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Changes in Lipoprotein Metabolism during a Supplemented Fast and an Ensuing Vegetarian Diet Period

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ABSTRACT

The effect on lipoprotein metabolism of a 2-week modified fast and an immediately ensuing 3-week period on a vegetarian diet was studied under metabolic ward conditions in 21 non-obese female and 6 male patients. The very low calorie diet induced reductions of the cholesterol concentration in all serum lipoprotein classes. In the female patients, who were all normolipoproteinaemic, the triglycerides in serum showed a slight increase during the fast, reflecting small changes in very low (VLDL) and low density lipoprotein triglycerides. This may probably be explained partly by simultaneous significant reductions of both the adipose tissue and skeletal muscle tissue lipoprotein lipase activities (LPLA). In contrast, in the male patients who had a higher VLDL level at admission, the VLDL triglycerides decreased without significant changes of high density lipoprotein (HDL) cholesterol and of LPLA in muscle.

The female patients, whose weights were stable during the vegetarian diet, ended up with a lower HDL cholesterol than at the start of the trial. This effect was probably partly due to the high content of polyunsaturated fatty acids in the vegetarian diet.

It is concluded that the changes of lipoprotein metabolism during supplemented fasting are quantitatively and qualitatively different in several respects in females and males.

INTRODUCTION

The reported effects of low-calorie diets on the lipoprotein pattern have varied considerably. For example decreased (24), unchanged (28) and increased (22) levels of high density lipoprotein (HDL) cholesterol have all been

found during this treatment. There is a need for elucidation of the reasons for these discrepancies. We have carried out an investigation on the effects of a supplemented fast and an ensuing period on a vegetarian diet on the clinical condition in patients with different diseases (12). The present report deals with the lipoprotein changes that were observed in this group of non-obese females and males under metabolic ward conditions during the supplemented fast. In addition, analyses of lipoprotein lipase activities gave an opportunity for mode of action studies. Some aspects of the changes that occurred during the period on the vegetarian diet are also reported and discussed.

MATERIALS AND METHODS

Subjects

Twenty-seven patients, 21 females and 6 males, were studied. Two subjects (one female, one male) deteriorated clinically during the fasting period and interrupted the treatment trial. The mean age of the women was 46 years and of the men 41 years (ranges 24-64 and 23-55, respectively). Eight patients had psoriasis with arthritis, 4 had rheumatoid arthritis, one had spondylarthritis deformans, one had lupus erythematosus disseminatus, 4 had pustulosis palmaris et plantaris, 3 had atopic eczema, 3 had acne rosacea and 3 had a non-specific arthritis. None of the patients was febrile. One was being treated with corticosteroids in a low, constant dose. Treatment with other anti-inflammatory drugs was kept constant throughout the treatment period. For further information concerning the clinical conditions and other details see (12).

Experimental Procedure

The whole study was carried out under metabolic ward conditions. During the first day the ordinary hospital diet was given. After the second day's test the patients were given the vegetarian diet at a low energy level (5.0 MJ/day) for 2 days. They then fasted for 11 days, during which time only vegetarian beverages and herbal teas were given (0.9 MJ/day). After the fast was broken the energy intake was gradually increased during the first 3 days (1.1, 1.7 and 2.6 MJ/day), after which it was adjusted according to the patient's body weight (150 and 130 kJ/kg body weight for males and females respectively) aiming at a stable body weight during the last 2 weeks.

On admission, clinical examinations and laboratory tests were performed on the first and second days. All studies made on admission were repeated at the

end of the fast and at the end of the vegetarian diet period. All tests were carried out in the morning after an overnight fast.

Diets

During the fasting period vegetarian beverages and herbal teas were given every 2 to 3 hours. The beverages were made from different root vegetables, such as beetroot, carrots and potatoes, and from blueberries and strawberries.

A 7-day menu was used throughout the vegetarian diet period. The composition of the food was calculated from food tables (13). The fat content and composition were analysed as described earlier (27). All animal protein (meat, fish, egg, milk) was excluded, which made the diet rather low in protein content (Table 1). The energy derived from fat and carbohydrates was percentually similar to that in ordinary Swedish food (Table 1). The vegetarian diet was based on fresh vegetables, root vegetables, millets, lentils and buckwheat. Sunflower oil (with a high content of polyunsaturated fatty acids) was used in dressings etc. The average ratio of polyunsaturated to saturated fatty acids (P/S-ratio) was 4.3 ± 0.3 (mean ± SD). No extra salt or sugar was allowed. The only simple carbohydrates available were in the berries and fruits that were included; these were permitted in a controlled and limited amount. The average fibre content was 21.5 ± 1.9 g/4.2 MJ.

Table 1

Contents of Protein, Fat and Carbohydrates Calculated per 1.0 MJ in Grams and as Per Cent of Total Energy (E%).

	Vegan diet		Ordinary Swedish diet*		Swedish nutrition recommendation
	content	E%	content	E%	E%
Protein	5.5±0.5	9	7.6	13	10-15
Fat	10.5±0.5	42	10.3	40	<35
Carbohydrate	29.9±1.2	51	27.5	48	50-60

*J Agricult Econ 42:7-8, 1980 (food balance sheet).

Statistics

A 3-way analysis of variance was used to differentiate between the influences of sex, treatment and individuals on the results. Results are expressed as mean ± SEM. Significant changes are indicated by * = $p<0.05$, ** = $p<0.01$ and *** = $p<0.001$. When a change was different in females and males, this is indicated in the figures by ≠ followed by the sign for the level of significance.

Laboratory Procedures

A detailed description of the analysing procedures for lipoproteins and apolipoproteins has been published previously (26). In summary, very low density lipoproteins (VLDL) in serum were isolated from the top fraction after ultracentrifugation at a density of 1.006 in a Beckman LKB-65 ultracentrifuge, using a 40.3 rotor. Low density lipoproteins (LDL) were precipitated from the bottom fraction after the ultracentrifugation step using a heparin-manganese-chloride solution, and HDL were isolated from the supernatant after the precipitation. The concentrations of triglycerides and cholesterol were determined in whole serum, VLDL, HDL and the bottom fraction. The lipid concentrations in LDL were calculated as the differences between the concentrations in the bottom fraction and HDL. A Technicon Auto Analyzer II was used for determination of triglycerides and cholesterol (18). The concentrations of apolipoprotein (apo) B and apo A-I in whole serum were determined by an electroimmunoassay technique. Details of these analyses and of the antibody production, purification and standardization have been presented (26).

Specimens of abdominal adipose tissue and of the lateral vastus muscle were taken and used for determination of LPLA as earlier described (9,10) with slight modifications. Thus a triolein-phospholipid emulsion (11) and a heparin concentration of 2 g/l (about 240 IU/ml) were applied for both adipose and skeletal muscle tissue. The activity is expressed in mU/g (1 mU = 1 nmol fatty acid released per min).

RESULTS

All patients were of normal body weight (relative body weight <1.30) at admission. The body weight (Fig. 1) decreased significantly in both sexes by about 10% during the 2-week fast. During the subsequent vegetarian diet period it did not change significantly. However, some of the young men did not reach a new stable body weight during the last 2 weeks.

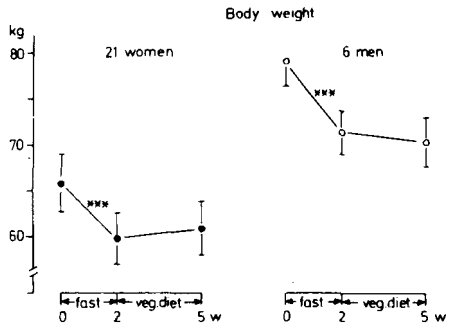

Fig. 1: Body weight in women and men on admission, after a 2-week vegetarian fast and after a 3-week vegetarian diet.

The serum cholesterol concentration (Fig. 2a) decreased during the fast and in the women it remained at the lower level until the end of the vegetarian diet. The pattern was similar among the men, but the decrease also continued during the vegetarian diet period.

The serum triglyceride concentration (Fig. 2a) changed in different ways among the women and the men ($p<0.05$). In the women it increased slightly during the fast but returned to pretreatment values during the vegetarian diet period. In the men the initial value was 1.9 $\pm$ 0.1 (mean SEM). After the vegetarian diet it was 1.2. This value was close to that found in women (1.1 $\pm$ 0.1).

In VLDL (Fig. 2b) neither the cholesterol nor the triglyceride concentrations changed significantly in the women during either period. However, women and men differed significantly with regard to the effects on VLDL during the fast, mainly due to the fact that in the men both cholesterol and triglycerides in VLDL decreased in parallel, whereas in the women the VLDL triglyceride concentration tended to increase.

In LDL (Fig. 2c) the cholesterol concentration decreased during the vegetarian diet period in both sexes following a reduction (significant among women) during the fast. During the fasting period the LDL composition changed towards a triglyceride enrichment relative to cholesterol (significant among women).

In HDL (Fig. 2d) the pretreatment cholesterol concentration was higher in females than in males. It decreased significantly among women from 1.5 to 1.1 mmol/l during the fast. The concentration at the end of the vegetarian diet period (1.2 mmol/l) was significantly lower ($p<0.01$) than the initial value. In males the pattern was similar to that in females, but the changes were less pronounced.

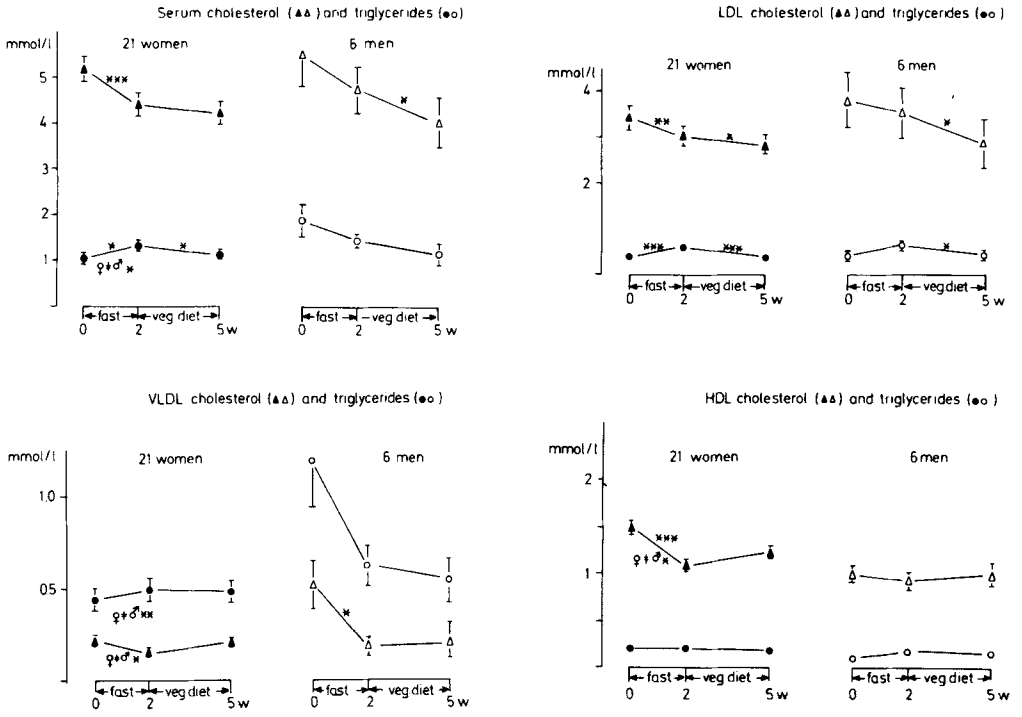

Fig. 2: Cholesterol and triglyceride concentrations (mmol/l) in serum, in VLDL, in LDL and in HDL.

The serum apo B concentration (Fig. 3a) showed similar reductions in women and men, with a pronounced reduction during the vegetarian diet period in both sexes, but small and non-significant reductions during the fast.

The serum apo A-I and A-II concentrations (Fig. 3a) altered in similar ways. In women they decreased during the fast and then increased during the vegetarian diet period to levels that were not significantly different from those at the start of the treatment. In men the changes were similar but less pronounced.

LPLA in adipose tissue and skeletal muscle decreased significantly in the women during the fast and then increased during the vegetarian diet period (Fig. 3b). In adipose tissue (but not in muscle) the enzyme activity was at a lower ($p<0.05$) level at the end of the vegetarian diet than before treatment. The change in LPLA was similar in men and women except during the vegetarian diet, when adipose tissue LPLA altered in different directions in the sexes. However, the unstable body weight in some men may have contributed to this difference.

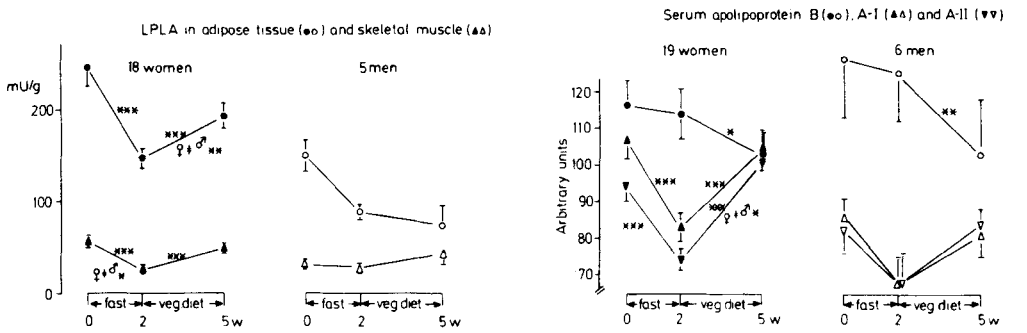

Fig. 3: Serum apo B, A-I and A-II concentrations in serum and LPLA in adipose tissue and skeletal muscle. Statistics as in Fig. 2.

DISCUSSION

The effects of different low calorie diets on lipoprotein metabolism have been studied in several investigations, but the results have varied considerably. This is probably due to the fact that many factors are important for the findings. For example it may be suggested that the results differ between sexes (2 and present study), between hyper- and normo-lipoproteinaemic subjects (8), and between very low and low calorie diets (to be published). In addition, different length of the study period have been shown to influence the results (24). The conditions in the present study differ in some respects from those in most others. Thus, none of the patients was obese. Women were in the majority (21/27) and were normolipoproteinaemic. Only 2 of the male patients had hyperlipoproteinaemia type IV.

The lipoprotein alterations in the 6 male patients included parallel decreases in VLDL cholesterol and triglycerides, and very slight, non-significant decreases in both LDL and HDL cholesterol.

The results in the 21 females differed in several respects from those in the males. Thus, the serum triglycerides increased significantly in females but decreased non-significantly in males. In VLDL, the pattern of change in triglycerides differed ($p<0.01$) between the sexes. The increases in triglycerides in VLDL (n.s.) and in LDL ($p<0.01$) in the female group were similarly large and could together have explained the increase in serum triglycerides ($p<0.05$). The lower LPLA in both adipose and muscle tissue after the very low calorie diet (confirming (24)) indicates a decreased capacity for peripheral hydrolysis of VLDL. In the females it is likely that the production of VLDL was normal from the beginning of the trial. Higher rates may have been in-

duced under the influence of the higher concentrations of free fatty acids during starvation-induced lipolysis (7) but during fasting triglyceride production is decreased and positively correlated only to the plasma insulin level, not to the level of free fatty acids (23). The fasting insulin concentration decreased significantly in the present study (to be published). Therefore, it seems likely that most of the increased level of VLDL triglycerides was due to a decreased LPLA and not to an increased production rate of VLDL. In contrast to the women, some of the men, with higher initial mean triglyceride levels, may have had an increased rate of VLDL production already at the start (17). Furthermore, their decrease in muscle LPLA was less pronounced than in the women. These factors may partly explain the observed differences between men and women.

The HDL-cholesterol concentration in the women was normal at the start of the trial. Both the HDL cholesterol and the apo A-I and A-II concentrations decreased during the fast. Hepatic lipase, which is probably involved in the degradation of HDL in the liver (14), is decreased during fasting (1,9), an effect which should lead to an increased HDL level. The reduction in HDL (cholesterol and protein) in the female subjects must therefore have been due to a lower production rate of HDL. It seems likely that the rate of synthesis of HDL in the intestine is decreased during this dietary-fat deprivation, since fat stimulates this synthesis (20). However, the reduced capacity for VLDL-triglyceride hydrolysis may partly account for the decrease in HDL cholesterol (25). The male group showed only a small change (n.s.) in HDL cholesterol, but they differed from the female group in their pre-trial level and also exhibited a smaller change in muscle LPLA, factors which could account for the observed difference in the change in HDL cholesterol. In a group of grossly obese women subjected to a 4-week fast of a type similar to that in the present study, Sörbris et al. found an increase in HDL cholesterol (22). In their study the subjects were encouraged to take part in regular, extended physical activities of moderate intensity which may have contributed to the increase in cholesterol in HDL.

The results of the vegetarian diet period are difficult to interpret, owing to the design of the study. This, however, was adopted from the one used at many health resorts in Sweden in an effort to critically evaluate the claimed therapeutic effect of such treatments particularly in subjects with skin and joint diseases. This evaluation comprised clinical condition, biochemical changes and side effects. During the vegetarian diet period, not only the dietary

change but also the accomplished reduction of body weight may have contributed to the final lipoprotein values.

Some young male patients did not conform to the metabolic ward conditions and did not eat their rations of vegetarian diet and therefore continued to decrease in body weight. This fact invalidates any comparisons between female and male reactions in this part of the study. In contrast to the males, the females maintained a steady body weight during this part of the study at a lower level than at the start. The women's post-trial values differed significantly in three respects from their pre-trial values, namely in the HDL cholesterol and in the apo B concentrations and the adipose tissue LPLA. Of these, the decrease in apo B may partly be related to the lower body weight, since LDL cholesterol is lower (5,6), whereas both HDL cholesterol (4,5,16) and adipose tissue LPLA (21) would rather be expected to be at a higher level after a body weight reduction than before.

The lower HDL-cholesterol value may therefore most likely be attributable to the vegetarian diet, which would be in accordance with the finding that female vegetarians have lower HDL cholesterol levels than female control subjects (3, 19). A substitution of polyunsaturated fats for saturated is associated with a decrease in HDL cholesterol (6,26). The very high P/S ratio in the vegetarian diet therefore probably accounted for part of the change in our study. In fact, some of the decrease in apo B may also be attributable to the use of polyunsaturated fats in the vegetarian diet (27), whereas the decrease in adipose tissue LPLA is not (27).

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