Changes in Lactate Dehydrogenase, LDH Isoenzymes, Lactate, and Pyruvate as a Result of Feeding Low Fat Diets to Healthy Men and Women


A study was conducted to evaluate the effects on blood lipids and lipoproteins of feeding 21 healthy volunteers, 40–60 yr old, foods commonly eaten in the United States for two 40-day periods. Activities of lactate dehydrogenase (LDH) and LDH isoenzymes, lactate, and pyruvate were monitored. Results showed that LDH activity was significantly lower in all subjects at the end of the 25% fat-calorie period (period I) than at the beginning of the study, but rose above initial levels at the end of the 35% fat-calorie period (period II). While total LDH fell during period I, relative activity of M type subunits of LDH rose significantly in relation to H type in both sexes. This rise is probably indicative of an increase in glycolytic activity as a consequence of the increased intake of dietary carbohydrate. In period I, lactate and pyruvate decreased significantly in males (pyruvate greater than lactate) but not in females. Values for males returned to near initial levels in period II. The ratio of lactate/pyruvate was elevated in both sexes after period I. The greater change in pyruvate relative to lactate with increased dietary carbohydrate suggests increased Krebs Cycle activity. There was a statistically significant positive correlation between lactate, pyruvate, and serum triglyceride for males after they ate the 25% and 35% fat-calorie diets and for females after they ate the 35% fat-calorie diet, but not between lactate, pyruvate, and serum cholesterol for either sex.

RESULTS of studies of some investigators have indicated that human subjects may show changes in plasma lactate dehydrogenase (LDH), lactate, pyruvate, and lactate/pyruvate (L/P) ratios in response to changes in type and level of carbohydrate in the diet. In addition, elevated levels of lactate and/or pyruvate have been found in human subjects with heart disease and hypertension.

To test the dietary recommendations of the Inter-Society Commission on Heart Disease Resources to moderately reduce blood lipids by lowering fat and cholesterol in the diet, we fed meals containing either 25% or 35% fat calories to healthy men and women. Because the reduction in fat calories in the diet resulted in an increase in carbohydrate calories, it was desirable to measure not only the blood lipids, but also to monitor certain parameters of carbohydrate metabolism to investigate their usefulness in evaluating effects of changes in diet on metabolic pathways.
Carbohydrate induction of triglyceride synthesis has been demonstrated both in healthy individuals and in individuals genetically predisposed to hyperlipoproteinemia.\textsuperscript{7-15} The elevated plasma triglycerides are accompanied by elevated levels of prebeta lipoproteins, the primary transport mechanism for triglycerides.\textsuperscript{9,15} While the induction of triglyceride synthesis with high carbohydrate diets has not been well defined, the major route of synthesis probably resides in the increased rate of glycolysis via the Emden–Meyerhof pathway. In addition, an increased conversion of pyruvate to acetyl Co-A could result in increased fatty acid synthesis.

Recently, the possibility was suggested that macronutrients, as well as micronutrients, can play a major role in the rate of utilization of metabolites such as pyruvate. For example, pyruvate can act not only as a substrate, but, by preventing kinase inactivation of pyruvate dehydrogenase, it can increase its utilization as well.\textsuperscript{16} This mechanism may explain, in part, the transient nature of carbohydrate induction of triglyceride synthesis in healthy man.

This paper reports the changes in LDH, LDH isoenzymes, lactate, pyruvate, and L/P ratios with changes in diet to reduce the intake of fat calories, and provides baseline values for these parameters in healthy man in the age group of 40–60 yr. Details on the design of the study, composition of the diets fed, results for the blood lipids, and other variables studied are reported in separate papers.\textsuperscript{17-19}

**MATERIALS AND METHODS**

Briefly, the study was divided into two main periods of 40 days each. During the first period (I), 21 healthy volunteers, 10 men and 11 women, 40–60 yr old, were fed diets containing 25% calories from fat, 60% calories from carbohydrate, and <300 mg cholesterol. During the second period (II), the same subjects received diets containing 35% calories from fat and 50% calories from carbohydrate, with an average of about 300 mg cholesterol. Depending on individual calorie intakes, the cholesterol varied from <300 mg in the 1600 kcal meals to 500 mg in the 3600 kcal meals. Protein was maintained at 15% of calories throughout the 80-day study.

**Subjects.** The participants were given physical examinations prior to the study. Most were USDA employees; a majority were scientists or technicians. They were permitted to maintain their normal activities at home and at work except that they ate three meals a day in a dining room at Beltsville on work days and took meals home for consumption during the weekend. The subjects were not receiving vitamin pills or medication and, except for only a few instances, did not receive medication during the study.

**Diets.** The diets were calculated from food composition data in Table 1 of USDA Handbook No. 8,\textsuperscript{20} including updated information on fatty acids and cholesterol on tape,\textsuperscript{21} to contain either 25% (period I) or 35% (period II) calories from fat. Changes in fat calories were made at the expense of carbohydrate. All food eaten by the participants was either weighed or measured.

Ten different daily menus of typical USA foods were planned, each of which was designed to contain an adequate supply of the nutrients known to be essential for the “reference” man and woman.\textsuperscript{22} The meals, in addition to specific calorie allowances of fat, carbohydrate, and protein, had polyunsaturated (linoleic acid) to saturated fatty acids (total) in a ratio of one. Calorie intakes of the subjects were adjusted throughout the study to maintain their initial body weights. Calorie levels varied from 1600 to 3600 in increments of 400 kcal each.

The diets contained simple (mono- and disaccharides) to complex (polysaccharides) carbohydrate in the ratio of one. The highest calorie levels had average ratios of slightly <one. Fruits, milk, some desserts, jams, and jellies were considered sources of simple carbohydrate. Vegetables, breads, and cereals were sources of complex carbohydrate. Desserts such as cakes,
gingerbread, cookies, etc., were divided between simple and complex carbohydrate according to a typical recipe used in their preparation.

Food intakes of the subjects and the menus were reported in a separate paper. Calculated prestudy intakes were, on the average for all subjects, 17.9% protein, 41.6% fat, and 39.9% carbohydrate calories. Total carbohydrate intake was 216.7 ± 17.3 g. This intake can be compared with the 335.6 ± 10.8 and 339.8 ± 17.9 g carbohydrate all subjects (men and women) consumed during the 25% and 35% fat-calorie periods, respectively. Results of analyses of the diets confirmed the validity of the calculated composition and nutrient intakes of the subjects.

Collection of samples. Venous blood was drawn after an overnight fast of at least 12 hr. Eleven samples were taken at given time intervals during the study. Two blood samples were taken a week apart prior to the study. The second blood sample was taken the morning of the start of the first day of the study. For the purpose of this report, the second sample was designated as the "initial" sample. Determinations made on the two samples were averaged for cholesterol and served as the predietary or initial value. All analyses were done in serum or plasma. All of the data reported are for serum except for one intermediate collection during period II. Blood was collected in chilled 50 ml plastic centrifuge tubes and placed in an ice bath immediately. All samples were kept cold until analyzed. LDH, LDH isoenzymes, and pyruvate were analyzed the same day the blood was drawn. Protein-free filtrates were prepared for determination of lactate on the next day. Hemolyzed serum was discarded.

LDH. Total activity of LDH was determined according to the method of Wroblewski and LaDue as adapted by Boehringer-Mannheim: LDH converts pyruvate and NADH to lactate and NAD+. Enzyme activity is measured by the decrease in absorbance of NADH at 340 nm. Levels of enzyme activity are reported in milliunits/min/ml plasma. One unit is the amount of enzyme that converts one micromole of substrate in 1 min under the conditions of the assay at 25°C.

LDH isoenzymes. The isoenzymes of LDH were separated by disc gel electrophoresis on a 5.5% polyacrylamide gel as adapted from the methods of Davis and Dietz and Lubrano.

The bands were stained by a specific stain for LDH at 37°C in an incubation medium containing 1M sodium lactate, 0.1M NaCl, 5 mM MgCl₂, 10 mg/ml phenazine methosulfate, 0.5 M phosphate buffer (pH 7.4), 10 mg/ml NAD, and 1 mg/ml nitro blue tetrazolium; scanned in a spectrophotometer; and the percentages of isoenzyme bands calculated after the peaks were triangulated. M-type LDH was calculated by assuming that band 5, the slowest moving band, contained all M-type subunits; the fastest moving band (band 1) contained all H-type subunits; band 2 contained 3/4 H and 1/4 M; band 3 contained 1/2 H and 1/2 M; and band 4 contained 1/4 H and 3/4 M.

Lactate and pyruvate. Lactate was assayed enzymatically during its oxidation to pyruvate by LDH and NAD⁺ according to the method of Hohorst. Perchloric acid (PCA) precipitated serum was incubated with NAD⁺ and LDH under conditions favoring complete oxidation of lactate to pyruvate (pH = 9, excess NAD⁺ and hydrazine to trap the pyruvate). The lactate concentration was determined by measuring the equimolar formation of NADH at 340 nm.

Pyruvate was assayed enzymatically by a method adapted from Mattenheimer after its reduction to lactate by LDH and NADH. After the serum was precipitated with 0.6 M PCA, phosphate buffer was added to an aliquot of the supernatant so that the final pH was near neutrality. The concentration of pyruvate was determined by measuring the decrease in NADH which is oxidized in equimolar amounts.

Statistical analysis of the data was by analysis of variance and the t test. Correlation coefficients were calculated by standard procedures.

RESULTS

Table 1 shows the mean values for LDH, LDH isoenzymes, lactate, and pyruvate for males and females. Figure 1 shows the data calculated according to the percentage change for individuals from their initial values. The responses of men and women were similar for some of the variables but significant sex differences in responses occurred for others.
Table 1. Mean Values for LDH, LDH isoenzymes, Lactate, and Pyruvate in Men and Women Fed Diets Containing 25% or 35% Fat Calories

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men</th>
<th>Women</th>
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<tbody>
<tr>
<td></td>
<td>Initial (Day 1)</td>
<td>After 25% Fat Calories (Day 39)</td>
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<tr>
<td>LDH activity, µU/ml</td>
<td>102.5 ± 6.5&lt;sup&gt;2&lt;/sup&gt;</td>
<td>72.8 ± 4.5</td>
</tr>
<tr>
<td>LDH isoenzymes, %</td>
<td></td>
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<tr>
<td>LDH-1</td>
<td>38.1 ± 2.2</td>
<td>32.4 ± 0.9</td>
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<tr>
<td>LDH-2</td>
<td>46.2 ± 1.5</td>
<td>45.9 ± 1.4</td>
</tr>
<tr>
<td>LDH-3</td>
<td>12.9 ± 1.3</td>
<td>17.0 ± 1.2</td>
</tr>
<tr>
<td>LDH-4</td>
<td>1.9 ± 0.2</td>
<td>3.5 ± 0.5</td>
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<tr>
<td>LDH-5</td>
<td>0.8 ± 0.3</td>
<td>1.1 ± 0.1</td>
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<tr>
<td>M-type LDH</td>
<td></td>
<td></td>
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<tr>
<td>%</td>
<td>20.3 ± 0.9</td>
<td>23.8 ± 0.6</td>
</tr>
<tr>
<td>Total</td>
<td>20.8 ± 1.7</td>
<td>17.4 ± 1.3</td>
</tr>
<tr>
<td>Ratio [(4 + 5)/(1 + 2)] × 10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>3.31 ± 0.50</td>
<td>6.05 ± 0.76</td>
</tr>
<tr>
<td>Lactate, mg/100 ml</td>
<td>13.90 ± 1.51</td>
<td>10.53 ± 0.83</td>
</tr>
<tr>
<td>Pyruvate, mg/100 ml</td>
<td>0.494 ± 0.06</td>
<td>0.272 ± 0.05</td>
</tr>
<tr>
<td>Ratio L/P</td>
<td>29.6 ± 3.0</td>
<td>46.3 ± 6.0</td>
</tr>
</tbody>
</table>

<sup>1</sup>Ten males and 11 females per group except as indicated.
<sup>2</sup>Mean ± standard error.
<sup>3</sup>Nine values per group.
<sup>4</sup>Ten values per group.
Total activity of LDH fell by almost 30% in both sexes during period I when the highest amount of carbohydrate calories (60%) was fed. The difference was statistically highly significant ($p < 0.001$). During period II, when fat calories were increased to 35% and carbohydrate calories were reduced to 50%, LDH activity rose, not only to its initial activity, but demonstrated an overshoot of about 20% of the initial activity. The sharp decrease in total LDH activity in men after the 25% fat-calorie diet was associated with a change in percentage composition of the isoenzymes (Table 1)—a fall in LDH-1, and a rise in LDH-3 and LDH-4. Percentage composition of the enzyme in men after the 35% fat-calorie diet was almost identical to that of the prestudy (initial) period. No change in percentage composition of the enzyme was seen in women with a change in diet.

While the total activity of LDH fell during period I, percentage of M-type LDH activity was 20% higher in men than initial values ($p < 0.005$). The rise in the percentage of M-LDH in men accounted for the significant rise in this form if data for all subjects are combined. Levels of M-type LDH returned to initial levels during period II. The magnitude of the change in M-type LDH with respect to H-type was seen when the areas of the bands 4 + 5, which
contained most of the M-LDH were related to areas of bands 1 + 2 which contained the predominately H subunit. Both sexes showed about a twofold increase (men higher) in the ratio of bands 4 + 5 to bands 1 + 2 after period I; the rise was statistically significant for men ($p < 0.01$), for women ($p < 0.005$), and for all subjects, ($p < 0.001$). These values did not quite achieve their initial levels after period II. Initial levels of LDH-1 and LDH-3 were different for males and females but the sex differences disappeared when both sexes were eating the same diets.

The lactate and pyruvate responses to changes in diet were dramatically different for men and women. Lactate in men fell by 20% ($p < 0.10$) during period I and rose almost to initial levels after period II. In women, however, lactate levels were more variable but rose by 20% during period I, (not statistically significant) and then fell to initial levels during period II. The sex by diet interaction for lactate response was statistically significant ($p < 0.05$). If the changes for all subjects are combined, very little difference in the lactate response with diet is apparent because of the difference in the response of the men and women to the diets.

A marked change of $-41\%$ in pyruvate was seen in the males after period I ($p < 0.005$). At the end of period II, the value was about 20% less than the initial value but it was not significantly different from the value achieved at the end of period I. Measurements taken about 10 days prior to the end of period II were intermediate between those for periods I and II but they were not significantly different from values for either periods I or II.

Women showed no significant changes in their pyruvate levels with changes in diet and the significant change ($p < 0.05$) seen during period I in the combined value for all subjects is caused by the change in the value for men.

The ratio of lactate to pyruvate—a possible indicator of the redox state of the tissues—rose dramatically in both sexes—$+73\%$ ($p < 0.025$) for men and $+45\%$ ($p < 0.05$) for women—after they ate the high carbohydrate, low fat diet in period I. The value for all subjects was $+59\%$ ($p < 0.005$) after period I. The ratios were still elevated by $+32\%$ and $+24\%$ over initial levels for men and women, respectively, after reduction of dietary carbohydrate in period II.

Simple correlation coefficients reflect the sex differences in response to changes in fat in the diet. In men, LDH had a direct relationship with lactate initially ($r = 0.715, p < 0.05$) and after period I ($r = 0.661, p < 0.05$). With cholesterol there was a similar direct relationship with LDH initially ($r = 0.747, p < 0.05$), and after period I ($r = 0.668, p < 0.05$). In women, these correlations were low and not statistically significant. These differences in responses between the sexes occurred even though mean values for LDH were not vastly different. Similarities in the sex response did occur with respect to changes in the ratio of M-type to H-type LDH with pyruvate (men, $r = 0.702, p < 0.05$; women, $r = 0.782, p < 0.01$), after period I. Although it is not known whether these relationships have any biologic significance, the relationships between the ratio of M-type to H-type LDH and L/P ratio differed for the sexes after period I (men, $r = -0.636, p < 0.05$; women, $r = 0.728, p < 0.05$). Except for the initial values in men pyruvate showed a significant direct association with lactate in both sexes (men, initial, $r = 0.478$ (NS), period I, 0.814, period II,
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0.887; women, initial, \( r = 0.773 \), period I, 0.799, period II, 0.860, \( p < 0.01 \) for all). Pyruvate was highly correlated with serum triglyceride in men after they ate both levels of fat calories (pyruvate versus triglyceride, period I, \( r = 0.760 \), period II, \( r = 0.754, p < 0.05 \)), and in women after they ate the 35% fat-calorie diet (\( r = 0.860, p < 0.01 \)). Lactate was highly correlated with triglyceride in men for all three measurements (initial, \( r = 0.646, p < 0.05 \); period I, 0.884, \( p < 0.01 \); period II, \( r = 0.903, p < 0.01 \)), and in women after they ate 35% fat-calorie diet (period II, \( r = 0.882, p < 0.01 \)). Correlations between lactate or pyruvate with serum cholesterol were not significant for either sex.

**DISCUSSION**

Although the average values for serum LDH in our subjects were within the normal range for the method used, it is difficult to compare our values with published values obtained by other methods. We did not observe the sex differences shown by Meyer et al. However, age differences of subjects in the studies, 40–60 yr in our study and 20–24 yr in the study of Meyer et al., could account for some differences in results. Increasing levels of LDH with increasing age were found by McQueen et al. Length of time of the study and time of measurement—immediate response to a test meal versus longer term response—could also produce important differences in results as a consequence of adaptive mechanisms produced during the longer periods.

Our results did agree with those of Meyer et al. with respect to the finding of a higher initial level of LDH-1 in men than in women and of a higher proportion of LDH-3 in women than in men, except during period I when the highest level of carbohydrate was eaten. No sex differences were found in levels of LDH-4 or LDH-5 in the present study. Differences in LDH isoenzymes of young men and women found by Cohen et al. were attributed to the action of sex hormones in controlling the assembly of the isoenzymes. The sex differences disappeared in older subjects. Total LDH was the same for both sexes.

High levels of pyruvate have been demonstrated to inhibit LDH-1 in some tissues of diabetic rats. Inhibition was removed by the administration of insulin. No sex differences were found in initial pyruvate levels in our study when LDH-1 levels were different.

The decrease in LDH activity from initial levels after period I was unexpected. Rather, an increase in activity was expected after an expected increase in glycolysis with a high carbohydrate diet. Such an increase in LDH activity was seen in studies in young men after the ingestion of 85% carbohydrate calories from sucrose but not after starch or glucose. A similar rise in LDH activity was seen in young women after they were given diets with 42% of the calories as sucrose as compared with the levels when they consumed the control diets. It should be remembered that one-half of the carbohydrate in our study was simple (mono- and disaccharides) and one-half complex (polysaccharides). Under these conditions, the flooding of glycolytic pathways may not have been as great as it would have been if only simple carbohydrate had been used because of the longer time necessary for the digestion and assimilation of polysaccharides.

Our results with healthy subjects confirm the significant positive correlation
between serum levels of lactate and pyruvate reported previously by Kelsay et al. The increase in L/P ratios was also seen when a load dose of various carbohydrates, particularly sucrose and fructose, was given. High L/P ratios are thought to be indicative of a reductive environment favorable for fat synthesis and have been demonstrated to be associated with increased synthesis of fat in arterial tissue. In our study, a transient increase in the level of plasma triglyceride, although not statistically significant, was seen during period I and was found to be directly related to the concentration of lactate and pyruvate. In men, however, the increased L/P ratio may be attributed to the larger fall in pyruvate levels in relation to lactate, while in women, the lactate rose and the pyruvate fell by only small amounts. Although alternative explanations may be equally acceptable, it appears that there was a greater oxidative utilization of pyruvate in men than in women which was not unexpected in view of the well known higher basal metabolic rate for men.

The dynamic changes in carbohydrate metabolism seen after a low fat, high carbohydrate diet were expected in view of an expected increase in glycolysis and subsequent oxidation of by products in healthy individuals. The elevation in the proportion of M-type LDH supports the increase in the rate of glycolysis since this type of LDH has been shown to function more efficiently than the H-type LDH as a means for oxidizing NADH in a rapidly glycolyzing system. In view of the previously cited report of elevated levels of lactate and pyruvate in patients with heart disease, we consider the lowering of pyruvate in men in our study, while they were eating a low fat diet, an effect indicative of an increase in Krebs cycle activity with subsequent oxidation of glucose and fat. The transient increase in triglyceride levels observed in subjects after they had eaten the 25% fat-calorie diet in our study (which returned to initial levels after the 35% fat-calorie diet) was probably due entirely to an increase in triglyceride synthesis since in healthy man no problem with mobilization or accumulation would be expected to occur. In these healthy subjects, the transient nature of the increase in plasma triglycerides appears to support the concept of an increase in synthesis which cannot be verified in our study. An increase in synthesis of triglycerides in man was demonstrated to occur after a test dose of C-labeled sucrose was given. It has been shown that a return to initial levels of plasma triglyceride, after exposure to a high carbohydrate diet, may take longer in older individuals than in younger ones. Rate of removal has been suggested as an important factor in triglyceride accumulation in abnormal subjects after they have eaten high carbohydrate or high fat diets.

Under the conditions of our study, the 35% fat-calorie diet, recommended by the Inter-Society Commission, produced acceptable levels of plasma triglycerides, appeared to have less lipogenic potential, if L/P ratios are considered, and maintained the lower levels of pyruvate produced by the 25% fat-calorie diet. The 35% fat-calorie diet would appear to be the diet of choice, if levels of lactate and pyruvate are lowered first by feeding the 25% fat-calorie diet. While the diet of 25% fat calories of commonly eaten foods in the United States may be beneficial to healthy individuals who can respond with an increase in oxidative activity as a consequence of increased glycolytic activity, such a diet may not have a desirable influence on individuals, such as potential diabetics, in...
whom abnormal levels of metabolites may be induced if they are maintained on it for prolonged periods. A possible block in pyruvate metabolism has been shown in diabetic subjects, in genetically obese mice, and in obese man. Levels of pyruvate and lactate which are readily determined by modern and specific methods may be considered useful in identifying those individuals on high carbohydrate diets whose levels are high but not abnormal or those who have abnormalities in or impairment of pyruvate metabolism.

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