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Basic Research

The Fatty Acid Composition of Blood Plasma and Arterial Wall in Atherosclerosis

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Abstract

The aim of this study was to assess the fatty acid balance in the blood plasma, in the fragments of the intact vessels and the vessels showing signs of atherosclerosis. ***Material and Methods***: The article presents the results of the examination of the blood plasma samples of patients with coronary heart disease and coronary atherosclerosis. The control group consisted of 16 healthy individuals. Also, the fragments of the abdominal aorta and the common carotid artery with varying degrees of atherosclerotic lesions were studied. Fatty acid analysis was conducted using capillary gas-liquid chromatography. ***Results:*** A reduction in the relative linoleic acid level with an increase in almost all the saturated fatty acids and polyunsaturated dihomo-γ-linolenic acid in the plasma was noted in patients with coronary heart disease and atherosclerosis. The relationships between certain fatty acids in patients with atherosclerosis and myocardial ischemia showed changes. In patients with atherosclerosis, a marked imbalance was observed between the monounsaturated and correspondingly the saturated fatty acids (with the same number of carbon atoms) in the abdominal aorta with significant atherosclerotic lesions, as well as in the intact common carotid arteries. These disorders are probably related to the relatively low content of the linoleic acid in the blood plasma. The reasons for the increased activity of the fatty acid synthase in vessels with significant atherosclerotic lesions are described. It is concluded that most of the fatty acids of the plaque were formed due to the synthetic processes in the smooth muscle cells, and not as a result of their intake from the blood plasma.

Key words: *atherosclerotic plaque, coronary heart disease, abdominal aorta, common carotid artery.*

Introduction

For many years, there has been a great interest in the problem of lipid accumulation in the arterial wall in patients with atherosclerosis. However, some aspects of the atherosclerotic lesions of the vessels and causes of dyslipidemia remain unclear [1,2]. Questions related to the role of the individual fatty acids (FA), including polyunsaturated FA (PUFA), in the development of atherosclerosis warrant further research.

It has been shown that cholesterol (as well as compounds possessing fatty acid) is the predominant lipid of the atherosclerotic plaques (AP) [3]. It is accepted that cholesterol almost wholly enters from the blood as a component of lipoproteins, and is not a result of local synthesis [4]. However, a study of the lipids in the AP and lipid spots revealed that the plaques contain significantly more free cholesterol relative to the cholesterol esters (CE) [3]. This fact opposes the concept of the origin of the atheromatous lipids from the plasma, as most part of the plasma cholesterol is represented by the CE [5]. The mechanism of the lipid penetration through the fibrous cap of the plaque remains unclear. According to one theory, the AP growth is due to the lipid accumulation coming from the vessels growing into the plaque [6]. However, it has been shown that the cells of the arteries synthesize the FA *de novo*, and that the FA are synthesized in greater quantity in the atherosclerotic arteries than in the intact arteries [7].

Thus, although the study of atherosclerosis is the subject

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of numerous publications [1-7], to date, several questions still remain unanswered regarding the different stages of atherogenesis that warrant further study. Also, the recent data recorded on the metabolism of the fibrous plaque are still insufficient.

The aim of this study was to assess the fatty acid balance in the blood plasma, in the fragments of the intact vessels and the vessels showing signs of atherosclerosis.

Material and Methods

The objects in this study were fragments (weight 2-3 g) of the abdominal aorta and the common carotid artery from the dead bodies of nine men (average age at death was 50 ± 6.7 years). Also, 3-4 mL of blood was taken from each corpse.

It was shown [8] that the AP was most often localized in the wall of the abdominal aorta. The common carotid artery showed more resistance to atherosclerotic lesions. In this work, all the samples of the common carotid artery studied showed no atheromatous changes. All the samples of the abdominal aorta had varying degrees of atherosclerotic lesions. Six of them revealed the 4th type (control), three showed the 5-6th type of atherosclerotic lesions (experimental group). The atherosclerotic lesions were assessed based on the classification developed by H.C. Stary [8].

The blood plasma samples of 16 patients (57.1 ± 1.4 years) with coronary heart disease (CHD), coronary atherosclerosis, angina II-III functional class, and hypertension were studied. The blood plasma samples of 16 healthy volunteers served as the control (37.7 ± 3.2 years).

As an adequate model of atherosclerosis cannot be obtained in rats with high-cholesterol diets [9], we examined the FAs present in the blood plasma of 20 rats and in the abdominal aorta of 4 rats.

The preanalytical phase of the study involved the isolation of the cellular component of the plasma by centrifugation. To obtain the ethyl esters of the fatty acids of the plasma lipids, derivatization was performed in 1.5 M HCL in ethanol at a temperature of 60 ° C for 1 hour. The ethyl esters of the fatty acids thus obtained were extracted from the reaction mixture using hexane. The fatty acid derivatives of the blood vessels were obtained after lipid extraction from the homogenized samples using ethanol. Next, homogenization of the blood vessel fragments was performed, until smooth. They were first ground with a porcelain pestle in a mortar with ground glass and a small amount of ethanol. Similarly, acidic ethanolysis was done and the ethyl esters of the fatty acids were extracted using hexane.

To analyze the FA in the hexane extracts, gas-liquid chromatography was employed and registration of the identified chemical compounds was done using a flame ionization detector [10]. The measurements were done on the gas chromatograph GC-1000, Tsvet-800 (Russia). Separation of the analytes was performed using a capillary column 60 m in length and 0.56 mm inner diameter with a silicone stationary phase (SE-30), and a 0.25 micron sorbent membrane thickness. Nitrogen was used as the carrier gas. Chromatographic conditions included injector temperature 280°C, detector temperature 290°C, carrier gas consumption at 60 cm^3/min and partition coefficient of the carrier gas flow 1:12. The chromatograms were produced by using the nonlinear heating program of the column thermostat. In the first stage, separation was performed for 30 minutes under isothermal conditions at 150°C. Then the temperature of the column thermostat was raised in several stages (2 to 4 deg/min) to 260°C.

Final identification of the analytes was done by using chromatography-mass spectrometry method. To achieve this we used a gas chromatograph/mass-spectrometer Finnigan DSQ II (USA) equipped with a similar chromatographic capillary column. Ionization mode: 70 eV.

Quantitative assessment of the content of the individual fatty acids was performed by the normalization method (a peak on the chromatogram corresponded to a specific FA and was determined as a percentage of the total area of the peaks of all the fatty acids). The content of each FA matched to its weight percentage of the total amount of FA.

All the data was processed employing the variation statistics methods using the software Microsoft Office Word Excel 2007, Statistica 8. Analysis of the distribution of the values obtained was performed using the Kolmogorov-Smirnov test and Shapiro-Wilk test. The mean (M) and standard error of the mean (m), confidence intervals were deduced. The Mann-Whitney (U Test) was used to compare the differences between the two independent groups (for nonparametric data). Pearson's Correlation Coefficient (r) was used to determine the strength of the relationship between the two continuous variables. P value less than 0.05 was considered significant.

The study was conducted in accordance with the standards of Good Clinical Practice, the principles of the Declaration of Helsinki, and with minimal invasive procedures for the subjects.

Result and Discussion

The analysis of the FA composition in the carotid arteries and abdominal aortas showed that the relative content of the arachidonic acid in the walls of the common carotid arteries was significantly lower than in the fragments of the abdominal aortas of the corresponding dead bodies (0.37±0.17% vs 0.84±0.31%, $p<0.05$) regardless of the extent of damage. The arachidonic acid levels in the aortas with advanced atherosclerotic lesions had a tendency to increase when compared with the aortas without major defects (Table 1). Thus, in the vessels with atherosclerosis, increasing of the PUFA predisposed to peroxidation was marked. This could indicate their higher susceptibility to oxidative degradation.

Table 1
Fatty acid composition of the abdominal aorta and the carotid artery

Fatty acids	Control		Experiment	
(wt% of the total FA)	Carotid artery	Abdominal aorta	Carotid artery	Abdominal aorta
Lauric	0.34±0.14	0.39±0.28	0.36±0.08	0.55±0.14
Myristoleic	0.19±0.05	0.19±0.07	0.33±0.06*	0.46±0.07*
Myristic	2.72±0.53	2.83±0.81	2.85±0.57	3.70±0.46
Palmitoleic	5.75±1.36	5.34±1.25	7.92±0.81*	9.40±2.57*
Palmitic	26.55±2.11	27.41±2.49	22.40±2.35*	23.29±2.48*
Linoleic	10.27±3.75	10.22±1.86	11.44±4.52	12.27±4.14
Oleic	42.82±2.05	40.55±5.04	44.04±0.64	40.34±0.43
Stearic	6.89±1.14	7.96±2.17	5.31±0.62*	4.76±1.78*
Arachidonic	0.24±0.19	0.73±0.54	0.63±0.28	1.07±0.52
Arachidic	0.89±0.30	0.94±0.36	0.73±0.04	0.58±0.09*
Docosahexaenoic	0.19±0.18	0.29±0.25	0.32±0.20	0.24±0.02

Note: **- statistical significance: $p < 0.05$*

Fragments of the aortas in the experimental group were also characterized by a reduction of the ratio of the saturated FA to the monounsaturated acids (MUFA) with the same number of carbon atoms (Fig. 1). This ratio was also lower in the carotid arteries of the experimental group than the control group, although they showed no signs of atherosclerosis. The ratio of the saturated FA to the MUFA in the aortas of the experimental and the control groups were 8.04 and 14.89 ($p<0.05$) respectively for myristic and myristoleic acids; 2.48 and 5.13 ($p<0.05$) for palmitic and palmitoleic, and 0.12 and 0.2 ($p<0.05$) for stearic and oleic acid; in the common carotid arteries : 8.64 and 14.32 ($p<0.05$), 2.83 and 4.62 ($p<0.05$). 0.12 and 0.16 ($p<0.05$) respectively.

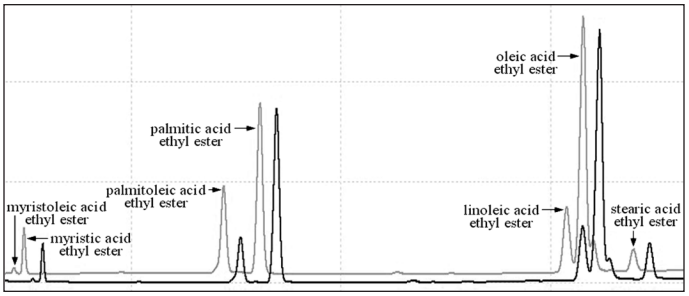

Figure 1.
Chromatogram of the saturated and the monounsaturated FA extracted from the fragments of the abdominal aorta with a slight atherosclerotic lesion (below) and a significant defect caused by atherosclerosis (top).

The results obtained testify to the activation of the 9-desaturase, which catalyzes the formation of the MUFA from the saturated FA and participates in the regulation of the response to environmental condition changes by maintaining the fluidity of the cell membranes. The presence of a sufficient amount of the PUFA in the food is known to inhibit the expression of the 9-desaturase 11]. However, it is known that the FA are synthesized with the participation of fatty acid synthase (FAS) leading to the formation of palmitic acid [12]. When the FAS activity is normal, a significant part of the palmitic acid is converted into stearic and arachidic acids. The over-expression of the enzyme is accompanied by increasing the palmitic acid content relative to the stearic acid [11]. It is shown that this process is always accompanied by the active proliferation of the tumor cells while an inhibition of the FAS (for example, with PUFA) leads to its cessation [11,13]. The decrease in the palmitic acid by 15.0%, stearic acid by 40.2%, and arachidic acid by 38,3% in the aortas with advanced atherosclerotic lesions could be an indication of the increased activity of the FAS in the AP cells. The palmitic, and stearic and levels in the carotid arteries were reduced almost to one magnitude, relative to the control.

Patients with CHD and atherosclerosis showed a significant change in the FA composition of the plasma lipids when compared with healthy individuals (Table 2). The fraction of the saturated FA, such as myristic, palmitic and stearic acids significantly increased. The PUFA content due to the linoleic acid was significantly reduced in the patients of the experimental group. Herewith, an increase in the endogenous dihomo-γ-linolenic acid was noted. It is known that the synthesis of this acid is activated under the conditions of a dietary deficit of PUFA.

Table 2
The fatty acid composition of the plasma lipids in CHD and atherosclerosis

Fatty acids (wt% of the total FA)	Control, %	Experiment, %
Myristic	0.67±0.12	1.29±0.31*
Palmitoleic	1.62±0.31	1.52±0.39
Palmitic	26.81±1.64	31.72±1.05*
Linoleic	30.38±2.35	20.65±2.26*
Oleic	16.73±1.28	16.61±1.09
Stearic	12.02±0.70	14.51±0.75*
Arachidonic	6.04±0.59	6.80±0.51
Dihomo-γ-linolenic	1.20±0.18	1.91±0.26*
Docosahexaenoic	2.10±0.38	2.55±0.28

Note: X± Δx (p=0.05); *- *statistical significance: $p < 0.001$*

The data obtained about the participation of the linoleic acid in atherogenesis are confirmed by the analysis of the postmortem blood plasma samples. In cases when the abdominal aorta had significant atherosclerotic lesions, the linoleic acid level was only 15.87 ± 1.92%. However, the content of the linoleic acid was 21.92 ± 2.75% ($p<0.05$), when only the small atheromas were present.

According to the statement mentioned prior, it can be assumed that the deficit of linoleic acid identified in the blood plasma in atherosclerosis patients may be the factor that stimulates the expression of the desaturases in the smooth muscle cells (SMC) against the background of high activity of the FAS, which ultimately promotes the myocyte proliferation.

It should be noted that the relative monounsaturated oleic acid levels in the abdominal aortas of the rats (animals resistant to the development of atherosclerosis) is lower (32.9±1.81%, $p<0.05$) than that in humans [9]. Besides, the saturated palmitic acid level in the abdominal aortas of rats compared with the human abdominal aorta is higher (34.09±2.79%, $p<0.05$). While the relative arachidonic PUFA level (11.55±1.01%, $p<0.001$) in the rat plasma is higher, and the relative level of endogenous dihomo-γ-linolenic PUFA (0.47±0.07%, $p<0.001$) is lower than those in healthy human subjects. Thus, the low level of the monounsaturated oleic acid (lower than in humans) may be due to the high content of arachidonic PUFA in rat plasma.

A significant decrease in the relative level of the linoleic acid in the plasma without a significant change in the level of the other essential FA can be explained as follows. It could be that the absorption of free FA and monoglycerides formed after influence of the pancreatic lipase free FA (mostly saturated) are vigorously absorbed by the mucosal cells of the small intestine [9]. In this case, the monoglycerides containing considerable amounts of the linoleic acid are less absorbed than in a healthy organism. Herewith, the rate of absorption of the other PUFA contained in the phospholipids and cholesterol esters is not changed, and therefore, the levels of these acids in the plasma lipids do not deviate from the normal values.

A study of the correlation between the levels of the individual fatty acids has revealed a close negative correlation between the relative content of the palmitic acid and linoleic acid of the plasma lipids in patients with CHD ($r=-0.87$, $p<0.001$) and that of the control group ($r=-0.80$, $p<0.001$). Moreover, the

average negative correlations between the oleic and stearic acids were marked in the experimental and control groups (r=-0.59, p<0.05 and r=-0.53, p<0.05).

In patients with CHD and atherosclerosis, the correlations between the individual fatty acids of the plasma lipids show a more complex characteristic. They showed a positive correlation between the stearic and linoleic acids (r=0.45, p<0.1), as well as a strong positive correlation between the palmitic and oleic acids (r=0.90, p<0.001). The correlation between the palmitic and stearic acids was negative (r=-0.62, p<0.05), although the levels of both saturated fatty acids were significantly increased. A strong negative correlation was observed between the linoleic and oleic acid levels(r=-0.92, p<0.001).

The increase in the proportion of phospholipids [5] with a high content of palmitic, stearic and linoleic acids against the triglycerides containing more palmitic and oleic acids than the linoleic acid may be a reason to change the correlations between the FA of the plasma lipids [14].

It should be noted that the balance of the FA in large AP is largely similar to the ratio of the FA in the same blood vessels with a normal texture (Table 3), and is significantly different from the spectrum of the FA of the plasma lipids (especially of healthy individuals).

Table 3
The fatty acid composition of the two fragments of the abdominal aorta with a typical texture, the two AP, and two blood plasma samples from the same dead bodies

	#1			#2		
Fatty acids (wt% of the total FA)	**Vessel without AP**	**AP**	**BP**	**Vessel without AP**	**AP**	**BP**
Lauric	0.30	0.68	0.13	0.26	0.44	0.15
Myristoleic	0.25	0.51	0.08	0.28	0.42	0.14
Myristic	2.71	4.13	1.02	2.40	3.27	1.72
Palmitoleic	6.81	8.71	4.48	9.91	11.30	6.75
Palmitic	21.34	24.52	31.25	20.90	25.07	36.95
Linoleic	11.01	8.88	15.27	17.63	10.25	14.75
Oleic	45.49	42.51	29.81	36.98	40.34	23.61
Stearic	6.26	5.43	8.89	4.06	3.59	7.92
Arachidonic	0.92	0.55	4.38	2.72	0.46	3.69
Arachidic	0.90	0.72	<0.1	0.47	0.50	<0.1
Docosa-hexaenoic	0.22	0.24	1.71	0.53	0.25	1.19

Note: *BP -blood plasma.*

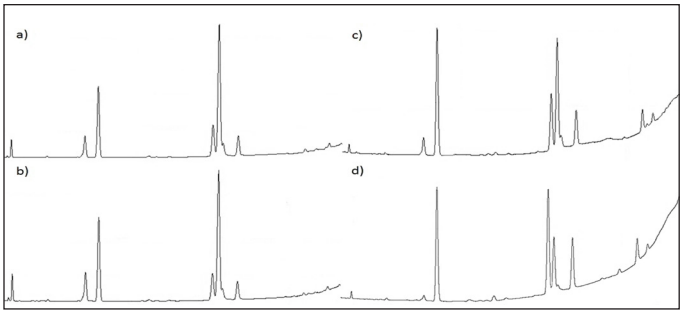

Figure 2.
Spectrum of the FA: (a) - the part of the abdominal aorta with a normal texture; (b) - AP, (c) - BP of the same dead body, (d) - BP of a healthy individual.

However, this indicates the presence of an active metabolism of the cellular elements of the AP directed toward maintaining homeostasis, while on the other hand this indicates that most part of the fatty acids of the AP are formed by the enzymatic processes in the SMC and that this is not the result of their intake from the blood flow (Fig. 2).

Conclusion

Based on our data, the following conclusions can be drawn. Reduction in the relative content of the arachidonic acid in the intact common carotid arteries compared with that of the postmortem material of the abdominal aortas (regardless of the degree of atherosclerotic lesions) indicates their lower susceptibility to the effects of the reactive oxygen species. In the case of a significant atherosclerotic lesion of the abdominal aorta, the ratio of the saturated FA to the MUFA with the same number of carbon atoms is reduced in the abdominal aortas and the corresponding common carotid arteries, which indicates the increased activity of cellular desaturases. When there are advanced atherosclerotic lesions, a more significant reduction in the relative levels of stearic and arachidic saturated FA, when compared with the saturated palmitic acid, may indicate the activation of FAS synthesis. The FA balance from the large AP resembles the ratio of the FA in the fragments of the same vessels with normal consistency and is significantly different from the spectrum of FA in blood plasma of both healthy individuals as well as those with severe atherosclerotic process.These data indicate that the origin of the FA of the AP may be a result of the synthetic processes in the plaque cells. Blood plasma in patients with atherosclerosis and myocardial ischemia is characterized by an increase in the relative level of the saturated FA and a reduction in the linoleic PUFA. This can lead to increased levels of MUFA in the arterial vessels by the activation of the cellular desaturases. Evidence of this can also be seen as an increase in the fraction of the dihomo-γ-linolenic acid in the blood plasma.

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