# Approaches to Aging Control

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# SUMARY

Nº 16 - SEPTEMBER 2012

# Editorial

Among the intervention strategies against aging the only one that had succeeded in increasing the life span was the caloric restriction. Experimentally, a caloric restricted diet means to eat 30% less calories but supplementing 30 % with micronutrients. It was thought that if it worked in lower animals (nematodes and mouse), it should work in higher animals and everybody were looking forward for the results of the NIA-funded monkey study (USA). However, they have seen that caloric restriction does not seem to lengthen lifespan in primates.

In some how, the results are not surprising because it is logical to think that animals with a short life span have a less "sophisticated" biochemical processes than those who already live much longer, such as humans. Also, the evolution is clever and, as gerontologist Dr. Richard Cutler says, "if caloric restriction worked for primates and especially human, then why did not caloric restriction evolve as a key mechanism during the evolution of human longevity? Human would have evolve a longer lifespan and also require less calories to live on! Instead humans actually show no evidence of being in a caloric restriction state yet they are the longest lived of all primates".

Besides the cause of why caloric restriction has failed, the clear thing is that research on aging is a very dynamic area. In the last two years there have been interesting paper showing that aging is a much more complex process than it was initially though. Within this context, some scientists' opinion is that the genetics is important; others think it is the diet and life style. The composition of the diet also plays an important role in life-extension. As can be seen, there are different opinions that make it difficult the development and implementation of life-extension therapies. To some extent, this scenario affects to Anti Aging Medicine, because the anti-aging strategies should never go faster than aging research.

Although we all are convinced that the human life span is not fixed and that aging rate can be modulated, we need high quality aging research whose results can be translated to the clinic. In SEMAL we are convinced of this and one of our main goals is to present the most recent human-life-extension advances in the 2012 meeting held in Barcelona (Spain)

# Nutraceutical management of stress-induced redox imbalance and mitochondrial damage

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**Key words:** Aging, Natural and Nutritional Antioxidants, Stress, Oxidative Damage

**Abstract:** We propose that stress may contribute to aging acceleration and age-associated degenerative disease by generating oxidants in the mitochondria, causing oxidative damage to lipids, proteins, and nucleic acids, weakening antioxidant defense systems, and further affecting the homeostasis of numerous mediators of defense reactions including hormones, neurotransmitters, and immune cytokines, all of which, like oxidants, are dangerous in excess. Because mitochondria are the source and also the targets of oxidants and play a key role in stress response, protecting mitochondrial from stress-induced dysfunction should be an effective strategy to fight against stress and stress-associated aging acceleration and degenerative diseases. We have identified a group of mitochondrial protective nutrients which can enhance antioxidant defense system, scavenge oxidants, improve mitochondrial function, inhibit oxidative damage, and consequently, regulate the balances of all stress mediators. We reviewed the recent evidence of stress-induced oxidative damage by different stress models in different organs, especially in the brain, and also summarized the neuroprotective effects of mitochondrial targeting nutrients, including vitamins A, C, E, estrogen, dehydroepiandrosterone, glutathione, acetyl-carnitine, and some herbal extracts.

## Introduction

Stress can affect many aspects of physiology, and has an important influence on health and disease. In the cardiovascular system, stress stimulates the release of catecholamines, which excite β-receptors, thus increasing heart rate, myocardial contraction, cardiac output and blood pressure. In the digestive system, stress induces either stimulation of chewing and eating, which is the inducing factor of obesity, inhibition of appetite, or neuro-anorexia. In the blood system, stress causes an increase in white blood cell numbers, platelet numbers, viscosity, fibrinogen, as well as anticoagulating factors V and VIII. In the reproductive system, stress generally disrupts reproductive function. The central nervous system is the regulating center of stress, but at the same time its function is affected by stress. The hypothalamus-pituitary-adrenal (HPA) system, which releases glucocorticoids, and the sympathetic adrenomedullary system which releases catecholamines, acts as integrating units controlling the physiological and behavioral responses. At the same time, stress causes serious psychological changes, associated with response of the brain limbic system, including the hippocampus and olfactory bulb, which are closely associated with hypothalamus. Different stressors cause different emotional reactions; strong and persistent emotional reactions may induce disorders of the central nervous systems, including psychological disorders and psychosis.

The glucocorticoid hypothesis of brain aging was proposed in the late 1970s, when it was recognized that the hippocampus and some other brain regions are rich in glucocorticoid receptors (Landfield and Eldridge, 1994; Lupien and Meaney, 1998; McEwen, 2002; McEwen et al., 1990; Munck and Naray-Fejes-Toth, 1992). This hypothesis suggests that brain aging may be mediated by long-term exposure to glucocorticoids. It is now further understood that the cellular mechanisms of glucocorticoid-mediated neuronal toxicity may be the altered calcium homeostasis via excitatory amino acids and N-methyl-D-aspartate receptors. That is, glucocorticoid-mediated increases in calcium influx may impair neuronal function, whereas, over the long-term, the cumulative affects of higher calcium influx may result in structural deterioration and impair hippocampal-dependent explicit learning and plasticity (Landfield and Eldridge, 1994; McEwen, 2002; Munck and Naray-Fejes-Toth, 1992; Sapolsky, 2003).

In recent years, a variety of oxidative damage induced by stress has been demonstrated in the brain and other organs of several animal models. Meanwhile, protective effect of antioxidants is a current topic not only in stress-related disorders but also in other age-associated degenerative diseases such as cancer, Parkinson's and Alzheimer's disease. In 1994, the Liu's group published a paper, in the International Journal of Stress Management entitled "Involvement of reactive oxygen species in emotional stress: A hypothesis based on the immobilization stress-induced oxidative damage and antioxidant defense changes in rat brain, and the effect of antioxidant treatment with reduced glutathione" (Liu and Mori, 1994). In 1999, it was further modified the hypothesis with more findings in stress and oxidative stress studies (Liu et al., 1996b) and proposed an "Oxidative damage hypothesis of stress-associated aging acceleration" (Fig. 1), published in the Neurochemical Research (Liu and Mori, 1999). The present review summarizes the recent advances in stress and oxidative stress studies in supporting this hypothesis, with a focus on the anti-stress and neuroprotective effects of some natural and nutritional antioxidants.

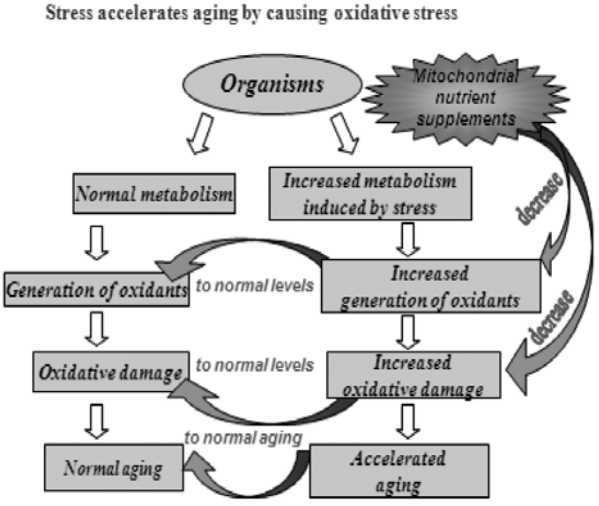



Figure 1. Schematic representation of oxidative hypothesis of stress-induced aging acceleration and age-associated diseases, and also the effects of natural and nutritional antioxidant intervention (shown as curved arrows).

**Oxidative damage hypothesis of stress-associated aging acceleration**

In the 1950s and 1960s, researchers hypothesized that old age results from the accumulated stress experienced during an organism's life span (Bortz, 1955; Platner, 1961). Pare (Pare, 1965) found that rats chronically exposed to 48 days of mild-shock stress exhibited changes similar to those which occur during aging. For example, stressed animals were found to fatigue significantly sooner on swimming trials than non-stressed controls. Moreover, animals that were allowed a 130-day rest period following this treatment, continued to exhibit fatigue, indicating that the effects of this stress are persistent. Two implications follow this: 1) the aging process is accelerated by repeated

exposures to stressful stimuli; and 2) old organisms cannot adapt to stress as readily as the young ones. But there are some problems with this hypothesis: 1) this hypothesis assumes that stress is the sole contributor to aging, and 2) it did not give a mechanism. For instance, what is "accumulated" by stress?

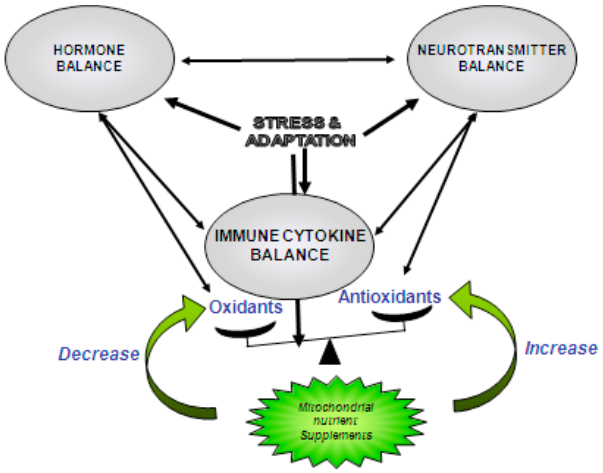
Glucocorticoids in vitro reduce glucose uptake and energy metabolism in neurons (Sapolsky, 1985; Sapolsky et al., 1985); however, it is not clear that stress or stress hormones reduce brain energy metabolism in vivo, and stress is known to increase brain metabolism (Landfield and Eldridge, 1994). Under stress, the metabolic rate increases. For example, the energy requirement for normal humans is about 2000 kcal/day, however, a seriously burned patient may need as high as 5000 kcal/day. This high energy is provided by the stress-induced release of catecholamines, glucocorticoids and some inflammation mediators, stimulating the release of energy substrates from muscle, fat tissue and liver, while simultaneously suppressing anabolic processes, including growth, digestion, reproduction, and immune function (Johnson et al., 1992). Under stress, glycogen degrades, but gluconeogenesis, blood sugar, fatty acids and amino acids increase. In addition, cells respond to stress by inducing the synthesis of a specific set of stress proteins. Examples are the heat shock proteins and the glucose-regulated proteins. The induction of these proteins can provide either immediate stress protection, or participate in cellular repair processes (Ushakova et al., 1996).

Metabolism, like other aspects of life, involves trade-offs. Oxidative by-products of normal metabolism cause extensive damage to lipids, proteins and DNA (Ames et al., 1993). The life span of mammalian species is related to the rate of oxygen consumption. The tissue and cellular concentration of oxygen and the levels of free radical reactions increase as oxygen utilization rises. Hence, the life span is associated with metabolic rates (Harman, 1981). It has been argued that this normal metabolism-induced oxidative damage (the same as that produced by radiation) is a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, immune-system decline, brain dysfunction and cataracts (Ames et al., 1993; Shigenaga et al., 1994a).

The purpose of increased glucocorticoids and catecholamines are for the needs of increased energy production and consumption, which are accompanied by augmented metabolism of sugars, lipids and proteins. However, chronic activation of the catabolic processes induced by stress may be ultimately destructive and damaging. Normal metabolism, which uses oxygen, generates oxidants such as superoxide, hydrogen peroxide and hydroxyl radical, which cause oxidative damage. Based on the experimental data of stress-associated oxidative damage and the evidence that oxidative damage caused by the trade-offs in normal metabolism is a major contributor to aging, it would appear that stress could stimulate numerous pathways leading to an increased production of oxidants. Thus, stress may add to the oxidant burden associated with normal aerobic metabolism and its consequent damage to lipids, proteins and RNA/DNA. The relationship between stress and increased oxidant production suggests a critical role for increased oxidant production in stress-associated aging acceleration and age-related diseases. This increased oxidative damage may be a major, albeit not the only, contributor to aging acceleration and age-related diseases (Liu and Mori, 1999).

The stress-related changes in hormones, neurotransmitters, oxidants and other related mediators are shown in Fig 2. These changes suggest that the stress and adaptation homeostasis may be

governed by the balances between oxidants and antioxidants, between excitatory and inhibitory neurotransmitters, between stress and anti-stress hormones and between other mediators such as immune cytokines, as well as by the interactions of these substances (Liu and Mori, 1999).Figure 2




2. Schematic representation of stress-associated hormone, neurotransmitter, and oxidant balances as well as their interactions, and the effects of antioxidant supplementation on oxidant balance and the stress-adaptation homeostasis.

**Stress and brain oxidative damage**

Increasing evidence supports the hypothesis that stress could induce oxidant generation, decrease antioxidant defense, increase oxidative damage to lipids, proteins, and nucleic acids in many organs, particularly in the brain (Liu and Mori, 1994; Liu et al., 2001; Liu et al., 1996b; Liu et al., 1998). Some recent examples are listed below.

Immobilization stress induces oxidative damage in rat brain by decreasing the activities of superoxide dismutase (SOD), glutathione-S-transferase, catalase and glutathione levels, while increasing lipid peroxidation (Zaidi and Banu, 2004). Chronic and sub-chronic stress induce different changes in lipid peroxidation and total radical-trapping potential of the lung of rats (Torres et al., 2004). Hydroxyl radical seems to be the major causative factor in stress-induced gastric ulceration because quantitative measurement indicates that cold plus restraint stress causes a fivefold increase in the generation of hydroxyl radical, which correlates well with the increase in ulcer index and the progress of stress (Das et al., 1997).

Cold stress also induces oxidative damage. Gumuslu et al. (Gumuslu et al., 2002) studied the influences of different stress models (cold stress, immobilization stress, and cold + immobilization stress) on the antioxidant status in erythrocytes of rats. Cu, Zn-SOD activity and thiobarbituric acid-reactive substances (TBARS) are increased after cold and immobilization stresses and catalase and glutathione peroxidase activities and GSH levels are decreased. Immobilization stress decreases the activity of glucose-6-phosphate dehydrogenase. The activities of glucose-6-phosphate dehydrogenase, catalase and glutathione peroxidase (GPx), and the level of GSH are decreased by cold + immobilization stress. Cu, Zn-SOD activity and levels of TBARS are increased by cold plus immobilization stress. These results show that all stress models tested cause oxidative stress.

Footshock stress results in an increase in dopamine metabolism in the prefrontal cortex. Dopamine is metabolized by monoamine oxidase with hydrogen peroxide as a product. Footshock stress increases glutathione peroxidase activity in the prefrontal cortex and the striatum, indicating that increased dopamine metabolism induced by footshock stress is probably responsible for the increase of glutathione peroxidase activity (Gonenc et al., 2000).

Exposure to physical or psychological stress-induced oxidative damage is associated with behavioral dysfunction. Chronic stress is shown to induce an increase in cerebral cortex in oxidative stress (Manoli et al., 2000), overproduction of

nitric oxide (NO) and related oxidative-nitrosative compounds via expression of the inducible NO synthase (iNOS) (De Cristobal et al., 2002; Madrigal et al., 2002), an increase in plasma glutamate and brain TNF-α, induction of oxidative indicators in brain and a fall in brain ATP levels (De Cristobal et al., 2002). Acquisition and memory performances are negatively correlated with plasma corticosterone level, nitrite, and TBARS levels of hippocampus and frontal cortex (Abidin et al., 2004). An 8 hours of immobilization results in impairment of passive avoidance test (memory retrieval deficit) and increased latency to start locomotion in an open-field test. The oxidative damage of lipids, proteins and nuclear DNA is increased significantly and the activity of glutamine synthetase is increased by immobilization stress whereas exercise abolishes these harmful changes in behavior and oxidative damage, suggesting that oxidative damage of macromolecules is associated with impaired cognitive function (Radak et al., 2001).

In addition, cellular, molecular and morphological changes induced by stress might be accelerated when there is a pre-existing strain upon their already compromised adaptive responses to internal or external stimuli, similar to what may occur with uncontrolled diabetes mellitus (Reagan et al., 2000). The deleterious actions of diabetes and stress may increase oxidative stress in the brain, leading to increases in neuronal vulnerability. Stress/diabetes is shown to mediate 4-hydroxy-2-nonenal protein conjugation of the neuron specific glucose transporter, GLUT3, from hippocampal membranes of diabetic rats subjected to stress (Reagan et al., 2000). Oxidative stress and modulation of antioxidant enzymes contribute to the deleterious consequences of diabetes mellitus and to the effects of chronic stress in the CNS. Restraint stress or hyperglycemia, or the combination, produces similar increases in oxidative stress markers 4-hydroxy-2-nonenal and malondialdehyde throughout the hippocampus and regulates the expression of two anti-oxidant enzymes, Cu/Zn-SOD and Mn-SOD in a time- and region-specific manner in the rat hippocampus (Grillo et al., 2003).

Cortisol levels during human aging predict hippocampal atrophy and memory deficits (Lupien et al., 1998). Direct evidence of glucocorticoids causing oxidative stress has been extensively studied. Corticosterone exacerbates excitotoxicity, oxidative injury, and amyloid β-peptide toxicity in hippocampal neurons (Goodman et al., 1996) and mouse clonal hippocampal HT22 cells (Behl, 1998). Glucocorticoids enhance the hippocampal neurotoxicity of reactive oxygen species and decrease the activity of the antioxidant enzyme, glutathione peroxidase in primary hippocampal cultures, including glutathione levels, NADPH level and GPx activity over a range of glucose concentrations (Patel et al., 2002). Thus, glucocorticoids have both energetic and non-energetic effects in nature, upon steps in GPx biochemistry that, collectively, may impair hippocampal antioxidant capacity (Patel et al., 2002).

Mitochondrial decay has been suggested to be an important factor in the pathogenesis of aging and age-associated neurodegenerative disorders (Ames, 2003; Ames et al., 1995; Shigenaga et al., 1994b). Mitochondrion is the source and also the target of oxidants. Chronic corticosterone affects brain weight, and mitochondrial, but not glial volume fraction in hippocampal area CA3. The relative reduction in mitochondria may indicate a reduction in bioenergetic capacity that, in turn, could render CA3 vulnerable to metabolic challenges (Coburn-Litvak et al., 2004). Stress is shown to inhibit the activities of the first complexes of the mitochondrial respiratory chain. Administration

of the preferred iNOS inhibitor aminoguanidine protects against the inhibition of the activity of complexes of the mitochondrial respiratory chain as well as prevents NO(x) accumulation, lipid peroxidation and glutathione depletion induced by stress, suggesting that a sustained overproduction of NO via iNOS is responsible for the inhibition of mitochondrial respiratory chain caused by stress and that this pathway also accounts for the oxidative stress found in this situation (Madrigal et al., 2001). Cyclooxygenase-2 and inducible nitric oxide synthase isoforms (NOS-2) are up-regulated and account for oxidative damage in brain after immobilization stress indicating a possible adaptive role for cyclooxygenase-2 and NOS-2 pathways in stress response (Madrigal et al., 2003). These findings may provide possible therapeutic targets by administering mitochondrial antioxidants in the context of neuropsychiatric and other disorders related to stress.

**Protection against oxidative damage by natural and nutritional antioxidants**

It has been suggested that mitochondria should be the source and also target of oxidants generated during stress (Liu and Mori, 1999), therefore, it should be more rational and effective to use mitochondrial targeting agents to ameliorate stress-induced oxidative damage. Liu et al. and other groups (Ames et al., 2002; Liu and Ames, 2005) have defined mitochondrial nutrients as those nutrients that can 1) prevent oxidant production or scavenge free radicals to prevent mitochondrial oxidative stress ( e.g. iron chelating agents and enzymatic and non-enzymatic radical scavengers); 2) enhance antioxidant defense (e.g. phase-2 enzyme inducers); 3) protect mitochondrial enzymes and/or stimulate enzyme activity by elevating substrate and cofactor levels; and 4) enhance mitochondrial metabolism, for example, by repairing less damaged but degrading more damaged mitochondria, and increasing mitochondrial biogenesis. Mitochondrial nutrients have been classified into three groups: 1) Antioxidant defense agents, such as coenzyme Q10 and α-lipoic acid; 2) Energy enhancers, such as carnitine/acetyl-L-carnitine, creatine, pyruvate, and choline; and 3) Cofactors and precursors of cofactors, such as α-lipoic acid, coenzyme Q10, and B-vitamins (B1, B2, B3, B5, B6, B7, B11, B12). Some mitochondrial nutrients may have several functions, and some may have the same function. Increased evidence demonstrates that some mitochondrial nutrient supplementations delay aging and age-associated diseases, such as cancer and neurodegenerative diseases (Ames, 1998; Ames, 2004; Ames et al., 2003; Liu et al., 2002a; Melov, 2002; Melov et al., 2000). Because stress accelerates aging by increasing oxidant generation from mitochondria and oxidative damage to mitochondria, therefore, mitochondrial protective nutrient supplementation should be effective in protecting against stress-induced oxidative damage, relieving stress, preventing stress-associated aging acceleration and diseases for a normal aging (Fig. 1). A few examples are given below.

L-Carnitine, an essential cofactor in the mitochondrial transfer of fatty acids, has been described as a conditionally essential nutrient for humans. L-carnitine facilitates entry of long-chain fatty acids into mitochondria for utilization as fuel and facilitates removal from mitochondria of short-chain and medium-chain fatty acids that accumulate as a result of normal and abnormal metabolism (Liu et al., 2002a). Experimental data demonstrate an age-associated decrease of tissue levels of L-carnitine in animals, including humans, and an associated decrease in the integrity of the mitochondrial membrane. L-Carnitine and its acyl esters may act as an oxidant either having a primary antioxidant activity (inhibiting free radical generation, scavenging the initiating free radicals, and terminating the radical propagation reactions),

or more likely functioning as a secondary antioxidant (repairing oxidized polyunsaturated fatty acids esterified in membrane phospholipids). In addition, acetyl-L-carnitine protects mitochondrial complex III ubiquinol cytochrome c reductase, perhaps as an iron chelators (Liu et al., 2002a). Acetyl-L-carnitine has protective effects against stress damage in aging rats (McEwen et al., 1990). Cold-restraint stress caused a significant decrease in gastric mucin and PGE2 content while L-carnitine prevents the occurrence of mucosal lesions by strengthening the gastric mucosal barrier and by reducing the products of lipid peroxidation against noxious factors that cause elevation of lipid peroxidation (Izgut-Uysal et al., 2001). Long-term acetyl-L-carnitine administration in rats increases dopamine output in mesocorticolimbic areas (Tolu et al., 2002) and protects against the disrupting effect of stress on the acquisition of appetitive behavior (Masi et al., 2003).

Vitamin E is the mostly tested natural antioxidant in fighting against stress-induced oxidative damage (Armario et al., 1990; Das et al., 1997; Yargicoglu et al., 2003; Zaidi and Banu, 2004). Uric acid, SOD, albumin, ethanol (Vladimirov et al., 1992), vitamin A and C (Zaidi and Banu, 2004), estrogen (Goodman et al., 1996), and dehydroepiandrosterone (Hu et al., 2000), as well as exercise (Radak et al., 2001) are also tested in different stress models and showed protective effects. B-vitamins, amino acid tyrosine, phosphatidylserine, and plant sterol/sterolin combinations have also been proposed to be possibly to allow individual to sustain an adaptive response and minimize some of the systemic effects of stress (Kelly, 1999).

Stress causes depletion of endogenous antioxidants GSH (Liu and Mori, 1994; Liu et al., 1994; Liu et al., 2001; Madrigal et al., 2001). Administration of GSH protects immobilization stress-induced stomach bleeding, oxidative damage and disorders in antioxidant defenses in the rat brain (Liu and Mori, 1994) and inhibits restraint-cold stress-induced increase in lipid peroxidation and inactivation of gastric peroxidase in rats (Das et al., 1997). On the other hand, treatment of rats with buthionine-S,R-sulfoximine, an inhibitor of glutathione biosynthesis, causes a depletion in glutathione and an increase in oxidative damage to lipids, proteins and DNA in the brain of stressed rats (Liu et al., 1996a). Dietary supplements of vitamins with antioxidant properties modify gene expression induced by heat shock in vivo and protect rat tissues against oxidative stress by enhancing the level of endogenous antioxidants and inducing hsp-70 gene expression (Ushakova et al., 1996).

Both synthetic and natural antioxidants, similar to mitochondrial protective nutrients, have been shown to protect the stress-induced behavioral changes and oxidative damage. Specific free radical scavengers such as benzoate or dimethylsulfoxide, free radical traps such as α-phenyl-N-tert-butyl nitrone, and desferrioxamine (a nontoxic transition metal chelator), significantly inhibit gastric ulceration (Das et al., 1997). Increased level of lipid peroxidation and the inactivation of gastric peroxidase are also prevented by desferrioxamine (Das et al., 1997). Ionol (2,6-di-tert-butyl-4-methylphenol), an antioxidant, is an effective anti-stress agent (Meerson, 1984; Meerson et al., 1991; Shamkulashvili et al., 1992). Administration of iNOS inhibitor protects against stress-induced inhibition of the activity of mitochondrial complexes of the respiratory chain as well as prevents NO(x) accumulation, lipid peroxidation and glutathione depletion (Madrigal et al., 2002),. Aspirin, a NSAID with neuroprotective actions, inhibits stress-induced increase in plasma glutamate, brain oxidative damage and ATP fall in rats (De Cristobal et al., 2002).

Natural herbal extracts have also been suggested and used to protect oxidative damaged induced by stress (Kelly, 1999). Vietnamese ginseng saponin (VG saponin) and its major component majonoside-R2 (MR2) on psychological stress-induced lipid peroxidation in the mouse brain are studied (Yobimoto et al., 2000). Psychological stress exposure using a communication box system for 4 h significantly increases the level of TBARS in the brain. Pretreatment with VG saponin and MR2 attenuated the psychological stress-induced increase in TBARS in the brain. The aglycone of MR2, at the equivalent dose of MR2, also produces a suppressive effect on the increase in TBARS (Yobimoto et al., 2000). Manda, a fermented natural food with antioxidant activity, suppresses immobilization stress-induced stomach ulcers and inhibits lipid peroxidation in rat brain (Kawai and Matsura, 1997; Kawai et al., 1998). Liu's group has shown that an extract of medicinal plants is an effective anti-stress agent. Yi-Zhi-Yi-Shou (YZYS) is a fine extract prepared from 16 medicinal herbs, a prescription of Dowager Cixi's Yanling-Yishou-Dan in Qing Dynasty (Liu et al., 2001). It inhibits free radical-induced peroxidation of brain homogenate, microsomes, mitochondria, amino acids, deoxyribose and DNA. The in vivo study with immobilization-induced emotional stress in rats, showed that YZYS effectively inhibits stress-induced stomach ulcers and oxidative damage in plasma and in the brain by inhibiting lipid peroxidation, protecting enzyme inactivation, maintaining antioxidant defenses, membrane structures and functions. In addition, YZYS was found to be non-toxic in both acute and chronic toxicity tests. These studies demonstrate that YZYS is a potent natural antioxidant and offers theoretical evidence for the beneficial effect of YZYS on stress (Liu et al., 2001). It would be interesting to test whether these herb extracts could enter into mitochondria and work as mitochondrial protective nutrients.

It has also been proposed that a combination of various mitochondrial nutrients should be more effective by working on different mechanism and showing complementary protection (Liu and Ames, 2005). Some combinations have been tried in aging studies, For example, combination of CoQ and $\alpha$-tocopherol (Lass and Sohal, 2000; McDonald et al., 2005) or $\alpha$-lipoic acid and acetyl-L-carnitine (Hagen et al., 2002; Liu et al., 2002a; Liu et al., 2002b; Liu et al., 2002c) has been shown to improve learning and memory, decrease oxidative damage, enhance antioxidant defense, and improve mitochondrial function in old rats. However, no studies have been carried out in stress model. Therefore, studying the combination of various anti-stress agents is a promising and interesting direction for stress study.

**Conclusions**

Stress is associated with increase in oxidant production and oxidative damage via imbalances of hormones, neurotransmitters, oxidants (mainly from mitochondria) and other stress mediators such as immune cytokines, as well as the interactions of these stress-related mediators. All of the imbalances are resulted from stress-induced changes in metabolic rate/catabolic processes and all of the mediators are dangerous in excess. The increased accumulation of oxidative damage to biomolecules critically contributes to aging acceleration and age-related diseases, such as neurodegenerative diseases. Future interdisciplinary research is greatly needed to support this hypothesis. For example, if social epidemiologists are capable of defining subgroups in initially healthy middle-aged populations that are at risk of accelerated stress-mediated diseases, we may demonstrate that enhanced endogenous oxidative damage is critical in triggering aging acceleration

and disease developments by measuring biomarkers of oxidative damage. In addition, based on the discussion above, there are several possible stress management strategies for slowing stress and aging-associated neural degeneration. The strategies include interventions to maintain or restore hormone, neurotransmitter and oxidant balances, such as reducing endogenous excitatory amino acid levels, blocking NMDA receptors, reducing glucocorticoid levels, inhibiting oxidant formation and oxidative damage. Among these interventions, dietary supplementation of mitochondrial protective nutrients may be especially important for preventing or slowing stress-associated aging acceleration and stress-associated diseases because they directly target the mitochondria, which play a key role in stress response in regulating various mediator balances and homeostasis. In addition, many of the mitochondrial protective nutrients can be supplemented by modifying nutrition, such as having more fruit and vegetables.

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# Folic acid supplementation: some practical aspects

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## Abstract

Since 1956, when Harman first postulated the free radical theory of aging, numerous studies have been carried out to test the protective action of antioxidants. One of these protective compounds used in antioxidant therapy is folic acid (FA). Folate deficiency can lead to several pathologies and its protective role is very well known. Because the negative effects of the synthetic form on the metabolism of folates and the controversy about the role of folic acid in cancer, the question is whether or not folic acid is good for everyone. In this paper we summarize some aspects of the biochemistry of folic acid and we show some precautions that should be taken into consideration when supplementing with this compound.

## Introduction

The biological aging can be defined as the progressive loss of the optimal functions as a function of the age of an organism because of intrinsic factors [1,2]. Although several hypotheses exist that try to explain the causes of the aging, only a few are widely accepted, which does not mean that they are the correct ones. Among these hypotheses, the theory of Harman [3], which is more than 50 years old and continuously reviewed [4], postulates that the macromolecular damage induced by the reactive oxygen species (ROS) is the main causal factor of the aging process. The ROS are produced during the course of the normal metabolism, mainly in the reactions of detoxification by the microsomal cytochrome P-450 system [5,6] and in the electron transfer in mitochondria [7]. Within this context, the oxidative damage of specific protein has been considered like one of the mechanisms by which oxidative stress is related to the loss of physiological functions that takes place in the aging and neurodegenerative diseases. In this sense, it is necessary to mention that all the proteins are not equally susceptible to the oxidative damage. Also, the effects of ROS are not either general or indiscriminate.

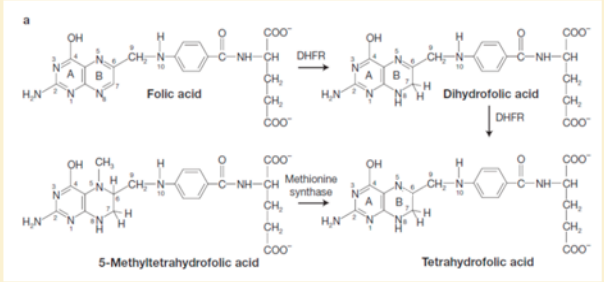
According to the free radical theory, it is reasonable to think that the reduction of oxidative stress by antioxidants can prevent the situation caused by ROS and, therefore, antioxidants should have "anti-aging" effects. This is the reason why people use antioxidants along with the fact that it is generally accepted that antioxidants vitamins are good for the health and that epidemiologic studies relate fruit intake with less incidence of diseases. Unfortunately, nowadays, none of the antioxidant formula allows us to "escape" from getting old and the clinical utility and health benefits of antioxidants are even in doubt.

Besides this fact, it is true that several pathologies have been associated with the oxidative damage produced by ROS and many studies have demonstrated the beneficial effects of different protective, antioxidant compounds. One of these protective compounds used in antioxidant therapy is folic acid (FA), which has been extensively by our research group.

## Structure, digestion and absorption of folates/folic acid

Folate/folic acid is a generic term for a family of compounds, all of them have a common structure (Fig. 1) containing the pteridine ring, p-amino benzoic acid, and glutamic acid. The pteridin-benzoic acid skeleton can be conjugated with one or more L-glutamic acid residues. The pteridine ring can be reduced either with hydrogen at the 5,6,7, and 8 positions (tetrahydrofolate-THF) or with hydrogen at the 7 and 8 positions (dihydrofolate-DHF). These reduced derivatives are the active forms and can accept one carbon unit at the 5 and 10 positions (Methyl-THF or Methyl-DHF).

FIGURE 1.- CHEMICAL STRUCTURES OF FOLIC ACID




Folate = Natural Form
Folic Acid = Synthetic Form

Folic acid is synthetic form used in dietary supplements and in fortified foods. It contains a single glutamic residue, it is totally oxidized and, therefore, it is very stable. Folate is the natural form of the vitamin found in foods, and contains several glutamic residues. Leafy green vegetables (like spinach-140 μg/100 g), fruits (like citrus fruits and juices-20-40 μg/100 g), dried beans (180 μg/100 g) and liver (182 μg/100 g) are all natural sources of folate. Also, fortified cereals have become a very important contributor of folic acid to the diet (50-200 μg/100 g). As folic acid is a water-soluble vitamin it is lost (up to 90 %) during cooking. This can be reduced by avoiding over-cooking, and steaming or microwaving vegetables instead of boiling.

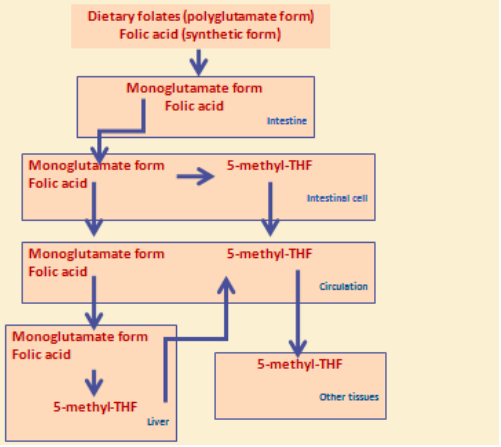
Polyglutamate form must be digested to the monoglutamate form for absortion (Fig. 2). The hydrolysis is performed by the enzyme pteroylpolyglutamate hydrolases, which is zinc-dependent and, therefore, zinc deficiency impairs the digestion and absortion of folate. Folic acid does not need to undergo digestion because it is already present in the monoglutamate form. Therefore, it has a higher bioavailability (> 90 %) than natural folates (~ 50 %), being rapidly absorbed into intestinal cells. Chronic alcohol ingestion diminishes the absorption of this vitamin.

Inside the intestinal cell, the mono-glutamic molecules (coming from the natural and synthetic forms) are reduced and methylated to form 5-methyl-THF by the participation of the enzyme NADPH dependent dihydrofolate reductase, whose activity is really low in humans.

Folate is absorbed by both saturable and unsaturable mechanisms. The saturable processes are specific and tend to occur in the upper small intestine. These processes mediate the absorption of a variety of folates, usually after the polyglutamated forms have been hydrolyzed to monoglutamates. After entering the intestinal cell, folates are usually

converted to 5-methyl-THF. The entire process of specific absorption takes approximately 1 h from the time of ingestion.

FIGURE 2.- ABSORTION- DISTRIBUTION OF FOLIC ACID AND FOLATES




Non specific, unsaturable absorption predominates in the ileum and allows nearly all folate reaching that site to be absorbed in linear proportion to the amount presented. This mechanism assumes importance whenever ingested folate exceeds the limited capacity of specific jejune absorption (200 μg). This is particularly relevant for the synthetic form (folic acid) since this process can deliver large amounts of unreduced folic acid from supplements that can reach the liver directly. In fact it has been postulated that liver instead of intestinal cells is the main tissue for the synthetic form (folic acid). This process can deliver large amounts of unreduced folic acid from supplements into the bloodstream without modification producing a chronic presence of non metabolized folic acid in blood. It has been found that non metabolized folic acids can be found after intake of 260-280 μg.

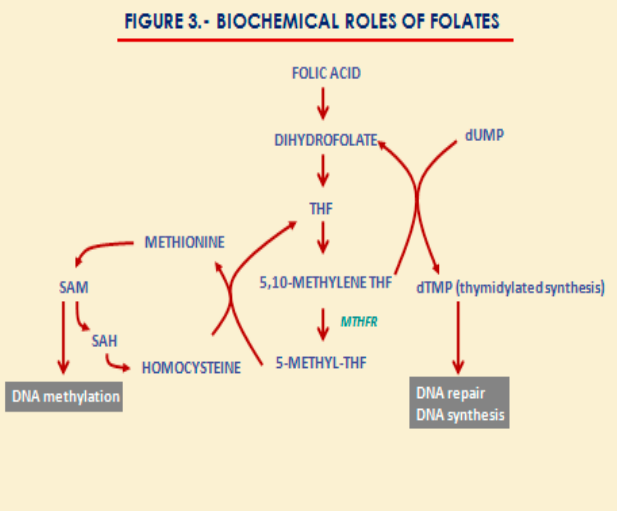
The enzyme methyl reductase is also present in liver, so that both monoglutamate forms and folic acid can be transformed to 5-methyl-THF, which in turn goes into the bloodstream. As can be seen, 5-methylTHF is the main form of folate found in plasma. However, when supplementing with folic acid, and due to the low activity of both intestinal and hepatic reductase, it can be found a significant proportion of non metabolized folic acid in the bloodstream.

Most folate circulates in the blood attached to albumin and other proteins, although one third circulates unattached. Then, folates are distributed to tissues, mainly to those of high cellular turnover such a bone marrow, intestinal mucosa, because these tissues need folic acid for DNA synthesis.

The presence of unmetabolized folic acid is an important issue because this form elicits and antifolate effect via competitive interaction with carriers, receptors and enzyme within the body. Thus, it has been described that folic acid can compete with the entrance of 5-MTHF into the brain [8]. Also, the excess of folic acid in the bloodstream has been inversely correlated with the activity of natural killer lymphocytes, which are one of the first lines of defense against cancer. In addition, unmetabolized form can diminish the levels of cellular methionine. In summary, large increases in the concentrations of FA can inhibit several folate-dependent enzymes.

### Biochemical functions and biological activity

In the circulation, folate is mainly present as 5-MeTHF, which is taken by the cells via specific carriers or receptors. In the cell, folate donates one-carbon units for use in methylation reactions and in purine and thymidine synthesis. The utilization of 5-MeTHF starts with the transfer of a methyl group to homocysteine, which is converted in methionine. Then, the THF formed gets a methyl group from serine, before the participation in several biochemical interconversions involved in purine and thymidine synthesis (Fig. 3)




## Deficiency of folates

Folate deficiencies are observed in some population groups under some special circumstances. For instance, in the elderly caused by inadequate intake; in pregnancy as a consequence of an increase of the requirements; intestinal diseases, where folate absorption is diminished; in chronic alcoholism, because alcohol affects folate absorption; in spite of the fortification policy, typical folate intakes are found to be sub-optimal in the diet of many people [9]. This widespread under-provision of folate is generally attributed to the poor bioavailability of natural food folates.

## Folic acid and health

As we have seen, folate plays an important role in the biosynthesis of thymidine and de novo generation of methionine for genomic and non-genomic methylation reactions. They are also required for purine synthesis as well as serine-glycine interconversion. This means that folate is important for the structural integrity of DNA. Folate deficiency may cause accelerated telomere shortening [10]. Folate is also critical for maintaining homocysteine levels within a non-pathological range. High blood plasma levels of homocysteine have been linked to many major disease conditions such as cardiovascular disease, Alzheimer, dementia and certain cancers [11,12]. These effects can be due to either a neurotoxic effect of homocysteine either to a lower availability of s-adenosylmethionine, which can lead to lower methylation levels of brain tissues. The levels of homocysteine can be elevated in the case of methylenetetrahydrofolate reductase (MTHFR) polimorfisms.

There is a big list of studies that implicate folate status with a range of chronic developmental diseases [8]. The most affected tissues are those with a high cellular turnover such as hematopoietic precursors, epithelial cells, etc. This is the reason why the clinical symptoms of acute effect of antifolate compound such a methotrexate are digestive, epithelial and hematologic problems. In addition, folate has a well established protective role against both occurrence and recurrence of neural tube defects (NTD). More recently, it has been described that low folate status is associated with poor cognitive function and dementia in the elderly [13]. On the contrary, the supplementation with folic acid over a 3-year period appears to reduce the rate of cognitive decline in older adults [14].

Other protective aspects of folic acid base are less known. Our groups have been working on the antioxidant properties of folates [15-17]. Some results from these studies are: folic acid supplementation improves the levels of oxidative stress markers in individuals with hypertension, overall in those patients whose initial parameters values were highest; Folic acid is useful in the prevention of damage and health problems of indivuduals born from mothers that have alcohol during pregnancy and lactation; the antioxidant

capacity of folic acid is similar to vitamine E, so that folates can be useful in protecting the aqueous part of the cells; folic acid protects very important pathways form oxidation such as rho guanine nucleotide dissociation inhibitor -RhoGDI-1- (an important protein involved in signal transduction), protease ER60 (involved in assembly of major complex histocompatibility and chaperone function) and gelsonin (involved in the response to stress and cellular differentiation).

In addition, it is worthy to note the dual role of folate on cancer. Folate play a key role in cellular differentiation and proliferation as well as in DNA repair, so that it is necessary for tissue maintenance and regeneration [18]. Low folate status is associated with DNA strand breaks, and impaired DNA repair, increased mutations and aberrant methylations. Folate is critically required for cell division and growth because it is a cofactor in the novo synthesis of purines and thymidylate and thus in nucleic acid synthesis. However, in cancer cells, where DNA replication and cell division occur at a rapid rate, removal of folate or a blockade of its metabolism causes inhibition of tumor growth. This is the basis of the use of antifolate drugs in cancer chemotherapy (methotrexate).

Consequently, the timing and dose of folate intervention are critical: if folate supplementation is started before the establishment of neoplasic process, the development and progression of the tumor is suppressed. On the contrary, if supplementation is started once the tumor is present, it can enhance its growth and progression [18,19]

In summary, it is very well known that folic acid protects important biological process and prevent some important diseases. However, it is necessary to avoid dairy doses higher than 200 μg of synthetic form for a long time and, ideally, it is recommendable to be sure that a neoplasic process has not started before supplementation.

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# Antiglycating agents: a short review

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## Introduction

Glycation of proteins involves non-enzymatic reaction of a sugar, or reactive aldehyde derived from these, with a nucleophilic group on the protein. Aldehydes (like methylglyoxal, glyoxal and glycoaldehyde), formed via glucose autooxidation, amino acid and lipid oxidation, are particularly potent glycating agents. Glycation of amino acids leads to the generation of a hetereogenous group of adducts known as advanced glycation end products (AGE). Glucose and other aldehydes, both free and protein-bound, can also undergo autooxidation reactions that contribute to AGE formation.

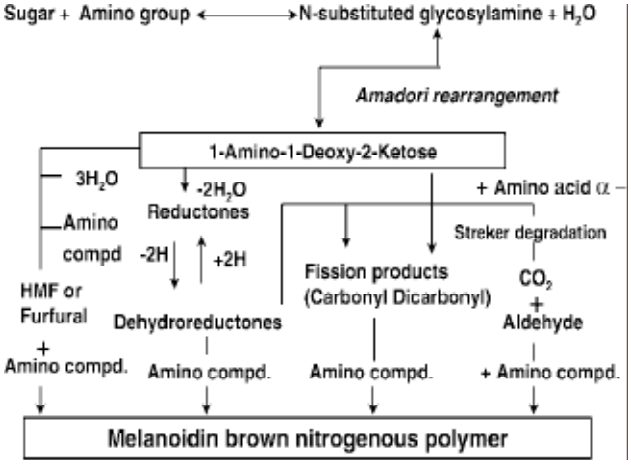


Figure 1. Initial steps of glycation.

Glycation reactions have been linked with the development of diabetes-associated cardiovascular diseases and other complications arising from them. Several antiglycating agents have been discovered that prevent this process of glycation, thereby serving to improve quality of life and increasing life span. The anitiglycating agents discussed in this chapter include lipoic acid, carnosine, and benfotiamine.

## 1. Lipoic acid

Lipoic acid or α-lipoic acid was first called pyruvate oxidation factor after the observation that it functioned as an essential growth factor for Enterococci, which lack the ability to synthesize lipoate. It was isolated from bovine liver in 1950. In the following years, its chemical structure was established and confirmed by synthesis.

### 1.1 Biochemical role

Amide of lipoic acid is a coenzyme of E2 subunit of multienzymatic mitochondrial complexes catalyzing oxidative decarboxylation of pyruvate, ketoglutarate and branched-chain ketoacids, formed during transamination of leucine, isoleucine and valine. These reactions share the mechanism consisting in ketonic group transfer onto coenzyme A. Lipoic acid is also an essential element of mitochondrial complex of four proteins participating in glycine synthesis and degradation (glycine cleavage system).

### 1.2 Therapy of degenerative diseases

Numerous experimental and clinical studies proved lipoic acid-containing drugs to be efficacious in the treatment of diseases, in which pro- and antioxidant

balance is disrupted (e.g. diabetes, neurodegenerative diseases, acquired immune deficiency syndrome (AIDS), tumors). Efficiency of lipoate has been attributed to unique antioxidant properties of lipoate/dihydrolipoate system, its reactive oxygen species (ROS) scavenging ability and significant effect on the tissue concentrations of reduced forms of other antioxidants, including one of the most powerful, glutathione (thus lipoate is called an antioxidant of antioxidants). Lipoic acid has been shown in cell culture experiments to increase cellular uptake of glucose by recruiting the glucose transporter GLUT4 to the cell membrane. There has also been evidence of participation of lipoic acid in processes of cell growth and differentiation.

### 1.2.1 Diabetes

The reaction of glucose with oxygen leads to formation of superoxide radical anion and glucose radical. The high glucose level in diabetic patients, leads to the formation of the superoxide radical anion in dramatically large quantities that leads to oxidation of hemoglobin to methemoglobin, cysteine to cysteinic acid and methionione to sulfoxides. It is also known that in the presence of transition metal ions (e.g. copper ions), glucose stimulates lipid peroxidation, biodegeneration of proteins and modification of amino acid residues, which frequently change activity of enzymes. The presence of oxidative stress in diabetes is corroborated by the fact that diabetic patients have significantly lower cellular and plasma levels of antioxidants, particularly glutathione (GSH), vitamin C and vitamin E in comparison with healthy people. The elevated level of N-carboxymethyllysine (CML) and pentosidine, the products of nonenzymatic glycosylation (glycation) of proteins is also one of proofs of systemic oxidative stress in diabetes. Neuropathic changes, cataract, neurological disturbances, degenerative joint disease are some of the frequent complications of diabetes, while atherosclerosis, cardiovascular diseases (heart attack, embolism, and stroke) are the main causes of death of diabetic patients. Many of these problems are connected with glycation of three long-lived proteins: myelin, collagen and crystalline.

Studies on an influence of lipoic acid on glycation of animal proteins showed that formation of advanced glycation end products (AGEs) was markedly reduced by lipoic acid and other antioxidants. These studies were later confirmed by human studies. It was demonstrated that lipoic acid lowered protein glycation rate and decreased CML level. Cataract is one of complications of diabetes mellitus, in which AGEs play a certain role. Cells of eye lenses maintain high level of GSH to protect themselves from glycation and reactions related to free radicals. However, in diabetes, glutathione concentration in eye lenses is considerably reduced. Research conducted on an animal model of cataract produced by the use of a known GSH synthesis inhibitor, L-buthionine-(S,R)-sulfoximine (BSO), revealed that administration of lipoic acid to animals caused a rise in cellular level of GSH, vitamin C and vitamin E, and contributed to an increase in activity of glutathione peroxidase, catalase and ascorbate reductase in the lenses. These results prompted some researchers. to make an attempt to evaluate efficiency of lipoic acid in cataract induced by high glucose level. The experiments were carried out on normal rat lenses at normal and elevated glucose concentration. Opacification of the lenses at high glucose level was significantly weaker in the presence of lipoic acid in comparison with opacification observed in lenses in the absence of lipoate. At present, medications preventing chronic complications of diabetes, like neuropathy and nephropathy, are intensively searched for because currently used therapies are not satisfactorily efficient in combating these complaints.

### 1.2.2 Neurodegenerative diseases

A potential of lipoic acid to exert protective effect on nervous cells was first mentioned in a comprehensive

review on oxygen toxicity published as early as in 1968. Brain damage due to stroke, heart arrest, and hemorrhage or head injury is a result of sudden re oxygenation of tissues (reperfusion) after a period of more or less enhanced hypoxia. Administration of lipoic acid to animals subjected to ischemia-reperfusion (e.g. by occlusion of cerebral artery) alleviate the effects of reperfusion, *viz*. level of ROS in the brain cells was lower, extent of the damage was reduced and survival time of animals was decidedly longer in comparison with the control group. Administration of lipoic acid to pregnant women, particularly during parturition helps to mediate the sudden oxygenation of the newborn and prevent the high loss of neurons in a child.

*Post mortem* studies of the brains of *Parkinson's disease* patients demonstrated an elevated dopamine (DA) turnover that results in the enhanced production of $H_2O_2$, thereby increasing concentration of the neurotoxic hydroxyl radical OH-. Lipoic acid aids in the preservation of glutathione levels in the cells of the central nervous system (CNS) that scavenge these free radicals.

*Alzheimer's disease* is a progressive neurodegenerative disorder that result in loss of memory and cognitive capacities, accompanied by personality disintegration. It is believed that reactive oxygen species (ROS) also contribute to the course of this disease. Histopathological hallmark of this disease is the presence of extra cellular deposits of amyloid (amyloid plaques, senile plaques), which is formed from a precursor called amyloid precursor protein (APP). Under physiological conditions, APP is proteolytically split by several types of secretases yielding soluble amyloid peptide composed of 40 amino acids (A40). A change in activity of secretases leads to formation of insoluble peptides containing 42 (A42) and 43 (A43) amino acids. These peptides are the main constituents of senile plaques in Alzheimer's disease, and are a source of ROS. Lipoic acid applied in patients diagnosed with dementia inhibited progress of the disease, which was confirmed by two neuropsychological tests, minimal mental state examination (MMSE) and Alzheimer's disease assessment scale, cognitive subscale (ASAScog). This indicates a potential neuroprotective effect of lipoic acid in the course of Alzheimer's disease. It has been shown to increase acetylcholine (Ach) production by activation of choline acetyltransferase and increases glucose uptake, thus supplying more acetyl-CoA for the production of Ach. Lipoic acid chelates redox-active transition metals, thus inhibiting the formation of hydroxyl radicals and also scavenges ROS, thereby increasing the levels of reduced glutathione. Furthermore, lipoic acid can also scavenge lipid peroxidation products such as hydroxynonenal and acrolein. The variety of properties of lipoic acid can successfully interfere with pathogenic principles of Alzheimer's disease.

### 1.2.3 Cardiovascular diseases

Oxidative stress constitutes a major causative factor in atherosclerosis. Oxidative modifications to LDL cholesterol increase atherogenicity by altering cell receptor uptake of these particles, particularly cells in the intima of blood vessels. Furthermore, oxidized LDL is taken up by scavenger receptors on monocytes, smooth muscle cells and macrophages in an uncontrolled process leading to the accumulation of lipid and formation of foam cells, an early feature of atherosclerotic plaques. Within this early atherosclerotic lesion, increased oxidative stress evokes inflammatory events that further generate peroxides, superperoxides and hydroxyl radicals within the endothelium. The inflammatory events in turn continue the cycle of damage to the vasculature. In light of these mechanisms, current research focusing on the effect of antioxidants exhibiting a protective effect on the oxidation of LDL cholesterol may lead to the mitigation of the atherosclerotic process. Alpha lipoic acid has the capacity to recycle endogenous

antioxidants and has been shown to be effective in recycling vitamin E by interacting synergistically with vitamin C (Fig. 2) thus contributing to enhanced antioxidant protection of LDL.

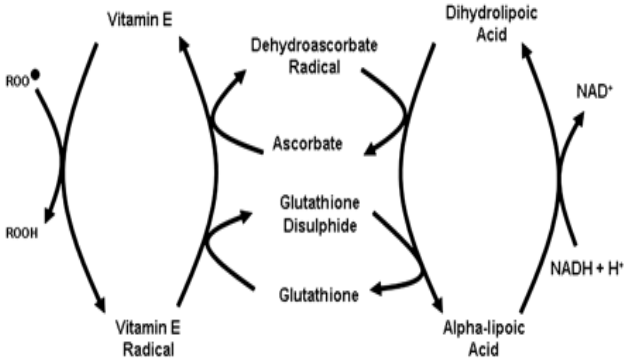


Figure 2. The role of α-lipoic acid in the recycling of other antioxidant systems.

Dietary ALA of 1 mg/kg of diet not only reduced the levels of total cholesterol and lipoproteins in the serum and aortic tissue of rabbits, but also intensified tissue respiration in the heart, liver and blood vessels. More recently, supplementation with ALA at 300 mg/kg body weight/day caused a decrease in plasma triglyceride concentrations in diabetic rats. This decrease in plasma triglycerides could possibly facilitate improved endothelial function, which could prove beneficial in CVD. Alpha lipoic acid has also been shown to exert hypertensive effects mediated by an attenuation of oxidative stress in the aortic vessel and the preservation of glutathione peroxidase activity.

### 1.2.4 Obesity

Obesity shortens life expectancy and is a risk factor for hypertension and Type 2 diabetes, hypertension, hyperlipidemia, and cardiovascular diseases. Lipoic acid has been shown to reduce food intake and body weight and food intake and increase whole-body energy expenditure by suppressing hypothalamic AMP-activated protein kinase. It also inhibits the differentiation of preadipocytes to adipocytes by attenuating the expression of adipocyte-specific fatty acid-binding protein and lipoprotein lipase.

### 2. Carnosine

Carnosine is an endogenous dipeptide that reacts rapidly with aldehydes and detoxifies aldehyde-modified proteins. Also known as L-carnitine, it was discovered over 100 years ago by two Russian scientists, Gulevitch and Amiragdibi, as a naturally occurring protein found predominantly in long-lived tissues including the brain, innervated muscle, and the lens in surprisingly high amounts (up to 20 mM in human muscle). It appears to possess antiglycating, antioxidant, and free-radical scavenging activity. The imidazolium group of histidine or carnosine stabilizes adducts formed at the primary amino group and may play an important role as an anticrosslinking agent. β-Alanine, a component of carnosine with neurotransmitter activity, has chaperone-like activity to suppress thermally-induced inactivation of lactate dehydrogenase, suggesting that it may play a cellular role in the preservation of enzyme function. It also inhibits inactivation and cross linking of enzymes, including superoxide dismutase, aspartate aminotransferase, esterase and catalase by glycation and oxidation.

### 2.1 Atherogenesis

Glycation of LDL by reactive aldehydes, such as glycoaldehyde can result in the cellular accumulation of cholesterol in macrophages thus directing towards the therapeutic potential of carnosine and its constituent amino acids in preventing-induced atherosclerosis. Carnosine and its constituents inhibit glycation of low-density lipoproteins that promotes foam cell formation. Such cholesterol-laden "foam-cells" are a hallmark of atherosclerosis and play a critical role in the development of cardiovascular disease and its associated complications. This decrease in LDL modification results in complete protection

results in complete protection against the pro-atherogenic effects of the aldehyde-modified LDL in terms of promotion of intracellular cholesterol and cholesterol ester accumulation.

### 2.2 Cataract

α-Crystallin, a major structural protein in the lens, prevents heat and oxidative stress-induced aggregation of proteins and inactivation of enzymes by acting as a molecular chaperone. The role of α-crystallin as a molecular chaperone may explain how the lens stays transparent for long. Modification of α-crystallin by some posttranslational modifications results in conformational changes that decrease chaperone activity, which may contribute to cataractogenesis in vivo. Carnosine has been shown to protect the chaperone activity of α-crystallin against glycation induced by sugars like fructose-6-phosphate and ribose; it also reacts with these sugars by forming adducts in addition to inhibiting cross linking by glycation. This anti-glycating action of carnosine provides protection to the lens.

## 3. Benfotiamine

In February of 2003, the news media broke with a story that is now bringing hope to millions of sufferers of neuropathy and other such problems. This news story featured a "new" chemical compound which is a derivative of vitamin b-1. This compound is called, benfotiamine. Benfotiamine was invented by Japanese scientists who patented the process in 1962. Although it has been used successfully for over 12 years in Europe for the prevention and relief of symptoms in people already suffering with various neuropathies, etc., it is just now being introduced into the United States. Benfotiamine is a lipid (fat) soluble form of vitamin B-1 (thiamin), belonging to the family of compounds knows as "allithiamines." It is physiologically active than thiamin and raises the blood level of thiamin pyrophosphate (TPP, the biologically active co-enzyme of thiamin. It stimulates transkotelase that directs glucose substrates to the pentose phosphate pathway, thereby controlling the formation of advanced glycation end products.

Benfotiamine is found in very small quantities within roasted, crushed garlic and other allium genus vegetables such as onions, shallots, and leeks. Researchers have known for years that thiamine could help in neuropathy, but being water soluble, not enough could be kept in the body without having to take toxic amounts, but as it is lipid soluble, it builds up to therapeutic amounts in a relatively short amount of time.

### 3.1 Benfotiamine and Glucose Metabolism: Benfotiamine normalizes cellular processes fueled by glucose metabolites.

The bulk of the cell's glucose supply is converted to pyruvic acid, which serves as substrate for production of acetyl CoA, the primary fuel for the Krebs cycle. In the presence of elevated glucose levels, the electron transport chain, the final ATP-generating system in the mitochondrion, produces larger than normal amounts of the oxygen free radical "superoxide." This excess superoxide inhibits glyceraldehyde phosphate dehydrogenase (GAPDH), as key enzyme in the conversion of glucose to pyruvic acid, resulting in an excess of intermediate metabolites known as "triosephosphates." Increased triosephophate levels trigger several cellular mechanisms that result in potential damage to vascular tissue. Cells particularly vulnerable to this biochemical dysfunction are found in the retina, kidneys and nerves.

Benfotiamine has been shown to block three of these mechanisms: the hexosamine pathway, the diaglycerol-protein kinease C pathway and the formation of Advanced Glycation End-poducts. As discussed below, benfotiamine does this by activating transketolase, a key thiamin-dependent enzyme. Benfotiamine stimulates tranketolase, a cellular enzyme essential for maintenance of normal glucose

metabolic pathways. Transketolase diverts the excess fructose-6-phosphate and glyceraldehydes-3-phosphate, (formed by the inhibition of GAPDH, as mentioned above), into production of pentose-5-phosphates and erythrose-4-phosphate and away from the damaging pathways. Benfotiamine activates transketolase activity in bovine aortic endothelial cells incubated in glucose.

### 3.2 Benfotiamine and Protein glycation: Benfotiamine controls formation of Advanced Glycation End-products (AGEs)

AGEs have an affinity for proteins such as collagen, the major structural protein in connective tissue. AGEs are formed through abnormal linkages between proteins and glucose. This occurs via a non-enzymatic glycosylation reaction similar to the "browning reaction" that takes place in stored food. At high glucose concentrations, glucose attaches to lysine, forming a Schiff base, which in turn forms "early glycosylation products." Once blood glucose levels return to normal levels, the amount of these early glycosylation products decreases, and they are not particularly harmful to most tissue proteins. On long-lived proteins such as collagen, however, early glycosylation products are chemically rearranged into the damaging Advanced Glycation End-products. AGE formation on the collagen in coronary arteries causes increased vascular permeability. This vessel "leakiness" allows for abnormal cross-linking between plasma proteins and other proteins in the vessel wall, comprising vascular function and potentially occluding the vessel lumen. A number of other potentially harmful events may also occur, including production of cytokines that further increase vascular permeability. Endothelin-1, a strong vasoconstrictor, is over produced, increasing the possibility of thrombosis and generation of oxygen free radicals is stimulated. It is vitally important to support normal glucose metabolic pathways so that formation of AGEs is minimized. Benfotiamine, in vitro prevents AGE formation in endothelial cells cultured in high glucose by decreasing the glucose metabolites that produce AGEs. Endothelial cells make up the membranes that line the inner walls of organs and blood vessels. In a rat study, comparing the effects of Benfotiamine with water-soluble thiamin, benfotiamine inhibited AGE formation in diabetic rats while completely preventing formation of "glycooxidation products," which are toxic by products of chronic elevated blood glucose. AGE levels were not significantly altered by thiamin. Benfotiamine also normalized nerve function in the animals. After three months of administration, "nerve conduction velocity (NCV)," a measure of nerve function, was increased by both benfotiamine and thiamin. Benfotiamine has also proved to be a potential treatment for improving micro- and macrovascular endothelial dysfunction and oxidative stress.

## 4. Conclusion

Glycation is an adverse reaction that leads to several degenerative diseases that occur as a result of alteration of protein structure and function. Presence of increased amounts of glucose in the blood promotes glycation, and the AGEs formed as a result of enhanced glycation increase oxidative stress and affect the quality of life. Antiglycation agents prevent or retard the rate of glycation thereby sparing vital body proteins for life-saving functions and maintaining internal and external homeostasis. Further research is warranted in this area in order to be able to manufacture more potent drugs that can prevent glycation without appreciable and significant side-effects.

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# Genomics disease susceptibility analysis in middle eastern population: its effects on anti-aging medicine practice

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## Introduction

The field of clinical genomics has been expanding recently and data is being always updated, it is the scientific gate of personalized medicine where the general guidelines of screening for diseases or for their treatment will no more be applicable; there will be a personal medical guideline for each person as per his/her genome. Personalized medicine is an advancing field of healthcare that is based on individualized unique clinical, genomic and environmental information.

The difference between classical genetics which is taught in medical schools and the genomics is large and maybe beyond this paper; however it can be summarized in the following differences: First, in clinical genomics, the genetic variants that are being tested are Single Nucleotide Polymorphisms (SNPs); polymorphism by definition is a genetic variant that has two alleles in a population at a frequency greater than one percent. SNPs are the most common form of genetic variation and there are an estimated 10,000,000 SNPs in the human genome, luckily they tend to come in groups so it's not needed to test all the SNPs, lesser quantity of SNPs testing can carry the needed information. This is in contrast to genetics which test gene alleles and mutations that are less in number but are more penetrant regarding disease expression which leads us to the second important difference between genetics and genomics which lies in the penetrance of the genetic variations and their effect on individual and on community levels. While the medical genetics deal mainly with rare diseases, the genomics deals more with the common chronic diseases such as diabetes, hypertension, coronary artery disease…. However; the multigenic nature of the common diseases, the modest effect of the genetic variant(s), and the confounding effects of environmental influences have made them exceedingly difficult to validate in multiple populations. A predictive genetic test for a complex disease would likely consist of a panel of multiple SNPs combined with other genomic and clinical information in contrast to a single gene mutation that is directly expressed medically in the classical genetics[1]. But because of the high prevalence of common diseases and their associated SNPs, genomics is gaining major importance in health maintenance both on individual as well as community levels.

One good example of this discussion would be mutations related to Alzheimer's disease. Genetically it's known that rare mutations in presenilin 1, presenilin 2, or the -amyloid precursor protein gene are highly penetrant causes of early-onset Alzheimer's disease, yet these mutations play a part in fewer than 1 percent of cases of Alzheimer's disease. In contrast, the apolipoprotein E 4 allele also increases the risk of

late-onset Alzheimer's disease (and atherosclerosis), but more subtly. Nonetheless, because approximately 26 percent of the U.S. population is heterozygous and 2 percent is homozygous for the apolipoprotein E 4 allele, this genetic factor has a role in many more cases of Alzheimer's disease than do the mutations in the genes for presenilin 1, presenilin 2, and -amyloid precursor protein combined. To add more to this picture, the apolipoprotein E4 allele relation to Alzheimer's disease in the U.S. population cannot be extended to other populations as other studies have suggested[2].

Therefore the application of clinical genomics would be of major importance in the different fields of medicine and would bring multiple advantages such as: the ability to make more informed medical decisions, higher probability of desired outcomes thanks to better-targeted therapies, reduced probability of adverse side effects, focus on prevention and prediction of disease rather than reaction to it, earlier disease intervention, and even reduced healthcare costs on community level if this genomic testing was done in a planned directed manner. Our study will illuminate on the importance of clinical genomics, but more thoroughly will focus on its application in different populations. We use that data we obtained from Middle Eastern population and compare it to similar data obtained from other populations and analyze accordingly if generalizations and therefore applications can be done worldwide, or it has to be adjusted as per ethnic and individual differences.

## Method

The genomic analysis presented in this paper will be focused on 7 SNPs (ApoE4, GSTT1, GSTM1, CYP2D6, CYP2C9, CYP2C19, and NAT2). The role of this SNPs and their importance will be discussed in details in the subsequent section (Discussion). The analysis was done on saliva samples from 160 subjects. All the samples were collected at Retrieve Health and Aesthetic Center, part of Specialized Medical Center Hospital in Riyadh KSA. The samples were transferred to Barcelona, Spain where they were analyzed at Sabater Analisis Laboratories. All the subjects who provided the samples did it on voluntary basis after coming to do general genomic analysis and signed on an informed consent agreeing to enroll in this study analysis.

The number of subjects was divided between 86 males (54%) and 74 females (46%). The age of subjects varied between 8 years as youngest and 80 years as eldest with the mean age of 43.2 years. Note is made that majority of subjects are in the age group of fourties and fifties (Figure 1). All the subjects were from Middle East area, mostly from Saudi Arabia with the nationalities varying as such: Subjects form Saudi Arabia are 137, from Lebanon are 13, from Qatar are 4, from Syria are 3, from Tunisia are 2, and from Bahrain 1. (Figure 2)

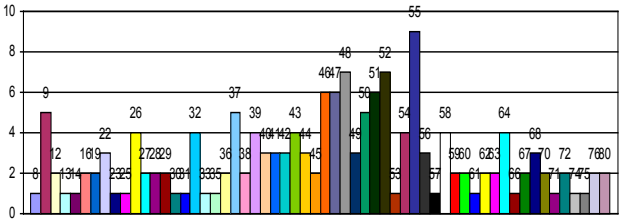


Figure 1. Frequency of subjects enrolled in study (Y-axis) vs. Age of subjects upon enrolling

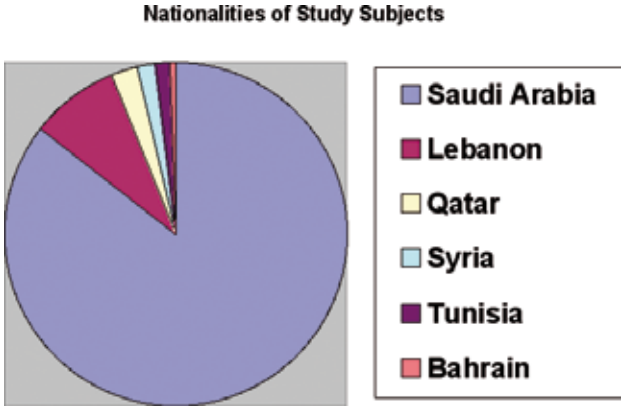


Figure 2. Subjects enrolled in the study by their nationalities

## Results

As mentioned earlier, the study analysis focuses on 7 important SNPs: ApoE4, GSTT1, GSTM1, CYP2D6, CYP2C9, CYP2C19, and NAT2. The definition and role of each one of these SNPs will be stated in addition to showing the results of this study. Important comparisons to other studies will be made under the discussion section.

First of the 7 SNPs analyzed is ApoE4. Apolipoprotein E (ApoE) is a major protein in lipid metabolism and exists in three isoforms: ApoE2, ApoE3 and ApoE4. The isoforms differ by one amino acid substitution, and in their binding affinity to the four Apo E receptors. Apo E polymorphism is a significant risk determinant for the development of cardiovascular and late-onset Alzheimer diseases, the prevalence of the epsilon 4 allele being high in both kinds of patients compared to control subjects. Furthermore, the prevalence of the epsilon 4 allele differs among populations[3].

The results of the study analysis for ApoE4 are:

Table 1. ApoE4 Alleles Frequency in Population of Retrieve Study

| **Apolipoprotein Alleles Presentation** | **Frequency** |
|---|---|
| Apolipoprotein E 4 heterozygous allele | 12.6% |
| Apolipoprotein E 4 homozygous allele | 0% |

Second SNP analyzed is GSTT1. GSTT1 (Glutathione S-transferase T1) is a member of GST supergene family (phase II of biotransformation) and is has important role in determining susceptibility to certain diseases associated with exposure to different industrial chemicals. Metabolism of chemicals such as synthetic halomethanes, methyl bromide, ethylene oxide, and methylene chloride in humans has been directly affected by the type of genetic polymorphism of GSTT1. It has two different alleles: GSTT1*active (wild) and GSTT1*0 (zero). The presence of two GSTT1*0 alleles (null type) results in a gene deletion and therefore in a non-active form of the enzyme associated with a reduced detoxification capacity[4]. The frequency of GSTT1 deficiency is about 20% in Caucasians and 80% in Asians[5]. The highest level of GSTT1 enzyme expression is found in the liver and the kidney. GSTT1 is also involved in detoxification processes in the large intestine.

Next SNP analyzed belongs also to the GST family and is GSTM1. GSTM1 (Glutathione S-transferase M1) is a biomarker and a risk modifier for various environmentally exposure-induced diseases. It is also a member of Glutathione S-transferase (GST) supergene family and determines the activity of the GSTM1 enzyme, (phase II of biotransformation), which detoxifies different electrophilic compounds, oxidized lipids, DNA and catechol products generated by reactive oxygen species. The GSTM1 gene is polymorphic and the presence of two 0 (zero) alleles (null type) corresponds to an impaired activity of the GSTM1 enzyme[6]. Different case-control studies have demonstrated correlations between GSTM1 deficiency and an increased relative risk for lung, bladder and colon cancer, basal cell carcinoma of the skin and other environmentally induced pathologies.

The results of the study analysis for GSTT1 and GSTM1 are:

Table 2. GSTT1 and GSTM1 Null Genotypes Frequency in Population of Retrieve Study

| **GSTT1 and GSTM1 Alleles Presentation** | **Frequency** |
|---|---|
| GSTT1 null genotype | 16.8% |
| GSTM1 null genotype | 55.6% |

| | |
|---|---|
| Concomitant GSTT1 and GSTM1 null genotypes | 10.1% |

The next 3 SNPs are pharmacogenomic and they are drugs metabolism modulators working through cytochrome P450. CYP2C9 is one of the key pharmacogenomic markers, which functions in interaction with CYP2C19 and CYP2D6. The CYP2C9 enzyme has important roles in metabolism of oral antidiabetic agents, anticoagulant drugs, antihypertensive drugs and some nonsteroidal anti-inflammatory drugs[7]. The gene is polymorphic. The presence of "slow" alleles can significantly influence the drug metabolism processes.

CYP2C19 is involved in phase I of biotransformation and plays an important role in drug metabolism. The CYP2C19 gene codes the S-mephenytoin hydroxylase, which is involved in metabolism of epileptic drugs, proton pump inhibitors, antidepressants, antipsychotics, oral contraceptives and platelet inhibitory drugs (Clopidogrel)[8]. Polymorphic effects of the CYP2C19 gene significantly influence the drug metabolism. The frequency of poor metabolizers (low enzyme activity) is 13-23% in Orientals and 2-5% in Caucasians[9].

CYP2D6 is involved in phase I of biotransformation and is a major pharmacogenetic marker. Its gene polymorphisms affect metabolism of more than 30 drugs and environmental chemicals, including about 20% of all commonly prescribed drugs (anti-depressants, antipsychotics, antiarrythmics, antihypertensives, morphine derivatives, tamoxifen). It's also involved in metabolism of neurotransmitters such as dopamine and dopamine-related neurotransmitters which potentially can provoke drug extrapyramidal side effects[10]. CYP2D6 activity is low in poor metabolizers (5 - 10% in Caucasians and less than 1 % in Asians) and significantly increased in ultrarapid metabolisers[11].

CYP19, known as aromatase or estrogen synthetase, is a cytochrome P450 enzyme which catalyzes the formation of aromatic C18 estrogens from C19 androgens. The enzyme CYP19 is responsible for the conversion of androgen precursor steroids to estrogens and may, therefore, have a role in regulating adipose tissue mass and its distribution. Accordingly, polymorphic effects of the CYP19 gene may influence the susceptibility to osteoporosis, breast cancer, and hormonal replacement therapy applications[12].

The results of the study analysis for CYP2C9, CYP2C19 and CYP2D6 as per their allele presentations effect on drugs metabolism rates.

Table 3. CYP2C9, CYP2C19 and CYP2D6 Genotypes as per Metabolism Rate Frequency in Population of Retrieve Study

| Genetic Polymorphism | Fast Metabolizers | Intermediate Metabolizers | Slow Metabolizers |
|---|---|---|---|
| CYP2C9 | 61.9% | 20% | 18.1% |
| CYP2C19 | 78.7% | 21.3% | 0% |
| CYP2D6 | 98.7% | 0.6% | 0.6% |

The last, but not the least, SNP being analyzed is NAT2. The NAT2 (N-acetyltransferase 2) gene - encodes the N-acetyltransferase enzyme 2 which is responsible for acetylation processes and is involved in phase II of detoxification and biotransformation. NAT2 gene is highly polymorphic and has about 20 different alleles. Polymorphic effects of this gene reflect on the phenotype level by slow, rapid and ultra-rapid acetylation. NAT2 is involved in activation/inactivation reactions of many xenobiotics, including aromatic and heterocyclic amines. NAT2 polymorphic effects significantly contribute to susceptibility[13] changes for urinary bladder, colorectal,

breast, head and neck, lung, and prostate cancer, and influence drug metabolism and susceptibility. The frequencies of NAT2 slow acetylators (SA)/ versus rapid acetylators (RA) differ significantly according to population groups. There are about 40 - 70 % of NAT2 SA in Central Europe and only 10-30% in Chinese and Japanese[14]. The results of the study analysis for NAT2 as per its allele presentations effect on drugs metabolism rates is in table 4.

Table 4. NAT2 Genotypes as per Metabolism Rate Frequency in Population of Retrieve Study

| Genetic Polymorphism | Fast Metabolizers | Intermediate Metabolizers | Slow Metabolizers |
|---|---|---|---|
| NAT2 | 8.1% | 35.6% | 56.3% |

## Discussion

The importance of the data presented on the different SNPs alleles and their prevalence in the population studied in this paper lies in comparing them to similar data from other populations, and observe how much variance in expression of gene alleles is present and does it affect the recommendation of the clinical practice of personalized medicine in the light of the quick advances in genomic medicine.

Starting with the ApoE4, the comparison is made on 2 main alleles ApoE3/E4 as heterozygous allele and ApoE4/E4 homozygous allele known to be related to Alzheimer's disease in different studies in the U.S. population as mentioned earlier. The comparison in the prevalence of alleles is summarized in Table 5

Table 5. Comparison of ApoE4 Alleles Frequency in Multiple Populations

| ApoE4 Alleles | Prevalence in Current study (Middle East Population) | Prevalence in U.S. population |
|---|---|---|
| ApoE3/E4 | 12.6% | 26% |
| ApoE4/E4 | 0.0% | 2% |

Does this variance in the expression of ApoE4 alleles reflect less prevalence of Alzheimer's disease in Middle East, Saudi Arabia mainly? The answer is not simple, because ApoE4 accounts for only part of the genetic risk for late-onset Alzheimer's disease expression in addition to the major roles of other genetic factors and potential promoting environmental factors. Late onset Alzheimer's disease occurs over the age of 65 and is the most common form of Alzheimer's disease, accounting for over 99 per cent of cases. A family history of dementia, regardless of *APOE-4* status, can increase the risk for developing Alzheimer's disease as well. Specifically, persons with a first-degree relative with dementia have a 10-30% increased risk of developing the disorder[16].

Since recent studies of asymptomatic *APOE-4* carriers show that these persons are more likely to display subtle abnormalities on brain scans, such as positron emission tomography (PET) or magnetic resonance imaging (MRI) scans and it has been shown that ApoE4 enhances amyloid beta clumping as a main ethiopatogenic effect causing late-onset AD, the relevance of having ApoE4 as genetic risk factor for late-onset Alzheimer's Disease is highly valid and in front of a ApoE4 carrier you have to do close monitoring, considering the patient age as an indicator[17].

Furthermore, and as per Saudi Alzheimer's Disease Association, in Saudi Arabia, there is a complete lack of information pertaining to estimates of current prevalence and cost of care as well as other aspects of the disease, such as care settings, treatment, detection, diagnosis and the current understanding of the disease[18]. This makes conclusions difficult, but at least it signifies the need for more data in this field especially in Middle East and it suggests that the risk

factors for developing Alzheimer's Disease in Middle East maybe different in importance than that of other populations.

As for GSTT1 and GSTM1, Glutathione S-Transferase theta and Glutathione S-Transferase mu genes control the corresponding enzymes involvement in the second phase of xenobiotic metabolism. Null genotypes of GSTT1 and/or GSTM1 are associated with increased risk of developing various kinds of neoplastic diseases, including cancers of bladder, colon, skin, lung and stomach. The comparison of this study findings and findings from multiple studies done among different populations is summarized in Table 6.

Table 6. GSTT1 & GSTM1 Null Genotypes Frequency in Multiple Populations

| **Populations** | **GSTT1 Null Genotype Prevalence** | **GSTM1 Null Genotype Prevalence** |
|---|---|---|
| Middle Eastern Population (Retrieve Center data) | 16.8% | 55.6% |
| North Americans[19] | 15% | 51% |
| Egyptians[17] | 14.7% | 44% |
| Indians[20] | 21% | 54% |
| East Asians[21] | 62.3% | 50.5% |
| Africans[17] | 21.8% | 32% |
| Caucasians[17] | 20.4% | 50.5% |

It's noted that the distribution of null genotypes of GSTT1 and GSTM1 among different populations is, more or less, similar; except for the East Asians who have clearly higher prevalence of the null genotype of GSTT1 and for the Africans who have less prevalence of the null genotype of GSTM1. These differences especially regarding the East Asian population who have high null GSTT1 phenotypes have been of major interest regarding possible relation with the well known high incidence of gastric cancer among the same population. Several studies had been done regarding this subject; and even though none was fully conclusive, findings were not favoring a clear relationship between GSTT1 and gastric cancer incidence in Eastern Asian population. Though, most of the studies recommend larger scale studies regarding this subject because of its major importance.

Next is the field of pharmacogenomics. Pharmacogenomics differs from pharmacogenetics; it's broader and more extensive. Pharmacogenetics, by definition, is the study of inherited differences in drug metabolism and response, while pharmacogenomics is the general study of the many different genes that determine drug behavior. Phrmacogenomics try to answer the genetic component of the drug dosing variability among individuals (the other component is environmental and its discussion is beyond this paper). The genes control the effect of drugs by controlling the drug metabolizing enzymes expression, which in turn plays a major role in the inter-individual variability in drug response. The drug metabolizing enzymes work on 2 phases of drug metabolism: phase I which includes oxidation, reduction and hydroxylation of drugs (occurs in Cytochrome P450). Phase II includes conjugation reactions (thiopurine methyltransferase TPMT, n-acetyltransferase (NAT)[22]. The main clinical problem occurs with patients who are slow metabolizers for certain drugs (have SNPs that cause certain drug metabolizing enzyme to work slow), as they may end with drug toxicity and lot of drug side effects while they are just taking the standard dosage of this drug. Three of the SNPs analyzed in this study act through phase I of drug metabolism: CYP2D6, CYP2C9, and CYP2C19; while one SNP analyzed works on phase II of drug metabolism is NAT2.

CYP2D6 is involved in metabolism of 20% of known medicines. CYP2D6 slow metabolizers in this study

were found to be 0.6%. The significance of this result in comparison to results of other studies concerning different population is shown in table 7:

Table 7. Comparison of CYP2D6 Slow Metabolizers Frequency in Multiple Populations

| Population studied | CYP2D6 Slow Metabolizers |
|---|---|
| Middle Eastern Population (Retrieve Center Data) | 0.6% |
| U.S.A.[23] | 7.7% |
| British[24] | 8.9% |
| Turkish[25] | 1.5% |
| West Africans[26] | 2.5% |
| Japanese/Chinese[27] | 0% |
| Saudi Arabian[28] | 1-2% |
| Caucasians[29] | 5-10% |
| South East Asians[30] | 1-2% |

The percentage of slow metabolizers in our study is consistent with findings of similar populations in Middle East (Saudi Arabia and Turkey) and is in general low in comparison to other populations of the world, mainly American and European. This minimizes possibilities of drug toxicities especially with certain drugs as morphine derivatives, tamoxifen, SSRIs, antipsychotics and antiarrythmics.

CYP2C9 is very important as it has an important role in controlling metabolism of Warfarin, among other drugs, because warfarin is very well known for its narrow therapeutic range and serious side effects upon high dosing. The percentage of slow metabolizers in comparison to other populations is shown in table 8.

Table 8. Comparison of CYP2C9 Slow and Intermediate Metabolizers Frequency in Multiple Populations

| Populations studied | CYP2C9 Intermediate Metabolizers | CYP2C9 Slow Metabolizers |
|---|---|---|
| Middle Eastern Population (Retrieve Center Data) | 20% | 18.1% |
| Caucasians[31] | 12% | 8.3% |
| Chinese[29] | 0% | 3.3% |
| Africans[32] | | 1.25% |

The comparison shows clearly that the percentage of CYP2C9 slow metabolizers in our study are significantly more common than in studies on other populations. This necessitates further larger studies, which in case had confirmatory results, would recommend starting at lower doses of warfarin on people from Middle East area.

CYP2C19 is of major importance, as among its metabolized drugs is Clopidogrel. Clopidogrel (Plavix) is a major drug used to treat cardiac patient post myocardial infarction and post PTCA. Two big studies showed that certain SNP variations in CYP2C19 makes it intermediate metabolizer of Clopidogrel and render this drug ineffective in the clinical outcome.' Many of the cardiac patients taking this medicine will not benefit from it and will end with high morbidity and mortality rates. The results of Retrieve study and comparison with other studies' findings are shown in table 9.

Table 9. Comparison of CYP2C19 Slow and Intermediate Metabolizers Frequency in Multiple Populations

| Populations studied | CYP2C19 Intermediate Metabolizers | CYP2C19 Slow Metabolizers |
|---|---|---|
| Middle Eastern Population (Retrieve Center Data) | 21.3% | 0% |
| Caucasians[29] | 18% | 0-2% |
| East Asian[35]s | 28% | 6% |
| Africans[36] | 20% | 0.3% |

Other study also showed that The CYP2C19*2 polymorphism occurs in 30% of individuals of European ancestry, 40% of individuals of African ancestry, and 50% of individuals of Asian ancestry.[32] The result of Retrieve study put the Middle Eastern population in line with the other populations in the high percentage of potential patient who would not benefit from Clopidogrel, and which would necessitate more awareness of this issue especially among physicians, namely cardiologists.

NAT2 polymorphism analysis has been historically done prior to other SNPs involved in pharmacogenomics because of its potential relationship with increased predisposition to multiple cancers due to its vital role in phase II detoxification. Slow metabolizer polymorphism have been found in generally higher in percentage among Caucasians and Central European ancestors in comparison to east Asians mainly. The results of this study shows that Middle Eastern population probably belongs to the group with high percentage of slow metabolizers also as shown in table 10. This calls for major attention by doctors regarding advice for cancer prevention behaviors as well as starting by low dosage medicines such as amiodarone or isoniazide among others to minimize potential toxicities and side effects.

Table 10. Comparison of NAT2 Slow Metabolizers Frequency in Multiple Populations

| Populations studied | NAT2 Slow Metabolizers |
|---|---|
| Middle Eastern Population (Retrieve Center Data) | 56.3% |
| U.S.A.[37,38] | 40-70% |
| Japanese/Chinese[14] | 10-30% |
| Caucasians[39] | 40-60% |
| South Indians[40] | 74% |
| Egyptians[41,42] | 60% |
| Emirates[43] | 82% |

## Conclusion

These data call for international efforts to make some drug dosage criteria that is pertinent to different ethnicities especially for those drugs that carry serious side effects and with minimal therapeutic range or that have critical role in patient treatment outcome. As an example, the genomic variation effect and its potential harmful results on Clopidogrel use for coronary disease indications mandates a quick research to find an easy, cheap, accessible test to identify patients with CYP2C19*2 polymorphisms that alter the metabolism and thus the desired effects of this drug. Another example would be further investigations on CYP29 slow metabolizers percentage in certain populations (as Middle Eastern population), and accordingly state recommendations regarding initial dosing of warfarin to be lower in populations that have more prevalence of CYP2C9 slow metabolizers. The clinical genomics could make a new regimen of dosing for several important medicines per

ethnicity to minimize as much as possible toxicities, side effects, undesired outcomes, or even non-benefit from the treatments. Of course, the individualized treatments, which is the ultimate goal of genomic testing will always be the ideal solution for the problems of medicine dosing to attain best outcome with minimal side effects.

Furthermore, and apart from pharmacogenomics, the study suggest possible interactions and trends between certain polymorphisms prevalence in certain ethnicities (GSTT1, GSTM1, and NAT2) and some cancers that are more prevalent in these ethnicities. Of course, the picture is more complicated but these findings could be of benefit in the process of understanding the complexity of cancer and its pathogenesis and demographic distribution. The genomics and its clinical applications is a huge new branch of medicine that has the potential to change clinical practice and give it a new perspective. Research is continuing, and data needs to be obtained from different populations all over the world. Middle East area in particular is still lagging in these studies and data collection, and I hope this study will be of value for future more extensive studies.

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# Effect of smoking on aging: extrapulmonary pathology

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## Introduction

Smoking is a chronic recurrent and addictive disease that affects 28% of the population. The age group with the highest overall prevalence is between 35-44 years. Aging is a progressive and universal process during which there are a series of morphological and physiological changes that occur as a result of the action passing of time on life, representing a decrease of resilience in each of the organs , apparatus and systems, as well as at the ability to respond to harmful agents that affect the individual. The combustion of the tobacco produces numerous oxidizing substances that may accelerate the aging process. One in every 6 deaths is produced as a direct result of exposure to tobacco smoke. There are numerous works in the scientific literature where the relationship of tobacco with the onset and aggravation of pulmonary pathology is documented,... but this is not the goal of this article.

## Aging and oxidative stress theory

Ageing is caused by the influence of the environment and the adaptive response by the body. The reactions caused by free radicals leads to regression of cellular and extracellular structures[1]. The consumption of oxygen as evolution of body's degree of activity influences the deleterious effects of oxidation the magnitude of wich depends on the regulatory capacity of the antioxidant enzyme system[2].

Aerobic cells are in continuous production of oxidants or ROS (reactive oxygen species). The most important are hydroxyl radical, superoxide radical and hydrogen peroxide. Most production occurs in the mitochondrial respiratory chain. The ageing rate would occur, therefore, depending on the intensity of mitochondrial ROS generation[3].

## Cardiovascular pathology

Epidemiological studies suggest that the ageing process itself increases the risk of developing hypertension, atherosclerosis and coronary artery disease and that along with the harmful consequences of smoking, would lead to increased morbidity and mortality from cardiovascular causes.

Cigarettes contain soluble components pro-oxidants (quinones, acrolein, aldehydes etc.), resulting in an increased intracellular ROS. These components are capable of activating leukocytes and platelets, that reach the systemic circulation and contribute to oxidative stress in the vascular bed. The whole of this inflammatory process justifies more rapid cardiovascular aging in smokers at the expense of

producing a generalized endothelial dysfunction[4]. Smoking can also promote endothelial dysfunction induced by hypercholesterolemia[5].

Likewise, it is believed that these components are capable of damaging the DNA of fibroblasts and endothelial cells through oxidative reactions, which reinforces the hypothesis that smoking may cause injury of the DNA of the lung and other tissues as well as promote carcinogenesis and other cardiovascular disorders[8].

It is well known that smokers have a 20% greater rate of progression of atherosclerosis compared to patients without exposure to environmental smoke or passive smokers. Cigarette smoke can also cause inflammation because it induces the production of proinflammatory cytokines such as TNF-α, IL-6, IL-1β. These play a key role in the process underlying the formation of the atheroma plaque. Furthermore, cigarette smoking activates the sympathetic nervous system leading to an increased heart rate and blood pressure. Similarly, cutaneous and coronary vasoconstriction is triggered, not only increasing the risk of atherosclerosis but also favouring the occurrence of ischemic heart disease.

It is known that nicotine is not directly related to the process of atherosclerosis but increases cardiac expense, heart rate and blood pressure, wich can lead directly to acute endothelial dysfunction in smokers[7].

### Diabetes and obesity

As we have seen previously, smoking provokes oxidative stress, causes inflammation and reduces blood flow to the muscles, progressively contributing to the development of insensitivity to insulin and therefore to the onset of Diabetes Type-II[8].

There is a risk of getting directly proportional, long-term diabetes mellitus type-II on a larger index of year age / pack of cigarettes. This may be due in part to the effect of nicotine on secretion and insulin sensitivity. It is known that nicotine increases the serum concentration of catecholamines. These alter the effect of insulin and can induce insensitivity to it with the resulting increase in blood glucose. There are documented reports that, at the cellular level, catecholamines alter the pathways that are related to the production of insulin as well as, the activity and synthesis of proteins that deliver glucose to the cells[9].

Furthermore, nicotine is capable of activating a surface receptor of lipolytic cells located in the adipose tissue, inducing lipolysis and an increase in free fatty acid levels in blood. Consequently, the chronic increase in levels of fatty acids would adversely affect the secretion and sensitivity to insulin via its direct effect on the muscles, liver and pancreas.

It has also been suggested that chronic smoking may have a direct impact on the distribution of body fat. Several studies support this hypothesis, showing that chronic smokers have a dysfunction in the hypothalamus, an area associated with weight gain and obesity. The accumulation of fat in the abdominal region is associated with increased risk of developing insulin insensitivity or alteration of glucose tolerance[10].

Similarly, it has been shown, that there is an increased risk of developing type 2 diabetes shortly after topping tobacco consumption, perhaps due in part to weight increase. However, quitting

smoking reduces the risk of diabetes after some years of abstinence[11].

### Dementia

Advanced age is the main risk factor for the onset of dementia, mainly Alzheimer's disease. Data on the impact of smoking on the central nervous system are contradictory.

Several prospective studies have suggested that smoking in middle age and old age is associated with an increased risk of dementias, however, there is a study that suggests that nicotine improves cognitive performance in the short term and reduces the formation of amyloid[12]. A meta-analysis of 19 studies with a minimum of 12 months follow-up concluded that elderly smokers have an increased risk of Alzheimer's disease, vascular dementia and other types of dementia, with greater annual declines in the scores of the Mini-Mental Status Examination (MMSE)[13].

ApoE apolipoprotein e4 may explain, at least in part, these conflicting results; it is also involved in the development of atherosclerosis, Alzheimer's disease and inadequate cognitive development. In two population cohorts which did not have this gene, smoking was associated with decreased memory, whether Alzheimer's disease was present or not. Furthermore, Alzheimer's patients who are ApoE e4 carriers, have fewer nicotinic receptors, which further suggests that it could be a direct biological change of the effects of tobacco consumption by ApoE e4[14].

### Genitourinary system

Aging increases the risk of incontinence and urinary tract infection, erectile dysfunction and dyspareunia. There are studies that link smoking with erectile dysfunction, being more likely to be present as much tobacco consumption increases[15].

Smoking is an independent risk factor even in the absence of comorbidities that may justify erectile dysfunction such as hypertension, diabetes, neuropathy, etc...

### Visual system

Maculopathy is one of the major causes of blindness in people over 65. Smoking is related to the appearance of large soft drusen, which are predictors of age-related maculopathy. The hypothesis of its pathogenesis is the disruption of blood flow in the choroidal arteries due to the effect of nicotine on these. Studies in both men and women, related smoking with an increased risk of developing maculopathy, which rises to highest rate of year(age) / pack of cigarettes. [16],[17].

### Osteoporosis

As we have seen, smoking causes inflammation. It is in this environment, where healthy elderly, have loss of bone substance in both the cortical (peripheral skeleton) and the axial skeleton (trabecular substance). There is also a progressive decrease in the number of osteoblasts and osteoclasts although their activity does not change with age.

Cigarette smoking accelerates bone loss. Smoking a pack a day during adulthood was associated with a reduction of 5-10% in bone density. In addition, different meta-analyses have shown that smoking is associated with an increased risk of fracture. This also increased in people with an early history of smoking, yet remains greater for active smokers[18]. Smoking, too, may override the beneficial effect of oestrogen therapy in postmenopausal women[19].

### Skin

During aging, the skin has a lower repair capacity. Skin atrophy occurs with loss of elasticity, progressive dryness and the appearance of staining. Women, by nature of their skin, are more likely to form wrinkles earlier than men[20]. The epidermal layer becomes thinner and the dermal-epidermal junction is flattened, producing a much more fragile skin. This decreases the area available to transfer nutrients and the amount of protective lipids of the stratum corneum, inducing a dry skin (xerosis).

Smoking is presumed to be an etiological factor of certain skin diseases such as lupus erythematosus, psoriasis, palmoplantar pustulosis, squamous cell carcinoma, hidradenitis suppurativa and the genital warts.

The aging of the skin subordinate to smoking affects both men and women. Changes are evident from as early as 35 years of age. Free radicals produced by tobacco smoke have deleterious effects on inflammatory cell function, attenuating phagocytosis and its bactericidal effect. Similarly, it causes increased proteolytic enzymes such as elastases[21], decreased collagen synthesis and its deposition in the extracellular matrix[22].

Nicotine cigarettes cause a reduction of the blood supply that leads to a chronic decrease in oxygenation and dehydration of the skin, thus favouring the apparence of wrinkles and paler complexion.

The appearance of wrinkles is directly proportional to quantity of habit of cigarettes in the habit of smoking. It is unknown whether the process is reversible after a patient stops smoking. It appears that tobacco smoke reduces the amount of vitamin A in the skin, which has a protective role against free radicals. Other authors believe that wrinkles could also be due to the effects of snuff on the cutaneous microcirculation, cicatrisation, oxidative phenomena, increase of serum concentrations of retinol and the proteolytic activity due to the effect of matrix metalloproteinases.

Macroscopic changes in the skin that give rise to a characteristic phenotype, "smoker's face"[23], have been described ; these consist of: plethoric skin, either pink or orange, prominence of bony prominences, particularly the cheeks, rough skin, with a greyish tint, deep lines at the lower jaw and cheek, fine wrinkles on the upper lip and eyelid corners ("crow's feet"), and appearance of pimples, nodules and grooves in the periorbital area (Favre-Racouchot Syndrome). On the other hand, is well known that nicotine can leave a yellowish tint impregnation on the thumb nail, beard and hair, and accelerate the fall of these.

### Oral cavity, taste and smell

Smokers, as well as patient with neoplastic processes which we'll discuss later, usually have gingival melanosis and show an increased risk of gingivitis, lingual leukoplakia, gingivostomatitis and lichen planus. Tobacco is likewise responsible for the appearance of keratosis "tablet" generally in the lower lip, but can also appear at the top. Tars and nicotine, will blacken teeth and induce dental mobility and early tooth loss. The senses of taste and smell also diminish with age. Studies show that smoking, at present, increases the risk of impaired olfactory function, but does not seem to increase the risk of gustatory dysfunction. Only heavy smokers of 20 or more cigarettes a day may be in a position of increased risk of deterioration in both senses. [24].

**Smoking and cancer**

The mere fact of aging is the first risk factor for morbidity and mortality for malignant tumours. About 60% of all tumour processes occur in people over the age of 65. Cancer is, in this age group, the second leading cause of death after cardiovascular disease. Smoking is, in the elderly, is a major cause of mortality especially for lung and bladder cancer, these being the first and fourth leading cause of cancer death, respectively, in elderly men. Cigarette smoke contains many substances with mutagenic and carcinogenic characteristics.

The risk of cancer of any type descends in ex-smokers, however, this risk remains higher than for non- smokers even 20 years after they gave up smoking; it has long been recognized that the risk is quite close to that of the non-smoker. The pattern of this decline is almost inappreciable in the first five years, and there is a gradual but slow fall thereafter.

There are several theories that relate the ageing process with the development of neoplastic disease. Simply living longer would be associated with a prolonged exposure to various carcinogenic agents. The genes described in the carcinogenesis of tobacco are c-ras and c-myc and tumour suppressor genes p53 and rb. Amplification of proto-oncogenes and loss of tumor suppressor genes can cause the development of neoplastic disease. Furthermore, the onset of ageing and increased free radicals impairs the ability to repair our genetic material besides mitochondrial DNA damage. These alterations are transmitted in the process of cell replication and thereby induce the appearance of tumours[25].

Smoking and skin cancer. Tobacco is associated with the appearance of squamous cell carcinoma. However, cigarette smoke is not a causative factor for melanoma, although it is known that smokers have a lower survival rate and an increased risk of metastasis in this tumour.

Smoking and head and neck cancer. The most common tumour in this area is squamous cell carcinoma. The risk of occurrence relates to the dose and time of exposure. There also appears to be an increased risk in non-smokers who have suffered an intense passive exposure to it, especially women[26]. It is estimated that the concomitant abuse of tobacco and alcohol would be responsible for up to 80% of cases of squamous cell carcinoma in the head and neck. Epidemiological studies suggest that tobacco consumption either through smoking cigarettes or smoking a pipe is associated with an increased incidence of head and neck cancer. However, tobacco for chewing and snuff (snuff tobacco prepared to consume nasally) present an increased risk of cancer of the oral cavity and pharynx. The permanence of snuff in the oral cavity produces dysplasia and leukoplakia. In ten years, between 1-20% of these precancerous lesions progress towards squamous cell carcinoma[27]. In addition, tobacco smoke is an etiological factor for the development of tumours of the nasal cavity. These are two times more common in men, with an age of onset between 60 and 65 years compared to women. Studies examining risk factors have generally combined nasal cavity cancer with tumours arising in the paranasal bosoms.[28].

Smoking and cancer of the digestive tract. Tobacco is related to the occurrence of neoplasms in the oesophagus, stomach, colon and pancreas. There is a direct relationship between the smoke of the tobacco and squamous carcinoma of the oesophagus. It seems that pipe smokers have a lower risk of suffer it. Tobacco and alcohol can

increase risk synergistically[29]. Likewise, there is also an increased risk of developing adenocarcinoma in smokers, especially in those associated with Barrett's oesophagus[30]. Approximately 18 percent of gastric cancer cases were attributed to the consumption of snuff[31]. The risk of colon cancer increases with age. In addition to prolonged exposure to potential carcinogens such as tobacco, ageing is associated with increased proliferation and decrease of apoptosis in the colonic mucosa. Smoking has been associated with an increased incidence and mortality of colorectal cancer. In terms of incidence and mortality, the association with tobacco is stronger for rectal cancer than for colon cancer. Smoking is also a risk factor for virtually all types of colonic polyps. Similarly, smoking increases the risk of colorectal cancer in Lynch syndrome patients[32]. Cohort and case-control studies have shown an increased risk of developing pancreatic adenocarcinoma among smokers. This increases to more cigarettes[33].

Smoking and cancer of the cervix. Smoking and HPV infection have synergistic effects on the development of carcinoma in situ and cervical cancer, especially in heavy smokers. The degradation products of cigarette smoke are concentrated in the cervical mucus, which can induce cellular changes in the epithelium and decrease local immunity, allowing persistence of HPV[34].

Smoking and cancer of the urinary tract. There are over 60 existing substances in cigarette smoke which may be responsible for the appearance of transitional cell carcinoma in both men and women and, particulary, in those who smoke a pipe or cigars. The risk of this tumour is somewhat lower in those who smoke cigarettes. Some studies have shown that snuff produces alterations in the p53 gene[35]. Passive smoking also appears to be a risk factor for developing bladder cancer in women[36]. Smoking is associated with an increased risk of renal cell carcinoma, proportional to the magnitude of exposure. If this is intense, the presentation of the tumour tends to be more serious[37].

Smoking and breast cancer. The scientific evidence supports an association between active and passive smoking and an increased risk of breast cancer, especially in premenopausal women. The relationship between smoking and breast cancer has been interfered by the interaction of the consumption of tobacco with alcohol as well as with hormonal endogenous influences[38].

## Conclusion

There is ample evidence that the smoke of the tobacco, through oxidative reactions, causes a systemic inflammation responsible for accelerating the normal ageing process. In the specific case of the elderly, who have spent decades smoking, quitting this harmful habit can prevent or reduce the risk of various diseases like heart disease, cancer and respiratory diseases. The stop smoking at any age, produces significant benefits in the health of patients It is our role as health professionals to insist on patients giving up smoking in order to increase their life expectancy and quality.

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# Potential use of adipose tissue stem cells in the control of aging

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## Abstract

Cell therapy with adult stem cells is a new battle front for the control of aging. Before being used for this purpose, we need to answer several basic questions about the biochemistry and physiology of these cells. This paper presents some aspects and preliminary results obtained in our laboratory using stem cells from adipose tissue.

## Introduction

Various strategies are used to control the effects of aging on the normal functions of an organism. Antioxidants, diet, exercise and even activating enzymes of phase II detoxification appear to delay the decay function to a greater or lesser extent. However, none of these strategies prevent biological aging. Hence, to control the effects of aging it will be necessary to emphasize the need to explore the mechanisms of tissue repair and regeneration as a complementary methodology to other prevention strategies. In this sense, Regenerative Medicine may represent a fascinating alternative to control the aging process.

One of the aspects of Regenerative Medicine is based on the regenerative capacity of adult stem cells are in most tissues and contribute to this homeostasis and repair. After tissue damage several intracellular and intercellular pathways are activated in a coordinated attempt to restore the tissue integrity. With aging occurs general decline in the regeneration potential. Besides this loss of regenerative capacity is produced by alterations of the stem cells with aging, it is thought that stem cells transplanted into damaged or aged tissues may have therapeutic and restorative capacity. In fact, stem cells represent a huge promise in the therapy of many aging-related degenerative disorders aging some diseases such diabetes, heart disease, stroke, Parkinson, etc [1-4]

### Stem cells

Stem cells have the ability to self-renew through symmetric divisions and the potential of differentiating into several different cell types depending on their degree of multipotentiality through asymmetric division [1-3,5-8]. It is now known that most of the tissues have a very specific population of adult stem cells that allow their regular renewal or regeneration [6,8]. Thus, it has been described the existence of these SC in tissues such as muscle, skin, liver, pancreas, brain [9], intestines, fatty tissue [10,11].

In higher animals, stem cells have been classified into two groups: embryonic stem cells and organ-specific stem cells or stem cells from adult tissue. The latter are able to originate cells of a particular organ in the adult. Our work focuses on adult stem cells. The activity of these stem cells varies greatly from one organ to another: those of the bone marrow that form blood cells are very active and are continually dividing, while those which are, for example, in the small intestine are more inactive. Some adult stem cells are capable of differentiating into more than one cell type as mesenchymal stem cells.

### Adipose tissue mesenchymal stem cells (ADSC)

Within the adult stem cell group are the mesenchymal stem cells (MSC). The MSC belong to the mesenchyma, which by a differentiation process will lead to the blood vessels, smooth muscle, mesothelium, lymphatic system and connective tissue itself. These cells can be obtained from different organs including fetal liver, umbilical cord, and bone marrow [12,13]. Another important source is adipose tissue, which contains progenitor cells called adipose tissue stem cells (ADSC) [2,10,14,15].

### Advantage of ADSC

The easy access to the subcutaneous fat by liposuction, allows ADSC to be obtained under local anesthesia and with minimal discomfort to the patient. In addition, its high abundance with not ethical problems associated with its use, make adipose tissue an important source of MSC [16-18]. Another advantage is that the proportion of MSC is 500 times higher [19,20] in adipose tissue than in the bone marrow, so that a large number of cells can be obtained without a large number of passes, decreasing the risk of chromosomal abnormalities induced senescence in cultures [21]. An additional advantage is its potential to differentiate into bone, cartilage, tendons, skeletal muscle, fat, endothelial tissue and macrophages when grown under specific conditions of each lineage [11,13,22]. Surprisingly, the ADSC not only have the potential to differentiate into cells of mesodermal origin and organs, but also they have the ability to differentiate into neurons, endocrine cells of the pancreas, hepatocytes, endothelial cells and cardiomyocytes [10].

ADSCs can repair and regenerate the tissues by several mechanisms: First, the ADSC transplanted into a damaged or diseased tissue can secrete cytokines and growth factors that stimulate the recovery in a paracrine manner. The ADSC could modulate the host stem cell niche by stimulating the recruitment of endogenous stem cells to a particular site and promote their differentiation into the required lineage. Also, ADSC can provide antioxidants so that toxic substances released into

the local environment are eliminated, thereby promoting the recovery of the surviving cells. Another mechanism is thought their in vitro differentiation into the desired line, prior to their autologous transplantation [2]. They can also act as immune modulators [23].

Today it is possible to isolate and culture adult stem cells for therapeutic use. Once transplanted, these cells can be used to rejuvenate damaged tissue by aging or other causes. However, no one knows for sure the consequences of the stem cells treatments because there are many basic questions about the biology of these CM that must be answered before its therapeutic use. Obviously, these cells have to survive and perform their function in unfavourable conditions as the damaged tissue microenvironment, where many factors needed for stem cells are lacking. Under these adverse conditions, the survival of the stem cells will depend on his "molecular health" and its responsiveness when implanted in the tissue. Therefore, stem cells must have a robust repair mechanisms and resistance in order to participate in tissue regeneration. It is likely that this molecular health and stress response depends on many biochemical and physiological aspects related to donor age, lifestyle, etc. As these cells are continuously receiving "on-off" signals to divide, it could be that old cells are less sensitive to these signals. Consequently, the therapeutic applications of stem cells in adult tissue repair requires a better understanding of the biology of these cells, of the environment of the damaged tissue or both.

The work we are carrying out in our laboratory try to answer several of these basic questions about ADSC.

First of all, we focused on the isolation methods. These methods are based on magnetic cell sorting (Miltenyi Biotech), where magnetic microparticles linked to antibodies recognize surface antigen in the stem cells. The separation is performed on a column inserted in an extremely powerful magnet to retain the labeled cells. This methodology allows us to build a cell bank of patients of different ages that meet the criteria of "Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy."

The first criteria is that cells must be adherent to the plastic. The photographs of Figure 1 show that the human ADSC are adherent to the culture flask without addition of any substrate.

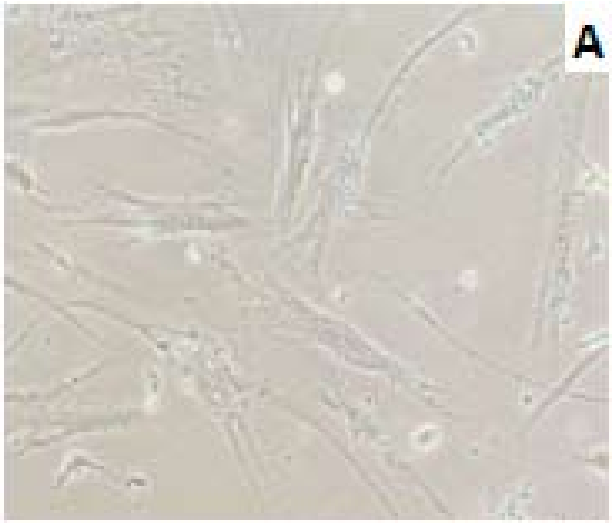
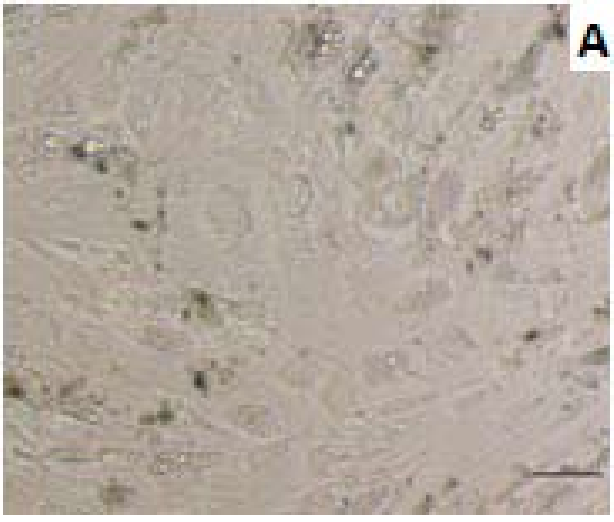
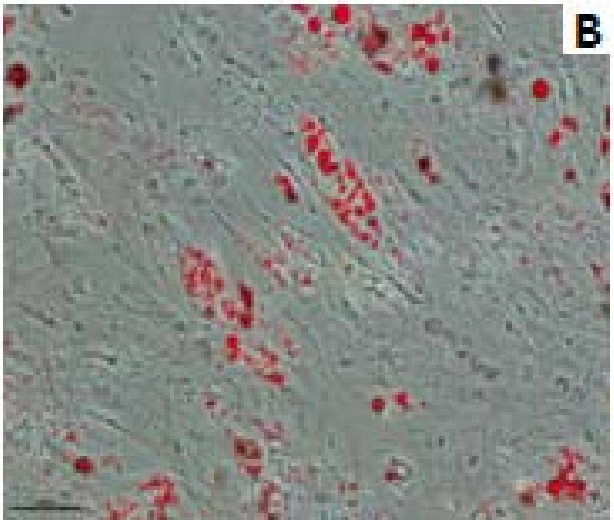



Figure 1. Human ADSC (42 yrs) in culture flask. 10x magnification.

A prerequisite for considering the cultured ADSC as MSC, is to demonstrate that they have the potential to differentiate into at least two different cell types [11,13,22,24]. For this, cells were cultured in specific differentiating media. Figure 2 shows photographs taken before and after

differentiation into adipocytes, where it can be observed fusiform cells containing lipid vacuoles. The red staining of these vacuoles (Fig. 2 and B) shows the differentiation into adipocytes ADSCs. Furthermore, the presence of black-purple shown by the presence of alkaline phosphatase in osteoblasts generated by ADSC differentiation (Fig. 2C)



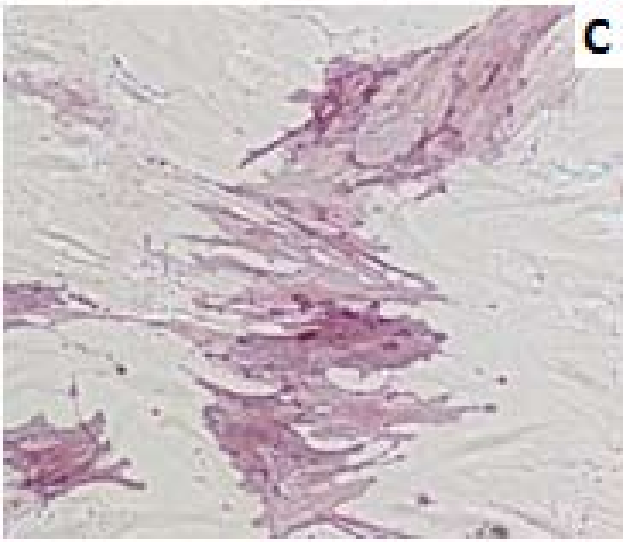
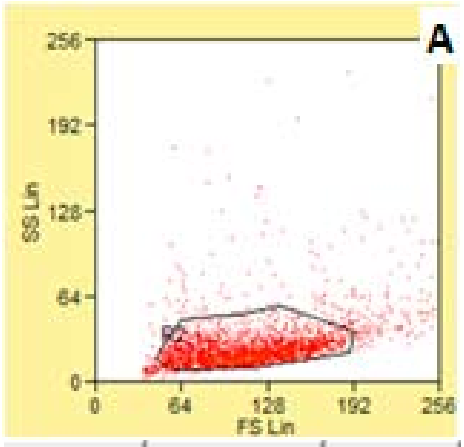



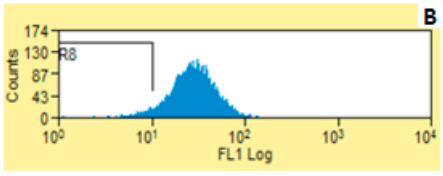

Figure 2. Differentiation of human ADSC into adipocytes and osteoblasts. A) Picture of human adipocytes obtained from ADSC (patient of 37 years) prior to staining, 10X magnification. B) Photograph after fixation and staining process. C) Photograph of osteoblasts after fixing and staining 20X magnification.

In addition to the ability of adherence to plastic and differentiation, the results provided by flow cytometry serve to confirm the nature of the cells obtained. Figure 3 show the results obtained from a cell population of human ADSC. These graphs represents side scatter (Side Scatter, SS), which gives information about the existence of different cell populations. The forward scatter (forward scatter, FS), gives an insight on cell size. As can be seen, the sample contains a unique cell population, reflected in the graph as a single cloud.

To verify that the obtained cells meet the minimum standards required by the committee of mesenchymal stem cells and tissues of the international cell therapy, cells must have the surface markers CD73, CD90 and CD105. On the contrary, they should not contain CD14, CD34, CD45, CD19, CD79$\alpha$ and human leukocyte antigen (HLA)-DR.




| Region | Count | % Hist |
|---|---|---|
| Total | 4139 | 100.00 |
| R2 | 3690 | 89.15 |




| Region | Count | % Hist |
|---|---|---|
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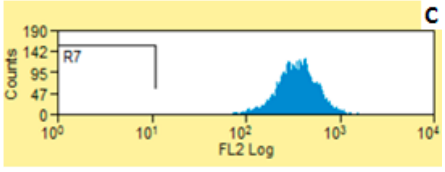
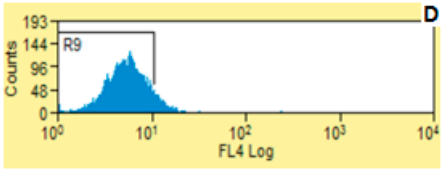
| R8 | 202 | 5.47 |




| Region | Count | % Hist |
|---|---|---|
| Total | 3690 | 100.00 |
| R7 | 13 | 0.35 |




| Region | Count | % Hist |
|---|---|---|
| Total | 3690 | 100.00 |
| R9 | 3323 | 90.05 |

Figure 3. Study of the degree of cellular homogeneity and phenotyping of human ADSC. Data were obtained from the flow cytometer FC-500 (Beckman-Coulter). The results of positive events to the fluorophore are in %.

The histograms represent the number of events counted on each exposed channel. In our case, the channel FL1, FL2 and FL4 are set to excite the fluorophores FITC, PE, PerCP, respectively. Considering the antibodies used, (CD14-PerCP, CD20-PerCP, CD34-PerCP, CD45-PerCP, CD105-FITC and CD90-PE), FL1 channel gives us information on the number of cells expressing CD90 marker (Fig. 3B); FL2 channel gives information about the proportion of CD105-expressing cells (Fig. 5C) and FL4 channel indicates the percentage of positive cells is any of the following markers: CD14, CD20, CD34 and CD45 (Fig. 3D). The first histogram (Fig. 3B) indicates that 94.53% of the sample is positive for the CD90 marker. The second histogram (Fig. 3C) shows that almost 100% of the sample expressed the marker CD105. Only 9.5% of the cells have one of the following markers: CD14, CD20, CD34, CD45.

Finally, we have used a method for labeling and tracking that allow us to monitor stem cells once they are injected in vivo. This method is based in transfecting the ADSC with the luciferase gene by using lentivirus. After intraperitoneal injection of luciferin, cells carrying the luciferase gen can be observed by using an in vivo imaging system. Figure 4A shows a culture of transfected ADSC in a 25 cm 2 flask prior to administration of 3 luciferin used as a control of bioluminescence. Figure 4B shows the same culture after the administration of D-luciferin.

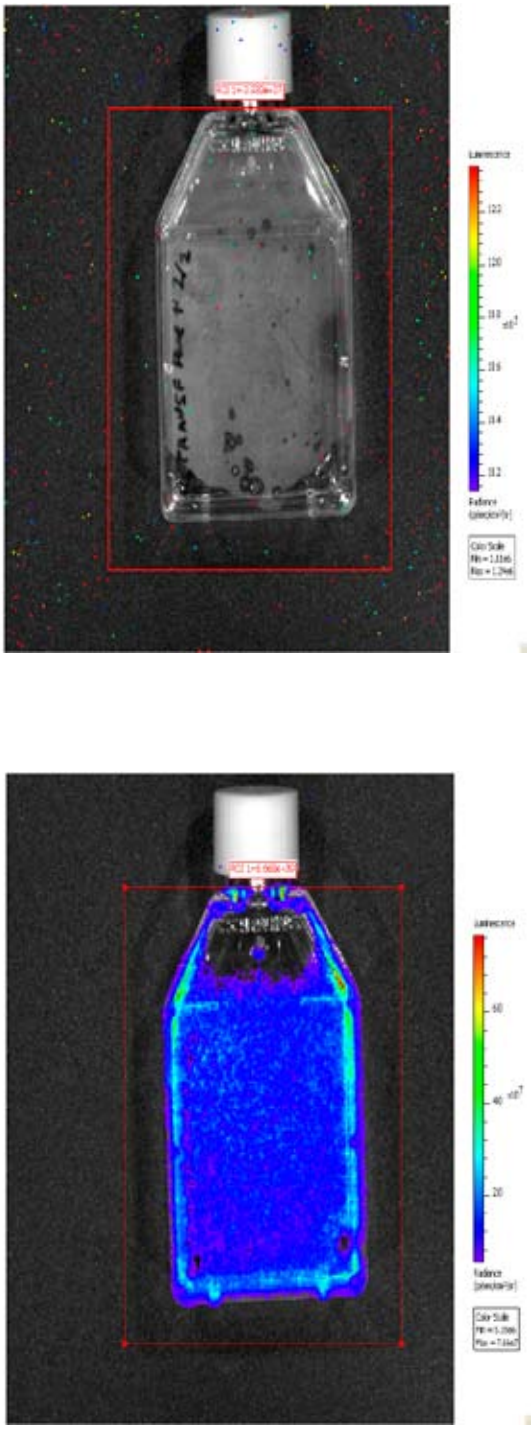


Figure 4. ADSC cells transfected with lentivirus containing the luciferase gene. A) vial containing the cells prior to the addition of luciferin. B) Same bottle treated with luciferin.

## Conclusion

In summary, we can say that cell therapy open a new battle front in the control and treatment of aging related diseases. However, there are many basic questions about the biology of these stem cells that should be answered to predict the therapeutic potential of these cells, some of which are going to be known after the completion of the the present study because. The main objective of this work is to identify the factors affecting the "robustness" of the biochemistry and physiology of ADSC.

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# Sociedad Española de Medicina Antienvejecimiento y Longevidad

El objetivo y finalidad de la Sociedad es fomentar y llevar a cabo, en interés público y sin ánimo de lucro, la Medicina Antienvejecimiento (Anti-Aging) como procedimiento terapéutico, procurando la cooperación y la unión de especialidades médicas y de todos los profesionales de la salud, farmacéuticos, psicólogos, biológos, odontólogos, etc..., que por su actividades y dedicaciones, manifiestan expresamente su interés en la Medicina Antienvejecimiento, que básicamente es un sistema integral preventivo y curativo, que a partir del estudio del envejecimiento natural, descarta los factores perjudiciales que producen un envejecimiento prematuro, proponiéndose un sistema de vida de promoción de la salud, aplicando técnicas correctoras de los signos estéticos y orgánicos de decaimiento corporal.

Para cumplir estos objetivos, vamos a proporcionar a los miembros, la información necesaria sobre la práctica y avances de las técnicas que nos ocupan, mediante congresos, symposium, cursos y actos (siempre en unas condiciones especiales para sus miembros), además recibiras la Approaches to Aging Control, órgano oficial de la S.E.M.A.L.

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## SALA ESTÉTICA — SÁBADO 6 de Octubre de 2012

## MEDICINA DEL CABELLO

**Moderadores: Dr. Ramón Vila-rovira - Dr. Fabio Rinaldi**

9:00 "Láser de baja potencia en alopecias"
Dr. Mariano Vélez

9:20 "Tratamientos no quirúrgicos de la caída del cabello"
Dr. Luis Berrocal

9:40 "Revisión de los tratamientos en función de las diferentes enfermedades de pelo"
Dra. Roge Navarro

10:00 "F.U.E, nuestra técnica"
Dra. Begoña Barros

10:20 "Posibilidades terapeúticas en patología del cabello: desde los factores de crecimiento hasta el latanoprost"
Dr. Fabio Rinaldi

10:40 "Terapias capilares, mis tratamientos"
Dra. Elizete Nikoluk Kaffer

**11:00 PAUSA CAFÉ Y VISITA A LA EXPOSICIÓN COMERCIAL**

## DERMATOLOGÍA ESTÉTICA Y LÁSER

**Moderadores: Prof. Joaquín Calap - Dr. Pedro Jaén**

11:30 "Rejuvenecer con naturalidad. Resurfacing no ablativo fraccional en 3D"
Dr. José Luis Cisneros Vela

11:50 "Optimización de aparatos en la consulta"
Dr. Daniel Brualla

12:10 "Rejuvenecimiento con nuevos láseres no ablativo de 1064 microsegundos y sus combinaciones"
Dr. Rubén Del Río

12:30 "Terapia fotodinamica en el envejecimiento cutáneo"
Dr. Pedro Jaén

12:50 "Lipoplastia con láser"
Dr. Fernando Carvalho

13:10 "Rejuvenecimiento facial con radiofrecuencia fraccionada e Infrarrojos. Un aporte novedoso"
Dr. César Arroyo

13:30 "Rejuvenecimiento integral con láser de CO2 fraccionado y otras indicaciones"
Dra. Montserrat Planas

13:50 DISCUSIÓN

**14:00 DEGUSTACIÓN DE JAMÓN IBERICO POR CORTESIA DE D.O. HUELVA Y CERVEZA POR CORTESÍA DEL C.I.C.S**

**14:30 ASAMBLEA GENERAL DE MIEMBROS DE SEMAL 1ª Convocatoria. 15:00 2ª convocatoria.**

## CIRUGÍA ESTÉTICA

**Moderadores: Prof. Jose Mª Serra Renom - Dr. Angelo Rebelo**

16:00 "Cirugía estética genital y envejecimiento"
Dr. Esteban Sarmentero

16:20 "Rejuvenecimiento periorbitario"
Dr. Jesús Benito Ruiz

16:40 "Otoplastia, mi técnica"
Dr. Angelo Rebelo

17:00 "Exorinoplastia y smash extendido en lift facial"
Dr. Juan Santamaría

17:20 "Envejecimiento del pecho femenino , como rejuvenecerlo"
Dr. Julio Millán

17:40 "Células madre mesenquimales y factores de crecimiento en el enriquecimiento de los injertos grasos: evaluación in vivo de las dos técnicas"
Dr. Joan Fontdevila

**18:00 PAUSA CAFÉ Y VISITA A LA EXPOSICIÓN COMERCIAL**

## CIRUGÍA ESTÉTICA

**Moderadores: Dr. Jorge Planas - Dr. Jaume Masià**

18:30 "Manejo del dorso nasal con injertos en punta y bioplastia en radix"
Dr. Jorge Hidalgo

18:50 "Rejuvenecimiento del cuello con tratamiento del vector negativo facial"
Prof. José María Serra Renom

19:10 "Doble plano en rejuvenecimiento mamario"
Dr. Agustín Blanch

19:30 "Rejuvenecimiento corporal en la edad menopáusica"
Dr. Ramón Vila-rovira

19:50 "Cirugía estética genital: Dos décadas de experiencia"
Dra. Yhelda De Alencar Felicio

20:10 "Revisión de la técnica de hidroliposucción en los últimos 5 años, complicaciones"
Dr. Víctor Hugo Flores

20:30 DISCUSIÓN

***CIRCUNSTANCIAS IMPREVISTAS PUEDEN PRODUCIR ALGÚN CAMBIO EN EL PROGRAMA**

## SALA ESTÉTICA — VIERNES 5 de Octubre de 2012

## MEDICINA ESTÉTICA

**Moderadores: Dra. Mercedes Eguiluz - Dr. Jean Paul Osores**

9:50 "Experiencia del uso de extractos de placenta en psoriais y rejuvenecimiento facial"
Dra. Vicenta Llorca

10:10 "Insatisfacción corporal en (adultos o en el ciclo vital): repercusiones psicosociales"
Dra. Carmina Saldaña

10:30 "Endopeel, ácido hialuirónico y radiofrecuencia en el lifting facial no quirúrgico"
Dra. Samia Guerbaa

10:50 DISCUSIÓN

**11:00 PAUSA CAFÉ Y VISITA A LA EXPOSICIÓN COMERCIAL**

## MEDICINA ESTÉTICA

**Moderadores: Dr. Victor García - Dr. Julián Bayón**

11:30 "Mesoterapia e inyecciones intradérmicas con productos sanitarios con marcado CE"
Dr. Fernando Galcerán

11:50 "Relleno facial con técnica de pilares"
Dra. Patricia J. Erazo

12:10 "Actualización en esclerosis de varices"
Dr. Justo M. Alcolea

12:30 "Racionalización en el empleo de los factores de crecimiento plaquetario a la luz de la ciencia y de la experiencia"
Dr. Victor García

12:50 "Retensado y suspensión facial con hilos"
Dr. Jesus Sierra

13:10 DISCUSIÓN

13:30 CONFERENCIA MAGISTRAL: "Testosterona: Beneficios en la mujer y el hombre"
Dr. Edwin Lee

**14:00 COCKTAIL BIENVENIDA. Lugar: Terraza COEC**

## MEDICINA ESTÉTICA

**Moderadores: Dra. Petra Mª Vega - Dr. Jorge Hidalgo**

16:00 "Resultados y técnicas comparativas en lifting facial. Nuestra experiencia"
Dr. Víctor Hugo Flores y Dra. Natalie Flores

16:20 "Actualización en mesoterapia"
Dr. José Folch

16:40 "Armonización Facial mediante sustancias de relleno"
Dr. Fernando Carvalho

17:00 "Suplementos para la piel"
Dra. Montse Folch

17:20 "Indicaciones esteticas de toxina botulinica asociada a otros tratamientos"
Dr. Rafael Serena

17:40 DISCUSIÓN

**18:00 PAUSA CAFÉ Y VISITA A LA EXPOSICIÓN COMERCIAL**

## MEDICINA ESTÉTICA

**Moderadores: Dra. Pilar Rodrigo - Dra. Patricia J. Erazo**

18:30 "Suero autólogo antienvejecimiento: resultados preliminares"
Dr. Hernán Pinto

18:50 "Tratamientos menos habituales con toxina botulínica"
Dr. Juan Carlos López

19:10 "Levantamiento facial no quirurgico : técnica de despegamiento facial con gas carbonico infusional"
Dra. Patricia J. Erazo

19:30 "Rejuvenecimiento en 5 dimiensiones, optimización de la toxina botulínica"
Dra. Elizete Nikoluk kaffer

19:50 DISCUSIÓN

**21:30 CENA DE CLAUSURA -Restaurante CAN CORTADA. Salida BUS 21:15 desde Hotel Balmoral. INSCRIPCIÓN EN SECRETARÍA**

## SALA ANTI-AGING — SÁBADO 6 de Octubre de 2012

### SUPLEMENTOS NUTRICIONALES EN MEDICINA ANTIENVEJECIMIENTO

**Moderadores: Prof. Mónica de La Fuente - Dr. Julián Bayón**

9:00 "Prevención de los cambios en la visión a través de la edad"
**Dr. Octavio Viera**

9:20 "Acidos grasos omega 3, ¿son todos iguales?, diferentes formas y diferentes aplicaciones"
**Dra. Gloria Sabater**

9:40 "Resveratrol, estilvenos y envejecimiento"
**Dr. José Juan Rodríguez**

10:00 "Actualidad en suplementos nutricionales"
**Dra. F. Trindade**

10:20 "El Jamón en la dieta mediterránea: estudio nutricional"
**Dr. Francisco Martín Florido**

10:40 **DISCUSIÓN**

**11:00 PAUSA CAFÉ Y VISITA A LA EXPOSICIÓN COMERCIAL**

### ENVEJECIMIENTO Y CANCER

**Moderadores: Prof. Antonio Ayala - Dr. Jaume Masiá**

11:30 "Actualización en prevención, diagnóstico y tratamiento del cancer de próstata"
**Dr. Gilberto Chechile**

11:50 "SNPs y riesgo de cáncer de mama"
**Prof. Juan Sabater**

12:10 "Prevención del cáncer hereditario"
**Dr. Gabriel Capella**

12:30 "Cáncer de mama y calidad de vida"
**Dr. Jaume Masià**

12:50 "Estilo de vida y cáncer de mama"
**Dr. Máximo Izquierdo**

13:10 "Envejecimiento y Cáncer: Alteraciones por solapamiento de mecanismos moleculares"
**Dr. Joao Pedro de Magalhaes**

13:30 "Calcio y vitamina D: ¿es necesaria la suplementación?"
**Dr. Pascual García Alfaro**

13:50 **DISCUSIÓN**

**14:00 DEGUSTACIÓN DE JAMÓN IBERICO POR CORTESIA DE D.O. HUELVA Y CERVEZA POR CORTESÍA DEL C.I.C.S**

**14:30 ASAMBLEA GENERAL DE MIEMBROS DE SEMAL 1ª Convocatoria. 15:00 2ª convocatoria.**

### HORMONAS Y ENVEJECIMIENTO

**Moderadores: Prof. Darío Acuña - Dra. F. Trindade**

16:00 "El manejo de la melatonina en la clínica humana"
**Prof. Darío Acuña**

16:20 "Nutrición y hormonas antienvejecimiento:La conexión bioquímica"
**Dr. Jorge Hidalgo**

16:40 "Las claves para mantener la vida sexual del hombre en la edad avanzada"
**Dr. José María Pomerol**

17:00 "Efectos neurologicos de la progesterona. Su uso en PMDD y traumatismo cerebral"
**Dra. Angeli Akey**

17:20 "Nuevas formulaciones de la melatonina para la protección de la piel y mucosas"
**Dra. Germaine Escames**

17:40 "Alopecia masculina: tema importante en la práctica de la medicina antienvejecimiento"
**Dra. Anna Moldeska**

**18:00 PAUSA CAFÉ Y VISITA A LA EXPOSICIÓN COMERCIAL**

### SALUD CARDIOVASCULAR, CEREBRAL Y ENVEJECIMIENTO

**Moderadores: Prof. Juan Sabater - Dr. Iván Ibáñez**

18:30 "¿Es el rejuvenecimiento arterial hoy día posible?"
**Dr. José Sabán**

18:50 "Control factores de riesgo cardiovascular y envejecimiento"
**Dr. Xavier Pintó y Dr. Lluis Asmarats**

19:10 "Reclasificación del riesgo cardiovascular mediante tests genéticos: edad cronológica versus edad cardiovascular"
**Dr. Eduardo Salas**

19:30 "Estrés oxidativo y neuroinflamación en la prevención del declive cognitivo"
**Dr.Damiano Galimberti**

19:50 "Alteraciones cognitivas precoces por yatrogenia y drogas"
**Dr. Javier Aizpiri**

20:10 "Impacto de los cordales en el S.N.C. y el envejecimiento, rehabilitación mediante terapia electroneuromedular"
**Dr. Osvaldo Font**

20:30 **DISCUSIÓN**

***CIRCUNSTANCIAS IMPREVISTAS PUEDEN PRODUCIR ALGÚN CAMBIO EN EL PROGRAMA**

## SALA ANTIAGING — VIERNES 5 de Octubre de 2012

8:30 Entrega Documentación
9:00 Acto Inaugural

## GENÓMICA Y ENVEJECIMIENTO

**Moderadores: Dr. José Ignacio Lao - Dr. José Serres**

9:30 "Retraso del envejecimiento por la restricción calórica: genes y rutas implicadas "
**Dr. Joao Pedro de Magalhaes**
9:50 "Valor de la genómica dentro de la estrategia para alcanzar una longevidad activa y saludable"
**Dr. José Ignacio Lao**
10:10 "Epigenética y envejecimiento"
**Dr. Manel Esteller**
10:30 "Telomerasa, envejecimiento y longevidad"
**Científico del equipo de la Dra. María Blasco**
10:50 **DISCUSIÓN**

**11:00 PAUSA CAFÉ Y VISITA A LA EXPOSICIÓN COMERCIAL**

## ESTILO DE VIDA Y ENVEJECIMIENTO

**Moderadores: Prof. Manuel J. Castillo - Dr. Georges Debled**

11:30 "Alteración en la permeabilidad intestinal, flora, inmunidad e inflamación"
**Jean-Michel Philippart de Foy**
11:50 "La Ozonoterapia: una nueva estrategia para combatir el envejecimiento y mejorar la calidad de vida"
**Dra. Silvia Menendez**
12:10 "Valoración integral y diferencial del agotamiento mental, depresión y fatiga crónica (CFS)"
**Prof. Alfred Wolf - Florian Wolf**
12:30 "Estudio Predimec. Lecciones para vivir más y mejor: El caso de las bebidas fermentadas"
**Dr. Ramón Estruch**
12:50 "Qué comer, qué beber, que hacer…para estar mejor (y creéselo)"
**Prof. Manuel J. Castillo**
13:10 **DISCUSIÓN**
13:30 **CONFERENCIA MAGISTRAL: "Testosterona: Beneficios en la mujer y el hombre"**
**Dr. Edwin Lee**

**14:00 COCKTAIL BIENVENIDA. Lugar: Terraza COEC**

## DIAGNÓSTICO METABÓLICO EN MEDICINA ANTIENVEJECIMIENTO

**Moderadores: Prof. August Corominas - Dra. Gloria Sabater**

16:00 "Hormonas esteroideas y desarrollo del síndrome metabólico"
**Prof. Marià Alemany**
16:20 "Genes relacionados con la Obesidad: relación con dieta y ejercicio"
**Prof. Juan Sabater**
16:40 "La utilidad del estudio del metabolismo en reposo"
**Dr. Iván Ibáñez**
17:00 "La aplicación de la prueba de esfuerzo metabólica en el envejecimiento"
**Dr. Jorge Ibáñez Palomo**
17:20 "Metabolómica. Utilidad en Medicina Antienvejecimiento"
**Dra. Cristina Andrés Lacueva**
17:40 **DISCUSIÓN**

**18:00 PAUSA CAFÉ Y VISITA A LA EXPOSICIÓN COMERCIAL**

## DIETOTERAPIA EN MEDICINA ANTIENVEJECIMIENTO

**Moderadores: Dr. Ramón Vila-Rovira - Dr. Lluis Asmarats**

18:30 "Salud osteoarticular y colágeno asimilable en la dieta"
**Lda. Mª Teresa Figueres**
18:50 "Detoxificación"
**Dra. F. Trindade**
19:10 "Detoxificación hepatica a traves de la nutrición"
**Dra. Carolina Hernández**
19:30 "Seguimiento de dietas en adultos: repercusiones físicas y psicosociales"
**Dra. Carmina Saldaña**
19:50 **DISCUSIÓN**

**21:30 CENA DE CLAUSURA -Restaurante CAN CORTADA. Salida BUS 21:15 desde Hotel Balmoral. INSCRIPCIÓN EN SECRETARÍA**

**JUEVES, 4 DE OCTUBRE DE 2012**

## CURSOS PRE-CONGRESO

## "Optimización de la terapia hormonal en mujeres y hombres: Individualice su enfoque"

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Este curso internacional, está dirigido por tres de los más sobresalientes expertos en Medicina Antienvejecimiento. Centrándose en los temas más importantes relacionados con las hormonas bioidénticas tanto en mujeres como en hombres, la mañana del curso se dedicará a la parte teórica y la tarde al estudio práctico de casos clínicos tanto en mujeres como en hombres.

Objetivos del Curso:

1. Proporcionar una visión integrativa del manejo de la andropausia, perimenopausia, menopausia y de las disfunciones sexuales producidas por desequilibrios hormonales.

2. Optimizar la salud sexual en las mujeres y los hombres durante el envejecimiento, comprendiendo el manejo de las hormonas bioidénticas.

**JUEVES, 4 DE OCTUBRE DE 2012**

## "Curso Teórico - Práctico de Carboxiterapia"

**Dra. Patricia Erazo, Brasil, Dr. Juan López, Brasil, Dra. Elizete Nikoluk, Brasil.**

HORARIO: 16 A 20 HORAS
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Este curso será realizado por tres grandes expertos: la Dra. Patricia J. Erazo, cirujano plástico, el Dr. Juan López, médico estético y la Dra. Elizete Nikoluk, Dermatólogo, que abordarán las técnicas de infiltración de CO2 de forma multidisciplinaria. Será un curso teórico-práctico donde se realizarán tratamientos en pacientes seleccionados para los distintos tipos de terapias.

# XI CONGRESO DE LA SOCIEDAD ESPAÑOLA DE MEDICINA ANTIENVEJECIMIENTO

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