized in **Table 3**. Fiber intake increased by 27 g/d with the HF diet; changes in fiber intake with the other diets were not significant. Total fat intake was 9% higher and available carbohydrate intake 7–10% lower with the MUFA diet than with the other diets. The difference in fat intake was accounted for by an increase in MUFA intake (4). There were no significant changes in intakes of ethanol (1–2% of energy) or cholesterol (210–250 mg/d) (data not shown).

There were no significant differences in body weight, fasting glucose, or glycated hemoglobin at baseline between the diet groups and no significant changes in body weight (**Table 4**) or glycemic control (data not shown) by diet (4). The reason for the main effect of diet on body weight in the absence of a significant difference at baseline was presumably due to the tendency of the LF group, which had the lowest mean weight at baseline, to lose weight during the study. This difference cannot be considered significant, however, because of the lack of a significant diet × time interaction.

Mean serum total cholesterol concentrations at baseline and throughout the study were significantly higher in the MUFA group than in the HF and LF groups (**Table 5**). Mean serum triacylglycerol did not differ significantly between the 3 diet groups at baseline, but there was a main effect of diet because the values tended to rise in the HF group, which also tended to have the highest baseline mean. However, the lack of a diet × time interaction indicates that serum triacylglycerols did not change significantly with time for any diet. HDL cholesterol rose significantly in the MUFA group and fell significantly in the LF group, with a nonsignificant decrease in the HF group. The ratio of total to HDL cholesterol did not differ significantly between the 3 groups at baseline. After 3 mo, the ratio of total to HDL cholesterol was significantly higher in the LF group than in the MUFA group, but the difference was not significant after 6 mo. In the HF group, the ratio of total to HDL cholesterol did not change significantly over the first 3 mo but tended to rise between 3 and 6 mo (NS).

TABLE 3
Composition of the study diets[1]

				P (ANOVA)		
	LF diet	HF diet	MUFA diet	Diet effect	Time effect	Diet × time interaction
Energy (MJ)						
Baseline	8.0 ± 0.4	7.4 ± 0.2	7.5 ± 0.3			
Study	8.4 ± 0.4	7.8 ± 0.3	7.9 ± 0.4	0.005	0.003	NS
Protein (% of energy)						
Baseline	19.1 ± 0.8^{a,b}	18.6 ± 0.6^{a,b,c}	18.1 ± 0.5^{b,c,d}			
Study	17.0 ± 0.5^{d}	19.7 ± 0.5^{a}	17.8 ± 0.6^{c,d}	NS	NS	<0.001
Total fat (% of energy)						
Baseline	31.7 ± 1.4^{b}	30.5 ± 1.0^{b,c}	31.1 ± 1.3^{b}			
Study	28.9 ± 1.1^{c,d}	28.3 ± 1.0^{d}	37.2 ± 1.1^{a}	NS	NS	<0.001
Carbohydrate (% of energy)						
Baseline	48.1 ± 1.3^{b}	49.3 ± 0.8^{b}	49.0 ± 1.7^{b}			
Study	53.5 ± 1.1^{a}	50.1 ± 0.9^{b}	43.2 ± 1.2^{c}	NS	NS	<0.001
Dietary fiber (g)						
Baseline	25.2 ± 1.7^{b}	23.2 ± 1.3^{b}	24.0 ± 0.7^{b}			
Study	23.1 ± 1.3^{b}	50.3 ± 1.4^{a}	23.5 ± 1.2^{b}	NS	NS	<0.001

[1] $\bar{x}$ ± SEM. LF, low-fiber; HF, high-fiber; MUFA, monounsaturated fatty acids. Values for the same nutrient with different superscript letters are significantly different, $P < 0.05$.

TABLE 4
Body weight of the subjects by diet group and time[1]

Group	Baseline	3 mo	6 mo
		kg	
LF	77.4 ± 3.2	76.9 ± 3.1	76.2 ± 3.0
HF	77.6 ± 3.6	77.1 ± 3.6	77.3 ± 3.6
MUFA	78.8 ± 2.8	79.0 ± 2.8	78.9 ± 2.9

[1] $\bar{x}$ ± SEM. LF group, received a low-fiber breakfast cereal; HF group, received a high-fiber breakfast cereal; MUFA group, received monounsaturated fatty acids. There was a main effect of diet on body weight, $P < 0.004$.

The distribution of mean 0–8-h plasma triacylglycerol concentrations during the metabolic profile tests was normalized by log transformation. At baseline, the mean 0–8-h triacylglycerol concentration did not differ significantly between diet groups: LF, 2.10 ± 0.19 mmol/L; HF, 2.56 ± 0.27 mmol/L; and MUFA, 2.10 ± 0.26 mmol/L. However, after 6 mo, the mean 0–8-h triacylglycerol concentration of the HF group, 2.85 ± 0.38 mmol/L, was significantly greater than that of the LF group, 1.88 ± 0.23 mmol/L, with the value for the MUFA group, 2.06 ± 0.16 mmol/L, being intermediate.

At baseline, the HF group tended to have the highest serum SCFA concentrations and the LF group the lowest, but these differences were not significant. However, because of the tendency for SCFAs to rise with time in the HF group and decrease in the LF group, subjects in the HF group had significantly higher fasting serum SCFA concentrations (average of baseline, 3-, and 6-mo values) than did those in the LF group, with subjects in the MUFA group being intermediate (**Figure 1**). Serum acetate did not change significantly over the first 3 mo with any diet, but between 3 and 6 mo tended to rise with the HF diet by 7 ± 4 μmol/L and fall with the LF diet by 14 ± 4 μmol/L (Figure 1). Although there was no significant effect for propionate, the diet × time interaction was significant for serum butyrate, with an increase between 3 and 6 mo of 0.65 ± 0.18 μmol/L with the HF diet and no significant change with the other 2 diets. The acetate-to-propionate ratio was similar in all groups at baseline and at 3 mo but between 3 and 6 mo increased with the HF diet by 6% and decreased with the LF diet by 12%, with no significant change with the MUFA diet (**Table 6**).

Changes in total fiber intake were significantly related to changes in serum butyrate but not to changes in acetate and propionate (**Figure 2**). Intakes of total, soluble, insoluble, and psyllium fiber from the high-fiber cereals were not significantly related to changes in serum SCFA concentrations.

Between 0 and 3 mo, changes in serum acetate were not related to changes in serum total ($r = 0.02$) or HDL ($r = -0.02$) cholesterol. However, changes in serum acetate between 3 and 6 mo tended to correlate positively with changes in total cholesterol ($r = 0.28$, $P = 0.08$). Changes in acetate were not significantly related to changes in HDL cholesterol ($r = -0.17$). Changes in serum acetate were positively related to changes in triacylglycerol and to the ratio of total to HDL cholesterol between 3 and 6 mo, but not between 0 and 3 mo (**Figure 3**). Changes in the mean 0–8-h triacylglycerol concentration during the metabolic profile test were positively related to changes in both fasting serum acetate and the acetate-to-propionate ratio (**Figure 4**).

Changes in serum propionate and butyrate between 3 and 6 mo were not significantly related to changes in fasting or mean 0–8-h triacylglycerol concentrations nor to changes in the ratio of total to HDL cholesterol. However, changes in serum propionate between 3 and 6 mo were positively and significantly correlated with changes in serum cholesterol ($r = 0.323$, $P = 0.042$), with the correlation between changes in butyrate and changes in cholesterol being nearly significant ($r = 0.308$, $P = 0.056$).

DISCUSSION

These results suggest that long-term adaptation of blood lipid responses occurs when type 2 diabetic subjects alter their carbohydrate and dietary fiber intakes, and that this adaptation may be

TABLE 5
Fasting serum lipids of the subjects by diet group and time[1]

				P (ANOVA)		
Lipid and group	Baseline	3 mo	6 mo	Diet effect	Time effect	Diet × time interaction
Total cholesterol (mmol/L)						
LF	5.13 ± 0.20	5.21 ± 0.22	5.00 ± 0.25	<0.001	NS	NS
HF	4.96 ± 0.13	5.01 ± 0.12	5.06 ± 0.14			
MUFA	5.75 ± 0.19	5.81 ± 0.18	5.88 ± 0.21			
Triacylglycerol (mmol/L)						
LF	2.45 ± 0.37	2.42 ± 0.28	2.59 ± 0.55	0.006	NS	NS
HF	2.71 ± 0.32	2.78 ± 0.39	3.16 ± 0.49			
MUFA	2.13 ± 0.34	1.75 ± 0.17	1.95 ± 0.18			
HDL cholesterol (mmol/L)						
LF	1.10 ± 0.08^{a,x}	1.04 ± 0.08^{b,x}	1.03 ± 0.07^{b,x}	0.004	NS	0.003
HF	0.97 ± 0.04^{a,y}	0.96 ± 0.04^{a,y}	0.92 ± 0.03^{a,y}			
MUFA	1.17 ± 0.08^{a,z}	1.25 ± 0.09^{b,z}	1.24 ± 0.09^{b,z}			
Total:HDL						
LF	5.14 ± 0.41^{a,x}	5.58 ± 0.48^{a,x}	5.26 ± 0.45^{a,x}	NS	NS	0.018
HF	5.30 ± 0.22^{a,x}	5.38 ± 0.24^{a,x,y}	5.65 ± 0.25^{a,x}			
MUFA	5.27 ± 0.32^{a,x}	4.96 ± 0.27^{a,y}	5.10 ± 0.32^{a,x}			

[1] $\bar{x}$ ± SEM. LF group, received a low-fiber breakfast cereal; HF group, received a high-fiber breakfast cereal; MUFA group, received monounsaturated fatty acids. Values in the same row with different superscript letters (a and b) are significantly different, $P < 0.05$. Values in the same column with different superscript letters (x, y, and z) are significantly different, $P < 0.05$. Superscripts are not shown when there was no significant diet × time interaction.

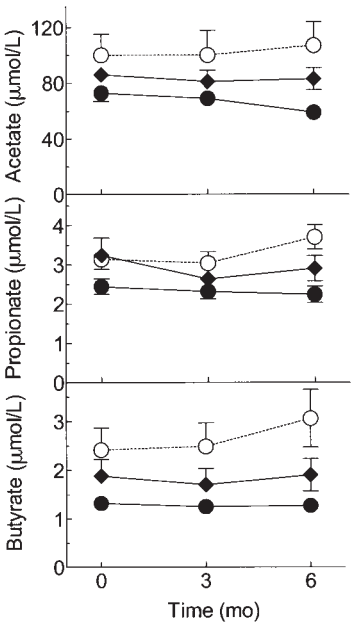

FIGURE 1. Mean (±SEM) fasting serum acetate, propionate, and butyrate concentrations of subjects with type 2 diabetes before and after 3 and 6 mo of a high-carbohydrate, high-fiber diet (HF; ○); a high-carbohydrate, low-fiber diet (LF; ●); or a low-carbohydrate, high–monounsaturated fatty acid diet (MUFA; ◆). Numbers of subjects at 0, 3, and 6 mo, respectively, were as follows: HF, 19, 21, and 18; LF, 18, 21, and 11; and MUFA, 17, 20, and 11. Time × diet interactions: acetate, $P = 0.08$; propionate, NS; butyrate, $P = 0.001$.

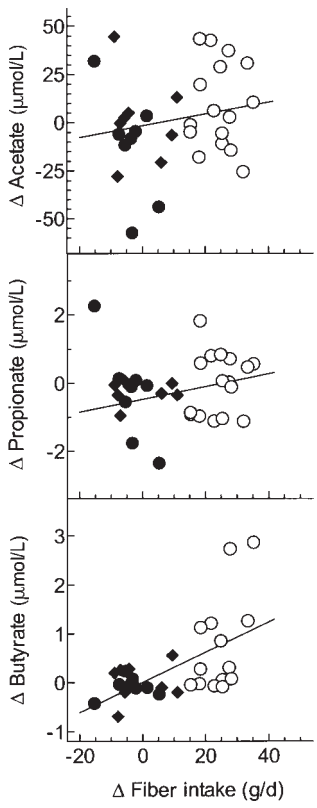

FIGURE 2. Relations between change (Δ) in dietary fiber intake and changes in serum acetate, propionate, and butyrate concentrations between 0 and 6 mo in subjects with type 2 diabetes consuming a high-carbohydrate, high-fiber diet (○); a high-carbohydrate, low-fiber diet (●); or a low-carbohydrate, high–monounsaturated fatty acid diet (◆). Regression lines are shown. Significance of correlations: acetate, NS ($n = 32$); propionate, NS ($n = 32$); butyrate, $r = 0.57$, $P < 0.001$ ($n = 31$).

due, at least in part, to a long-term adaptation in SCFA production from colonic fermentation of unabsorbed dietary carbohydrates. This is based on the fact that significant changes in blood SCFA concentrations took longer than 3 mo to occur and that changes in serum acetate were significantly related to changes in serum concentrations of cholesterol and triacylglycerol between 3 and 6 mo but not between 0 and 3 mo.

We must be cautious in attributing the changes in blood lipids we observed with time to true physiologic adaptation. Most studies suggest that fasting blood lipids respond maximally to dietary changes within 2–4 wk, with no change after that time for 3–4 mo (16, 17). In dietary studies lasting longer than 3–4 mo, blood lipids may change with time as a result of seasonal effects, loss of compliance (18, 19), or weight change (20). However, the classic studies of Antonis and Bersohn (21), in which the diets of prisoners were manipulated, suggest that long-term adaptation of blood lipids to changes in diet can occur. In that study, increasing dietary carbohydrate from 45% to 70% of energy increased serum triacylglycerols to a maximum in 4–6 wk, followed by a gradual return to baseline between 3 and 8 mo. Unfortunately, serum total and HDL cholesterol were not measured. In the present study, there were no significant changes in body weight, so this was not a confounding variable. Subjects reported no changes in intake of the treatment foods over the course of the study, nor was there any change in the macronutrient content of the diets calculated from diet records. Diet records are not sensitive measures of compliance. However, the persistent changes in HDL cholesterol and gradual changes in SCFA provide evidence that the differences in carbohydrate and fiber intakes were sustained over the 6-mo study period.

In the face of nonsignificant changes in fasting triacylglycerols, the importance of their association with changes serum acetate could be questioned. Statistical significance will not be achieved if there is high variability, too few subjects, or small effect sizes. The reduction in carbohydrate intake with the MUFA diet compared with the LF diet was only 10%. However, the mean reduction in triacylglycerol with the MUFA diet between 0 and 3 mo, 0.38 mmol/L (18%; Table 5), is of the same magnitude as the 0.36-mmol/L (19%) mean reduction in 10 other studies (1). All of these studies had larger changes in carbohydrate intake (13–25%) and most had fewer subjects (1). Similarly, between 3 and 6 mo

TABLE 6
Ratio of fasting serum acetate to propionate by diet group and time[1]

Group	Baseline	3 mo	6 mo
LF	31.5 ± 2.1^{x}	32.1 ± 1.7^{x}	27.8 ± 1.7^{x}
HF	30.5 ± 3.1^{x}	31.6 ± 3.0^{x}	32.4 ± 3.1^{y}
MUFA	30.5 ± 2.3^{x}	33.2 ± 2.8^{x}	$31.1 \pm 2.7^{x,y}$

[1] $\bar{x} \pm$ SEM. LF group, received a low-fiber breakfast cereal; HF group, received a high-fiber breakfast cereal; MUFA group, received monounsaturated fatty acids. Values in the same column with different superscript letters (x and y) are significantly different, $P < 0.05$. There were no significant differences within diets by time. There was an overall diet × time interaction, $P < 0.046$.

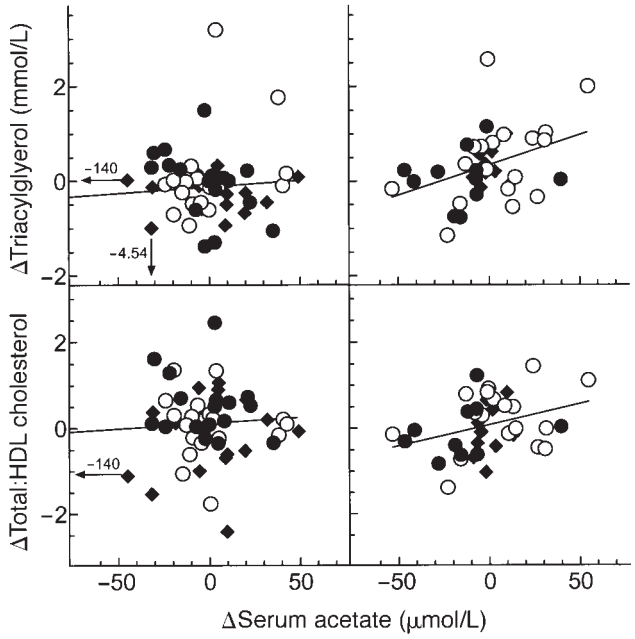

FIGURE 3. Relations between change (Δ) in serum acetate and changes in serum triacylglycerols and the ratio of total to HDL cholesterol between 0 and 3 mo (left) and between 3 and 6 mo (right) in subjects with type 2 diabetes consuming a high-carbohydrate, high-fiber diet (○); a high-carbohydrate, low-fiber diet (●); or a low-carbohydrate, high–monounsaturated fatty acid diet (◆). Regression lines are shown. The correlations for changes between 0 and 3 mo were not significant (n = 54). Significance of correlations for changes between 3 and 6 mo: triacylglycerol, r = 0.39, P = 0.013 (n = 40); total:HDL cholesterol, r = 0.33, P = 0.041 (n = 39).

of the HF diet, fasting serum triacylglycerol increased by a mean of 0.38 mmol/L. Thus, because both the n and the effect size are similar to those in the literature, high variation may explain the lack of significance of the changes in serum triacylglycerols. The correlation coefficient indicates that 15% of the variability in change in triacylglycerol between 3 and 6 mo was explained by changes in serum acetate. If colonic acetate does raise blood lipids, high variation in adaptive colonic acetate supply could increase the variation in long-term triacylglycerol responses compared with that seen in shorter studies.

SCFAs in blood can come from colonic fermentation or endogenous metabolism. Colonic fermentation is generally considered to be the major source of blood acetate (22, 23), but under conditions of increased fat oxidation, such as prolonged starvation (23) and diabetes (24), endogenous acetate production is increased. Fasting serum propionate and butyrate are increased in insulin-resistant subjects, possibly because of increased endogenous production (25). However, changes in endogenous production are unlikely to explain the differences in butyrate we observed because fasting glucose and insulin were not affected by the dietary treatments (4).

Changes in blood acetate reflect, at least qualitatively, changes in colonic acetate production. Short-term starvation reduces serum acetate (23), whereas infusion of acetate into the rectum causes a dose-dependent increase in blood acetate (26). In addition, the peaks of serum acetate after lunch and dinner (25) correspond to periods when the rate of flow of glucose and starch into the cecum, and hence the provision of fermentation substrates, is maximal (27). Although changes in fasting serum acetate tended to differ between dietary treatments, the effect was not statistically significant. There was high individual variation in acetate in response to changes in carbohydrate and fiber intakes, which may be explained by large differences in the rate of SCFA production and absorption by different individuals. Large differences in the rate of SCFA production have been seen when the same substrates were fermented in vitro by using fecal bacteria harvested from different subjects (28, 29). There is indirect evidence that SCFA absorption rates vary in different individuals, with methane producers absorbing colonic SCFA more rapidly than methane nonproducers (30).

It is generally assumed that peripheral blood butyrate concentrations do not reflect colonic butyrate production. Butyrate is the preferred fuel of colonocytes (31) and is selectively extracted by them, resulting in relatively low butyrate concentrations in portal blood (32). Studies in rats indicate that the liver extracts nearly 90% of the butyrate present in portal blood (33), resulting in even lower peripheral blood butyrate concentrations (32). Nevertheless, some colonic butyrate should reach peripheral blood, and the present results are consistent with this notion. In addition, we showed that acarbose, a drug that increases the delivery of starch to the colon, increases serum butyrate in glucose-intolerant subjects (34).

Our results are consistent with studies in experimental animals suggesting that it takes ≥6 mo for the colonic flora to adapt to an increased intake of resistant starch, with gradual increases in acetate and butyrate production occurring during this period (11). Consistent with this, we showed that in diabetic subjects treated with acarbose, it took 9 mo for the increase in fasting serum acetate to reach a plateau (35). The long time course of adaptation in SCFA production presumably reflects the complexity of interactions between the several hundred species of bacteria existing in the human colon (36).

Nearly 80% of the increase in fiber intake with the HF diet was from Bran Buds cereal (Table 1), which contains wheat bran and psyllium, fibers that are relatively poorly fermented (37, 38). Assuming that colonic fermentation increased, what, then, was the substrate being fermented? The increase in serum butyrate suggests an increased delivery of starch to the colon, because more butyrate is produced from in vitro fermentation of starch

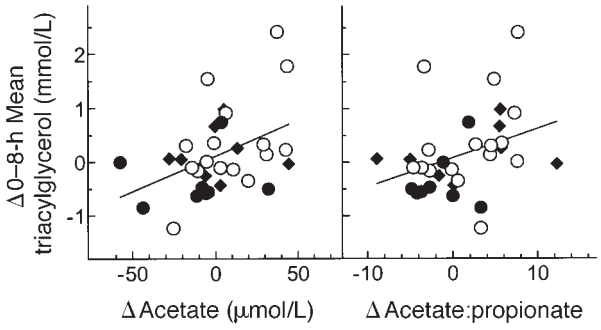

FIGURE 4. Relations between change (Δ) from baseline in serum postprandial triaclyglycerols and changes in serum acetate and the serum ratio of acetate to propionate between 0 and 6 mo in subjects with type 2 diabetes consuming a high-carbohydrate, high-fiber diet (○); a high-carbohydrate, low-fiber diet (●); or a low-carbohydrate, high–monounsaturated fatty acid diet (◆). Spearman rank correlation coefficients: acetate, r_s = 0.41, P < 0.05 (n = 32); acetate:propionate, r_s = 0.44, P < 0.02 (n = 32).

than of dietary fiber (39, 40). Wheat bran may increase the delivery of starch to the colon in 2 ways. It contains physically inaccessible starch that is incompletely digested in the small intestine. It may also reduce starch digestion and absorption by increasing the small-intestinal transit rate (41–43). Our results are consistent with studies showing that feeding wheat bran to human subjects raises fecal butyrate concentrations more than does feeding oat bran (44).

There is considerable evidence that increased availability of colonic acetate raises blood lipids. Infusion of acetate into the rectum acutely raises serum cholesterol and triacylglycerol concentrations (7, 26) and significantly more carbon atoms from [^{13}C]acetate appear in serum cholesterol and triacylglycerol after rectal than intravenous infusion of labeled acetate (6). These short-term studies are consistent with the results of the present study and are supported by data in experimental animals (45) and humans (8) that feeding lactulose, an unabsorbed sugar that produces primarily acetate when fermented in vitro (9, 10), raises fasting blood lipids. The reason for this is not entirely clear. Although acetate is the preferred substrate for hepatic fatty acid and cholesterol synthesis, it does not increase the overall rate of lipid synthesis (5). Nevertheless, the incorporation of acetate into cholesterol and fatty acids is inhibited by propionate (5), and the addition of propionate to rectal infusions of acetate reduces acetate incorporation into serum lipids, especially triacylglycerols, in humans (6). Thus, the ratio of acetate to propionate produced in the colon may be an important determinant of the effect of colonic SCFAs on blood lipids (14). The present results are consistent with this, in that the changes in serum triacylglycerol were significantly related to changes in the serum acetate-to-propionate ratio. We conclude that, in subjects with type 2 diabetes, changes in serum SCFAs in response to changes in carbohydrate and fiber intakes took many months to occur, and the changes in serum acetate were significantly related to the long-term adaptive changes in blood lipids.

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