ORIGINAL ARTICLE

Changes in adipose tissue and increased serum cholesterol of coronary patients following training

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Summary: A study was undertaken to investigate whether a physical training effect could be observed in the adipose tissue composition of coronary patients. The results indicated that there was an increase in the proportion of palmitic acid and a decrease in oleic acid. These changes were accompanied by an increase in blood cholesterol.

Résumé: *Les modifications de composition du tissu adipeux chez des coronariens, sous l'influence d'exercices physiques*

Nous avons entrepris d étudier si un programme d'exercices physiques était susceptible d'affecter la composition du tissue adipeux chez des coronariens. Cette étude a permis de découvrir une augmentation de la proportion d'acide palmitique et une diminution de l'acide oléique. Ces modifications étaient accompagnées d'une élévation de la cholestérolémie.

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Physical inactivity in modern man has perhaps created a greater need in his diet for polyunsaturated fatty acids. This interesting aspect of the relationship of physical activity and blood lipids has been raised by Simko *et al* as a result of studies on fat metabolism in rats submitted to intense physical exercise.[1] These authors observed "that epididymal fat triglycerides in exercising rats displayed, as compared to controls, a higher proportion of saturated fatty acids mainly because of a higher amount of palmitic acid. The proportions of monoenes and of linoleic acid were significantly lower in the depot fat triglycerides of exercising animals".

The purpose of the present study was to determine if such a phenomenon could be observed in coronary patients following a physical exercise program.

Subjects and methods

Ten male patients (aged 43.6 ± 3.9 years) were selected for this study, all of whom had had a myocardial infarction in the past one to three years, and four of whom suffered from angina pectoris.

Functional work capacity

Functional work capacity (FWC), as measured by oxygen consumption ($\dot{V}O_2$), was determined before and after training by means of a modification of the Balke multi-stage treadmill technique. A warm-up walk of 2 minutes at 2 m.p.h. was followed by a 3-minute rest period. The patient then walked at 2 m.p.h. on a 0% grade for 3 minutes with subsequent and continuous grade elevations of 3.5% every 3 minutes. FWC was defined as the $\dot{V}O_2$ (ml./kg.-min.) measured the minute preceding the attainment of one of the following criteria: the patient developed progressively severe angina, reached 85% of age-adjusted maximal heart rate, or exhibited premature ventricular beats in series. ST-segment depression was not an indication for terminating the test.

$\dot{V}O_2$ was continuously determined by means of an open-circuit technique in which the patient breathed through a low resistance valve. A portion of the well mixed expired air was withdrawn from an 8-litre chamber and analysed for O_2 and CO_2 concentrations by means of Beckman E2

and LB1 gas analysers, respectively. Minute ventilation was measured with a Parkinson-Cowan CD-4 dry gas meter.

In addition, the patients were also evaluated before and after training for heart rate response to a single upright exercise of submaximal intensity (200 to 400 kg.m./min.) on the bicycle ergometer.

Training program

The training program lasted 13 weeks and consisted of three medically supervised group-sessions per week in which the patients exercised on bicycle ergometers. During the first two weeks the patients performed 5 minutes of work of low intensity ($\bar{X}$ = 160 kg.m./min.), 21 minutes of moderately intense work ($\bar{X}$ = 300 kg.m./min.; mean heart rate = 114), followed by another 4 minutes at low intensity. This format was continued throughout the training program although the duration of the moderately intense work period was extended to 36 minutes (third week) and the work intensities were gradually increased according to the work capacities and training adaptation of each patient. During the 13th week, work intensity and heart rate averaged 732 ± 130 kg.m./min. and 136 ± 13 beats/min., respectively.

All 10 patients completed the training program with excellent adherence. Eight of the ten patients did not miss more than one scheduled session. Business commitments accounted for three and eight absences of the other two patients. Although readily available, serious emergency measures did not have to be employed. Early in the training program one patient was digitalized for paroxysmal atrial fibrillation; digoxin was stopped three days prior to the post-training work capacity evaluation. One patient took sublingual nitroglycerin prior to each exercise session. Nine patients took anticoagulants (Warfilone®) prior to the training program and continued to do so throughout the study.

Table I—Estimates of components of variation in fatty acid analysis of adipose tissue (σ^2)*

	Estimated variance		
Fatty acid**	Chromatogram	Sample	Subject
12:0	0.005	0.003	0.113
14:0	0.060	0.001	0.905
15:0	0.005	0.002	0.065
16:0	0.543	0.026	4.950
18:0	0.105	0.034	0.370
16:1	0.034	0.049	4.276
18:1	0.634	0.151	8.958
18:2	0.619	−0.179	17.657

*Analysis of variance based on necropsy specimens from ten individuals (see text), three samples per specimen and two chromatograms per sample.
**Fatty acids are numbered according to number of carbon atoms per molecule (number before colon) and double bond content (number after colon). 12:0 lauric; 14:0 myristic; 15:0 pentadecanoic; 16:0 palmitic; 18:0 stearic; 16:1 palmitoleic; 18:2 linoleic.

Table II—Effect of training on functional work capacity of coronary patients

	Heart rate†	$\dot{V}_{O_2}$ (ml./kg.-min.)	$\dot{V}_{O_2}$ (litres/min.)
Before	141 ± 15	20.6 ± 4.0	1.56 ± 0.36
After	141 ± 14	22.5 ± 4.0	1.75 ± 0.39
Difference	0 ± 3	*1.9 ± 1.7	*0.18 ± 0.20

Values are means ± standard deviations
†Heart rate was fixed according to criterion for work capacity evaluation
*Significant difference: $P < 0.05$

Biochemical determinations

Fasting blood and adipose tissue samples were collected during the week preceding the functional work capacity evaluation.

Adipose tissue was aspirated from about the midline of the anterior abdominal wall according to the method of Hirsch *et al.*[2] Serum total cholesterol and triglycerides were determined simultaneously by an automated method, as in a previous study.[3] Analysis of fatty acid from adipose tissue was carried out as described previously.[4] The triglycerides were methylated by refluxing them with methanol containing 2% (V/V) concentrated H_2SO_4 for 3 hours at 68°C.

Estimates of sources of variation in fatty acid analyses

To provide a quality control of the entire procedure of determination of fatty acid composition of adipose tissue, three components of variation were estimated, namely subject, sample and the chromatogram as described by Insull and Bartsch.[5] Blocks of about 4 g. of subcutaneous adipose tissue were obtained from 10 consecutive necropsies of men and women aged 20 to 70 years who had no peptic ulcer, cancer, cirrhosis or history of alcoholism.

Table I shows the estimated variances. The subject variance was much greater than that of the chromatogram or sample. The analytical protocol chosen was one sample and two chromatograms since the variance was generally greater for the chromatograms. When two chromatograms differed by more than $2\sqrt{2}$ (σ^2 sample + σ^2 chromatogram) they were repeated in duplicate. If the difference was still greater than the criterion of quality established above, a new extract of the same sample was prepared and processed for gas-liquid chromatography. The mean of duplicate chromatograms was used in the calculation of the data.

Estimates of variation in nutritional habits during the exercise program

The various lipid classes (cholesteryl esters, phospholipids and triglycerides) were isolated from one fasting blood sample withdrawn before the exercise program and from another sample taken at the end of the program. The lipid classes were isolated as described by Hirsch and Ahrens[6] on a total lipid extract using the procedure of Folch, Lees and Sloane-Stanley.[7] Absorbent silicic acid (Bio-Sil HA, Bio-Rad, Richmond, California) was used in the separating column which was maintained at 25 ± 0.5°C. throughout. The purity of each separation of the lipid classes was always checked on chromatography medium I.T.L.C. (Type 20 cm. x 20 cm., Gelman Instrument Co., Ann Arbor, Michigan). The development mixture contained isooctane/isopropyl ether (90/5)(V/V). The papers were air-dried and soaked in rhodamine 6G and subjected to ultraviolet lamp examination. Adequate controls were run simultaneously. When the lipid classes were contaminated the separation was repeated.

Gas chromatography

Essentially the same procedure and apparatus were used as described previously.[4] The fractions were methylated as described above. An estimate of variance was made for each lipid class as done for the triglycerides from adipose tissue. These results will be published elsewhere.

Results

Training results

There was a significant change in FWC with training (Table II), as indicated by the 9.2% increase in the mean $\dot{V}_{O_2}$ (20.6 to 22.5 ml./kg.-min.) at a constant mean heart

rate of 141 beats/min. A training effect was also observed as a result of the single submaximal bicycle ergometer evaluation. There was a significant decrease ($P < 0.05$) in heart rate (106 to 94 beats/min).

Biochemical results

Table III shows the serum lipid concentration and weight before and after the training program. A mean increase in cholesterol of 30 mg./100 ml. was observed following the training program, this difference being significant ($P < 0.05$). The triglyceride concentration and body weight remained the same. The increase in the mean cholesterol concentration cannot be attributed to a change in nutritional habits during the exercise program since the fatty acid composition of the various serum lipid classes did not vary (Tables IV, V and VI) although a slight change was noted in stearate and myristate percentages in the phospholipids and in the myristate proportion in the triglyceride fraction.

In the adipose tissue (Table VII) a significant modification was observed in the proportion of palmitic and oleic acids. The percentage of palmitic acid increased and that of oleic acid decreased, both changes being highly significant ($P < 0.005$).

Discussion

The difference in concentration of blood lipids in active versus sedentary subjects and the effect of physical training on blood lipids of sedentary people are not at all clearly stated in the literature.[8,9] Whether the application of a physical activity program is beneficial or not to coronary patients who were sedentary before the overt manifestation of their disease remains an open question.[9] Some experimental work with dogs[10] and rabbits[11] raises "the possibility that in arteries already hampered by sclerosis, *strenuous exercise* might act as a pathogenic stress".[8] These experi-

Table III—Serum lipid concentration before and after physical training program

Lipid (mg./100 ml.)	Initial	After	P*
Cholesterol	244 ± 51 (167 – 329)†	274 ± 58 (183 – 371)	< 0.05
Triglyceride	340 ± 167 (122 – 720)	330 ± 225 (107 – 917)	NS
Weight (kg.)	77.4 ± 7.7	78.3 ± 9.8	NS

*Paired t-test
†Range
NS = no significant difference

Table IV—Serum cholesteryl ester fatty acid composition before and after physical training program (% of total fatty acids)

Fatty acid	Initial	After	P*
Myristate (14:0)	0.9 ± 0.4	0.8 ± 0.3	NS
Palmitate (16:0)	12.0 ± 2.0	11.0 ± 1.4	NS
Stearate (18:0)	0.7 ± 0.4	0.6 ± 0.2	NS
Palmitoleate (16:1)	4.3 ± 2.4	4.3 ± 1.6	NS
Oleate (0) (18:1)	21.4 ± 5.6	19.6 ± 3.3	NS
Linoleate (L) (18:2)	55.5 ± 10.4	57.8 ± 7.0	NS
L/O ratio	2.9 ± 1.2	3.1 ± 0.8	NS
Total saturated	13.5 ± 2.4	12.3 ± 1.6	NS
Total monounsaturated	25.7 ± 7.4	24.0 ± 4.6	NS
Total polyunsaturated	60.8 ± 9.5	63.8 ± 6.0	NS

*Paired t-test
NS = no significant difference

Table V—Serum triglyceride fatty acid composition before and after physical training program (% of total fatty acids)

Fatty acid	Initial	After	P*
Myristate (14:0)	2.7 ± 1.1	1.8 ± 0.8	<0.025
Palmitate (16:0)	28.1 ± 3.2	27.1 ± 1.8	NS
Stearate (18:0)	4.5 ± 1.1	3.6 ± 0.9	NS
Palmitoleate (16:1)	5.9 ± 1.6	5.8 ± 1.0	NS
Oleate (18:1)	42.7 ± 4.6	44.1 ± 2.7	NS
Linoleate (18:2)	16.1 ± 7.5	17.7 ± 4.6	NS
Total saturated	35.0 ± 4.6	32.4 ± 2.6	NS
Total monounsaturated	48.6 ± 5.7	49.9 ± 3.4	NS
Total polyunsaturated	16.1 ± 7.5	17.7 ± 4.6	NS

*Paired t-test
NS = no significant difference

Table VI—Serum phospholipid fatty acid composition before and after physical training program (% of total fatty acids)

Fatty acid	Initial	After	P*
Myristate (14:0)	0.4 ± 0.1	0.3 ± 0.1	<0.005
Palmitate (16:0)	33.5 ± 2.9	34.6 ± 2.1	NS
Stearate (18:0)	16.6 ± 1.3	15.5 ± 1.1	<0.025
Palmitoleate (16:1)	1.1 ± 0.7	1.1 ± 0.3	NS
Oleate (18:1)	15.5 ± 2.7	14.8 ± 1.8	NS
Linoleate (18:2)	24.3 ± 4.7	24.1 ± 3.8	NS
Arachidonate (20:4)	8.5 ± 2.0	9.8 ± 2.4	NS
Total saturated	50.7 ± 2.7	50.3 ± 1.8	NS
Total monounsaturated	16.6 ± 3.1	15.9 ± 2.0	NS
Total polyunsaturated	32.8 ± 4.2	33.8 ± 3.1	NS

*Paired t-test
NS = no significant difference

Table VII—Adipose tissue fatty acid composition before and after physical training program (% of total fatty acids)

Fatty acid	Initial	After	P*
Laurate (12:0)	0.3 ± 0.3	0.2 ± 0.2	NS
Myristate (14:0)	3.1 ± 0.7	3.0 ± 1.3	NS
Pentadecanoic (15:0)	0.6 ± 0.2	0.6 ± 0.3	NS
Palmitate (16:0)	24.5 ± 2.5	25.4 ± 2.6 ↑	<0.005
Stearate (18:0)	4.0 ± 0.8	4.4 ± 0.9	NS
Palmitoleate (16:1)	5.8 ± 1.5	6.1 ± 1.5	NS
Oleate (18:1)	51.4 ± 2.2	49.5 ± 1.9 ↓	<0.005
Linoleate (18:2)	10.3 ± 3.2	10.8 ± 3.8	NS

*Paired t-test
NS = no significant difference

mental data leave open the question of what is a strenuous physical activity for a coronary patient.

The results of the present investigation with exercising coronary patients partly confirm the observations made on rats by Simko *et al* quoted in the introductory paragraph.[8,1] The proportion of linoleate we found in adipose tissue, however, did not vary in exercising coronary subjects and the serum cholesterol increased. Unpublished data from this laboratory show that the mean initial blood cholesterol of 233 mg./100 ml. increased to 253 mg. among 14 coronary patients who participated twice a week for one year in an exercise program in a gymnasium ($P < 0.02$). In a recent communication Landry *et al*[12] reported that in coronary-prone subjects a significant increase in serum cholesterol was apparent following a 10-week exercise program even though the subjects lost weight. Results of a study of the effect of a supervised activity program on three principal high risk factors, including serum cholesterol, in a group of high-risk men free of coronary heart disease, were recently presented by Taylor.[13] One conclusion was that supervised exercise does not influence serum cholesterol. The training regimen used by Taylor was not the same as ours and the difference may explain why the cholesterol concentration did not increase; but the important point is that this constituent did not decrease, as the results of some studies indicate.[9] Conflicting results of investigations of the effect of training on blood cholesterol may arise from failure to take into account differences in body build of the subject, as suggested by Campbell and Lumsden.[14]

The significant change in the percentage of palmitic and oleic acids in the subcutaneous fat of coronary patients after such a short training period (13 weeks) seems a striking phenomenon. It is known that the response of adipose tissue to even a drastic change in diet becomes apparent only after a much longer period of time.[15] Moreover, a change in diet cannot be implicated since the composition of the circulating lipids and the weight did not change after training.

Whether an increment in the proportion of palmitic acid in the adipose tissue of coronary patients is a beneficial effect or not is not known. Insull, Houser and Littell[16] have suggested that there might be a metabolic link between the concentration of serum cholesterol and the proportion of palmitic acid in adipose tissue. The present results support the hypothesis that such a link exists.

It is of interest to mention also that in human diets palmitic acid is one of the important hypercholesterolemic fatty acids.[17]

It has recently been shown that "plasma free fatty acid composition during exercise tends to resemble more closely that of adipose tissue".[18] It might be that the composition of the fatty acids released into the circulation when mobilization from adipose tissue occurs is different in trained and untrained individuals. Therefore, a relative increase in the circulating free palmitic acid might favour an increase in the serum cholesterol concentration in coronary patients submitted to physical training.

In conclusion, it is suggested that exercise programs for coronary patients should be accompanied by dietary intervention to treat hyperlipoproteinemia according to the individual type of dyslipidemia.

An increment of a risk factor already present (such as high blood cholesterol concentration) in coronary patients is not desirable.

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