Metabolic consequences of feeding frequency in man 1,2,3

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Metabolic activities of hepatic cells are influenced by the types (1) and quantities (2) of nutrients available to them. Beneficial effects have been claimed for a more frequent eating pattern in that a more constant energy supply is provided by the diet, thereby reducing the stress associated with the variable flux of energy concomitant with a gorging pattern. These effects include lowering of blood glucose levels in diabetics (3), lowering of serum triglyceride and cholesterol levels of hyperlipidemics (4, 5), and when hypocaloric diets were fed to "resistant obese" subjects, weight loss was effected (6). Similar changes have been observed in normal subjects when they were switched from gorging to nibbling regimens (7–9).

The effects of saturated and polyunsaturated fats on serum lipids, particularly cholesterol, have been widely discussed since the early report of Keys, Anderson and Grande (10). The combined effects of polyunsaturated fats and nibbling on decreasing fasting serum cholesterol were shown by Jagan-nathan et al. (11) and Hashim and co-workers (12).

The dynamics of the adaptive responses to these changes seemingly would be better explained by studies of these parameters following a glucose challenge rather than measurements obtained during the fasting state. Reported here are observations of the interrelationship of adaptations to feeding frequency and dietary fat as reflected by blood glucose, serum triglycerides, immunoreactive insulin (IRI), free fatty acid (FFA) levels during a glucose tolerance test (GTT), and also fasting serum cholesterol and phospholipid levels of young men.

Methods

Subjects

Healthy, male university students between 20 and 30 years of age were selected on the basis that their weights were within 10% of their ideal body weight according to their height, and that they had no history of diabetes in their immediate families. Six subjects participated in one experiment and seven in the other.

Diet

The energy intake required by each subject was calculated according to the formula: calories = 725 + 31 x weight in kilograms (13). The common dietary items in both experiments were turkey, bread, apples, oranges, and whole milk with Dextri-Maltose. Raisins replaced part of the Dextri-Maltose to increase the acceptability of the diet in the second experiment. Either corn oil or butter oil was added to the basal diet to bring the caloric contribution from fat to 40% of the total energy intake. Protein and carbohydrate provided 13% and 47% of the total calories, respectively. The fatty acid composition, cholesterol content, and complex:simple carbohydrate ratio for the two diets are given in Table 1. On the basis of the dietary fat, these two experiments will be referred to as CO and BO.

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TABLE 1
Lipid and carbohydrate characteristics of the diets

<table>
<thead>
<tr>
<th>Added fat source</th>
<th>Fatty acid composition</th>
<th>Cholesterol, mg/day</th>
<th>Complex to simple carbohydrate ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percent saturated</td>
<td>Percent mono-unsaturated</td>
<td>Percent linoleic acid</td>
</tr>
<tr>
<td>Butter oil</td>
<td>70.5</td>
<td>21.6</td>
<td>4.0</td>
</tr>
<tr>
<td>Corn oil</td>
<td>37.3</td>
<td>36.1</td>
<td>26.6</td>
</tr>
</tbody>
</table>

* Mayonnaise was prepared with corn oil.

Meal size and frequency

Three eating patterns were used in the experiments. For the first week, all the subjects were introduced to the diet with a regular three-meal pattern in the proportion of 2/8:3/8:3/8 eaten at 8:00 AM, 12:00 Noon, and 5:00 PM, respectively. For the first experimental period of 4 weeks, one-half the subjects from each group consumed the total food allowed per day in 1/8:7/8 portions, 1/8 at 8:00 AM and 7/8 at 5:00 PM. This pattern will be referred to as gorging. The rest of the subjects followed a pattern of eight small isocaloric meals every 2 hr from 8:00 AM to 10:00 PM for the 4-week period, which will be referred to as nibbling. For the second experimental period, the subjects switched the eating patterns, gorgers became nibblers and nibblers became gorgers.

Analytical procedure

Blood drawn from the antecubital vein into Vacutainer tubes at fasting was analyzed for blood glucose (14), serum triglycerides (15), free fatty acids (16), and cholesterol (17) using semiautomated procedures. Phospholipids (18) and total lipids (19) were determined gravimetrically. At 0.5, 1, 2, and 3 hr after a 100-g oral glucose load, determinations were made for blood glucose, serum triglycerides, and free fatty acids in both experiments. Serum IR1 levels during the glucose tolerance test were determined in the CO experiment according to the procedure of Hales and Randle (20) using the Insulin Immunosay Kit (Wellcome Research Laboratories, Research Triangle Park, North Carolina 27709).

All the data were subjected to analysis of variance. Because no differences were observed between groups due to the order of presentation of the two feeding patterns, the sequence sums of squares were added to the error sums of squares before calculating F values.

Results

Fasting levels of blood glucose and serum lipid components

Variance analyses of the fasting levels of serum components of subjects fed two types of dietary fats on two feeding patterns indicate that the type of fat influenced all serum components except phospholipids. Only blood glucose levels were affected by the feeding pattern and an interaction between fat and frequency of feeding was observed only for glucose. The sequences in which the two feeding patterns were presented had no effect on the levels of fasting serum components.

The data in Table 2 represent only the dietary fat effect on fasting serum components. The mean serum triglyceride level (135.2 ± 8.0 mg/100 ml) of the CO group was significantly higher than that (75.3 ± 9.8 mg/100 ml) of the BO group. This latter value was confirmed by using a gravimetric procedure (18). All other components were found in elevated levels in the sera of the latter group as follows: FFA, 568.2 ± 51.4 μEq/liter and 501.4 ± 58.2 μEq/liter; cho-

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Fasting levels of blood glucose and serum lipid in subjects fed corn oil or butter oil diets</th>
</tr>
</thead>
</table>
| Serum components, mg/100 ml | M Eq/liter | Corn oil | P*
| Glucose (blood) | 99.4±3.9 | 86.4±1.4 | <0.001 |
| Triglyceride | 75.3±9.8 | 135.2±8.0 | <0.001 |
| Free fatty acid, pEq/liter | 568.2±51.4 | 501.4±58.2 | 0.001 |
| Cholesterol, pEq/liter | 268.7±10.4 | 288.1±11.9 | NS |
| Total protein, pEq/liter | 701.2±35.7 | 587.0±33.8 | 0.05 |

* All P values are for 1 and 22 degrees of freedom for treatment and error sums of squares.
* Mean ± se of combined values of gorgers and nibblers.
TABLE 3
Fasting levels of blood glucose and serum lipid components of subjects fed on gorging or nibbling regimen

<table>
<thead>
<tr>
<th>Serum components, mg/100 ml</th>
<th>Gorging</th>
<th>Nibbling</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (blood)</td>
<td>99.0±3.5</td>
<td>85.7±1.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>115.0±12.7</td>
<td>101.0±13.8</td>
<td>NS</td>
</tr>
<tr>
<td>Free fatty acids, µEq/liter</td>
<td>420.5±39.7</td>
<td>501.4±58.2</td>
<td>NS</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>292.5±12.9</td>
<td>265.7±8.7</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>198.1±12.1</td>
<td>206.2±16.8</td>
<td>NS</td>
</tr>
<tr>
<td>Total lipids</td>
<td>665.5±34.9</td>
<td>613.8±40.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

* All P values are for 1 and 22 degrees of freedom for treatment and error sums of squares.

** Mean ± SEM of combined values for BO and CO groups.

Lesterol, 236.7 ± 15.2 mg/100 ml and 172.6 ± 7.6 mg/100 ml; total lipids, 701.2 ± 35.7 mg/100 ml and 587.0 ± 33.8 mg/100 ml; and glucose, 99.4 ± 3.9 mg/100 ml and 86.4 ± 1.4 mg/100 ml, respectively, for the BO and CO groups. No significant difference was observed in the mean serum phospholipid levels of subjects due to the type of dietary fat. These values, reported in Tables 2 and 3, also include small amounts of compounds eluted from the column between the free fatty acid and the phospholipid fractions. These unidentified compounds were present in both fractions and contributed less than 10% error to the phospholipid values as judged by the intensity of the spots following charring after TLC separation.

The mean fasting serum levels in gorging and nibbling subjects are presented in Table 3. The mean blood glucose level of the subjects was significantly higher when they gorged (99.0 ± 3.5 mg/100 ml) than when they nibbled (85.7 ± 1.6 mg/100 ml). For the other serum components, the effects of feeding frequency, if present, were small and variances were large. However, trends seemed to be apparent for certain components. Mean triglyceride, phospholipid, and total lipid levels were 13, 10, and 8% higher, respectively, when subjects were on the gorging regimen than when on the nibbling regimen. Feeding frequency had no effect on serum cholesterol levels.

Variance analysis showed a significant fat times frequency interaction on the fasting blood glucose levels. Mean levels of BO gorgers were strikingly higher (110.3 ± 3.1 mg/100 ml) than those of BO nibblers (88.5 ± 3.2 mg/100 ml), CO gorgers (89.4 ± 2.1 mg/100 ml), and CO nibblers (83.3 ± 0.9 mg/100 ml). No other interactions were noted.

Blood glucose levels during GTT

The blood glucose levels of BO subjects at 0.5 (195.5 ± 10.6 mg/100 ml) and 1 hr (199.2 ± 14.5 mg/100 ml) were significantly higher than levels observed at 0.5 (167.5 ± 12.9 mg/100 ml) and 1 hr (139.9 ± 15.3 mg/100 ml) in CO subjects. Post-glucose levels were also significantly affected by the frequency of feeding. The mean values of gorgers were 208.9 ± 8.8 mg/100 ml and 200.8 ± 13.6 mg/100 ml and those of nibblers were 151.9 ± 10.4 mg/100 ml and 133.8 ± 14.9 mg/100 ml at 0.5 and 1 hr, respectively. A significant fat times frequency interaction was also noted at these two times. As shown in Fig. 1, the nibbling pattern of food intake primarily affected the blood glu-

FIG. 1. Blood glucose levels of BO and CO gorgers and nibblers during glucose tolerance test (mean ± SEM).
Serum triglyceride levels of CO but not BO subjects. The 0.5- and 1-hr blood glucose levels of BO gorgers, BO nibblers, and CO gorgers were similar to those of a diabetic, according to the criteria of Fajans and Conn (21). For CO nibblers, the blood glucose levels at 0.5 and 1 hr were 58.8% ($P < 0.005$) and 47.3% ($P < 0.005$), respectively, of the levels observed for the gorgers. The 0.5- and 1-hr levels observed for BO nibblers were 86.7 and 83.3%, respectively, of those observed for gorgers.

Serum triglyceride levels during GTT

Serum triglyceride levels of the CO group were consistently higher than those of the BO group. In the former group, maximum serum triglyceride levels were observed at 0.5 hr (170.1 ± 10.2 mg/100 ml) and in the latter group at 1 hr (107.0 ± 13.9 mg/100 ml) following glucose loading. The serum triglyceride levels of gorging subjects at 0.5 (157.0 ± 15.6 mg/100 ml) and 1 hr (158.0 ± 18.4 mg/100 ml) were significantly higher than those of nibbling subjects at 0.5 (122.8 ± 9.2 mg/100 ml) and 1 hr (118.0 ± 10.3 mg/100 ml). The feeding frequency effect had disappeared by 2 hr. A significant fat times frequency interaction is suggested by the data in Fig. 2. The feeding frequency had little effect on the serum triglyceride levels of BO subjects but CO gorgers had strikingly higher serum triglyceride levels at 0.5 and 1 hr than did the nibblers.

Serum FFA levels during GTT

Although variance analyses showed no significant treatment effect on serum FFA levels following glucose loading, the mean FFA levels during GTT in the sera of BO subjects were approximately 50% higher than those of CO subjects. The serum FFA levels of CO subjects declined by 51.5% of the fasting levels during the first 2 hr and then increased slightly. In these subjects no trends related to feeding frequency were apparent. However, in BO subjects the levels of serum FFA declined throughout the tolerance test. After 3 hr, the mean FFA levels of BO nibblers and gorgers were decreased by 62 and 45%, respectively. This reflects primarily the difference in the fasting FFA levels, however.

Serum IRI levels during GTT

The serum IRI levels of CO subjects were considerably affected by the frequency of feeding. After gorging their meals for 4 weeks, the subjects responded to oral glucose loads with delayed and exaggerated increases in serum IRI levels (Fig. 3). The maximum rise in serum IRI level occurred at 1 hr for gorgers and at 0.5 hr for nibblers. The 0.5-, 1-, and 2-hr levels were 96.1 ± 33.4, 105.8 ± 7.4, and 68.1 ± 15.2 μU/ml, respectively,
for gorgers and 50.7 ± 18.2, 33.8 ± 10.9, and 17.5 ± 4.9 μU/ml, respectively, for nibblers. The differences in serum IRI levels for gorgers and nibblers were significant at 1 hr (P < 0.01) and 2 hr (P < 0.05).

The ratios of serum IRI (μU/ml) to blood glucose (mg/100 ml) (insulinogenic index) that relate the increase in serum IRI to the corresponding glycemic stimulus (22) were higher for gorgers throughout the 3-hr GTT (Fig. 4). The difference between the 2-hr insulinogenic indices of gorgers (0.71 ± 0.14) and nibblers (0.21 ± 0.053) was significant (P < 0.05).

Body weight changes

No significant differences due to feeding frequency were observed in the body weight of subjects fed the BO diet. However, when fed the CO diet, gorgers gained 1.97 ± 0.81 lb and nibblers lost 2.7 ± 0.75 lb. These differences, although of small magnitude, may be of considerable importance considering their constancy over the 4-week experimental periods.

Discussion

In the evolutionary process, higher organisms develop adaptive mechanisms that allow them a degree of independence from their environment. In this report, we describe apparent metabolic adaptations brought about by a change in the energy flow in the presence of constant proportions of protein, carbohydrate, and fat and also an adaptation brought about by the two types of fats differing in fatty acid chain length and saturation.

In man, these adaptations occur primarily in the liver. Dependent on the energy flux, excesses of amino acids and carbohydrates are utilized for the synthesis of fatty acids. Short- and medium-chain fatty acids, found in butter oil, pass as free fatty acids into the portal circulation and are taken up by the liver. Subsequently, chain elongation takes place. Long-chain fatty acids entering systemic circulation as components of chylomicrons are removed from circulation by adipose tissue and by the liver. Fatty acids synthesized or elongated by the liver are subsequently released as triglyceride components of pre-β-lipoproteins for transport to the adipose tissue.

Two types of adaptive response are suggested by these studies. One, similar to that observed in meal-eating rats involves increased absorptive capacity (23), increased lipogenic capacity (2), and increased gluconeogenic capacity (24) of the system to tide over the long period of external food energy deprivation. An additional adaptive response was elicited by the inclusion of butter oil in the diet that required chain elongation processes in the liver prior to triglyceride synthesis and storage. The saturated long-chain fatty acids of butter oil may also have had an effect different from that of the more unsaturated long-chain fatty acids of corn oil.

Adaptation was apparent in the levels of certain circulating metabolites at fasting as well as after glucose loading. Fasting blood glucose and serum triglyceride levels of gorgers were higher than those of nibblers, but the difference in glucose levels between BO gorgers and BO nibblers was 21.8 mg/100 ml as compared with the levels of CO gorgers and CO nibblers at 4.4 mg/100 ml. Furthermore, subjects in the BO group retained less nitrogen during gorging (unpublished observations).
Correspondingly, both BO and CO gorgers responded to oral glucose loads with an increase above fasting levels greater than 100% within 0.5 hr. Blood glucose levels remained higher through 1 hr for CO gorgers, whereas BO gorgers had abnormally high levels throughout the GTT as compared with the respective nibblers. That the hormonal regulation of the glucose homeostatic mechanism was impaired is evident from the higher circulating serum IRI levels (Fig. 3). Moreover, a delayed response of serum IRI to glucose load in gorging subjects suggests that decreased sensitivity of peripheral tissue to insulin may be a contributory factor to greater than normal circulating blood glucose levels. This hypothesis is further substantiated by the significantly higher insulogenic index at 2 hr following glucose load (Fig. 4).

Serum triglyceride levels of gorgers were also higher than those of nibblers throughout the GTT. The maximum value after glucose loading was 38% above the fasting level in CO gorgers. This observation is in agreement with the higher glucose and insulin levels also observed in these subjects, as it has been suggested that mild glucose intolerance, which results in excessive insulin secretion, causes increased hepatic triglyceride synthesis. Triglyceride clearance from serum mediated by lipoprotein lipase may also have been impaired due to decreased sensitivity of peripheral tissues to insulin (25). The glucose load produced much smaller increments above fasting levels for glucose and triglycerides in the CO nibblers (Figs. 1 and 2). The insulogenic index curves (Fig. 4) indicate that the insulin sensitivity of the peripheral tissue of the nibbling subjects increased even above that observed during the pre-experimental period. The 15% increase in serum triglycerides of CO nibblers in response to glucose load may indicate that either the hepatic triglyceride synthesis was depressed or the triglyceride removal was more efficient due to increased insulin sensitivity of peripheral tissues compared with the gorgers.

How a gorging regimen produces such abnormalities in the circulating levels of these metabolites is not known. It is suggested that in a system conditioned to accept a large load of nutrients at a single meal, the gastric emptying rate or the absorptive capacity of the gastrointestinal tract may be increased or there may be enhanced secretion of humoral factors which mediates insulin secretion during glucose absorption (23, 26, 27).

This adaptive capacity of the system to metabolic stress may lead to the pathological implications of abnormal glucose tolerance curves, serum IRI levels, and serum triglyceride levels that have been observed in obese subjects (28), patients with adult onset diabetes (29), or ischemic heart disease (IHD) (30). Fabry observed an inverse relation between meal frequency and IHD occurrence rate in an elderly Czech population (31). That a gorging pattern of eating may be an additional risk factor in the development of these conditions can only be suggested but cannot be proved from our short-term studies. These metabolic changes are readily reversed. The observations regarding CO nibblers indicate that such a regimen may be effective in delaying the onset of pathological conditions or even alleviating the symptoms of decreased glucose tolerance, hyperinsulinemia, and hypertriglyceridemia.

Summary

Normal male subjects adapted to a meal-eating pattern for 4 weeks with caloric intake limited to two meals, 1/8 at 8:00 AM and 7/8 at 5:00 PM (gorgers), exhibited abnormal insulin responses to 100-g oral glucose loads. Insulogenic indices were abnormally high 2 hr after a glucose load in these subjects. These subjects then consumed isocaloric diets in eight equal meals (nibblers) during the hours of 8:00 AM to 10:00 PM. Both insulin responses and insulogenic indices were returned to normal within 1 week and after 4 weeks values were below pretest levels. In these studies, corn oil (CO) was the major fat source. In a subsequent study, butter oil (BO) was used in place of CO to determine the effect of the dietary fat on carbohydrate and lipid metabolism. At fasting, CO subjects had higher serum triglyceride levels, whereas BO subjects had higher serum cholesterol, free fatty acids and total lipids, and
higher blood glucose levels. Phospholipids were not affected by the diets. In the gorging subjects blood glucose levels were higher. Following oral glucose loads, serum triglycerides increased sharply in subjects adapted to CO gorging; in CO nibblers, the triglyceride response was considerably dampened. The feeding pattern did not influence the serum triglyceride of BO subjects following oral glucose loading. Also, blood glucose levels were not greatly affected by the feeding pattern in these subjects, whereas in CO subjects, gorging caused a striking increase. It may be important that blood glucose levels of both BO gorgers and nibblers resembled that of CO gorgers.

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