Metabolic Effects of Alterations in Meal Frequency in Type 2 Diabetes

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OBJECTIVE — The effects of altering meal frequency on measures of glucose and lipid metabolism in type 2 diabetes were examined by comparing isocaloric dietary regimens in which daily food intake was provided by three or nine meals each day.

RESEARCH DESIGN AND METHODS — A total of 13 free-living men and women with type 2 diabetes or persistently impaired glucose tolerance participated in a randomized crossover study in which three- and nine-meal regimes were followed for 4-week periods. Fasting plasma lipid and lipoprotein, glucose and insulin concentrations were measured at weekly intervals and glucose, insulin, and triglyceride responses following a 75-g glucose load at weeks 2 and 4 of each diet period. Dietary intake was also recorded during these weeks.

RESULTS — Nutrient intakes and all measures of carbohydrate and lipid metabolism were similar on the three- and nine-meal regimes.

CONCLUSIONS — This longer-term study could not confirm the potential benefits of increased meal frequency suggested by comparable 4-week studies in type 2 diabetic individuals and acute experiments in individuals with diabetes. However, as there were no adverse effects of consuming nine meals per day, it would seem appropriate that meal frequency in those with type 2 diabetes should be left to personal choice, provided that energy balance is maintained.

Several studies in people who do not have diabetes suggest that those with the condition, especially people with type 2 diabetes, might benefit from increased meal frequency. Healthy normolipidemic individuals have been shown to have a more favorable glucose response to an oral carbohydrate load, reduced levels of insulin, and reduced levels of total and LDL cholesterol and apolipoprotein B when meal frequency is increased without an associated increase in total energy over periods of several weeks (1,2).

Two studies have contrasted the effects of two or three large meals with six to 13 small meals per day in type 2 diabetic subjects each studied on two separate days (3,4). Both studies showed reductions in insulin and glucose levels on the days with increased meal frequency. One of the studies also showed reduction in urinary C peptide excretion (3), and the other showed lower average free fatty acids during the day on which more frequent meals were consumed (4). The authors of these studies have concluded that increased partitioning of energy intake may be beneficial in patients with type 2 diabetes. However, thus far, there have been no published studies that have examined the longer-term effects of increasing meal frequency on glycemic control and cardiovascular risk indicators in people with diabetes.

We report the findings of a study in which the effects of three and nine meals per day have been compared in 13 subjects with type 2 diabetes or persistently impaired glucose tolerance. We chose to study the effect of nine meals rather than the greater number studied in other investigations of the effects of nibbling (2,3), since we found this level of meal frequency to be associated with several metabolic differences when contrasted with a gorging meal pattern (3 meals per day) in nondiabetic individuals (1) and since there is little likelihood of achieving long-term compliance with a regimen involving more than nine meals per day.

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RESEARCH DESIGN AND METHODS

Subjects

A total of 13 (4 men and 9 women) of 17 subjects initially recruited to the study completed the 8-week protocol; 11 subjects fulfilled the criteria for the diagnosis of diabetes, and 2 subjects had persistently impaired glucose tolerance (i.e., impaired response to a glucose load on at least two occasions). Their ages ranged from 46 to 70 years, and their mean (± SD) BMI was 29.9 ± 4.2 kg/m^2. Ten subjects were treated on diet alone, and three subjects were on diet plus oral hypoglycemic agents. All subjects gave written informed consent to the study, which had received ethical approval from the Ethics Committee of the Otago Area Health Board.

Experimental design

The participants followed isoenergetic regimens of three and nine meals per day in random order for 28 days each (8 subjects beginning with the three-meal-per-day diet and 5 with the nine-meal-per-day diet). Each subject was allotted a daily energy allowance or target based on the analysis of an initial prestudy 4-day dietary record. During the three-meal-per-day period, this energy allowance was divided during the day as follows: breakfast, 25%; lunch, 25%; dinner, ~50%; and a single small snack of ~150 kcal (or two small snacks of 75 kcal each) to be consumed when desired. During the nibbling period, each of the three meals from the meal eating regimen was divided into three smaller meals distributing the daily energy allowance in the following manner: early morning, 8.3%; breakfast, 8.3%; mid-morning, 8.3%; lunch, 8.3%; mid-afternoon, 8.3%; late afternoon, 8.3%; dinner, 16.6%; mid-evening, 16.6%; and late evening, 16.6%. The nine meals were spaced 1–2 h apart depending on the subject's schedule. The subjects remained free-living throughout the study, and the diets were entirely self-selected. Small amounts

of alcohol were allowed as the subject desired and were generally consumed in the evening. To aid with the adjustment to a different meal frequency, individual instruction was given to the subjects before commencing each diet. A personalized 7-day menu example was prepared for both the three- and nine-meal diets, and each subject was given a comprehensive list of foods with the portion sizes equivalent to 75 kcal. In addition to the detailed prestudy instructions, regular contact (at least weekly) between researchers and participants helped to maintain compliance.

Each subject completed a 3-day semiquantitative diet record (5) at the end of the 2nd and 4th weeks on each diet. These records were later analyzed for the mean intake of energy, fat, carbohydrate, protein, total sugars (lactose + glucose + sucrose + fructose), alcohol, and fiber on the three- and nine-meal diets. Nutrients were calculated using a computerized database. (Foodfiles, version 1, a computerized version of the New Zealand Food Composition Tables [6], was developed by Crop and Food in Palmerston North, New Zealand and released in 1993.) The database was accessed using Diet Cruncher, version 1, developed by Ross Marshall (Department of Human Nutrition, University of Otago) in 1991. Ingredients of recipes not listed in the database were obtained from the participants and entered as individual food items. There were no missing data for macronutrients, and no modifications were made to the database. Subject adherence to the appropriate meal frequency was assessed from self-reported records of the number of meals and snacks consumed each day.

Clinical and metabolic measurements

Subjects were weighed on an electronic balance before the study and after 2, 3, and 4 weeks on each diet. Seated blood pressure was measured before the study and at the end of the 3rd week of each dietary period, using a random zero sphygmomanometer. Fasting venous blood specimens were collected into EDTA vacutainers 1 and 4 days before the study and at the end of the 2nd, 3rd, and 4th weeks of each diet for subsequent lipid analysis. At the end of the 2nd and 4th weeks of each dietary regimen, glucose, insulin, and triglyceride concentrations were measured 5 min before (2 samples, 5 min apart) and after (0.5, 1.0, and 2.0 h) a 75-g oral glucose load. During the 4th week of each diet, a 24-h urine sample was collected for the measurement of creatinine and C-peptide. Glycated hemoglobin (HbA_1) was measured at the end of each diet period.

Laboratory analyses

Blood samples for lipid and glucose measurements were centrifuged after collection, and the plasma was stored at −25°C. Specimens for apolipoprotein and insulin analysis were left to clot for at least half an hour after collection before being centrifuged at 1,110*g*. The resulting sera, together with urine samples for the measurement of C-peptide and creatinine, were stored at −80°C. To eliminate day-to-day laboratory variance, all blood samples were analyzed in a single batch after completion of the study.

Plasma total cholesterol, HDL cholesterol, triglycerides, and glucose concentrations were measured enzymatically using kits and standards supplied by Boehringer Mannheim (Mannheim, Germany). HDL cholesterol was measured after precipitation of apo B–containing lipoproteins with heparin and manganese chloride (7). LDL cholesterol was calculated using the Friedewald formula, modified for molar concentrations (8). The laboratory participated in the quality control programs of the New Zealand Lipid Analysis Proficiency Programme and the Royal Australasian College of Pathologists, and the coefficient of variation for the cholesterol and triglyceride measurement during the experimental period was <1%.

Plasma glucose was measured using an enzymatic colorimetric method (Boehringer Mannheim) on blood collected into vacutainers containing a fluoride/oxalate anticoagulant. Serum apolipoprotein A-I and B were measured by immunoassay (kit and standards supplied by Boehringer Mannheim). All lipid, apolipoprotein, and glucose analyses were performed on a Cobas Fara II autoanalyzer (Roche Diagnostics, Basle, Switzerland). Serum insulin was measured by a solid-phase ^{125}I-radioimmunoassay using coated tube technology (Coat-A-Count, Diagnostic Products, Los Angeles, CA). Urinary C-peptide was measured by a double antibody competitive ^{125}I-radioimmunoassay, using polyethylene glycol to assist separation (Diagnostic Products). Urinary creatinine was measured colorimetrically using the Jaffé reaction. HbA_1 (normal range, 4–6%) was measured by affinity chromatography, using a hemolysate of blood anticoagulated with EDTA (kit supplied by Pierce, Rockford, IL).

Statistical analyses

The areas under the glucose, insulin, triglyceride, and insulin/glucose curves were calculated using the trapezoidal rule, excluding any area below the mean baseline measurement.

Body weight, blood pressure, and the lipid, lipoprotein, and apolipoprotein values obtained during each dietary regime were tested for the effect of treatment (three and nine meals per day), time (week of measurement), and the order of treatment with a repeated measures analysis of variance design, using the multiple analysis of variance procedure in SPSS (SPSS, Chicago, IL). A polynomial contrast was fitted on the time effect to give more information about the change over time. Where it was indicated that the results were not normally distributed, the data was subjected to log transformation before analysis. In a second analysis, the design was modified to include the baseline as a covariate.

The triglyceride, glucose, insulin, and insulin-to-glucose curves obtained during the glucose tolerance test were tested for the effect of treatment, time, and order, using the multiple analysis of variance procedure as described above, the time component referring to the number of minutes from consumption of the glucose. The areas under the triglyceride, insulin, and insulin-to-glucose curves were compared using the multiple analysis of variance procedure. Student's paired *t* test (two-tailed) was used to compare the reported dietary intake, the 24-h urinary C-peptide, and creatinine and HbA_1 values obtained on the three- and nine-meal-per-day regimens.

RESULTS — Reported dietary intake on the three- and nine-meal regimes is shown in Table 1. Because the data were similar at weeks 2 and 4 of each experimental period, the results presented are the mean values for the three- and nine-meal experimental diets. Fat intake was significantly lower on both experimental diets than at baseline, but the distribution of nutrients did not show any significant differences on the two experimental diets. Participants reported eating 3.2 ± 0.3 and 8.2 ± 0.5 meals, respectively, on the two diets. Body weight showed similar small, but statistically significant ($P = 0.002$), decreases on both experimental diets, compared with baseline (79.03 ± 8.16 kg to 78.91 ± 7.91 kg on three meals and 79.43 ± 8.27 to 79.00 ± 8.47 kg on nine meals). Fasting levels of lipids, lipoproteins, and glycated hemoglo-

Table 1—*Distribution of nutrient intake prior to the study and while consuming three and nine meals daily*

	Baseline	Three meals/day	Nine meals/day
Source of total energy (%)			
Fat	35.6 ± 7.2	32.5 ± 5.4*	32.0 ± 5.6*
Carbohydrate	46.1 ± 6.2	48.3 ± 5.6	51.3 ± 6.2
Sugars (lactose, glucose, fructose, and sucrose)	18.2 ± 2.0	17.6 ± 5.3	17.9 ± 6.2
Protein	17.5 ± 3.6	18.9 ± 3.6	16.4 ± 2.5
Alcohol	0.9 ± 2.5	0.4 ± 1.4	0.2 ± 0.7
Fiber (g/1,000 kcal)	14.4 ± 4.7	15.6 ± 3.2	14.6 ± 3.7

Data are means ± SD. *Significantly different ($P < 0.05$) from baseline.

bin and urinary C-peptide and creatinine are shown in Table 2.

Repeated measures analysis of variance indicated that there were no significant differences according to the number of weeks on each diet. Therefore, the data for each of the variables presented are based on the mean of the three measurements made at the end of the 2nd, 3rd, or 4th week on each of the three- and nine-meal regimes. There were also no differences among those treated with oral hypoglycemic agents and those on diet alone or when the data were examined excluding those with persistent impaired glucose tolerance. Glucose and insulin levels, insulin-to-glucose ratios, and triglyceride levels after a glucose load at weeks 2 and 4 of each regime were very similar on the two diets, with no statistically significant differences being observed at any of the sampling times. Glucose and insulin data are shown in Fig. 1.

CONCLUSIONS — Several sets of national dietary recommendations for people with diabetes have offered some comment regarding meal frequency. The Canadian recommendations suggested that a few large meals each day might be the most appropriate eating pattern for people with diabetes (9). British recommendations on the other hand advised increased meal frequency (10). More recent recommendations have not offered specific advice regarding optimal meal frequency (11,12). However, a series of fairly recent observations have led to the suggestion that more attention should be paid to the potential benefits of increased meal frequency. In various experiments carried out during a single day in people with diabetes, a range of measures of carbohydrate and lipid metabolism appears to be more favorable when meal frequency is increased (3,4). A number of studies of normolipidemic nondiabetic individuals carried out over a period of several weeks have demonstrated lower total and LDL cholesterol levels when the number of meals taken each day has been substantially increased (1,2). One study has shown a 25% reduction in daytime insulin levels on 17, as compared with 3, meals per day (2), a change which could explain a reduction in cholesterol synthesis (13) and potentially lead to long-term cardiovascular risk reduction in people with diabetes.

However, two longer-term studies could not confirm such metabolic benefits in normal weight hyperlipidemic individuals (14,15). In this first study in people with diabetes to be carried out over a period of weeks rather than days, we have not found any changes in fasting lipid, insulin, or glucose levels or in glucose, insulin, or triglyceride responses to a glucose load. It could be argued that the duration of the experimental diets (28 days) was too short to demonstrate potentially meaningful changes in glycated hemoglobin. However,

Table 2—*Blood and urine measurement at baseline and during three- and nine-meal experimental periods*

	Baseline	Three meals/day	Nine meals/day
Fasting blood measurements			
Total cholesterol (mmol/l)	5.68 ± 1.22	5.88 ± 1.14	5.88 ± 1.16
LDL cholesterol (mmol/l)	3.65 ± 0.92	3.98 ± 0.94	4.03 ± 0.94
HDL cholesterol (mmol/l)	1.10 ± 0.29	1.09 ± 0.13	1.05 ± 0.13
Triglyceride (mmol/l)	2.15 ± 1.02	1.86 ± 0.72	1.85 ± 0.77
Apolipoprotein B (mg/dl)	77.5 ± 25.1	86.5 ± 20.6	90.5 ± 19.2
HbA_1 (%)	7.86 ± 2.12	7.56 ± 2.19	8.08 ± 1.96
Glucose (mmol/l)	7.31 ± 1.69	7.48 ± 1.78	7.58 ± 1.75
Insulin (μU/ml)	21.36 ± 14.93	21.53 ± 12.11	21.01 ± 9.76
24-h urine measurements			
C-peptide (μg)	90.46 ± 34.72	90.09 ± 30.99	90.73 ± 42.46
Creatinine (g)	1.06 ± 0.48	1.06 ± 0.54	1.07 ± 0.36

Data are means ± SD.

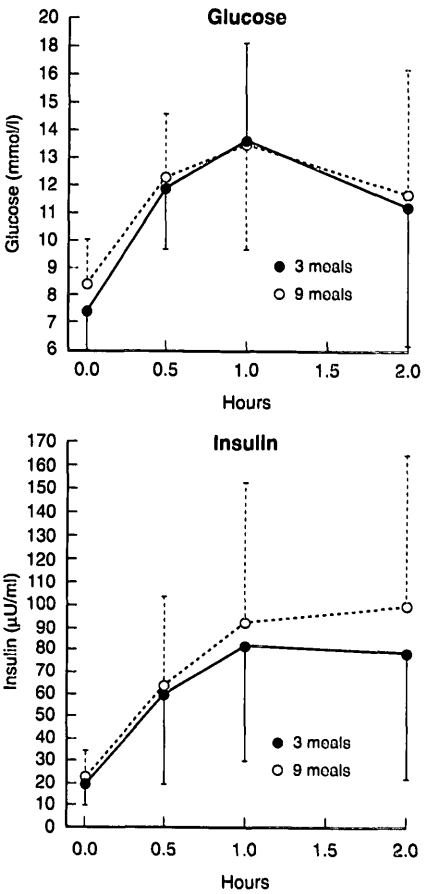

Figure 1—*Glucose and insulin responses to glucose load on three- and nine-meal regimes. Data given represent the means of the tests done at weeks 2 and 4 of each regimen.*

the virtually identical levels of fasting glucose and insulin and responses to the glucose load suggest that such change would be unlikely. The inclusion of two subjects with persistent impaired glucose tolerance in addition to those with type 2 diabetes could confound the results, but the findings were virtually identical when examining the data after excluding these individuals. Many of the studies have involved comparisons of three or fewer meals per day, with nibbling patterns involving as many as 17 snacks each day. It is conceivable that nine meals per day is insufficient to demonstrate a difference between frequent and infrequent meal patterns. To counter this potential criticism we would argue that we have been able to demonstrate differences in fasting lipid levels in normolipidemic nondiabetic individuals when contrasting three and nine meals per day (1) and further that increasing to beyond nine meals per day would reduce the practical application of the study. It is our experience that few people are prepared to eat more than 9 meals each day. It is conceivable that our subjects who were all free-living may not have complied with the dietary instructions despite reporting a high level of compliance. This is unlikely since they were all highly motivated volunteers in very regular contact with the research group and certainly much more likely to comply with such an eating pattern than would be the case in routine clinical practice.

Thus, we believe that we have demonstrated that little benefit is likely to accrue in a practical setting by suggesting that people with diabetes increase their meal frequency. It is conceivable that small, but clinically meaningful, benefits may have been missed because of insufficient power in this study to detect small changes. This issue can only be addressed by the further study of larger numbers of subjects. For the present, it seems appropriate to suggest that meal frequency should largely be a matter of personal choice, though people with type 2 diabetes should be encouraged to use self–blood glucose monitoring to assess whether meal frequency impacts their personal glycemic control. Our data do not suggest any adverse effects of nine meals per day, though for some, increased meal frequency may carry the risk of increased energy intake.

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