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G protein-coupled receptor systems and their lipid environment in health disorders during aging

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Summary

Cells, tissues and organs undergo phenotypic changes and deteriorate as they age. Cell growth arrest and hyporesponsiveness to extrinsic stimuli are all hallmarks of senescent cells. Most such external stimuli received by a cell are processed by two different cell membrane systems: receptor tyrosine kinases (RTKs) and G protein-coupled receptors (GPCRs). GPCRs form the largest gene family in the human genome and they are involved in most relevant physiological functions. Given the changes observed in the expression and activity of GPCRs during aging, it is possible that these receptors are directly involved in aging and certain age-related pathologies. On the other hand, both GPCRs and G proteins are associated with the plasma membrane and since lipid-protein interactions regulate their activity, they can both be considered to be sensitive to the lipid environment. Changes in membrane lipid composition and structure have been described in aged cells and furthermore, these membrane changes have been associated with alterations in GPCR mediated signaling in some of the main health disorders in elderly subjects. Although senescence could be considered a physiologic process, not all aging humans develop the same health disorders. Here, we review the involvement of GPCRs and their lipid environment in the development of the major human pathologies associated with aging such as cancer, neurodegenerative disorders and cardiovascular pathologies.

Organisms, cells and even proteins are subject to time-dependent degenerative processes. As such, aging is characterized by the accumulation of adverse changes in cells over time, which augments the risk of disease, and the breakdown of homeostatic control and death [1]. Moreover, the process of aging is also manifested as senescence, involving the deterioration of certain functions at the cell and tissue level as well as that of the whole organism. Since aging plays an important role in many biological processes including development, tumorigenesis and death, many attempts have been made to understand the fundamental features underlying aging [2].

Cell growth arrest and hyporesponsiveness to extrinsic stimuli are hallmarks of senescent cells [3, 4]. The influences of the external stimuli involved are mainly mediated by two different systems at the level of the cell membrane. One of these involves receptor tyrosine kinases (RTKs) that bind growth factors and activate signal cascades through the phosphorylation of tyrosine residues in the receptor. The other depends on G protein-coupled receptors (GPCRs) that make up approximately 5% of the genes in eukaryotic organisms [5, 6]. GPCRs constitute the main family of receptors for neurotransmitters, hormones and neuromodulators. Upon agonist binding, they undergo conformational changes that result in the activation of heterotrimeric G proteins and that modulate the activity of effector proteins (e.g. adenylyl cyclase [AC], phospholipase C [PLC], A_2 [PLA_2], guanylyl cyclase [GC] and some ion channels). These effectors regulate the cytosolic levels of second messengers, which in turn influence the activity of third messengers and so on. Finally, a single signaling event may originate short-, medium-, and long-term responses that regulate a cell's activity and its responses to environmental conditions through molecular events such as the regulation of gene expression, cross-talk and other complex phenomena. After

remaining active for a time, GPCRs are phosphorylated by G-protein receptor kinases (GRK) and other kinases, extinguishing the receptor's activity [7, 8].

Age-dependent changes can occur at several different levels, from the ligand-receptor interaction at the cell surface to the various downstream signaling cascades with which these receptors interact. GPCRs form the largest gene family in the human genome and they are involved in most relevant physiological functions. This fact and the changes observed in the expression and activity of GPCRs during aging, indicate that these receptors may be involved in aging and associated pathologies [4].

Membrane lipids have recently been shown to influence GPCR-associated signaling [9, 10] and aging [11, 12]. Some specific properties of cell membranes (i.e., fluidity, nonlamellar phase propensity, surface packing, thickness, surface charge, etc.) are critical for many membrane events, such as receptor and G protein localization, activity and sorting of G protein subunits upon activation, etc. Moreover, the structure and lipid composition of the membrane regulates the function and localization of several membrane signaling proteins [10, 13-17]. Changes in membrane lipid composition are frequently accompanied by alterations in the fluidity, lipid structure, and functionality of the membrane [18, 19]. Accordingly, modifications in the lipid composition and the physical properties of cell membranes, in cell signaling and in gene expression have been identified in different tissues from elderly subjects, including the brain [18, 20-23]. Interestingly, membrane fatty acid composition is an important determinant of the lifespan of different species [12]. In this context, alterations in membrane lipids can induce changes in the activity and localization of GPCRs in aged cells. Therefore, interventions that involve manipulating dietary lipids and lipid metabolism, including changes in diet or the administration of cholesterol-modifying agents and antioxidants, show great promise in slowing or possibly averting the development of some human

diseases associated with aging including some types of cancer, Alzheimer´s Disease (AD), hypertension and other cardiovascular disorders [24-32].

Although senescence could be considered a physiological process, not all aging individuals develop the same health disorders. Here we shall review the involvement of GPCRs in the development of major human pathologies in aging, such as cancer, cardiovascular and neurodegenerative disorders (mainly AD). We shall focus on the alterations of membrane lipid composition and structure associated with these pathologies, its influence on GPCR signaling, and the beneficial effects of some fatty acid-rich diets on healthy aging.

GPCRs and aging in cancers

In general, there is a positive correlation between age and the incidence of cancer, in particular in breast, lung, prostate, and colon cancers [33, 34]. Cancers originate through defects in the control of cell proliferation, usually associated with mutations in proto-oncogenes, tumor suppressors and other signaling proteins. Obviously, the probability that a cell might bear a mutation increases with age and indeed, many cancers require the accumulation of multiple mutations. For instance, p53 induces apoptosis of cells with genetic alterations that cannot be repaired and mutations in p53 have been observed in about 50% of all human tumors. However, p53 alone does not induce uncontrolled cell proliferation, but rather the deficiency in p53 function allows a cell with mutation in genes involved in proliferation to divide continuously. It is therefore clear that in most cancers, the probability of developing a tumor increases with age (Fig. 1) [35]. Nevertheless, it should be noted that exceptions to this rule do exist, as is the case for pilocytic astrocytoma (Fig. 1).

With respect to GPCRs and their related proteins, the role they fulfill in the control of cell proliferation is closely associated with their involvement in the development of certain cancers [36-39]. Indeed, changes in the expression and function of these receptors may result in cellular transformation (see the review by Gutkind et al. in this issue). Recent studies have demonstrated that GPCRs are implicated in tumorigenesis and metastasis [40]. Changes in the expression of GPCRs or mutations in the genes encoding these receptors have been observed in some cancers, indicating that certain GPCRs can behave as potent agonist-dependent oncogenes [41, 42]. One example of a growth-stimulatory role of GPCRs can be seen in small cell lung carcinoma (SCLC) cells, where neuropeptides like gastrin-releasing peptide (GRP), galanin, vasopressin, etc., activate GPCRs [41, 43-45]. SCLC constitutes approximately 25% of all lung cancers and it is characterized by a very low 5-year survival rate, despite the initial sensitivity that it displays to radio- and chemotherapy. In SCLC, neuropeptides activate GPCRs that are coupled to more than one G protein family (i.e., Gq/11 and G12/13 [41]) thereby stimulating phospholipase C [46] and ERK [47] activity, which promotes cell proliferation. Thus, through an autocrine feedback on GPCRs neuropeptides represent the principal mitogens of SCLC [44] and indeed, neuropeptide growth factor antagonists have been developed to treat SCLC [48].

The detection of activating mutations of the human thyrotropin (TSH) receptor in some types of thyroid carcinomas established a causal link between GPCRs and autonomous cell growth [49]. The human TSH receptor has an exceptionally broad profile of G protein coupling and it is able to interact with members of all four G protein families, Gs, Gi, Gq and G12 [50, 51]. The cAMP regulatory cascade has also been implicated in the control of growth and differentiation, whereas calcium and diacylglycerol (G_q/phospholipase C-β activation) are thought to stimulate iodination and

thyroid hormone synthesis [52]. In approximately 30% of hyperfuctional thyroid adenomas, GTPase-inhibiting mutations in Gαs are responsible for the increased cAMP synthesis characteristic of this tumor. Similarly, mutations in the THS receptor constitutively activate its ability to stimulate G- and AC-catalyzed cAMP synthesis [53-55]. Moreover, alterations in the desensitization of GPCRs in the thyroid gland play a crucial role in thyroid pathologies [56]. In differentiated thyroid carcinomas (where TSH acts as a mitogenic agent), changes in the levels of GRK5 are correlated with a decrease in the desensitization of the TSH receptor that provokes an increase in cAMP synthesis [57]. Likewise, an increase in GRK3 expression was detected in hyperfunctional thyroid nodules (HTNs), suggesting a potential role for this GRK as a negative feedback regulator for the constitutively activated cAMP pathway in these structures [56]. Similar alterations in GPCR and GRK activation and/or expression are associated with the development of breast cancer [58-61], with neoplastic transformation in the prostate [62-65] and in human colon cancer [66]. All these cancers become more prevalent with age, most probably due to their dependence on the accumulation of mutations.

Taken together, these results show that mutations in GPCRs, G proteins, their effectors and GRKs are all involved in the etiology of several human cancers, particularly those involving hormone dependence. Hence, it would appear that these proteins might be relevant pharmacological targets for selective strategies aimed at antagonizing distinct mitogenic stimuli. Tumor growth and metastasis are both processes that are affected by changes in membrane lipid composition and accordingly, lipid alterations might be involved in the development of some types of cancer. In this context, a wide variety of molecular entities that control cell proliferation and survival are membrane-associated proteins. Indeed, alterations in the levels of certain membrane

lipids have been observed in cell membranes from patients with cancer and from cancer cells that are resistant to chemotherapy [13, 67, 68]. Interestingly, compounds that modulate the membrane lipid structure (e.g. fatty acids) influence the cellular localization and activity of important membrane-associated signal transduction proteins [10, 14, 15, 69], thereby producing molecular and cellular alterations that affect cell signaling and division [70, 71]. Thus, it is not surprising that the activity of various anticancer drugs is associated with their ability to alter membrane lipid composition, thereby affecting membrane lipid structure. Accordingly, the possibility of using lipids as targets to overcome anticancer drug resistance has recently been highlighted [67, 72].

GPCRs and aging in the brain under normal conditions and in association with neurodegenerative disorders

A variety of age-related alterations in GPCRs have been observed in neuronal signaling systems [73]. While age-associated changes appear to be variable and dependent on the specific brain region, the expression of most GPCRs and G proteins decreases in the human brain with age [74-77]. Indeed, like most brain neurotransmitter receptors, the density of β-adrenoceptors decreases with age in the human brain [78]. This decline is largely associated with the degeneration of noradrenergic nerve terminals [79] and it is mainly due to the loss of high-affinity receptors [76]. The human brain contains all three subtypes of α_2-adrenoceptors identified by molecular cloning (α_{2A}, α_{2B}, and α_{2C}), the α_{2A}-adrenoceptor being the most abundant in the frontal cortex [75]. The density of α_{2A}-adrenoceptors and associated regulatory G proteins has also been shown to decrease with aging in the human brain [74, 75, 80]. The concomitant decrease in the density of α_{2A}- and β-adrenoceptors in the frontal cortex suggests a relationship between both these GPCRs [76]. Both adrenoceptors belong to a large family of membrane receptors that regulates AC activity through Gαs and Gαi proteins

[81-83]. Some studies reveal a decline of various Gα proteins and their signal transduction cascades within the aging human brain [75, 76, 84, 85]. This coordinated decrease in the density of β- and α_{2A}-adrenoceptors indicates the existence of a relationship between these receptors and their signaling proteins [76]. Furthermore, the muscarinic acetyl choline (ACh) receptor (mAChR) system loses its sensitivity to ACh stimulation both during aging and in AD [73, 86, 87]. In contrast to most receptors and signaling proteins, the presence of other receptors and signaling-related proteins may also increase with age, as seen for the imidazoline receptors and monoamine oxidase [88, 89]. This would indicate that the loss of such elements is not unspecific.

AD is currently the commonest cause of senile dementia among humans over 65, and it is characterized by a progressive cognitive decline coupled defined by the loss of memory, cognitive abilities, and even of personality. These changes are due to the progressive dysfunction and death of neurons responsible for learning and memory. AD is characterized by a variety of pathological features, such as the extracellular senile plaques formed by amyloid β peptides, the appearance of intracellular neurofibrillary tangles consisting of twisted filaments of hyperphosphorylated tau protein, the loss of neuronal subpopulations and cholinergic fibers, and brain atrophy [90-94]. The amyloid β precursor protein (APP) is a membrane protein and the proteolytic fragments generated during AD form deposits on the extracellular face of the cell membrane. It has recently been demonstrated that a gradual decline in the cellular processing and maturation of APP is one of the main pathogenic mechanisms for familial and age-associated AD [95]. GPCRs have recently been implicated in APP processing. In this context, the somatostatin receptor has been identified as a modulator that decreases amyloid β levels by increasing brain neprilysin activity [96], a rate-limiting peptidase involved in the physiological degradation of amyloid β in the brain [97]. Thus, reduced

neprilysin activity contributes to amyloid β accumulation and consequently, to AD development. There is evidence that neprilysin is down-regulated in the hippocampus and cerebral cortex with aging [98] and from an early stage of AD development [99-101], supporting the association of neprilysin activity with the etiology and pathogenesis of AD. Therefore, the up-regulation of neprilysin through the somatostatin-activated GPCR signaling pathway may represent a promising target for AD therapeutic and preventative strategies [102].

Postsynaptic M1 AChRs are found at high density in the neocortex and hippocampus, where they mediate cholinergic neurotransmission in a variety of CNS functions including learning and memory [103]. Of the neurotransmitter pathologies associated with AD, cholinergic dysfunction occurs early in the disease process [104] and it is thought to underlie much of the characteristic cognitive and neuropsychiatric symptoms [105-107]. In addition, the ability of the mAChR to form high affinity agonist-binding complexes with G proteins was impaired in AD [108] and phosphoinositide hydrolysis, as well as phospholipase C and PKC activity was reduced [104, 109, 110]. Recently, the reduction in M1 AChR/G-protein coupling has been related to the severity of cognitive symptoms in the neocortex of AD patients. Thus, that impairment of M1 AChR-mediated signaling through uncoupling of its G protein may be a neurochemical cause of cognitive decline in AD [111]. Potential mechanisms for the loss of GPCR-G protein coupling in AD may be aberrant phosphorylation or dephosphorylation of these receptors, which in turn may alter their association with G proteins and/or the levels or activity of G proteins (e.g., loss of GTPase activity). Accordingly, both G protein subunit concentrations and activity are altered in different regions of the AD brain, thereby modifying GPCR-mediated AC signaling. In fact, it has been reported that inhibition of AC mediated by Gs protein but not Gi is disrupted

in various brain regions in AD patients [112-116]. Furthermore, the interactions between the β_1-adrenoceptor and Gs protein are disrupted in the temporal cortex in AD [117] and β-adrenoceptor-stimulated cAMP production is reduced in fibroblasts from sporadic AD cases [118]. Finally, a deficit in cytosolic PKA has been associated with the accumulation of neurofibrillary changes and amyloid β deposits in the AD brain [119].

The fatty acid composition of neural tissue plays an important role in neurodevelopment, changing significantly during periods of rapid brain growth [120]. Indeed, neural tissue has the highest lipid content after adipose tissue. The levels of polyunsaturated (PUFA) and monounsaturated fatty acids (MUFA) in the cerebral cortex change from early childhood through late adulthood [121], and some studies suggest that neurocognitive function may be related to the fatty acid composition of developing brain [122-124]. Accordingly, alterations in brain fatty acid composition have been reported in several diseases associated with neurodegenerative disorders [125, 126]. Neurodegeneration in AD is accompanied by lipid alterations, such as changes in the phosphocholine-containing lipids in cerebrospinal fluid [127], and in the levels of phospholipids and fatty acid in blood plasma and brain membranes [128, 129]. Furthermore, several aspects related to the etiology of AD are associated with alterations of lipid membrane composition and structure. On the one hand, APP is a membrane protein and as mentioned, the resulting proteolytic fragment forms deposits on the extracellular side of the lipid bilayer. Recent results demonstrate that age-dependent membrane modifications are associated with alteration in the distribution of presenilin-1 and a beta-APP-cleaving enzyme, very likely affecting APP processing and leading to its accumulation [95]. On the other hand, some membrane alterations could in part explain the cognitive deficits reported in mice with neurodegenerative disorders

[19, 130-132], as well as the impaired neurotransmitter-associated signaling observed in AD and aging [132, 133]. These specific alterations in membrane lipids have led to studies on the effects of phospholipid and fatty acid supplementation on the mental status of patients with AD and in animal models of aging [28, 29, 134, 135]. Diets rich in carbohydrate [136], particularly those with a high glycemic index and those low in essential fatty acids (particularly ω-3 long-chain PUFAs) increase the risk of developing AD [137-140]. Likewise, the risk of AD may be increased through altered lipid metabolism and neuronal membrane lipid composition that may lead to changes in neurotransmission, antioxidant defenses, inflammatory responses, cerebral blood flow, and cognitive function [20, 27, 31, 141, 142]. It would be particular interesting to determine the beneficial effects of anti-oxidants and anti-inflammatory ω-3 PUFAs [27]. Similarly, essential components of the Mediterranean diet protect against age-related cognitive decline [143, 144], as well as protecting against atherosclerosis in an animal model of neurodegeneration [145]. Furthermore, there is evidence that this diet also benefits inflammatory and cardiovascular parameters [25].

GPCRs and aging in cardiovascular disorders

Adrenergic receptors are important regulators of cardiovascular physiology. The β-adrenergic receptor is activated by both noradrenaline and adrenaline, and it is a target for many medications prescribed to the elderly to treat hypertension, angina, post-myocardial infarction risk, congestive heart failure, glaucoma, tremor, and arrhythmias [146]. The β-adrenergic receptor also exhibits age-related change in its activity in the cardiovascular system.

In the heart

Human aging is associated with an increase in the activity of the sympathetic nervous system and with a decrease of cardiac neuronal uptake of catecholamines [147-150]. In this situation, the cardiac adrenoceptors are exposed to chronic stimulation and hence, desensitization of cardiac β-adrenoceptors could be expected with age (for a review see [151]). Numerous studies in animals show that the cardiac responses mediated by the β-adrenoceptor diminish with age. Although such changes in the density of the β-adrenoceptor in the myocardium with age are not consistent (decrease, increase and no changes have been reported), impaired coupling of the β-adrenoceptor to the Gs protein and to the catalytic unit of the adenylyl cyclase (AC) with age was consistently observed [147, 152, 153].

In humans and animals, a decrease in the levels of the β_1-adrenoceptor and Gs protein in the ventricular myocardium appear to be responsible for the reduction in β-adrenoceptor activity in aging [154]. In contrast, the increase in Gi protein levels and the reduction in the activity of the catalytic subunit of the AC lead to attenuated cyclic AMP formation in aged right atrial tissue [155]. However, irrespective of the underlying mechanism, the responses of the aged human heart to β-adrenoceptor stimulation and to stimulation by other receptors (i.e., serotonin 5-HT_4 receptors and histamine H_2 receptors) that evoke their effects through activation of AC are impaired. The density of muscarinic receptors also decreased with age in the human heart, and there was a significant inverse correlation between muscarinic receptor density and the age of the subjects. This decrease in receptor density was accompanied by an impairment in carbachol-induced inhibition of AC and an attenuation of the indirect negative inotropic effect of carbachol with aging [156, 157].

Taken together, it appears that autonomic receptor systems are altered in the aging human heart to protect the heart against a pronounced reduction in β-adrenoceptor responsiveness. Indeed, β-adrenoceptors and muscarinic receptors are both desensitized in parallel, thereby leading to an unaltered in vivo response of β-adrenoceptor stimulation. Furthermore, GRKs activity does not change [158] and therefore does not contribute to (or exaggerate) β-adrenoceptor desensitization.

In the vasculature

Hypertension, orthostatic hypotension, arterial insufficiency, and atherosclerosis are common disorders in the elderly, with a significant morbidity and mortality. It is generally understood that with advancing age, vascular tone shifts towards vasoconstriction and producing a hypertensive state. This shift is related to the precipitous decline of vasorelaxation with advanced age, stimulated by activation of Gαs-linked receptors (β-adrenergic, prostanglandin E_2, adenosine A_2, etc). Therefore, research has been focused on age-related changes in GPCR function, and specifically on the β-adrenergic receptor cascade [159, 160].

In vascular smooth muscle cells the generation of the vasodilatory agent, cAMP, by AC activation is due to agonist stimulation of GαsPCRs (β-adrenergic, prostanglandin-E_2, adenosine A_2, etc). Activation of PKA by cAMP initiates vasorelaxation through different pathways, all of which lead to a lowering of cytosolic Ca^{2+} [161]. During aging, β-adrenoceptor-mediated function and the subsequent generation of cAMP in the vasculature declines, leading to impaired vasorelaxation. Because cAMP is also an anti-proliferative agent, this age-related decrease may be associated with the progress of atherosclerosis. The accepted explanation for the age-related lack of β-adrenoceptor function is that GRK and β-arrestin expression is upregulated, leading to a higher basal phosphorylation and desensitization of the

receptors [162, 163]. Numerous studies demonstrate that Gαs may also undergo age-related changes that impair its coupling to β-adrenoceptor after agonist-binding, decreasing the proportion of high affinity receptors [164, 165]. However, the total β-adrenergic receptor pool [162, 166] and the expression of Gαs in the vasculature remains unchanged with age [162, 167-169]. Further studies of the changes in G protein function with age in vascular tissue are needed to determine whether changes in Gαs function underlie the loss of β-adrenoceptor-mediated vasorelaxation. A recent report has identified a novel regulator of G protein signaling (RGS)/GTPase activating protein (GAP) that acts on Gαs [170]. One possible explanation for the age-related alterations associated with Gαs is that RGS/GAP activity is enhanced in vessels from older animals. Under these conditions, agonist exposure would leave β-adrenoceptors in a low-affinity state due to their dissociation from Gαs. Thus, Gαs would not stimulate AC to produce cAMP, as Gαs signaling would be quenched due to the high GAP activity of the RGS/GTPase activating protein.

Alterations in the membrane content of cholesterol or phospholipid, in the phospholipid distribution, in the molecular species of particular phospholipid classes, and in the degree of fatty acid saturation have also been reported in hypertensive humans [171-173]. Interestingly, alterations in cell membrane lipid levels are associated with a reduction in the density of membrane-associated signaling proteins involved in the control of blood pressure, such as G proteins and PKC in elderly hypertensive subjects [23]. Therefore, it appears that one way to regulate GPCR signaling in the elderly would be through nutritional and pharmacologic interventions aiming at normalizing the abnormal lipid composition of the plasma membrane.

The interest in the potential cardiovascular health benefits of dietary MUFAs has increased in recent years [174-176]. It has been demonstrated that in hypertensive

normocholesterolemic and hypercholesterolemic subjects, high oleic acid intake (i.e. olive oil) lowers blood pressure [176]. Moreover, high oleic acid intake also normalizes certain alterations in erythrocyte membrane function in hypertensives, such as the distribution of the erythrocyte $Na(^{+})$-Li^{+} countertransport [172] and membrane cholesterol [177-179]. Normalization of these parameters by olive oil is concomitant with a reduction in blood pressure and strongly related to lipoprotein and membrane lipid modifications [30, 176, 180, 181]. In addition, oleic acid regulates the activity of adrenoceptor, G protein and adenylyl cyclase activities [71], and the GPCR pathway involved in the control of blood pressure.

In elderly hypertensive subjects, long-term olive oil consumption reduces the cholesterol/phospholipid ratio in erythrocyte membranes (before olive oil consumption 0.44 ± 0.00 and after 0.38 ± 0.01, $P<0.05$), normalizing these values to those of normotensives (0.37 ± 0.01). These decreases in the levels of cholesterol in membranes after olive oil consumption are associated with increased membrane fluidity [182]. Accordingly, a reduction in membrane fluidity (high cholesterol) has been associated with the development of hypertension [183] and with an age-related impairment of β-adrenergic-mediated vasorelaxation and Gαs coupling [22, 164, 184]. Olive oil consumption also induces significant changes in the levels of specific fatty acid moieties in phospholipids and cholesterol esters (Table 1). In both cases, MUFA levels significantly increase in elderly humans after long-term olive oil consumption, mainly due to a rise in the proportion of oleic acid (C18:1). This fact was reflected in a significant increase of the MUFA:SFA (saturated fatty acid) ratio (from 0.57 to 0.64 in the normotensive group and from 0.57 to 0.65 in the hypertensive subjects) and of the MUFA:PUFA ratio in membrane phospholipids (from 0.62 to 0.71 in normotensive subjects and from 0.57 to 0.73 in hypertensive subjects). In contrast, the PUFA:SFA

ratio did not markedly change under these circumstances. These changes induce important alterations in membrane lipid structure because oleic acid favors the formation of nonlamellar membrane structures in vitro (hexagonal H_{II} phases) [9, 185]. The H_{II}-phase propensity is an important physical property of cell membranes, since it influences the localization and activity of several membrane-associated proteins (e.g., G proteins and PKC) [10, 14, 70, 71]. Both the type and the quantities of free or esterified fatty acids and cholesterol influence the membrane fluidity and H_{II}-phase propensity [9]. In this context, the normotensive effects of olive oil could originate from the modulation of H_{II}-phase propensity (induced by changes in membrane lipids) and its influence on the interaction of G proteins and PKC, in addition to or alternative to membrane fluidity. Long-term high oleic acid intake significantly reduces the membrane levels of $G\alpha i_{1/2}$, $G\alpha s$, $G\beta$ and $PKC\alpha$ in elderly hypertensive subjects (Fig. 2). These effects are accompanied by a reduction in blood pressure (mean systolic and diastolic blood pressure values were 162.4 and 81.0 mm Hg, respectively before and 138.0 and 72.3 mm Hg after olive oil consumption, $P<0.05$), suggesting that the changes observed may in part account for the normotensive effects of olive oil. Moreover, these results support the hypothesis that the lower basal levels of membrane-associated $G\alpha i_{1/2}$, $G\alpha o$ and $PKC\alpha$ previously found in elderly hypertensive subjects [23] result from the compensatory adaptation to other changes that induce hypertension, rather than to the etiology of this pathology. Oleic acid has been shown to regulate α_2-adrenoceptors and related signaling proteins in 3T3 cells, whereas the chemically related analogues had no effect [71]. This effect has been related to the structural regulation of the membrane induced by this fatty acid and it could explain the effects induced by high olive oil consumption. Moreover, oleic acid derivatives have been found to exert a normotensive action in hypertensive animals [186].

Conclusions

There is currently strong evidence that the function and expression of GPCRs and other related signaling proteins are affected by age. Thus, altered GPCR signaling may be involved in many important age-related human diseases, either as a consequence of alterations suffered by senescent cells or due to its participation in the origin or the development of the disease. Furthermore, it has recently been hypothesized that aging could be initiated and modified at the plasma membrane, leading to the proposal that the membrane might act as a gate which modulates the signals that induce a senescent phenotype [12]. Thus, aging may alter the lipid composition and structure of biological membranes, which in turn regulate the activity of membrane proteins involved in cell signaling and important physiologic functions, such as growth, neurotransmission and blood pressure. In this context, small heat shock proteins can act as membrane lipid sensors capable of recognizing membrane lipid structures and of modulating them [187]. Moreover, the possibility of reversing the senescent phenotype by simply restoring the membrane signaling apparatus confirms the significance of membrane in the aging [4]. From these results, it might be hypothesized that the age associated decline of GPCR signaling could be initiated and modified at the membrane level and consequently, one way to restore the effects of this process in elderly humans would be to modify membrane lipid composition and structure. In this sense, fats are important in human diet and indeed, different types of food lipids have been associated with both positive and negative effects on human health. As such, the oleic acid component of Mediterranean diets (i.e., olive oil), has been shown to have beneficial effects in important human pathologies linked to age, such as hypertension and related cardiovascular disorders [30, 32, 176, 180, 181].

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Figure Legends

Figure 1. Age-related incidence of cancer. The figure shows an example of the increase in the rate of a specific cancer with age (meningioma), representing the usual trend for most cancers, and an example of a cancer whose prevalence decreases with age (pilocytic astrocytoma). Adapted from Wrensch et al. 2002.

Figure 2. G protein and PKCα concentrations in erythrocyte membranes of elderly subjects. The data shown are the mean ± sem of the G protein densities (n=10). CB indicates basal values in control (normotensive) subjects; COO, controls after olive oil consumption; HB, basal values in hypertensive subjects; HOO, hypertensive subjects after long-term olive oil consumption. Panels show the levels of Gαs, $G\alpha i_{1/2}$, Gαo, Gβ and PKCα. Quantification was performed by image analysis, using standard curves with four points (i.e., total protein loaded *vs* integrated optical density) of different protein contents loaded on the same gels as described [23]. Representative immunoblotting bands are also shown. About 36 μg of total protein was loaded for all subjects. **P*<0.05; ***P*<0.01; ****P*<0.001, vs CB; †*P*<0.01 vs HB.

Table 1. Fatty acid composition of phospholipids in erythrocyte membranes (mg/100mg)

	Normotensives		Hypertensives	
Fatty acid species	Basal	Long-term olive oil	Basal	Long-term olive oil
14:1	2.21 ± 0.26	2.49 ± 0.16	1.84 ± 0.22	2.62 ± 0.14†
16:0	22.93 ± 0.45	24.08 ± 0.86	24.00 ± 0.67	25.76 ± 0.49
16:1	0.81 ± 0.08	0.80 ± 0.07	0.85 ± 0.08	0.93 ± 0.08
18:0	16.88 ± 0.82	14.56 ± 0.63*	16.42 ± 0.29	13.31 ± 0.68†
18:1	17.49 ± 0.42	20.00 ± 1.01*	17.90 ± 0.79	19.30 ± 0.93
18:2	13.25 ± 0.49	12.05 ± 0.60	12.75 ± 0.79	12.43 ± 0.70
18:3	0.73 ± 0.03	0.42 ± 0.03†	0.68 ± 0.06	0.52 ± 0.05*
20:2	2.14 ± 0.17	2.35 ± 0.22	2.22 ± 0.14	2.14 ± 0.12
20:4	16.98 ± 0.44	16.02 ± 0.73	16.81 ± 0.34	15.47 ± 0.62*
22:5	3.47 ± 0.18	3.30 ± 0.21*	3.28 ± 0.24	2.86 ± 0.12
Others	3.12 ± 0.12	3.92 ± 0.16	2.40 ± 0.22	3.85 ± 0.32†
Total SFA	39.99±0.66	38.89±0.89	40.73±0.59	39.3±0.72
Total MUFA	22.91±0.54	25.36±0.42†	21.42±0.66	25.51±0.47†
Total PUFA	37.10±0.57	35.73±0.55†	37.85±0.68	35.18±0.72†
MUFA:SFA	0.57±0.07	0.65±0.06	0.52±0.05	0.65±0.06†
MUFA:PUFA	0.62±0.05	0.71±0.06*	0.57±0.04	0.72±0.06†
PUFA:SFA	0.93±0.04	0.92±0.03	0.93±0.03	0.90±0.04

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Values are expressed as the mean ± SEM (n=28). *P < 0.05, †P < 0.01, when compared to the corresponding basal group.

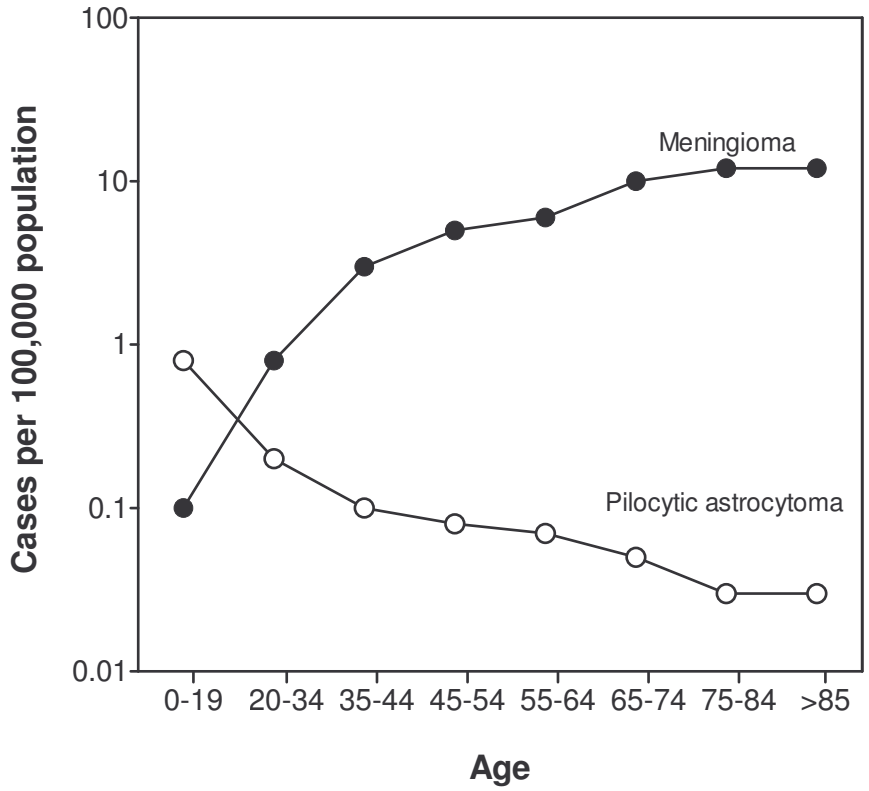

Figure 1 Alemany et al

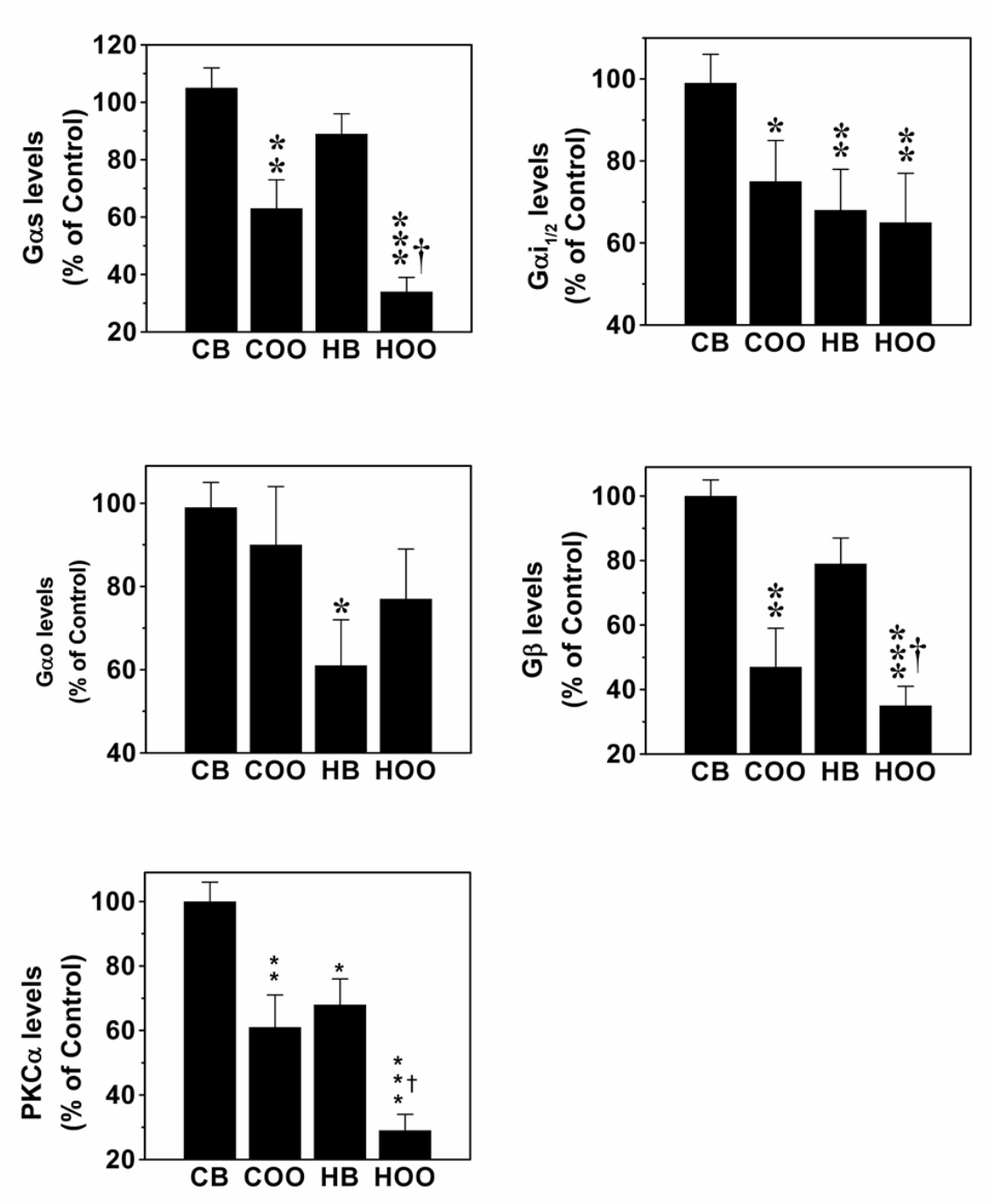

Figure 2 Alemany et al