Effect of Nibbling Versus Gorging on Cardiovascular Risk Factors: Serum Uric Acid and Blood Lipids

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Nibbling has been reported to decrease serum cholesterol under fasting conditions, as well as the incidence of cardiovascular disease. It has been suggested that these effects are partly attributable to reduced concentrations of serum insulin, which are also observed. However, data on the effects of nibbling on serum lipids throughout the day are not available, nor is it known how nibbling affects serum uric acid as a further insulin-related risk factor for cardiovascular disease. We have attempted to address these issues. Seven healthy men consumed identical diets in a randomized crossover design either as three meals daily (control) or as 17 meals daily (nibbling) for 2 weeks. On day 13, serum lipid levels were measured over the course of the day (12 hours) together with the 24-hour urinary excretion of mevalonic acid as an indicator of hepatic cholesterol synthesis. Concentrations of uric acid in serum and 24-hour urinary excretion of uric acid were also determined. Mean (±SE) percent treatment differences in day-long total, low-density lipoprotein (LDL), and non–high-density lipoprotein (HDL) cholesterol, and apolipoprotein (apo) B were significant, with lower values on the nibbling diet as compared with the control diet (8.1% ± 1.6%, $P = .002$; 12.2% ± 2.6%, $P = .005$; 10.1% ± 1.6%, $P < .001$; and 9.9% ± 2.6%, $P = .008$, respectively). No significant difference was seen in the total to HDL cholesterol ratio or in urinary mevalonic acid excretion. However, the percent difference between treatments in total cholesterol levels was directly related to the percent difference in urinary mevalonic acid excretion ($r = .94$, $P = .005$, n = 6). The mean fasting concentration of uric acid was significantly lower during the nibbling period as compared with the three-meal period (5.8% ± 1.8%, $P = .019$). Urinary uric acid excretion was also increased on nibbling (26.3% ± 7.9%, $P = .021$), and the treatment difference related to the reduction in serum insulin over the day ($r = -.83$, $P = .041$). We conclude that spreading the nutrient load over time reduces serum risk factors for cardiovascular disease. Our results suggest that these effects may be partly due to lower serum insulin concentrations. Lower insulin concentrations may lead to increased urinary uric acid excretion and possibly reduced hepatic cholesterol synthesis, since the treatment difference in urinary mevalonic acid excretion, although nonsignificant, related significantly to the treatment difference in serum cholesterol.

PREVIOUS STUDIES have demonstrated that increased meal frequency is associated with lower fasting serum cholesterol levels and is linked epidemiologically with reduced cardiovascular mortality.[1-7] However, the effect of food frequency on serum lipid and lipoprotein levels over the course of the day has not been assessed systematically. In addition, further evidence is required to support the proposed mechanisms responsible for the reduction of fasting lipids. These mechanisms include reduced hepatic cholesterol synthesis and a possible increase in bile acid pool size and fecal losses.

Recent evidence suggests that increased meal frequency results in a reduction in hepatic cholesterol synthesis in man.[8] A further physiologic accompaniment of increased meal frequency has been the reduction in day-long serum insulin levels, and this may provide part of the explanation for the decreased cholesterol synthesis.[7-9] In isolated hepatocytes, insulin has been shown to stimulate the activity of 3-hydroxy-3-methyl glutaryl coenzyme A (HMG CoA) reductase,[10] the rate-limiting enzyme in hepatic cholesterol synthesis. Reduced HMG CoA reductase activity is in turn reflected in lower rates of synthesis of mevalonic acid, the immediate product of this enzyme.[11] We have therefore measured the urinary excretion of mevalonic acid to determine the extent to which reduced hepatic cholesterol synthesis plays a role in the decreased fasting serum cholesterol levels observed.

Increased serum insulin concentrations have also been related to an increased risk of coronary heart disease (CHD)[12] and are associated with a number of other metabolic changes. These include higher serum uric acid concentrations, which result from reduced uric acid excretion.[13] At the same time, increased uric acid levels have been proposed as an additional independent risk factor for CHD.[14-18] It therefore seemed important to determine whether increased meal frequency, which results in decreased serum insulin levels, also leads to reduced concentrations of serum uric acid. These findings would further strengthen the case for spreading the nutrient load over the day through increased fiber consumption or changes in size and frequency of meals to reduce risk factors for cardiovascular disease.

The present study was originally designed to test the effect of food frequency on fasting serum lipids and insulin status. The fasting data have already been published in detail elsewhere.[7] The current report therefore focuses on

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day-long lipid changes in response to the two different feeding patterns and changes in fasting serum uric acid levels and urinary excretion of uric acid and mevalonic acid. These data have not been published previously.

SUBJECTS AND METHODS

The experimental details have been reported elsewhere.[7] Seven normal men with a mean age of 39.6 years (range, 31 to 51) and a mean weight that was 110% of ideal (range, 98 to 121)[19] were placed on two identical diets in which they ate the same food either as three meals (three-meal diet) or as 17 snacks given at hourly intervals throughout the day (nibbling diet). Each diet was of 2 weeks' duration. The study followed a randomized crossover design with three volunteers beginning with the three-meal diet and four with the nibbling diet for the first 2 weeks. The two 2-week periods were separated by a period of 20 ± 3 days (mean ± SE) during which the volunteers followed their usual diets.

The protocol for this study was approved by the human ethics committee of the University of Toronto, and informed consent was obtained from all volunteers.

Study Design

The studies were conducted on an outpatient basis. During the three-meal part of the study, daily food intake was distributed as 30% of the total caloric intake at breakfast (8 AM), 30% at lunch (1 PM), and 40% at dinner (7 PM). Lunch was eaten in the study kitchen, and breakfast and dinner were weighed and packed for consumption at home. During the nibbling part of the study, daily intake was divided into 17 packaged portions with approximately equal macronutrient and caloric content; at least one portion was eaten in the study kitchen. One of these "snacks" was eaten upon rising in the morning, and the other 16 were eaten at hourly intervals throughout the day. Pocket timers were provided to ensure punctuality. The diet consisted of common foods, except that meat and fish were replaced by dairy products. Further dietary details have been reported previously.[7] Caloric requirements were calculated according to the tables used in the Lipid Research Clinics Coronary Primary Prevention Trial, with allowances made for physical activity.[20] The calculated caloric intake required for the study group was 2,730 ± 189 kcal/d (mean ± SE). Diets meeting these requirements were planned using food-composition tables[21] and provided 33% fat (with a ratio of polyunsaturated to saturated fatty acids of 0.55), 15% protein, and 52% carbohydrate (with 15 g fiber/1,000 kcal). The same diet was followed each day. During the entire study, volunteers were asked to maintain the same level of physical activity, to record all foods and the times they were eaten in a food diary, and to return any uneaten food portions. Body weight was measured and blood samples were obtained after an overnight fast before breakfast on days 0, 7, 14, and 15 of each diet period.

Day Profile

On day 13 of each diet period, blood was obtained before breakfast and every 2 hours thereafter for 12 hours for determination of serum lipid, lipoprotein, and insulin concentrations. Samples for glucose analysis were collected hourly and also at 8:30 AM, 1:30 PM, and 7:30 PM (ie, 30 minutes after each of the three meals). The mean value for each 12-hour period was calculated from the values of all seven men.

Finally, two 24-hour urine samples were collected from six men on days 12 and 13, with the exception of one subject whose day-13 sample for one phase was no longer available, and a further sample collected on day 14 was used. Twenty-four-hour urine samples were collected separately as day (8 AM to midnight) and night (midnight to 8 AM) aliquots.

Analyses

Serum was obtained from fasting blood samples and stored at −20°C. Serum lipid levels were determined according to the Lipid Research Clinics protocol[22] in a laboratory certified as specified for standardization by the Centers for Disease Control–National Heart, Lung, and Blood Institute Lipid Standardization Program. Low-density lipoprotein (LDL) cholesterol level was derived using the formula reported by Friedewald et al.[23] Apolipoprotein (apo) AI and apo B levels were measured by a standard enzyme-linked immunosorbent assay technique[24,25] by using a modified coating buffer and timing individual steps to allow each assay to be completed in a single day. Glucose was assayed in capillary blood,[26] and insulin[27] level was measured in serum. The day and night urine samples, collected in containers without preservative, were assayed for urinary mevalonic acid.[28] Both serum and urine were assayed for uric acid[29] and creatinine.[30] Plasma clearance and filtration fractions were calculated using standard formulae[19]: clearance (C) = UV ÷ P, and filtration fraction = C (uric acid) ÷ C (creatinine) × 100, where U is urine concentration (mmol/L), V is urine volume (mL/min), and P is plasma concentration (mmol/L).

Mevalonic acid and insulin were analyzed in duplicate. Single samples were used in the other assays. All samples from each subject were analyzed in the same run. Where assays were performed on replicates, intraassay coefficients of variation were 10.3% for urinary mevalonic acid and 4.0% for insulin.

Values are expressed as the mean ± SE with adjustment for missing values.[31] The means of the two serum and urine values obtained at the end of each 2-week diet period and the mean values of measurements obtained for the day profile were used to calculate the significance of percent differences between the diet periods, [(three meal − nibbling) ÷ three meal] × 100, by Student's t test for paired data (two-tailed).[31] Simple correlation coefficients were calculated for the association between treatment differences in mean day-long insulin levels and the other variables measured. Differences in fasting and day-profile serum measurements across periods were assessed for the effect of treatment, period, and order by the General Linear Model (GLM) procedure.[32]

RESULTS

All subjects completed both parts of the study and adhered well to the timing of the meals. All food was eaten, and the same level of physical activity was maintained throughout the study periods. Mean body weight decreased slightly and by similar amounts during both metabolic periods (0.6 ± 0.2 kg on nibbling and 0.9 ± 0.2 kg on three meals), and the order in which the diets were followed had no effect on the results.

At the end of both treatments, serum lipids tended to be relatively constant over the course of the day (Fig 1). However, when expressed as the percent difference, mean levels of total, LDL, and non–high-density lipoprotein (HDL) cholesterol (from 8 AM to 8 PM) were significantly lower on nibbling as compared with three meals by 8.1% ± 1.6% ($P = .002$), 12.2% ± 2.6% ($P = .005$), and 10.1% ± 1.6% ($P < .001$), respectively. Mean apo B levels (8 AM to 8 PM) were also lower on nibbling as compared with three meals by 9.9% ± 2.6% ($P = .008$). No treatment difference was observed in HDL cholesterol, triglyceride, or apo AI, or in the ratios of total to HDL cholesterol, LDL:HDL cholesterol, or apo B:AI (Table 1). Where the percent differences were significant, absolute treatment differences

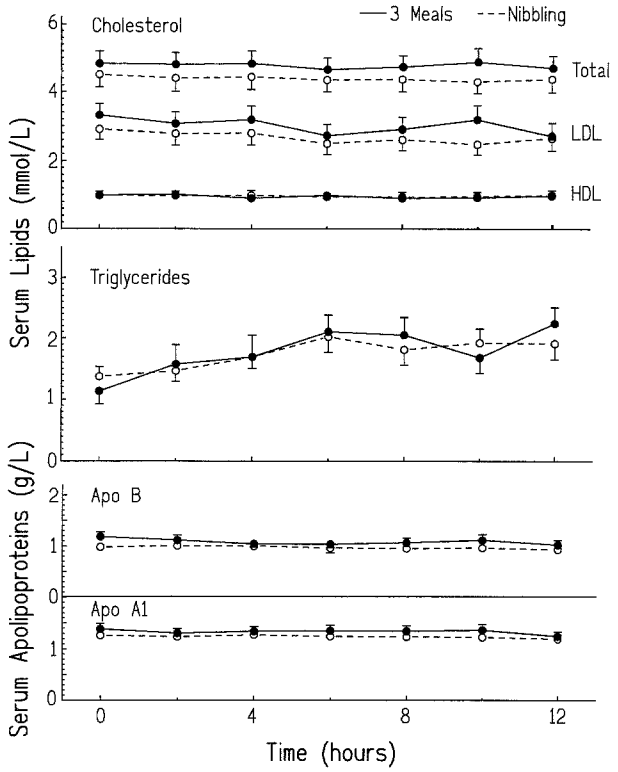

Fig 1. Mean ± SE serum lipids and lipoproteins over the course of day 13 in seven men. (●) Three meals; (○) nibbling.

in serum lipids were also significant using the GLM procedure.

Urinary mevalonic acid excretion was also measured to determine whether reduced hepatic cholesterol synthesis could explain the decreased serum cholesterol levels over the day. There was no significant treatment difference in 24-hour urinary excretion of mevalonic acid during the nibbling period as compared with the three-meal period (Table 2). However, the percent difference in total cholesterol was positively and significantly related to the percent difference between treatments in the 24-hour excretion of urinary mevalonic acid ($r = .94$, $P = .005$, n = 6; Fig 2).

Table 1. Mean Serum Lipid and Insulin Concentrations Measured Over 12 Hours (day profile) at the End of Each Metabolic Period (n = 7)

	Three Meals	Nibbling	Difference (%)	P*
Cholesterol (mmol/L)				
Total	4.77 ± 0.36	4.39 ± 0.37	−8.1 ± 1.6	.002
LDL	3.01 ± 0.36	2.63 ± 0.31	−12.2 ± 2.6	.003
HDL	0.95 ± 0.09	0.97 ± 0.13	0.5 ± 6.7	.947
Non-HDL	3.82 ± 0.30	3.43 ± 0.27	−10.1 ± 1.6	<.001
Triglyceride (mmol/L)	1.77 ± 0.26	1.74 ± 0.20	5.0 ± 12.2	.697
Total cholesterol to HDL cholesterol ratio	5.1 ± 0.4	4.8 ± 0.3	−6.6 ± 4.8	.213
LDL cholesterol to HDL cholesterol ratio	3.2 ± 0.2	2.8 ± 0.2	10.4 ± 5.5	.106
Apolipoprotein				
AI (g/L)	1.33 ± 0.10	1.23 ± 0.06	−6.0 ± 3.4	.125
B (g/L)	1.08 ± 0.09	0.97 ± 0.08	−9.9 ± 2.6	.009
Apo B:AI	0.82 ± 0.07	0.79 ± 0.05	−3.2 ± 4.8	.533
Glucose (mmol/L)	5.3 ± 0.1	5.1 ± 0.1	−3.8 ± 2.4	.088
Insulin (μmol/L)	153 ± 24	104 ± 10	−27.9 ± 6.3	.004

NOTE. To convert cholesterol, triglyceride, and creatinine to mg/dL, multiply by 38.7, 88.5, and 0.0113, respectively, and multiply by 0.1394 to convert insulin to μU/L.

*Significance of the percent difference was assessed by paired *t* test.

Table 2. Serum Uric Acid and Creatinine Concentrations at the End of Both Metabolic Periods (n = 7) Together With 24-Hour Urinary Excretion, Clearance, and Uric Acid Filtration Fraction (n = 6)

	Three Meals	Nibbling	Difference (%)	P*
Serum uric acid (μmol/L)	319 ± 17	300 ± 15	−5.8 ± 1.8	.019
Serum creatinine (μmol/L)	88 ± 4	89 ± 4	0.8 ± 2.6	.781
Urine volume (mL)	1,792 ± 35	2,204 ± 337	29.8 ± 16.4	.129
Uric acid excretion (mmol/24 h)	2,371 ± 269	2,955 ± 346	26.3 ± 7.9	.021
Creatinine excretion (mmol/24 h)	14.6 ± 1.3	14.6 ± 1.3	0.82 ± 3.8	.841
Mevalonic acid excretion (μmol/24 h)	1.49 ± 0.16	1.52 ± 0.14	3.2 ± 4.6	.525
Uric acid clearance (mL/min)	5.4 ± 0.8	7.1 ± 1.1	34.3 ± 9.1	.013
Uric acid filtration fraction (%)	4.6 ± 0.4	6.2 ± 0.8	34.8 ± 11.0	.025
Creatinine clearance (mL/min)	115 ± 10	116 ± 11	1.0 ± 5.6	.866

NOTE. To convert cholesterol, triglyceride, and creatinine to mg/dL, multiply by 38.7, 88.5, and 0.0113, respectively.

*Significance of the percent difference was assessed by paired *t* test.

Serum Uric Acid and Creatinine

The mean serum uric acid level at the end of the nibbling phase was significantly lower than the corresponding value

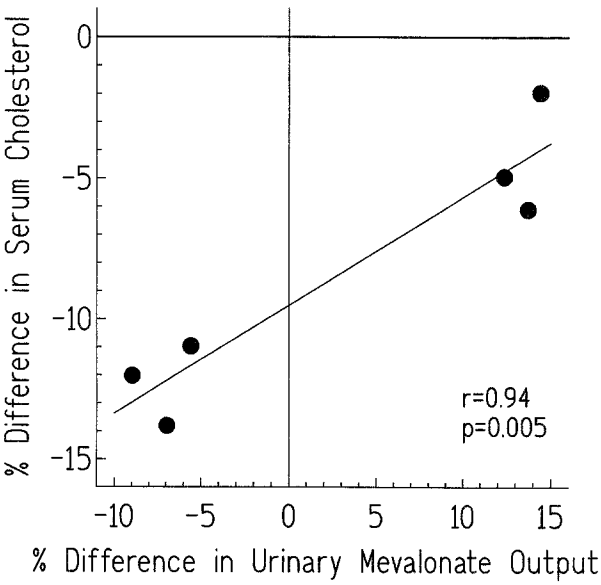

Fig 2. Association between the percent difference in serum total cholesterol for 6 men and the percent difference (three meals − nibbling) in urinary mevalonic acid output.

for the three-meal phase when expressed as the percent difference between treatments (5.8% ± 1.8%, n = 7, $P = .019$). However, assessment of the difference between absolute values failed to show significance at the 5% level using the GLM procedure ($P = .053$; Table 2). Mean serum creatinine levels at the end of both regimens were almost identical (Table 2).

Urine Volume, Uric Acid, and Creatinine Outputs

In assessing the percent difference between treatments, on nibbling there was a nonsignificant increase (29.8% ± 16.4%, n = 6, $P = .129$) in mean 24-hour urine volume for the last 2 days of the study in comparison to the corresponding three-meal value (Table 1). Urinary uric acid outputs were increased significantly by 26.3% ± 7.9% ($P = .021$). However, urinary creatinine outputs were identical on both the nibbling diet (14.6 ± 1.3 mmol/d) and three-meal diet (14.6 ± 1.3 mmol/d). In addition, renal clearance of uric acid was 34.3% ± 9.1% greater during the nibbling period as compared with the three-meal period (n = 6, $P = .013$). The filtration fraction for uric acid was also correspondingly increased during the nibbling period (34.8% ± 11.0%, $P = .025$, n = 6) (Table 2). Significance of the absolute treatment differences was confirmed using the GLM procedure for uric acid clearance ($P = .043$) and uric acid filtration fraction ($P = .031$). The absolute difference between treatments for uric acid output failed to reach significance ($P = .056$) (Table 2). No significant associations were found between percent differences in urine volume and percent differences in creatinine or uric acid output ($r = .24$, $P = .638$ and $r = .48$, $P = .361$, respectively).

Mean 12-Hour Blood Glucose and Serum Insulin Levels

No treatment difference was seen in mean 12-hour blood glucose levels. Day profiles showed that the mean 12-hour insulin level on nibbling was 27.9% ± 6.3% ($P = .004$) lower than the three-meal value (Table 1). These data have been reported in detail previously.[8] The only significant association between percent changes in serum insulin levels and treatment differences in the other serum and urinary measurements included a positive association with the apo B:AI ratio ($r = .88$, $P = .008$, n = 7) and a negative association with urinary uric acid excretion ($r = -.83$, $P = .041$, n = 6) (Table 3).

Table 3. Associations Between Percent Treatment Differences in 12-Hour Serum Insulin and Percent Treatment Differences in 12-Hour Serum Lipids, Fasting Serum Uric Acid, 24-Hour Urinary Uric Acid, and Mevalonic Acid Excretion

	% Treatment Difference in Serum Insulin	
% Treatment Difference in:	*r*	*P*
Total cholesterol	.35	.447
LDL cholesterol	.22	.631
Non-HDL cholesterol	.50	.250
HDL cholesterol	−.11	.816
Triglycerides	.57	.179
Apo B	.57	.177
Apo AI	−.72	.070
Total to HDL cholesterol ratio	.32	.487
LDL:HDL cholesterol	.27	.560
Apo B:AI	.88	.008
Serum uric acid	.13	.783
Uric acid excretion*	−.83	.041
Uric acid clearance*	−.75	.087
Mevalonic acid excretion*	.30	.559

*Data from 6 subjects only.

DISCUSSION

The present study indicates that serum uric acid levels are reduced when meal frequency is greatly increased. Previous studies demonstrated reduced concentrations of fasting serum total cholesterol when meal frequency was increased.[1-7] Our study extends these earlier reports on fasting serum lipids by demonstrating that mean postprandial concentrations of LDL and non-HDL cholesterol and apo B are also decreased throughout the day in response to increased meal frequency. Decreased serum lipids and uric acid would be expected to reduce the risk of CHD. The effects on CHD risk of reduction of LDL cholesterol, the LDL:HDL ratio, and apo B are well documented,[33-38] and there is a growing body of evidence to suggest that a high serum uric acid level is an independent risk factor for CHD.[14-18]

Part of the mechanism by which both of these risk factors are reduced in response to nibbling may relate to the decreased day-long insulin levels observed in this and other studies where meal frequency was increased.[7] Insulin has been shown to stimulate the activity of HMG CoA reductase, the rate-limiting enzyme in cholesterol synthesis, and to increase hepatic cholesterol production.[10,39] Recently, increased insulin levels during sepsis were associated with increased activity of HMG CoA reductase and hepatic cholesterogenesis.[40] In this way, decreased insulin levels that result from daily nibbling may reduce the stimulus for hepatic cholesterol synthesis and lead to reduced synthesis of apo B, enhanced receptor-mediated catabolism, and a resultant decrease in the serum concentration of total, LDL, and non-HDL cholesterol.[7] Recent 3-day studies of food frequency using stable-isotope incorporation reported reduced rates of cholesterol synthesis with increased food frequency.[8] This mechanism may also be relevant to the actions of dietary fiber, which reduces both serum lipids and postprandial insulin levels.[9]

HMG CoA reductase acts on HMG CoA to produce the water-soluble mevalonic acid.[11] If a reduced insulin stimulus to HMG CoA reductase was the reason for the decreased LDL cholesterol levels, then reduced production and ultimately excretion of mevalonate would be expected to occur.[41] However, there was no difference between treatments in the rates of urinary mevalonic acid excretion. One reason for the lack of significant effect may have been the lack of power to detect small changes in mevalonic acid associated with the relatively large variance in the percent treatment difference. With 43 individuals, we would have had an 80% chance of detecting a 5% treatment difference in mevalonic acid at the 5% significance level. With the six individuals we studied, we would have been able to detect

only a 17% difference. Nevertheless, a relationship was seen between the nibbling-induced reduction in cholesterol and the change in mevalonate excretion. This finding raises the possibility that factors that alter cholesterol synthesis such as insulin may still be responsible, at least in part, for the lipid-lowering effect of increased meal frequency.

In addition, increased bile acid cycling through the gut, expanded bile acid pool size, and increased fecal sterol losses[42-44] may be part of the explanation for the lipid reduction seen with increased food frequency. These factors have already been proposed as the reason for the lipid-lowering effect of fiber.[42-44] However, if this mechanism were operative, it would be predicted that, by analogy with bile acid sequestrant therapy, hepatic cholesterol synthesis and the 24-hour urinary excretion of mevalonic acid would increase.[41] Such an effect could, if present, partly or completely obscure any potential inhibitory effects on cholesterol synthesis resulting from lower insulin concentrations. In this way, as noted here, these opposite effects would tend to neutralize each other, and no significant treatment difference in the urinary excretion of mevalonic acid would be seen.

The Friedewald method used for calculating LDL cholesterol level was not intended to be applied to lipid values derived from plasma containing lighter triglyceride-rich particles, such as chylomicra, found postprandially. Our justification for presenting calculated LDL cholesterol values and adding non-HDL cholesterol values as a further surrogate for measured LDL cholesterol lies in the result of a previous study. That study examined the difference between soluble and insoluble fiber in 43 dyslipidemic subjects, but used a similar day-profile protocol to the one used here. The fasting lipid and bile acid data have already been reported together with preliminary day-profile data on 11 subjects.[45] This fiber study allowed us to compare measured with calculated LDL cholesterol and non-HDL cholesterol concentrations in fasting and postprandial plasma. The pooled soluble- and insoluble-fiber data on 36 subjects were used where triglyceride levels over the day were less than 4.5 mmol/L (400 mg/dL; 3.37 mmol/L was the maximum triglyceride level recorded in the present study). In the comparison of measured with calculated postprandial LDL concentrations at different time points over the day, the correlation was good (mean correlation coefficient, $r = .97$, slope = 0.98, and Y-intercept = −0.06). A similar relationship was seen for the comparison of measured LDL with non-HDL cholesterol ($r = .95$, slope = 0.89, and Y-intercept = −0.06). In view of the similarity between the day-profile protocol used here and that of the fiber study, we believed it was reasonable to report calculated LDL and non-HDL cholesterol values as surrogates for measured LDL cholesterol levels.

Increased insulin resistance has been shown to correlate positively with elevated serum uric acid.[13,46,47] Insulin has been suggested to increase serum uric acid levels through increasing renal reabsorption of uric acid.[13,46] One possible mechanism is that insulin acts to enhance sodium reabsorption[48] and that this could be accompanied by increased reabsorption of urate anion.[13,46,49,50]

An alternative mechanism is that decreased lactate or ketone body levels on nibbling lead to increased renal tubular uric acid secretion, which is considered the primary determinant of urinary uric acid output.[51-53] Increased insulin action increases blood lactate levels, and in the acute situation, intravenous lactate infusion reduces urinary uric acid output.[54] In obese patients placed on sucrose diets for 7 days, serum uric acid increased in association with increased serum lactate concentrations and reduced fractional clearance of uric acid.[55] Although the association between increased serum lactate and reduced renal clearance of urate is not seen in all situations,[55] it remains a possibility in a situation such as increased meal frequency where insulin levels are reduced. Decreased renal clearance of uric acid is also associated with ketonemia, although this association appears to be attenuated during fasting-induced ketonemia as the fast progresses.[56] In the present study, there was a tendency for serum ketone body concentrations to be decreased over the day when meal frequency was increased,[7] and this reduction in serum ketone bodies may also have contributed to the increased urinary urate losses.

To our knowledge, this is the first study to examine the effect that altering insulin levels in the same individual might have on uric acid excretion. A difference in mean insulin levels was achieved using a randomized crossover design with no change in the nature of the foods eaten and without administration of exogenous insulin. The results support the conclusion that reduced serum concentrations of insulin influence renal urate excretion and lead to an increased uric acid output,[13,46] although the exact mechanism has not been defined. Water intake was not regulated in our study, but it appears unlikely that the effect on uric acid was due to the increase in fluid output on the decreased-insulin limb (nibbling) of the study. There was no relation between the treatment difference in urine volume and uric acid excretion. Furthermore, most of the uric acid present in urine is thought to result from tubular secretion.[51-53] In addition, differences in completeness of urine collection did not explain the treatment difference seen in urinary uric acid output, since 24-hour urinary creatinine outputs were identical on both limbs of the study.

Increased uric acid levels also relate to other risk factors in addition to insulin[13,46,47] that are associated with CHD, including increased blood pressure, obesity, increased serum triglycerides, and low HDL cholesterol levels.[13,15-18,57-59] These risk factors, in turn, have been grouped together as part of the insulin-resistance syndrome.[60]

Weight loss occurred on both limbs of the study and might have been expected to reduce both serum lipids and 24-hour insulin secretion. However, the weight loss tended to be greater on the three-meal diet despite the higher fasting and postprandial LDL cholesterol concentrations and the higher mean postprandial insulin level.

With large numbers of comparisons, there is always an increased risk of false-positive results. This is relevant to the present study if we consider all 21 comparisons made in this report to have equal weighting. However, it is difficult to apply the appropriate adjustment to our data, since we

are dealing with paired comparisons of many different variables rather than many comparisons of the same variable. Furthermore, we had not considered that we were making comparisons between variables of equal importance. Our primary objective was to see if a state that reduced day-long insulin levels resulted in day-long reductions in LDL cholesterol and apo B and a reduced concentration of serum uric acid. Our comparisons of primary interest were therefore relatively few. Related to these measurements were associated measurements taken to provide ideas on mechanism (eg, mevalonic acid related to lipid metabolism and urinary measurements related to uric acid excretion). Therefore, we hope that this is not seen simply as looking for significance in any of the 21 variables assessed, but rather as examining two groups of data, each supporting a primary end point and including some comparisons that we would prefer not to see as significant (eg, body weight, serum HDL cholesterol, serum creatinine, and urinary creatinine output and clearance).

We conclude that a state characterized by decreased day-long insulin levels results in both decreased fasting serum uric acid levels and decreased postprandial cholesterol levels under both fasting conditions and over the course of the day. The current studies indicate that altered synthesis may be one of the mechanisms contributing to the observed cholesterol reduction seen during the nibbling phase of the study. The findings also suggest that reduced insulin action on the kidney may be responsible for the decreased serum uric acid levels seen during nibbling, and may be related to an increase in urinary uric acid excretion. Our results add further support to the early epidemiologic observations[6] that increased meal frequency may be associated with a reduced risk of CHD. Although the number of meals used makes direct application of the present study impractical, nibbling serves as an extreme model of spreading nutrient intake over the day for assessment of metabolic effects. This approach may have relevance to other similar approaches to decreasing the rate of nutrient absorption, including dietary fiber, low–glycemic-index foods, and enzyme inhibitors.

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