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ORIGINAL COMMUNICATION

Regular meal frequency creates more appropriate insulin sensitivity and lipid profiles compared with irregular meal frequency in healthy lean women

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Objective: To investigate the impact of irregular meal frequency on circulating lipids, insulin, glucose and uric acid concentrations which are known cardiovascular risk factors.
Design: A randomised crossover dietary intervention study.
Setting: Nottingham, UK—Healthy free-living women.
Subjects: A total of nine lean healthy women aged 18–42 y recruited via advertisement.
Intervention: A randomised crossover trial with two phases of 14 days each. In Phase 1, subjects consumed their normal diet on either 6 occasions per day (regular) or by following a variable meal frequency (3–9 meals/day, irregular). In Phase 2, subjects followed the alternative meal pattern to that followed in Phase 1, after a 2-week (wash-out) period. Subjects were asked to come to the laboratory after an overnight fast at the start and end of each phase. Blood samples were taken for measurement of circulating glucose, lipids, insulin and uric acid concentrations before and for 3 h after consumption of a high-carbohydrate test meal.
Results: Fasting glucose and insulin values were not affected by meal frequency, but peak insulin and AUC of insulin responses to the test meal were higher after the irregular compared to the regular eating patterns ($P<0.01$). The irregular meal frequency was associated with higher fasting total ($P<0.01$) and LDL ($P<0.05$) cholesterol.
Conclusion: The irregular meal frequency appears to produce a degree of insulin resistance and higher fasting lipid profiles, which may indicate a deleterious effect on these cardiovascular risk factors.
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Keywords: irregular meal pattern; regular meal frequency; insulin; lipids; cardiovascular risk

Introduction

Altered circulating total cholesterol, LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), triacylglycerol (Solberg & Strong, 1983; Grundy, 1998; Grundy *et al*, 1998), insulin (Despres *et al*, 1996) and uric acid (Waring *et al*, 2000) concentrations are recognised as risk factors for cardiovascular diseases. Meal pattern influences these parameters, with an increased meal frequency being associated with lower fasting total serum cholesterol and low-density lipoprotein levels (Gwinup *et al*, 1963; Jenkins *et al*, 1989; Arnold *et al*, 1993). A few epidemiological studies showed better glucose tolerance and lower fasting blood glucose level in healthy people consuming a higher number of meals per day (Fabry *et al*, 1964; Edelstein *et al*, 1992). Intervention studies, however, failed to find consistent responses of glucose and insulin to meal frequency variations (Fábry *et al*, 1966; Jenkins *et al*, 1989; Arnold *et al*, 1993), although a recent study (Jenkins *et al*, 1995) reported an inverse relationship between meal frequency and serum uric acid concentration.

It seems that Western populations are increasingly moving away from regular meals, because of wider food availability and eating more meals outside the home. Food technology development has increased the availability of ready prepared

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meals that can be stored and rapidly regenerated. Recent studies (Hoglund *et al*, 1998; Samuelson, 2000) report that the prevalence of an irregular meal pattern has increased in adolescents compared with previous decades. A recent article (Murata, 2000) also claims that Japanese adolescents' serum cholesterol concentration has increased during the last decades due to nation-wide life-style changes such as irregular food intake. To our knowledge, no studies have evaluated the association between irregular meal pattern and lipid–carbohydrate metabolism in adulthood. It is hypothesised that an irregular meal pattern may have a deleterious effect on lipid and carbohydrate metabolism. The purpose of this short-term intervention study was to investigate the impact of irregular meal frequency (ie chaotic eating) on lipid and carbohydrate metabolism and serum uric acid concentration in lean healthy women.

Methods

Subjects

Nine healthy, lean women aged 18–42 y (mean 23.7, s.d. 7.4 y) with regular menstruation or on the oral contraceptive pills, neither pregnant nor lactating and with no self-reported history of hypercholesterolaemia, hyperglycaemia or any serious medical conditions were recruited from the students and staff of Queen's Medical Centre, Nottingham. Subjects were excluded if they reported that they were dieting (a score of more than 10 on The Eating Inventory (Garner & Garfinkel, 1979)), or experiencing depression (score of more than 30 on the Beck Depression Inventory (BDI) (Beck, 1969)). The mean body mass index (BMI) was 22.4 kg/m^2 (s.d. 2.4 kg/m^2). Ethical permission for the study was obtained from The University of Nottingham Medical School Research Ethics Committee.

Design

The randomised crossover trial consisted of three phases over a total of 43 days for each subject. Subjects attended four laboratory visits in addition to the screening session over the study period. In Phase 1 (14 days), subjects were asked to eat and drink similar things to their normal diet, but to either consume them on six occasions per day (regular meal pattern) with regular intervals between meals or follow a chaotic meal pattern (irregular meal pattern). To achieve the irregular meal pattern, subjects were asked to have a predetermined meal frequency with between 3–9 meals/day for 14 days, in which each number of meals per day was repeated twice (average of 6 meals/day). Subjects were asked to have their usual diet on 7, 4, 9, 3, 5, 8, 6, 5, 9, 8, 3, 4, 7 and 6 occasions per day, during the 14 days of the irregular meal pattern period, respectively. After Phase 1, subjects continued their normal diet and eating pattern for 14 days as a 'wash- out' period. In Phase 2 (14 days), subjects were finally asked to follow the alternative meal pattern to that which they followed in Phase 1. A 'meal' was defined as providing more than 0 kcal, with an interval between two eating occasions of more than 1 h. The participants remained free-living during the study and the diet content was self-selected. Each volunteer came to the laboratory on the first and the last days of Phase 1 and 2, to give a total of four visits. Each visit lasted up to 4 h.

The participants were given training in recording their intake diary on the basis of a semiquantitative record before the start of the study. An instruction booklet with a day menu example was also given to each subject before each phase. The subjects were asked to record their food intake for 3 days (1 weekend and 2 weekdays) before the start of Phase 1. Throughout the regular phase, 6 meals were eaten per day and the subjects were asked to provide a food record for 3 of the 14 days in order to measure their adherence to this diet. The subjects were also asked to record their dietary intake on 3 separate days during the irregular meal frequency period when they were eating 9, 6 and 3 meals/day. Food diary records for the subjects' habitual, regular and irregular meal patterns were analysed using the 'Microdiet' Package (Downlee Systems Limited, 2000, Edition 1.2).

Laboratory visits

Subjects were asked to fast overnight for at least 10 h and take no exercise other than walking required for the activities of daily living for 48 h prior to each visit. On arrival at the laboratory, the hand was warmed in a heated ventilated perspex box (50–55°C) for 15–20 min to open the arteriovenous anastomoses (McGuire *et al*, 1976). A 20-gauge cannula was inserted into a dorsal hand vein retrogradely, with a slow running infusion of Saline (154 mmol sodium chloride/l) to keep the cannula patent. Blood samples were withdrawn via a three-way tap with the first 2-ml being discarded to avoid contamination with saline. Two baseline blood samples were tested for fasting blood glucose, serum insulin, cholesterol (HDL, LDL), triacylglycerol and uric acid. Then, a milk shake test meal containing by percentage of total energy 50% carbohydrate, 35% fat and 15% protein was provided. Subjects were given a volume of test meal on the basis of their weight. A measure of 10 kcal (41.8 kJ) was given per kg body weight. Subjects were asked to consume the drink over 10 min. After the test meal, blood samples were taken every 15 min for 3 h. These blood samples were analysed for blood glucose, plasma insulin and serum triacylglycerol.

Analysis

Blood glucose was measured immediately using a B-Glucose Analyser (Hemocue AB, Angelholm, Sweden). Blood samples for insulin were left to clot for at least 30 min after the collection before being centrifuged for 10 min at 3000 r.p.m. The serum samples were then sealed and stored at −80°C for future analysis. Blood samples for cholesterol and triacylglycerol were taken into lithium heparin tubes and for uric acid

into potassium EDTA tubes. The lipids and uric acid blood samples were then kept in an icebox until the end of each visit, before being centrifuged for 10 min at 3000 r.p.m. The plasma was transferred into a fresh tube with 25 μl EGTA for lipids and uric acid. The tube was then sealed and stored at −80°C for later analysis.

Insulin measurements were performed by a solid-phase ^{125}I radioimmunoassay (RIA) method using coated-tube technology (Count-A- Count TK INX, DPC, USA). The intra-assay coefficients of variation was 3.8 and 4.1% for blood glucose and insulin, respectively.

The Clinical Chemistry Laboratory of Queen's Medical Centre measured plasma lipids and uric acid concentrations. Plasma total cholesterol and triacylglycerol were measured enzymatically by using kits and standards supplied by VITROS, Ortho-Clinical Diagnostics (Rochester, USA). High-density lipoprotein cholesterol (HDL-C) was measured after precipitation of apo B-containing lipoproteins with heparin and manganese chloride (Burstein *et al*, 1970) using EZ HDL™ Cholesterol Kit (SIGMA DIAGNOSTICS, USA). LDL-C was calculated by using the Friedewald formula (Friedewald *et al*, 1972).

Plasma uric acid concentration was measured enzymatically (Kageyama, 1971; Trivedi *et al*, 1978) by using kits supplied VITROS, Ortho-Clinical Diagnostics (Rochester, USA). The intra-assay coefficients of variation were 2.5, 2.2, 1.8 and 1.4 % for total cholesterol, HDL-C, triacylglycerol and urea, respectively.

Homeostasis model assessment (HOMA) was used to assess insulin resistance when the subjects were in the fasting state. The HOMA values for the subjects were calculated by using the following formula: fasting serum insulin (μIU/ml) × fasting blood glucose (mmol/l)/22.5 (Matthews *et al*, 1985).

Statistical analysis

The statistical package SPSS version 10 (SPSS, Chicago, USA) was used for data entry and analysis. All data are reported as means ± their standard deviation (s.d.). Data were tested for normality (Kolmogorov–Smirnov statistic with Lillefors correction). Comparisons of the fasting results between the pre- and post-intervention periods were performed using Student's paired *t*-test (two-tailed). Statistical analysis of the results was also performed by repeated-measures analysis of variance (ANOVA) with two within-subjects factors (pre- and post-intervention, meal pattern), or with three within-subjects factors (time after the test meal, meal pattern, pre- and post-intervention) as appropriate. When ANOVA indicated a significant main effect, the level of significance for pairwise comparisons of each was obtained. Statistical significance was set at $P<0.05$ for all statistical tests.

Results

Energy intake

All subjects reported having adhered to the appropriate meal patterns. There was no significant difference in the percentage of protein, fat and carbohydrate between the two eating patterns. There was also no significant difference in the mean total energy intake measured from the 3-day food diary record over each phase (Table 1). The mean daily energy intake before the start of the study was more variable compared with the two interventional periods, which might be related to differences in energy intake between weekdays and weekends.

Table 1 Nutrient intake before the study and during the regular and irregular meal pattern[a]

	Before study	*Regular meal pattern*	*Irregular meal pattern*
Energy (MJ/day)	8.368 ± 1.014	8.014 ± 0.497	8.415 ± 0.488
Protein (%)	17.8 ± 3.4	14.2 ± 2.6	14.7 ± 1.8
Fat (%)	34.9 ± 5.2	39.2 ± 6.1	38.2 ± 5.3
Carbohydrate (%)	44.8 ± 3.5	44.0 ± 5.9	43.3 ± 5.6

[a]Mean ± s.d.

Mean body weight showed no significant differences during regular (63.6 ± 8.0 before, and 63.2 ± 7.2 kg after) and irregular (62.7 ± 7.7 before, and 63.0 ± 7.9 kg after) meal pattern periods. All subjects completed all parts of the study.

Blood glucose and serum insulin

All blood glucose and serum insulin concentrations were not significantly different in the pre-interventional visits (visits 1 and 3). Fasting blood glucose did not change across the phases of the study, and there were no significant differences in fasting blood glucose between the post-regular and post-irregular meal patterns (Table 2). Blood glucose concentration rose significantly after the test meal in all visits (Figure 1). There were no significant differences in the peak values of blood glucose between the visits at the end of each of the 14 days on each eating pattern (visits 2 and 4). The peak of blood glucose values at the start of each phase also showed no significant difference (Table 3). Areas under the curve (AUCs) for blood glucose above the baseline were measured over the post-test meal period by using the trapezoidal method. There were no significant differences in AUC between the post-regular and irregular meal pattern visits (16.4 ± 0.9 and 17.0 ± 0.8 mmol/l h, respectively). There were also no significant differences between the change of post-test meal AUC for blood glucose across the regular and irregular phases.

No significant differences were observed in the HOMA index between the pre-meal pattern periods (visits 1 and 3). The two meal patterns created no significant changes in fasting serum insulin concentration. However, the HOMA value was larger after the irregular meal pattern ($P=0.02$, Table 2). A significant increase in serum insulin concentration occurred after the test meal in all visits. The peak of insulin concentration showed a significant meal pattern by phase interaction ($P=0.001$, ANOVA), such that it fell

Table 2 Fasting blood glucose, serum insulin, plasma lipids and uric acid[a]

	Regular meal pattern		*Irregular meal pattern*	
	Pre-diet visit	*Post-diet visit*	*Pre-diet visit*	*Post-diet visit*
Blood glucose (mmol/l)	4.99±0.41	4.71±0.34	5.19±0.35	5.0±0.46
Serum insulin (pmol/l)	24.9±5.16	22.2±5.64	25.92±6.9	24.72±5.82
HOMA IR	0.91±0.20	0.77±0.19	1.00±0.35	0.91±0.24*
Plasma total cholesterol (mmol/l)	3.54±0.59	3.51±0.52	3.56±0.60	3.82±0.66†
Plasma HDL-C (mmol/l)	1.44±0.35	1.56±0.46	1.45±0.42	1.57±0.46
Plasma LDL-C (mmol/l)	1.97±0.50	1.82±0.26	1.98±0.14	2.10±0.40‡
Plasma triacylglycerol (mmol/l)	0.63±0.17	0.66±0.21	0.68±0.18	0.71±0.23
Plasma uric acid (mg/l)	213±43	215±52	215±40	223±38

[a]Mean±s.d., $n=9$.
*Significantly different from the regular meal pattern: $P=0.022$ (paired *t*-test).
†Significantly different from the regular meal pattern: $P=0.01$ (paired *t*-test).
‡Significantly different from the regular meal pattern: $P=0.028$ (paired *t*-test).

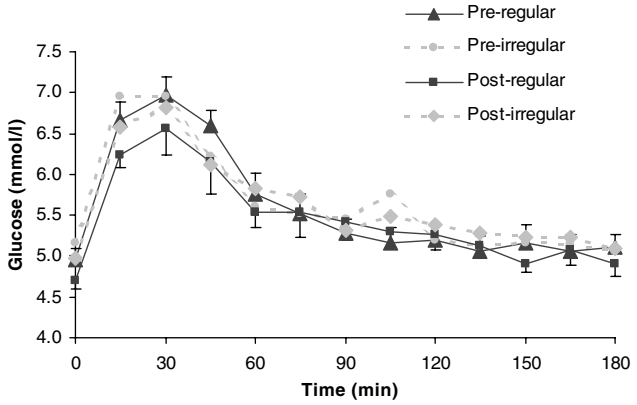

Figure 1 Mean (±s.e.m.) blood glucose concentrations in nine healthy lean women in the fasting state and after a high carbohydrate test meal in the pre- and post-regular meal patterns. No significant differences were observed in blood glucose profiles over the experiment. For the clarity, s.e.m. values only presented for two of the profiles.

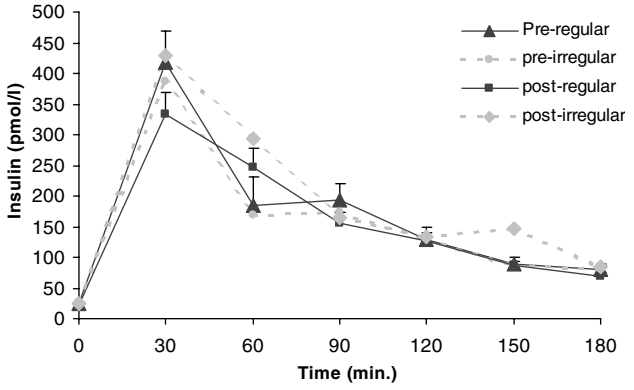

Figure 2 Mean (±s.e.m.) serum insulin concentration in nine healthy lean women in the fasting state and in response to a high-carbohydrate test meal in the pre- and post-regular meal patterns. The insulin response profiles were significantly different between the post-regular and irregular meal patterns ($P=0.015$). For clarity, s.e.m. values only presented for two of the profiles.

Table 3 Mean peak of glucose and insulin concentrations[a]

Visit	*Peak of blood glucose (mmol/l)*	*Peak of serum insulin (pmol/l)*
Pre-regular meal pattern	7.31±0.55	421.2±151.2
Post regular meal pattern	6.83±0.79	332.4±107.4
Pre-irregular meal pattern	7.09±0.66	389.4±148.2
Post -irregular meal pattern	7.04±0.39	428.4±150.0

[a]Mean±s.d., $n=9$. A significant difference was shown between the peak of serum insulin response responding to the high-carbohydrate test meal between the post regular and irregular meal patterns ($P=0.003$).

between pre- and post-regular eating, but rose over the irregular eating periods (Table 3). The peak insulin levels showed significant differences between the post- regular and irregular meal pattern visits (visits 2 and 4), with the lower values after the regular period (332.4±107.4 pmol/l after the regular meal pattern compared with 428.4±150.0 pmol/l after the irregular one, $P=0.003$, paired *t*-test). Insulin profiles, for the 3 h after the test meal, also showed a significantly higher response after the irregular compared with after the regular period ($P=0.015$, ANOVA) (Figure 2). AUC above the fasting value for insulin response to the test meal was measured by the trapezoid method. There was also a significant difference in the change in insulin AUC before and after the two meal pattern periods ($P=0.001$, ANOVA), (Figure 3). Meal pattern had a significant effect on insulin AUC, with higher response after the irregular period compared to after the irregular period ($P=0.007$, paired *t*-test).

Plasma lipids

Table 2 also shows the mean values of plasma total cholesterol (LDL-C), HDL-C and triacylglycerol concentra-

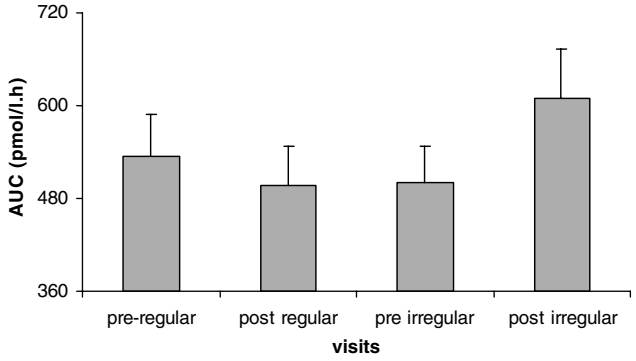

Figure 3 Mean (±s.e.m.) AUC (above the baseline) for the serum insulin profiles in nine healthy lean women responding to a high-carbohydrate test meal before and after the regular and irregular meal patterns. There was a significant interaction between pre- and post-meal pattern conditions ($P=0.001$, ANOVA), and a significant difference between the AUC of the regular and the irregular meal patterns ($P=0.007$, paired *t*-test).

tion in all visits. All of the lipid values were not significantly different in the preinterventional visits (visits 1 and 3). There was a significant meal pattern difference by pre- and post-phase interaction in total serum cholesterol levels ($P=0.037$, ANOVA). Total plasma cholesterol was also significantly higher after the irregular meal pattern compared with the regular meal pattern (visits 2 and 4, $P=0.01$). There was a significant meal pattern difference by pre- and post-phase interaction in plasma LDL concentration ($P=0.008$, ANOVA). In addition, at the end of irregular meal pattern, the plasma LDL value was significantly higher than after the regular meal pattern ($P=0.028$). No significant differences were, however, observed in plasma HDL concentration over either the regular or the irregular meal patterns. Plasma triacylglycerol concentration also showed no significant difference over the experiment. AUC for triacylglycerol profile after the test meal was measured by using the trapezoidal method. No significant differences in AUC of triacylglycerol profile were observed over the experiment.

Plasma uric acid

There were no significant changes in plasma uric acid over the experiment (Table 2).

Discussion

The aim of this study was to investigate the impact of irregular meal frequency on indices of carbohydrate and lipid metabolism and plasma uric acid level. To our knowledge, no studies have evaluated the association between irregular meal pattern and lipid–carbohydrate metabolism and serum uric acid concentration in adulthood.

The present study indicates that the irregular meal frequency for 14 days produced lower fasting insulin sensitivity (higher HOMA-IR) and a greater insulin response to a test meal compared with the regular meal pattern. It also reports that irregular meal frequency causes serum total cholesterol and LDL to be higher than a regular meal pattern. Plasma total cholesterol, LDL-C, HDL-C and triacylglycerol levels have been identified as risk factors for cardiovascular diseases (Solberg & Strong, 1983; Grundy, 1998; Grundy *et al*, 1998). High serum insulin (Despres *et al*, 1996) and serum uric acid levels (Waring *et al*, 2000) have also been related to the increase of the risk of cardiovascular diseases.

Meal frequency has been recognised for over 30 y as influencing these risk factors for cardiovascular diseases (Fabry *et al*, 1968). The opportunity to consume food irregularly has increased over the last decades due to accessibility of ready prepared foods, fast food outlets and differences in work patterns. Although no study has evaluated the effect of irregular meal pattern on these biochemical risk factors for cardiovascular diseases, a number of studies attempted to assess the impact of meal frequency on these factors. These studies compared low with high meal frequencies which, within an intervention, were constant. Previous studies have shown that meal frequency might be associated with fasting total plasma cholesterol and low-density lipoprotein levels and glucose tolerance in healthy people (Gwinup *et al*, 1963; Jenkins *et al*, 1989; Arnold *et al*, 1993). However, the results are not conclusive. A recent study (Jenkins *et al*, 1995) reports an inverse relationship between meal frequency and serum uric acid concentration. One of the main criticisms of these studies is the poor and inconsistent definition of the principal variables, which hampers interpretation of the results and prevents comparison between different studies. For example, to define the degree of obesity, authors sometimes quantified with weight measurement; some used BMI and in some cases estimated either total fat or percentage body fat. Furthermore, the definition of 'meal' which might be one of the principal variable in meal frequency studies—some studies did not explain any definition and the definition in others was not consistent which may cause difficulties in comparing the results. In the present study, a 'meal' was defined as a food or snack containing energy and the interval between two 'meals' was to be more than 1 h. Subjects had received enough instruction in order to record their food intake appropriately. To overcome these confounding factors, subjects were asked to have between 3 to 9 meals/day during the irregular diet period, such that the average was the same as 6 meals/day used during the regular meal pattern period.

The present study reports no significant difference in fasting blood glucose and glucose profile after the test meal. These results were expected since during short-term interventions in healthy subjects, blood glucose is likely to be maintained constant with any compensation occurring via changes in insulin secretion. No significant differences furthermore appeared in baseline insulin level over the experiment. However, the HOMA index was larger after the irregular meal pattern compared with the regular one, which

might represent a degree of fasting insulin resistance after the irregular meal pattern. It should be mentioned that the subjects were healthy and the HOMA values changed within the normal limits. The peak of postprandial insulin response was significantly lower after the regular meal pattern compared with the irregular meal pattern. AUC of insulin profile after the test meal furthermore showed a significantly higher response after the irregular meal pattern, compared with the regular meal pattern period. The present study confirms the hypothesis that an irregular meal frequency may create fasting insulin insensitivity and a higher insulin response to a test meal compared with the regular meal pattern. Overall, it suggests that irregular meal frequency may create inappropriate carbohydrate metabolism following insulin insensitivity in lean people.

Regarding the changes in plasma lipids, the present study indicates that plasma total cholesterol and LDL levels increased after the irregular meal pattern compared with the regular meal pattern period. Fasting plasma HDL-C and triacylglycerol concentration, however, showed no significant differences between the regular and irregular meal patterns. Previous studies have shown that higher meal frequency is associated with lower fasting total serum cholesterol and low-density lipoprotein levels. A recent epidemiological study (Murata, 2000) study claims that an irregular meal pattern may be associated with elevated serum total cholesterol level in adolescents. However, there was no exact definition of irregular meal pattern in that study.

The present study failed to find any significant differences in serum uric acid concentration in terms of irregular meal pattern. A recent previous study (Jenkins *et al*, 1995) reports an inverse relationship between meal frequency and serum uric acid levels.

In relation to the insulin insensitivity after the irregular meal pattern, it seems likely that the insulin secretion process can usually cope with stable food-intake intervals, so that the insulin response controls carbohydrate metabolism optimally. However, an irregular meal pattern would disturb such regular circadian variations of insulin secretion. In addition, irregular meals may alter other hormonal responses such as sympathoadrenal activity and circulating adiponectin, which could also affect insulin sensitivity.

Insulin stimulates hydroxy methyl glutaryl- Co-A (HMGCoA) reductase, one of the rate limiting enzymes in cholesterol synthesis. Lower total and LDL cholesterol after the regular meal pattern might be due to a lower insulin stimulus to HMGCoA reductase. The results of this study have revealed the importance of meal pattern in addition to amount and composition of food in influencing carbohydrate and lipid metabolism. Further comprehensive studies measuring the above factors and other possible influences are required.

These findings suggest that a regular meal pattern would optimise lipid and carbohydrate metabolism and may be of value in improving insulin sensitivity and reducing total and LDL cholesterol concentrations. This could then become a useful part of the advice given to those at risk of developing cardiovascular diseases and diabetes.

In conclusion, we observed insulin insensitivity and higher fasting serum total and LDL cholesterol after the irregular meal frequency compared with the regular one, which may indicate the deleterious impact of an irregular meal pattern on these cardiovascular risk factors. Particularly significant was the fact that these participants were free-living with a self-selected diet. Finally, it is necessary to evaluate these effects in further larger and more comprehensive studies not only in normal populations but also in obese people and diabetic patients, in order to identify the public health relevance of these observations.

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