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antioxidant activity (TAA) was measured by the $ABTS^{+\cdot}$ decolourization method. Serum α- and γ-tocopherol and malondialdehyde (MDA) were determined by HPLC. *COMT* Val108/158Met polymorphism was detected by tetraprimer ARMS-PCR. Specifically the *COMT* high activity allele (Val) was associated with a decreased serum antioxidant status at E2max respecting E2min, expressed by: (a) increased serum oxidation rates in the lag- and propagation phases, and a decreased lag time; (b) decreased TAA; (c) reduced α- and γ-tocopherol content; and (d) increased MDA. We conclude that the *COMT* low activity genotype (Met/Met) confers antioxidant protection respecting the high activity allele during the ovarian stimulation achieved in IVF.

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P-010

Protection of anthocyanins against human LDL oxidation and their structure–activity relationship: A key component in the French paradox

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An increased interest in anthocyanins and their biological effects has emerged in the last years. They are a sub-group of flavonoids responsible for the colour and most of the benefits of moderate consumption of red wine. The present study was designed to evaluate and compare the antioxidative properties of four structurally related anthocyanins—pelargonidin, cyanidin, malvidin and malvidin-3-glucoside—against human LDL oxidation promoted either by AAPH-generated peroxyl radicals, or two physiologically relevant oxidants, ferrylmyoglobin and peroxynitrite. Their ability to recycle α-tocopherol (α-TOH), the most abundant LDL-lipophilic antioxidant, was also studied. When LDL oxidation was initiated either by AAPH or ferrylmyoglobin, as determined by the fluorescence decay of incorporated *cis*-parinaric acid and conjugated dienes formation, those anthocyanins strongly inhibit LDL oxidative damage, cyanidin and malvidin being far more efficient as compared with pelargonidin. Also, malvidin-3-glucoside exhibited a stronger antioxidant activity than malvidin, the non-glycosilated derivative. Peroxynitrite-promoted LDL apoprotein modifications, as evaluated by apoB net surface charge alterations, were efficiently inhibited by cyanidin, malvidin or malvidin-3-glucoside, while almost no effects were observed with pelargonidin. Moreover, all the anthocyanins significantly decreased peroxynitrite-mediated carbonyl groups formation in LDL. EPR measurements of α-tocopheroxyl radical showed that the anthocyanins strongly reduce the signal intensity of that radical pointing to their highest abilities to recycle α-TOH, although malvidin-3-glucoside was far less effective. Our results corroborate the relevance of patterns of hydroxyl or methoxyl substitution and glycosylation to the modulation of antioxidant activities of anthocyanins. Also, they suggest that the consumption of anthocyanins through the intake of red wine may greatly contribute to protect LDL from oxidative damage and, therefore, may be a key component in the French paradox.

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P-011

Antioxidant status of human follicular fluid: Implications in female infertility

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The aim of this work was to determine the antioxidant status in follicular fluid and assess its involvement in woman infertility. ORAC (oxygen radical absorbance capacity) and TAC (total antioxidant capacity) were measured in follicular fluid aspirated from follicles during oocyte pickup from women enrolled in IVF therapy ($n = 30$) and were compared with the activities in follicular fluid aspirated from healthy control donors ($n = 30$). ORAC was measured by assessing the area under the fluorescence decay curve (AUC) of fluorescein with AAPH as free radical initiator in the presence of the sample as compared to that in the blank in which no antioxidant is present. The ORAC value was also determined in the soluble fraction after acetone deproteinization. TAC was measured by the $ABTS^{+}$ radical cation decolourization method. The follicular fluid of subfertile women exhibited a significant lower ORAC value compared with control donors (5783 ± 1237 vs 6492 ± 1066 μM Trolox, $p = 0.021$). No differences in either the ORAC value in deproteinized samples or TAC were found between both groups. In conclusion, the reduced antioxidant activity in the follicular fluid suggests a role for free radicals in women infertility, probably contributing to impairment of reproduction in these patients.

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P-012

Free radical scavenging activity of different almond (*Prunus dulcis*) varieties

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Reactive oxygen species are known to be implicated in many cell disorders and in the development of many diseases including cardiovascular diseases, atherosclerosis, cataracts, chronic inflammation or neurodegenerative diseases such as Alzheimer's or Parkinson's disease. Thus, synthetic antioxidants are widely used in the food industry, but, because of their toxic and carcinogenic effects, their use is being restricted. The pursuit for novel natural sources of bioactive compounds, namely those who present antioxidant activity, has been acquiring higher significance, since these compounds may contribute to the prevention of diseases in which free radicals are implicated. In this study, the antioxidant properties of different almond varieties (*Casanova*, *Duro Italiano*, *Ferraduel*, *Ferranhês*, *Ferred Star*, *Guara*, *Molar*, *Orelha de Mula* and *Pegarinhos*) were evaluated through several biochemical assays: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, reducing power, inhibition of β-carotene bleaching, inhibition of oxidative haemolysis in erythrocytes, induced by 2,2′-azobis(2-amidinopropane)dihydrochloride (AAPH) and inhibition of lipid peroxidation in pig brain tissue through formation of thiobarbituric acid reactive substances (TBARS). For all the methods, EC_{50} values were calculated in order to evaluate the antioxidant efficiency of each variety. The total phenols and flavonoid contents were also obtained and correlated with antioxidant activity. *Ferred Star* and *Duro Italiano* revealed better antioxidant properties, presenting lower EC_{50} values, particularly for lipid peroxidation inhibition in TBARS assay. The highest antioxidant contents (phenols and flavonoids) were also found for these varieties.

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P-013

Free radical scavenging activity and bioactive compounds of five *Agaricus* sp. edible mushrooms

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Reactive oxygen and free radicals play an important role in cellular injury and the ageing process and also are considered to induce the lipid peroxidation that causes the deterioration of foods. Although organisms have endogenous antioxidant defences produced during normal cell aerobic respiration against the reactive oxygen species, other antioxidants are taken from the diet, both from natural or synthetic origin. Thus, synthetic antioxidants are widely used in food industry, but because of their toxic and carcinogenic effects, their use is being restricted. Individual tocopherol profile of five *Agaricus* mushroom species, widely consumed in Portugal, was obtained by high performance liquid chromatography coupled to a fluorescence detector

(HPLC/fluorescence). Bioactive compounds such as phenols, flavonoids, ascorbic acid, β-carotene and lycopene were also determined. The lipid peroxidation inhibition capacity of the edible mushrooms was accessed by biochemical assays used as models for the lipid peroxidation damage in biomembranes, namelly inhibition of β-carotene bleaching in the presence of linoleic acid radicals, inhibition of erythrocytes haemolysis mediated by peroxyl radicals and inhibition of thiobarbituric acid reactive substances (TBARS) formation in brain cells. Their antioxidant properties were also evaluated through the reducing power determination and radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. *A. silvaticus* revealed better antioxidant properties (lower EC_{50} values) than the other *Agaricus* species (*A. arvensis*, *A. bisporus*, *A. romagnesii*, *A. silvicola*) which is in agreement with the higher content of bioactive compounds found in the first specie. α-tocopherol and β-tocopherol were found in the samples, while γ- and δ-tocopherols were not detected.

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P-014

Effects of two new di(hetero)arylamines on prevention of oxidative stress induced in two different biological models

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We investigated the antioxidant properties of two new diarylamines from organic synthesis (MJQ1 and MJQ2), whose basic structure is similar to others, with reported antioxidant capacity, assessed by chemical tests, and biological activity against microorganisms. In this study we induced lipid peroxidation, in isolated rat liver mitochondria with ADP/Fe^{2+} and the diarylamine effects were examined by oxygen consumption and by TBARS method. The anti-peroxidative effect was maximal for MJQ1 at 50 nM (higher than the one reported for trolox) and for MJQ2 at 60 µM. At these same concentrations none of them depressed the transmembrane potential ($\Delta\Psi$) developed by mitochondria, neither the RCR nor the ADP/O ratio values. For 2-fold concentrations both diarylamines were effective in the prevention of mitochondrial $\Delta\Psi$ collapse observed on respiring mitochondria, with the TPP^+ electrode, which means a stabilization action on mitochondrial inner membrane. The results obtained were confirmed in whole cells. The compounds did not show toxicity to the L929 cell line, evaluated by the MTT reducing test and clearly protected from lipid peroxidation, induced by the oxidant pair ascorbate/Fe^{2+}, to the PC12 cell model, at the concentrations where maximal antioxidant effect was observed in mitochondria. The new diarylamines were revealed as very good antioxidants at very low concentrations, both in mitochondria and in whole cells. The results suggest a specific action site, for MJQ2, at mitochondrial complex I level. We are further exploring other intracellular targets for these new compounds that seem very promising against pathologies where oxidative stress is involved.

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P-015

Nitric oxide regulates Foxo3a activity to modulate mitochondrial ROS production

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It has long been established that, depending on the biological context, nitric oxide (NO) can play either a pro-oxidant or an anti-oxidant role; however, the underlying molecular mechanisms have only recently begun to be elucidated. Our studies have shown that NO can both positively and negatively regulate the expression of the main proteins involved in mitochondrial ROS detoxification. This regulation involves NO-mediated modulation of the mRNA levels of the transcriptional coactivator PGC-1α via the activation of the sGC/PKG pathway [1]. PGC-1α is the master transcriptional regulator of mitochondrial function and ROS protection systems [2]. Our current work focuses on understanding the mechanism that mediates NO-induced down-regulation of PGC-1α expression in the vascular endothelium. Since some NO effects are known to be mediated via activation of the PI3K/AKT pathway, we have examined the effect of inhibiting PI3K. Pre-treatment of primary endothelial cells with PI3K inhibitors prevented down-regulation of PGC-1α expression in response to the NO donor DETA-NO or the PKG activator 8-Br-GMPc, indicating that PI3K acts downstream of PKG. Similarly, pre-treatment with the AKT inhibitor AKT IV blocked NO-induced PGC-1α down-regulation, whereas DETA-NO increased the phosphorylation (activation) of AKT, supporting a role for the PI3K/AKT pathway in NO regulation of PGC-1α expression. Point mutation in a functional IRS box of the mouse PGC-1α promoter cancelled NO activity, suggesting the involvement of a FOXO transcription factor as a mediator of NO action. Importantly, FOXO factors are phosphorylated and inactivated by AKT. In further experiments we have shown that treatment with DETA-NO leads to phosphorylation and inactivation of the FOXO factor Foxo3a. To determine whether Foxo3a is in fact a transcriptional regulator of PGC-1α we have used a series of experimental approaches. Over-expression of a constitutive form of Foxo3a dramatically upregulated PGC-1α levels, whereas a siRNA directed against Foxo3a had the opposite effect. ChIP analysis showed that Foxo3a directly associates with the PGC-1α promoter and transient transfection assays indicated that Foxo3a activates the PGC-1α promoter through the same binding site that is required for NO activity. We therefore conclude that NO down-regulates PGC-1α expression through the PI3K/AKT/Foxo3a pathway.

References

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P-016

Bilirubin induces oxidative stress in neurons, which is prevented by nNOS inhibition

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High levels of unconjugated bilirubin (UCB) can be toxic to the central nervous system and oxidative stress is emerging as a relevant event in the mechanisms of UCB encephalopathy. We investigated if exposure of rat neurons to UCB leads to disruption of the redox status by a mechanism involving nNOS activation. Rat cortical neuronal cultures at 8-days *in vitro* were exposed to 50 or 100 µM UCB, for 4 h, at 37°C. To assess the role of NO, neurons were co-incubated with 100 µM UCB and 100 µM L-NAME, an inhibitor of nNOS. All the experiments were performed in the presence of 100 µM human serum albumin, used to solubilize UCB. Formation of protein carbonyls was assessed by slot blot analysis, reduced glutathione (GSH) by an enzymatic recycling assay and cell death was determined by quantification of LDH release using a commercial kit. NO production was evaluated by quantification of nitrite levels using Griess reagent, while the activity of nNOS was estimated by Western blot. Neuronal exposure to 50 or 100 µM UCB led to protein oxidation ($p < 0.05$) and decrease in GSH content ($p < 0.05$), resulting in enhanced cell death ($p < 0.01$). Oxidative disruption was accompanied by nNOS activation ($p < 0.05$), with consequent increase in NO production ($p < 0.01$). All UCB-induced effects were significantly counteracted by co-incubation with L-NAME. This study reinforces the involvement of oxidative stress in neuronal damage induced by UCB and demonstrates that cell lesion is mediated by nNOS activation, therefore, pointing to NO as a key element.

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P-017

Resveratrol prevents peroxynitrite-induced endothelial cells apoptosis by disrupting the mitochondrial pathway

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