

Changes in lecithin concentrations in human blood with aging

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Abstract: Lecithin is a phospholipid and a major structural component of the cell membrane. The aim of this study was to determine the lecithin concentrations (LC) in the human blood depending on age. Lecithin was measured in the human plasma (807) and whole blood (787) of men and women aged 0.6 to 90 years, by using a commercially available enzymatic kit, which is based on the spectrophotometric determination. All samples were divided into four aging groups: children (0-12 years), adolescents (13-20 y.), work-active group (21-60 y.), and older group (>60 y.). The plasma LC of all males (397) were very similar to those of females (410) (mean 3.01 ± 0.91 mmol/l, and 2.99 ± 1.02 mmol/l, respectively). However, within both gender, the plasma LC increased with aging. The LC in whole blood increased with aging in males, but in the work-active group, and in older group of women, the whole blood lecithin decreased without statistical significance. Also, the LC in whole blood of older women were statistically lower than the LC in older men ($p^{***} < 0.001$, Mann-Whitney test), while the LC in whole blood of other groups of both gender were mainly uniform. The lecithin ratio in plasma and whole blood was $\approx 1:2$ in all aging groups.

INTRODUCTION

Phospholipids (PL) are the major structural components of the cell membranes. Numerous data indicate that in cells throughout life, *in vivo*, the damage accumulates, leading to a gradual loss of differentiated function and the degree of growth (Boyer, 2006). These changes lead to breakdown of normal homeostatic mechanisms. Gradual reduction of cellular functions and reducing the capacity of the cell growth with aging, are based on the discoordination of interactive pathways in the cells themselves, as well as between cells and tissues (Bourne, 2012). PL are unevenly distributed in the blood. In plasma, they make up about 35-40% of total lipids, 60-65% in erythrocytes, leukocytes 50%, and platelets up to 70% of total lipids. In the plasma, approximately 50% of total PL are in the form of lecithin. Plasma containing normal 2.3-4.0 mmol/l PL (Straus, 1992). With aging of the organism, red blood cells (RBC) also change their

shape, deformability, fragility and fluidity of their membranes (Marin et al., 1990). Aging of the RBC can easily be understood in two ways. The first, is to have a life cycle of the individual RBC, which lasts about three months, and the second: the change in erythropoiesis of the older RBC. In the first case, the reduced fluidity of RBC membranes of elderly in particular relating to the account of reduction in the ratio of lipid/protein, reduced levels of ATP and changed the shape of RBC (Shiga et al., 1979).

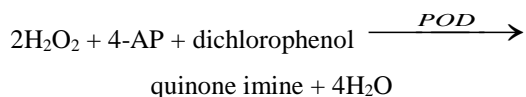
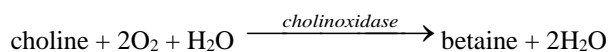
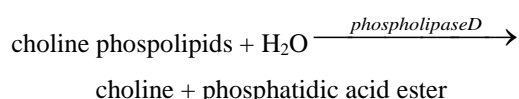
In the second case, reduced fluidity is associated with a reduced ratio of phosphatidylserine/sphingomyelin, which increases rigidity. Also, the impact of the changes in membrane lipid peroxidation (LP) with aging has great importance and contributes to higher rigidity of the membrane (Marin et al., 1990). Increased LP increases the risk of atherosclerosis and other inflammatory diseases (Stefan et al., 2007; Halliwell and Gutteridge, 1984).

Increased amounts of transmembrane proteins has resulted in the creation of protein-lipid domains, which reduce membrane dynamics (Subczynski and Wisniewska, 2000). The aim of this study was to determine the LC in both human blood plasma and in whole blood in different aging groups of people.

MATERIAL AND METHODS

A total of 807 blood plasma samples and 787 whole blood samples of both gender aged from 0.6 to 90 years were analysed by using an enzymatic-spectrophotometric method, (Takayama et al, 1977) with a commercial kit (Chronolab Systems, Spain) on UV/VIS spectrophotometer (Perkin Elmer Lambda 25).

The enzymatic method uses a combination of two purified enzymes, phospholipase D and cholinoxidase that through a sequence of reactions produce H₂O₂. The resulting H₂O₂ reacts with the mixture of 4-aminoantipyrine and dichlorophenol (reaction is catalysed by peroxidase, POD) to give quinone imine, which absorbs at 505 nm. The absorption of quinone imine is proportional to the concentration of phospholipids:



For the determination of lecithin in whole blood, all specimens were diluted with double volume of 0.9% NaCl solution. Statistical analysis was performed using Descriptive Statistics (Kruskal-Wallis test and Mann-Whitney test), using the statistical program SPSS 19.0 for Windows.

Ethics

All specimens sampled in the Laboratory for Clinical Chemistry and Biochemistry of the Clinical Centre University of Sarajevo and approved by the Ethics Committee of the Clinical Centre University of Sarajevo (Nr. 0305-33560 on December 18, 2006.).

RESULTS AND DISCUSSION

The results of the concentration of lecithin in the blood plasma and in whole blood in human subjects of different aging groups are shown in Table 1.

Table 1. The lecithin concentrations in the plasma and in whole blood in different aging groups of people

Age (y.)	n	Plasma lecithin (mean±S.D.) (mmol/l)	n	Whole blood lecithin (mean±S.D.) (mmol/l)
≤12	92	1.57±0.61	94	4.39±0.74
13-20	95	2.71±0.65 ^{***}	95	5.70±0.70 ^{***}
21-60	351	3.22±0.87 ^{***a}	346	5.76±1.09 ^{***}
>61	269	3.30±0.82 ^{***}	252	5.73±1.11 ^{***}
	Total (n=807)	3.00±0.96	Total (n=787)	5.58±1.11

^{***} Significantly higher concentrations of lecithin in comparison to the youngest group of subjects (p<0.001, Mann-Whitney test)

^a Significantly higher concentrations of lecithin in comparison to the adolescent group of subjects (p<0.01, Mann-Whitney test)

Results of this study showed that the concentration of lecithin in the whole blood and in plasma of total subjects (without taking into account the gender) statistically significantly increases after 12 years of age (p^{***}<0.001).

In aging group 21-60 y. (total, without taking into account the gender), the lecithin concentration in the plasma increases (p^{***}<0.001), and after age 60 (older group, >60 y.) the concentration of lecithin was slightly increased in plasma, and slightly decreased in whole blood in comparison to the work-active group. The ratio of lecithin in plasma and whole blood of both gender was ≈ 1:2 and that relationship did not change during aging.

The results of the concentrations of lecithin in the blood plasma of males and females of different aging groups are shown in Table 2.

Table 2. The lecithin concentrations in the plasma of different aging groups for both gender

Age (y.)	n	Plasma lecithin (mean±S.D.) (mmol/l)	n	Plasma lecithin (mean±S.D.) (mmol/l)
		Men		Women
≤12	46	1.62±0.64	46	1.52±0.58
13-20	47	2.61±0.69 ^{***}	48	2.81±0.60 ^{***}
21-60	169	3.25±0.78 ^{***}	182	3.19±0.94 ^{***}
>60	135	3.31±0.71 ^{***b}	134	3.29±0.92 ^{***}
	Total (n=397)	3.01±0.91	Total (n=410)	2.99±1.02

^{***} Significantly higher concentrations of plasma lecithin in comparison to the youngest group of subjects (p<0.001, Mann-Whitney test)

^b Significantly higher concentrations of plasma lecithin in comparison to the adolescent group of subjects (p<0.01, Mann-Whitney test)

The concentrations of lecithin in the blood plasma of total male subjects ($n=397$, mean 3.01 ± 0.91 mmol/l) were not significantly different from the concentrations of lecithin of total female subjects ($n=410$, mean 2.99 ± 1.02 mmol/l).

The concentrations of plasma lecithin in subjects of both gender were statistically significantly increased after 12 years ($p^{***}<0.001$, Mann-Whitney test). Also, the concentrations of plasma lecithin in the work-active and older group of subjects of both gender were statistically significantly increased in comparison to the adolescent group ($p^{***}<0.001$, and $p^{**}<0.01$ for older group of women to adolescent group; Mann-Whitney test).

There were no statistically significant differences in the plasma lecithin concentrations of male and female subjects in the same aging groups.

The results of the concentrations of lecithin in the whole blood of males and females of different aging groups are shown in Table 3.

Table 3. The lecithin concentrations in the whole blood of both gender of different aging groups

Age (y.)	n	Whole blood lecithin (mean \pm S.D.) (mmol/l)	n	Whole blood lecithin (mean \pm S.D.) (mmol/l)
		Men		Women
≤ 12	49	4.39 ± 0.73	45	4.38 ± 0.76
13-20	47	$5.58\pm 0.70^{***}$	48	$5.82\pm 0.69^{***}$
21-60	171	$5.83\pm 1.18^{***}$	175	$5.69\pm 1.01^{***}$
>60	125	$6.03\pm 0.96^{***c}$	127	$5.44\pm 1.18^{**d}$
	Total (n=392)	$5.67\pm 1.12^{**e}$	Total (n=395)	5.48 ± 1.08

^{***} Significantly higher concentrations of lecithin in whole blood in comparison to the youngest group of subjects ($p<0.001$, Mann-Whitney test)

^{**} Significantly lower concentrations of lecithin in comparison to those of the youngest group of female subjects ($p<0.01$, Mann-Whitney test)

^c Significantly increased in comparison to the adolescent group ($p<0.01$, Mann-Whitney test)

^d Significantly lower concentrations of lecithin in whole blood in comparison to the same aging group of men ($p<0.01$, Mann-Whitney test)

^{**e} Significantly higher than LC of total female subjects ($p<0.01$, Mann-Whitney test).

The concentration of lecithin in whole blood of total male subjects ($n=392$, mean 5.67 ± 1.12 mmol/l) were statistically higher in comparison to those of total female subjects ($n=395$, mean 5.48 ± 1.08 mmol/l) ($p^{**}<0.01$, Mann-Whitney test).

The whole blood LC in all groups of subjects of both gender were statistically significantly higher in comparison to the youngest group of subjects ($p^{***}<0.001$). Also, the whole blood LC in the older group of male subjects were statistically significantly increased in comparison to the adolescent group ($p^{**}<0.01$, Mann-Whitney test). Decreasing of the whole blood LC in the

older group of female subjects was marginally significant in comparison to the adolescent group ($p=0.058$, Mann-Whitney test).

Also, the whole blood lecithin concentrations of older female subjects (>60 y.) were statistically significantly lower than those in the same aging group of males ($p^{***}<0.001$, Mann-Whitney test), while the whole blood lecithin concentrations in other aging groups of both gender were generally uniform.

Satisfactory results were obtained, which correlate with other previously used reference methods. By separating the plasma lipids using by agarose gel electrophoresis and measured by reference methods, it was found that in infants at birth, the concentration of PL in the blood plasma is very low (1.36 ± 0.18 mmol/l) (Gurantz et al., 1981). After about one month of life, plasma phospholipid concentrations reach values twice as high as the initial value (Wissenschaftliche Tabellen Geigy, 1986).

The earlier studies have shown that during the first year of life, plasma PL concentrations continuously increasing, it would have reached puberty, values that are common in young adults (Wijnberger et al., 2003). For boys younger than 15, the plasma PL concentrations were lower compared to girls of the same age. From age 16-50 the concentrations of plasma PL were approximately the same in both gender with a tendency of gradual increase during aging. In this age period, the PL concentrations ranged from 2.36 to 2.98 mmol/l for both gender. After age 50 for both gender, the concentration of plasma PL ranged from 3.28-3.30 mmol/l (Wissenschaftliche Tabellen Geigy, 1986). According to data from the American Heart Association (Heistad, 2006), 4-5 people who died of heart diseases are over 65 years old. Heart diseases commonly occur more often in younger men, than in women. The risk for them is increasing especially after 45 years of age. In women, the risk for these diseases increases sharply after age 55 (Knight, 2000). The reason for this protective effect (in comparison to 45 y. for men) is estrogen, but when women reach menopause when estrogen secretion is reduced, they are much more prone to cardiovascular diseases (CVD). Estrogen changes the concentration of lipids in the blood, affects the coagulation and fibrinolytic systems, antioxidant systems and the production of other vasoactive molecules such as prostaglandins, prostacyclins, tromboxanes etc. As for arterial hypertension, which is the most common cause of CVD, investigations suggest that the cause can easily be variability of erythrocyte membranes. Studies have shown that people with hypertension have less fluid membranes (Tsuda et al., 2003). After menopause, the use of hormone replacement therapy (HRT) may have a protective effect against CVD in women (Canadian Medical Association Journal, 2004). Estrogen stimulates the opening of calcium activated K-channels, K^+ out of cells, the membrane becomes hyperpolarized, leading to vasodilatation (Grant and Beastall, 1983).

CONCLUSIONS

The concentration of lecithin in the whole blood and plasma of total subjects (regardless of gender) statistically significantly increases after 12 years of age ($p^{***}<0.001$). After 20 years, the concentration of lecithin in whole blood and plasma of subjects regardless of gender, is growing, but without statistical significance between age groups. The plasma lecithin concentrations of total male subjects were not significantly different from those of total female subjects (3.01 ± 0.91 mmol/l, and 2.99 ± 1.02 mmol/l respectively). There were no statistically significant differences in the plasma lecithin concentrations of the same aging groups of male and female subjects. The concentration of lecithin in whole blood of total male subjects was statistically higher in comparison to the lecithin concentration of total female subjects (5.67 ± 1.12 mmol/l, and 5.48 ± 1.08 respectively; $p^{**}<0.01$, Mann-Whitney test). The concentrations of lecithin in the whole blood of older female subjects (>60 y.) were statistically significantly lower than those in the same aging group of males ($p^{***}<0.001$, Mann-Whitney test). The ratio of lecithin in plasma and whole blood in subjects of both gender was $\approx 1:2$, and it was found that this ratio did not alter during aging. The changes of lecithin concentrations in the blood during the aging processes can be partly explained by cell membrane dysfunction and provide insight into the metabolic and biochemical disturbances in the process of aging. Because that, we can reasonably say that the changes of lecithin concentrations in the plasma and whole blood during of aging are very important risk factor of various cardiovascular, neurodegenerative, diabetic and malignant diseases, general diseases of aging etc. The changes could be used as a diagnostic biochemical indicator of the above-mentioned pathochemic states, as well.

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Summary/Sažetak

Lecitin je fosfolipid i glavna strukturna jedinica ćelijske membrane. Cilj ovog istraživanja je bio odrediti koncentracije lecitina (KL) u ljudskoj krvi u ovisnosti od starosne dobi. KL je određivana u krvnoj plazmi (807) i punoj krvi (787) muškaraca i žena starosne dobi 0,6-90 godina, spektrofotometrijskom metodom uz komercijalno dostupni enzimatski reakcioni kit. Svi analizirani uzorci podijeljeni su u sljedeće starosne kategorije: djeca (0-12 godina), adolescenti (13-20 godina), radno-aktivnu grupu (21-60 godina) i grupu starijih osoba (>60 godina). KL u krvnoj plazmi muškaraca (397) i žena (410) je vrlo slična ($3,01 \pm 0,91$ mmol/l i $2,99 \pm 1,02$ mmol/l respektivno). Međutim, kod oba spola, KL krvne plazme je rasla sa povećanjem starosne dobi. KL u punoj krvi je rasla sa povećanjem starosne dobi kod muškaraca, dok u radno-aktivnoj grupi i grupi starijih žena KL opada bez statističke značajnosti. Pored toga, KL u punoj krvi grupe starijih žena je statistički značajno niža u odnosu na KL u punoj krvi grupe starijih muškaraca ($p^{***} < 0.001$, Mann-Whitney test), dok je u ostalim kategorijama KL u punoj krvi kod oba spola bila uglavnom ujednačena. Odnos KL u krvnoj plazmi i punoj krvi u svim starosnim grupama bila je $\approx 1:2$.

