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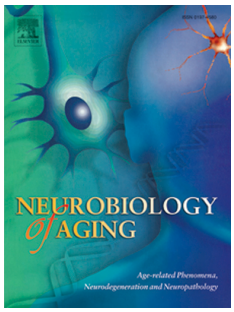

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'LIPID RAFT AGING' IN THE HUMAN FRONTAL CORTEX DURING NON-PATHOLOGICAL AGING: GENDER INFLUENCES AND POTENTIAL IMPLICATIONS IN ALZHEIMER'S DISEASE

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ABSTRACT

Lipid rafts are highly dynamic membrane domains featured by distinctive biochemical composition and physicochemical properties compared to the surrounding plasma membrane. These microstructures are associated not only with cellular signalling and communication in normal nerve cells but also with pathological processing of amyloid precursor protein in Alzheimer's disease. Using lipid rafts isolated from human frontal cortex in non-demented subjects aging 24-85 years, we demonstrate here that lipid structure of lipid rafts undergo significant alterations of specific lipid classes and phospholipid-bound fatty acids as brain cortex correlating with aging. Main changes affect levels of plasmalogens, polyunsaturated fatty acids (especially docosahexaenoic acid and arachidonic acid), total polar lipids (mainly phosphatidylinositol, sphingomyelin, sulphatides and cerebrosides), and total neutral lipids (particularly cholesterol and sterol esters). Besides, relevant relationships between main fatty acids and/or lipid classes were altered in an age-related manner. This "lipid raft aging" exhibits clear gender differences and appear to be more pronounced in women than in men, especially in older (postmenopausal) women. The outcomes led us to conclude that human cortical lipid rafts are modified by aging in a gender-dependent fashion. Given the central role of bilayer lipid matrix in lipid rafts functionality and neuronal signalling, we hypothesize that these findings might underlie the higher prevalence of cognitive decline evolving towards Alzheimer´s disease in postmenopausal women.

Keywords: Aging, gender differences, lipid rafts, membrane lipids, plasmalogens, sphingolipids, long-chain polyunsaturated fatty acids, cholesterol.

Introduction

Cerebral aging is generally acknowledged as a complex process involving multiple factors which converge to reduce cognitive functions, eventually leading to neuronal vulnerability and the development of pathological conditions that favour neurodegenerative diseases. Amongst these factors, nutritional habits, genotype, and hormonal status are increasingly recognized in the likelihood of developing neurodegenerative pathologies. For instance, cholesterol-rich and high saturated fat (prevalent in the Western diet), are regarded as risk factors for brain aging and dementia (Collin et al 2016; Davidson et al., 2013; Park et al., 2013; Uranga et al., 2010), as well as to higher incidence of Alzheimer's disease (AD) (Julien et al., 2010; Refolo et al., 2000). Indeed, compelling evidence accumulated over the last two decades have led to the notion that lipid alterations in the brain parenchyma affect the organization of the neuronal membrane, particularly in the structure and functionality of specific microdomains, such as lipid rafts (recently reviewed in Collin et al., 2016). As lipid composition impact phase separation of membrane domains, it appears evident that age-associated variations in lipid composition would lead to modifications of the physicochemical properties of membranes. This fact provides a mechanistic link for age-related disturbances of lipid raft functionality, particularly in synaptic membranes, neurotransmission, neurotransmitter signalling and transduction, protein clustering, potentially contributing to neuropathological events (Sebastião et al., 2013). For instance, aged mice hippocampi have decreased density of TrkB, glutamatergic AMPA and NMDA receptors in lipid rafts, as well as decreased density of caveolin-1, a cholesterol binding protein relevant for lipid raft organization (Head et al., 2010: Martín et al., 2010a). These lead to reallocation of glutamatergic receptors to non-raft domains which favour excitotoxic events (Collin et al., 2016; Liu et al., 2007). The importance of perturbing lipid rafts or membrane lipid composition in the functionality and modulation of several ionotropic and metabotropic receptors, GABAergic, cholinergic, serotonergic, purinergic, dopaminergic and neurotrophic factor receptors has been well established (reviewed in Sebastião et al., 2013). Importantly, we have recently found that changes in the lipid composition and biophysical properties of neuronal membrane domains in human brains, particularly the reduction of cholesterol and polyunsaturated fatty acids (PUFA) in lipid rafts, facilitates the clustering and interaction of proteins involved in the amyloidogenic cascade, thereby formation and aggregation of Amyloid β peptides (Díaz et al., 2015a; Fabelo et al., 2014).

In close connection with brain lipid metabolism, it has been solidly established that the presence of the apolipoprotein E4 alelle is a primary genetic risk for sporadic AD. The evidence is clear: although only 20% of humans carry this allele (ApoE4), about 60% of them progress to AD (reviewed in Mayeux, 2003). However, is spite of intense research, the mechanistic connection between this genetic feature and AD pathogenesis is not clear. A recent study by Zhu et al. (2015) have revealed that the ApoE4 isoform is a critically determinant of phospholipid homeostasis, and that its presence reduces levels of phosphatidylinositol (which are particularly abundant in the inner leaflet of lipid rafts) secondary to increased expression of PIP_2-degrading enzyme, thereby affecting lipid signalling (Zhu et al., 2015). In addition, other (epi)genetic mechanisms appear to be involved. Such is the case of age-related changes in DNA methylation in neuronal and glial genomes, which have been reported to be responsible for aging-induced changes in gene expression, including genes involved in brain lipid metabolism (Steegenga et al., 2014). However, a cause-effect relationship has yet to be demonstrated.

Cumulative epidemiological and basic research evidences have involved age-related changes in hormonal status in brain lipid changes. Amongst best studied hormonal influences, the decline of estrogens during menopause has received great attention. Indeed, postmenopausal reduction of circulating estrogens affect lipid metabolism both at the systemic (mainly liver) and brain parenchyma levels. In our recent study, using wild type and transgenic APP/PS1 mice (a model of familial AD), we demonstrated that estrogen deprivation leads to significant changes in hippocampal lipid composition and lipid-related gene expression, including *Acat* family of genes (responsible for generation of steryl/cholesteryl esters), *HMGCoAR* (involved in *de novo* synthesis of cholesterol), and *Scd1* and *Scd2* (encoding for Δ9-desaturases) (Díaz et al. 2015b). Noteworthy, the effects of estrogen deprivation/restoration were tightly linked to diet, and particularly to the amount of DHA included therein. Paralleling these findings, it has been recently demonstrated that estrogen deprivation in humans, i.e postmenopausal women, leads to the disruption of estrogen receptors(ER)-containing signalosomes (mER/IGF1Rβ/VDAC1/Caveolin-1) located in lipid rafts (Canerina-Amaro et al., 2017). The alterations of this protein multicomplex may be a consequence of lipid raft lipid membrane disarrangements. Indeed, the relevance of this signalosome disruption is enormous since it has been demonstrated to participate in the neuroprotective actions of estrogens and IGF1 (García-Segura et al., 2000; 2007).

In the present study we have aimed to endeavour a complete analysis of lipid raft lipid composition in human frontal cortex from non-demented (healthy) subjects, with particular emphasis in the effects of aging and gender. We have also analysed potential interactions between these factors. To the best of our knowledge, this represents the first overall study on the influence of aging and gender in the lipid composition of human cortex membrane microdomains.

Materials and Methods.

Human Brain tissue and isolation of lipid rafts. Brain tissues were obtained from the Institute of Neuropathology Brain Bank from Hospital Universitario de Bellvitge (Barcelona, Spain) following the guidelines of the ethics committee. Twenty five cases of Caucasian individuals neurologically normal and without neuropathological traits at autopsy were analysed. Groups were established according to their age and gender as young men (Men < 60, average age 42.86 ± 1.93, ranging 38-52 years), young women (Women < 60, average age 42.20 ± 4.11, ranging 24-49 years), aged men (Men > 60, average age 73.29 ± 3.0, ranging 64-85 years), and aged women (Women > 60, average age 71.67 ± 2.38, ranging 65-82 years). A summary of cases including post-mortem delays, primary disease and cause of death is shown in Table 1. Frontal cortex grey matter was used for the isolation of tissue homogenates and lipid rafts. Cases were processed following standard sampling, staining and immunohistochemistry protocols as described previously (Fabelo et al., 2014; Martín et al., 2010a). At autopsy, half of the brain was fixed in formalin and the other half was cut in coronal sections of selected brain regions and immediately frozen and stored at -80ºC until membrane isolation. Cortical homogenates and Lipid raft fractions were isolated following the protocols previously described for human brain in sucrose gradients centrifuged at 150,000xg for 18 h at 4ºC (Fabelo et al., 2011; 2014; Martín et al., 2010a). Two ml fractions were collected from the top to the bottom from the ultracentrifuge tubes, re-suspended in 200 µl of homogenization buffer and frozen at -80 ºC until analysis.

Lipid analyses. Total lipids from lipid rafts fractions were extracted with chloroform/methanol (2:1 v/v) containing butylated hydroxytoluene (BHT, 0.01%) as antioxidant. Lipid classes were separated by one-dimensional double development high performance thin layer chromatography using methyl acetate/isopropanol/chloroform/methanol /0.25% KCl (5:5:5:2:1.8 volume basis) as developing solvent system for polar lipid classes, and hexane/diethyl ether/acetic acid (22.5:2.5:0.25 volume basis) as developing solvent system for neutral lipids. Lipid classes were quantified by scanning densitometry after charring plates with 3% (w/v) aqueous cupric acetate containing 8% (v/v) phosphoric acid, using a Shimadzu CS-9001PC dual wavelength spot scanner. Lipids from lipid rafts were subjected to acid-catalyzed trans-methylation using 1 mL of toluene and 2 ml of 1% sulfuric acid (v/v) in methanol for 16h at 50ºC. The resultant fatty acid methyl esters (FAME) and dimethylacetals (DMA) were purified

in thin layer chromatography (TLC), and quantified using a TRACE GC Ultra (Thermo Fisher Scientific, Waltham, MA, USA) gas chromatograph equipped with a flame ionization detector. Individual FAME and DMA were identified by reference to a multi-standard mixture (Supelco PARK, Supelko, Bellefonte, USA), and confirmed using a DSQ II mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA).

Statistical methods. Lipid variables were initially assessed by one-way analyses of variance (ANOVA-I) followed by Tukey's or Games-Howell post hoc tests, where appropriate. Kruskal-Wallis and Mann-Whitney U tests were used in cases where normality was not achieved. Data were afterwards analysed by two-way or multiple analyses of variance (ANOVA-II) in order to determine main effects between the different factors, and to assess the existence and degree of interactions. Lipid classes and main fatty acids were additionally submitted to multivariate analyses using principal components analysis (PCA), in order to obtain the extraction coefficient matrixes of lipid components and their contributions to overall variance and weights in group segregation. Factor scores from principal component 1 (PC1) were used to obtain the lipid profile of each experimental group. Factor scores were further analysed by two-way ANOVA to evaluate the main effects of diet, hormonal status and genotype, as well as their interactions, in the lipid signatures of the different groups. Pearson and partial correlation analyses as well as lineal regression analyses were performed in order to assess the significance of bivariate relationships between different lipids, and to explore for the effects of age and gender factors in setting their relationships.

Results

Lipid profiles in lipid rafts isolated from women and men as a function of aging. We first analysed the lipid classes and fatty acid composition of lipid rafts from women and men aging 24-85 years. Results in Table 2 revealed significant differences extending to both lipid classes and fatty acids. Within younger individuals, most lipid variables remained similar regardless gender, although women displayed lower (or null) levels of dimethylacetals 16:0 DMA, 18:1n-9 DMA, 18:1n-7 DMA, total n-3 LCPUFA, unsaturation index (UIx) and, peroxydability index (PIx). Significant lower levels of 20:4n-6 and 22:5n-6, and higher levels of 22:0, 22:2n-6 and sulphatides were observed in aged individuals in both sexes. Cholesterol was reduced by average 8.9% in older individuals, though this reduction was not statistically significant (p=0.072). Older men (but not women) displayed lower levels of 18:1n-9 DMA, 18:1n-7 DMA, UIx, PIx indexes as well as higher levels of 22:0, 23:0, 18:1n-7 and 24:1n-9. Conversely, compared to aged men, aged women exhibited higher contents of 18:1n-9 (partly reflected in total monoenoic fatty acids), sulphatides and cerebrosides, and lower amounts of 18:1n-9 DMA, 18:1n-7 DMA (but not total DMA).

Given that group ages span a wide range (24-85 years), and that a number of differences were close to achieve statistical significance, we next performed correlation analyses between all lipid variables and age. We used both Pearson correlation analyses on pooled samples (women and men), as well as partial correlations to detect changes controlled by age. The results illustrated in Figure 1 and Table 3, revealed that while some regression lines followed the same trends in both sexes, other relationships were markedly different. Thus, total n-3 LCPUFA fatty acids (r=0.707), n-3/n-6 ratio (r=0.714), UIx (r=0.611), PIx (r=0.562), 20:0 (r=0.638), phosphatidylethanolamine (PE, r=0.533) increased with age in women but not in men (Fig. 1A and Table 3). Likewise, levels of 16:0 DMA, 18:1n-9 DMA and 18:1n-7 DMA were undetectable in women, instead all plasmalogens in women appear to contain only the more saturated 18:0 DMA. Lipid rafts from men exhibited significant relationships for 18:1n-7 (r=0.559), 24:1n-9 (r=0.687) and sphingomyelin (r=0.476) with age, which were not observed in women (Fig. 1B and Table 3). Correlations of pooled data showed that 18:2n-6 (r=-0.363), 20:3n-6 (r=-0.385), 22:2n-6 (r=0.403), 22:5n-3 (r=0.370), total n-6 (r=-0.343), cholesterol (r=-0.357), sulphatides (r=0.309), TNL (r=-0.416) and TPL (r=0.416), exhibited identical significant trends in both sexes indicating that these alterations are mostly associated with aging (Fig. 1C and Table 3). This

assumption was confirmed by using partial correlation analyses controlling for gender (Table 3), which revealed that all these relationships remained with little or no modifications.

Lipid profiles in frontal cortex. In order to evaluate whether changes in lipid rafts resemble alterations in the cortical parenchyma, we analysed the lipid profiles in frontal cortex grey matter. The results are summarized in Table 4. We observed that only small changes, mostly affecting minor fatty acids were detected between groups. Nonetheless, it is worth mentioning that some clear age-related trends were observed for the reduction in the levels of cholesterol and total n-6 LCPUFA in women, but not in men, while 20:4n-6 and DHA levels exhibited a trend to decrease, and saturates to increase in both sexes. As expected, levels of TG and DAG were undetectable, as were those of LPC, indicating the absence of lipid peroxidation in frontal cortex samples

Multivariate analyses of lipid rafts lipid profiles. Effects of gender, aging and interactions. We next attempted to determine the set of overall variables that might differentiate between age-gender groups. We used a variable reduction strategy based on principal component analyses (PCA). Given the requirements of PCA analyses of lipid classes and fatty acids were analysed separately and the results are illustrated in Figures 2 and 3.

We found that three principal components were able to explain most overall variance (73.85% for lipid classes and 65.51% for fatty acids). In the rotated component matrix, PC1 (34.51% of total variance) was positively related to PS and PI (more abundant in women) and negatively to sulphatides (more abundant in men) (see Table 2). PC2 (20.41% of total variance) was positively associated to PE and SE in both cases, and negatively to SM (all exhibiting opposite trends in women and men) and cholesterol (more abundant in younger subjects); while PC3 (12.39% of total variance) was positively correlated to PC (less represented in younger men) and negatively to cholesterol (more abundant in younger subjects) and free fatty acids (more abundant in older subjects). Plots of factor scores 1 and 2 revealed that age and gender factors are distributed in different regions of the 2D plots (Fig. 2 A and B), though a significant overlapping exists. Factor score plots for the whole dataset in the 3D space are illustrated in Fig. 2C. It can be observed a certain degree of segregation between groups, with groups Women > 60 and Men < 60 the ones most clearly differentiated. In order to detect main factors and interactions between gender and age in the distribution of groups we performed two-way ANOVA on the

three factor scores. It turned out that age (F=8.43, p=0.008) and gender (F=3.33, p=0.082) were the main factors in determining factor score 1 (Fig. 2D). No interaction was detected between age and gender for this factor score. As gender only reached poor statistical significance, this indicates that most variance (attributed to PC1) is explained by age management. Conversely, for factor score 2, age (F=5.71, p=0.026) and gender (F=4.69, p=0.042) were significant as main determinant factors, but interestingly, a strong interaction was detected between them (F=6.32, p=0.020) (Fig. 2E). No significant effects were observed in factor score 3 (not shown)

When applied to fatty acids, PCA revealed again that three principal components explained a large proportion of variance (65.51%), with PC1, PC2 and PC3 accounting for 33.89%, 20.59 and PC3 11.36%, respectively. The component matrix indicated that PC1 was positively related to 16:0 DMA, 17:0, 18:1n-9 DMA, 18:1n-7 DMA, 20:4n-6 and 22:6n-3 (more abundant in women), and negatively to 23:0, 22:2n-6, 20:2n-6 and 16:2 (more abundant in men). PC2 was related to saturates 14:0, 15:0, 22:0 and monoenoic 16:1 (generally more abundant in older subjects) and negatively to polyunsaturated 20:2n-6, 20:3n-6 and saturate 18:0 (more abundant in women). Finally, PC3 was positively related to main saturates 16:0, 18:0 and to 22:5n-3 (more abundant in women), but negatively to 18:1n-9 and 20:1 (more abundant in men). Plotting factor scores 1 and 2 for age and gender conditions showed a complex scatterplot, especially for women and younger subjects (Fig. 3A and B). However, in the 3D space, plotting of factor scores for the whole dataset revealed a clear distribution of individual groups with a neat segregation between Women > 60 and Men < 60 (Fig. 3C), as it was observed in the case of lipid classes described above. Analyses of main factors and interaction using two-way ANOVA, indicated that for factor score 1 the main significant determinant was the interaction between age and gender (F=3.037, p=0.096) (Fig. 3D). Conversely, for factor score 2, both gender (F=7.376, p=0.013) and age (F=12.12, p=0.002) as main factors, as well as their interaction (F=9.735, p=0.005) were highly significant.

Regression analyses of lipid profiles in in lipid rafts from women and men as a function of gender. We next performed regression analyses between relevant fatty acids and lipid classes in both sexes. The results shown in Fig. 4 and Supplementary Table 1 indicate that a number of relevant relationships between lipid classes and/or fatty acids and indexes are present which are statistically significant. For instance, DHA (22:6n-3) is positively correlated to PI and PS (Fig. 4A and 4B) but this occurs only in men. However, arachidonic acid (20:4n-6) is significantly

related to PI in both sexes, but not to PS (Fig. 4C and Supplementary Table 1). Likewise, plasmalogens (as estimated from dimethylacetals) are positively related to 20:4n-6 and 22:6n-3 in men but not in women (Fig. 4E and 4F). In this same lipid class, it turns out that saturated 16:0 DMA and monounsaturated 18:1n-7 DMA and 18:1n-9 DMA, were totally excluded form women's lipid rafts, where the only dimethylacetal species detected was 18:0 DMA (Table 2). Further, in men, DHA levels displayed a negative relationship with 18:1n-9, which was not observed in women. Likewise, sulphatides and cerebrosides exhibited a very significant positive covariation in men that was absent in women (Fig. 4I). Despite these bivariate differences, a number of relationships are shared by both sexes. Some of them are physiologically relevant. Such is the case of the negative relationship between 18:1n-9 and 18:0 (Fig. 4D), the negative association between LCPUFA and 18:1n-9 (Fig 4G), and the positive and negative relationships between PC and saturates and unsaturates, respectively (Fig. 4K and 4L). Of particular interest is the association between SM and long chain saturates 23:0 and 24:0 (Supplementary Table 1), which has structural impact in transbilayer stability.

Discussion

In the present study, we demonstrate for the first time that non-pathological aging is accompanied by alterations in the lipid matrix of lipid rafts in the human frontal cortex. These alterations affect both lipid classes and fatty acids. These changes appear to develop gradually as demonstrated when lipid data were plotted against subject age (Figure 1 and Table 3). Thus, there appear to occur a progressive decline in the levels of total n-6 LCPUFA, 18:2n-6, 20:3n-6, 22:5n-6, TNL and cholesterol as well as a gradual increase in the levels of 22:5n-3, 22:2n-6, sulphatides and TPL. Altogether, these data indicate that normal aging associates with remodelling of lipid contents in cortical lipid rafts. Interestingly, these results are in close agreement with recent observations in mouse frontal cortex, where aging was demonstrated to alter the lipid matrix of lipid rafts, including cholesterol reduction, thereby giving rise in 2012 to the ´lipid raft aging´ hypothesis (Fabelo et al., 2012). Amongst all lipid changes detected in this study, the reduction of cholesterol contents appears to be the most commonly reported effect of aging in the brain, and it has been observed in human cortex, hippocampus and cerebellum, mouse synaptosomes or cultured hippocampal neurons (Martín et al., 2010a; Söderberg et al., 1990; Svennerholm, 1994). It is widely accepted that cholesterol synthesis is very high in the developing brain, but this rate declines at low and constant levels during adulthood (Dietschy and Turley, 2004). Cholesterol plays an essential role in establishing physicochemical properties of lipid rafts and is largely responsible for its liquid-ordered state (Brown and London, 2000; Díaz et al., 2012). We and others have reported that lipid alterations in lipid rafts severely impact protein-protein interactions and protein clustering during signal activation, and thereby modify transduction pathways affecting neuronal physiology (Colin et al., 2016; Frisardi et al., 2010; Martín et al., 2010; Marín et al., 2013; Ohno-Iwashita et al., 2010). For example, it has been shown the recruitment of NMDA receptors to lipid rafts (where specific kinases that phosphorylate its subunits - NR1, NR2A, NR2B- are present in larger proportion) in the rat hippocampus and insular cortex during spatial memory formation. This process is associated to receptor lateral diffusion within the membrane (Delint-Ramirez et al., 2008), which, in turn, rely on the composition of lipid rafts. Also, in the CA1 hippocampal area, both LTP and LTD become impaired by raft disruption as a consequence of cholesterol depletion, which correlates with a decrease of synaptic efficiency by pre-synaptic and post-synaptic inhibition of transmission due

to modifications of AMPA and NMDA responses (Frank et al., 2008; Koudinov and Koudinova, 2001).

Most evidences on the deleterious effects of raft disruption on synaptic transmission and plasticity reported up to now, derive from artificial manipulations of membrane cholesterol (with little specificity for membrane domains). Thus, the observation reported here that lipid rafts cholesterol contents are reduced as part of the normal aging program, at least in the frontal cortex, is relevant and provides a biochemical support for the modifications on protein-protein dynamics and protein clustering in lipid raft underlying alterations in synaptic plasticity and memory retention and learning during aging.

Several reports have demonstrated that besides cholesterol, other important lipids undergo a selective depletion with aging in both human brain and animal models. Amongst the most important, long chain polyunsaturated fatty acids (LCPUFA) are particularly relevant since they are highly concentrated in brain membranes. Both n-6 LCPUFA (mainly arachidonic acid) and n-3 LCPUFA (mainly DHA) have been found to be reduced during physiological aging in human brain (particularly in cortex and hippocampus) (Cutuli, 2016; McNamara et al., 2008). However, a recent exhaustive lipid profiling of human frontal cortex demonstrates that fatty acid contents are basically preserved through the adult lifespan but decay at advanced ages (Cabré et al., 2017). The onset of lipid alterations occurs at advanced ages, therefore changes do not occur gradually, and depends on the group of fatty acids. Thus, the increase in saturated fatty acids begins in the decade following age 70, but the decay in main LCPUFA (arachidonic acid and DHA) occurs in the decade following age 80 (Cabré et el., 2017). In agreement, in the present study, the increase in saturates in total phospholipids was observed in older men and women, but the decline in LCPUFA was only apparent in older women. This is likely due to the age range used in this study (24-85 years), with only two subjects above the age of 80. However, our demonstration that these essential fatty acids are affected by aging in frontal cortex lipid rafts indicates that these microdomains are particularly sensitive and that alterations in their biogenesis represent early events during normal aging. These findings are clearly in line with our previous findings demonstrating a dramatic reduction of both n-3 and n-6 LCPUFA in cortical lipid rafts in different neurodegenerative diseases, namely Alzheimer's disease, Dementia with Lewy Bodies and Parkinson`s disease (Díaz et al. 2013; Fabelo et al., 2011; Marín et al., 2017; Martín et al., 2010b). Similar effects have also been observed in the

double transgenic (APP/PS1) mice model of familial Alzheimer's disease (Fabelo et al., 2012). Paralleling the age-associated changes of LCPUFA in non-pathological human brain, lipid raft alterations are also accompanied by increased sulphatide levels (and total polar lipids) as well as reduction in cholesterol contents. This is interesting since these features are signs of lipid raft aging in mice frontal cortex, being this effect exacerbated in the mice model of familial Alzheimer's disease (Fabelo et al., 2012). Comparatively, these observations led us to hypothesize that it may be the depletion of n-3 and n-6 PUFA, cholesterol and probably plasmalogens, together with the increase of sulphatides, the features that determine the onset of the neurodegenerative process, at least at the lipid raft level.

Noteworthy, several studies have disclosed alterations in the activity and/or gene expression of proteins involved in lipid metabolism. For instance, *ELOVL2* (encoding for the ELongase Of Very Long fatty acids 2 protein, one of the critical enzymes involved in the elongation of n-3 and n-6 polyunsaturated fatty acids), undergoes an increase in its methylation level which strongly correlates with age in healthy volunteers (Steegenga et al., 2014). Further, It has been reported for the brain cortex that levels of plasmalogens decrease with age, and that the activity of DHAP-AT (Dihydroxyacetone phosphate acyltransferase, involved in the first step of plasmalogens biosynthesis) decreases during aging, along with increased activation of Pls-PLA2s (a specific plasmalogens degradation enzyme, endowed with phospholipase A2 activity (Andrè et al., 2006). These effects appear to be mediated by epigenetic mechanisms. On the other hand, protein expression of HMGR (3-hydroxy 3-methyl glutaryl coenzyme A reductase, the key regulatory enzyme in cholesterol biosynthesis) and LDLr (the low density lipoprotein receptor, responsible of cellular cholesterol uptake), undergo age-related alterations in different brain areas, including frontal cortex and hippocampus (Segatto et al., 2013). Interestingly, this same study demonstrated an age- and sex-dependent reduction of HMGR, with total HMGR protein levels being lower in female rats than age-matched males, secondary to reduction of transcriptional processes.

One relevant observation in the present study is the demonstration that lipid modifications are qualitatively and quantitatively linked to gender. These gender differences extend to both lipid classes (plasmalogens, sterol esters), fatty acids (saturates 15:0, 22:0, 16:0 DMA, 22:0, 23:0, monoenes 18:1n-7, 18:1n-9, 18:1n-9 DMA, 18:1n-7 DMA, polyunsaturated 22:6n-3, 22:5n-6, 20:4n-6) and indexes (total n-3 LCPUFA, UIx and PIx). Further, bivariate relationships using age

as independent variable are also different between sexes. These differences were further confirmed by using partial correlation analyses controlling for gender (Table 3). We used a multivariate approach in a 3D space using these differential variables to obtain a representative lipid fingerprint for the lipid rafts from the different groups. The analyses revealed that largest differences between young and older individuals was more notorious in the case of women, being the group Women > 60 the best segregated, both for fatty acids and lipid classes. Further, the outcomes of ANOVA-II analyses applied to factor scores demonstrate significant interactions between age and gender for fatty acids in both factor scores, and for factor score 2 for lipid classes, which agrees with the fact that most differential features between sexes occurred in fatty acids. Furthermore, some relevant bivariate relationships between fatty acids and lipid classes were also altered by the effect of aging in a gender-related manner, indicating a pronounced alteration of phospholipid acylation-reacylation in older women, while others, perhaps more critical for membrane homeoviscosity and raft stability (Fig. 4D, G and H), remained unaltered.

The existence of gender differences in lipid raft composition and the ascertainment that these alterations are more prominent in older women might be seminal to explain why progression from mild cognitive impairment to AD is higher in postmenopausal women than in age-matched men (Alzheimer's Association, 2014; Henderson, 2008; Rettberg et al., 2016). Indeed, according to Alzheimer's Association (2014), women have a 2-fold greater lifetime risk of developing AD. Noteworthy, comparison of our present results with those of lipid rafts from early stages of Alzheimer disease (Fabelo et al., 2014) reveal that, although lipid alterations in AD brains were much more severe, similar trends to destabilize lipid matrix of lipid rafts were observed in the frontal cortex from aged brains, particularly in women. Although the biological basis for gender differences in AD remains to be established, basic science and epidemiological data indicate that the menopausal transition and decline in estrogen adversely affect brain and whole-body metabolism (Brinton et al., 2015; Henderson and Brinton, 2010; Henderson, 2008; Rettberg et al., 2016). Presumably, these lipid alterations will impact synaptic function and protein interactions and dynamics in signalosomes residing in lipid rafts (see Hara et al., 2015 for a comprehensive review). In line with this, we have recently observed that aging disrupts the neuroprotective multiprotein complex estrogen-receptor (ERα)/IGF-1R/VDAC1, which interacts with caveolin-1 in a signalosome within lipid rafts in the frontal cortex of aged women

(Canerina-Amaro et al., 2017). Such disruption displaces protein components towards non-raft domains in parallel with modifications of raft lipid matrix and leave plasmalemmal VDAC out of the control of ER, thereby favouring its pro-apoptotic actions (Fernandez-Echevarría et al., 2014; Herrera et al., 2011a; 2011b).

In summary, our lipid profiling study of lipid rafts reveals that aging is accompanied by a substantial alteration of lipid rafts lipid matrix. This phenomenon, termed "lipid raft aging" exhibits clear gender differences and appear to be more pronounced in women than in men, especially in older (postmenopausal) women. The outcomes led us to conclude that human cortical lipid rafts are modified by aging in a gender-dependent fashion. Given the central role of bilayer lipid matrix in lipid rafts functionality and neuronal signalling, we hypothesize that these findings might underlie the higher prevalence of cognitive decline evolving towards Alzheimer´s disease in postmenopausal women.

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1 Table 1. Summary of cases

Case	Age	Gender	PM delay	SNC	Primary diseases	Immediate diseases	Cause of death
1	38	M	18h	NL	Diverticulitis, peritonitis	Surgical resection	
2	40	M	9h15min	NL	Sarcoidosis, lung stage IV	Respiratory insufficiency	Cardiac failure
3	39	M	3h30min	NL	AHT, dyslipidemia, obesity	Pneumonia	Respiratory failure
4	47	M	4h50min	SBVD	Ischemic cardiopathy	Coronary bypass	Massive cardiac infarction
5	40	M	5h10min	NL	INA	INA	INA
6	44	M	6h40min	SBVD	AHT, diabetes, obesity	Pulmonary thromboembolism	Respiratory failure
7	52	M	3h	NL	Pancreatic carcinoma	Multiple metastasis	Hepatic failure
8	78	M	2h15min	SBVD	Lung carcinoma	Multiple metastasis, pneumonia	Respiratory failure
9	79	M	7h	SBVD	Cardiomegaly	Cardiac infarction	Multi-organic failure
10	85	M	5h45min	SBVD	Temporal arteritis; ischemic cardiopathy	Coronary bypass	Respiratory failure
11	70	M	13h	NL	AHT, diabetes, obesity, alcoholism, dyslipidemia	Ischemic heart disease	Cardiac and respiratory failure
12	69	M	7h35min	AAII	AHT, alcoholism, anemia, hepatic cirrhosis	Hepatorenal transplantation	Respiratory failure
13	71	M	12h	NL	Gastrectomy	Prostate carcinoma	Respiratory failure
14	61	M	3h55min	NL	AHT, dyslipidemia, COPD	Acute hepatitis	Multi-organic failure
15	45	F	14h40min	NL	AHT, dyslipidemia, COPD	Metastatic carcinoma	Respiratory failure
16	24	F	6h	NL	Ovaric dysgerminoma	Renal insufficiency	Septic shock
17	46	F	14h5min	NL	Diabetes, cardiomyopathy	Renal insufficiency	Cardiac failure
18	49	F	7h	NL	Paraganglioma	Small cell carcinoma of the lung	Respiratory failure
19	47	F	9h35min	NL	INA	INA	INA
20	74	F	5h30min	NL	Renal insufficiency	Infectious endocarditis	Massive digestive hemorrhage
21	82	F	11h	NL	INA	INA	INA
22	75	F	3h	SBVD	Ischemic heart disease	Acute heart infarction	Cardiac failure
23	69	F	2h30min	NL	Alcoholism	Double mitral heart disease	Cardiac failure
24	66	F	8h	NL	Valvular heart disease	Intervention	Cardiac arrest
25	65	F	4h	NL	Tachyarrhythmia	Pulmonary thromboembolism	Respiratory failure

2

NL: no lesions; SBVD: small blood vessel disease; AHT: arterial hypertension; AAII: Alzheimer astrocyte type II; COPD: chronic obstructive pulmonary disease. INA: Information not available.

1 Table 2. Summary of the results of lipid rafts lipid composition in younger (<60) and older (>60)
2 Women and Men.

	Men < 60	Men > 60	Women < 60	Women > 60
Age	42,86 ± 1,93	73,29 ± 3,00	42,20 ± 4,11	71,67 ± 2,38
FATTY ACIDS				
14 : 0	0,56 ± 0,05	0,50 ± 0,04	0,51 ± 0,08	0,53 ± 0,05
15 : 0	0,61 ± 0,19	0,82 ± 0,18	1,21 ± 0,31	0,98 ± 0,14
16 : 0 DMA	0,59 ± 0,28 a	0,03 ± 0,03 b	0,00 ± 0,00 c	0,00 ± 0,00 c
16 : 0	26,66 ± 1,48	24,86 ± 1,19	24,57 ± 1,52	22,95 ± 1,84
16 : 1	0,90 ± 0,07	0,94 ± 0,08	0,99 ± 0,15	1,17 ± 0,11
16 : 2	0,12 ± 0,04	0,17 ± 0,03	0,23 ± 0,03	0,23 ± 0,02
17 : 0	0,39 ± 0,04	0,32 ± 0,01	0,32 ± 0,01	0,30 ± 0,02
18 : 0 DMA	4,14 ± 0,13	4,23 ± 0,19	4,13 ± 0,18	4,15 ± 0,12
18:1 n-9 DMA	0,14 ± 0,07 a	0,01 ± 0,01 b	0,00 ± 0,00 c	0,00 ± 0,00 c
18:1 n-7 DMA	0,24 ± 0,12 a	0,02 ± 0,02 b	0,00 ± 0,00 c	0,00 ± 0,00 c
18 : 0	22,22 ± 0,34	22,50 ± 0,42	21,75 ± 0,53	21,51 ± 0,54
18:1 n-9	16,02 ± 0,74 b	16,38 ± 1,07 b	17,77 ± 0,63 b	20,16 ± 1,33 a
18:1 n-7	4,12 ± 0,16 b	5,39 ± 0,42 a	5,00 ± 0,54 ab	4,79 ± 0,35 ab
18 : 2 n-6	0,95 ± 0,07	0,90 ± 0,12	1,10 ± 0,13	0,78 ± 0,09
18 : 3 n-3	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00
20 : 0	0,25 ± 0,03	0,20 ± 0,01	0,18 ± 0,01	0,21 ± 0,01
20 : 1	0,99 ± 0,14	1,07 ± 0,27	1,28 ± 0,27	1,22 ± 0,20
20 : 2 n-6	0,05 ± 0,04	0,17 ± 0,05	0,20 ± 0,08	0,13 ± 0,06
20 : 3 n-6	0,58 ± 0,06	0,50 ± 0,01	0,59 ± 0,08	0,56 ± 0,03
20 : 4 n-6	4,71 ± 0,36 a	3,90 ± 0,15 ab	3,66 ± 0,14 ab	3,49 ± 0,25 b
20 : 5 n-3	0,03 ± 0,03	0,07 ± 0,04	0,12 ± 0,05	0,11 ± 0,04
22:0	0,12 ± 0,05 b	0,20 ± 0,08 a	0,13 ± 0,05 b	0,25 ± 0,04 a
22 : 1	0,15 ± 0,09	0,36 ± 0,13	0,35 ± 0,05	0,32 ± 0,11
22 : 2 n-6	0,27 ± 0,11 b	0,63 ± 0,11 ab	0,56 ± 0.08 ab	0,73 ± 0,10 a
23:0	1,55 ± 0,57 b	2,39 ± 0,10 a	2,41 ± 0,19 a	2,39 ± 0,19 a
22 : 5 n-6	0,88 ± 0,11 a	0,59 ± 0,08 ab	0,63 ± 0,16 ab	0,42 ± 0,07 b
22 : 5 n-3	0,07 ± 0,03	0,15 ± 0,04	0,04 ± 0,04	0,11 ± 0,03
22 : 6 n-3	7,91 ± 0,36 a	6,83 ± 0,62 ab	6,28 ± 0,31 ab	6,59 ± 0,25 b
24:0	0,22 ± 0,09	0,12 ± 0,05	0,10 ± 0,06	0,36 ± 0,15
24 : 1 n-9	0,69 ± 0,11	1,37 ± 0,23	1,47 ± 0,34	1,20 ± 0,23
Totals and indexes				
Saturates	57,30 ± 1,27	56,16 ± 1,57	55,32 ± 1,53	53,63 ± 2,11
Unsaturated	40,25 ± 0,96	39,82 ± 0,96	40,06 ± 0,85	41,25 ± 1,25
DMAs	5,10 ± 0,40	4,29 ± 0,19	4,13 ± 0,18	4,15 ± 0,12
n-9	16,86 ± 0,70	17,76 ± 1,26	19,24 ± 0,93	20,36 ± 1,33
n-3	8,01 ± 0,33 a	7,05 ± 0,67 ab	6,04 ± 0,50 b	6,80 ± 0,26 ab
n-6	8,66 ± 0,86 a	6,75 ± 0,26 ab	6,74 ± 0,30 ab	6,11 ± 0,29 b
n-3 LCPUFA	7,98 ± 0,35 a	6,98 ± 0,65 ab	5,92 ± 0,47 b	6,69 ± 0,26 ab
n-3/n-6	0,96 ± 0,07	1,05 ± 0,09	0,91 ± 0,09	1,12 ± 0,06

18:1n-9/n-3 LCPUFA	2,04 ± 0,16 b	2,59 ± 0,40 ab	3,13 ± 0,34 a	2,87 ± 0,20 ab
Monoenoic	23,40 ± 0,88	25,84 ± 1,41	27,05 ± 1,37	28,11 ± 1,39
PIx	90,11 ± 5,65 a	74,90 ± 5,61 b	66,71 ± 4,34 b	70,63 ± 2,54 b
UIx	104,09 ± 5,18 a	91,97 ± 3,50 b	86,87 ± 2,07 b	90,16 ± 2,13 b
saturates/unsaturates	1,43 ± 0,06	1,42 ± 0,07	1,39 ± 0,07	1,32 ± 0,09
saturates/n-3	7,23 ± 0,32 b	8,43 ± 0,68 ab	9,40 ± 0,60 a	7,98 ± 0,52 ab
saturates/n-9	3,45 ± 0,22	3,31 ± 0,32	2,92 ± 0,21	2,75 ± 0,29
LIPID CLASSES				
LPC	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00
SM	8,95 ± 1,90	12,93 ± 1,08	12,40 ± 1,71	9,78 ± 2,01
PC	5,63 ± 0,33	5,32 ± 0,67	4,30 ± 0,65	5,26 ± 0,46
PS	7,36 ± 0,29	7,09 ± 0,70	6,35 ± 0,23	6,30 ± 0,44
PI	2,47 ± 0,14	2,20 ± 0,27	2,12 ± 0,10	2,02 ± 0,13
PG	0,90 ± 0,16	0,79 ± 0,09	0,53 ± 0,14	0,61 ± 0,14
PE	20,87 ± 0,51	20,24 ± 0,30	19,51 ± 0,97	21,58 ± 0,87
Sulphatides	8,57 ± 1,03 b	9,94 ± 0,92 ab	10,12 ± 0,83 ab	11,44 ± 0,57 a
Cerebrosides	3,97 ± 1,04 a	3,89 ± 0,52 ab	4,86 ± 1,16 ab	6,27 ± 1,05 a
DAG	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00
Cholesterol	36,92 ± 2,14	33,91 ± 1,13	36,79 ± 2,25	33,01 ± 1,78
FFA	1,87 ± 0,32	2,29 ± 0,33	1,89 ± 0,25	1,96 ± 0,33
TG	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00
SE	2,47 ± 0,90	1,37 ± 0,72	1,13 ± 0,62	1,74 ± 0,74
Totals				
TNL	41,25 ± 2,06	37,57 ± 1,20	39,82 ± 1,51	36,71 ± 2,13
TPL	58,72 ± 2,05	62,41 ± 1,21	60,18 ± 1,51	63,27 ± 2,12

1

2 Results are expressed as mean ± SEM. Different letters in the same row indicate statistical differences with *p*-
3 values < 0.05. DMA: Dimethylacetals, LCPUFA: Long-chain polyunsaturated fatty acids, PIx: Peroxydability
4 index, UIx: unsaturation index LPC: Lyso-phosphatidylcholine, SM: Sphingomyelin, PC: Phosphatidylcholine, PS:
5 Phosphatidylserine, PI: Phosphatidylinositol, PG: Phosphatidylglycerol, PE: Phosphatidylethanolamine, DAG:
6 Diacylglycerides, FFA: Free fatty acids, TG: Triglycerides, SE: sterol esters, TNL: Total neutral lipids, TPL: Total
7 polar lipids.

8

Table 3. Correlation analyses for all lipid variables in pooled data (Women + Men), partial correlations (controlling for sex), and Pearson correlations for separated gender.

			Gender	
Fatty acid	Total Pooled Pearson	Partial Correlations	Men Pearson	Women Pearson
14 : 0	-0,147	-0,147	-0,359	0,042
15 : 0	-0,025	-0,029	0,099	-0,166
16 : 0 DMA	-0,282	-0,298	-0,399	(a) #
16 : 0	-0,176	-0,180	-0,261	-0,098
16 : 1	0,134	0,140	0,012	0,242
16 : 2	0,116	0,127	0,261	-0,139
17 : 0	-0,265	-0,280	-0,386	-0,049
18 : 0 DMA	0,158	0,158	0,197	0,103
18:1 n-9 DMA	-0,235	-0,246	-0,33	(a) #
18:1 n-7 DMA	-0,262	-0,277	-0,371	(a) #
18 : 0	-0,028	-0,027	0,057	-0,107
18:1 n-9	0,161	0,175	0,09	0,425
18:1 n-7	0,127	0,127	0,559 *	-0,402
18 : 2 n-6	-0,363 *	-0,367 *	-0,135	-0,602 #
20 : 0	-0,146	-0,150	-0,362	0.,638 *
20 : 1	-0,011	-0,012	0,143	-0,206
20 : 2 n-6	0,083	0,083	0,45	-0,279
20 : 3 n-6	-0,385 *	-0,389 *	-0,421 #	-0,361
20 : 4 n-6	-0,242	-0,271	-0,415 #	-0,002
20 : 5 n-3	0,141	0,145	0,243	0,044
22:0	0,233	0,233	0,092	0,492
22 : 1	0,063	0,062	0,277	-0,324
22 : 2 n-6	0,403 #	0,425 #	0,459 #	0,379
23:0	0,193	0,197	0,288	-0,019
22 : 5 n-6	-0,321	-0,341 *	-0,431	-0,232
22 : 5 n-3	0,370 *	0,375 *	0,325	0,444
24:0	0,063	0,063	-0,289	0,347
22 : 6 n-3	-0,110	-0,117	-0,316	0,365
24 : 1 n-9	0,162	0,165	0,687 *	-0,378

Lipid Index	Total Pooled Pearson	Partial Correlations	Men Pearson	Women Pearson
Saturates	-0,148	-0,152	-0,214	-0,092
Unsaturates	0,091	0,091	0,039	0,15
DMAs	-0,192	-0,204	-0,323	0,103
n-9	0,173	0,188	0,212	0,163
n-3	0,062	0,070	-0,27	0,693 *
n-6	-0,343 *	-0,373 *	-0,424	-0,331
n-3 LCPUFA	0,052	0,060	-0,29	0,707 *
n-3/n-6	0,418 #	0,418 #	0,184	0,714 *
18:1n-9/n-3 LCPUFA	0,021	0,020	0,303	-0,424
Monoenoic	0,198	0,216	0,397	-0,003
PIx	-0,095	-0,104	-0,385	0,562 #

UIx	-0,107	-0,116	-0,369	0,611 #
saturates/unsaturates	-0,113	-0,114	-0,124	-0,104
saturates/n-3	-0,151	-0,159	0,312	-0,736 *
saturates/n-9	-0,120	-0,127	-0,147	-0,102

Lipid classes	Total Pooled Pearson	Partial Correlations	Men Pearson	Women Pearson
LPC	NA	NA	NA	NA
SM	0,110	0,110	0,476 *	-0,315
PC	0,054	0,057	-0,237	0,409
PS	-0,011	-0,009	-0,057	0,082
PI	-0,202	-0,209	-0,229	-0,194
PG	0,053	0,059	-0,149	0,318
PE	0,216	0,216	-0,299	0,533 #
Sulphatides	0,309 #	0,326	0,287	0,411 #
Cerebrosides	0,143	0,150	-0,021	0,316
Cholesterol	-0,357 *	-0,358 *	-0,28	-0,446
FFA	0,083	0,084	0,11	0,046
SE	-0,162	-0,162	-0,322	0,086
TNL	-0,416 *	-0,419 *	-0,404 #	-0,437 *
TPL	0,416 *	0,419 *	0,405 #	0,437 *

Statistical differences: * $p<0.01$, # $p<0.05$

Results are expressed as mean ± SEM. Different letters in the same row indicate statistical differences with *p*-values below 0.01 (*) or 0.05 (#). DMA: Dimethylacetals, LCPUFA: Long-chain polyunsaturated fatty acids, PIx: Peroxydability index, UIx: unsaturation index LPC: Lyso-phosphatidylcholine, SM: Sphingomyelin, PC: Phosphatidylcholine, PS: Phosphatidylserine, PI: Phosphatidylinositol, PG: Phosphatidylglycerol, PE: Phosphatidylethanolamine, FFA: Free fatty acids, SE: sterol esters, TNL: Total neutral lipids, TPL: Total polar lipids.

1 Table 4. Lipid composition of frontal brain cortex from young (< 60 years, n=10) and older (>
2 60 years, n=11) Men and Women.

3

	Men < 60	Men > 60	Women < 60	Women > 60
FATTY ACIDS				
14 : 0	0,35 ± 0,05	0,34 ± 0,03	0,34 ± 0,04	0,40 ± 0,02
15 : 0	0,56 ± 0,10	0,69 ± 0,22	0,59 ± 0,08	0,63 ± 0,06
16 : 0	18,89 ± 1,15	19,78 ± 0,35	20,19 ± 2,55	22,30 ± 1,51
16 : 1[1]	1,32 ± 0,21	1,00 ± 0,14	1,25 ± 0,21	2,70 ± 0,86
16 : 4	4,33 ± 0,45 a	4,45 ± 0,39 a	4,48 ± 1,00 a	2,86 ± 0,80 b
18 : 0	21,57 ± 0,69	23,98 ± 0,91	23,92 ± 1,29	25,06 ± 1,61
18: 1 n-9	14,64 ± 2,12	13,45 ± 1,54	16,81 ± 2,46	18,09 ± 2,53
18: 1 n-7	7,29 ± 0,77	6,77 ± 0,69	7,31 ± 1,46	7,34 ± 1,00
18 : 2 n-6	0,56 ± 0,14	0,87 ± 0,41	0,78 ± 0,49	0,43 ± 0,07
20 : 1[2]	1,26 ± 0,47	0,90 ± 0,30	1,34 ± 0,66	1,36 ± 0,50
20 : 3 n-6	0,71 ± 0,09	0,56 ± 0,03	0,45 ± 0,27	0,44 ± 0,13
20 : 4 n-6	5,06 ± 0,78	4,01 ± 0,71	3,23 ± 0,70	2,37 ± 0,76
20 : 5 n-3	0,16 ± 0,09	0,85 ± 0,32	0,38 ± 0,40	0,23 ± 0,05
22: 0	0,37 ± 0,15 b	1,30 ± 0,42 a	0,60 ± 0,68 ab	0,27 ± 0,11 b
22 : 2 n-6	0,62 ± 0,12 b	1,97 ± 0,59 a	0,89 ± 1,02 ab	0,39 ± 0,06 b
22 : 4 n-6	3,69 ± 0,17	3,25 ± 0,42	2,68 ± 0,69	2,09 ± 0,55
22 : 5 n-6	0,73 ± 0,19	0,46 ± 0,22	0,37 ± 0,11	0,18 ± 0,17
24: 0	0,37 ± 0,08	0,32 ± 0,05	0,43 ± 0,20	0,36 ± 0,09
22 : 6 n-3	7,46 ± 1,09 a	5,63 ± 1,13 ab	6,41 ± 1,36	5,57 ± 1,01
24 : 1 n-9	1,24 ± 0,38	1,13 ± 0,33	1,36 ± 0,61	1,34 ± 0,47
Totals and indexes				
Saturates	42,50 ± 1,64	46,87 ± 1,22	46,48 ± 3,29	49,55 ± 3,03
n-9	15,88 ± 2,32	14,44 ± 1,48	18,17 ± 2,91	19,44 ± 2,82
n-3	7,81 ± 1,17	6,70 ± 0,86	7,34 ± 1,18	7,49 ± 0,86
n-6	11,56 ± 1,11 a	11,34 ± 1,27 a	9,02 ± 1,50 a	6,86 ± 1,68 b
n-3 LCPUFA	7,81 ± 1,17	6,70 ± 0,86	6,79 ± 1,18	5,80 ± 0,86
n-3/n-6	0,68 ± 0,08	0,59 ± 0,13	0,81 ± 0,14	1,09 ± 0,10
18:1/n-3 H	1,88 ± 0,53	2,01 ± 0,07	2,48 ± 1,06	3,12 ± 1,08
Saturates/n-3	5,45 ± 0,38	7,00 ± 1,02	6,34 ± 0,69	6,62 ± 0,83
Saturates /n-9	2,68 ± 0,25	3,25 ± 0,35	2,56 ± 0,29	2,55 ± 0,32
LIPID CLASSES				
LPC	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00
SM	3,52 ± 0,30	4,20 ± 0,50	3,74 ± 0,72	3,80 ± 0,60
PC	9,29 ± 1,04	8,32 ± 0,73	8,03 ± 1,93	8,33 ± 1,44
PS	8,18 ± 1,33	8,93 ± 0,83	7,99 ± 1,70	9,22 ± 0,73
PI	2,98 ± 1,21	3,23 ± 0,35	2,11 ± 0,75	3,60 ± 0,74
PG	0,73 ± 0,42	1,41 ± 0,22	1,37 ± 0,53	0,68 ± 1,52
PE	22,62 ± 1,23	21,17 ± 0,78	21,60 ± 1,54	22,91 ± 1,88

Sulphatides	12,07 ± 2,17	11,85 ± 1,78	12,16 ± 2,71	12,26 ± 1,13
Cerebrosides	5,60 ± 1,77	6,09 ± 1,51	6,79 ± 2,58	6,94 ± 1,85
DAG	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00
CHO	29,64 ± 1,01	30,07 ± 1,63	31,26 ± 0,97	27,77 ± 1,76
FFA	3,21 ± 0,73	3,05 ± 0,68	3,74 ± 0,75	1,61 ± 1,15
TG	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00
SE	2,16 ± 1,08	1,68 ± 0,51	1,22 ± 0,43	2,87 ± 1,47
Totals				
TNL	52,68 ± 3,02	52,73 ± 0,97	55,17 ± 4,91	51,45 ± 2,48
TPL	47,32 ± 3,02	47,27 ± 0,97	44,83 ± 4,91	48,55 ± 2,48

Results are expressed as mean ± SEM. Different letters in the same row indicate statistical differences with *p*-values below 0.05. [1] Contains n-9 and n-7 isomers. [2] Contains n-11 and n-9 isomers. Totals include some minor components not shown. DMA: Dimethylacetals, LCPUFA: Long-chain polyunsaturated fatty acids, PIx: Peroxydability index, UIx: unsaturation index LPC: Lyso-phosphatidylcholine, SM: Sphingomyelin, PC: Phosphatidylcholine, PS: Phosphatidylserine, PI: Phosphatidylinositol, PG: Phosphatidylglycerol, PE: Phosphatidylethanolamine, DAG: Diacylglycerides, FFA: Free fatty acids, TG: Triglycerides, SE: sterol esters, TNL: Total neutral lipids, TPL: Total polar lipids.

Figure 1. Age-associated changes in lipid rafts in a subset of lipid groups, indexes and lipid classes in healthy (control) women and men. A and B: Examples of bivariate relationships that differ between gender. C: Regression lines for lipid groups not affected by aging in neither sex.

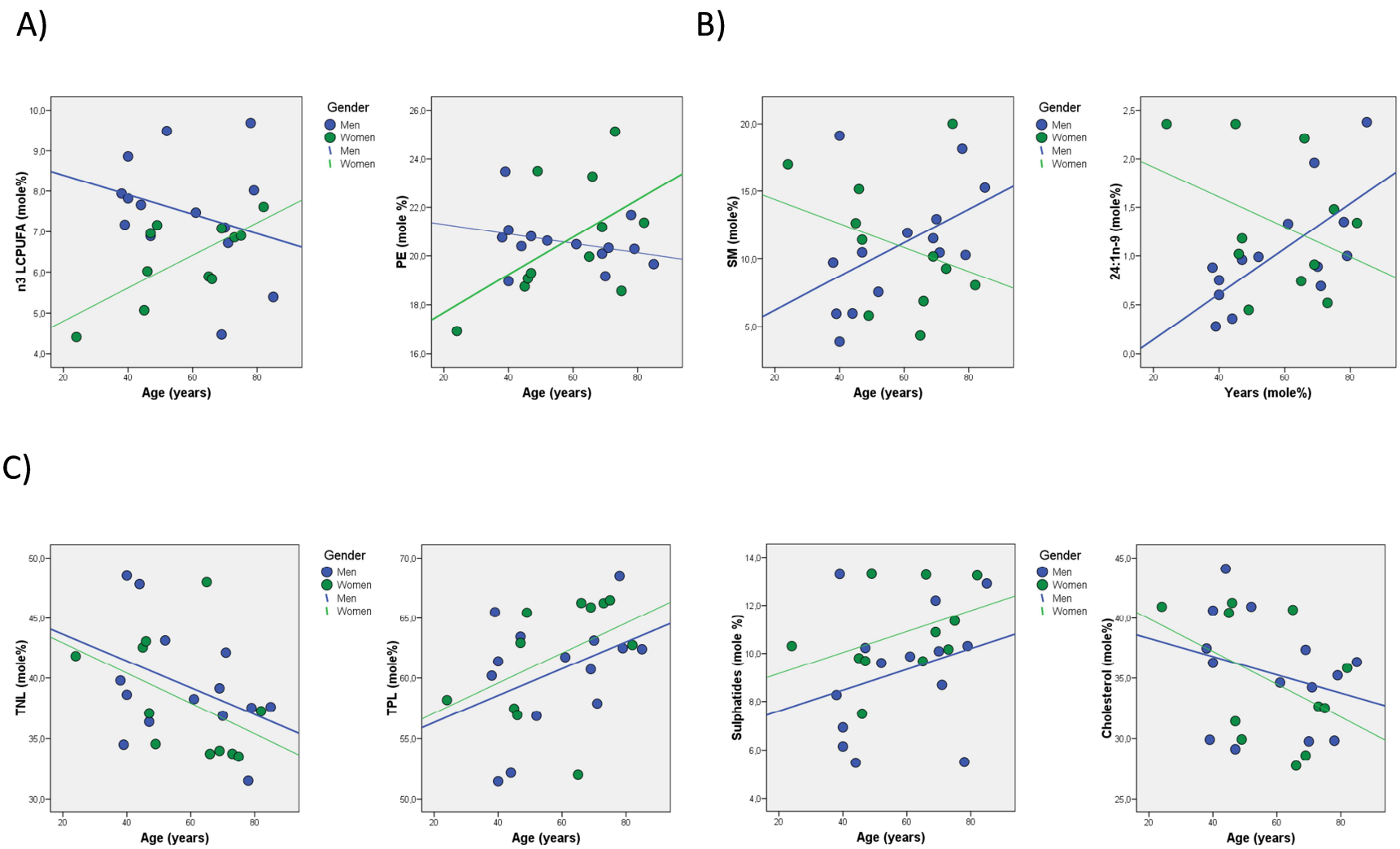


Figure 2. Score plots from Principal Component Analyses for lipid classes in all groups according to age-gender pairs (A: Age and B: Gender, for PC1 and PC2) and C for all groups in a 3-D space. D and E analyses of age-gender interactions in factor scores 1 and 2. For details see results.

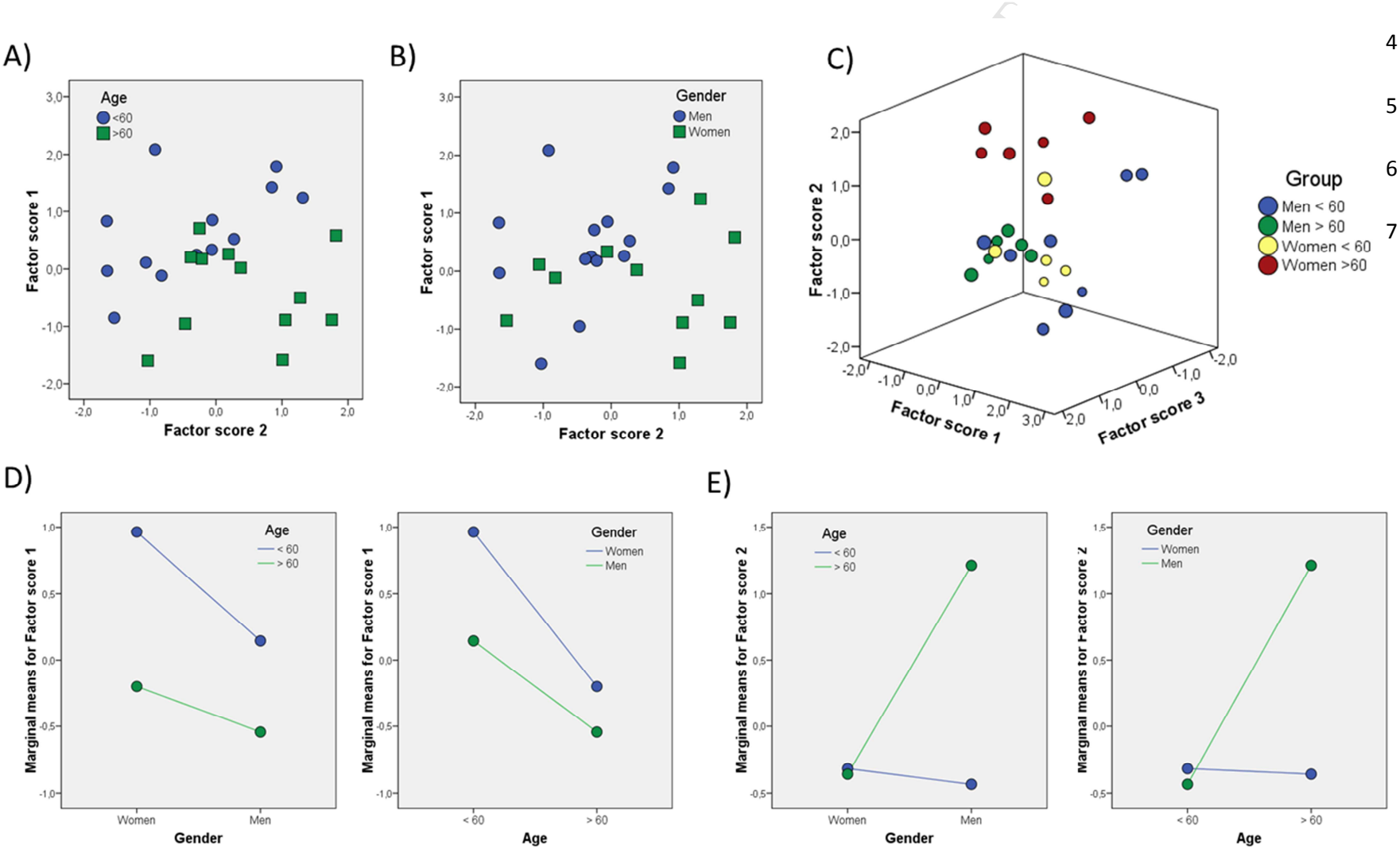


Figure 3. Score plots from Principal Component Analyses for fatty acids in all groups according to age-gender pairs (A: Age and B: Gender for PC1 and PC2) and C for all groups in a 3-D space. D and E analyses of age-gender interactions in factor scores 1 and 2. For details see results.

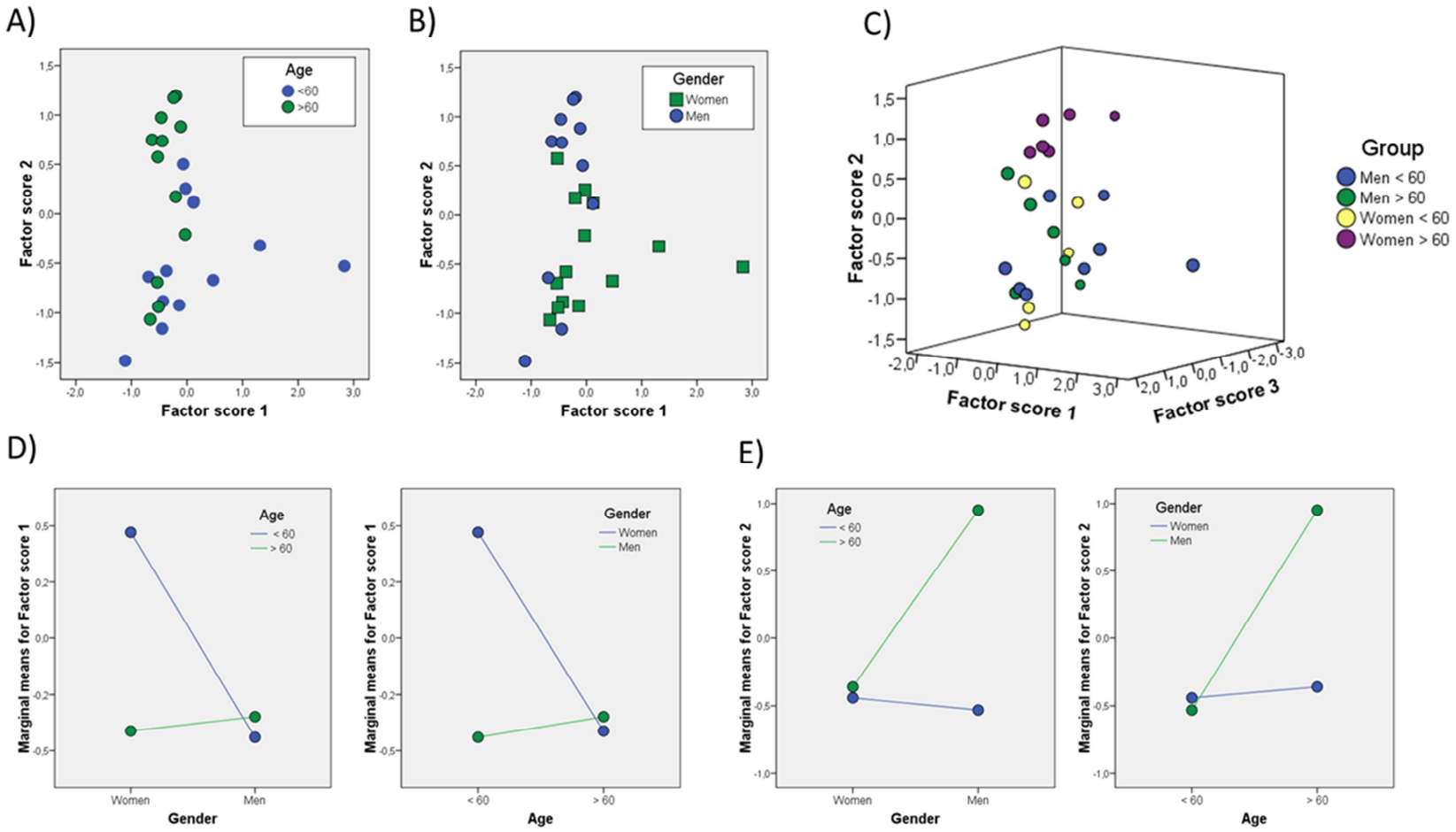
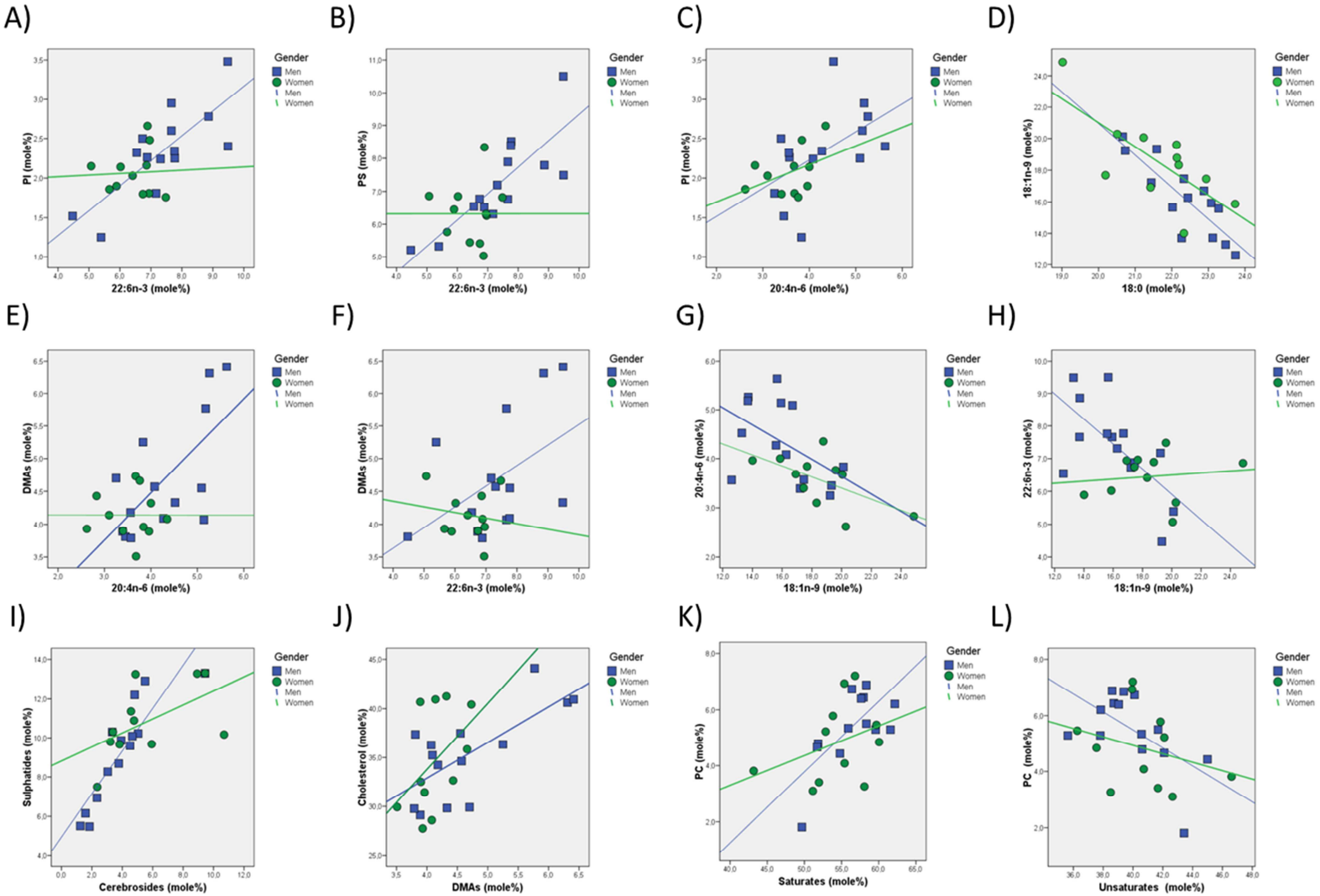


Figure 4. Bivariate regression lines between different groups of lipids from frontal cortex lipid rafts in women and men. For details see results.



Supplementary Table 1. Pearson correlation matrixes for totals and indexes, fatty acids and lipid classes in lipid rafts from women and men

	Pearson Correlations	Women: Saturates	Unsaturates	DMAs	n-9	n-3	n-6	n-3 LCPUFA	n-3/n-6	18:1n-9/n-3HUFA	Monoenes	PIx	UIx	sat/unsat	saturates/n-3	saturates/n-9	TNL	TPL
Men	Saturates	1,000	-,973(**)	-0,544	-,916(**)	0,145	0,566	0,164	-0,270	-,739(**)	-,947(**)	0,337	-0,083	,984(**)	0,277	,922(**)	0,288	-0,289
	Unsaturates	-,771(**)	1,000	0,534	,953(**)	-0,057	-0,573	-0,075	0,336	,704(*)	,947(**)	-0,263	0,170	-,996(**)	-0,343	-,970(**)	-0,440	0,442
	DMAs	-0,048	,548(*)	1,000	0,537	-0,174	0,103	-0,162	-0,186	0,461	0,450	-0,108	0,166	-0,559	-0,059	-0,548	0,222	-0,222
	n-9	-,896(**)	,581(*)	-0,194	1,000	-0,033	-,665(*)	-0,069	0,398	,722(*)	,926(**)	-0,262	0,143	-,949(**)	-0,358	-,989(**)	-0,489	0,490
	n-3	0,458	-0,007	0,401	-,656(*)	1,000	-0,077	,994(**)	,791(**)	-,698(*)	-0,313	,933(**)	,905(**)	0,120	-,903(**)	0,070	-,630(*)	,629(*)
	n-6	0,191	0,434	,833(**)	-0,374	,589(*)	1,000	-0,046	-,659(*)	-0,398	-,669(*)	0,272	0,084	0,562	0,339	,608(*)	0,553	-0,555
	n-3 LCPUFA	0,452	0,008	0,434	-,661(*)	,998(**)	,615(*)	1,000	,762(**)	-,728(*)	-0,334	,940(**)	,906(**)	0,136	-,885(**)	0,095	-0,597	0,596
	n-3/n-6	0,242	-0,495	-0,516	-0,227	0,325	-,560(*)	0,297	1,000	-0,264	0,196	0,531	,637(*)	-0,280	-,897(**)	-0,334	-,813(**)	,814(**)
	18:1n-9/n-3 LCPUFA	-,737(**)	0,338	-0,333	,879(**)	-,910(**)	-0,491	-,913(**)	-0,362	1,000	,855(**)	-,816(**)	-0,512	-,741(**)	0,370	-,738(**)	0,073	-0,072
	Monoenoic	-,876(**)	0,489	-0,250	,930(**)	-,786(**)	-0,512	-,789(**)	-0,191	,932(**)	1,000	-0,540	-0,143	-,959(**)	-0,095	-,936(**)	-0,304	0,306
	PIx	0,365	0,210	,649(*)	-,587(*)	,934(**)	,835(**)	,946(**)	-0,017	-,823(**)	-,738(**)	1,000	,903(**)	0,315	-,756(**)	0,281	-0,388	0,387
	UIx	0,186	0,418	,759(**)	-0,427	,841(**)	,906(**)	,857(**)	-0,189	-,679(**)	-,584(*)	,973(**)	1,000	-0,117	-,896(**)	-0,137	-0,555	0,554
	saturates/unsaturates	,943(**)	-,935(**)	-0,299	-,793(**)	0,239	-0,114	0,229	0,368	-,572(*)	-,725(**)	0,083	-0,120	1,000	0,290	,967(**)	0,377	-0,379
	saturates/n-3	-0,270	-0,104	-0,436	0,472	-,951(**)	-0,509	-,952(**)	-0,387	,826(**)	,638(*)	-,877(**)	-,805(**)	-0,077	1,000	0,316	,730(*)	-,731(*)
	saturates/n-9	,927(**)	-,660(*)	0,142	-,981(**)	0,523	0,298	0,526	0,174	-,794(**)	-,885(**)	0,458	0,297	,855(**)	-0,326	1,000	0,484	-0,485
	TNL	0,362	0,063	,682(**)	-0,400	0,076	,660(*)	0,114	-,634(*)	-0,253	-0,364	0,337	0,397	0,181	0,013	0,430	1,000	-1,000(**)
	TPL	-0,361	-0,064	-,679(**)	0,396	-0,071	-,657(*)	-0,109	,637(*)	0,248	0,360	-0,333	-0,393	-0,180	-0,019	-0,427	-1,000(**)	1,000

* p<0.05 ** p<0.01

Supplementary Table 1. Continued.

		Women																		
	Pearson Correlations	14 : 0	15 : 0	16 : 0 DMA	16 : 0	16 : 1	16 : 2	17 : 0	18 : 0 DMA	18:1 n-9 DMA	18:1 n-7 DMA	18 : 0	18:1 n-9	18:1 n-7	18 : 2 n-6	20 : 0	20 : 1	20 : 2 n-6	20 : 3 n-6	20 : 4 n-6	20 : 5 n-3
Men	14 : 0	1,000	0,6	(a)	,650(*)	,874(**)	-,773(**)	0,044	-,829(**)	(a)	(a)	-0,135	-0,362	-0,468	0,321	0,451	-,683(*)	-,849(**)	-0,233	0,198	0,374
	15 : 0	0,49	1,000	(a)	0,337	0,556	-0,238	0,112	-0,547	(a)	(a)	-0,375	-0,31	-0,203	0,165	-0,083	-0,54	-,645(*)	-0,374	0,218	-0,101
	16 : 0 DMA	0,05	-,560(*)	1,000	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)
	16 : 0	0,447	-0,353	0,504	1,000	0,365	-,805(**)	,666(*)	-,635(*)	(a)	(a)	0,555	-,860(**)	-0,338	0,583	0,312	-,699(*)	-0,599	-0,032	0,576	-0,066
	16 : 1	,648(*)	,699(**)	-0,311	0,067	1,000	-0,568	-0,086	-,607(*)	(a)	(a)	-0,428	-0,039	-0,523	0,074	0,53	-0,436	-,846(**)	-0,254	-0,065	0,307
	16 : 2	-0,202	0,331	-,744(**)	-,740(**)	-0,066	1,000	-0,322	0,561	(a)	(a)	-0,248	0,453	,703(*)	-0,35	-0,459	,688(*)	,775(**)	0,381	-0,506	-0,434
	17 : 0	0,267	-0,447	,921(**)	,633(*)	-0,235	-,681(**)	1,000	-0,226	(a)	(a)	,604(*)	-,653(*)	-0,138	0,561	-0,069	-0,299	-0,118	0,199	0,353	-0,402
	18 : 0 DMA	-0,383	0,117	-0,363	-,681(**)	-0,207	0,465	-0,519	1,000	(a)	(a)	-0,051	0,487	0,029	-0,509	-0,312	0,503	0,574	-0,046	-0,001	-0,152
	18:1 n-9 DMA	-0,179	-0,526	,880(**)	0,275	-0,243	-,671(**)	,749(**)	-0,304	1,000	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)
	18:1 n-7 DMA	-0,112	-,554(*)	,939(**)	0,359	-0,274	-,713(**)	,833(**)	-0,323	,985(**)	1,000	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)
	18 : 0	-0,087	-0,355	0,05	,628(*)	-0,059	-0,495	0,116	-0,352	0,052	0,071	1,000	-,726(*)	0,187	0,512	0,072	-0,239	0,207	0,395	0,53	-0,354
	18:1 n-9	-0,184	0,428	-0,425	-,811(**)	0,049	,771(**)	-0,494	0,398	-0,321	-0,374	-,841(**)	1,000	-0,075	-,607(*)	-0,145	0,589	0,298	-0,163	-0,598	0,393
	18:1 n-7	-0,149	0,304	-0,269	-0,152	0,049	0,095	-0,209	0,044	-0,31	-0,291	0,131	-0,02	1,000	0,101	-0,237	0,537	,659(*)	0,602	-0,427	-0,494
	18 : 2 n-6	0,207	-0,221	0	0,356	0,322	-0,306	-0,088	-0,282	0,019	-0,004	0,325	-0,324	-,533(*)	1,000	-0,269	-0,235	-0,038	0,501	0,228	-0,049
	20 : 0	0,486	-0,194	,613(*)	,549(*)	-0,181	-0,474	,658(*)	-0,31	0,208	0,337	-0,092	-0,295	-0,15	0,089	1,000	-0,237	-0,562	-0,24	-0,064	0,117
	20 : 1	-0,377	0,202	-0,085	-,817(**)	-0,274	,546(*)	-0,19	0,497	0,007	-0,025	-,828(**)	,820(**)	0,187	-,610(*)	-0,138	1,000	,651(*)	0,331	-,737(**)	-0,122
	20 : 2 n-6	-,734(**)	-0,139	-0,456	-,570(*)	-,535(*)	,617(*)	-,560(*)	0,424	-0,408	-0,433	-0,18	0,505	0,367	-0,31	-0,322	0,531	1,000	0,597	-0,308	-0,229
	20 : 3 n-6	-0,043	-0,504	0,374	,539(*)	-,623(*)	-0,249	0,456	-0,178	0,105	0,214	0,371	-0,381	-0,188	-0,135	0,505	-0,289	0,053	1,000	-0,292	-0,357
	20 : 4 n-6	-0,354	-,841(**)	,690(**)	0,481	-,579(*)	-,567(*)	,534(*)	-0,18	,664(**)	,685(**)	0,42	-0,501	-0,454	0,225	0,263	-0,328	-0,116	,628(*)	1,000	-0,137
	20 : 5 n-3	0,352	0,007	-0,296	0,186	0,448	0,092	-0,181	-0,196	-0,266	-0,283	0,071	-0,106	-0,304	,671(**)	-0,079	-0,39	-0,216	-0,357	-0,286	1,000
	22:0	,567(*)	,776(**)	-0,514	-0,023	,717(**)	0,01	-0,381	-0,067	-0,463	-0,492	0,113	-0,09	0,212	0,212	-0,134	-0,28	-0,351	-0,461	-,660(*)	0,32
	22 : 1	0,476	,853(**)	-0,438	-0,176	,703(**)	0,017	-0,352	0,151	-0,393	-0,418	-0,122	0,067	0,37	-0,052	-0,124	-0,003	-0,272	-,572(*)	-,754(**)	0,178
	22 : 2 n-6	0,102	,693(**)	-,734(**)	-0,521	0,33	0,453	-,676(**)	0,345	-,659(*)	-,701(**)	-0,23	0,346	0,404	-0,055	-0,262	0,263	0,288	-,535(*)	-,819(**)	0,238
	23:0	-0,159	0,466	-,959(**)	-,577(*)	0,134	,772(**)	-,944(**)	0,499	-,865(**)	-,920(**)	-0,086	0,465	0,18	-0,003	-,554(*)	0,155	,550(*)	-0,255	-,555(*)	0,149
	22 : 5 n-6	-0,332	-0,512	,551(*)	-0,094	-0,417	-0,25	0,351	-0,119	,723(**)	,665(**)	-0,178	0,036	-,545(*)	0,226	0,115	0,219	-0,055	0,044	,584(*)	-0,165
	22 : 5 n-3	0,108	0,137	-,571(*)	0,282	0,419	0,09	-0,461	-0,167	-0,509	-,541(*)	,613(*)	-0,312	0,107	0,388	-0,458	-,682(**)	-0,042	-0,096	-0,17	0,491
	24:0	,590(*)	,587(*)	-0,042	-0,191	,626(*)	0,029	0,061	0,026	0,081	0,048	-0,327	0,139	-0,246	0,022	-0,102	0,064	-,666(**)	-0,51	-0,442	0,188
	22 : 6 n-3	-0,037	-,647(*)	0,497	0,475	-0,156	-0,495	0,449	-0,176	,571(*)	,561(*)	0,5	-,645(*)	-,571(*)	0,446	0,014	-,572(*)	-0,515	0,227	,714(**)	0,185
	24 : 1 n-9	-,651(*)	-0,095	-0,306	-,566(*)	-0,275	0,41	-0,434	0,359	-0,169	-0,218	-0,322	0,479	0,373	-0,182	-0,325	,588(*)	,794(**)	-0,343	-0,26	0,086
	SM	-0,314	-0,037	-,618(*)	-0,144	-0,203	0,421	-,586(*)	0,194	-,542(*)	-,594(*)	0,159	0,116	-0,076	0,221	-0,321	-0,106	0,529	0,046	-0,132	0,403
	PC	0,463	-0,124	-0,036	,624(*)	0,394	-0,23	0,1	-,571(*)	-0,148	-0,108	,583(*)	-0,506	-0,201	,635(*)	0,091	-,820(**)	-0,442	0,144	0,124	0,497
	PS	-0,06	-,665(**)	0,197	0,472	-0,148	-0,293	0,176	-0,113	0,154	0,175	,635(*)	-,671(**)	-0,285	,550(*)	0,008	-,671(**)	-0,233	0,219	0,53	0,37
	PI	0,283	-,562(*)	0,36	,796(**)	-0,06	-,538(*)	0,466	-0,44	0,241	0,293	,674(**)	-,837(**)	-0,384	,575(*)	0,319	-,835(**)	-0,512	0,404	0,522	0,456
	PG	0,183	-0,461	0,173	,561(*)	-0,217	-0,095	0,321	-0,215	-0,126	-0,001	0,275	-0,341	0,018	0,044	0,409	-0,346	0,028	,603(*)	0,257	0,211
	PE	0,251	0,054	0,059	-0,267	0,112	0,142	0,069	0,275	0,085	0,086	-0,217	-0,009	-0,13	-0,001	-0,017	0,129	-0,335	-0,305	-0,148	0,07
	SULFATIDES	-0,107	,630(*)	-0,405	-,820(**)	0,29	,615(*)	-0,451	0,372	-0,226	-0,3	-,738(**)	,833(**)	0,325	-0,492	-0,445	,785(**)	0,302	-,680(**)	-,688(**)	-0,181
	CEREBROSIDES	0,212	,704(**)	-0,272	-,709(**)	0,411	0,491	-0,287	0,352	-0,142	-0,201	-,772(**)	,714(**)	0,056	-0,36	-0,254	,653(*)	-0,065	-,651(*)	-,650(*)	-0,127
	CHO	-0,332	-,566(*)	,769(**)	0,313	-,613(*)	-,537(*)	,617(*)	-0,169	,619(*)	,681(**)	0,115	-0,26	0,062	-0,253	0,503	0,095	0,081	,589(*)	,720(**)	-,690(**)
	FFA	0,52	,723(**)	-,654(*)	0,126	,778(**)	0,12	-0,491	-0,177	-,638(*)	-,655(*)	0,237	-0,026	0,216	0,225	-0,264	-0,429	-0,214	-0,28	-,594(*)	0,36
	SE	,587(*)	0,257	0,379	0,394	0,516	-,565(*)	0,516	-0,306	0,36	0,415	0,092	-0,345	-0,038	0,032	0,262	-0,246	-,754(**)	-0,071	-0,093	0,106

Supplementary Table 1. Continued.

		Women																			
Pearson Correlations		22:0	22 : 1	22 : 2 n-6	23:0	22 : 5 n-6	22 : 5 n-3	24:0	22 : 6 n-3	24 : 1 n-9	SM	PC	PS	PI	PG	PE	SULFATIDES	CEREBROSIDES	CHO	FFA	EE
Men	14 : 0	0,566	0,515	-0,504	-0,588	-0,071	0,53	0,187	0,153	-0,449	-,625(*)	0,586	0,228	0,051	0,138	0,404	0,404	0,431	-0,569	,648(*)	,854(**)
	15 : 0	0,511	0,526	-0,284	-0,328	0,19	-0,158	0,291	0,162	-0,374	-0,358	0,573	-0,009	0,349	0,148	0,058	-0,117	0,229	-0,276	0,535	,727(*)
	16 : 0 DMA	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)
	16 : 0	0,179	0,129	-,609(*)	-,704(*)	0,351	0,445	-0,551	0,003	-0,212	-0,2	0,365	0,444	-0,15	0,41	-0,205	0,162	-0,253	-0,091	0,505	0,557
	16 : 1	,673(*)	0,444	-0,4	-0,485	-0,236	0,504	0,41	0,175	-0,272	-,769(**)	,703(*)	0,124	0,022	0,294	0,581	0,592	,602(*)	-,698(*)	,684(*)	,839(**)
	16 : 2	-0,277	-0,053	0,55	0,483	-0,213	-,688(*)	0,189	-0,025	0,347	0,394	-0,51	-,616(*)	-0,097	-0,453	-0,24	-0,315	-0,103	0,482	-,648(*)	-0,586
	17 : 0	-0,271	-0,331	-0,502	-0,565	0,52	0,202	-,656(*)	0,151	0,029	0,226	0,134	0,161	-0,267	,607(*)	-0,395	0,023	-0,396	0,072	0,088	0,027
	18 : 0 DMA	-0,295	-0,447	0,446	,802(**)	0,136	-0,464	-0,052	-0,165	0,332	0,184	-0,289	0,149	0,123	-0,009	-0,131	-0,276	-0,326	0,467	-0,32	-,646(*)
	18:1 n-9 DMA	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)
	18:1 n-7 DMA	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)
	18 : 0	-0,403	-0,197	-0,29	-0,208	0,469	0,124	-,800(**)	-0,071	0,065	0,405	-0,176	0,38	-0,198	0,095	-,614(*)	-0,23	-,771(**)	0,462	-0,133	-0,158
	18:1 n-9	-0,067	-0,284	0,446	0,523	-0,512	-0,021	,624(*)	0,123	0,129	-0,023	-0,14	-0,312	0,133	-0,174	0,539	0,228	0,575	-0,334	-0,325	-0,431
	18:1 n-7	-0,334	0,206	0,319	0,027	-0,218	-0,502	-0,12	-0,255	0,416	0,584	-,642(*)	-,637(*)	-0,243	-0,554	-0,55	-0,366	-0,323	0,551	-0,551	-0,35
	18 : 2 n-6	-0,485	0,208	-,855(**)	-0,447	0,445	0,144	-0,479	0,057	0,073	0,227	0,011	0,167	0,073	0,071	-0,389	-0,102	-0,288	0,05	0,105	0,174
	20 : 0	,662(*)	0,173	0,149	-0,375	-0,297	0,523	0,169	-0,196	-0,112	-0,507	0,305	0,121	-0,369	0,289	0,384	0,464	0,21	-0,333	0,475	0,527
	20 : 1	-0,469	-0,3	0,358	0,272	-0,431	-0,263	0,032	-0,371	,794(**)	0,466	-,603(*)	-0,518	-0,18	-0,125	-0,173	0,022	-0,006	0,192	-0,458	-,624(*)
	20 : 2 n-6	-,716(*)	-0,32	0,346	0,475	-0,06	-0,447	-0,214	-0,008	0,347	,653(*)	-,773(**)	-0,394	-0,183	-0,521	-0,369	-0,322	-0,316	0,595	-,870(**)	-,896(**)
	20 : 3 n-6	-0,522	0,028	-0,27	-0,194	-0,134	-0,026	-0,402	0,18	0,338	0,323	-0,409	-0,348	-0,456	-0,366	-0,355	0,145	-0,234	0,362	-0,564	-0,351
	20 : 4 n-6	0,059	-0,044	-0,342	0,079	,662(*)	0,023	-0,447	0,162	-0,395	-0,057	0,443	,832(**)	0,426	0,207	-0,329	-0,331	-0,563	0,191	0,311	0,245
	20 : 5 n-3	0,059	0,108	-0,035	0,177	-0,116	0,35	0,448	-0,056	-0,368	-0,363	-0,075	0,119	0,143	-0,168	,727(*)	0,184	,630(*)	-0,449	0,179	0,056
	22:0	1,000	0,469	0,271	-0,185	-0,238	0,104	0,477	-0,019	-0,42	-,784(**)	0,521	0,036	-0,099	0,102	0,481	0,268	0,396	-0,282	0,578	,737(**)
	22 : 1	,891(**)	1,000	-0,106	-0,036	0,09	-0,34	0,397	-0,264	-0,253	-0,493	0,087	-0,106	0,12	-0,412	0,123	-0,248	0,153	0,137	0,441	,665(*)
	22 : 2 n-6	,735(**)	,772(**)	1,000	0,435	-0,386	-0,368	0,369	-0,229	-0,036	0,069	-0,44	-0,445	-0,245	-0,353	0,163	-0,182	0,127	0,264	-0,362	-0,371
	23:0	0,406	0,316	,687(**)	1,000	0,219	-0,585	0,305	-0,136	-0,032	0,057	-0,251	0,191	0,464	-0,377	0,059	-0,531	-0,146	0,395	-0,229	-0,448
	22 : 5 n-6	-0,419	-0,504	-0,384	-0,422	1,000	-0,359	-0,287	-0,097	-0,225	0,064	0,131	0,541	0,404	0,275	-0,334	-,655(*)	-0,546	0,372	0,306	0,096
	22 : 5 n-3	0,388	0,198	0,151	0,45	-,591(*)	1,000	-0,116	0,363	-0,138	-0,18	0,384	0,225	-0,239	0,371	0,392	,791(**)	0,361	-,730(*)	0,137	0,202
	24:0	,564(*)	,557(*)	0,247	-0,038	0,02	-0,122	1,000	0,113	-0,389	-0,432	0,184	-0,329	0,251	-0,239	,737(**)	0,026	,752(**)	-0,453	0,143	0,252
	22 : 6 n-3	-0,277	-0,449	-,646(*)	-0,465	0,42	0,151	0,079	1,000	-0,532	-0,033	0,444	0	0,05	-0,057	0,264	0,354	0,295	-0,422	-0,368	-0,068
	24 : 1 n-9	-0,249	-0,033	0,389	0,293	0,01	-0,121	-0,457	-0,483	1,000	0,419	-0,335	-0,153	-0,127	0,22	-0,513	0,068	-0,36	0,248	-0,083	-0,305
	SM	0,12	0,025	0,432	,628(*)	-0,19	0,492	-0,413	-0,147	0,443	1,000	-0,486	-0,219	0,107	-0,104	-,679(*)	-0,397	-0,495	0,278	-,622(*)	-,665(*)
	PC	0,212	-0,129	-0,274	-0,046	-0,218	,627(*)	0,044	0,424	-,553(*)	-0,058	1,000	0,522	0,415	0,528	0,234	0,309	0,154	-0,59	,608(*)	,692(*)
	PS	-0,221	-0,447	-0,457	-0,173	0,093	0,416	-0,203	,817(**)	-0,292	0,045	,645(*)	1,000	0,504	0,383	-0,11	-0,097	-0,422	-0,021	0,502	0,269
	PI	-0,039	-0,302	-0,445	-0,392	0,094	0,352	-0,082	,782(**)	-0,504	0,039	,699(**)	,834(**)	1,000	0,039	-0,108	-0,494	-0,137	-0,165	0,293	0,175
	PG	-0,385	-0,452	-0,429	-0,203	-0,299	0,166	-0,452	0,142	-0,114	-0,019	0,449	0,432	0,512	1,000	-0,025	0,272	-0,101	-0,379	0,547	0,273
	PE	0,153	0,084	0,055	-0,015	0,19	-0,259	,619(*)	0,3	-0,316	-0,464	0,087	0,285	0,119	-0,109	1,000	0,494	,890(**)	-,627(*)	0,185	0,222
	SULFATIDES	0,152	0,39	0,465	0,362	-0,124	-0,245	0,369	-,637(*)	0,427	-0,125	-,543(*)	-,694(**)	-,890(**)	-,542(*)	0,163	1,000	0,545	-,621(*)	0,043	0,158
	CEREBROSIDES	0,315	0,482	0,422	0,255	-0,019	-0,339	,707(**)	-0,433	0,051	-0,305	-0,418	-,602(*)	-,705(**)	-,596(*)	0,462	,885(**)	1,000	-,715(*)	0,064	0,206
	CHO	-,655(*)	-,554(*)	-,631(*)	-,641(*)	0,428	-,551(*)	-0,513	0,151	-0,04	-0,413	-0,242	0,012	0,043	0,201	-0,253	-0,328	-0,399	1,000	-0,289	-0,37
	FFA	,809(**)	,684(**)	0,509	0,51	-,678(**)	,710(**)	0,287	-0,328	-0,24	0,239	0,441	-0,166	-0,031	-0,07	-0,195	0,096	0,117	-,655(*)	1,000	,849(**)
	SE	0,35	0,405	-0,086	-0,52	-0,093	-0,131	,589(*)	0,133	-0,455	-,583(*)	0,184	-0,106	0,218	0,065	0,17	-0,119	0,095	-0,021	0,237	1,000

Highlights:

- Aging alters lipid contents of Lipid rafts from frontal cortex in normal subjects
- Alterations involve plasmalogens, polyunsaturates, and total polar/neutral lipids
- This “Lipid raft aging” exhibits gender differences
- Interaction Aging and gender modify lipid alterations in lipid rafts