

**EFFECTS OF TESTOSTERONE ON ANTIOXIDANT SYSTEMS IN MALE SECONDARY
HYPOGONADISM**

Short title: Antioxidants in male hypogonadism

ANTONIO MANCINI¹, ERIKA LEONE¹, ROBERTO FESTA¹, GIUSEPPE GRANDE¹, ANDREA SILVESTRINI², LAURA DE MARINIS¹, ALFREDO PONTECORVI¹, GIULIO MAIRA³, GIAN PAOLO LITTARRU⁴, & ELISABETTA MEUCCI²

¹Chair of Endocrinology, ²Institute of Biochemistry and Clinical Biochemistry, ³Institute of Neurosurgery, Catholic University of The Sacred Heart, Rome; ⁴Institute of Biochemistry, University “Politecnico delle Marche”, Ancona; Italy

Correspondence:

Antonio Mancini, M.D.

Largo G. Vidari 7, 00135 Rome, Italy.

Phone number: +39 06 3380925

Fax number: +39 06 35500486

E-mail: mancini.giac@mclink.it

Abstract

Oxidative stress is involved both in metabolic syndrome and male infertility. Hypogonadism is also associated with increased risk for cardiovascular disease. To investigate the role of gonadal steroids in systemic antioxidants regulation, we determined plasma CoenzymeQ₁₀ (CoQ₁₀) and Total Antioxidant Capacity (TAC) in post-surgical hypopituitary patients. Twenty-six patients, aged 28-55 ys, were studied 6-12 months after operation. CoQ₁₀ levels were measured by HPLC; TAC by spectroscopy using the system mioglobin-H₂O₂, which, interacting with the chromogen 2,2¹-azinobis-(3-ethylbenzothiazoline-6-sulphonate), generates a radical after a latency time (LAG) that is proportional to antioxidant content. Sixteen patients presented low testosterone values; in 10 patients hypogonadism was isolated, while in 6 patients also hypothyroidism was present. CoQ₁₀ levels were significantly lower in isolated hypogonadism than in normogonadism. Testosterone treatment, performed in those patients with isolated hypogonadism, induced a significant enhancement both in CoQ₁₀ level and LAG. CoQ₁₀ and LAG values significantly correlated, suggesting an inter-relationship between different antioxidants. Our data suggest that hypogonadism could represent a condition of oxidative stress, in turn related with augmented cardiovascular risk.

Keywords: Androgen, Oxidative stress, Coenzyme Q₁₀, Total antioxidant capacity.

Introduction

From cross-sectional studies in healthy men, lower plasma total testosterone levels seem to be associated with hyperinsulinemia, decreased glucose tolerance, and a higher level of cardiovascular risk factors (Simon *et al.*, 1992; Haffner^a *et al.*, 1994; Haffner^b *et al.*, 1994). A relatively low blood concentration of testosterone in the older men may have adverse effects promoting atherosclerosis and explain the higher incidence of coronary heart disease in the male (Channer and Jones, 2003). Therefore, male hypogonadism can be associated with metabolic syndrome as well as increased risk for cardiovascular disease.

Oxidative stress, due to an imbalance between reactive oxygen species (ROS) and antioxidant defense, can underlie both these phenomena. However the role of gonadal steroids in the regulation of systemic antioxidants is not known.

It has already been recognized that seminal Total Antioxidant Capacity (TAC), which reflects non enzymatic antioxidants, significantly correlates with FSH, LH, free-T3 (fT3), but not with testosterone (Mancini *et al.*, 2005). In previous works we have demonstrated alterations of plasma coenzyme Q₁₀ (CoQ₁₀), a lipidic antioxidant, also endowed with bioenergetic properties, in pituitary diseases, such as acromegaly or secondary hypothyroidism. In particular, in patients with acromegaly, plasmatic value of CoQ₁₀ was low; in hypothyroidism CoQ₁₀ levels were higher than controls, showing a significant inverse correlation with thyroidal hormones, fT3 and free-T4 (fT4) (Mancini *et al.*, 1987; Mancini *et al.*, 1991; Mancini *et al.*, 1992).

Finally, a relationship between sex hormones and plasmatic TAC was already observed (Demirbarg *et al.*, 2005). In fact, estradiol correlated with TAC, that showed lower levels in post-menopausal women than in pre-menopausal ones. Similarly in men testosterone correlated with TAC, that was lower in hypogonadal men than normogonadal ones. The exact molecular mechanism of this action is not known, but estrogens are potent antioxidants both in vitro and in vivo (Avres *et al.*, 1996), especially in terms of protection of fatty acid peroxidation. Both testosterone and estradiol were shown to increase the effects of antioxidant enzymes, such as glutathione peroxidase (Massafra *et al.*, 2000). In vivo, significant cycle-phase related changes in this enzyme were observed in cycling women with positive correlation between estradiol and erythrocyte glutathione-peroxidase (Massafra *et al.*, 1998).

In order to investigate the role of gonadal steroids in the regulation of systemic antioxidants, we determined blood plasma CoQ₁₀ and TAC in a group of hypopituitary patients, following a trans-sphenoidal removal of non-secreting pituitary adenoma or craniopharyngioma. The first objective was to compare hypogonadal patients to normogonadal ones. However, due to the complexity of this postoperative model and the involvement of different pituitary-dependent axes, in order to explain the confounding effect of hypothyroidism on antioxidants, patients with hypothyroidism and hypogonadism were compared to patients with isolated hypogonadism. Finally, the effect of testosterone replacement therapy was evaluated in patients with isolated hypogonadism.

Subjects, Materials and Methods

Twenty-six male subjects, aged 28-55 years, entered this study, after they had given an informed consent. The study was conducted in accordance with the guidelines in the Declaration of Helsinki. The patients were studied at 6-12 months after neurosurgical operation via trans-sphenoidal route, with removal of a non-secreting pituitary adenoma or craniopharyngioma. All patients were hypopituitary, with replacement therapy for thyroidal (ranging from 50 to 100 mcg of L-thyroxine daily according to body weight) and adrenal (20-30 mg of hydrocortisone daily) axes. Patients were classified as normo- or hypogonadal according to their testosterone levels; no androgen replacement therapy had been previously performed before the study. Exclusion criteria for our study included diseases with well-known decreased levels of CoQ₁₀: cardiac, metabolic, cerebral, neuromuscular and mitochondrial diseases (Thomas *et al.*, 2001; Singh and Narankar, 2003; Yalcin *et al.*, 2004; Koroshetz *et al.*, 1997; Beal and Russel, 1997). In the second step, when comparing data before and after androgen therapy, a further added exclusion criterion was the persistence of abnormal thyroid hormones values, because of the demonstrated predominant confounding effect of both hypo- and hyperthyroidism (Mancini *et al.*, 1991).

A blood sample was collected at 08.00 am, for the determination of testosterone (T), estradiol (E2), dihydrotestosterone (DHT), sex hormone binding globulin (SHBG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), free-T3 (fT3), free-T4 (fT4), thyroid-stimulating hormone (TSH), prolactin (PRL), insulin-like growth factor 1 (IGF-1), CoQ₁₀, total cholesterol levels, and TAC in blood plasma. After centrifugation at 2000 g for 10 min, plasma aliquots were immediately stored at -80°C, until assayed. Finally, urinary cortisol (F_U) was assayed in a sample from 24-hour collection.

Moreover, patients with isolated hypogonadism were also studied after a six-month treatment with testosterone enantate (250 mg/im every 3 weeks). Blood collection in testosterone-treated group was performed at the 7th day after the last testosterone enantate injection, according to the kinetic profile of the drug.

Testosterone, estradiol, prolactin and thyroid hormones were assayed in duplicate by RIA, using commercial kits by Radim (Pomezia, Italy). LH, FSH and SHBG were assayed by immunoradiometric method on solid phase (coated tube), which is based on a monoclonal double-antibody technique. Dihydrotestosterone and urinary cortisol were assayed by RIA, using commercial kits by Chematil (Angri, Italy). Plasma IGF-1 was measured by immunoradiometric assay method using kits from Medgenix Diagnostix SA (Fleurus, Belgium); soluble IGF-1 was separated from interfering binding proteins using the acid-ethanol procedure of Daughaday (Daughaday *et al.*, 1980). Free testosterone (free-T) was calculated using the formula proposed by Vermeulen (Vermeulen *et al.*, 1999).

Normal values of the studied hormones were for testosterone: 12.14-34.67 nmol/L; free-T 174-902 pmol/L; estradiol: 36.7-128.45 pmol/L; dihydrotestosterone: 1.04-2.94 nmol/L; SHBG: 15-65 nmol/L ; LH: 2.5-10 UI/L; FSH: 2.5-11 UI/L; fT3: 3.54-6.46 pmol/L; fT4: 10.93-19.94 pmol/L; TSH: 0.35-2.80 mUI/L; PRL:

3.5-15.5 µg/mL; F_U: 99.3-377.9 nmol/d; IGF-1 80-330 µg/L. The intra-assay coefficients of variation were 6.1% for T, 2.3 % for E2, 5.1% for DHT, 6.9% for SHBG, 5.6% for LH, 6.9% for FSH, 4.5% for TSH, 4.1% for fT4, 3.8% for fT3, 2.1% for PRL, 5.3% for F_u, 4.1% for IGF-1. The interassay coefficients of variation were 9.3% for T, 3.5% for E2, 8.9% for DHT, 8.5% for SHBG, 9.1% for LH, 8.4% for FSH, 3.4% for TSH, 4.9% for fT4, 3.9% for fT3, 3.1 for PRL, 8.9% for F_U, 9.6% for IGF-1.

CoQ₁₀ levels were measured by a well recognized HPLC (high-performance liquid chromatography) method (Mosca *et al.*, 2002). The method is based on oxidation of CoQ₁₀ in the sample by treating it with *para*-benzoquinone followed by extraction with 1-propanol and direct injection into the HPLC apparatus. Preoxidation of the sample ensures quantification of total CoQ₁₀ by U.V. detection. This method achieves a linear detector response for peak area measurements over the concentration range of 0.05–3.47 µM. Diode array analysis of the peak was consistent with CoQ₁₀ spectrum. Supplementation of the samples with known amounts of CoQ₁₀ yielded a quantitative recovery of 96–98.5%. The method showed a level of quantitation of 1.23 nmol per HPLC injection (200 µl of propanol extract containing 33.3 µl of plasma). A good correlation was found with a reference electrochemical detection method ($r = 0.99$, $p < 0.0001$). Within run precision showed a CV% of 1.6 for samples approaching normal values (1.02 µM). Day-to-day precision was also close to 2%. Reference values of CoQ₁₀ are 810.74 -1158.20 nmol/L (Tomasetti *et al.*, 1999). Moreover, CoQ₁₀ values were related to plasma cholesterol concentration, measured by a cholesterol-oxidase enzymatic test.

Total Antioxidant Capacity (TAC) was evaluated as previously described (Meucci *et al.*, 2003), with a modification of the method developed by Rice-Evans and Miller (Rice-Evans and Miller, 2004). The method is based on the antioxidants inhibition of the absorbance of the radical cation 2,2¹-azinobis (3-ethylbenzothiazoline-6 sulphonate) (ABTS⁺) formed by interaction between ABTS (150 µM) and ferrylmyoglobin radical species, generated by activation of metmyoglobin (2.5 µM) with H₂O₂ (75 µM). Aliquots of the frozen plasma were thawed at room temperature and 10 µl of the samples were tested immediately. The manual procedure was used with only minor modifications, i.e. temperature was set at 37°C, to be in more physiological conditions, and each sample was assayed alone to carefully control timing and temperature. The reaction was started directly in a cuvette through H₂O₂ addition after 1 min equilibration of all other reagents (temperature control by a thermocouple probe, model 1408 K thermocouple, Digitron Instrumentation Ltd, Scunthorpe, United kingdom) and followed for 10 min under continuous stirring, monitoring at 734 nm, typical of the spectroscopically detectable ABTS⁺. The presence of chain-breaking antioxidants induces a lag time (the LAG phase) in the accumulation of ABTS⁺ whose length (sec) is proportional to the concentration of this type of antioxidants. In the LAG mode, the assay mainly measures non-protein and non-enzymatic antioxidants that are primarily extracellular chain-breaking antioxidants, such as ascorbate, urate and glutathione (Meucci *et al.*, 2003). Trolox, a water-soluble tocopherol analog, was used as a reference standard and assayed in all experiments to control the system.

Absorbance was measured with a Hewlett-Packard 8450A UV/Vis spectrophotometer (Palo Alto, CA) equipped with a cuvette stirring apparatus and a constant temperature cell holder. Measurements of pH were made with a PHM84 Research pH meter (Radiometer, Copenhagen, Denmark,); the electrode response was corrected for temperature. Unless stated differently, experiments were repeated two to three times; intra- and inter-assay coefficients of variation were < 8%.

Distribution of data was estimated by the test of Kolmogorov-Smirnov. Since data were not normally distributed, comparison among the groups was made using Mann-Whitney U test and comparison between the same patients, before and after therapy, was performed using Wilcoxon-Runk Sum test. Linear correlation analysis was also employed. The Software Arcus Quickstat (Software Publishing Biomedical version 1.2) was used for the statistical analysis.

Results

Sixteen of the twenty-six hypopituitary patients presented low levels of testosterone, which were instead normal in the remaining ten patients. CoQ₁₀ and LAG were lower, although not significantly, in hypogonadal patients than in normogonadal ones (see Table 1).

The 16 hypogonadic patients also needed replacement therapy for secondary thyroidal and adrenal deficiencies; normal Igf-1 values indicated preservation of GH-Igf-1 axis. Despite thyroidal replacement therapy, at the time of the study, only ten hypogonadal patients exhibited a normalization of thyroidal values, while six hypogonadal patients remained hypothyroidal (see Table 2); all the patients showed normal cortisol levels under replacement therapy. When we divided hypogonadal patients in normo- and hypothyroidal, we found a significantly lower CoQ₁₀ value in isolated hypogonadism (see Table 2); CoQ₁₀ in isolated hypogonadism was also significantly lower than in normogonadal subjects ($p < 0.05$). When the concentrations of CoQ₁₀ were normalized to cholesterol values, the trend was the same, but the difference among the groups was not significant (see Table 2).

CoQ₁₀ and CoQ₁₀/Cholesterol significantly correlated with LAG values ($r = 0.5$, $p < 0.005$ and $r = 0.7$, $p < 0.001$, respectively), suggesting an inter-relationship between different antioxidants in the whole group of patients (see Figure 1).

Because of the confounding role of thyroid hormones, the study continued only on patients with isolated hypogonadism, who underwent testosterone enantate replacement therapy. Testosterone treatment, by restoring a normogonadal state, induced a significant change both in CoQ₁₀ concentration (937.62 ± 32.79 nmol/L) and in LAG values (78 ± 3 s) (see Figure 2). CoQ₁₀/Cholesterol ratio did not significantly change, confirming that CoQ₁₀ variations were not only due to alteration in CoQ₁₀ transport in plasma lipoproteins.

Discussion

The physiology of testosterone is complex. Its blood concentration shows a circannual and circadian variation and the biologically active moiety is affected by both the amount of sex hormone binding globulin (SHBG) and albumin. The concept of “bioavailable” testosterone, which comprises free testosterone (1-2% of total) and that component loosely bound to albumin, has led to a more accurate assessment of the androgen status. Moreover, because testosterone is converted to estrogen by the aromatase enzyme in adipose tissue, an individual’s concentration is affected by body habitus and weight (Channer and Jones, 2003). There is a higher incidence of men with low testosterone concentrations as age progresses (Vermeulen *et al.*, 1972).

Epidemiological observations suggest a relationship between hypogonadism and cardiovascular diseases. Recent studies have shown that men with coronary artery disease (CAD) have significantly lower concentrations of bioavailable testosterone than men with normal angiograms (English *et al.*, 2000). The prevalence of hypogonadism in a population of men with CAD is about twice that observed in the general population (Morris *et al.*, 2002). Hypotestosteronemia is associated with an atherogenic lipid profile (elevated low density lipoproteins and triglycerides, decreased high density lipoprotein), high fibrinogen with a hypercoagulable state, an increase in insulin resistance and hyperinsulinaemia, and higher systolic and diastolic blood pressure (English *et al.*, 1997).

Also experimental data reinforce the concept of a positive effect of exogenous testosterone administration. In an animal model, castration increased aortic atheroma formation and testosterone replacement ameliorated this effect (Alexandersen *et al.*, 1999). In addition, testosterone has direct vasoactive properties, which directly affect the vascular smooth muscle, not mediated by the nuclear androgen receptor, as the effect is too rapid and is not reduced by flutamide, a nuclear androgen receptor blocker (English *et al.*, 2001; English *et al.*, 2002; Deenadayalu *et al.*, 2001). When testosterone is instilled into the left coronary artery, vasodilatation ensues and coronary flow increases (Webb^b *et al.*, 1999). More importantly, acute administration of intravenous testosterone improves exercise tolerance and reduces angina threshold in men with CAD (Rosano *et al.*, 1999; Webb^a *et al.*, 1999). These positive effects seem to be related to the non-genomic action of testosterone on vascular smooth muscle cells (Jones^a *et al.*, 2003; Jones^b *et al.*, 2003).

Oxidative stress can underlie the above mentioned clinical conditions. As demonstrated by statistical meta-analysis, low testosterone and androgen deficiency were associated with increased risk of developing metabolic syndrome over time (Kupelian *et al.*, 2006). However, the pathophysiological details of these changes in atherosclerosis (Von Eckardstein and Wu, 2004) and implications in testosterone replacement therapy (Nieschlag *et al.*, 2004) are still under investigation. Moreover the role of gonadal steroids in the regulation of systemic antioxidants is not known. We therefore investigated the role of CoQ₁₀, a lipidic antioxidant, and of TAC of blood plasma in secondary male hypogonadism.

Coenzyme Q₁₀ is well defined as a crucial component of the oxidative phosphorylation process in mitochondria. It can undergo oxidation/reduction reactions in other cell membranes such as lysosomes, Golgi

or plasma membranes. The presence of high concentrations of quinol in all membranes provides a basis for antioxidant action either by direct reaction with radicals or by regeneration of tocopherol and ascorbate (Crane and Frederick, 2001). Evidence for a function in redox control of cell signalling and gene expression can be found in studies on coenzyme Q stimulation of cell growth, inhibition of apoptosis, control of thiol groups, formation of hydrogen peroxide, and control of membrane channels (Groneberg *et al.*, 2005). Thyroid hormones exert a profound influence on CoQ₁₀, as previously demonstrated (Mancini *et al.*, 1991). Hyperthyroid patients exhibit extremely low CoQ₁₀ levels. The possible mechanisms include: a) decreased synthesis, related to the competition for tyrosine, which is common substrate for CoQ and thyroxine synthesis (Olson, 1983), even if this hypothesis is disconfirmed by experimental data in animals (Ikeda *et al.*, 1984; Horrum *et al.*, 1986); b) increased CoQ₁₀ utilization, due to the increased stimulation of energy metabolism; c) increased degradation; d) decreased levels of carriers in serum, since it has been demonstrated that the VLDL release from liver is decreased in hyperthyroid states (Keyes *et al.*, 1981). The opposite mechanisms could explain higher CoQ₁₀ levels in hypothyroidism.

Another important parameter of the antioxidant defense of body is plasmatic TAC, which is studied with greater frequency. Representing the functional sum of antioxidants present in plasma, it is a measure of the extracellular antioxidant barrier (Prior and Cao, 1999; Bartoz, 2003; Chevion and Chevion, 2000). In a recent work, TAC was determined during cardiovascular bypass surgery in patients with coronary heart disease: TAC decreased during surgery, but no further decrease in TAC was observed during reperfusion, indicating that it is a relatively stable parameter of the antioxidant barrier of the body (Kedziora-Kornatowska *et al.*, 2003). Finally, a relationship between sex hormones and plasmatic TAC had already been observed (Demirbag *et al.*, 2005). In this paper TAC was measured with a novel automatized method (Erel, 2004). When expressed as mmol Trolox equivalent/L, it significantly correlated with total testosterone in male subjects and also with estradiol in a group of pre- and post-menopausal women (Demirbag *et al.*, 2005). No effect of androgens was observed on erythrocyte antioxidant systems in cycling women (Massafra *et al.*, 2000).

Conflicting results do not allow unequivocal conclusions on the role of androgens in coronary artery disease, as recently reviewed by Wu and Liu (Wu and von Eckardstein, 2003; Liu *et al.*, 2003). Many confounding factors contribute to making this question a very complex one. Studies on endogenous androgen levels depend on different mechanisms, such as gender-specific gene expression, distribution of body fat, vascular factors, and adaptation to ageing. Similarly, studies on exogenous androgen administration are influenced by dose, route of administration, duration of treatment and again gender, age, condition of recipients. Moreover, the field of androgen effects on cardiovascular system, concerning genomic and non-genomic effects, aromatization-mediated or not actions, anti- and pro-atherogenic mechanisms is rapidly growing, but it still does not allow a conclusive picture. Therefore, data on antioxidant regulation by steroids can be useful to clarify molecular mechanisms of testosterone action.

Even though the relationship between systemic and seminal antioxidants, as well as systemic regulation of seminal antioxidants, are still poorly understood, our data suggest that CoQ₁₀ levels are significantly lower in secondary isolated hypogonadal vs normogonadal patients with the same physiopathological postoperative condition. However, since thyroid hormones have an important role in modulating CoQ₁₀ levels and metabolism (Mancini *et al.*, 1991), when coexistent, thyroid deficiency could be more important in influencing antioxidant levels than hypogonadism. Other explanations such as thyroid influence on gonadal hormones are unlikely in this particular clinical model. It is well known that hypothyroidism *per se* reduces the clearance of testosterone and increases SHBG (Larsen and Davies, 2003), but these mechanisms are not relevant in our model where both axes are depressed due to the pituitary surgery. The same consideration concerns the possible effects of hypothyroidism on GnRH secretion (Toni *et al.*, 2005), and on Leydig cell steroidogenesis (Mendis-Handagama and Siril Ariyaratne, 2005). The influence of pituitary-adrenal axis and of growth hormone, which can affect antioxidant levels (Mancini *et al.*, 2005; Brown-Borg *et al.*, 1999) were excluded on the basis of normal cortisol and IGF-1 levels.

Testosterone therapy reported values toward the same levels observed in normogonadic patients, with a significant increase in CoQ₁₀ concentrations. TAC also, expressed as LAG, which exhibited a trend toward lower values in hypogonadal subjects, was significantly increased by testosterone treatment.

In conclusion, our data reinforce the concept that hypogonadism could represent a condition of oxidative stress. Although the small number of patients studied does not allow definitive conclusions, lower levels of CoQ₁₀ were discovered in isolated hypogonadal vs normogonadal patients. To our knowledge this is the first report of testosterone effect on antioxidant systems in humans. Further studies can clarify the relationship of this datum with the augmented cardiovascular risk in such patients.

References

- Alexandersen P, Haarbo J, Byrjalsen I, Lawaetz H, Christiansen C. Natural androgens inhibit male atherosclerosis. A study of castrated, cholesterol fed rabbits. *Circ. Res.* 1999;84:813-819.
- Avres S, Tang M, Subbiah MT. Estradiol-17beta as an antioxidant: some distinct features when compared with common fat-soluble antioxidants. *J. Lab Clin Med* 1996;128(4):367-375.
- Bartoz G. Total antioxidant capacity. *Adv. Clin. Chem.* 2003;37:219-292.
- Beal MF, Russel TM. Coenzyme Q₁₀ in the Central nervous System and its Potential Usefulness in the Treatment of Neurodegenerative Diseases. *Mol. Aspects Med.* 1997;18:169-179.
- Brown-Borg HM, Bode AM, Bartke A. Antioxidative mechanisms and plasma growth hormone levels: potential relationship in the aging process. *Endocrine* 1999;11(1):41-48.
- Channer KS, Jones TH. Cardiovascular effects of testosterone: implications of the "male menopause"? *Heart* 2003;89:121-122.
- Chevion S, Chevion M. Antioxidant status and human health. Use of cyclic voltammetry for the evaluation of the antioxidant capacity of plasma and of edible plants. *Ann. N. Y. Acad. Sci.* 2000;899:308-325.
- Crane P, Frederick L. Biochemical Functions of Coenzyme Q₁₀. *J. Am. Coll. Nutr.* 2001;20:591-598.
- Daughaday WH, Mariz IK, Blethen SL. Inhibition of access of bound somatomedin to membrane receptor and immunobinding sites: a comparison of radioreceptor and radioimmunoassay of somatomedin in native and acid-ethanol-extracted serum. *J. Clin. Endocrinol. Metab.* 1980;51:781-788.
- Deenadayalu VP, White RE, Stallone JN, Gao X, Garcia AJ. Testosterone relaxes coronary arteries by opening the large-conductance, calcium-activated potassium channel. *Am. J. Physiol. Heart Circ. Physiol.* 2001;281:1720-1727.
- Demirbag R, Yilmaz R, Erel O. The association of total antioxidant capacity with sex hormones. *Scand. Cardiovasc. J.* 2005;39:172-176.
- English K, Steeds RP, Jones TH, Channer KS. Testosterone and ischaemic heart disease: is there a link? *QJM* 1997;90:787-791.
- English KM, Jones RD, Jones TH, Morice AH, Channer KS. Gender differences in the vasomotor effects of different steroid hormones in rat pulmonary and coronary arteries. *Horm. Metab. Res.* 2001;33:645-652.
- English KM, Jones RD, Jones TH, Morice AH, Channer KS. Testosterone acts as a coronary vasodilator by a calcium channel antagonist action. *J. Endocrinol. Invest.* 2002;25:455-458.
- English KM, Mandour O, Steeds RP, Diver MJ, Jones TH, Channer KS. Men with coronary artery disease have lower levels of testosterone than those with normal coronary angiograms. *Eur. Heart J.* 2000;21:890-894.
- Erel O. A novel-automated method to measure total antioxidant response against potent free radical reactions. *Clin. Biochem.* 2004;37:112-119.
- Groneberg DA, Kindermann S, Althammer M, Klapper M, Vormann J, Littarru GP, Doring F. Coenzyme Q₁₀ affects expression of genes involved in cell signalling, metabolism and transport in human CaCO-2 cells. *Int. J. Biochem. Cell. Biol.* 2005;37:1208-1218.
- Haffner SM, Karhapaa P, Mykkanen L, Laakso M. Insulin resistance, body fat distribution, and sex hormone in men. *Diabetes* 1994;43:212-219.
- Haffner SM, Valdez RA, Mykkanen L, Stern MP, Katz MS. Decreased testosterone and dehydroepiandrosterone sulphate concentrations are associated with increased insulin and glucose concentrations in non-diabetic men. *Metabolism* 1994;43:599-603.
- Horrum MA, Tobin RB, Ecklund RE. Thyroxine-induced changes in rat liver mitochondrial ubiquinone. *Biochem. Biophys. Res. Commun.* 1986; 138(1):381-386.
- Ikeda S, Hamada N, Morii H, Inaba M, Yamakawa J. Serum and tissue coenzyme Q₉ in rats with thyroid dysfunctions. *Horm. Metab. Res.* 1984; 16:585-588.
- Jones RD, Pugh PJ, Jones TH, Channer KS. The vaso-dilatory action of testosterone – a potassium channel opening or a calcium channel antagonistic action. *Br. J. Pharmacol.* 2003;138:733-744.

- Jones RD, Ruban LN, Morton IE, Roberts SA, English KM, Channer KS, Jones TH. Testosterone inhibits the prostaglandin F_{2α}-mediated increase in intracellular calcium in A7r5 aortic smooth muscle cells: evidence of an antagonistic action upon store operated calcium channels. *J. Endocrinol.* 2003;178:381-393.
- Kedziora-Kornatowska K, Bartosz M, Mussur M, Zaslonka J, Kedziora J, Bartosz G. The total antioxidant capacity of blood plasma during cardiovascular bypass surgery in patients with coronary heart disease. *Cell. Mol. Biol. Lett.* 2003;8:973-977.
- Keyes WG, Wilcox HG, Heimberg M. Formation of the very low density lipoprotein and metabolism of [1-¹⁴C]-oleate by perfused livers from rats treated with triiodothyronine or propylthiouracil. *Metabolism* 1981; 30:135-146.
- Koroshetz WJ, Jenkins BG, Rosen BR, Beal MF. Assessment of energy metabolism defects in Huntington's disease and possible therapy with coenzyme Q₁₀. *Ann. Neurol.* 1997;41:160-165.
- Kupelian V, Page ST, Araujo AB, Travison TG, Bremner WJ, McKinlay JB. Low sex hormone-binding globulin, total testosterone, and symptomatic androgen deficiency are associated with development of the metabolic syndrome in nonobese men. *J. Clin. Endocrinol. Metab.* 2006;91:843-850.
- Larsen PR and Davies TF. Hypothyroidism and thyroiditis. In: Larsen PR, Kronenberg HM, Melmed S, Polonsky KS, eds. *Williams Textbook of Endocrinology*, tenth edition. Philadelphia: Saunders; 2003. p 423-455.
- Liu PY, Death AK, Handelsman DJ. Androgens and cardiovascular disease. *Endocr. Rev.* 2003;24(3):313-340.
- Mancini A, Bianchi A, Fusco A, Sacco E, Leone E, Tilaro L, Porcelli T, Giampietro A, Principi F, De Marinis L, Littarru GP. Coenzyme Q10 evaluation in pituitary-adrenal axis disease: preliminary data. *Biofactors* 2005;25:197-199.
- Mancini A, Calabrò F, Fiumara C, Conte G, Oradei A, Lippa S, De Marinis L, Littarru GP. Plasma coenzyme Q10 determination in acromegaly. *Exp. Clin. Endocrinol. Life Sci. Adv.* 1992;11:55-60.
- Mancini A, De Marinis L, Calabrò F, Sciuto R, Oradei A, Lippa S, Sandric S, Littarru GP, Barbarino A. Evaluation of metabolic status in amiodarone-induced thyroid disorders: plasma Coenzyme Q10 determination. *J. Endocrinol. Invest.* 1989;12:511-516.
- Mancini A, De Marinis L, Calabrò F, Fiumara C, Goglia A, Sofo L, Lippa S, Oradei A, Rabitti C, Littarru GP. Physio-pathological relevance of coenzyme Q10 in thyroid disorders: CoQ10 concentrations in normal and diseased human thyroid tissue. In: Folkers, K.; Littarru, G. P.; Yamagami, T., eds. *Biomedical and Clinical Aspects of Coenzyme Q*. Amsterdam: Elsevier; 1991. p 441-448.
- Mancini A, Milardi D, Bianchi A, Meucci E, Pontecorvi A, De Marinis L. Hormonal regulation of total antioxidant capacity in seminal plasma. In: Kim, S.; Grootegeod, J. A.; Chemes, H. E., eds *Proceedings of the 8th International Congress of Andrology*, Seoul, Korea, Free Papers. Bologna: Medimond; 2005. p 59-62.
- Massafra C, De Felice C, Gioia D, Buonocore G. Variations in erythrocyte antioxidant glutathione peroxidase activity during the menstrual cycle. *Clin. Endocrinol.* 1998; 49:63-67.
- Massafra C, Gioia D, De Felice C, Picciolini E, De Leo V, Bonifazi M, Bernabei A. Effects of estrogens and androgens on erythrocyte antioxidant superoxide dismutase, catalane and glutathione peroxidase activities during the menstrual cycle. *J. Endocrinol.* 2000;167:447-452.
- Mendis-Handagama SM, Siril Ariyaratne HB. Leydig cells, thyroid hormones and steroidogenesis. *Indian J Exp Biol.* 2005; 43(11):939-962.
- Meucci E, Milardi D, Mordente A, Martorana GE, Giacchi E, De Marinis L, Mancini A. Total antioxidant capacity in patients with varicoceles. *Fertil. Steril.* 2003;79(3):1577-1583.
- Morris P, Pugh PJ, Hall J. The relationship between smoking, statin therapy and testosterone in men with coronary artery disease. *Endocrine Abstracts*; 2002. p 248.
- Mosca F, Fattorini D, Bompadre S, Littarru GP. Assay of coenzyme Q(10) in plasma by a single dilution step. *Anal. Biochem.* 2002;305:49-54.
- Nieschlag E, Behre HM, Bouchard P, Corrales JJ, Jones TH, Stalla GK, Webb SM, Wu FCW. Testosterone replacement therapy: current trends and future directions. *Hum. Reprod.* 2004;10:409-419.
- Olson RE, Rudney H. Biosynthesis of ubiquinone. *Vitam. Horm.* 1983; 40:1-43.

- Prior RL, Cao G. In vivo total antioxidant capacity: comparison of different analytical methods. *Free Radic. Biol. Med.* 1999;27:1173-1181.
- Rice-Evans C, Miller NJ. Total antioxidant status in plasma and body fluids. *Methods. Enzymol.* 1994;234:279-293.
- Rosano GMC, Leonardo F, Pagnotta P, Pelliccia F, Panina G, Cerquetani E, Della Monica PL, Bonfigli B, Volpe M, Chierchia SL. Acute anti-ischaemic effect of testosterone in men with coronary artery disease. *Circulation* 1999;99:1666-1670.
- Simon D, Preziosi P, Barrett-Connor E, Roger M, Saint-Paul M, Nahoul K, Papoz L. Interrelation between plasma testosterone and plasma insulin in healthy adult men: the Telecom Study. *Diabetologia* 1992;35:173-177.
- Singh RB, Narankar SF. Effect of coenzyme Q₁₀ on risk of atherosclerosis in patients with recent myocardial infarction. *Mol. Cell. Biochem.* 2003;246:75-82.
- Thomas ST, Leichtweis SB, Pettersson K, Croft K. Dietary cosupplementation with vitamin E and CoQ₁₀ inhibits atherosclerosis in apolipoprotein E gene knockout mice. *Arterioscler. Thromb. Vasc. Biol.* 2001;21:585-593.
- Tomasetti M, Alleva R, Solenghi MD, Littarru GP. Distribution of antioxidants among blood components and lipoproteins: significance of lipids/CoQ₁₀ ratio as a possible marker of increased risk for atherosclerosis. *Biofactors* 1999;9:231-240.
- Toni R, Della Casa C, Castorina S, Cocchi D, Celotti F. Effects of hypothyroidism and endocrine disruptor-dependent non-thyroidal illness syndrome on the GnRH-gonadotroph axis of the adult male rat. *J Endocrinol Invest.* 2005;28(11 Suppl Proceedings):20-27.
- Vermeulen A, Rubens R, Verdonck L. Testosterone secretion and metabolism in male senescence. *J. Clin. Endocrinol. Metab.* 1972;34:730-735.
- Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J. Clin. Endocrinol. Metab.* 1999;84:3666-3672.
- Von Eckardstein A, Wu FCW. Testosterone and cardiovascular disease. In: Nieschlag, E. and Behre, H. M., eds. *Testosterone: Action, Deficiency, Substitution*. 3rd Ed. Cambridge: University Press; 2004. p 297-331.
- Webb CM, Adamson DL, de Zeigler D, Collins P. Effect of acute testosterone on myocardial ischaemia in men with coronary artery disease. *Am. J. Cardiol.* 1999;83:437-439.
- Webb CM, Mc Neill JG, Hayward CS, De Zeigler D, Collins P. Effects of testosterone on coronary vasomotor regulation in men with coronary heart disease. *Circulation* 1999;100:1690-1696.
- Wu FCW, von Eckardstein A. Androgens and coronary artery disease. *Endocr. Rev.* 2003;24(2):183-217.
- Yalcin A, Kilinc E, Sagcan A, Kultursay H. Coenzyme Q₁₀ concentrations in coronary artery disease. *Clin. Biochem.* 2004;37:706-709.

Figure legends

Figure 1. Scattered plotting of linear correlation analysis between CoQ₁₀ values or CoQ₁₀/Cholesterol ratio and LAG. $r =$ Spearman's coefficient.

Figure 2. Mean \pm SEM levels of testosterone, CoQ₁₀, CoQ₁₀/Cholesterol and LAG in patients with isolated hypogonadism, before and after testosterone treatment.

* $p < 0.05$ vs pre-treatment values.

Table 1. Mean \pm SEM hormone and antioxidant levels in our patients, classified in two groups according to testosterone levels. * $p < 0.05$ vs normogonadal patients.

	Hypogonadal patients <i>n=16</i>	Normogonadal patients <i>n=10</i>
T (nmol/L)	4.90 \pm 0.92 *	21.20 \pm 3.04
free-T (pmol/L)	98.44 \pm 18.31 *	440.24 \pm 72.03
E2 (pmol/L)	53.90 \pm 5.14 *	133.33 \pm 18.52
DHT (nmol/L)	1.23 \pm 0.14 *	5.94 \pm 0.76
SHBG (nmol/L)	27.96 \pm 1.74 *	43.52 \pm 2.83
LH (IU/l)	3.84 \pm 1.50	4.49 \pm 0.63
FSH (IU/l)	6.17 \pm 2.35	4.78 \pm 0.82
IGF-1 (μ g/L)	142.44 \pm 14.50	125.20 \pm 18.89
PRL (μ g/L)	18.36 \pm 3.18	13.32 \pm 1.75
fT3 (pmol/L)	4.31 \pm 0.28	4.59 \pm 0.31
fT4 (pmol/L)	14.75 \pm 0.98	17.53 \pm 1.07
TSH (mUI/L)	0.80 \pm 0.19	0.71 \pm 0.15
Fu (nmol/d)	261.41 \pm 165.54	331.08 \pm 82.52
CoQ₁₀ (nmol/L)	963.67 \pm 114,85	1077,69 \pm 117.79
CoQ₁₀/Chol. (nmol/mmol)	200.30 \pm 12.35	214.63 \pm 17.23
LAG (s)	70 \pm 6	76 \pm 10

Table 2. Mean \pm SEM hormone and antioxidant levels in patients with hypogonadism, classified in two sub-groups according to thyroid hormone levels. * $p < 0.05$ vs isolated hypogonadism

	Isolated Hypogonadism <i>n=10</i>	Hypogonadism & Hypothyroidism <i>n=6</i>
T (nmol/L)	5.54 \pm 1.10	3.84 \pm 1.68
free-T (pmol/L)	110.51 \pm 21.96	78.32 \pm 33.24
fT3 (pmol/L)	4.93 \pm 0.16	3.28 \pm .47 *
fT4 (pmol/L)	16.45 \pm 0.74	11.93 \pm 1.88 *
CoQ₁₀ (nmol/L)	768.62 \pm 75.73	1288.75 \pm 233.46 *
CoQ₁₀/Chol. (nmol/mmol)	190.84 \pm 13.05	216.06 \pm 25.09
LAG (s)	71 \pm 4	69 \pm 14



