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Journal of Pharmaceutical and Biomedical Analysis
24 (2001) 1157–1162

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Short communication

Bioanalysis of age-related changes of lipid metabolism in nonagenarians

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Received 16 May 2000; received in revised form 6 November 2000; accepted 8 November 2000

Abstract

The aim of the present study was the bioanalysis of lipid metabolism in the aged patients and to study the relationship between these biochemical markers and longevity. Eleven nonagenarians, nine women and two men, aged 94 ± 3 years and ten control patients, six women and four men, aged 84 ± 5 years, followed at the Department of Metabolic Care and Gerontology, Charles University, Teaching Hospital entered the study. All subjects were self-sufficient, without major illnesses and free living. At the start of the project the free fatty acids (FFA), thiobarbituric reactive substances (TBARS), retinol, alpha tocopherol, ascorbic acid, cholesterol, triacylglycerols, phospholipids in serum, in lipoprotein fractions and fatty acids (FA) and phospholipids in erythrocyte membrane were determined. We used capillary gas chromatography for determination of fatty acids. Retinol and alpha tocopherol were analysed by reversed-phase high-performance liquid chromatography, other parameters were determinated spectrophotometrically or spectrofluorometrically. We found significantly higher LDL polyunsaturated fatty acids (PUFA) 22:4$n-3$ ($P = 0.028$) and 22:6$n-3$ ($P = 0.018$) and a significant increase of HDL alpha tocopherol/cholesterol ratio ($P = 0.034$) in nonagenarians. There were not any significant differences in erythrocyte membrane fatty acids and phospholipids. In serum we found significantly higher level of TBARS (3.22 ± 1.22 vs 1.98 ± 0.71 μmol/l, $P = 0.012$) in nonagenarians, other parameters were not changed significantly. The higher concentration of PUFAs in LDL and alpha tocopherol in HDL might be parameters related to longevity.

Keywords: Aging; Lipid metabolism; Nonagenarians; Antioxidant; Lipoproteins; Fatty acids

1. Introduction

With aging, the population becomes more heterogeneous with regard to health status, due to the increasing prevalence of chronic illnesses and

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PII: S0731-7085(00)00577-X

disability. Deterioration in health and functional status often induces important modifications in several biological parameters, including lipids. Among biochemical parameters usually used to assess the existence and the severity of specific diseases the lipid metabolites such as cholesterol, fatty acids (FA) and lipoperoxidation has long been recognized as an indicator of general health and nutrition and/or increased morbidity and mortality. The aim of the present study was the bioanalysis of lipid metabolism in the aged patients and to study the relationship between these biochemical markers and longevity.

2. Materials and methods

2.1. Study group

Eleven nonagenarians, nine women and two men, aged 94 ± 3 yr and 10 control patients, six women and four men, aged 84 ± 5 yr, followed at the Department of Metabolic Care and Gerontology, Charles University, Teaching Hospital entered the study. All subjects were self-sufficient, without major illnesses and free living. At the start of the project a sample of peripheral blood was obtained for analysis of lipid metabolism parameters. The collection of blood samples was repeated at 3 months interval.

2.2. Bioanalysis

Blood samples were drawn from the peripheral vein after 12 h overnight fasting and were collected in Na–EDTA tubes. The samples were centrifuged immediately. Plasma was separated and red blood cells were washed in a standard manner [1].

Serum lipoprotein fractions were prepared by density gradient ultracentrifugation, ultracentrifuge TL 100 (Beckman, Palo Alto, CA) was used. The lipoprotein fractions were distinguished in the following density ranges: very low-density lipoprotein (VLDL) < 1.006 g/ml; low-density lipoprotein (LDL) < 1.063 g/ml; high-density lipoprotein (HDL) > 1.063 g/ml [2].

Vitamin E (alpha tocopherol) and vitamin A (retinol) were analysed by reversed-phase high-performance liquid chromatography (HPLC) technique, system LC 200 (Perkin–Elmer, Norwalk). The mobile phase 100% methanol, flow rate 1.2 ml/min, column Pecosphere C18 4.6×150 mm, 5 μm, (Perkin–Elmer) were used. The vitamins were detected simultaneously after extraction with *n*-hexane using diode-array detector at 325 and 290 nm for vitamin A and vitamin E, respectively (Perkin–Elmer) [3,4]. Vitamin C (ascorbic acid) was determinated spectrophotometrically by Specol 11 (Carl Zeiss Jena, Germany) as colour Fe^{2+} complex after reducing Fe^{3+} by ascorbic acid with 2,2-dipyridyl at 525 nm wavelength [5].

Phospholipids in serum and erythrocyte membranes were analysed after mineralization with chloric acid as colour complex of inorganic phosphate and ammonium molybdate spectrophotometrically at 660 nm wavelength, Specol 11 (Carl Zeiss Jena) was used [6].

Total serum and lipoprotein fraction concentration of cholesterol [7] and triacylglycerols [8] were assessed enzymatically by conventional diagnostic kits (Lachema, Brno, Czech Republic) and spectrophotometer ULTROSPEC III (Pharmacia LKB Biotechnology, Uppsala, Sweden) was used. Cholesterol was detected at 540 nm and triacylglycerols at 520 nm wavelength.

Thiobarbituric acid reactive substances (TBARS) were determinated after extraction at *n*-butanol and reaction with thiobarbituric acid spectrofluorometrically using LS-5 spectrofluorimeter (Perkin–Elmer), excitation wavelength 528 nm, emission wavelength 558 nm [9].

Free fatty acids (FFA) in serum, FA composition of erythrocyte cell membranes, including monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), and FA of lipoprotein fractions were measured by capillary gas chromatography.

The red blood cells were haemolysed by the addition of an equivalent volume of water. Lipids were extracted according to the procedure described by Dodge and Phillips [10]. Plasma FFA were extracted after addition of internal standard (heptadecanoic acid) by modified Dole method [11]. Lipids from lipoprotein fractions were extracted following Bligh and Dyer [12]. The FA methyl esters were then formed by heating for 30

min with boron trifluoride–methanol. Profiling of fatty acid-methyl esters (FAME) was performed by on gas chromatograph 5890 II (Hewlett-Packard, Palo Alto, MA) equipped with flame ionisation detector and HP 3396 A integrator. FAME were separated on a SP-2330 Fused Silica Capillary Column 30 m × 0.25 mm i.d., 0.20 μm film (Supelco, Bellafonte, PA). Injection port and FID temperatures were both 250°C. Oven temperature was programmed to change from 120 to 230°C at 4°C/min. Helium was used as the carrier gas, splitting ratio was 1:100. Individual FAs were identified by relative retention times determined with known standards.

2.3. Statistical analysis

The significance of difference between group of nonagenarians and control group was examined by Mann–Whitney U test, statistical software NCSS 6.0.1 (Kaysville, UT, 1995) was used. The decision of significance was based on $P = 0.05$.

The study protocol had been accepted by local ethical committee, Charles University, Teaching Hospital, Hradec Králové, Czech Republic.

3. Results

3.1. Serum

We did not find any significant changes of vitamin A, E and C, cholesterol, triacylglycerols, phospholipids between group of nonagenarians and controls. These results are shown in Tables 1 and 2. There were not any significant changes of FFA (the results are not shown). The serum values of TBARS, which are markers of lipoperoxidation, were higher in the group of nonagenarians (3.22 ± 1.22 vs. 1.98 ± 0.71 μmol/l, $P = 0.012$; Fig. 1).

3.2. Lipoproteins

We did not find any significant changes of vitamin E, cholesterol and triacylglycerols in lipoproteins (Tables 1 and 2). The same results were obtained for saturated FA and MUFA (the results are not shown). In LDL we found significant higher concentrations of PUFA C22:4n3 ($P = 0.028$) a C22:6n3 ($P = 0.018$) in group of nonagenarians (Figs. 2 and 3). Moreover, there were

Table 1
Cholesterol and triacylglycerols in serum and lipoproteins (VLDL, LDL, HDL), serum LDL/HDL cholesterol ratio and phospholipids in serum and erythrocyte membranes in nonagenarians and control group

Variable	Unit of measurement	Nonagenarians		Controls		*P*-value
		n	Mean (±S.D.)	*n*	Mean (±S.D.)	
Total cholesterol	mmol/l	11	5.34 (1.00)	10	5.52 (1.49)	n.s.
VLDL cholesterol	mmol/l	11	1.26 (0.45)	10	1.14 (0.39)	n.s.
LDL cholesterol	mmol/l	11	2.78 (0.66)	10	2.92 (0.82)	n.s.
HDL cholesterol	mmol/l	11	1.07 (0.30)	10	1.25 (0.28)	n.s.
LDL/HDL cholesterol		11	3.11 (2.56)	10	2.38 (0.57)	n.s.
Triacylglycerols	mmol/l	11	1.59 (0.64)	10	1.43 (0.46)	n.s.
VLDL triacylglycerols	mmol/l	11	0.99 (0.50)	10	0.73 (0.33)	n.s.
LDL triacylglycerols	mmol/l	11	0.41 (0.16)	10	0.47 (0.15)	n.s.
HDL triacylglycerols	mmol/l	11	0.12 (0.06)	10	0.13 (0.1)	n.s.
Serum phospholipids	g/l	11	2.44 (0.66)	10	2.20 (0.57)	n.s.
Ery phospholipids	g/l	11	2.83 (0.21)	10	2.59 (0.41)	n.s.

Table 2
Serum vitamin A, serum vitamin E, vitamin E in lipoproteins (VLDL, LDL, HDL), vitamin C in serum, vitamin E/HDL cholesterol in groups of nonagenarians and controls

Variable	Unit of measurement	Nonagenarians		Controls		*P*-value
		n	Mean (±S.D.)	*n*	Mean (±S.D.)	
Age	yr	11	94 (3)	10	84 (5)	0.0001
Vitamin A serum	μmol/l	11	2.1 (0.9)	10	2.2 (0.8)	n.s.
Vitamin E serum	μmol/l	11	27.6 (7.0)	10	26.8 (8.0)	n.s.
Vitamin E VLDL	μmol/l	11	8.3 (4.3)	10	7.9 (3.4)	n.s.
Vitamin E LDL	μmol/l	11	11.3 (3.5)	10	11.7 (3.4)	n.s.
Vitamin E HDL	μmol/l	11	7.2 (2.8)	10	5.0 (3.1)	n.s.
Vitamin C serum	μmol/l	11	64.3 (37.3)	10	54.7 (26.2)	n.s.
Vitamin E/chol HDL	μmol/mmol	11	6.6 (1.4)	10	4.4 (2.1)	0.034

significantly higher concentrations of vitamin E/cholesterol ratio in group of nonagenarians (6.6 ± 1.4 vs. 4.4 ± 2.1 μmol/mmol, $P = 0.034$) in HDL (Table 2).

3.3. Erythrocyte membranes

We did not find any significant changes of any parameters of lipid metabolism. The results of phospholipids are shown in the Table 1, other results are not shown.

4. Discussion

Aging is a complex biological phenomenon, and many of the contributing mechanisms have not yet been identified. With increasing age there is an increased risk of development of a number of disorders, e.g. coronary heart disease, stroke and cancer. Numerous theories have been proposed to explain the association between old age and increased frequency of these disorders, e.g. molecular cross-linking [13] changes in immunology function [14], damage by free-radical reactions [15], and to senescence genes [16]. While no single theory is generally accepted, lipid pathology is thought to play an important role in the pathogenesis of disorders associated with aging.

4.1. Cholesterol and lipoproteins

It has been demonstrated that hypocholesterolemia is associated with a multitude of acute and chronic illness of the aged [17,18], but it remains unclear whether low serum cholesterol is the consequence of chronic diseases rather

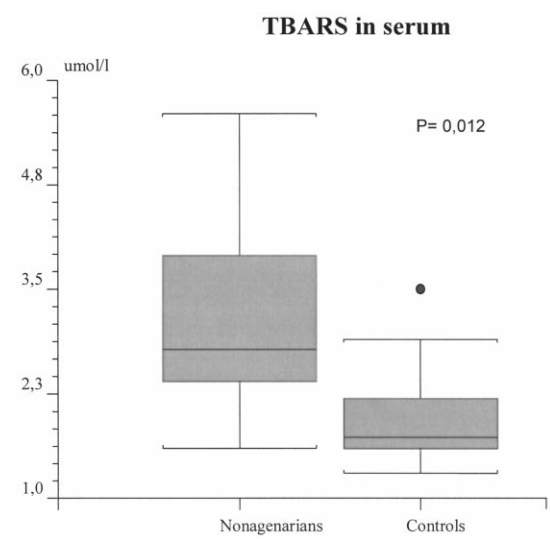

Fig. 1. TBARS in serum of nonagenarians and group of controls. Nonagenarians ($n = 11$) 3.22 ± 1.21 μmol/l, median 2.8 μmol/l, ranges 1.6–5.6 μmol/l; controls ($n = 10$) 1.98 ± 0.71 μmol/l, median 1.75 μmol/l, ranges 1.3–3.5 μmol/l. Significance was established as $P < 0.05$.

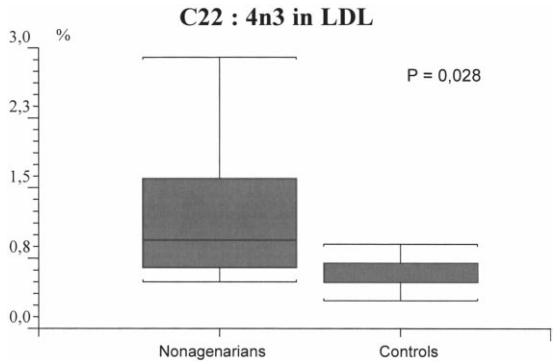

Fig. 2. Dodecatetraenoic acid (C22:4n3) in LDL of nonagenarians and group of controls. Nonagenarians ($n = 8$) $1.23 \pm 0.80\%$, median 0.95%, ranges 0.5–2.9%; controls ($n = 9$) $0.58 \pm 0.19\%$, median 0.50%, ranges 0.3–0.9%. Significance was established as $P < 0.05$.

than reflects other factors present in an elderly population characterised by a high prevalence of comorbidity, physical disability, and malnutrition. A potential limitation of these studies is the lack of the main determinant of serum cholesterol levels (i.e., genetic factors). It is well known, for example, that persons with long-standing, stable hypocholesterolemia, principally attributable to genetic causes, are in general good health [19]. However, the variance of serum cholesterol levels, comor bidity, disability, and malnutrition should be taken into account to understand the clinical value of cholesterol levels in the elderly. In conclusion, data show that common markers of frailty in the elderly are independently related with lower levels of serum cholesterol; and although are not able to clarify the direct–inverse causality of this association, data suggest that low cholesterolemia could be used as a marker of frailty in the aged. In the present study we did not find any changes of cholesterol in control group and nonagenarians. In these groups the levels of serum cholesterol were normal and we found the normal ranges of cholesterol in lipoproteins VLDL, LDL and HDL. It is in consensus with good health status of these patients and other authors [20]. Our patients were self-sufficient, without major illnesses and free living.

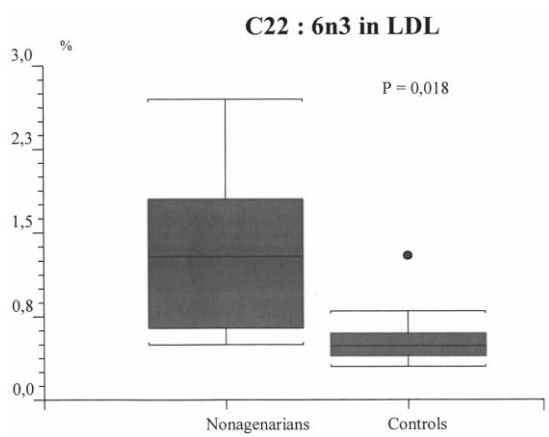

Fig. 3. Dodecahexaenoic acid (C22:6n3) in LDL of nonagenarians and group of controls. Nonagenarians ($n = 8$) $1.34 \pm 0.77\%$, median 1.3%, ranges 0.5–2.6%; controls ($n = 9$) $0.60 \pm 0.30\%$, median 0.5%, ranges 0.3–1.3%. Significance was established as $P < 0.05$.

4.2. Fatty acids

The most of the degenerative diseases of aging, including cardiovascular disease, cancer, diabetes, obesity, multiple sclerosis, arthritis, fatty deposits in inner organs, and even some behavioural problems, involve FA denegeration [21]. In our study we found the significantly higher concentrations of PUFA C22:4n3 ($P = 0.028$) a C22:6n3 ($P = 0.018$) in group of nonagenarians vs. controls in LDL fraction. Any other FA did not change significantly. The higher levels of dodecahexaenoic and dodecatetraenoic acids might be connected with longevity. Other authors [22] found the same significant increase of PUFA of the erythrocyte membrane in the group of centenarians.

On the other hand, we have found the significantly higher levels of TBARS in serum. The same results are from the study of Carrera and Estrada [23], which had shown the increase of TBARS and other parameters during aging. The higher concentrations of TBARS in serum in the nonagenarians can be connected with higher concentrations of PUFA in LDL, which are substrates for oxidation.

4.3. Lipoperoxidation, antioxidants and vitamin E

In recent years, experimental evidence has highlighted the role of free-radical-induced damage in the pathogenesis of many disorders. This is of importance since treatment by antioxidants could,

by inhibiting or reducing free radical toxicity, alleviate or delay the symptoms of aging and chronic disease [15]. According to the free radical theory of aging, these reactive species, which are produced continuously during normal oxidative metabolism, eventually accumulate damaging DNA and other macromolecules. This is due to the progressive defects in the defense systems against reactions that generate free radicals. The result is the appearance of degenerative lesions and cellular death leading to the aging and ultimate death of the organism [24]. Among other disorders, free radicals are thought to play an important role in cancer, atherosclerosis, essential hypertension, and senile dementia of the Alzheimer type [15]. In the present study, we found significantly higher vitamin E/cholesterol ratio in group of nonagenarians vs. controls in HDL. There were no changes in VLDL and LDL and in serum. The increase of vitamin E in HDL might be one of the factors concerning longevity.

5. Conclusions

We found the higher concentrations of PUFA–dodecatetraenoic acid (C22:4n3) and dodecahexaenoic acid (C22:6n3) in group of nonagenarians in LDL. In HDL there were significant changes of vitamin E/cholesterol ratio in the group of nonagenarians. We conclude that higher concentrations of PUFAs in LDL and alpha tocopherol in HDL might be parameters concerning longevity.

Acknowledgements

Supported by grants IGA MH Czech Republic No. NG/1-3, NC 6171-3, 5205-3. We thank to Marie Burešová, Drahomíra Jánská, Marie Mejtská and Iveta Svobodová for excellent technical assistance.

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