

# Approaches to Aging Control

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# Editorial

In this issue, we are proud to announce our 10th international congress, where once again we congregate many experts in the field of aging control. In the last 10 years, SEMAL has been working in the integration of different disciplines because fighting aging will necessarily require multidisciplinary teams of clinicians and basic scientists, working in cooperation with the pharmaceutical industry. A better understanding of the aging process itself can lead to improved and earlier interventions to prevent the negative effects of the aging process. As some projections suggest in relation to life-style related diseases, failing health in younger years can be even more burdensome than failing health in old age.

In recent decades, scientists have learned enough about the biological processes that many believe it will become possible to slow aging in human. Also, every year, new molecules are reported to modulate life span extension (for instance, AMP-activated protein kinase associated with mitochondrial biogenesis). As a consequence of this, we already know that certain life-style related practices like eating habits, physical and mental activity, stress reduction, etc. can contribute to general health improvements and healthier aging.

However, we must have a realistic view of this interesting and difficult field because there are still critical knowledge gaps about the root cause of aging. Of course, we know that Life style/environment as well as genetic factors plays an important role in healthy aging but aging as a rule remains a fact of life.

Nowadays, why we age is an unanswered question like many other big and basic questions in Biology and Medicine such as what is the biological basis of the consciousness, how the memories are stored and retrieved, what cause Parkinson´s disease, etc. In spite of this, patients and media demand to Anti-aging professionals a fast solution to prevent or reverse aging by giving a magic recipe but everybody knows that this formula does not exist. In this sense, our goal is to give to the patients reliable information and stay away of the danger of false promises of anti-aging products. For that, we must know the possibilities and the limits of the science of aging, some of which are addressed in our meeting in Madrid.



# Using Pomegranate, from Anti-aging to treating cancer

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## Introduction

The pomegranate, fruit of the *Punica granatum* tree, is native to the Himalayas in northern India, and Iran. Since antiquity its cultivation has spread to the Mediterranean countries, India, China, Japan and Russia, as well as areas of the United States and Afghanistan. The pomegranate's medicinal properties have been known for thousands of years, mention being made in the Old Testament, the Jewish Torah, and the Talmud of Babylon. It was used in the ceremonies and mythology of the Ancient Egyptians, Greeks and Romans. Ayurvedic medicine regards the pomegranate as a pharmacy in its own right, using it against parasites, diarrhoea, diabetes, and to cure ulcers. In South America, pomegranate bark, peel and petals are chewed to treat dysentery, and mouth and gum problems<sup>1</sup>.

## Phytochemical constituents of the pomegranate

Phytochemicals are plant secondary metabolites having health-giving benefits, although they are not considered essential nutrients. Generally, phytochemicals are produced by the plant as part of its defence mechanism against external dangers, such as ultraviolet radiation, pathogens, etc<sup>2</sup>. Diets rich in phytochemicals are associated with a reduced risk of developing illnesses such as certain types of cancer, and inflammatory, cardiovascular or neurodegenerative diseases. Although the greatest source of the pomegranate's phytochemicals are found in the fruit, other parts of the tree, such as leaves and seeds also contain them. More than 100 phytochemical compounds have been isolated in the pomegranate. Those detected most frequently are

the polyphenols, which include: a- *flavonoids* such as the anthocyanins and anthocyanidins (cyanidin, delphinidin, pelargonidin); b- *flavonols* such as luteolin, quercetin and kaempferol; c- *hydrolysable tannins* such as the ellagitannins, punicalagins and gallotannins. Hydrolysable tannins are responsible for 92% of the antioxidant activity of pomegranate juice and the punicalagins are responsible for half of this antioxidant effect<sup>3</sup>. Pomegranates also contain catechins such as those found in green tea, and steroids such as estradiol, estriol, estrone, testosterone and ursolic acid. The oil obtained from pomegranate seeds contains fatty acids, the most common of these being punიცic acid (>60%). The structural variations between the polyphenols extracted from the various components - the fruit, juice or other parts of the pomegranate including the tree - are numerous.

## The pharmacokinetics of pomegranate juice

Ellagitannins are hydrolysed rapidly in the body, becoming ellagic acid, of which no trace is found in the circulatory system after five hours<sup>4</sup>. Once absorbed, ellagic acid is metabolised by enzymes, such as glucuronosyltransferase and sulphotransferase, which increase its excretion and detoxification by increasing its water solubility. Intestinal microflora transform ellagic acid into two principal metabolites, urolithin A and B, which can remain in urine for up to three to four days after ingesting pomegranate juice; this may explain the beneficial effects of its chronic administration<sup>5,6</sup>. González-Sarrias *et al* have shown the presence of urolithin A and traces of urolithin B in the prostate of men who had previously received pomegranate juice or walnuts for three days before surgery<sup>7</sup>.



### Antioxidant effects of pomegranate

Recent research suggests that oxygen-dependent free radicals are the first step in physio-pathological mechanisms of chronic illness and the aging process<sup>8</sup>. The increase of nitric oxide (NO) and nitric oxide synthase (NOS), associated with an excess in O<sub>2</sub> production, produces the formation of high levels of peroxynitrite (ONOO<sup>-</sup>)<sup>9</sup>. This compound causes direct toxic effects, such as lipid peroxidation, protein oxidation and DNA damage, as well as the induction of various transcription factors, including the nuclear factor kappa B (NF-KB) and the activator protein-1 (AP-1), which lead to cytokine-induced chronic inflammation. As a result of the latter mechanism, nitro-oxidative stress is transformed into an inflammatory process as these cytokines spread the inflammatory message via blood circulation, thus causing continuing cell damage (for example, endothelial cell dysfunction)<sup>10</sup>. DNA exposure to ONOO<sup>-</sup> or NO plus O<sub>2</sub> causes breaks in the chains<sup>11</sup>. Furthermore, the ONOO<sup>-</sup> renders various enzymes inactive that are important in repairing damaged DNA. Due to all these effects, ONOO<sup>-</sup> induces apoptosis if oxidation is moderate, or cell necrosis if oxidative stress is severe<sup>12</sup>. The antioxidant activity of pomegranate juice is three times higher than red wine or green tea<sup>13</sup>. Consuming 250 ml of pomegranate juice for four weeks has been proven to eradicate free radicals from the body, and significantly increase plasma antioxidant capacity in older people when compared to those consuming apple juice<sup>14</sup>. Rosenblat and Aviram proved that pomegranate juice contains a total higher concentration of polyphenols (5 mmol/l) and a greater antioxidant activity than other fruit juices (kiwi, apple, grape, orange, pineapple, pear, peach), which contain 1.3 – 4 mmol/l of total polyphenols<sup>15</sup>. These properties have a potential use as a complement to anti-aging treatment in both sexes.

Oxidation of LDL cholesterol is considered to be an initial marker of atherogenesis. Natural antioxidants can inhibit this oxidation via several mechanisms: a – free-radical reduction or quelation of metal ions; b – protection of artery wall cells from oxidative damage; c – preservation of certain serum enzymes (paraoxonases) that hydrolyse

specific lipid peroxides<sup>15</sup>. The flavonoids present in pomegranate juice are powerful antioxidants against LDL cholesterol oxidation<sup>16</sup>. A daily consumption of 240 ml of pomegranate juice for one year significantly increases paraoxanase 1 (PON1) activity, and reduces LDL and HDL cholesterol oxidation by 60%. The majority of these effects are obtained during the first month of consuming pomegranate juice<sup>16</sup>. Supplementing hypercholesterolemic mice under oxidative stress with pomegranate juice reduces the size of arteriosclerotic lesions by 20%. The advance of the illness was seen to be detained after 24 weeks' treatment with pomegranate juice in another group of mice which had been induced with arteriosclerotic disease for six months<sup>17</sup>. Disturbed blood flow harms the vascular endothelium as the cells increase the production of free radicals capable of causing oxidative stress, and the activity of nitric oxide synthase (NOS) is reduced with the subsequent drop in the production and action of NO<sup>18</sup>. De Nigris *et al* studied the *in vitro* and *in vivo* effects of punicalagin-rich pomegranate extract on oxidation-sensitive genes (ELK-1 and p-CREB) and NOS<sup>18</sup>. Use of pomegranate extract or juice corrects the pro-atherogenic imbalance by increasing NOS activity, which leads to a rise in NO production and activity, thus reducing oxidative stress<sup>18</sup>.

Other *in vitro* and *in vivo* research has shown that prolonged administration of pomegranate flower extract to obese diabetic mice reduces triglyceride levels in myocardial cells, lowers plasma total cholesterol levels, improves postprandial hyperglycaemia, and glucose tolerance<sup>19,20</sup>.

These properties can be used in the prevention and treatment of arteriosclerosis, diabetes-related cardiovascular complications, in reducing plasma levels of lipids and glucose, and in improving irrigation of cardiac muscle.

### Anti-tumour effects of pomegranate

#### 1- Antiproliferative effects and proapoptosis

**a- Prostate cancer.** Several studies have shown that different parts of the pomegranate (arils, pericarp, seeds etc.), fresh or fermented, have antiproliferative effects. Albrect *et al*<sup>21</sup> showed *in vitro* that extracts derived



from pomegranates inhibited the proliferation of several prostate cancer cell lines - hormone-sensitive (LNCaP) as well as hormone refractory (PC-3 and DU 145). However, normal prostate cells are not affected. Malik *et al*<sup>22</sup> assessed the antiproliferative effect and proapoptosis of pomegranate extract in aggressive hormone-refractory prostate cancer cells (PC-3), and observed a dose-dependent inhibition in cell-growth and apoptosis induction. This effect came about due to the decrease in expression of the anti-apoptotic Bcl-2 gene protein and the increase in expression of the pro-apoptotic Bax gene protein. In one *in vivo* experiment in which athymic mice were implanted with hormone-sensitive prostate cancer cells, tumour growth was observed to be slower in animals that had been administered with pomegranate extract as their sole source of liquid compared to those drinking only water. Furthermore, the animals receiving pomegranate extract showed a significant reduction (up to 85%) in PSA production<sup>23</sup>. Seeram *et al*<sup>23</sup> obtained similar results from pomegranate juice with respect to growth inhibition of prostate cancer cells *in vitro* and *in vivo*. They also observed that the urolithins (ellagic acid metabolites) were localised in the prostate, inhibiting growth of both hormone-sensitive and hormone-refractory cancer cells. Recently, Koyama *et al* have shown that pomegranate juice induces apoptosis in prostate cancer cells by inhibition of IGF<sup>24</sup>. These results suggest that consuming pomegranate may delay the growth of prostate cancer, which could lengthen and improve the patients' quality of life.

**b - Breast cancer.** Similar results have been seen with breast cancer cells in *in vitro* and animal models. Metha *et al*<sup>25</sup> saw that pomegranate seed oil had anti-tumour effects in breast cancer cells. Jeune *et al*<sup>26</sup> studied MCF-7 breast cancer cells and found that the extract obtained from whole pomegranate had a cytotoxic effect caused by induction of apoptosis and this effect was dose-dependent. The effect was greater with the whole fruit extract than when different parts of the pomegranate were used separately. Kim *et al*<sup>27</sup> showed *in vitro* that pomegranate seed oil, as well as natural and fermented juice, inhibited estrogen synthesis and aromatase activity between 60 and 80%. The greatest effect was found in estrogen-dependent

MCF-7 cells, a lesser effect in the estrogen-independent MB-MDA-231 cells, and a very low effect in normal human breast epithelial cells, MCF-10. Furthermore, in a murine breast cancer model, fermented pomegranate juice inhibited the formation of tumours induced by the carcinogen DMBA by 47%<sup>29</sup>. These observations support the therapeutic use of pomegranate in human breast cancer.

**c- Colon cancer.** Pomegranate seed oil, with a conjugated linolenic acid content of more than 70%, was shown to suppress colon carcinogenesis<sup>29</sup>. All components of the pomegranate have been shown to induce apoptosis in colon cancer cells (HT-29)<sup>30</sup>.

**d- Lung cancer.** Treatment with pomegranate produces a reduction in the viability of lung cancer cells (A549) while only minimally affecting normal human bronchial epithelial cells (NHBE)<sup>30</sup>. In another study, Khan *et al*<sup>31</sup> showed that pomegranate extract significantly reduced the number of tumours developed by mice exposed to two different carcinogens: benzo[a]pyrene (B[a]P) and N-nitrosotris-chloroethylurea (NTCU). Eighty-four days after being exposed to the carcinogen (B[a]P), the animals receiving pomegranate extract displayed a 54% reduction in the number of tumours while at 140 days the reduction was 62%. At 240 days after exposure to the carcinogen NTCU, the animals receiving pomegranate extract displayed a 66% reduction in the number of tumours<sup>31</sup>.

**d- Skin cancer.** Hora *et al*<sup>32</sup> observed that pomegranate seed oil reduced the incidence and number of skin tumours in cancer models in mice.

## 2- Effects on the nuclear kappa B (NF-kB)

NF-kB forms part of a family of transcription factors and is activated as a response to various stimuli: cytokines, carcinogens, chemotherapies, endotoxins, chemical or physical stress, radiation, hypoxia, and inflammation. Activated NF-kB is found in several tumours and it has been shown to regulate the expression of more than 200 genes with different functions that participate in regulating the immune system, carcinogenesis, cell proliferation and adhesion, anti-apoptosis, angiogenesis, invasion and metastasis<sup>31</sup>. NF-kB activity is regulated by an



inhibiting protein that binds onto it, retaining it in the cytoplasm. When the NF- $\kappa$ B route is activated, the inhibiting protein degrades by phosphorylation, releasing NF- $\kappa$ B, which translocates to the nucleus where it acts as a transcription factor<sup>35</sup>. Shishodia *et al* have shown that pomegranate juice, as well as pomegranate tannins and punicalagin, suppress the activation of NF- $\kappa$ B in colon cancer cells<sup>29</sup>. Various studies conducted by Khan *et al* have demonstrated that pomegranate extract inhibits NF- $\kappa$ B activation in lung and breast cancer cells and in an *in vivo* model using athymic mice implanted with lung cancer cells<sup>30,31,34</sup>.

Prostate cancer is one of the tumours in which NF- $\kappa$ B activation has been shown to be present and represents an independent risk factor of tumour recurrence after radical prostatectomy<sup>36,37</sup>. Rettig *et al* have demonstrated *in vitro* that pomegranate juice, as well as pomegranate extract, inhibit NF- $\kappa$ B and cell viability in prostate cancer cells. In one *in vivo* model, pomegranate was seen to delay the appearance of hormone resistant prostate cancer<sup>37</sup>. NF- $\kappa$ B inhibition is a necessary mechanism to obtain the maximum pro-apoptotic effect from pomegranate juice.

### 3- Effects on angiogenesis

Hypoxia is the principal mechanism in the progression of more than 70% of tumours via the activation of angiogenesis, an essential factor for a tumour to be able to grow more than 200 micras<sup>38</sup>. However, in contrast to what happens with the vascularisation of normal tissue, the tumoural microvessels formed through angiogenesis are extremely disorganised. Thus greater hypoxia is produced with the subsequent activation of transcription factors associated with cellular hypoxia, such as hypoxia-inducible factor 1- $\alpha$  and 1- $\beta$  (HIF-1 $\alpha$  and HIF-1 $\beta$ ); these in turn activate different genes related to angiogenesis, leading to greater progression and invasion<sup>39</sup>. Tumour-induced angiogenesis is regulated by factors produced by macrophages, neutrophils, and by the tumoural cells themselves such as the vascular endothelial growth factor (VEGF). In prostate cancer, for example, it has been shown that androgens, which play an important role in tumour aetiology and progression, activate the

expression of HIF-1 $\alpha$  and VEGF<sup>40</sup>. Toi *et al* analysed the anti-angiogenesis potential of pomegranate seed oil or fermented pomegranate juice on estrogen-sensitive (MCF-7) or estrogen-resistant (MB-MDA-231) breast cancer cells, observing a significant reduction<sup>41</sup>. Sartippour *et al* carried out *in vitro* studies on the effect of pomegranate peel extract (standardised to 37% ellagitannins and 3.5% free ellagic acid) on hormone-sensitive prostate cancer cells (LNCaP) and human umbilical vein endothelial cells<sup>42</sup>. Pomegranate extract inhibited the proliferation of the endothelial cells under both normoxic and hypoxic conditions, and inhibited the proliferation of LNCaP cells under hypoxic conditions. Under hypoxic conditions, a reduction was also observed in the concentration of HIF-1 $\alpha$  protein and VEGF in both cell groups. In an *in vivo* experiment, human prostate cancer cells (LAPC4) were implanted into mice with severe combined immunodeficiency (SCID); the animals then received either pomegranate extract or a liquid serving as control by mouth five days a week for four weeks. The pomegranate extract the animals received was the equivalent of a human intake of 320 ml of pomegranate juice. After four weeks, tumour volume was observed to be significantly smaller ( $199 \pm 37$  mm<sup>3</sup> compared to  $1179 \pm 106$  mm<sup>3</sup>) in those animals that had received the pomegranate extract. Furthermore, VEGF concentration was significantly higher in those animals receiving the control liquid, while HIF-1 $\alpha$  staining and blood vessel density were reduced significantly in those animals receiving pomegranate extract<sup>43</sup>. Khan *et al* observed that the density of microvessels decreased by 78% in mice with primary lung cancer and which received pomegranate extract; VEGF expression was also reduced<sup>31</sup>.

### 4 – Effects on tumoural invasion.

For tumours to be able to infiltrate surrounding tissue, tumour cells need to secrete proteolytic enzymes, such as metalloproteinases, in order to digest the extracellular matrix. Pomegranate extract has been proven to be effective in inhibiting metalloproteinase expression by inhibition of NF- $\kappa$ B in human chondrocytes<sup>43</sup>. In another study, several constituents of pomegranate (ellagic acid, caffeic acid, luteolin and punicalic acid) were examined *in*



*in vitro* for their potential inhibiting effect on human hormone-refractory prostate cancer cell (PC-3) invasion through an artificial membrane<sup>44</sup>. Although each of the substances separately and significantly inhibited invasion, when used together, a supra-additive effect was seen. Albrecht *et al* had similar results with the same type of prostate cancer cell<sup>21</sup>. Khan *et al* observed a dose-dependent reduction in cell invasion (up to 46%) using pomegranate extract in an *in vitro* model of breast cancer<sup>34</sup>.

### Anti-inflammatory effects of pomegranate

Approximately 15% of all tumours are related to the presence of chronic inflammation. Epidemiological studies have shown that men with a history of prostatitis and sexually-transmitted diseases are at greater risk of prostate cancer<sup>45</sup>. Cyclooxygenase (COX) is an enzyme that participates in converting arachidonic acid to prostaglandin, an inflammatory mediator. There are two isoforms of the enzyme: COX-1 is found in the majority of tissues and is responsible for maintaining normal physiological functions, while COX-2 is related to cutaneous inflammation, cell proliferation and tumour promotion<sup>46</sup>. COX-2 also produces resistance to apoptosis and promotes angiogenesis and metastasis. Inhibition of COX-2 is important, not only in reducing inflammation, but also in cancer prevention. Pomegranate seed oil has been shown to inhibit COX-2 by 37% *in vitro*, and lipooxygenase (which catalyses the conversion of arachidonic acid into leukotrienes) by 75%<sup>47</sup>. Adam *et al* studied the effects of pomegranate on the inflammatory cells in HT-29 colon cancer cells, observing that COX-2 protein expression was inhibited by 79% with pomegranate extract, by 55% with total pomegranate tannins, and by 48% with punicalagin<sup>48</sup>. These results suggest that pomegranate juice could be a potential anti-inflammatory agent thanks to its COX-2 inhibiting capacity.

### Clinical applications of pomegranate juice

All parts of the pomegranate have been used to treat a variety of illnesses for over a thousand years. However, it was not until the early 90s that the first *modern* experimental and clinical trials began<sup>1</sup>.

### 1- Prostate cancer

Pantuck *et al*<sup>49</sup> undertook a phase II clinical trial with 46 men with prostate cancer who had been treated by surgery, radiotherapy or cryotherapy, and whose PSA levels had increased. The inclusion criteria were a Gleason score of  $\leq 7$  and PSA between 0.2 and 5 ng/ml. Treatment consisted of 240ml of pomegranate juice a day until the illness progressed. None of the patients had metastasis nor had they received hormonal treatment. Follow-up was carried out every three months and PSA levels were determined then. The aim of the investigation was to study the variation in PSA figures, such as doubling time. Concurrently an *in vitro* study of cell proliferation was undertaken in which patients' serum was incubated with a culture of hormone-sensitive prostate cancer cells (LNCaP). Of the 46 patients, 16 (35%) showed a reduction in PSA values. In four cases PSA dropped by more than 50%. PSA doubling time (PSADT) increased significantly, from an average of 15 months at the beginning of the study up to 54 months ( $p < 0.001$ ). In the *in vitro* study, after nine months a 12% reduction in prostate cancer cell proliferation was observed, and a 17% increase in apoptosis. Results from the patients who had continued the treatment with pomegranate juice were presented at the 2008 Annual Congress of the American Society of Clinical Oncology (ASCO), the findings showing that PSADT increased at 68 months<sup>50</sup>. These results suggest that pomegranate juice is effective in delaying the progression of prostate cancer in patients whose initial therapies had been unsuccessful. There is currently a multi-centre phase III clinical trial under way to evaluate the benefits of pomegranate juice compared to placebo in prostate cancer patients. There are presently no results on the effects of pomegranate juice in patients with other tumours

### 2- Andrology

Patients with erectile dysfunction caused by vascular conditions show an accumulation of oxidative products in the corpora cavernosa, for this reason oxidative stress may be of great importance in the aetiology of arteriogenic erectile dysfunction. Antioxidants may be useful in preventing erectile dysfunction and fibrosis of the corpora cavernosa. Azadoi *et al*<sup>51</sup> examined the



antioxidant activity of several antioxidant beverages, such as pomegranate juice, cranberry juice, orange juice, green tea, and red wine. Pomegranate juice showed the highest capacity for scavenging free radicals, reducing low-density lipoprotein oxidation, and for inhibiting oxidative stress in macrophages. Furthermore, in a model of erectile dysfunction in rabbits, the authors noted that using pomegranate juice over an extended period increased blood flow in the corpora cavernosa, improved erectile response and smooth muscle relaxation in animals with erectile dysfunction and in the control group. No significant effects were observed in the expression of nitric oxide synthase. In the group of animals with erectile dysfunction, prolonged consumption of pomegranate juice prevented fibrosis of the corpora cavernosa<sup>51</sup>. Forest *et al*<sup>52</sup> undertook a pilot study with 53 men with mild to moderate erectile dysfunction, who were given pomegranate juice or a placebo for two four-week periods separated by a two-week period without treatment. Assessment was carried out via a questionnaire on sexual activity (IIEF) and a global assessment (GAQ). Of the 42 patients reporting improvement, 25 had consumed pomegranate juice. The symptoms analysed in the IIEF questionnaire improved more in the pomegranate juice cohort; however, statistical significance was not achieved ( $p=0.058$ ), probably due to the small sample size<sup>52</sup>.

Turk *et al*<sup>53</sup> looked at the effects of pomegranate juice on different semen parameters in 28 healthy male rats, which were divided into four groups of seven animals. Each group was treated daily for seven weeks with either pomegranate juice (at different concentrations) or water in the case of the control group. At the end of the study all the animals were sacrificed, their sexual organs were weighed and studies made of semen quality, spermatogenic cell density, antioxidant enzyme activity, and testosterone levels. The animals that had received pomegranate juice showed an increase in: a - epididymal sperm concentration, b- sperm motility. c - spermatogenic cell density, d- diameter of seminiferous tubules, e- germinal cell layer thickness. A rise in antioxidant enzyme activity (glutathione peroxidase and catalases) was also observed<sup>53</sup>.

### 3- Diabetes

Diabetes is associated with high levels of oxidative stress and the development of atherosclerosis; antioxidants could thus be useful in treating sufferers of this illness. In several studies using diabetes-induced rats, various components of pomegranate were used (flowers, seed oil, skin or juice) and their effects noted<sup>54-58</sup>. Xu *et al* treated diabetic rats with pomegranate flower extract and observed a reduction in fat in the liver, produced, at least in part, by the activation of hepatic expression of the genes responsible for the oxidation of fatty acids<sup>54</sup>. McFarlin *et al* studied the effects of pomegranate seed oil in mice fed on a high-fat diet (60%), noting a lesser accumulation of body fat and an improvement in insulin sensitivity, which would reduce the risk of developing type 2 diabetes<sup>55</sup>. Bagri *et al* treated diabetes-induced mice for 21 days with an aqueous solution obtained from pomegranate flowers. The results showed a significant reduction in plasma glucose levels, total cholesterol, triglycerides, LDL and VLDL cholesterol, and an increase in the antioxidant enzymes glutathione peroxidase, glutathione reductase, glutathione transferase, superoxide dismutase, and catalase<sup>56</sup>. Parmar and Kar used pomegranate skin extract to treat diabetes-induced mice, observing a normalisation in glucose levels and the peroxidation of lipids in hepatic, cardiac and renal tissue<sup>57</sup>.

Several studies have been made with human diabetic patients, confirming the results obtained from animal models. Esmailzadeh *et al* studied 22 patients with diabetes as well as hyperlipidemia, who were treated with 40 g of concentrated pomegranate juice per day for eight weeks. They found a significant reduction in total cholesterol and LDL cholesterol, although no variations were seen in HDL cholesterol or in triglycerides<sup>58</sup>. Rock *et al* treated 30 patients with diabetes type 2 with either 50 ml of pomegranate juice or 5 ml of pomegranate extract per day for four weeks, observing a 35% drop in oxidative stress<sup>59</sup>. Fenercioglu *et al* treated 114 non-smoking diabetics of both sexes without complications for three months with either a placebo (58 cases) or with a supplement rich in antioxidant polyphenols (56 cases) containing pomegranate extract, green tea extract and ascorbic acid<sup>60</sup>. Those patients treated with the antioxidant



supplement showed lower LDL cholesterol levels and an increase in HDL, both statistically significant when compared to the control group. A decrease in oxidative stress and peroxidation of lipids was also observed. The results from this research suggest that pomegranate juice could be beneficial in preventing cardiovascular problems in patients with diabetes.

#### 4- Cardiovascular disease

There is an inverse relation between eating food rich in polyphenols and cardiovascular disease. This effect is attributed to the capacity of polyphenols to inhibit LDL cholesterol oxidation and atherosclerosis. The polyphenols in pomegranate juice projected against LDL cholesterol oxidation in two ways: 1) a direct interaction with the lipoprotein, and 2) an indirect mechanism through the accumulation of polyphenols in arterial macrophages. These antioxidant and antiatherogenic effects of pomegranate juice have been proven both *in vitro* and *in vivo* in atherosclerotic apolipoprotein e-deficient mice and in humans<sup>61</sup>. Treating atherosclerotic mice with the product obtained from the whole pomegranate after juice extraction produces up to a 57% decrease in the size of atherosclerotic lesions, while lipid peroxidation is reduced by up to 42%<sup>62</sup>.

When patients with carotid artery stenosis consumed pomegranate juice for a year, a reduction of up to 30% was seen in atherosclerotic lesion size; arterial lesions in the patients who did not take pomegranate juice, however, increased by 9 %. Those who continued taking pomegranate juice for another three years saw no further reductions in the size of atheromatous plaques<sup>63</sup>. Aviram *et al*<sup>64</sup> compared the effects produced by either a placebo or by six preparations obtained from different parts of the pomegranate on arterial lesions in atherosclerotic apolipoprotein e-deficient mice. After three months of treatment, the preparation obtained from pomegranate flower extract produced up to a 70% reduction in arteriosclerotic lesions. Davidson *et al*<sup>65</sup> carried out a double-blind clinical trial in which patients of both sexes at moderate risk of being afflicted with coronary disease and carotid intima-media thickening were treated. Pomegranate juice was given to 146 participants and a control beverage to another 143 participants over an

18-month period. The progression of atheromatous plaques was assessed, with the observation that although globally there were no differences between the two groups, those participants with higher base rates for triglycerides, HDL cholesterol and apolipoprotein, and who received pomegranate juice showed a slower carotid lesion progression rate than those receiving the control beverage. Sumner *et al*<sup>66</sup> studied the effect pomegranate juice had on myocardial perfusion in patients with ischaemic heart disease and saw that after three months of treatment, stress-induced ischaemia had increased in the control group but had gone down in the group receiving treatment. A 50% decrease in episodes of *angina pectoris* was found in those receiving the pomegranate juice compared to a 38% increase in the placebo group. The administration of pomegranate juice to patients with arterial hypertension (AHT) produced a significant reduction in blood pressure and a 36% decrease in angiotensin converting enzyme (ACE) activity<sup>67,68</sup>. Chronic administration of pomegranate juice reduced mean blood pressure in diabetic rats and vascular reactivity to various catecholamines, probably reducing ACE activity<sup>68</sup>.

#### 5- Mouth diseases

The components of the pomegranate possess properties that can be used to improve aspects of oral health, such as reducing dental plaque and the risk of gingivitis, and the treatment of infection from candidosis. Menezes *et al*<sup>69</sup> studied the effects of a hydroalcoholic extract of pomegranate on the microorganisms in dental plaque in healthy participants of both sexes aged 9 to 25 who used orthodontic appliances. The participants were organised into three groups of 20 people, each using distilled water, chlorhexidine or pomegranate extract as a mouthwash. Samples of dental plaque were taken before and one minute after rinsing the mouth with 15 ml of the respective solutions for each group. The samples collected were diluted in a saline solution, incubated at 37°C for 48 hours, then colony forming units were counted per millilitre (CFU/ml). Pomegranate extract was found to be very effective against the germs in dental plaque: results showed an 84% reduction in CFU/ml. Similar results were observed with the chlorhexidine (a 79% inhibition of CFU/ml), while



distilled water produced an inhibition of just 11% CFU/ml. Sastravaha *et al*<sup>70</sup> used biodegradable chips impregnated with pomegranate and *Centella asiatica*, or chips with no impregnation as a placebo, in patients with periodontal disease. Significant improvement was seen in all the clinical parameters used in those patients using the treated products when compared with the placebo. Inflammatory markers, such as interleukin-1 $\beta$  (IL-1 $\beta$ ) and IL-6 were also analysed, finding a significant reduction at three and six months compared to the results obtained before treatment. Vasconcelos *et al*<sup>71</sup> carried out a phase II clinical trial with 60 patients with candida stomatitis. The participants were randomly distributed into groups and treated with either a miconazole oral gel (Daktarin<sup>®</sup>) (group A) or a gel obtained from pomegranate bark (group B). The treatments were applied three times daily for 15 days. Forty-eight hours after terminating treatment, the patients were re-examined and samples taken for mycological analysis. The clinical results demonstrated a satisfactory and regular response in 27 patients in group A, and 21 patients in group B. In 25 subjects in group A and 23 in group B, the cultivations were negative for candida, suggesting that pomegranate can be used as a topical antimycotic in the treatment of candida stomatitis. DiSilvestro<sup>72</sup> *et al* treated 32 young adults of both sexes with mouth rinses of pomegranate extract dissolved in water three times a day for four weeks. They observed changes in saliva measures that were relevant to oral health. The investigators noted the following changes: a reduction in total proteins, which is correlated with plaque-forming bacteria; a reduction in aspartate aminotransferase, an indicator of cell injury; an increase in the activity of antioxidant enzymes, such as ceruloplasmin alpha, which protects against oral oxidative stress. The use of a placebo did not alter the parameters.

The results suggest that pomegranate might be incorporated into oral hygiene products, such as toothpaste or mouthwashes.

### **Potential therapeutic applications of pomegranate juice**

*In vivo* research has been carried out with the aim of finding new uses for pomegranate juice.

**Obesity:** Obese, dyslipidemic mice were administered either 400 or 800 mg/kg/day of pomegranate leaf extract, leading to a significant loss of body weight and adipose pad weight, a decrease in intestinal fat absorption and appetite, and a drop in serum levels of cholesterol, triglycerides and glucose<sup>73</sup>. Abidov *et al*<sup>74</sup> studied the effects a product (Xanthigen) composed of 300 mg of pomegranate seed oil and 300 mg of marine algae containing 4.2 g of fucoxanthin had on non-diabetic obese women, some of whom had non-alcoholic fatty liver. Some 151 women were tested and of these, 113 showed high levels of liver fat (>11%) and 38 had normal levels of liver fat. After 16 weeks of treatment with the product, there was a significant reduction in body weight in both groups of women (5.5 kg  $\pm$  1.4 kg y 4.9  $\pm$  1.2 kg respectively). Additionally, a reduction in waist circumference, a fall in body and liver fat content, and a drop in triglyceride and C-reactive protein levels in serum were observed.

**Alzheimer's disease:** In a model with transgenic mice with Alzheimer's disease, use of pomegranate juice reduced amyloid deposition in the hippocampus by 50% when compared to other animals receiving only sugar water. This suggests pomegranate products may act as neuroprotectants given that the animals improved on water maze tasks and swam faster than control animals<sup>75</sup>.

### **Ongoing clinical trials using pomegranate juice**

There are 25 clinical trials using pomegranate juice at different stages of progression (completed trials, those including patients, or those active but in the pre-recruitment phase, etc) currently underway around the world<sup>76</sup>. Eight of the investigations are related to prostate cancer, five of which are recruiting participants with high PSA levels after the failure of the initial treatment with radical prostatectomy or radiotherapy. In another study patients are treated with pomegranate juice before undergoing radical surgery. Another trial treats patients with localised prostate cancer who have not yet received any treatment, and the final study evaluates supplementing prostate cancer patients' diets with phytochemicals and polyunsaturated fatty



acids. The effects of pomegranate juice in diabetic patients is the focus of five studies. Three further studies consider infection by rhinovirus, influenza, swine flu, and the common cold. The nine remaining investigations are looking into: benign prostatic hyperplasia, carotid intima-media thickness, chronic cardiomyopathy with renal failure, brain injury in foetuses with intrauterine growth restriction, risk of cardiovascular disease, lymphomas, oxidative stress in patients in haemodialysis, physical and mental function in middle-aged adults, and the oxidative capacity of diet.

### Conclusions

The properties of the pomegranate have been known for more than a thousand years; however, it has only been in the last couple of decades that the number of *in vitro* and *in vivo* trials analysing its various components (especially the juice) and their effect on different pathologies has increased. Likewise, over the last few years several multi-centre clinical trials have been designed and are currently in progress; when their results have been analysed, they will be able to offer us a great deal of information about the therapeutic effects of pomegranate. For the time being, its potent antioxidant activity, similar to or greater than green tea, has been proven; it could thus be used as an adjuvant in anti-aging treatments. In oncology, its antiproliferative, pro-apoptotic and angiogenesis effects have been widely studied in animal models and are pending confirmation from human studies. The capacity of pomegranate to regulate plasma levels of glucose, cholesterol and triglycerides, and to reduce blood pressure opens an ample therapeutic potential for patients with diabetes and cardiovascular disease. The possible use of pomegranate juice in other fields, such as neurology and contagious diseases, needs further research.

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# Prevention during menopause to improve life quality

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## Abstract

Due to an increase in population life expectancy, particularly in the female population, we face a consequent increase in the number of women in menopausal age.

This population aging implies an increment of several potential problems such as osteoporosis, cardiovascular diseases, cancer and dementia that can affect life quality and length.

Professionals involved in woman's total health should make an effort towards preventive medicine in order to improve life quality. Menopause is a stage in which we can face up to strategies that allow us to develop a good preventive health program.

Actions to improve life quality should include:

1.- Promotion of healthy life styles, putting special emphasis on introducing changes in diet, doing physical exercise, eliminating toxic habits and preventing accidents (falls).

2.- Early diagnosis and treatment of chronic diseases.

Specific evaluations of particular pathologies will be performed, such as for arterial hypertension and hypercholesterolemia, in order to prevent cardiovascular diseases. It would be advisable to perform a bone mass measurement, diagnosing osteoporosis and using instruments that permit the evaluation of fracture risk. Cognitive functions will be evaluated, in order to find changes that could lead to the main pathologies of depression and dementia.

3.- Early diagnosis of those cancers which more frequently affect women at this stage of life, applying

screening programs that demonstrate their usefulness, as in breast and cervical cancer, also considering the possibility of applying other programs according to each woman's oncological risk.

4.- Treatment of pathologies linked to hormonal deficit, specifically in the genitourinary area. Preservation of pelvic floor muscles is important in order to avoid disturbances connected to urinary incontinence.

## Introduction

Menopause is defined as the final end of menstruation. It is a physiological fact determined by the complete exhaustion of follicular reserve. Beginning from the age of 40, in a different way for each woman, ovarian function undergoes different progressive transformations, ending with ovarian inactivity.

Menopause is something that happens during the climacteric or period of time in which a woman goes from reproductive to a non-reproductive phase.[1]

In western women menopause occurs at 50 on the average, waving between 45 and 55.

If it shows before 40, then it's called early menopause; on the other hand, if it starts from the age of 55, it's called late menopause.

Menopause age is influenced by several intrinsic and extrinsic factors.

## Intrinsic Factors:

- Heredity. Menopause appears at a similar age in women of the same family.



- Menarche. Women who had early menarche usually have menopause at a more advanced age.
- Length of cycles. When a woman has short menstrual cycles (less than 26 days) she'll have an earlier menopause.
- Giving birth. In women who have not given birth menopause appears approximately one year before women who have.

#### **Extrinsic Factors:**

- Socioeconomic. In countries with a high socioeconomic level, menopause is postponed.
- Nutrition. Nutritional deficits usually cause an early menopause.
- Climate. In countries with a warmer climate and high altitude menopause appears earlier.
- Tobacco. In smoker women a decrease of ovarian vascular flux is observed, as well as a hepatic metabolism increase, factors that can bring menopause forward one or two years.
- Alcohol. A high alcohol intake produces a harmful action on the ovary, but in moderate consumers it could even have a protective effect on menopause.[2]

If we consider that women life expectancy is now over 80 years, this means that she will spend one third of her life (between 25 and 35 years) in a hormonal deficit condition.

This hormonal deficit entails a symptomatology and clinic consequences that won't affect all women the same way.

#### **Menopause consequences are:**

- Short-term consequences: Neurovegetative and psychological alterations
- Medium-term consequences: Genitourinary and cutaneous alterations.
- Long-term consequences: Osteoporosis and cardiovascular pathology.[3]

It is therefore necessary to develop a good preventive health program in order to improve the life quality of climacteric women.

The activities that should be performed to improve life quality should cover the following aspects:

- 1.- Promotion of healthy life styles.
- 2.- Prevention of cardiovascular risk.
- 3.- Early diagnosis of those cancers that more frequently appear in this phase of the woman's life.
- 4.- Treatment of pathologies connected with hormonal deficit.

#### **Promotion of healthy life styles**

Promoting a life style change must start as early as possible (even during childhood and adolescence) in order to carry out a preventive medicine and, consequently, offer a higher quality of life.

The bases on which we build a healthy life style are diet, physical exercise and toxic habits elimination.

#### **Diet and bone mass**

A proper diet must be oriented towards each woman individual necessities. Nevertheless, as a general criterion, we could fix some basic nutritional recommendations: increasing vegetable and fruit intake, providing proteins with high biological value (legumes and fish), reducing the consumption of saturated fat and refined carbohydrates and avoiding sodium excess.

Due to aging, calcium necessities increase and its absorption diminishes, thus preserving bone mass will be a priority.

Bone mass is the total bone quantity of each person. Obtaining an excellent bone mass depends on some genetic factors (not modifiable) and on some other factors concerning life style, such as physical exercise and calcium and vitamin D intake, that are subject to our decision.

Bone mass triples after puberty and reaches its highest moment between 30 and 35 years, keeping steady up



to 40. After that, we face a constant loss that shows an acceleration in the post-menopause period.[5]

### **Daily Calcium need**

The National Health Institute (NHI) advises keeping daily doses of calcium of 1000mg/day in adults up to 65 years and after that it must be increased up to 1500mg/day.

Doses are to be taken as a complement to the diet in order to reach the right quantity, without risking increasing renal calculi formation in patients who are not predisposed to eliminate it through urine.

### **Vitamin D needs**

Vitamin D is necessary to keep the homeostasis of calcium plasmatic concentration. It can be obtained through 2 ways: vitamin D3 (cholecalciferol) synthesized in the skin after sunlight exposure or ultraviolet rays beginning from 7-dehydrocholesterol and vitamin D2 (ergocalciferol) that we have in food such as fish liver oil, egg yolks and butter.

Sunlight exposure is advisable for 10 -15 minutes, after that the reaction saturates. The use of sun protector factors (SPF) must be reconsidered, knowing that after SPF 8 there's no vitamin D synthesis in fotoexposed skins.

Vitamin D insufficiency in a sane post-menopausal population is a risk factor for osteoporosis connected to augmented bone remodelling and low bone mass. Its administration is recommended in post-menopausal women, especially if there's low sunlight exposure.

The National Osteoporosis Foundation recommends the administration of 800 UI/day in women at risk or with deficit and not to exceed the 2000UI/day dose.

### **Physical exercise**

We know that physical inactivity increases bone reabsorption and diminishes its formation. Compared to controls, exercise programs increase bone mineral density in postmenopausal women.

Physical exercise must be repetitive and constant (at least 3 times a week), in accordance with age, general conditions and previous sport habits.

Physical activity improves coordination, agility and self-sufficiency, it maintains muscular tone and strength, and it also produces a 25% reduction in the risk of falls.[6]

Trying to go at a speed faster than walk-speed, wearing comfortable shoes, at least 30 minutes per day, would be an advisable exercise.

### **Avoid toxic habits**

Alcohol increases bone mass loss and increases vasomotor symptoms, which is not advisable for obese persons, though a glass of wine's polyphenols may have cardio protector effects.

Tobacco increases the risk of cardiovascular diseases, osteoporosis, neoplasm and chronic obstructive pulmonary disease.

It could also bring forward menopause age of one or two years.

### **Prevention of cardiovascular risk**

Women usually face cardiovascular diseases at a more advanced age than men, equilibrating or increasing after 10 years of menopause.

Cardiovascular disease is the most frequent cause of death in industrialized countries. Main risk factors, according to Framingham Study data, are arterial hypertension, hypercholesterolemia and tobacco.[7]

### **Arterial hypertension**

Estrogen decreasing during menopause involves an increase in sympathetic tone and in Renin-angiotensin-aldosterone system, encouraging the establishment of arterial hypertension.[8]

Reduction of sodium intake, in normotensive as in hypertensive persons, entails a decrease in arterial tension, particularly if it is accompanied by increments in potassium, magnesium and calcium intake (DASH diet = Dietary Approaches to Stop Hypertension). Diet and physical exercise represent 80% of cardiovascular health. Weight reduction will



diminish arterial tension and it would be effective as an isolated treatment or accompanied by medicaments.

Recommending walking ½ hour daily would diminish the coronary disease risk.

The WHO recommends a control of arterial tension every 2 years when the value is <130/85 and every year if it exceeds this.

### **Hypercholesterolemia**

In post menopause an increase of total cholesterol, LDL and triglycerides is produced, with a decrease of HDL cholesterol.

A preventive diet suggests a daily cholesterol intake that does not exceeds 300mg/day. Consumption of fibre is important, especially the soluble kind because it joins fats and eliminates them through the faeces directly. We recommend the consumption of oily fish and a moderate quantity of dried fruits, avoiding saturated fats (fat meat, butters, pastries).

Using cold-pressed vegetable oils, such as virgin olive oil, better if raw, can improve the cholesterol profile. The intake of functional food, usually dairy food, which has been enriched with vegetable sterols, can diminish LDL cholesterol by 10-15%.

### **Early diagnosis in more frequent cancers**

#### **Breast cancer**

This is the most frequent cancer among women. About 70 – 75% of the cases are registered after the age of 50 and every day the number of younger women with it increases.

The early detection of breast cancer aims to improve disease prognosis and to augment survival. This detection is based on clinic breast exam and on mammography, which is the most effective detection method. The characteristics of breast screening varies depending on scientific societies recommendations, so that mammography is performed starting from the age of 40 (US Preventive Services Task Force, American Cancer Society) or from the age of 50 (Canadian Cancer Society).

Mammography screening programs showed a reduction of 40% mortality for breast cancer. One of the problems with mammography is related to augmented breast density, which diminishes the exam's sensitivity, thus a breast ultrasound would be advisable to complete the study. In case of high risk breast cancer we would recommend Magnetic resonance.

Concerning the use of Substitutive Hormonal Therapy (SHT) and breast cancer, we have gathered the following interesting tips from different studies:

- The current use of SHT carries a risk of causing breast cancer, especially in combined treatment with estrogens and progesterone, the risk being significantly higher during the fifth year of treatment.[9]
- Estrogens-only therapy doesn't involve an increase in breast cancer risk.
- The SHT produces an increment in breast density, which is transitory and reversible after stopping the treatment.

#### **Cervical cancer**

This is the second most common cancer in young women (15 to 44 years).

With the introduction, more than 50 years ago, of screening made through cervical pap smears, a reduction of 70-80% of the incidence of squamous cell carcinoma of the cervix was achieved.

There is clear evidence that a persistent infection of high risk human papillomavirus (HPV) is a cause of cervical cancer and its precursor lesions. This evidence permitted setting new strategies to prevent the cancer [10]. Currently we have two available prophylactic vaccinations against HPV which allows the realization of a primary prevention; these vaccinations have demonstrated their effectiveness, safety and vaccine efficiency. Secondary prevention could be performed by population screening with cervical pap smears (morphological study of exfoliated cells of exo and endocervix) and systems for detection and the ability to type HPV.



### **Endometrium cancer**

This cancer is increasing due to several factors, such as the increase of life expectancy, obesity and the use of tamoxifene as a breast cancer treatment. In Spain the incidence is 7-13 cases/100000 women/year. Its appearance frequency varies with age: it is less frequent in women younger than 40 (5%) and increases up to 75 years, with an average age of 66 years.

Even though population screening is not effective for this kind of cancer, in case of metrorrhagia during pre and post menopause, it justifies realizing additional exams such as vaginal ultrasound with Doppler, that helps find signs of malignancy [11], and hysteroscopy with biopsy that has a sensitivity >90% and a specificity >95%.

### **Ovarian cancer**

Although this represents 20-25% of gynaecological cancers, it is responsible for a high mortality rate, with a global survival after 5 years of less than 40%. It has an incidence with two main age peaks: the first in women of fertile age (between 20 and 30 years), with a germinal lineage predominance; the second in women between 50 and 70 years, with epithelial lineage predominance. Symptoms usually appear late, not allowing the possibility of an early diagnosis for this cancer.

Though, according to the evidence currently available, the population screening has not proved effective [12], strategies for an early diagnosis are being developed. A diagnostic approximation of suspected ovarian cancer can be realized through tumoral markers (Ca 125, Ca 19.9 and CEA) and vaginal ultrasound accompanied by Doppler in order to see ecographic malignity signs.

### **Colorectal cancer**

Compared to other cancers of the digestive system, the incidence of colorectal cancer has augmented during the last few years. Most of the cases are usually diagnosed after the age of 40. Factors in the cases are, chronic colon diseases, history of cancer and colon polyps in the family, a diet poor in fibre and rich in fat, tobacco, alcohol consumption and a

sedentary life all contribute to the colorectal cancer risk. If suspected symptomatology of colon cancer are faced, such as intestinal habits alteration, blood in faeces, rectal tenesmus sensation or abdominal pain, it would be advisable to proceed with a study of rectal touch, examination of blood in the faeces and a colonoscopy. In general, it is recommended to start screening examinations, beginning at age of 50 and repeating them every 5-10 years.

### **Treatment of pathologies connected to hormonal deficit**

#### **Neurovegetatives alterations**

In 75-85% of women neurovegetatives alterations (Climacteric Syndrome) arise: hot flushes, perspiration, palpitations, paresthesia, vertigo, insomnia, and migraine. The effect and intensity of these alterations is variable, presenting a severe symptomatology in one third of women and, in 20% of the cases, remaining for more than 5 years.

For women affected by Climacteric Syndrome a Hormonal Substitutive Therapy would help, with beneficial effects.[13]

#### **Genitourinary alterations**

In menopause, the estrogen deficit produces a reduction in vaginal epithelia thickness, glycogen and Döderlein bacillus, also muscles and pelvic floor collagen are affected. All these changes infer the appearance of atrophy, dyspareunia, urinary infections and pelvic static alterations that could permit pelvic organ prolapse and urinary incontinence.

Genitourinary symptomatology appears in 40% of women, even during perimenopause. Genitourinary atrophy represents a correct sign for suggesting Hormonal therapy.[14] It could be taken both orally and vaginally, but the vaginal consumption is more effective, its systemic absorption being low and it doesn't need gestagen.

### **Conclusions**

1.- Age is not a decisive factor to start preventive measures and take advantage of them.



2.- During menopause, every opportunity to diminish the magnitude of its cardiovascular risks must be taken into account.

3.- Early detection of frequent cancers in menopause increases the survival rate.

4.- Treating the symptomatology linked to estrogenic deficit improves women life quality, because it relieves Climacteric Syndromes, improves urogenital symptoms, improving their sexuality.

5.- Growing old does not mean getting ill.

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## Sleep: a necessary condition to avoid aging

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Sleep is a reversible self-regulating natural state characterized by a reduction in voluntary motor activity, a diminished response to sensory stimulation (higher threshold for arousal) and a stereotypical view (1), but unlike other conscious states such as wakefulness, hibernation, torpor, etc. The main function of sleep has not been identified, although we have been able to know and show most of the systems involved in its generation. It is clear that sleep has been selected as an evolutionary advantage in the animal kingdom; contrary to what one might think a priori, because when we immerse into that state of consciousness, we would be more vulnerable to predation. Then, why do we sleep?

### **CNS centers involved in stages of awake/sleep**

In 1916, Baron Constantin von Economo, a Viennese neurologist, began to see patients with a new type of encephalitis that specifically attacked regions of the brain that regulate sleep and wakefulness (2). This disorder, which was eventually called encephalitis lethargic and the virus that caused it was never identified, affected areas of the brain in which lesions caused specific alterations of wake-sleep regulation. Only during the past decade has the accuracy of his observations come to be appreciated, as key components of the wake-sleep-regulatory system were found to reside at the sites that von Economo first identified.

The majority of patients with encephalitis lethargic slept excessively. Many slept for 20 or more hours per day, arising only briefly to eat and drink (2). But a small percentage of the patients had an

opposite response (2). Rather than being sleepy, they became insomniac and slept for only a few hours each day. Typically, they were extremely tired, but found it difficult to fall asleep, could sleep for only a short time, and then awoke and were unable to fall back asleep. For the first group of patients, which slept excessively, von Economo found lesions at the junction of the midbrain and the diencephalon. He therefore proposed that there was an ascending reticular activating system originating in the brainstem that kept the forebrain awake.

This pathway has two major branches. The first branch is an ascending pathway to the thalamus that activates the thalamic neurons that transmit information to the cerebral cortex. The major source is a pair of acetylcholine-producing cell groups: the pedunculopontine and laterodorsal tegmental nuclei (PPT/LDT) (3). These neurons fire with higher frequency during wakefulness and REM sleep (rapid eye movement) and are much less active during non-REM (NREM) sleep, when cortical activity is slow.

The second branch of the ascending reticular activating system bypasses the thalamus, instead of activating neurons in the lateral hypothalamic area (LHA) and basal forebrain (BF), and throughout the cerebral cortex (4-6). This pathway has its origin from monoaminergic neurons in the upper brainstem and caudal hypothalamus, including the noradrenergic locus coeruleus (LC), serotonergic dorsal (DR) and median raphe nuclei (Raphe), dopaminergic ventral periaqueductal grey matter (vPAG) and histaminergic tuberomammillary neurons (TMN). The input to the cerebral cortex is augmented by



lateral hypothalamic peptidergic neurons (containing melanin-concentrating hormone (MCH) or orexin/hypocretin), and BF neurons (containing acetylcholine or GABA). Lesions along this pathway, especially in the LHA and rostral midbrain, can produce sleepiness or even coma (7-8). Neurons of the monoaminergic nuclei of this pathway fire a higher frequency during wakefulness, slowing down during NREM sleep and stopping during REM sleep (9-11). Orexin neurons in the LHA are, similarly, most active during wakefulness (12-14) whereas MCH neurons are active during REM sleep (15). Many BF neurons, including most cholinergic neurons, are active during both wake and REM sleep (16). These nuclei are found precisely in those areas that von Economo had studied, exactly at the junction of the midbrain and forebrain.

For the second group of patients, which rather than being sleepy, they became insomniac, slept for only a few hours each day, were always extremely tired, but had difficulty falling asleep, slept for a short time, and then, awoke and were unable to fall back asleep. These patients had lesions involving the basal ganglia and adjacent anterior hypothalamus. Later experiments that were performed in animals with similar insomnia showed that a lesion in the hypothalamic, specifically in the lateral preoptic area, produced the same effects (17-18). One such cell group, the ventrolateral preoptic (VLPO), was found to send outputs to all of the major cell groups in the hypothalamus and brainstem that participate in arousal (19). The VLPO neurons are primarily active during sleep, and contain the inhibitory neurotransmitters, galanin and GABA (20-22). These neurons form a dense cluster, as well as a more diffuse extended part of the nucleus (20-21). These observations suggested that damage to the VLPO might have caused the insomnia in von Economo's patients.

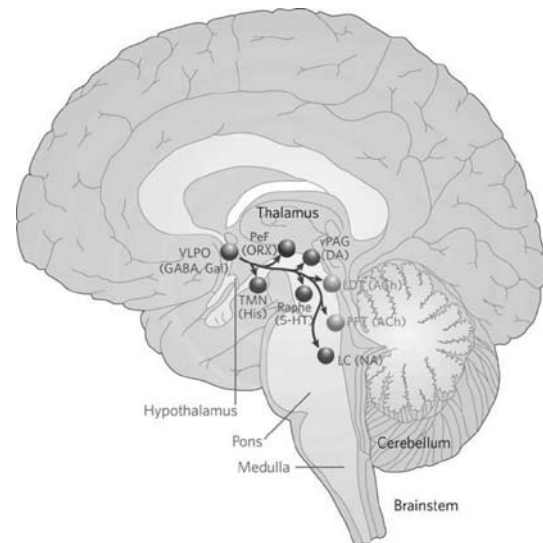
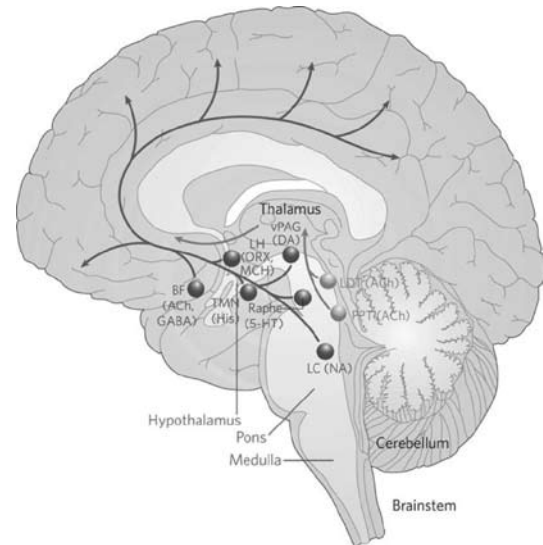
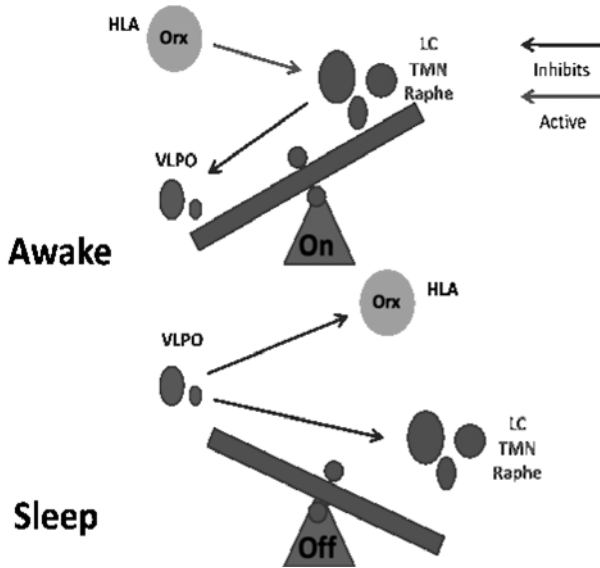


Image obtained from: Clifford B. Saper, Thomas E. Scammell and Jun Lu. Hypothalamic regulation of sleep and circadian rhythms. *Nature* 437, 1257-1263 (27 October 2005).

In conclusion, the VLPO inhibits the ascending reticular arousal system, and this in turn, inhibits the VLPO, by a mechanism called flip-flop switch. This explains why the states of wakefulness and sleep cannot happen simultaneously. One can only be awake or asleep in a normal physiological state. Many hormones like melatonin or orexin, or neurotransmitters such as GABA, ADP, and Galanin... involved in this regulatory process swing the balance toward waking or sleep (23-26).



### Circadian regulation of sleep

The suprachiasmatic nucleus (SCN) serves as the brain's clock. Neurons in the SCN fire in a 24-hour cycle that is driven by a transcriptional-translational loop, which can go on even when the neurons are dissociated from tissue and are introduced into the cell culture (27-28). Whether or not we destroy the SCN of animals the circadian rhythms are abolished, which will lead to a range of changes in behavior and physiological processes in them, including sleep. This happens because external sync cues are blocked (29). Under normal circumstances, the SCN is reset on a daily basis by light inputs from the retina during the day and by melatonin secretion from the pineal gland during the dark cycle (30-31).

The light signal is received from a specialized set of retinal ganglion cells that contain the photopigment melanopsin (32). These timing signals keep the clock in synchrony with the external day-night cycle. The SCN has relatively modest projections to the VLPO or the orexin neurons (33-34). However, the bulk of its output is directed toward the adjacent subparaventricular zone (SPZ) and the dorsomedial nucleus of the hypothalamus (DMH). The SPZ contains a ventral part, just above the SCN, and a dorsal part, just below the paraventricular nucleus (35). Lesions in regions of the ventral SPZ disrupt the circadian rhythms of sleep and wakefulness, as well as locomotor activity,

but have minimal effects on body-temperature rhythms. Conversely, lesions of the dorsal SPZ severely impair circadian rhythms of body temperature, but not wake-sleep or locomotor activity (33-34). Therefore, direct projections from the SCN to sleep or thermoregulatory regions are not sufficiently strong to maintain the circadian rhythms of these functions, and the relay neurons in the SPZ are required.

The SPZ also has, but relatively limited, projections to the VLPO, orexin neurons and other components of the wake-sleep-regulatory system (33-34). However, a major target is the DMH (37). This region receives input from many neurons in the SPZ and the SCN. Lesions in the DMH also profoundly diminish circadian rhythms of sleep and wakefulness, as well as locomotor activity, corticosteroid secretion and feeding (36). Interestingly, animals with DMH lesions sleep about one hour more each day and have much less locomotor activity, implying that the output of the DMH is mainly activating. This theme is also reflected in the corticosteroid levels, which, in animals with DMH lesions, remain at the lowest basal levels throughout the day. Body temperature retains a normal circadian variation, but is about 0.5 °C lower than in control animals (36).

The DMH is one of the largest sources of input to the VLPO and orexin neurons (33-34), and is fundamental to integrate and amplify the influence of the SNC to the wake-sleep-regulatory system. The DMH projection to the VLPO comes largely from GABA-containing neurons (that is, those that promote wakefulness by inhibiting sleep), and the projection to the LHA originates from neurons containing glutamate and thyrotropin-releasing hormone (which should presumably be excitatory and promote wakefulness). The DMH has relatively few direct outputs to the brainstem components of the ascending reticular arousal system, but the orexin neurons have extensive projections to these targets. Examination of Fos patterns in the DMH show that it contains many more active neurons during wakefulness than during sleep (37).

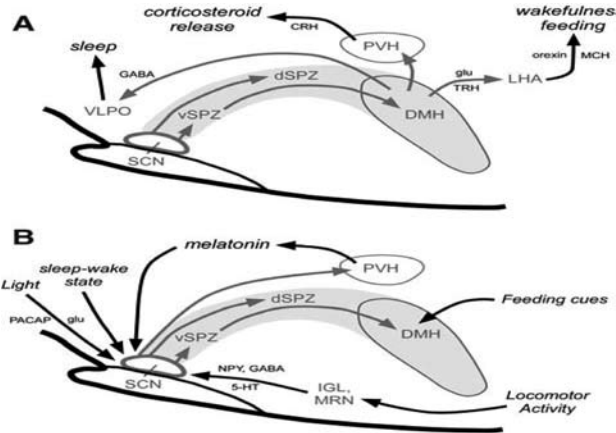


Image obtained from: Fuller PM, Gooley JJ, and Saper CB. Neurobiology of the Sleep-Wake Cycle: Sleep Architecture, Circadian Regulation, and Regulatory Feedback. *J Biol Rhythms* 2006; 21; 482.

In conclusion we can say that the DMH seems to integrate clock information from the SCN and SPZ with feeding, temperature, social and other cues, providing animals with the flexibility to adapt their behavioral and physiological cycles to the environment, thereby maximizing their chances of survival.

**Sleep-cycle basics**

In mammals, there are two extremely different sleep stages —rapid eye movement (REM) and non-REM (NREM). They are defined mainly in terms of electrophysiological signs that are detected with a combination of electroencephalography (EEG), electroculography (EOG) and electromyography (EMG), the joining measurement of these parameters in humans is termed Polysomnography (38). REM sleep (also known as paradoxical, active or ‘desynchronized’ sleep) is characterized by the following: wake-like and ‘activated’ (high-frequency, low-amplitude, or ‘desynchronized’) activity in the EEG; singlets and clusters of REMs in the EOG; and low muscle tone (atonia) in the EMG (39). Note that the term ‘desynchronized’ for the activated states of waking and REM has been rendered obsolete by the discovery of highly synchronized gammafrequency (30–80 Hz) activity in these states (40).

NREM sleep is divided into three stages, corresponding to increasing depth of sleep, as indicated by progressive dominance of the EEG by high-voltage, low-frequency (‘synchronized’) wave activity. Stage I is not particularly different from wakefulness. Stage II NREM is characterized by distinctive sleep spindle and K-complex waveforms, as well as a slow (<1 Hz) oscillation, which influences their timing. Such low-frequency waves dominate the deepest stages of NREM (also termed slow-wave sleep).

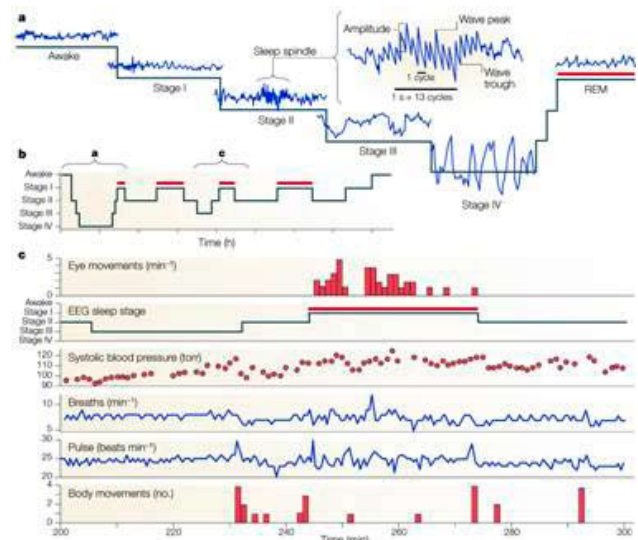


Image obtained from Pace-Schott and Hobson. *The Neurobiology of sleep: Genetics, Cellular physiology and Sub-cortical networks*.

Pace-Schott and Hobson (2002) in their review show a panel which explains quite well the different stages of sleep and some physiological states during sleep stages. Panel a) shows the characteristic waveforms of the different sleep stages; panel c) shows changes in peripheral physiology associated with these stages.

NREM and REM sleep alternate in each of the four or five cycles that occur in each night of adult human sleep. Early in the night, NREM sleep is deeper and occupies a disproportionately large amount of time, especially in the first cycle, when the REM epoch might be short or aborted. Later in the night, NREM sleep is shallow, and more of each cycle is devoted to REM (red bars). Panel b illustrates these changes over



the course of a night's sleep. Panel a depicts, in detail, features of an early-night sleep cycle in which NREM reaches its greatest depth at stage III and IV (delta) sleep (currently, stage III and stage IV is considered as stage III or slow-wave sleep, this change was decided in 2007 by the American Academy of Sleep Medicine), whereas panel c depicts a late-night cycle in which NREM descends only to stage III. The constant period length of the NREM-REM cycle indicates that it is timed by a reliable oscillator (for a discussion of ultradian alternation of REM and NREM sleep, see the main text), the amplitude of which varies according to extrinsic factors. The cyclic organization of sleep varies within and between species. The period length of each REM-NREM epoch increases with brain size across species, and the depth and proportion of the NREM phase in each cycle increases with brain maturation within species. NREM sleep complexity is a function of brain systems, such as the thalamocortical circuitry, that reach their maximum development in mature humans only to decline in post-mature age. It can therefore be concluded that the differentiation of sleep is a function of brain differentiation, a rule that indicates both mechanistic and functional links between sleep and other brain functions.

### **The consequences of sleep loss and its importance in aging**

Most people cannot survive without sleeping as we cannot be without drinking water for a long time, although there are a few exceptions (41). Total sleep deprivation can lead to early death, so much that some studies had to be stopped prematurely because the changes that occurred in the subjects were so dangerous that they became life-threatening. Sleep deprivation consists either of a complete lack of sleep during a certain period of time or a shorter than optimal sleep time (42). It is precisely the latter, "a shorter sleep time than optimal", that those involved in sleep studies are paying more attention lately. Especially because of the fact that throughout our lives a lack of sleep can affect the morbidity of diseases associated with aging. What still remains unclear, and recent studies are exploring possibilities, is whether the

optimal sleep loss is a consequence or cause of these diseases. It seems that this is a reciprocal process, and that's what we'll see in this section, common diseases of old age being linked directly or indirectly to sleep disorders.

The late nineteenth century saw the beginnings of the first experiments to evaluate the effects of long-term sleep deprivation. Three American volunteers were subjected to a 90-hour sleep deprivation study during which one person experienced hallucinations (44). It was not until the decade of the 60s when they began to do a series of clinical trials in humans which examined the effects of sleep deprivation (44-45).

Symptoms of sleep deprivation are different and affect all systems, mainly the nervous system, the appearance of these symptoms depending on idiosyncrasies of each person since not everybody needs the same number of sleep hours, this demand varying from individual to individual. Some people need only 3-5 hours of sleep, whereas others need at least 8 hours to maintain work effectiveness. Also, data will depend on the type of study being conducted and how much sleep deprivation the individuals are subjected to. When talk is of sleep deprivation, the term 'deprivation' applies only to the cases when impaired functioning due to sleep loss can be observed. The extent to which one experiences the effects of sleep deprivation depends on individual needs. Generally, when this happens possibilities are: a longer reaction time, distractedness, disturbances in attention and concentration, forgetting known facts, difficulty in memorizing new information, and making mistakes and omissions (42). A higher level of stress is observed; tiredness, drowsiness and irritability increases; work effectiveness decreases and usually motivation as well. Reasoning slows down not only during the night of sleep deprivation but also on the following day. Work effectiveness decreases, particularly at the low points of the circadian rhythm and when the subjects perform long, difficult, compulsory, monotonous, sitting activities in an unchanging environment with limited lighting, little supply of sound, and low



motivation or little interest on the part of the participants (42).

These are the most evident symptoms, easy enough to observe when a subject goes a long time without sleep, but there are other symptoms not so obvious that could jeopardize the subject's long-term health. It is important to know if and when severity of the symptoms increases to a point where the damage can be irreversible.

### **Sleep and Hormonal changes**

All or almost all hormones are related to a rhythm, such as ultradian, circadian, infradian, stationary... and if one fails, another will also be affected. Perhaps the hormones most associated with sleep are the melatonin, which are released during the night, and orexin is released during the day, and if mutated in the receptor can cause sleep disorders such as narcolepsy; GH is released during NREM sleep and prolactin is released during REM sleep. Glucocorticosteroids, estrogens, TSH, LH, FSH, ACTH, renin, natriuretic peptide also fluctuate during the day. An interruption in some of these rhythms can alter the sensitivity of the hormonal homeostasis system.

### **Melatonin**

Melatonin is synthesized and secreted in a strict circadian manner, peaking during the night, thereby acting as a signal for the length of day and night. But despite its rhythmicity, many mechanisms of melatonin are still unclear. In addition to diurnal variations in melatonin levels, the secretion is significantly altered as a function of age (46). This reduction in amplitude and loss of clear peak in aging has been associated with numerous age-related conditions such as fragmented sleep and poor sleep quality (47), ocular degeneration (48), increased risk of cancer formation, among others. For this reason, maintaining good sleep habits throughout life can help prevent the diseases related to deregulation of the melatonin hormone, diseases that have been generated by the ongoing deregulation of the sleep-wake cycles during youth, and then in old age, an individual does not have the capacity to counteract these imbalances. Studies

are still missing that elucidate, in a quantitative way, how poor sleep habits acquired during a life time affect a person in old age.

### **Orexin**

Orexin, together with melatonin, are the two main hormones involved in the wake-sleep cycle. As happens in both, prior thought followed the line that their main involvement in the wake-sleep cycle was due to greater frequency and rhythmicity they show during the photoperiod, their functions only related to maintenance of waking and sleeping, when actually their involvement in the human physiology goes beyond this limiting thought. Orexin, also known as hypocretin, is secreted by the HLA orexigenic neurons and its primary mission is to excite the nuclei ascending reticular activating system, among others, whose ultimate goal is a general release of dopamine, norepinephrine, histamine and acetylcholine. This plays an important role in the stabilization of insomnia and sleep.

A mutation in the orexin receptor causes sleep disorders such as narcolepsy (49) that causes a frequent and rapid permutation between sleep and insomnia. This disease is characterized by a daily lack of sleep and an inability to maintain wakefulness during the day (and sleep at night) with more or less frequent episodes of cataplexy (loss of muscle tone). In humans, narcolepsy is associated with a specific variant of the complex of human leukocyte antigen (HLA). Ultimately, these genetic abnormalities cause the autoimmune system to attack and kill orexigenic neurons. Hence the absence of orexin production in narcoleptic could be the result of an autoimmune disorder (50).

Orexin is closely related to hormones that regulate the homeostasis of appetite and satiety. Orexinergic neurons on the one hand are inhibited by leptin, a hormone that is released by adipocytes and reports on the internal situation of the energy state, but on the other hand are activated by ghrelin, a hormone whose levels increase before food intake and decrease after eating. A deregulation of this triad of



hormone Orexin-Leptin-Ghrelin is closely related to metabolic syndrome in the elderly.

A study by Kang et al (2009) (51) shows sleep disorders may be related to neurodegenerative diseases, particularly Alzheimer's disease. In their study, monitored APP transgenic mice expressed a mutated form of human amyloid protein APP, and levels of A $\beta$  protein were found in the brain interstitial fluid (ISF) causing diurnal variations. The average levels during the light period were 75% of the average levels of A $\beta$  during the dark period. ISF A $\beta$  levels were significantly correlated with the amount of time awake. By contrast, ISF A $\beta$  levels were negatively correlated with the amount of time spent sleeping. This negative correlation was even stronger with NREM sleep. Kang et al. reasoned that if A $\beta$  levels were correlated with wakefulness, they could conduct a manipulation of sleep to alter ISF A $\beta$  levels. And so it was, Mice were forced into wakefulness for 6 hours at the beginning of the second 12-hour light period when they would naturally be asleep. During sleep deprivation A $\beta$  levels were significantly higher compared to A $\beta$  levels during the normal light period 24 hours previously. Following sleep deprivation, the mice spent more time sleeping and had an immediate reduction in A $\beta$  levels. Thus, the state of wakefulness, and not time of day, was associated with increased ISF A $\beta$  levels.

One might ask which molecular mechanism could mediate the diurnal variation in levels of A $\beta$ . As we saw above, orexin is the molecule that regulates wakefulness. An examination was done of whether administration of orexin modulates A $\beta$  levels. During an infusion of orexin, A $\beta$  levels were significantly increased compared to the levels of A $\beta$  measured during the light period of the previous day. Also investigated was whether chronic sleep deprivation could ultimately affect the deposition of A $\beta$  plaques in the brain. APP transgenic mice were subjected to chronic sleep restriction for 20 hours a day for 21 days. Sleep-restricted animals showed significantly higher A $\beta$  plaque deposition compared to control littermates of the same age.

In conclusion, this study showed the diurnal variation in levels of A $\beta$  in the brain of awake and active animals. Disturbances in both orexin signaling and the sleep-wake cycle had severe effects on the A $\beta$  dynamics. Furthermore, chronic sleep restriction accelerates the A $\beta$  plaque burden, while improving sleep by blocking the orexin receptors markedly inhibits A $\beta$  plaque accumulation. Sleep disorders can exacerbate a fundamental process that leads to neurodegeneration, and optimization of sleep time may inhibit the aggregation of toxic proteins and slow the progression of Alzheimer's disease.

### Thyroid Hormones

Thyroid-stimulating hormone (TSH or thyrotropin) is a hormone synthesized and secreted by thyrotrope cells in the adenohypophysis which regulates the endocrine function of the thyroid gland. From TSH stimulation thyroxine (T4) and triiodotironine (T3) are released. These hormones have a catabolic effect in the human organism; in addition to an increment of cardiac output, heart rate, ventilation rate, basal metabolic rate and involvement in brain development. They also have a synergic effect on melatonin and orexin in metabolic rate and thermoregulation.

It is obvious that the plasma concentrations of the thyroid hormones are modified from lack of sleep, but it is not clear yet in what measure due to contradictory studies about this modification. Total deprivation or selective deprivation of REM sleep produced a considerable decrease in both T4 and T3 (52). This decline is surprising in view of the increased metabolic rate and body temperature in the sleep-deprived animals (42). However, humans, post 24-h sleep deprivation produced an increase in T3, T4 and TSH (53).

### Corticosteroid

After 72 hours of sleep deprivation in animal experiments, the corticotropin-releasing hormone (CRH) suffered change in its plasmatic levels. This resulted in an increase of ACTH and corticosteroid, and consequently, higher plasmatic levels of noradrenaline. This would partially explain the



increased energy expenditure (54) in animals sleep deprivation.

The circadian rhythm of cortisol is relatively well established with a clear peak in cortisol secretion during the morning (55), but in humans a 24-hour hour wakefulness resulted in a slight increase in plasma cortisol level, cortisol being a corticosteroid hormone that is involved in the response to stress. Cortisol increases blood pressure and blood sugar levels and suppresses the immune system. It works as a physiological antagonist to insulin by promoting gluconeogenesis, breaking down lipids and proteins leading to higher glucose concentrations in serum. It also increases blood pressure and when suppressing the immune system it alleviates inflammatory responses in cells like the inflammation of eczema for example. Cortisol also lowers bone formation.

This does not mean that cortisol is totally bad for health, because its effects are vital to fighting stress, but an increase could lead to a deregulation, producing an exacerbated and chronic response. This can also explain why sleep loss is associated with glucose intolerance and an increased risk of diabetes. The effects of sleep restriction on insulin sensitivity have not been established, but sleep restriction (5 h / night) for 1 week significantly reduced insulin sensitivity, raising concerns about the effects of lack of sleep in chronic disease processes associated with insulin resistance (56). Other studies claim that even partial sleep deprivation for one night induces insulin resistance in multiple metabolic pathways in healthy subjects (67).

### **Estrogens**

Estrogens is normally considered to be mainly a hormone related to sex characteristics and sexual behavior, but estrogen receptors exist in both the male and female brain as well as in retinas of both sexes. The diurnal rhythm of estrogens is most likely driven by the SCN, and is integrated with signals from the hypothalamic GnRH and the pituitary gonadotropin secretion profiles (58-60). This is important in animals whose females need to coordinate their estrogen cycle with the circannual

rhythm to give birth in the most favorable season. In human females further research is needed, although it appears that estrogens play a minor role in chronobiology. Anyway, estrogens have some connection to light and are able to modulate at least, to some extent, the SCN (61). This light connection should be the subject of future study.

### **Growth Hormone (GH)**

As the human organism ages, inevitably it will produce less GH and without sufficient sleep it would produce even less. The influence of 24-hours of wakefulness on GH release is particularly interesting. GHs have a typical maximum release peak, normally occurring during the first NREM sleep, but in subjects with 24-hours of wakefulness, it will disappear, or at least could not be seen; however, the total GH release remained unchanged (62-63). The physiological significance of the GH release peak at early nocturnal hours has not been elucidated. It also remains to be shown whether the lack of GH peak in the sleep-deprived subjects might be compensated simply by an increase in the daily release of the hormone. Such considerations are justified by the findings indicating that during the rebound sleep, the GH release peak appeared earlier and achieved a higher level than the values obtained for the controls (64).

Dermal effects have been reported in animals with serious sleep deprivation. The lack of dermal symptoms in humans associated with sleep deprivation can be due to the fact that such drastic conditions do not happen in humans (65). In rats the major effects, such as alteration of skin and hairless spots, are mainly localized in the tail and paws. It is possible that these effects might be linked either to the change in the release pattern of the growth hormone (GH), namely, the absence of the nocturnal maximal GH release in the sleep-deprived animals (42,62-63), or to a tendency for such animals to become infected with their own migrating bacterial flora (42,66). Although these results have not been reported in humans, the frequent lack of sleep in older people produces a decrease in growth hormone release that could harm a skin in good condition, or at least, wouldn't



help if there was already a skin disease. Also during old age our immune system is weakened, which can lead to the appearance of bad skin conditions. Sleep is also involved in the immune system (see below).

In conclusion we can say that with aging although less GH is produced it does not mean that its lack ceases to be important. For this reason, maintaining good sleep habits is vital to a healthy state of being.

### **Prolactin**

Prolactin is a hormone whose name describes the function by which it was discovered. This function is to stimulate milk production in the mammary glands and the synthesis of progesterone in the corpus luteum, but among all the functions perhaps the least important but the best known and studied is that of the relationship between prolactin and sleep. The hormone is released during REM sleep. There are few human studies to show whether there really is a link between sleep quality and the release of prolactin.

In humans, there are other functions related to prolactin such as fluid and electrolyte balance, growth, development, metabolism, behavior, reproduction, immune regulation and protection ... In animals, variety of the functions of prolactin, increases. In the year 2000 alone 300 different actions were collected. The vast majority of them produced different behavior in the animals that were studied, but they all kept to a pattern, a circadian rhythm, circannual ... that regulated them.

### **Sleep and the Immune system**

It is clear that when there is an infection, sleep time is prolonged as a sign of healing. At first it was thought to be the result of wear caused by the immune system's response to the infectious agent. Nowadays the idea of "consequence" has changed and has become an idea of "condition". It was presumed that cytokines, somehow might also be involved in the sleep regulation processes (67-68). Most studies have been done on animals, few

on humans with the result that human studies are not as conclusive as those of animal studies and have even been inconsistent and sometimes contradictory, creating all kinds of problems. (69).

There are numerous cytokines, chemokines, and growth factors that have been shown to alter sleep when administered to laboratory animals (and some cases human volunteers) include IL-1 $\beta$ , IL-2, IL-4, IL-6, IL-8, IL-10, IL-13, IL-15, IL-18, interferon- $\gamma$  (IFN $\gamma$ ), interferon- $\alpha/\beta$ , TNF- $\alpha$ , TNF- $\beta$ , MIP-1 $\beta$ , GM-CSF, FGF, NGF, BDNF, GDNF and TGF- $\beta$ 1 (70), but only few of them have been studied in depth.

A lot of the data demonstrate that at least two cytokines, IL-1 $\beta$  and TNF- $\alpha$ , are involved in sleep regulation. Slow wave activity increases during periods of NREM sleep enhancement that follow administration of IL-1 $\beta$  and TNF- $\alpha$ . Local application of TNF- $\alpha$  to the surface of the somatosensory cortex under the dura mater increases slow wave activity during NREMs of rats without altering the amount of time spent in wakefulness (70-71). These cytokines, at effective doses, increase NREM sleep in mice, rats, rabbits, cats, and sheep (human subject on IL-1 $\beta$  therapy complain of fatigue and sleepiness) These cytokine-induced increased in NREM sleep occur irrespective of whether they are administered centrally or peripheral (70). Microinjections into the dorsal raphe nucleus increase NREM sleep (72). Low doses of IL-1 $\beta$  or TNF- $\alpha$  need not affect REM sleep, but most concentrations that consistently increase NREM sleep also suppress REM sleep, irrespective of timing of administration. As such, these cytokines and their receptors are situated in brain regions important for regulation of sleep-wake behavior (73), where they function in the absence of immune challenge.

Evidence suggests that sleep alteration during infection is important. It is not known if increased sleep facilitates, or sleep loss impairs, recovery from infection (70). In one experiment the probability of survival of microbial infection was demonstrated using *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. Survival was greater for



rabbits that develop increased NREM sleep and slow wave activity during NREM sleep than for those animals that underwent prolonged periods of NREM sleep suppression (74-75). Such a result should indicate that promoting NREM sleep during an infectious challenge may be beneficial.

Lack of sleep is one constant complaint in older adults, also in healthy seniors, with prevalence rates of diagnostic insomnia exceeding 20-30% (76). Indeed, during ageing sleep is associated with decline in subjective quality, increased sleep fragmentation, and decreases of deep slow-waves sleep (77). These sleep abnormalities are thought to contribute to daytime fatigue, depression, and impairments in health functioning. Elderly persons suffer greater changes in their immune system than younger persons, after acute or prolonged sleep deprivation. Like acute sleep deprivation, aging is associated with a reduced T-cell function that includes a shift, promoting the synthesis of type 2 cytokine instead of type 1, and in some studies has been found also to be associated with decreased production of IL-2 and IFN $\gamma$  (78-80). Regarding innate immune functions, aging, like sleep deprivation, leads to diminished NK cell activity when considered on a per cell basis (81). Blood counts of activated T-cells (HLA-DR) have likewise been found to be increased after short periods of sleep deprivation as well as in the aged. One study showed that healthy elderly, in comparison with young subjects, increased the production of IL-1 $\beta$  and TNF- $\alpha$ , which appeared to be particularly pronounced during nocturnal sleep (82). In addition, nocturnal levels of IL6 are lower during SWS as compared to Stages 1-2 sleep, and the deficits of SWS in older adults may be associated with overall increases in circulating levels of this pro-inflammatory cytokine. However, other changes in the aged, like the reduced number of circulating lymphocytes thought to originate from a thymic involution, are clearly opposite to what is observed following sleep deprivation. Accordingly, caution should be used when considering alterations in immune function in the aged as a consequence of a "chronic sleep deprivation", since the effects of poor sleep in

elderly persons interact with a number of other physiological conditions not related to sleep.

### **Sleep and Sensory, Cognitive and Mental functions**

Those symptoms that appear most frequently when there is lack of sleep will now be treated. Many of them are not dangerous in and of themselves, but they can make the organism more vulnerable when something needs to be brought under control.

When sleep deprivation is not long, the symptoms that appear are a consequence of an inaccurate image formation on the retina: the images become dim, there is double vision and disruption of visual perception, tunnel vision... (83-84), but if the period of sleep deprivation is long (85-86), the number of visual errors and hallucinations increase with the duration of wakefulness (42). Hyperesthesia or limb numbness may occur, as well as an increased sensitivity to pain, whereas the sense of temperature remains unchanged (42).

In the course of prolonged wakefulness, concentration of attention becomes impaired, thoughts are distracted and the microepisodes of sleep are longer (83). Such effects lead to decreased accuracy and effectiveness of work performance as well as impaired cognitive processing (42). It is plausible that during a stressful event, the motivation compensates for the effects of weariness (83). This is because the release of adrenergic neurotransmitters that stimulate the ascending reticular activating system and production of catabolic hormones that prevent going into the sleep phase are temporarily postponed. What is not clear yet, it is how long optimal waking conditions can be maintained only with releasing neurotransmitter and catabolic hormones.

A study realized in humans with a 50-hour sleep deprivation, showed decreased emotional intelligence and deteriorated interpersonal relations, lower assertiveness, empathy and positive thinking (87). Other studies demonstrated that a 55-hour sleep deprivation induced intense frustration and aggression in subjects, along with an increase in the subjective perception of affective symptoms



of psychopathology (anxiety, depression, mania, insanity) (88) Survey studies conducted on male teenagers revealed a correlation between sleep deficiency and elevated aggression (89). Moreover, an improvement in the quality of sleep mitigated the emotional problems.

### Conclusion

This review, “sleep and its importance in aging”, has not attempted to reach a consensus about which is the main function of sleep. It has been an attempt to discuss to what extent sleep is involved in and affects the human organism. There are a few examples shown of species that have been investigated such as humans, rats, mice and monkeys, but sleep is different in the animal kingdom and so different among the various species that to attempt to reach a consensus would be ludicrous. What can be said is that sleep performs a vital function for any species that has been molded by environment and behavior of each species on its evolutionary path. For this reason, to understand the essential function of sleep it is necessary to carry out an exhaustive evolutionary study of sleep. Meanwhile, health sciences could go a step further in terms of sleep physiology and not treat sleep disorders as mere symptoms, but as a global alteration that requires care to avoid breaking the balance that is established during the circadian rhythms. During old age, sleep disorders also require paying attention to because it is one stage of life in which our systems are altered, reason enough to prevent the disruption of circadian rhythms intensifying the changes that occur during old age.

We have mentioned in our review that studies have shown that maintaining good sleep habits prevents hormonal disruption. This control is important because it could be preventing the premature appearance of physiological aging, since both the melatonin and estrogen hormones are considered antioxidants. Proper sleep would be avoiding, or delaying, cognitive dysfunction, a characteristic of old age, and the immune system would be stronger. An added benefit is that sleep brings happiness if a person can relax after a long

day’s work. This fact is not trivial, it is the means to activate the reward pathway that exist in the brain because sleep is vital for life.

In conclusion we can assure that sleep is involved, directly or indirectly, in all biological processes of the human organism. Good sleep habits must be valued and the benefits brought to the attention of the world population.

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# Evolving concepts in nutrition: from functional foods to nutrigenomics: the paradigmatic example of fermented papaya preparation

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## Functional Food: recent research with historical beginnings

“Functional Foods” represent an emerging opportunity and if put on a timeline they will certainly play a consistent and important role in the future. Such a new perspective entirely depends on the growing attention paid by nutritionists to the development of new innovating solutions aimed at acting upon organic systems as well as on more general topics related to good consumer health. Different from the past, when mainly retrospective epidemiological studies or empirical experiences were carried out on single nutrients, such a new and growing interest by the scientific community follows research deeply oriented to clinics supplemented by an accurate study on nutrients, genomics and single nutritional requirement diagnostics. Already in 1993, the leading journal *Nature* published a report “Japan is exploring limits between food and medicine” (Swinbanks 1993). Clearly the success of “Functional Foods” depends on the food industry capacity, too, of developing new effective products which on the one hand meet any consumer request and on the other hand must have positive effects on health, supported and validated by scientific research and therefore far beyond simple positive properties, as recently underlined in a meeting, organised by a non-profit non-governmental international association.

## Definition and needed features

Such a new philosophy in the last few years has led to constant changes in Functional Food definition which an authoritative scientific European panel

defined as follows in 1999 “ A nutrient can only be easily considered functional if it was satisfactorily proved that it can positively change one or more target functions, besides nutritional effects, as to consistently improve health, well-being while reducing any affection risk. A Functional Food should ideally be a nutrient and should not change its efficacy when entering into a diet, it should not be either a pill or a capsule”. It was then agreed that, from a practical view point, a Functional Food should comply with the following features:

- 1) be a natural food;
- 2) a food which was simply supplemented by a component;
- 3) a food which was no longer holding a component;
- 4) a food which the nature of one of more components has been changed;
- 5) a food which one or more component availability has been changed;
- 6) a combination of the previous features.

It was then underlined how, besides its nutritional properties or physiological effects, it was necessary to offer a consistent administration safety profile. Such a condition is nothing but a prerequisite to further develop any Functional Food. From the recommendations of such a European commission, it is possible to come to the conclusion that “The design and development of a Functional Food is a key factor, besides a scientific challenge, which should be mainly based on consistent scientific



knowledge in terms of target functions and their possible modulations by nutritional components”. And therefore it is further stressed that “... while Functional Foods are not universal, therefore a nutritional-specific approach would be no longer enough. But mainly and universally a basic specific scientific approach only applies”.

It is important to underline a new concept within nutrition on the role played by “Functional Foods science”, which is the only one to be followed to get to useful clinical inferences (Roberfroid, 2002).

An ancient Chinese proverb specifies that “medicine and food are isogenic” and it is not by chance that in 1984, in Japan, a unique national study group was set up, under the patronage of the Ministry of Education, Science and Culture (MESC), aiming at exploring the interface between nutrition and science. Scientists for some time studied and defined a series of foods and nutrients which were officially listed in the category “foods to be specifically administered for health-care” (Food for Specified Health Use, FOSHU), stressing and recognising their nutritional value, after undergoing a consistent bio-fermentation process. Such a classification is still a legally-binding tool against media communication of wrongly defined natural products, misleading or simply generally recalling generic data in literature but not followed by specific validations of the product itself.

### **Synergies, markers and development strategy leading to nutrigenomics**

A biochemistry and molecular biology specific development, together with biotechnological methods, were enhanced so as to support the hypothesis that some nutrients could modulate the body functions playing a role in its general good health conditions as well as in the reduction of contagion risks depending on life style. Such assessments had to be in line with consistent marker identification, both directly connected (functional factors) to the process to be modified as well as indirectly liable (indicators). Suitable marker selection mainly supported the development of genomics. In fact from the human

genoma project conclusion (Venter JC 2001), the post-genomic era started, which should mainly be correlated with Functional Foods, profiting from sophisticated technologies such as the DNA chip technology and some others, which lead to nutrigenomics (DellaPenna, 1999). Such a word was only recently introduced and represents a leap forward in comparison with observation studies which were mainly based on research in the bioactive nutritional component field. Nutrigenomics mainly aims at studying genetic and epigenetic interactions with a nutrient so as to lead to a phenotype change and therefore to the cell metabolism, differentiation or apoptosis (Fafournoux 2000). Furthermore to stress the scientific research importance and mainly, as far as natural products are concerned, the simple fact that research is effectively carried out on the nutrient which apparently is “functionally” effective, it is necessary to define the minimum effective quantity leading to the above-mentioned changes. There are in fact many pre-clinical studies which use a bioactive nutritional component at concentrations which cannot practically be administered. More recently papers suggest that cells are able to adapt themselves when exposed to excessive quantities of nutrients. As previously stated, it would be highly confused, if there were no scientific application, to enforce any approach to a natural product:

- 1) which is only nutrient-specific;
- 2) and even more, if generally referring to properties simply derived from literature, but with no specific validation or bioavailability study.

What is more, a series of far-sighted companies and food industries are consistently sponsoring independent validation studies on natural products, even when not imposed by the regulation in force;

- 3) taking into account the negative effect of the variable efficacy of the nutrient according to the different formulation (lyophilised products, dehydration processes at low or high temperature, extracts, etc.) or associations. Isoflavones and soy proteins stand out among all of them, where the role of each single component is not clear yet, as



well as the effects of any possible association or the best formulation of soy itself (Crouse 1999).

As for new generation studies, however, it is too early yet and still many interactions are to be assessed between nutrients and host and among nutrients themselves, and possibly many mechanisms will play an important role when the dust is settled.. Biological answers in the presence of a Functional Food would shortly be anti-oxidant (followed by a series of possible genomic sequences mediated by an increased transcriptional rate by: cytochrome P450s, glutathione-S-transferase, NAD(p)H:kinone-reductase, UDP-glucuronosyltransferase, microsomal hydrolysis, aphta-toxin B1-aldehyde reductase, dihydrodiol-dehydrogenase, aldehyde-dehydrogenase, glutathione-reductase, etc.), supporting the detoxigenic enzymes, carcinogen build-up and metabolism block, hormonal homeostasis change, delaying the cell division or inducing apoptosis.

#### **Fermented Papaya Preparation history: an example of the rational and evidence-based biotechnological study.**

That being said, it is far more interesting to further and briefly analyse the study and development process, still in progress of fermented papaya preparation (FPP) a specific product derived from the technologically advanced and controlled bio-fermentation process of *Carica Papaya Linn*, in the absence of genetic manipulation, within a Japanese research institute carried out in compliance with every quality control and environmental-friendly validated standards.

It has been well-know for a long time that the anti-oxidant natural papaya properties, mainly depending on vitamins (A & C) and amino acids were consistent both in the fruit and derived from the papain enzyme (Arginine among others). Papain plays a digestive role, but such an activity is no longer present in the FPP. A long fermentation, by means of yeasts, is the unique process, supporting the preservation of papaya anti-oxidant properties while offering important new immune-modulating features. Fermentation deeply modifies, within the product, the ratio between complex carbohydrates

and proteins, which in lyophilised papaya accounts for about 10:1, increased up to 10:0.03 in the case of FPP, that is 30 times higher. In the final fermented product and not in the fresh fruit, many new classes of oligosaccharides are present at a different polymerisation as well a monomers similar to the basic structure of  $\beta$  1-3 D-glucan. Such oligosaccharides, mainly oligosaccharides exhibiting a low molecular weight, exhibit a wide spectrum of immune-modulating activity.

After a series of initial reports by Japanese scientists on a series of populations living in the Philippines and eating large amount of papaya on a daily basis, over 20 years ago, a research institute was set up committed to the study of "functional" properties of a series of specific compounds within a fruit - and vegetable-based diet. Special attention was paid to *Carica Papaya Linn*, which was collected in the Philippines and was further processed in Japan with other exotic fruits through a long fermentation process according to organic methods.

#### **Basic research: a compulsory process to follow the development of biotechnologies**

From extraction of the final product, a series of experimental scientific activities and studies were carried out by the Neuro-science Department, Molecular Biology Institute at the Okayama University in Japan, directed by Prof. Mori (Santiago 1991). Such studies, carried out with sophisticated methods, among which Electron Spin Resonance, highlighted that such a product consisting of fermented papaya exhibited a powerful anti-oxidizing activity on *in vitro* cerebral cells (Santiago 1993) as well as on the *in vivo* epilepsy experimental model, where the epileptogenic monoamine neutral release was consistently reduced (Santiago 1993). Prof. Mori's group also proved the capacity of fermented papaya to reduce the increase of free radical concentration as well as superoxide dismutase at the brain level in elderly rats followed by the reduction of experimental ischemia-reperfusion induced cerebral damage. The consistent *in vitro* resistant anti-oxidizing product capacities was furthermore highlighted even when tested for one hour at high temperatures



(100°C) and acid pH (1,2). What is more, such features were confirmed after long-term storage. Such potential neuroprotective effects of FPP are at the moment the issue of a clinical study on Parkinson's disease patients by the group of Dr. Nordera in northern Italy. which is showing some preliminary promising results especially in rigidity symptoms. Interestingly, some still uncontrolled data from Prof. Barbagallo, chief of Geriatrics unit at the University of Palermo pointing towards a significant decrease of plasma oxidative stress parameters in FPP-supplemented patients with varying degree of dementia.

Then, after thoroughly refining the product and getting certification by the governmental body (table 1), two important studies were carried out with international institutes so as to further assess the topic such as its possible effects on the immune system together with the Kyoto Pasteur Institute (Kishida 1994) as well as its effects on the oxidizing stress in co-operation with the Molecular Biology Department at UC at Berkley directed by Prof. Packer, a widely recognised authority on the subject, leading to a better assessment of its activity mechanisms. Such successful studies, still in progress, lead to a series of extremely interesting *in vitro* and *ex vivo* evidence. The group from the Pasteur Institute in Kyoto, starting from the evidence of positive effects of FPP on the Natural Killer population of a sarcoma experimental model proved its capacity to affect the  $\gamma$ -interferon production on human beings. Such data was further proved by studies supporting the positive activity of FPP on the macrophage function on rats (Marcocci 1996) and human beings as well. In the same time period, the working group co-ordinated by Prof. Mori proved the consistent protection effect by FPP on oxidizing stress on isolated rat hearts (Haramaki 1995) when undergoing a severe effect such as ischemia/reperfusion in the clinical practice, the unique epiphenomenon present during a myocardial stroke. Such data have recently been confirmed and have gained further insights from Aruoma et al. (2006) who has shown the ability of FPP to modulate oxidative DNA damage due to  $H_2O_2$  in rat pheochromocytoma (PC12)

cells and protection of brain oxidative damage in hypertensive rats.

The same Mori group also led to important scientific results proving the connection of the immune-modulating activity of FPP to its anti-oxidising features. In fact, on a rat macrophage line, important experimental evidence was put forward on how FPP can adjust the nitric acid production induced by interferon- $\gamma$  upward. FPP (Kobuchi 1997) would then exhibit a nutrigenomic effect able to change the messenger RNA expression both of inducible nitric acid and of TNF- $\alpha$  and of interleukin 1 $\beta$ .

Such an activity was further assessed when two different fractions were arbitrarily separated, according to their different molecular weight (cut off: MW 3.000), both confirming the previous results as well as the new important evidence of their action on the NF- $\kappa$ B binding to DNA as a clear explanation of the transcriptional increase of inducible nitric acid gene. The two different fractions, however, proved a series of differences in terms of macrophage stimulation and anti-oxidising scavenging activity. It is therefore possible to prove, for example, that a different immune-modulating activity could depend on the different (1-3)- $\beta$ -D-glucan concentrations, which represents the most representative portion of some peculiar yeasts, used in the FPP bio-fermentation process.

### **Clinical evidence supported by research: a needed evolution from the empirical**

Supports offered by scientific evidence and a series of works on human beings represented a foundation to plan a series of clinical studies. In 1995 in fact a oncological- haematologic Russian study group (Korkina 1995) proved, on young subjects undergoing radiotherapy against severe myelo- and lympho-leukaemia, how the administration of FPP, as proved in the previous experimental studies of Prof. Mori, managed to significantly reduce clinical side effects (encephalopathy score: anorexia, nausea, vomiting, convulsions, dizziness) and bio-humoral effects (change of the redox state due to erythrocyte glutathione depletion and



leukocyte SOD increase, deficit of the monocyte bactericidal activity). During the same time period a group of Italian, French and Japanese scientists co-ordinated a series of studies on the alcoholic liver disease which proved how FPP allows the reduction of alcoholic oxidative stress (reduction of plasma and erythrocyte level of malonyldialdehyde as well as of plasma lipoperoxides) both during the initial phases of withdrawal, when it is possible to observe a persistence of the microsomal system activation leading to ethanol oxidation (with a consequent maintenance of the pro-oxidative state) and during chronic alcoholic abuse. More precisely, taking into account the low clinical practice compliance in the case of withdrawal, it was proved how the administration of FPP to alcoholics led to the following effects:

1. a significant improvement of haemorheology (reduction of the whole blood viscosity, recovery of the erythrocyte deformability and increase of blood filtration capacity through a specific membrane). Such a consistent increase of the malonylaldehyde concentration in the erythrocytes in the case of chronic alcoholics leads to, through lipoperoxidising effects, a lipid asymmetry destabilisation (Marotta et al. 2001). Part of these data have recently been confirmed in a small group of generally healthy elderly individuals (Marotta et al, 2006). In a different setting of chronic liver disease unrelated to alcohol, i.e. HCV-related, the same research group has then shown that A significant improvement of redox status was obtained by both alpha-tocopherol 900 IU/day or 9 g/day of a FPP regimens. However, only FPP significantly decreased 8-OHdG and the improvement of cytokine balance with FPP was significantly better than with vitamin E treatment. A few years later, a similar group of patients was further studied (Marotta 2010) and it was found that patients with liver cirrhosis showed a significantly time-dependent upregulated TNF- $\alpha$  production from ex-vivo LPS-stimulated monocyte, this effect being more pronounced in more advanced stages of the disease together with a higher serum level of thioredoxin (Trx). Again, FPP showed a normalization of Trx and a partial but significant downregulation of TNF- $\alpha$  mRNA.

2. The previously mentioned haematological data also proved to be interesting for an authoritative (CORRECT) Israeli group led by Prof. Rachmilewitz (2002, Amer 2008) which has shown that *in vitro* treatment of blood cells from beta-thalassemic patients with FPP increased the glutathione content of red blood cells, platelets and polymorphonuclear leukocytes, and reduced their reactive oxygen species, membrane lipid peroxidation and externalization of phosphatidylserine. These effects result in (a) reduced thalassemic RBC sensitivity to hemolysis and phagocytosis by macrophages; (b) improved PMN ability to generate oxidative burst - an intracellular mechanism of bacteriolysis, and (c) reduced platelet tendency to undergo activation, as reflected by fewer platelets carrying external phosphatidylserine. Oral administration of FPP to beta-thalassemic mice (50 mg/mouse/day for 3 months) and to patients (3 g x 3 times/day for 3 months), reduced all the above mentioned parameters of oxidative stress (Fibach 2010). Quite recently, this group has studied the effect of FPP on two groups of beta-thal patients: beta-thal, major and intermediate, (in Israel) and E-beta-thal (in Singapore). The results indicated that in both groups FPP treatment increased the content of reduced glutathione in red blood cells, and decreased their reactive oxygen species generation, membrane lipid peroxidation, and externalization of phosphatidylserine, indicating amelioration of their oxidative status. Further corroborative hints come from a concomitant case report of a beneficial administration of FPP to a patient with paroxymal nocturnal haemoglobinuria (Ghoti 2010).

3. a significant recovery of the latent malabsorption of vitamin B12 due to the interference of alcohol-induced oxidising effects on the gastric mucus at the binding site level between the intrinsic factor and cyanocobalamin (Marotta 2000).

Such evidence on the efficacy of FPP on oxidising stress induced by alcohol on the gastric mucus was also based on the associated evidence of the significant protective effect (macro and microscopic and biochemical as well) on healthy subjects, after



being administered a test-dose of ethanol (40 ml 80% ethanol) (Marotta 1999).

According to the previous results on the antigenotoxic effect and on the DNA *in vitro* protection by FPP from the group of Prof. Mori and more recently of Prof. Packer's group (Rimbach 2000) who highlighted the iron chelating effect, a new clinical trial was carried out on the gastric pre-cancerous lesions. A group of Italian and Japanese scientists proved in fact in a controlled and randomised study carried out for a six month period on patients suffering from chronic atrophic gastritis without the presence of *Helicobacter pylori* that both a multivitamin anti-oxidant mixture and high dosage vitamin E and FPP led to the reduction of a series of mucosal markers related to oxidative stress. However, FPP only managed to significantly reduce the two markers used as an expression of a pre-mutagenic biochemical changes, that is ornithine decarboxylase and 8-oxoguanine. This is one of the most frequently used biochemical markers relating to the DNA oxidative damage, since being a mutated base, it can lead to severe replication errors and anaplastic transformation) (Marotta 2004).

At the same time as the first clinical trials by the Kyoto Pasteur group on the immuno-modulating FPP effects and related reports (increase of the CD8+ and QOL score), on the positive beneficial effect which HIV-affected patients could benefit from (Mimaya 1998), a series of studies were started by Prof. M. Weksler of Cornell University in the USA (2002) and Prof. L. Montagnier, former director of the virology laboratory of the Pasteur Institute in Paris and present chairman of the World AIDS Research and Prevention Foundation. In a preliminary study, which is going to be enlarged further, it was proved that FPP administration for 3 weeks before the anti-flue vaccination in 10 hospitalised elderly patients consistently improved their specific antibody response in comparison to a control group which was only administered the vaccine. What is more, Prof. Montagnier's group (2003) carried out a study on the administration of FPP to poor immunological-responder HIV-positive patients and data from the open preliminary

research proved how such a compound, when associated with the anti-retroviral treatment, could significantly improve the CD4+ concentration as well as hemoglobineamia, weight increase and cenaesthesia. Such immune-modulating effects of FPP are now under consideration in a clinical research project aimed at ascertaining its potential properties in reducing the upper respiratory tract infections in the overall population and, principally, in elderly subjects (Marotta 2010)

Taking into account the overall previously mentioned data, one can also suggest that either the antioxidant effect of FPP and its beneficial microrrheological and macrophage activity-enhancing properties must have played a role in the successful study of the Comprehensive Wound Center, Department of Surgery at Ohio State University Medical Center, USA. Indeed, Drs Collard and Roy studied (2010) the effects of FPP on wound healing in adult obese diabetic (db/db) mice and found that FPP supplementation improved respiratory-burst function as well as inducible NO production together with a higher abundance of CD68 as well as CD31 at the wound site, suggesting effective recruitment of monocytes and an improved proangiogenic response. Interestingly, the authors also noted that FPP blunted the gain in blood glucose and this somehow parallels the intriguing clinical findings of the Italian researcher Danese (2006) who, by administering 3 grams of FPP daily, during lunch, for two months to 25 patients affected by type-2 diabetes mellitus under treatment with glybenclamide and to 25 controls, noticed a significant decrease in plasma sugar levels in both groups. This data needs further confirmation in a larger study but it may open new avenues to an integrated medical approach.

It goes without saying that it is extremely important to promote a diet rich in organically-grown vegetables, which if correctly enforced, offers the availability of micro-nutrients and anti-oxidants which are sufficient to comply with the body requirement in the case of normal health conditions and in the absence of important psychological and physical burdens. What simply



depended on common sense, was underlined a long ago by an authoritative international non-profit institute which stressed how a healthy diet should not be replaced by a non-controlled diet rich in supplements or food-like compounds such as vitamins, extracts or lyophilised products, mainly when the variability of such products in each single batch is uncontrolled or even worse, when no certified titration was carried out. However, the absence of specific and referenced studies on each single nutraceutical attempt cannot be counterbalanced by general data from the literature. Legislation and standards are still open about fortified foods supplemented by specific nutrients which deserve a discussion of each one. As previously underlined by Prof. Packer during an international congress (2003), we are facing a consistent evolution of anti-oxidants, implying the study of some of them from a simple scavenger function are instead able to interact in a complex way with the redox balance and immune-modulating network through a genomic adjustment.

In particular, a polymorphism-profile designed placebo-controlled study (Marotta 2006) carried out in 54 elderly patients without major diseases has shown that only the GSTM1 (-) subgroup was the one that, under FPP treatment, decreased lymphocyte 8-OHdG. Such preliminary data show that FPP is an advisable nutraceutical for improving antioxidant defences even without any overt antioxidant-deficiency state while helping explain some inconsistent results of prior interventional studies. A further study (Marotta 2007) showed that in a similar group of patients, there may occur a proinflammatory profile acting also as a downregulating factor for inducible Hsp70, particularly if Interleukin-6 promoter -174 G/C-negative while FPP supplementation at the dosage of 9g/day sublingually (a preferable route) proved to normalize such phenomena. The understanding of the complex intracellular/epigenomic mechanisms of FPP still needs further investigations and posttranscriptional/translation protein modifications that also occur need to be unfolded as Prof. Migliore from Pisa University in

addressing her research studies stated. Nonetheless, a recent small pilot study showing FPP-induced upregulation of gene expression of leukocyte GPx, SOD, catalase and hOGG1 (Marotta 2010) seems to suggest that a transcriptomic modification of key redox and DNA repair genes may offer further insights when attempting to interrelate “nutragenomics” to clinical phenomena.

FPP certainly represents a Functional Food, highly compliant with the novel features of the new nutrigenomic-driven action plan strategy aimed at disease risk reduction and successful integration within specific pharmacological treatments.

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Fermented Papaya Preparation (100 gr)

FPP/100g Composition. (Japan Food Res.Lab, Tokyo)

Carbohydrates	90.7 g	Arginine	16 mg
Moist	8.9 g	Lysine	6 mg
Proteins	0.3 g	Hystidine	5 mg
Fats	absent	Phenylalanine	11 mg
Ashes	0.1 g	Tyrosine	9 mg
Fibres	Absent	Leucine	18 mg
Vitamin B6	17 mcg	Isoleucine	9 mg
Pholic acid	2 mcg	Methionine	5 mg
Niacin	240 mcg	Valine	13 mg
Calcium	2.5 mg	Glycine	11mg
Potassium	16.9 mg	Proline	8 mg
Magnesium	4.6 mg	Gluthamic acid	37 mg
Copper	14 mcg	Serine	11 mg
Zinc	75 mcg	Treonine	8 mg
		Aspartic acid	27 mg
		Tryptophane	2 mg



# “Coaching Anti-aging, training for Rejuvenation and Longevity”

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Apart from its meaning as training, the word coaching originates in the term for a carriage, a vehicle. It is the coach that takes us from where we are in life to where we want to go.

As a personal trainer, my activity consists of helping clients achieve their personal and professional goals. To this end, I use the fastest, most powerful and effective methodology currently known for obtaining such results. This methodology is coaching, **a personal training process** that works with people's potential to create new versions of themselves capable of attaining the objectives they wish to reach.

In order to do so, **coaching helps to change belief systems, habits and behaviour that keep people anchored to the situations they are trying to overcome**. It is often said that if we go on doing what we have also done before, we will go on getting the same results as before. It is not reasonable to expect a different outcome if we are doing the same as always, so people learn in the course of their training to assimilate and integrate the new thoughts and skills they need to project themselves to where they want to go.

During sessions, coaches use tools that help clients become aware of their current situation, their difficulties and limitations, their options and opportunities. Then they select, from among these options, the ones that are most suitable for the action plan established in the form of a previously designed strategy. The clients have to undertake to achieve these steps before the next session. Thus, they are engaged in a process of learning by doing

that forms a feedback loop session after session until the final goal is achieved. In other words, **coaching promotes action, action generates learning, learning becomes new habits, and new habits build new identities**.

At the core of any coaching process is the realization and harmonious balance of the individual. Around this, there is an alliance between coaches and their clients an alliance based on trust, confidentiality and commitment. A commitment to carry out the actions identified in the strategy designed to reach the set target.

The goals of a coaching session can be as diverse as the people participating in it search of assistance: to quit smoking, write a book, change jobs, control stress, balance personal and professional lives, set up a business, keep fit, feel younger, etc. Any personal or professional goal someone may want to set but is difficult to achieve by him/herself.

In the years I have spent devoted to this profession, I have more and more often run into cases in which feeling younger has been the direct or indirect goal of the individuals I was working with. This has led me to design an **anti-aging coaching programme** using an anti-aging protocol as its reference framework, in other words **a suite of actions of demonstrated efficacy that are aimed at rejuvenation and longevity and that work comprehensively with the body, the mind and the emotions**.

The reason for wanting to achieve the goal of rejuvenation is not merely the need to respond to



the demands of a society like ours, which places enormous value on youthfulness, but rather the intimate desire of people to achieve physical wellbeing, calmness and mental clarity, a feeling of plenitude that is maintained throughout an ever longer lifetime.

While we await the progress of biogerontology over the next two decades, with the help of the appropriate technology, to be able to manipulate the distinctive factors of cellular aging, we already have available today a number of actions that have been shown to be effective in the realms of science, technology and body-mind integration therapies that allow us to design powerful anti-aging protocols capable of delaying and in many cases reversing the aging process.

This is the goal a growing number of people are setting for themselves and that is why it is important for professionals working in the area of anti-aging and longevity to join forces so that the diversity of steps aimed at achieving this common goal can be implemented safely, effectively and with the greatest co-ordination.

In this sense, **coaching has been shown to be the ideal procedure through which interested parties can change the beliefs and the behaviour that keep them clinging to the current paradigm of aging and then undertake in an orchestrated fashion the actions of the anti-aging protocol that best fit their circumstances.**

**Let us see how coaching does this:**

First of all, **by working with the belief that rejuvenation and staying young longer is possible.** The German scientist George Lintenberg said way back in the 18th century that nothing made us age faster than intensely thinking that we are getting old.

Nowadays, based on the latest discoveries of neuroscience that have shown the extraordinary power of our thoughts, we can state that **aging depends, in part, on our expectations.** If we expect our physical and mental abilities to

decline over time then this is very probably what will happen, but if we start believing that we will remain strong and with our full capacity to live longer and better then this in all likelihood will be what we will achieve. Beliefs can change biology, knowing that is the prelude to learning how, as can be inferred from the work of highly renowned neuroscientists such as Sam Harris, Antonio Damasio or Richard Davinsson.

**Secondly, coaching provides an appropriate, safe space in which people participating in anti-aging training can discover, explore, select and put into practice the possibilities offered by science, technology and body-mind integration therapies.**

It is important to highlight here the holistic nature of coaching that allows it to work in a co-ordinated and harmonious way with the mind, the body and the emotions as a single whole. These aspects are interrelated and affect each other to provide powerful synergies that should be taken advantage of.

**Aspects such as nutrition and detoxication, physical exercise and beneficial rest, mental stimulus for better specific cognitive functions such as memory, attention, concentration, language and reasoning, the anti-aging related medical treatments available in all specialities, safer and more effective technologies, stress control, managing emotions, self-esteem, neuroplasticity, bio-energetics, sexuality, attitude and image, creativity, positive thinking, sense of humour, empowerment and leadership of one's own life are, among others, the material with which we work during the anti-aging coaching sessions.**

**Together with the best exercises, treatments and therapies, at coaching sessions we also seek and select the most recommendable professionals in each area depending on**



**the interests, needs and possibilities of each client.** This is where the synergy of professionals working with a shared goal is multiplied: the regeneration of the body, of the mind and the revitalization of the spirit.

As well as helping to change limiting beliefs around aging and selecting the most appropriate actions and the most suitable professionals, **coaching thirdly provides the focus, structure and motivation needed to carry out all the actions and learning required** as anti-aging protocols are usually extensive and demanding programmes, making them difficult to implement successfully without the aid of professional trainers.

**A large dose of enthusiasm is needed to implement an anti-aging protocol. It can sometimes seem heavy going, a break with routine, giving up doing things that have been done for many years, it's a challenge that requires effort, determination and great confidence that may perhaps be deflated in a couple of weeks, just like so many things that have been started and left unfinished, good intentions that fell by the wayside. That's why having a coach is what marks the difference here.**

In other words, from my point of view the fundamental added value provided by coaching for this matter of anti-aging and longevity.

**At each stage of the process, the coach maintains motivation with highly practical information, feedback and support, helping clients to clarify their ideas, take decisions and design a realistic action plan that fits their lifestyle.**

Coaches drive their clients' process of change and transformation throughout a programme that lasts from three to six months, accompanying them at all times, **offering encouragement in the face of difficulties, identifying and neutralizing their internal saboteurs, acknowledging their improvements and consolidating their achievements, making it easier for these to be assimilated and integrated into their daily life.**

Although the effects of coaching start to be noted shortly after the start of training, a minimum of twelve weeks is normally needed to integrate these new lessons and articulating them with one another until the final outcome is reached. We give time to the body to respond and subsequently change.

Bodies behave holistically. When an improvement occurs in a biological or cognitive marker, it triggers a domino effect on the rest. Thus, if muscles are developed, the bone structure also improves, if we boost aerobic capacity it improves the immunological system, our psychological and biological states affect each other, etc.

**At the end of training, coaches propose clients should enter into a conscious pact with themselves, a commitment to their health and wellbeing, which will lead them henceforth to a new way of living their lives.**

If we reflect on what has been said, **it all implies an improvement in people's ability to be happy** as the actions in the anti-aging protocol trained during the coaching process for rejuvenation and longevity are designed to furnish health, physical wellbeing, clarity and peace of mind, beauty, creativity, enthusiasm and personal progress.

The fact that more and more people have access to the proposals of anti-aging coaching will help define and consolidate **the new paradigm in which the idea of aging will be replaced by the idea of longevity. Long life as a consequence of the advance of humankind.**

Evolved individuals understand their bodies, their minds and emotions and know how they work.



They have also learned to remain connected to their spiritual side. If someone in this frame of mind wishes to slow down aging and maintain vitality, this will be achieved.

**In this sense, devoting time, attention and resources and achieving rejuvenation has nothing to do with a frivolous, superficial fad but rather constitutes a more aware and happier philosophy of life for people who wish to live a full life for many years.** It is the responsibility of our generation to confront this paradigm shift with the technical and human resources provided by the current state of the art in science and knowledge.

I should like to conclude by mentioning that the welfare industry, focused on diminishing the advance of illness and reducing the effects of aging, will grow by 500% in the United States in 2010, according to the economist Paul Zane Pilzer.

Baby boomers, the generation now between 37 and 55 years of age, have generated great purchasing power. They represent 30% of the US population and 50% of the country's gross domestic product. These figures lead us to think that health, anti-aging and welfare may well represent the next revolution. With its vocation to accompany people and organizations in their processes of change and progress, coaching will play more than important role in that scenario.

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# High throughput assays for microbial detection in stem cell transplantation

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## Abstract

In the field of medicine, stem cell research is now the most exciting opportunity for the treatment of some diseases that until now have been incurable. The discovery of the regenerative properties of stem cells, both embryonic and adult, has been one of the main advances in Biology in the past years. This fact has contributed to the increase in the number of projects of human stem cell line derivation. In the process of obtaining stem cell lines for research or clinical application purposes, there are still some problems that should be resolved. Apart from the difficulties of cell line generation, the problems with the culture media and the possibility of tumoral degeneration, one of the main complications of stem cell cultures is the possibility of contamination. The main sources for this to occur are the stem cells themselves, the laboratory workers and the environment in which these cell lines are being handled. Due to the large quantity of microorganisms that could contaminate these cultures (e.g. bacteria, fungi, viruses and prions), all the stem cell research centres should incorporate an exhaustive microbiological and environmental program in order to both detect the possible pathogens and to avoid the transmission to the recipients. Any microbial contamination during the manufacturing process presents a serious hazard to recipients. In order to establish the sterility of stem cell cultures, there are several methods to study the possible presence of pathogens both in cell cultures and in the environment

of the manufacturing cell factories. Until now, the methods used for this purpose are mainly standard protocols based on the culture in liquid or solid growth media and incubated at different temperatures, reflecting conditions for human pathogen culture and environmental microorganisms. Other techniques used are PCR and RT-PCR methods, electron microscopy and in vitro inoculation and in vivo animal inoculation. However, some of these tests are long standing techniques and can take a number of weeks and can prove difficult to implement in the scheduling for release testing of time critical products. In this sense, the technologies based on the use of hybridization chips, using microarrays of immobilized oligonucleotides or antigens/antibodies/genes for microorganisms can provide a rapid and useful methodology for the identification of contaminants.

This review will discuss the methodology that could be used in stem cell research centres to assure the quality and biosafety of cell lines and biotechnological products and avoid the transmission of pathogens like bacteria, virus and prion particles.

## Production

The recent advances with respect to the regenerative capacity of stem cells are one of most important facts in the past years in Biology and Medicine. The discovery of the regenerative properties [1] of both, adult and embryonic stem cells, to provide a solid base in research for the future treatment of some diseases that until now have been incurable



(e.g. Parkinson disease, degenerative osteoarticular diseases, myocardial infarctation, etc). Since the main purpose of these kinds of cells is the application in human beings, the quality and safety of cultures for transplantation should be assured. This should be achieved by means of the standardization of processes involved and the implementation of quality control programs and methods which reflect best practices. However, these possible new treatments still have several problems such as the possibility of chromosomal alterations, the difficulties of obtention, culture and differentiation, ethical problems in embryonic stem cells and potential for tumorigenicity [2]. In this sense, one of the main problems of these cultures is the possibility of contamination and the transmission of pathogens to the recipients of these cell products. The most common potential forms of contamination are bacterias (including mycoplasma), yeasts and fungi. These microorganisms can be diagnosed on a routine basis [3, 4]. Moreover, some stem cell lines may contain an endogenous virus or can be contaminated with exogenous viruses or prion particles. Viral and prion contamination of cell cultures and feeder cells is the most challenging and serious outcome to diagnose, due to the difficulty involved in virus and prion detection and the potential to cause serious disease in the recipients. Thus, to ensure the provision of safe and reliable cells for these purposes, it is necessary to regulate the obtention, processing, testing, storage and distribution of all the cells that will be transplanted in the human body [5, 6]. Stem cell research and in particular stem cell banks must assure the quality and safety of the cell products in order to avoid transmissible diseases caused by all the above mentioned microorganisms. These centres should implement adequate quality assurance programs like the microbiological control program.

So, accredited stem cell research centres should standardize procedures and protocols used for microbiological testing and in validation programs to assure both the quality and safety of the cells. The microbiological control program should be dynamic and should include upgraded protocols related to the testing of all microorganisms that could be found in stem cell cultures. The three key areas in this microbiological control are:

(a) Clean room environmental control and plans for processing and storage areas.

(b) Microbiological control of stem cell lines, “feeder” cells, and other biotechnological products and reagents used in cell culture manufacturing.

(c) Microbiological screening of donors of biological material (e.g. embryos, bone marrow, umbilical cord, etc) in order to avoid the transmission of infectious agents. This chapter will discuss the most appropriate methodology that could be used in stem cell research centres in order to assure the safety and quality of cell and biotechnological products, and intends to give an overview of the microbiological controls that should be carried out on the cell lines in order to avoid the transmission of pathogens to the recipients.

### **Microbiological contamination sources in stem cell cultures bacterial, yeast and fungi contamination**

The most important source of contamination for bacterias, yeasts and fungi are the contaminated cells that are used as the primary starting material for cell culture. The cell lines recently imported and introduced in the culture laboratory represent the greatest source of contamination, depending on their culture history and past exposure to microorganisms. For this reason, providers of stem cell lines should be able to provide details of passage history and appropriate testing [7] and a “quarantine status” should be established. Apart from other stem cell lines, another important source of contamination are the laboratory workers who handle the cell cultures. Thus, the most prevalent contaminants will be the microorganisms present in the human skin flora (e.g. staphylococcus, grampositive bacilli, Candida). Moreover, these microorganisms are dispersed into the air [8], so another potential source of contamination is the environment in which the cultures are carried out, because these microorganisms can be deposited into the cultures. Finally, other potential sources of microbial contamination in stem cell cultures include culture media and reagents, biological products (bone marrow, embryos, and blood umbilical cord), glassware or apparatus (e.g. storage bottles and pipettes) and break-down in aseptic procedures. Viral contamination

The primary sources of potential viral contamination in cell cultures are infected animal products used to prepare biological reagents and culture media, contamination during manipulation by laboratory workers and biological products from donors used to obtain the stem cell lines (e.g.



embryos, bone marrow, blood umbilical cord, etc.). Until this moment, in embryonic stem cell cultures the use of “feeder” layers both of animal and human origin is necessary to maintain undifferentiated growth. This requirement provides intimate contact between the embryonic stem cells and the “feeder” cells; this contact could be used as the means to transmit pathogens or bioactive molecules in the final cell product. With respect to the use of “feeders” of animal origin, above all of murine origin, certain mouse viruses like lymphocytic choriomeningitis virus (LCMV), reovirus-3 and Hantaan virus have been diagnosed in mouse colonies [9] and these viruses have caused serious infection and include fatalities in laboratory staff [10, 11] and may also be transmitted in cell lines and reagents [12]. Moreover, there is also evidence that other mouse viruses like Sendai virus and lactic dehydrogenase virus are capable of infecting human or primates [13]. Other murine viruses like ectromelia virus, Toolan virus, Kilham rat virus, mouse adenovirus, mouse cytomegalovirus, etc., are capable of replicating *in vitro* in cells of human or primate origin although it is not known that cause serious human disease [13] (Table 1).

On the other hand, in the case of use of “feeder” cells of human origin [14], there are numerous microorganisms that could be transmitted to the recipient due to the fact that the human “feeder” cells can be infected by viruses and other pathogens. Some viruses can be transmitted and should be screened like HIV-1/II, HBV, HCV and CMV. Other viruses susceptible of testing are HTLV-I/II, HAV, HEV, and other potentially viral agents that could be transmitted and cause diseases include human herpesviruses (HHV-6, HHV-7, HHV-8, EBV, and HSV), parvovirus B19, TTV virus and human polyomaviruses (JC and BK virus). These viruses could remain latent and later some of these viruses could be involved in oncogenic transformation [15]. Moreover, depending on the geographical origin of human “feeder” cells, some pathogens can be transmitted to the recipients and could be susceptible of testing. Several examples of these microorganisms can be the lymphocytic choriomeningitis virus [16], the coronavirus (severe acute respiratory syndrome-SARS), HTLV-3/4 [17] and rabies virus [18]. In this sense, any new pathogen should be considered as a risk factor and a testing should be performed in order to avoid the possible transmission to human biological products.

### Prion particles contamination

The American and European regulatory agencies [19, 20] have identified the risk of prion particle transmission with the use of all products derived from ruminants (e.g. bovine foetal serum). For this reason, the introduction of techniques for testing the prionic protein (PrP<sup>Sc</sup>) is necessary. Cell cultures could be an adequate medium to allow the replication of PrP<sup>Sc</sup> and to maintain the infectivity [21]. On the other hand, several products that can be used to obtain stem cell lines (peripheral blood, bone marrow) could transmit the prion protein to the recipients of the final products [22].

### Control of donors of biological products

All human cells (including reproductive cells) could transmit any infectious diseases. So, the procedures and technical criteria to assess are the eligibility of the donors, the laboratory tests required and the criteria for acceptance of cells. In the recent past, donors have been screened for the human immunodeficiency virus (HIV-1 and 2), the hepatitis B virus (HBV) and the hepatitis C virus (HCV) [23]. However, current regulations require that donor screening includes tests for a range of viruses causing serious human diseases and that are known to be transmitted by blood and tissues (Table 2). For this reason, additional viruses should be included in the screening like HTLV-I/II, CMV, hepatitis A virus (HAV), hepatitis E virus (HEV) which is considered “cell associated virus”. This list could be different in each country where the national guidelines might vary. Although this list may be expanded even further in light of developing knowledge and technology, it is inevitable that a balance will be drawn between the associated risk of infection and the resources and time required to perform an ever-increasing list of virus tests. It is known that some viruses, such as herpesviruses [herpes simplex virus (HSV), Epstein-Barr virus (EBV), human herpesvirus (HHV-6, 7, 8), human Polyomaviruses (JC and BK viruses), parvovirus B19 and transfusion transmitted virus (TTV)] remain latent and detectable in humans from early childhood and are potential contaminants of cells from normal, healthy individuals [24-28]. As these viruses are so ubiquitous, there may be no clinical impact of their presence in transplanted tissues and cells for the majority of patients, and in certain cases, such as parvovirus B19, contamination of blood products up to a maximum limit (currently 10<sup>5</sup> genome equivalents per dose for B19) is acceptable for use in humans. However,



in some instances, these agents can prove to be of concern. Some microbial agents have marked variation in their geographical distribution, producing infectious epidemics in different areas like the lymphocytic choriomeningitis virus in USA [29] and the very recent outbreak of severe acute respiratory syndrome (SARS) virus in humans in South East Asia. Furthermore, two new retroviruses (HTLV-3 and HTLV-4) have been recently identified among African bush-meat hunters [30] and recent cases of transplant transmitted disease due to rabies virus in USA and Germany [18] have been reported, which clearly shows the potential emergence of new serious pathogens or the re-emergence of known pathogens. Accordingly, all infectious agents should be considered as potential pathogens and any new entity that arises should be considered as a contamination risk factor, and a risk balance for the use of contaminated products established, which should include consideration of the recipient's prior exposure and competence to fight infection. Moreover, specific tests may be required, and these may be developed for surveillance initiatives, and in the case of SARS, for which detection methods are being developed for the causative coronavirus agent [31]. With respect to non-viral agents, currently donor screening may include *Treponema pallidum* [32], and it is inevitable that there will be a requirement to test for a transmissible spongiform encephalopathies (TSEs), including Creutzfeldt-Jakob Disease (CJD), as sensitive and clinically validated assays become available [23, 33]. Moreover, the Food and Drugs Administration is proposing to require that donors of reproductive cells and tissue be tested for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* (which are known to have been transmitted through artificial insemination) and screened for other sexually transmitted and genitourinary disease that could contaminate reproductive cells and tissue during recovery and then be transmitted to the recipient of those cells or tissues (Table 2). The screening of the majority of microorganisms has been carried out using currently available tests based on the detection of donor antibodies to viral infection, but more recent research has shown that the detection of antibodies exclusively runs the risk of samples for tests being taken during an antibody-negative window period of these infections, where an individual has been exposed to viral infection and indeed can be viraemic [34]. This finding has led to the introduction of nucleic acid amplification techniques such as polymerase chain reaction (PCR) and the retrotranscriptase

polymerase chain reaction (RT-PCR) in which the presence of viruses can be observed by means of the amplification of sequences of viral genome. The addition of nucleic acid test methods to the screening of tissue donors, and to the testing of cell lines, could reduce the risks of these infections among recipients of stem cells [35].

### **Microbiological diagnosis in stem cell lines**

Cell lines may become contaminated with a large variety of bacterial, yeasts and fungal agents. Potential sources of contamination include other cell lines, laboratory conditions and workers poorly trained in core areas such as aseptic techniques and good laboratory practice. Most of these contaminants become grossly obvious if the cells are grown in medium without antimicrobial agents. However, the routine use of such agents should be avoidable with good sterile cell culture techniques and clean cultures. The use of cells and reagents of known origin and quality alone is not sufficient to guarantee the quality of product (cell stock or culture products); it is necessary to demonstrate quality throughout the production process and also in the final product. Screening aids the early detection of contamination since all manipulations are a potential source of contamination. For any reasonable quality control program involving cell lines it is absolutely necessary to include routine culture tests for bacteria, yeast, fungi, virus and prion particles. However, the fundamental element in avoiding contamination and laboratory infection is aseptic technique and this should be a major component of the training in any tissue and cell culture laboratory. Moreover, one of the most important approaches to avoid contamination problems is to avoid the routine use of antimicrobials. These are not a substitute for aseptic techniques and may only mask contamination which may reappear at a later date. Finally, providers of cell lines should be able to provide details of passage history and appropriate testing [36]. Once the cell lines have been obtained from a reliable source, it is important at the earliest stage to establish a master bank and apply appropriate tests to rule out microbiological contaminants and to confirm the authenticity of cell culture [7]. Bacterial, yeast and fungal contamination

All these contaminants are generally visible to the naked eye and detected by an increase in turbidity and colour change of the culture medium; this change of colour is due to a change in pH. Early detection of contamination is possible due to daily



microscopic observation of cell cultures as part of a routine screening in the laboratory. The presence of contamination enables appropriate action to be taken as soon as the signs of contamination become apparent to avoid contamination of other cultures. In addition to daily culture observation, specific tests for the detection of bacteria and other contaminants must be used as part of a routine quality control screening procedure. It is necessary to detect low level of contamination, so samples from the stem cell cultures and their products should be inoculated either into liquid (e.g. fluid thioglycollate medium, tryptone soya broth) or onto solid (e.g. blood agar, Sabouraud's dextrose agar, malt extract agar) growth media. European and United States Pharmacopeia have given standard protocols for such testing [3, 4, 37]. These media should be incubated at different temperatures, reflecting conditions for human pathogen culture (e.g. 32-35¼ C) and environmental microorganisms with lower growth temperature optima (e.g. 25-30¼ C). These, should be incubated for 7-14 days in microbiological culture incubators, depending on the specific testing standard used. Moreover, the media should be tested using reference strains of potential contaminants. Later, all these microorganisms isolated should be identified and confirmed using confirmatory tests (e.g. Gram stain) (Figure 1). Microbial identification systems are either manual (conventional procedures) or automated. Automated systems offer the advantage of hands-off approach, allowing more time for the laboratory technician to carry out other duties; manual methods offer the advantage of using the analytical skills of the technologists for reading and interpreting the tests. These procedures should be carried out in the microbiology laboratory isolated from the cell culture laboratory. Mycoplasma contamination

Mycoplasma is a generic term given to microorganisms of the order Mycoplasmatales that can infect cell cultures. Those that belong to the families Mycoplasmataceae (*Mycoplasma*) and Acholeplasmataceae (*Acholeplasma*) are of particular interest. These microorganisms are the smallest free-living selfreplicating prokaryotes (0.3 µm in diameter) and can be observed as filamentous or coccid forms. They lack a cell wall and lack the ability to synthesize one. The first observation of mycoplasma infection of cell cultures was by Robinson et al in 1956 [38]. Although mycoplasma contamination of primary cultures and continuous cell lines has been known since this date and for several decades, it still represents a significant problem in cell

culture. This might be due to the inability of workers to detect these contaminants by microscopic observation. Mycoplasma contamination will also fail to be detected during routine sterility testing for other bacterial, fungi or yeast contaminations due to their fastidious growth requirements. The incidence of such infection has since been found to vary from laboratory to laboratory. At present, the surveys of cell culture laboratories and cell banks substantiate that on average 15-35% of all cell cultures may be contaminated with mycoplasma [39, 40]. Mycoplasma contamination is usually caused in 98% by seven species: *Mycoplasma hyorhinis*, *Mycoplasma arginini*, *Mycoplasma orale*, *Mycoplasma salivarium*, *Mycoplasma fermentans*, *Mycoplasma hominis* and *Acholeplasma laidlawii* [41]. In cell cultures, Mycoplasmas have shown several effects, including induction of chromosome aberrations [42], induction of morphological alterations (including cytopathology) [43], interference in the rate of growth of cells [44], influence of nucleic acid and amino acid metabolism [45, 46] and induction of membrane alteration and even cell transformation [47, 48]. The contamination mainly spreads from one culture to another, transmitted by aerosols or by poor cell culture practice. Therefore, good laboratory practice and frequent monitoring of the cell lines is mandatory for every laboratory engaged in research using cell cultures [49, 50]. A range of assay techniques is available for the detection of mycoplasma contamination, and it is usually

recommended to use at least two techniques for testing cell banks to ensure optimum sensitivity and specificity. These include culture, PCR, indirect DNA staining and non-isotopic detection system.

#### **New methods on the future: microarrays, protein arrays and biosensors**

DNA arrays consist of nucleic acid targets immobilised on a substrate of glass, nitrocellulose or nylon membrane. The arrays of high density can have thousands of probes per cm<sup>2</sup> and are known as microarrays. The microarrays synthesised on silicon surface are known as DNA chips. Microbial diagnostic microarrays consist of nucleic acid probes, and each probe is specific for a strain, species or genus. These platforms can be used as a complement of culture methods for identification of microorganisms [70]. There are two kinds of microarrays: the PCR product based DNA microarrays and the oligonucleotide-based DNA



microarrays. In the microarrays based on the PCR product, the first step is the design of primers to amplify specific regions of interest. All probes on a microarray should be highly specific for their target, should bind efficiently to target sequences to allow the detection of low targets, and should display similar hybridization behaviour. After this, whole genome PCR amplification should be carried out. The purified PCR products are spotted onto membranes or coated glass slides. The DNA microarrays based on oligonucleotides use oligonucleotides synthesized on a glass surface. In this case, no reverse transcription or amplification steps are involved. The main advantages of these last microarrays are that there is less likelihood for contamination due to non specific amplification and mishandling, that there is a reduction in cross-hybridization, that it is easier to normalize the oligonucleotide concentrations and that high density oligonucleotide arrays enable high coverage of the genome. From the microbiological point of view, the main advantage of DNA microarrays for stem cell cultures is the possibility of detection and analysis of hundreds or thousands of microorganisms in a single experiment. Aside from their costs and the difficulties associated with designing and making a suitable array, the majority of the problems with their use are related to quality control, due to the difficulties of standardisation and reproducibility associated with the large number of probes on an array. Moreover, there may be contamination if PCR amplicons are used, as large amounts of these products will be made. There may also be problems with the hybridization reaction. Other similar new technologies include protein arrays and biosensors. Proteomics include different methods to identify all the proteins present in a cell or tissue at a given time. Protein arrays are prepared with antigens or antibodies bound to a solid phase and used to capture specific antibodies or antigens, respectively [71]. The slides are incubated with serum samples and later with fluorescently labelled secondary antibodies. A microarray platform of oligosaccharides on nitrocellulose has been developed for capturing carbohydrate proteins, and it may be possible to develop this for microbial diagnosis [72].

Biosensors are defined as small devices which use biological reactions to detect targets [73]. The target bind to a ligand immobilized on a solid phase, and the hybridization of the probe and target is detected by electronic means. Biocatalytic arrays involve an immobilized enzyme being used to

recognize the substrate of the enzyme which is the target of the array. The reaction might be recognized either by colorimetric means or by electronic transducer. Finally, the amount of data generated by microarray experiments is very large. These data require specialised software to assess the patterns. There is no single universally accepted method of statistically analysing microarray data and each method has advantages and disadvantages.

## Conclusions

Stem cell research centres are the establishments that must guarantee the existence of an appropriate source of cell lines in a standardized way for their use in research and/or human therapies through clinical trials [74, 75]. Moreover, these establishments must assure the quality and biosafety of biological products for use in cell therapy [76]. One of the main risks associated with the use of cell lines and biological products in stem cell cultures and cell therapy is related to cell contamination [77]. Potential sources of microbial contamination include reagents, laboratory environment and other cell lines. Routine screening of cell lines helps in the early detection of contamination, since any kind of manipulation is a potential source of contamination. The assurance of the quality of the cell lines requires authentication, characterization and accurate description and to test the possible presence of microorganisms like bacteria (include mycoplasma), virus, prions, fungi and yeast [76, 77]. Selecting and testing of stem cell lines and biotechnological products is one part of a strategy for establishing a microbiological safety program (Table 3). There are still several problems to be solved before the final and routine application in humans is carried out. While the technology to avoid the animal products in the culture processing is being developed, the safety of the animal

and/or human products used in the cell cultures with respect to microorganism contamination should be obtained for application of an exhaustive program of microbiological screening by means of the combination of the above mentioned diagnostic methods.

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#### FIGURE LEGENDS

Figure 1.-Gram stains in which it can observe: A.- Gram positive cocci (*Staphylococcus epidermidis*) B.-Gram positive bacilli (*Corynebacterium* spp) C.-Gram negative bacilli (*Escherichia coli*) D.- Yeast (*Candida albicans*)

Figure 2.-In this microphotograph, some strains of *Mycoplasma* spp with a characteristic “fried egg” appearance are shown (Magnification 10x).

Figure 3.-Electron microscopy of murine “feeder” cells, that shows a viral particle corresponding to Murine Leukemia Virus (Magnification 50,000x)



**Table 1.- Murine viruses that can be transmitted to human cells (Obtained from EMEA, 1998).**

Viruses with capacity for infecting humans	Viruses with no evidence for infecting humans
Sendai virus *	Ectromelia virus *
Lymphocytic Choriomeningitis virus *	Kilham rat virus *
Reovirus type 3 *	Minute virus of mice *
Lactic Dehydrogenase virus *	Mouse adenovirus *
Hantaan virus *	Mouse cytomegalovirus *
	Mouse encephalomyelitis virus
	Mouse hepatitis virus
	Mouse rotavirus *
	Polyoma virus
	Pneumonia virus of mice *
	Rat coronavirus
	Sialoacryoadenitis virus
	Toolan virus *
	Thymic virus

\* Viruses capable of replicating *in vitro* in cells of human origin

**Table 2.- Infections, screening tests and indications for donors of biological cell, tissues and organs (Modified from US Food and Drugs Administration, 1999).**

Microorganism	Indication	Test
HIV-1/2	Always	HIV-1/2 antibody; MT
Hepatitis B	Always	HBsAg <sup>1, 2</sup> ; anti-core HBC; MT
Hepatitis C	Always	HCV antibody; MT
<i>Treponema pallidum</i>	Always	Treponemal-specific antibody (TPHA)
HTLV-I/II	Donor risk factors	Anti HTLV-I/II
CMV	For solid organ, allogenic bone marrow donors	CMV antibody
Toxoplasma	Heart, liver and bone marrow donors	Toxoplasma antibody
Epstein-Barr virus	Donor risk factors	MT
Prions	Donor risk factors	ELISA, W-B, MT
<i>Neisseria gonorrhoeae</i>	Donors of reproductive cells	Bacterial culture
<i>Chlamydia trachomatis</i>	Donors of reproductive cells	Ig G anti- <i>Chlamydia</i>

**HIV:** Human immunodeficiency virus; **MT:** Molecular techniques (nucleic acid tests); **HTLV:** Human T lymphocytotropic virus; **TPHA:** Treponemal hemagglutination; **CMV:** Cytomegalovirus; **ELISA:** Enzyme linked immunoassay; **WB:** Western-blot

**Table 3.- Summary of the main contaminants and diagnosis methods in stem cell cultures.**

Microorganisms	Bacterias	Mycoplasma	Fungi	Yeasts	Viruses	Prion particles
<b>Diagnosis methods</b>	Culture in solid and liquid media Microarrays *	Culture in specific solid and liquid media (PPLO) PCR Indirect DNA staining (Hoechst stain) Non-isotopic detection system Microarrays *	Culture in specific solid and liquid media (Sabouraud agar) Microarrays *	Culture in specific solid and liquid media (Sabouraud agar) Microarrays *	Electron microscopy Cell culture Antigen viral detection Molecular methods In vivo animal inoculation Microarrays *	Detection of antibodies (ELISA, WB) Cyclic amplification of protein misfolding Microarrays *

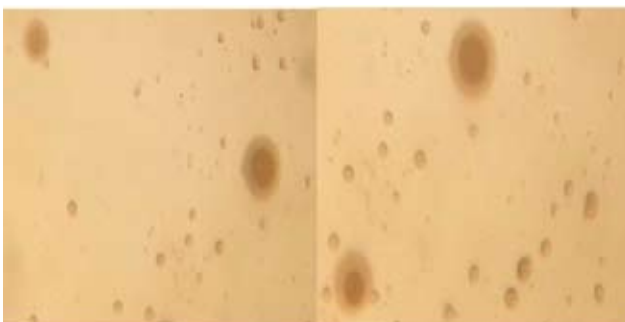
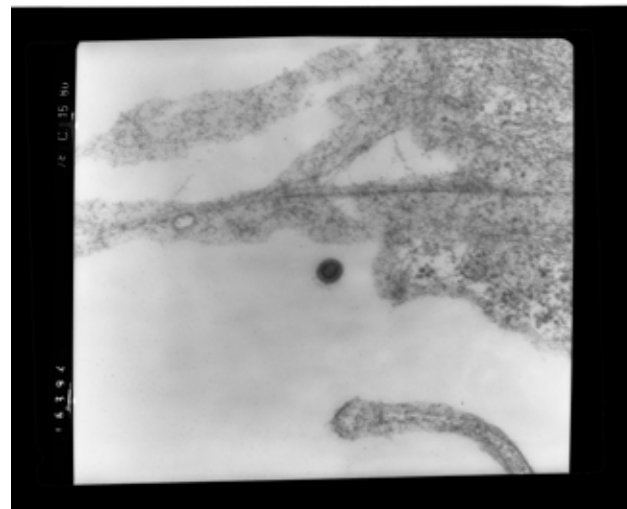
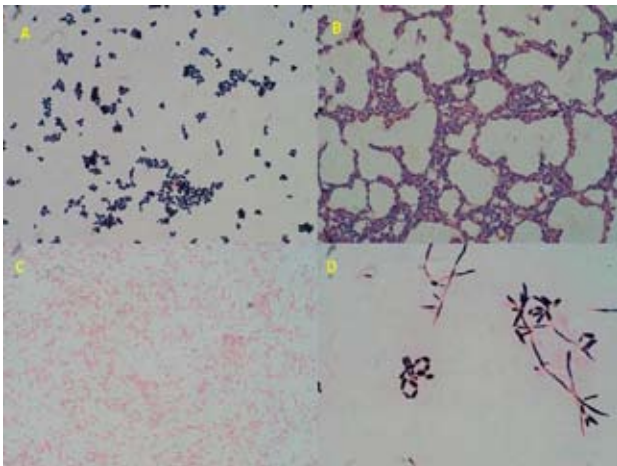
\* **Microarrays:** need validation for sensitivity and specificity.

**PPLO:** pleuropneumonia-like organism

**PCR:** polymerase chain reaction

**ELISA:** Enzyme linked immunoassay

**WB:** Western-blot





## The prevention and treatment of lung diseases in relation to the ageing processes

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Ageing is a gradual and adaptive process that is characterised by a relative decrease of homeostatic regulation due to morphological, physiological, biochemical, and psychological changes brought about by ageing and the accumulated wear and tear on the body over a person's lifetime in a given setting; hence, there is an increased risk of severe and chronic conditions that could develop<sup>1</sup>. These are due to morphological, physiological, biochemical and psychological changes that affect human beings as a result of the passage of time. (Binet & Bourlière, 1982).

Ageing is a characteristic of each species and is intrinsic to their habits and to their setting. It is not rare to find humans and Galapagos turtles living beyond the maximum life-span potential of 100 years, while mice live barely longer than three years of age.

The characteristics of ageing manifest themselves as an individualised process because not everybody ages in the same way; furthermore it is a multi-factor process caused by genetic, environmental and lifestyle factors. These characteristics also account for the body's vulnerability (susceptibility) to developing more severe and chronic conditions.

Even though more characteristics of the changes in the body brought about by age could be elaborated upon, they may be briefly summarised in the following:

1. - Changes in tissue composition and structure as age increases.
2. - A progressive weakening in the physiological function of the tissue.
3. - A reduction in the adaptive response to environmental stimuli.
4. - Greater susceptibility and vulnerability to illness.
5. - Increase in the mortality rate after the individual has attained maturity.

If we revise the theories on the ageing process, we find that there is no single theory that predicts longevity and ageing (there are human beings that have lived over the age of 122), nor is there one that explains, from a phenotype or genotype perspective, the variability shown by certain individuals in terms of ageing and longevity. Of noteworthy interest is the youthful and healthy appearance we can observe in some of the elderly, while others with similar ages present a decrepit aspect .

Genetic markers explain only 35% of the variability in longevity in people while longevity per se depends on an excess of 65% on environmental factors (lifestyle, habits, stress, etc.).

There are many theories that attempt to explain the ageing process. In 1961, Hayflick & Miihead proposed the so-called cellular clock that places a limit on the division of cells during a life time.



The proposal of error-catastrophe (Orgel 1963) attributed cellular death to the accumulation of errors in protein synthesis. Immunity Depression was an expression of the faulty functioning of the immunological and endocrine systems (Burnet 190) due to a modification of the T-lymphocytes, the weakening of immunity and senescence. Finally, The Free Radical Theory postulates that ageing is the result of damage to chromosomes and to certain macromolecules (collagen, elastin, mucopolysaccharides, lipids, etc.) by oxygen-free radicals over a prolonged period of time. (Denham Harman 1956–Jaume Miquel 1980).

### **Ageing and the respiratory function**

In the respiratory system, the changes caused by the ageing process can be explained by the slow and progressive reduction of various factors<sup>2</sup>

- a) A reduction of elastic retraction pressure in the lung attributed to changes in collagen configuration and the presence of elastin.
- b) A loss of thoracic wall compliance/insufflations caused by calcium-induced joint calcifications, particularly in the vertebrae-rib region and the intervertebral discs. Furthermore, the thoracic cavity changes in shape due to osteoporosis and vertebral collapse.
- c) A loss of strength in the respiratory muscles; hypoplasia of muscle fibres and a decrease in the number of peripheral neurons, interferences in the active transport of the calcium ion in the sarcoplasmic reticulum, a reduction in heavy chain myosin synthesis and a downturn in ATP mitochondria production.
- d) A reduction in the hypoxic response and to hypercapnia with an increased prevalence of sleep apnoea with age.
  - e) Reduction in the perception of increase of resistance in the airways. Elderly adults tend to reduce their perception of bronchial constriction caused by methacholine<sup>3</sup>
  - f) Decrease of purification in the mucociliary function.

These physiological changes that take place in the lungs of the elderly mean that in stress situations such as respiratory tract infections, coupled with failure in the left ventricle and where further ventilation is needed, the elderly with a low pulmonary reserve or chronic lung condition are at high risk of developing acute respiratory failure.

The prevention and treatment of lung conditions in relation to ageing

Respiratory illnesses are likely to play a greater role in the changes we understand as physiological ageing. Phenomena such as inflammation, the effects of excessive oxidant production in tissues, otherwise known as oxidative stress, as well as some repair mechanisms present in the lung, form part of many pneumological conditions<sup>4</sup>.

The incidence of inflammation and oxidation phenomena are common to virtually all respiratory conditions and it would be beyond the scope of this paper to deal with all of them; we have focused however on Chronic Obstructive Pulmonary Disease (COPD) and Obstructive Sleep Apnoeas (OSA) as paradigms of the conditions of interest to us.

### **Copd and its influence in ageing**

Chronic Obstructive Pulmonary Disease (COPD) is one of the most frequent causes of death and disablement today, but it was not until recently that there was renewed interest in the need to closely examine the most recently revealed cellular and molecular mechanisms that would fit in with an etiopathogenic perspective.

COPD is characterised by an inflammatory condition that leads to an obstruction that is mostly irreversible in the smaller airways; there is also damage to the alveolar walls (emphysema). In its pathogenesis, genetic and, above all environmental elements, in particular, the exposure to tobacco smoke intervenes. Various mechanisms are involved in the development of the disease: the inflow of inflammatory cells to the lung which in turn produce a chronic inflammation of the airways, an imbalance between proteolytic and antiproteolytic



activity that leads to tissue destruction and oxidative stress. More recently, the apoptosis of structural cells has been pointed out. These processes are involved in both the diseases as well as in processes that however physiological they may indeed be, result in non-desirable outcomes (e.g. ageing)<sup>4,5</sup>.

Chronic Obstructive Pulmonary Disease (COPD), according to the Spanish Society for Respiratory Pathology (known as SEPAR), is characterised by a virtually irreversible chronic obstruction of the airways and is associated with an anomalous inflammatory reaction, particularly when subjects are exposed to tobacco smoke. This etiological approach influenced by the effects of tobacco has offered doctors new scope and possibilities in the treatment and prevention of this disease<sup>6</sup>.

In Spain 15.1% of male smokers and 5.7% of female smokers in the 40 to 80 age bracket are afflicted with COPD. It is the 5th highest cause of mortality in Spain and the 4th highest in the world. It is believed that 73% of patients have not been diagnosed and it is the only cause of death that has risen over recent years compared to other serious causes such as cardio- and cerebro-vascular diseases<sup>7</sup>.

The functional diagnosis for a patient presenting compatible symptoms (a smoker) is carried out with a spirometer. A study carried out on a random population sample established an FEV<sub>1</sub>/FVC ratio greater than 0.70 in all age groups and this finding would validate the use of this numerical relationship in diagnosis of the illness. (Figure 1).

The non-smoking patient however has an annual loss of 35 ml. compared to smokers who lose 50 to 100 ml annually. As the forced expiratory volume in the first second (FEV<sub>1</sub>), the symptoms characteristic of the illness appear; there is a subsequent deterioration of quality of life related to health, respiratory failure develops and there is premature death<sup>8</sup> (Figure 2).

The inflammatory response to tobacco smoke toxins is crucial in the pathogenic mechanisms in chronic obstructive pulmonary disease (COPD).

The quantity of oxidants present in tobacco smoke, in addition to those generated by the inflammatory

activity mean that the capacity for the physiological mechanisms to afford antioxidant protection is overburdened, resulting in oxidative stress. Such stress can bring about direct damage to structural cells, increase the inflammation and favour the proteolytic degradation of tissues as the antiprotease systems are inhibited.

Furthermore, oxidative stress can negatively modify the regulation of genetic expression and can also interfere with the systems that remodel chromatin and hence a critical mechanism in steroid medication is blocked<sup>9</sup>.

As a consequence of the inflammatory and oxidative phenomenon, the patient with COPD presents a co-morbidity and systemic ailment that go beyond the simple functional malady resulting from a progressive reduction of FEV<sub>1</sub>. This is why there has been a recent tendency to abandon the traditional concept of COPD and hence the new understanding that we are dealing with a complex inflammatory and multidimensional disease that has opened up new and optimistic treatment expectations<sup>10</sup>. In a recent study, the increment in the systemic inflammation marker, which is the C-reactive protein, increased the risk of an eventual cardiovascular episode and is a predictor of mortality independent of the value of FEV<sub>1</sub><sup>11</sup>.

First and foremost, the role of prevention in potential patients is geared at their giving up the habit of smoking. Following from this, an early functional diagnosis is carried out in order to gauge the severity of the illness and then bronchodilating treatment is required to relieve symptoms.

### **Obstructive sleep apnoea and its influence on ageing**

Obstructive Sleep Apnoeas-Hypopnoea Syndrome (OSAHS or OSA) is characterised by the presence of respiratory pauses that are a consequence of the partial (hypopnoeas) or total (apnoeas) obstruction of the upper airways; this is due to blockage after the collapse of the softer portions of the throat during sleep; it causes a pause in breathing over 10 seconds long and such pauses may occur 10 or more times in the space of an hour. This situa-



tion almost always causes a reduction in the arterial saturation of oxygen<sup>11</sup>.

For these patients, the obstruction leads to a major decrease in the quantity of oxygen available in the blood and, consequently, to several unconscious occurrences of waking up. There is a resulting lack of restful sleep that is reflected in excessive somnolence and tiredness during the day.

OSA is quite a common disease that affects 4 to 6% of males and 2 to 4% of women in their middle age and its frequency increases with age<sup>12</sup>. Similarly, this syndrome occurs in 1 to 3% of children. It has been shown that OSAHS diminishes the quality of life, can cause arterial hypertension, cardio and cerebrovascular diseases and increases the risk of death: it also increases the likelihood of traffic, workplace and home accidents.

A conventional polysomnographic study (CPS) at night time is a standard procedure in the detection and evaluation of the severity of the syndrome of nocturnal apnoea. It involves the simultaneous registering of neurophysiologic and respiratory variables that enable us to evaluate the quantity and quality of sleep, as well as to identify the various respiratory episodes and their cardio-respiratory and neurophysiologic repercussions.

Respiratory Polygraphy (RP) is a simpler and cheaper diagnostic method that can be used in the patient's home; it consists of the analysis of both respiratory and cardiac variables without evaluating the neurophysiologic parameters. It is now an accepted approach in the diagnosis of obstructive sleep apnoea/hypopnoea syndrome. In non-complicated cases, pulse oximetry can offer a high degree of correlation with RP data (Figure 3).

It is clear, hence, that episodes of apnoea in OSAHS, which are associated with oxygen desaturation following from those of reoxygenation (Figure 3), are similar to the ischemia-reperfusion and its consequences (Oxidative Stress)<sup>13</sup> described in animals; this would explain the increased incidence of cardiovascular diseases suffered by these patients (arterial hypertension, hypercholesterolemia, coronary heart disease, etc)<sup>14</sup>.

In light of the risks and complications that these patients may face, the advised manner of proceeding would be an early diagnosis; it would otherwise confirm suspicions that hover over snorers who report daytime hypersomnolence and who present sleep apnoeas; such patients would be admitted to specialised units for a definitive diagnosis and a correction of those factors that may be common to them and which might impair their evolution such as obesity.

With a confirmed diagnosis, such patients should be treated with CPAP (Continuous Positive Airway Pressure) that will administer positive pressure on the airway, hence avoiding its closure and restarting the air flow (Figure 4). Should it be necessary, at this point concomitant anatomical factors can be corrected (tonsils, uvula hypertrophy, micrognathia, etc) that may contribute to a worsening of the condition.

### **The influence of ageing in respiratory infections: immunosenescence**

Community-based pneumonia is the main cause of death caused by infectious diseases in the elderly population; it ranks within the top 10 overall causes of mortality in this age group. It is well known that patients over 65 are presenting an increased incidence of pneumonias.

The greater predisposition in this population to endure these kinds of infections is explained by various mechanisms<sup>15</sup>:

1- Co-morbidities: Elderly patients tend to group together various co-morbidities that may be aggravated by infection or perhaps lead to some kind of negative change in the host's defence mechanisms, as well as the factors that depend on the medication assigned to this kind of chronic patients.

2- A negative change in local defence mechanisms: With ageing, the ability to expectorate diminishes and there are negative changes in mucociliary transport with a reduction of the bacteriologic clearing of the respiratory tract. There may also be a reduction of the cough reflex as well as an increase in oropharíngeaic aspirations in this population.



These patients may experience a greater incidence of oropharyngeal colonisation by *Staphylococcus aureus* and gram negative enterobacteriaceae; ensuing serious infections are also frequently associated with resistance to antibodies.

3- Immunosenescence: The immune system also goes through the effects of ageing and a reduction of cytotoxic T-lymphocytes (CTL) and of certain interleukins (IFN- $\gamma$ ) have been observed; furthermore there was a reduction in antibody production and a low affinity capacity in relation to antigenic stimulus as well as a reduction in the phagocyte function of the macrophages.

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**Figures:**

Figure 1: Spirometric readings indicating a typical obstruction.

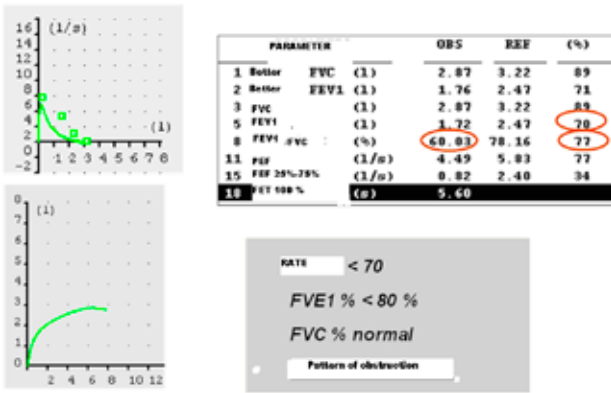


Figure 4: Correction of apnoeas with use of Continuous Positive Airway Pressure (CPAP).

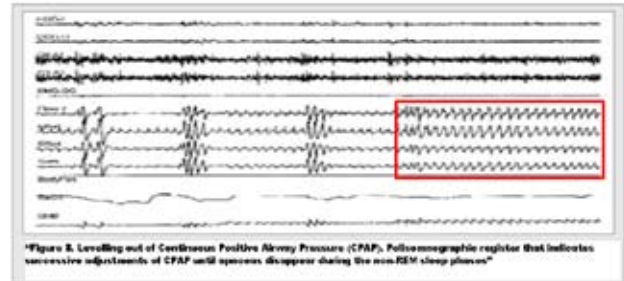


Figure 2: Reduction of FEV<sub>1</sub> and its prognosis.

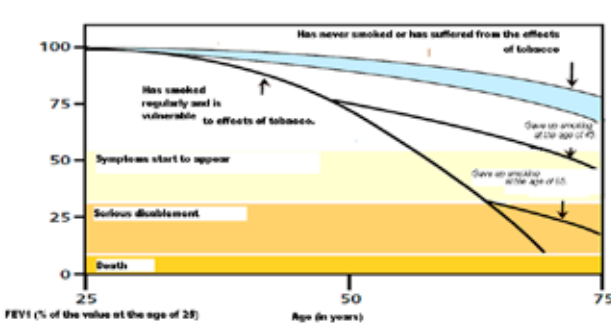
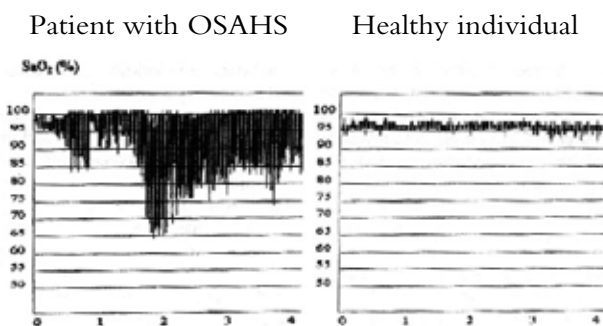


Figure 3: Continuous nocturnal oximetric register of a patient with Obstructive Sleep Apnoea-Hypopnoea Syndrome or OSAHS (de-saturations of (SaO<sub>2</sub>) show up and can reach a level as high as 65%) and a register of a healthy individual.





# Tapir syndrome

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## Keywords

**Syndrome, botulinic toxin, fillers, liftings, inexpressivity, tapir, proboscidea.**

## Abstract

The combination of using botulinic toxin and filling substances associated with ageing surgery in the maxillofacial and cervical oral fields [1][2][3][4], frequently shows optimum results, though not always, the procedures being used essentially for cosmetic purposes. This use is, and most likely, going to be on the increase even though the right correcting measures are not always taken into account in this often inappropriate mix. Caution about the mix should come fundamentally from alerted professionals, non professionals and from an informed society, forcing patients to see the possible inconveniences derived from a dubious scientific procedure of medicine and procedure. From what we observe in determined social and/or professional spheres no one wants his/her ageing to be or become visible to others. A wrinkle from the point of view of some can be as socially unacceptable as being untidy and from this same frame of mind that wrinkle can be a reason for day to day rejection of a person or an item that can even reduce the value of a person's CV. We have been able to acknowledge, without difficulty, especially from media examples, and also in our clinical practices, that features of preferred facial predominance are associated with specific psychosomatic characteristics of individuals in line for these procedures, which suggested to us the utility of joining clinical and psychosomatic characteristics into one syndrome.

## Material and methods

Syndromes primarily appear in clinical practices when resemblances are observed and noted among patients who often share a specific illness. A shared illness is not absolutely necessary for the circumstances but there should be enough points of reference that an observant physician can be aware of them and from there to be able to put in logical sequence the coincidences in a way that this physician would practically be forced to create a group of individuals with similar symptomatic data and pathologically similar features. This could be a group from which later more precise subgroups could be itemized that would serve to explain that various symptoms, or new discoveries, appear to match specific pathologies. It would be like a disaster drawer from where one could pull out easily obtained information that could deepen previous knowledge and bring to light heretofore unforeseen connections of what appears to be coincidence, and could then further develop the inherent possibilities of the material.

When we realized that patients who had had cosmetic facial treatments essentially developed an obvious look alike among them, the idea evolved of classifying them into a syndrome that called for a connecting family name. The first idea was that of naming it Botox Syndrome (a combo of botulin and toxin) as a starting point; meanwhile, the similarity of patients to a natural representative of the animal kingdom occurred to us: the Tapir. From there we created a huge group of individuals, not only patients from the clinics, but from social ambiances, even from the street and especially from television.



Along with patients, it is not difficult to see media characters, every day, with predominant facial characteristic of non-expressiveness and without dynamic that is shocking because it affects not only professionals but seems to be even more prevalent in common mortals of practically any age or social condition. For the moment, however, this condition seems to be found most often in young women and those affected seem to be younger all the time. The reader will most probably agree with us that in the case study we are about to present the problem is clear enough. It is not even worth the trouble of looking for a determined number of patients since more than a medical problem it is an evident social problem and “cases” can be seen everywhere. The mission of our work is to alert not only professionals and the public at large but the affected persons of this syndrome since they may not realize their aspect could be shocking to others causing interpersonal problems that seem to them to be ordinary, happening daily, seemingly natural when in fact their experience is massive rejection.

Since Cicero (106–43 B.C.) wrote what is considered a proverb *The face is a picture of the mind as the eyes are its interpreter*, there have been handed down variations on the theme from every culture with an oral tradition or a written language. To the point that nowadays we take for granted that the face is the mirror of the soul, reflecting with relative clarity the life history of every individual, showing each one of us has searched to some degree or another for our essence, our core existence, in order to possess a deep personal relationship, first with ourselves and then with others. In the case of the patients we are going to pin point, the personal relationships with others can become enormously deteriorated because of the lack of daily basic tools that could help them recognize immediately the innate and unique characteristics that should appear in the persons close to them, that seem not to be signaled and if they are signaled they are clearly not picked up.

As to the conceptual description of the Tapir Syndrome, which for the moment the bibliography covers only one case [10], it will be more detailed than at the beginning but for practical reasons will not be excessively explored due to the fact that by nature the syndrome term usually demands a large number of participants in the case study based on clinical, anatomophysiological and even psychosomatic symptoms in order to make valid compa-

rison. Because of that we should include in the same term diseases or developments such as the presence of angiomata and/or lymphoangioma-ta and other congenital malformations preferably situated in the nasal region and in the upper lip, places where other multiple temporary processes are or possibly what could be allergic reactions or edema, post-traumatic reactions or a parasitic procedure that can affect not only the soft parts of the mentioned region, but also neighboring areas that could determine neurofibronatotic profiles, or the affected area could possibly be systemic. In the same way, this can have a special importance in the exposition of our Syndrome, the known Melkersson-Rosenthal Syndrome (Dominant Autosomic Condition) where the neurological disorder and other symptoms mimic the real Tapir Syndrome, which we hope to show more clearly. Nevertheless, we want to insist on clarifying that our real intention when we tried to join them into a group of one Syndrome, the predominant facial manifestation along with social interplay, from what the clinic and the street offered us, was to reference aspects not only purely clinical but also the social habits involved. Because of the mal-use of the pretended botox and other similar therapies that produce the same negative effects, the idea was not to get lost in a never ending search for pathologies that remind us of Tapiarian faces with the proboscis shape, when the professional reader can surely see it in the same apparent way that we can.

## Methods

Primarily from our point of view and for the present, it is our wish to enrich our description of the Syndrome for the reader, with clinical proofs i.e., pysiopathological behavior, [5][6], etc., that can be measured.

Without further delay we believe we are able to demonstrate the symptoms and sufficient signs that permit the construction of a genuine Tapir Syndrome (TS) the origin of the disease basically caused by medical treatment, induced by drugs or surgery.

## The symptoms for now are the following:

**Social signs/symptoms:** normally a beautiful youthful woman, usually from the middle-middle-class or upper middleclass economical status with a variety of social and professional compromises, especially worried about her figure being in a proper organic form and if not organic then cosmeti-



cally, including several skin conditioners in which wrinkles can mean a symptom of self rejection or rejection from others.

**Physical signs/symptoms:** well-rounded face toward brilliant because of excessive use of creams and artificial colourings.

Lack of vital marks and expressivity tracks, such as frown marks, crow's feet, laughing and whistling marks.

Adinamic and inexpressive facial zones with fairly generalized limits.

Facial ptosis (drooping) fundamentally in the tender more fleshy superior part of the face.

Adinamia disorder, weight and increase in the volume of area bone structure and lip areas to various degrees (disorder in the modulation of words; decrease of lip occlusive capacity that can become a smile without meaning, scarce or nonexistent exposition of the gingivodental structures [8], with the consequent diminution of the expressive contrast between the white part of the sclerotic and the dental enamel; in serious cases saliva incontinence and some cases becoming sialorreas, making it difficult for alimentation forcing the person to eat with a lot of attention so as not to let food fall out of the oral cavity, which makes movements of the eater voluntarily quite slow). There is no naturalness or grace with this type of problem.

A resemblance among the several Tapirian syndromes is a well-known fact. The patients try to mimic their own general behavior with youthful aspects that are complicated and many times it does not work primarily because their behavior does not match reality, i.e. exaggerated movements, attitudes and clothing, none of which correspond to their age. At times people who have this Syndrome are seen by others as being rude and this puts them under social judgment that is frequently not particularly flattering. There is one possible favorable aspect and that is the condition could pass unnoticed by interested persons since it is often not blatantly apparent. Sometimes we are capable of noticing species of the Tapirian Syndrome from the formation of the inferior third part of a Belfic face that generally fits what we have described[1].

These phatophysiological aspects seem to be measured with good intentions and are fairly reliable. For now, the structures, craniofacial and cervical,

are not going to be treated clinically very well. We ask the reader's collaboration and concern for the purpose of reversing the poor treatments. Photographs, micro-graphs, electroencephalographs, along with biopsies, cytology and even bimolecular studies [9] can easily facilitate the confirmation that we are in fact faced with a real Syndrome (Figure 1).

## Conclusions

The existence of well-intentioned criteria, can result highly beneficial so that society will not lose the course of a "pre-ordained" evolutionary course and will stay oriented, keeping people within natural evolving parameters that could otherwise lead to an improper Avatar situation—an ever present temptation reflecting the times in which we live, possibly accepted in social gatherings but not practical for real life. Aside from genuine medical needs, what happens within the Syndrome, while attempting to heal an individual's wish to improve a physically unwanted aspect, can be a drastic dislocation of the eternal beauty canons that human beings enjoy genetically.

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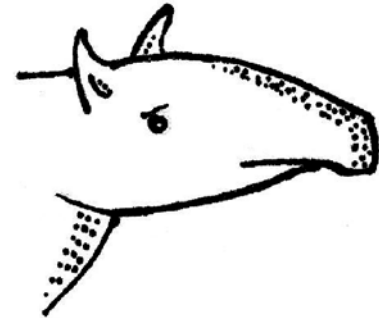
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**FIGURAS**

**Figura**



**Sociedad Española de Medicina Antienviejecimiento 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 Antienviejecimiento (Anti-Aging) como procedimiento terapéutico, procurando la cooperación y la unión de especialidades médicas y de todos los profesionales de la salud, farmacéuticos, psicólogos, biólogos, odontólogos, etc..., que por su actividades y dedicaciones, manifiestan expresamente su interés en la Medicina Antienviejecimiento, 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 recibirás la Revista Medicina Antienviejecimiento, órgano oficial de la S.E.M.A.L.

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Titular: Sociedad Española de Medicina Antienviejecimiento y Longevidad (S.E.M.A.L.)

Para formar parte de la sociedad, envíe este boletín de inscripción y el justificante de ingreso o transferencia, junto con una copia del título de Licenciado o Doctor en Medicina y Cirugía o de Especialista, a la siguiente dirección:  
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Avda. de la Borbolla, 47 - 41013 Sevilla  
Tlf.: 954 084 700 - Fax: 954 084 700  
e-mail: [info@sem.al.org](mailto:info@sem.al.org) · web: [www.sem.al.org](http://www.sem.al.org)

Moderadores: Prof. Joaquín Calap - Dr. Manuel Asín

9:00 "Dermatología Psicosomática"

Prof. Joaquín Calap, España.

9:20 "Efecto de la Oxigenación Hiperbárica en Medicina Antienvejecimiento"

Dr. Juan Carlos Méndez, Venezuela.

9:40 "Novedades en Tratamientos Estéticos y Dermatológicos"

Dr. Manuel Asín Llorca, España.

10:00 "Tratamiento integral y preventivo de la patología flebotómica mediante escleroterapia extensiva; comparativa con otras técnicas"

Dr. Manuel Ripoll, España.

10:20 "Técnicas no ablativas : Fraccional Microneedling and CO2 Injection"

Dr. Juan Carlos López, Brasil.

10:40 "Las edades de la boca"

Dra. Paloma Tejero, España.

11:00 PAUSA CAFÉ Y VISITA A LA EXPOSICIÓN COMERCIAL

## LÁSER Y NUEVAS TECNOLOGÍAS

Moderadores: Dra. Pilar Rodrigo - Paloma Tejero

11:30 "Estado actual en el tratamiento de las Hiperpigmentaciones postinflamatorias y el Melasma "

Dr. Mariano Velez, España.

11:50 "Cuidar bien la piel para envejecer mejor"

Dr. Miguel Aizpun, España.

12:10 "Avances del tratamiento de poliquilodermia y cuperosis"

Dr. Pablo Boixeda, España.

12:30 "Nuestra experiencia en más de 1000 pacientes con tratamientos de láser y luz pulsada"

Dr. Manuel Asín, España.

12:50 "Tratamiento de lesiones vasculares faciales con láser y luz pulsada"

Dra. Carmina Sierra, España.

13:10 "Novedades Tecnológicas en antienvejecimiento"

Dra. Elia Roó Rodríguez, España.

13:30 CONFERENCIA DE CLAUSURA (SALA ANTI-AGING)

"Reishi, sod y las teorías (y prácticas) del doctor Niwa"

D. Fernando Sánchez Dragó, España.

ESTE ES UN PROGRAMA PRELIMINAR Y ESTÁ SUJETO A CAMBIOS

Moderadores: Dra. Mercedes Eguiluz - Dr. Jorge Planas

9:00	"Cómo optimizar el remodelaje facial con las diferentes técnicas de inyección del ácido hialurónico STYLAGE"	Dra. Samia Guerbaa, Túnez.
9:20	"Utilidad de los injertos de grasa en multitud de situaciones como rejuvenecimiento facial o corporal"	Dr. Juan Monreal, España.
9:40	"Qué ofrece la mesoterapia al anti-aging"	Dr. Jose Folch, España.
10:00	"Abordaje terapéutico del tercio inferior del rostro"	Dr. Javier Anido, España.
10:20	"Estratamiento facial mediante hilos"	Dr. Helio Avelar, Brasil.
10:40	"Biorregeneración endodérmica mínimamente invasiva mediante microneedling con agujas de oro"	Dr. Jose Antonio González-Díaz, España.

11:00 PAUSA CAFÉ Y VISITA A LA EXPOSICIÓN COMERCIAL

MEDICINA ESTÉTICA REGENERATIVA

Moderadores: Dr. Miguel Ángel Santos - Dr. Julián Bayón

11:30	"Rejuvenecimiento cutáneo con factores de crecimiento inyectables- Protocolo Casys"	Dra. Viviane Ferreira De Campos, Brasil.
11:50	"Lipotransferencia enriquecida con PRFC"	Dr. Antonio González de Nicolás, España.
12:10	" PRFC y ozono "	
12:30	"Células madre en cirugía y medicina estética. Indicaciones, seguridad y marco legal"	Dr. Gerardo Ordiales, España.
12:50	"Estado actual de las células madre como tratamiento antiaging"	Dr. Jorge Planas, España.
13:10	DISCUSIÓN	Dr. Ramón Vila-Rovira, España.

13:40 DEGUSTACIÓN DE PRODUCTOS IBÉRICOS Y CAVA

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

CIRUGÍA ESTÉTICA

Moderadores: Prof. José M. Serra-Renom - Dr. Ezequiel Rodríguez

16:00	"Versatilidad del uso del láser en medicina y cirugía estética"	Dra. Alcátrix Jimenez Manauze, Venezuela.
16:20	"Laser lipólisis"	
16:40	"Cirugía del envejecimiento cervical"	Dr. Mario Trellés, España.
16:40	"Cirugía del envejecimiento cervical"	Dr. Enrique Monereo, España.
17:00	"Cirugía estética genital femenina"	Dr. Iván Mañero, España.
17:20	"Rejuvenecimiento Palpebral y Periorbitario"	Dra. Isabel de Benito Molina, España.
17:40	"Rejuvenecimiento centrorfacial con endosuspensión quirúrgica, lipoinjante y remodelación periorbitaria"	Dr. Francisco Navarro Viana, España.

18:00 PAUSA CAFÉ Y VISITA A LA EXPOSICIÓN COMERCIAL

CIRUGÍA ESTÉTICA

Moderadores: Dr. Rómulo Guerrero - Dr. Francisco Abril

18:30	"Reposición volumétrica como tratamiento del envejecimiento facial"	Dr. Rómulo Guerrero, Ecuador.
18:50	"Mamoplastia de aumento con prótesis, con grasa o con hialurónico. Cuando, cómo y por qué"	Dr. Ramón Vila-Rovira, España.
19:10	"Tratamiento del vector negativo facial. Importancia de la reconstrucción del óvalo facial en la cirugía antienvjecimiento"	Prof. José Mª Serra Renom, España.
19:30	"Rejuvenecimiento mamario. Enfoque personal"	Dr. Antonio Porcuna, España.
19:50	"Facial rejuvenation - lip rejuvenation surgery"	Dr. Bernard Cornette de Saint Cyr, Francia.

21:30 CENA DE CLAUSURA - INSCRIPCIÓN EN SECRETARÍA

## SALA ANTI-AGING

### TRATAMIENTOS ANTIENVEJECIMIENTO

Moderadores: Prof. Dario Acuña - Prof. Germaine Escames.

9:00 "Efectos de ondas biorresonantes en la regeneración del funcionamiento cerebral"

Dr. Juan Carlos Méndez, Venezuela.

9:20 "Extractos placentarios. Productos y tratamientos"

Dr. Jesús F. Ballesteros, España.

9:40 "Los efectos de la melatonina en la neurogénesis"

Prof. Germaine Escames, España.

10:00 "Valor terapéutico de la melatonina: Qué dosis usar y por qué"

Prof. Dario Acuña, España.

10:20 "Efectos específicos de las hormonas masculinas en la mujer"

Dr. Octavio Viera, España.

10:40 "Terapia de reemplazo hormonal en el hombre. Cómo verse de 5 a 8 años más joven"

Dr. Jean Paul Osores, Perú.

11:00 PAUSA CAFÉ Y VISITA A LA EXPOSICIÓN COMERCIAL

### MEDICINA ANTIENVEJECIMIENTO: LA PRÁCTICA

Moderadores: Dr. José Serres - Dr. Ramón Vila-Rovira

11:30 "Nuevas tendencias legales en responsabilidad médica"

Ldo. Ricardo Ibañez, España.

11:50 "Mi entrenamiento para llegar a los 120 años con vitalidad, agilidad y lucidez"

Dr. Ramón Vila-Rovira, España.

12:10 "Gestión en Medicina Anti-Envejecimiento: Experiencia crítica de 5 años"

Prof. Manuel J Castillo, España

12:30 "Estudio de 10 años de nuestra práctica en medicina anti-envejecimiento"

Dr. Manuel Sánchez, España.

12:50 "Medición de longitud telomérica como marcador de la edad biológica"

Dra. María Blasco, España.

13:10 DISCUSIÓN

13:30 CONFERENCIA DE CLAUSURA

"Reishi, sod y las teorías (y prácticas) del doctor Niwa"

D. Fernando Sánchez Drago, España.

## SALA TALLERES (ENTRADA GRATUITA)

VIERNES 7 de Octubre de 2011

9:30 "Medicina Personalizada Genómica: Bases científicas y aplicaciones prácticas en Medicina Antiaging"

Prof. Juan Sabater, España.

10:30 "Aplicación práctica de la medicina cuántica para la detección y corrección de las heridas emocionales y los trastornos de control que aceleran el envejecimiento" (PC TECNOLOGÍAS)

Dr. Ricardo Wohl, España.

11:30 "Importancia de la sincronización cerebral en la prevención y tratamiento de enfermedades neuro-degenerativas"

Dr. Juan Carlos Méndez, Venezuela.

12:30 "Influencias del envejecimiento sobre los niveles de consciencia y caso práctico"

Prof. Laurent Messéan, Francia.

13:30 "Mesoterapia facial de Mesoestetic - Productos Mesohyal"

Dr. Fernando Galceran Montal, España.

14:30 COCKTAIL BIENVENIDA

## SALA TALLERES (ENTRADA GRATUITA)

16:00 "La Carboxiterapia Indolora" (SKYMEDIC)

Dr. Juan Carlos López, Brasil.

17:00 "Rejuvenecimiento Facial Inmediato (Parte I). Toxina Botulínica y técnica de Meso-Botox"(ALLERGAN)

Dr. Juan Sopena, España.

18:00 "Rejuvenecimiento Facial Inmediato (Parte II). Restauración Indolora de contornos faciales con Voluma"

Dr. Juan Sopena, España.

19:00 "Versatilidad del uso del láser en medicina y cirugía estética" (SIX LASER)

Dr. Alcálitra Jiménez Manauze, Venezuela.

ESTE ES UN PROGRAMA PRELIMINAR Y ESTÁ SUJETO A CAMBIOS

20:30 VISITA GUIADA POR EL MADRID DE LOS AUSTRIAS. Inscripción Gratuita en Secretaría. Plazas limitadas.

SESIÓN INTERNACIONAL (Simultaneous translation) DIAGNOSTIC PROCEDURES

Moderadores: Dr. Jose Ignacio Lao - Prof. Damiano Galimberti

9:00	"Genomics disease susceptibility analysis in Middle Eastern Population: Its effects on Antiaging Medicine Practice"
9:25	"Nuclear Receptors and the relation between food longevity and cancer"
	Dr. Shafiq Kaidbey, Arabia Saudi.
9:50	"Detoxification in anti-aging; a new trend with genetic test"
	Prof. Damiano Galimberti, Italia.
10:15	"Muscle function and wellness"
	Dr. Claude Dalle, Francia.
10:40	DISCUSSION
	Prof. Damiano Galimberti, Italia.

11:00 PAUSA CAFÉ Y VISITA A LA EXPOSICIÓN COMERCIAL

SESIÓN INTERNACIONAL (Simultaneous translation) HORMONAL THERAPY

Moderadores: Prof. Manuel J Castillo - Prof. Michael Kientze

11:30	"Bioidentical Hormone therapy in a safe multi level basis"
	Dr. Michael Kientze, Alemania.
11:55	"The Use of Pregnenolone in an anti-aging practice"
	Dr. Filomena Trindade, EEUU.
12:20	"Centenarians secrets and hormonal balance"
	Prof. Vincenzo Aloisantonio, Italia.
12:45	"Beauty from the inside: Hormone applications and vitamins for the skin"
	Prof. Michael Kientze, Alemania.
13:10	"Melatonin: An Endogenous Tumor Cell Assassin"
	Prof. Russel Reiter, EEUU.
13:35	DISCUSSION

13:40 DEGUSTACIÓN DE PRODUCTOS IBÉRICOS Y CAVA

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

SESIÓN INTERNACIONAL (Simultaneous translation) TREATMENTS IN AGING

Moderadores: Dr. Julian Bayón - Dr. Claude Dalle

16:00	"Common Problems in Female Hormone Therapy"
	Dr. Claude Dalle, Francia.
16:25	"Interest of stilbens in Anti-Aging Medicine"
	Dr. James Betz, Austria.
16:50	"Biological therapy in cancer"
	Dr. Benno Wölfel, Alemania.
17:15	"Anti-Aging and Orthomolecular Medicine: Practical Concepts for the Prevention and Treatment of Aging diseases."
	Dr. Udo Böhm, Alemania.
17:40	DISCUSSION

18:00 PAUSA CAFÉ Y VISITA A LA EXPOSICIÓN COMERCIAL

SESIÓN INTERNACIONAL (Simultaneous translation) MECHANISMS IN AGING

Moderadores: Prof. Antonio Ayala - Prof. Walter Pierpaoli

18:30	"Metabolic energy homeostasis and neuro-immune endocrine triggers in chronic inflammatory diseases"
	Prof. Francesco Marotta, Italia.
18:55	"Mood Disorders: A Functional Approach"
	Dr. Filomena Trindade, EEUU.
19:20	"Melatonin and TRH: two life-generating and aging-reversing molecules"
	Prof. Walter Pierpaoli, Italia.
19:45	DISCUSSION

ESTE ES UN PROGRAMA PRELIMINAR Y ESTÁ SUJETO A CAMBIOS

8:30 Entrega Documentación

9:00 Acto Inaugural

**DIAGNÓSTICO GENÓMICO EN MEDICINA ANTIENTVEJECIMIENTO**

Moderadores: Prof. Juliana Farina - Dr. José Ignacio Lao

9:30 "La nutrigenómica como base para una micronutrición personalizada"

Dr. José Ignacio Lao, España.

9:50 "Utilidad del análisis genético en la prevención del envejecimiento cerebral"

Dr. José Félix Marcos Frías, España.

10:10 "Papel de la predisposición genética en el dolor generalizado y la fatiga en la edad avanzada"

Dr. Ferrán J. García Fructuoso, España.

10:30 "Envejecimiento, genética y disfunción endotelial. Implicaciones terapéuticas"

Dr. José Sabán, España.

10:50 DISCUSIÓN

11:00 PAUSA CAFÉ Y VISITA A LA EXPOSICIÓN COMERCIAL

**DIAGNÓSTICO EN MEDICINA ANTIENTVEJECIMIENTO**

Moderadores: Prof. Mónica de la fuente - Prof. Juan Sabater

11:30 "Estrategias de estilo de vida para conseguir una longevidad saludable. Utilización del sistema inmunitario para valorar su eficacia"

Prof. Mónica de la Fuente, España.

11:50 "Diagnóstico bioquímico y genético del estrés"

Dra. Gloria Sabater, España.

12:10 "Densitometría en Medicina Antientvejecimiento"

Dr. Albert Nadal, España.

12:30 "Medicina personalizada postgenómica: predicción-prevenición de efectos adversos por estatinas"

Prof. Juan Sabater, España.

12:50 DISCUSIÓN

**NUTRICION TERAPÉUTICA EN MEDICINA ANTIENTVEJECIMIENTO**

Moderadores: Dr. Efraín Olszewer - Dr. Julián Bayón

13:00 "Radicales libres y antioxidantes en las enfermedades cardiovasculares"

Dr. Efraín Olszewer, Brasil.

13:20 "Alcohol, Polifenoles y Salud"

Prof. Manuel J. Castillo, España.

13:40 "La nutrición grasa en los mecanismos de regulación endocrino-metabólica e inmunológica"

Dr. Vicente G. Villarrubia, España.

14:00 "Modulación nutricional de neurotransmisores y neuropéptidos en la obesidad"

Dr. Efraín Olszewer, Brasil.

14:20 DISCUSIÓN

14:30 COCKTAIL BIENVENIDA

**ENFERMEDADES RELACIONADAS CON EL ENVEJECIMIENTO**

Moderadores: Dr. Luis Asmarats- Dra. Mercedes Eguiluz

16:00 "Relación entre adiposidad y resistencia a insulina durante el envejecimiento"

Dr. Antonio Andrés Hueva, España.

16:20 "Estatinas de Nueva Generación. Su importancia en Medicina Antientvejecimiento"

Dr. Luis Asmarats, España.

16:40 "La inflamación en antientvejecimiento y su tratamiento con medicina bioreguladora"

Dra. Digna Vergara, Venezuela.

17:00 "Como minimizar el envejecimiento cerebral"

Dra. Odilza Vital, Brasil.

17:20 "Enfoque terapéutico antientvejecimiento de la depresión y la ansiedad "

Dr. Juan Carlos Méndez, Venezuela.

17:40 DISCUSIÓN

18:00 PAUSA CAFÉ Y VISITA A LA EXPOSICIÓN COMERCIAL

**PSICOLOGÍA Y MEDICINA ANTIENTVEJECIMIENTO**

Moderadores: Dr. Juan Carlos Méndez - Lda. Montserrat Márquez

18:30 "El arte de vivir en la 3ª edad"

Prof. Enrique Rojas, España.

18:50 "Efectos deletéreos del aislamiento social en la función cognitiva"

Dr. César Venero, España.

19:10 "Motivación y Mantenimiento en Coaching Antiajing"

Lda. Carmen Giménez- Cuenca, España.

19:30 "Motivational Interview y Anti-Aging"

Dra. Susana Cantón, España.

19:50 "Estrés y Envejecimiento"

Dr. José Antonio Carrobles, España.

20:10 "La poderosa influencia de la mente sobre el envejecimiento"

Dr. Ricardo Wohl, Francia.

20:30 VISITA GUIADA POR EL MADRID DE LOS AUSTRIAS. Inscripción Gratuita en Secretaría. Plazas limitadas.

## ¿Cómo incorporar una consulta de Medicina Antienviejimiento en tu práctica diaria?

**Dr. Michael Kientze**

Moderador: Prof. Manuel J Castillo

PRECIO CON INSCRIPCIÓN CONGRESO 150 €. SOLO CURSO 200€.

HORARIO: 9 A 14 HORAS

Se entregará diploma acreditativo

1. Análisis del Envejecimiento. Marcadores de Envejecimiento
2. Herramientas para detectar el envejecimiento de órganos y sistemas
3. Cómo tratar con Hormonas Bioidénticas:

A) Menopausia

B) Andropausia

C) Somatopausia

D) Problemas de la terapia hormonal

E) Tiroides

F) Fatiga Crónica: el cortisol y la DHEA

4. Pruebas de laboratorio, ¿que necesitamos?

6. ¿Cómo y donde encontrar ayuda?

7. Suplementos, ¿deben ser personalizados?

## MI práctica tras 15 años de Medicina Antienviejimiento

**Dr. Efraim Olszwer**

Moderador: Dr. Julián Bayón

PRECIO CON INSCRIPCIÓN CONGRESO 150 €. SOLO CURSO 200€.

HORARIO: 16.00 a 19:30 HORAS

Se entregará diploma acreditativo

1. Marcadores biológicos del envejecimiento y modulación hormonal (adrenopausia, andropausia, menopausia, somatopausia y tiroideopausia)
2. Nutri-genómica, epi-genómica, lipi-dómica, protei-nómica y restricción calórica en la modulación nutricional de los factores externos que actúan en el genoma. Control de la expresión del fenotipo.
3. Estrés oxidativo, estrés adrenal, inflamación, alteración endotelial y dislipidemias como factores que aceleran el envejecimiento.
4. Envejecimiento cerebral: De la disfunción cognitiva mínima a la demencia.
5. Modulación nutricional en el envejecimiento del sistema locomotor.

Durante el curso discutiremos:

La reposición hormonal: mitos y verdades basados en la ciencia.

Cómo tratamos nutricionalmente a los pacientes antes de padecer diabetes, arteroesclerosis, osteoporosis... y cómo modular factores que mimetizan la restricción calórica como factor que definitivamente prolonga la vida. Cómo actúan los radicales libres en el envejecimiento y su acción en el sistema cardiovascular. Cómo se mantiene nutricionalmente la actividad cerebral, factor de calidad de vida en el envejecimiento. Cómo controlar el desgaste articular, importante factor limitante en la senescencia.

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# 10th International Congress of Anti-aging Medicine X Congreso de la Sociedad Española de Medicina Antienvejecimiento y Longevidad

Madrid, 6th to 9th October, 2011