Summary

Objectives: Men die more often of coronary artery disease (CAD) than women. The higher incidence of CAD in men could be due to the higher levels of testosterone. There is evidence that testosterone is either neutral or has a beneficial effect on male cardiovascular disease. The role of oestrogens in male CAD has been less studied. This study was carried out with the purpose of evaluating the relationship among sex hormones levels and coronary artery disease.

Designer: Case-control study.

Participants: Men (aged 40-70 years) submitted to coronary angiography. A 70% occlusion of at least one major coronary artery defined the cases; subjects with ≤ 50% occlusion constituted the control group.

Measurements: Blood samples were collected for total testosterone, oestradiol, LH, FSH, SHBG, lipid profile and albumin measurements. Bioavailable and free testosterone, FAI and FEI were calculated. Oestradiol levels were examined as terciles, based on the whole study population.

Results: Of the 140 patients included, 72 were cases and 68 were controls. The baseline characteristics of the two groups were similar, except for the older age and lower LDL cholesterol in the cases. Oestradiol and FEI but not total, bioavailable and free testosterone and FAI correlated positively with CAD. The prevalence of CAD was significantly higher in the 3rd than in the 1st tercile of oestradiol.

Conclusion: This study suggests a deleterious effect of oestradiol and a neutral effect of testosterone on the incidence of CAD in men. Larger studies are needed to support these findings.

Introduction

When compared with women of similar age, cardiovascular disease (CVD) is more prevalent in men (1). Mortality and morbidity associated with CVD are lower in pre-menopausal women. Men are twice as likely to die of coronary artery disease (CAD) and display a greater incidence of myocardial infarction (MI) than women (2). This difference between genders regarding CVD may involve genetic, hormonal and lifestyle factors. A common explanation for the greater incidence of CAD in men than in women could be the elevated levels of testosterone in men, suggested to be pro-atherogenic, or the protective effect of oestrogens in women. Except for this indirect evidence, recent studies in the literature indicate that the endogenous androgens can have an either neutral and/or beneficial effect on the male cardiovascular system (3-6). Moreover, the endogenous oestradiol (E2) levels in men were correlated to atherosclerotic disease of the carotid artery (7) and of the lower extremities (8), risk of stroke in elderly men (9), and the treatment with high doses of oestrogens in men with prostate cancer (10) and male-to-female transsexuals (11) was associated with an increase in cardiovascular morbidity and mortality. This study was carried out with the purpose of evaluating the relationship among total testosterone (TT), bioavailable testosterone (BT) and free testosterone (FT), free androgen index (FAI), SHBG, E2, free oestrogen index (FEI), the oestradiol/testosterone (E2/T) ratio and the (FAI/FEI) ratio in patients with CAD and controls submitted to coronary angiography.

Materials and Methods

Study design

This is a case-control study. Levels of total, bioavailable and free testosterone, FAI, E2, FEI E2/T ratio, FAI/FEI ratio, LH, FSH and SHBG were measured in men submitted to coronary angiography. Individuals found to have ≥ 70% occlusion of at least one major coronary artery were defined as cases; those with ≤ 50% occlusion in all coronary arteries (free of significant stenosis) constituted the control group.

Study population

Men aged between 40 and 70 years were selected among consecutive patients admitted for coronary angiography for CAD diagnosis at the Hospital Dante Pazzanese de Cardiologia. The indication of the coronary angiography was for clinical follow up and all exams were elective. Subjects who had had MI, stroke or another major illness within the preceding 6 months, were taking any medications known to affect the sex hormone levels (ketoconazole, cimetidine, spironolactone, cyproterone and finasteride), were smokers, had a body mass index [(BMI) weight (kg)/height (m^2)] ≥ 40 kg/m2, creatinine blood levels > 2.0 mg/dL, or evidence of major liver disease upon clinical examination were excluded.

Written informed consent was obtained from all participants before the angiography, and the project was approved by the Ethics Committees of the two participating institutions.

Evaluation

Baseline data including age, history of risk factors for CAD and current medication used were recorded using standard questionnaires. Anthropometric measurements such as weight, height, waist circumference and BMI were also obtained.

Laboratory measurements

Blood samples were drawn between 8:00 a.m. and 10:00 a.m., after overnight fasting, immediately prior to coronary angiography. Glucose, total cholesterol, HDL cholesterol, triglycerides and albumin were measured at the laboratory of the Hospital Dante Pazzanese de Cardiologia immediately after the blood collection. LDL cholesterol was calculated using the Friedewald formula (12). The blood samples for hormone determinations were taken right away to the Steroid Laboratory of Escola Paulista de Medicina/UNIFESP, immediately centrifuged, and frozen to -21°C until laboratory studies were performed, within six months from the blood collection. All determinations were done in duplicate. The measurements of total, bioavailable and free testosterone were made according to the Endocrine Society Position Statement (13). Testosterone was measured by RIA (14) with local historical controls (15, 16) (limit of detection 0.35 nmol/L), SHBG was measured by immunofluorometric assay (IFMA) [Delfia PerkinElmer, limit of detection 0.5 nmol/L, intra-assay coefficient of variation (CV) 3.9, 4.9 and 3.3%; and inter-assay CV 2.3, 3.0 and 2.4% for 25.5, 63.9, and 138.0 nmol/L, respectively]. E2 was measured by the IFMA assay (Delfia PerkinElmer, limit of detection 0.05 nmol/L, intra-assay CV 6.9%, inter-assay CV 9.7%). FSH was also measured by IFMA (Delfia PerkinElmer, limit of detection 0.05 nmol/L, intra-assay CV 2.0, 2.8 and 2.2%, and inter-assay CV 1.8, 2.0 and 1.8% for 2.58, 11.5, and 44.8 UI/L, respectively); and LH was equally measured by IFMA (Delfia PerkinElmer, limit of detection 0.05 nmol/L, intra-assay CV 2.4 and 2.0% for 3.63 and 20.0 UI /L, respectively, and inter-assay CV 3.1%). Free and bioavailable testosterone were calculated according to Vermeulen et al, using total testosterone, SHBG and albumin (17). The Free Androgen Index and the Free Oestrogen Index were obtained using the formulae: total testosterone x 100/SHBG and total estradiol x 100/SHBG respectively (17).

Statistical analysis

The sample size was calculated based on the weighted mean of the standard deviations of the total testosterone levels of cases and controls in the study of English et al (3), with an 80% statistical power, resulting in a total of 66 cases and 66 controls. Categorical data were analyzed using Fisher's exact test. To calculate the normally distributed continuous variables (age, BMI, total cholesterol, LDL cholesterol, HDL cholesterol, SHBG, total, bioavailable and free testosterone, FAI, E_2/T ratio) we used the *t*-test for independent samples. The skewed continuous variables (waist circumference, triglycerides, LH, FSH, E_2, FEI and FAI/FEI ratio) were analyzed with the Mann-Whitney test. Oestradiol levels were examined as terciles, based on the whole study population. The comparison of the continuous variables between the oestradiol tercile groups was made using ANOVA. To determine the odds ratios (ORs) of CAD prevalence by oestradiol tercile group, we used logistic regression analysis. Data were analyzed using GraphPad Prism 5.0 and SPSS 9.0. Statistical significance was set at $p < 0.05$.

Results

When the 144 participants were considered eligible for the study, their degree of coronary occlusion at angiography was not known. Four patients were excluded because their coronary angiography showed < 70% occlusion of all coronary arteries and > 50% occlusion of at least one coronary artery.

After the coronary angiography results, the study subjects were divided into 72 cases and 68 controls. All 72 cases had undergone coronary angiography with symptoms of chest pain, 26 had been diagnosed with ischemic heart disease before the coronary angiography and 46 had not. Sixty-eight controls entered in the study, of which 57 had undergone coronary angiography with symptoms chest pain, 10 had valvular heart disease and one, arrhythmia. In

the control group, 63.24% had completely normal coronary arteries, 10.29% had minimal wall irregularities, and 26.47% had a 20-50% occlusion in at least one of the coronary arteries.

The individuals with ≥ 70% occlusion of at least one major coronary artery (cases) and those with ≤ 50% occlusion in all coronary arteries (controls) presented similar clinical characteristics (BMI, waist circumference, prevalence of hypertension, hyperlipidemia, diabetes mellitus and family history of CAD), except for age, since the cases were older than the controls (p=0.02) (Table 1). Two subjects were unable to inform about their family history of CAD, one in the control group and one in the case group; the latter was adopted. Waist circumference was not measured in one patient of the case group and in two controls.

With regard to the lipid profile measurements, the two groups were also similar, except for the LDL cholesterol (LDL-C) levels, which were significantly lower in the case group (p = 0.02) (Table 1). When we checked the use of cholesterol-lowering medications, we found that the use of statins was significantly higher in the case group (p < 0.001). LDL-C could not be calculated in two cases and two controls because of limitations imposed by the Friedewald formula.

No statistical differences were found in the LH, FSH, TT, BT, FT, FAI, SHBG levels, in the E_2/T ratio or in the FAI/FEI ratio of the patients with ≥ 70% and ≤ 50% occlusion on coronary angiography (Table 2). The oestradiol levels and FEI were significantly higher in the case than in control group (Table 2).

The oestradiol levels were analyzed as terciles, based on the whole study population. The baseline characteristics between the terciles were similar, except for family history of CAD (Table 3). The prevalence of CAD was found to be significantly higher in the 3rd tercile (highest) of oestradiol, as compared to the 1st tercile (lowest) [OR 2.0, (IC: 0.094 - 0.516)] (Table 4).

Discussion

In the present study, men with ≥ 70% occlusion of coronary arteries at angiography presented higher estradiol levels than those with ≤ 50% occlusion ($p< 0.001$). No statistically significant difference between the two groups was observed regarding lipid profile, TT, BT, FT, FAI, and SHBG levels, estradiol/testosterone ratio, or the FAI/FEI ratio.

Our data are in accordance with other prospective (4, 18, 19) and case-control (20) studies, which also failed to find association between testosterone levels and incidence of CAD in men. However, other studies reported a negative association between levels of testosterone and CAD, suggesting a protective effect of this hormone regarding CVD in men (3, 5, 6, 21, 22).

The variability in the results of the studies assessing the relation between testosterone levels and atherosclerosis may be due to an inappropriate selection of the control group, with different risk factors for CVD than the case group. In some studies, it was observed that the cases were older and showed a greater prevalence of smoking, obesity, diabetes mellitus, hyperlipidemia and hypertension than the controls, and it was argued that the testosterone levels might be only a consequence of the metabolic syndrome and/or visceral obesity (23, 24). Moreover, in several studies, the case group was evaluated by coronary angiography, whereas the control group was defined by the absence of clinical symptoms of CAD and/or by a normal treadmill test. However, in patients with CAD, the sensitivity of the treadmill test is approximately 68% and the specificity 77%, and in patients with one-vessel disease, sensitivity varies from 25% to 71% (25), which could represent a confounding factor in these studies. Furthermore, inappropriate testosterone assay techniques can make it difficult to determine the reference limits, affecting a refined analysis of the results (12). Testosterone has a diurnal rhythm, with peak blood levels in the morning and nadir in the evening (26), however some studies did not take the time of collection of the hormone into account. In our

study, the basic clinical characteristics of cases and controls were similar, except for the older age of the case group (59.04 ± 74 versus 56.12 ± 0.98, p = 0.02), all subjects underwent coronary angiography, which is the gold standard for evaluation of coronary atherosclerosis (27), and blood samples were obtained between 08:00 a.m. and 10:00 a.m. and measured by RIA (a method standardized by the Steroid Laboratory with historical controls) (15, 16).

This study found a positive association between oestradiol levels and CAD in adult men. The patients with severe CAD had higher oestradiol levels than the controls ($p < 0.001$). This association remained statistically significant when the whole sample was divided into terciles of oestradiol. The patients in the 3rd tercile (highest) of oestradiol showed an OR of 2.0 [IC (95%) 0.094 - 0.516] for the prevalence of CAD when compared with the patients in the 1st tercile (lowest). Our findings are in agreement with other studies suggesting a deleterious effect of endogenous oestrogens on the male cardiovascular system. One report of the Framingham Study found that 61 men with CAD had significantly higher oestradiol levels than 61 matched controls (28). Phillips et al (29) reported hyperoestrogenemia to be related to thrombotic occlusion of coronary arteries in MI. The mean serum oestradiol level in men who had had a MI [$(14.13 \pm 3.23) \times 10^{-2}$ nmol/L] was higher ($p = 0.002$) than the level in men who had not had a MI [$(11.7 \pm 2.6) \times 10^{-2}$ nmol/L]. High levels of endogenous oestradiol in men were related to atherosclerotic disease of the carotid artery (7) and of the lower extremities (8) and to risk of stroke in elderly men (9). However, our findings are in opposition to those of other authors who found a negative and/or neutral correlation of oestradiol levels with CAD (3, 4, 6, 20).

As observed with regard to testosterone, the variability in the results may be due to an inadequate selection of the control group and to an inadequate selection for CAD. In this study, although the cases were a little older than the controls, this difference disappeared when the data were analyzed as terciles of oestradiol, but the positive association between

serum oestradiol levels and CAD persisted. As previously mentioned, all the study subjects (cases and controls) underwent coronary angiography.

The reason why serum oestradiol levels correlate with CAD in men is not fully understood so far. Oestradiol is known to have beneficial effects on the male cardiovascular system, inhibiting the migration and proliferation of vascular smooth muscle cells (30), increasing HDL-C (31) and NO synthase activity, thus promoting vasodilatation (32). On the other hand, oestrogens can increase the risk of a thrombotic event (33, 34). Moreover, chronic inflammation has emerged as an important independent predictor of CAD (35), and a positive correlation between E_2 and C-reactive protein levels in middle-aged and elderly men was described (36).

The choice of the $\geq$ 70% and $\leq$ 50% coronary stenosis points for definition of cases and controls was based on studies that measured coronary flow reserve. The coronary flow reserve starts to decrease with 50% luminal diameter stenosis (37). The ability to increase flow during vasodilator stimulus is impaired when luminal diameter is reduced by 50% and abolished when the stenosis is > 70% (38).

The present study found higher values of LDL-C in the controls than in the cases ($p < 0,001$), but it should taken into account that the use of statins was highest in the cases ($p < 0,001$). Inhibition of cholesterol biosynthesis by statins could, in theory, adversely affect the male gonadal function because cholesterol is a precursor of steroid hormones. However, this hypothesis was not confirmed in several clinical studies (39, 40).

The results of this study should be interpreted with caution. It should be kept in mind that this was a case-control study, performed with adult male patients who received medical attention at a reference hospital in the city of São Paulo. These results should not be extrapolated to other populations, other age groups or gender.

In conclusion, in the present study, the laboratory markers for androgen status (total, bioavailable and free testosterone and free androgen index), SHBG, E_2/T ratio and FAI/FEI ratio were not associated with major coronary artery disease (arterial occlusion ≥ 70%) in adult men. Surprisingly, the oestradiol levels and FEI were higher in the patients with major coronary artery disease than in the controls. Further studies are needed to establish the role of endogenous E_2 in the prevalence of cardiovascular disease in men.

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Table 1 – Clinical and metabolic characteristics of the cases and controls groups

	Cases (n = 72)	**Controls (n = 68)**	**P**
Age (years)	59.04 ± 0.74	56.12 ± 0.98	**0.02**
BMI (kg/m^2)	27.71 ± 0.41	27.39 ± 0.55	0.64
Waist circumference (cm)	97.65 ± 1.11	98.75 ± 1.54	0.94
Hypertension (%)	91.67	85.29	0.29
Hyperlipidemia (%)	70.83	60.29	0.29
Diabetes mellitus (%)	29.17	16.18	0.07
Family history of CAD (%)	56.34	41.79	0.09
Total cholesterol (nmol/L)	4.43 ± 0.13	4.78 ± 0.14	0.08
LDL-cholesterol (nmol/L)	2.50 ± 0.11	2.88 ± 0.12	**0.02**
HDL-cholesterol (nmol/L)	1.07 ± 2.88(10^{-2})	1.12 ± 3.48(10^{-2})	0.28
Triglycerides (nmol/L)	1.94 ± 0.14	1.72 ± 0.13	0.22

Data are expressed as mean ± standard deviation. BMI, body mass index; CAD, coronary artery disease; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Table 2 – Sex hormone profile in men submitted to coronary angiography allocated in cases and controls groups.

	Cases (n = 72)	**Controls (n = 68)**	**p**
LH (UI/L)	4.36 ± 0.45	4.15 ± 0.40	0.86
FSH (UI/L)	6.11 ± 0.82	6.43 ± 0.60	0.43
SHBG (nmol/L)	44.29 ± 2.21	50.49 ± 3.34	0.14
Total testosterone (nmol/L)	17.37 ± 0.96	16.41 ± 0.99	0.49
Bioavailable testosterone (nmol/L)	7.47 ± 0.44	6.63 ± 0.48	0.19
Free testosterone (pmol/L)	321.7 ± 18.89	281.5 ± 20.22	0.15
Free androgen index	44.42 ± 2.89	37.91 ± 2.62	0.10
Oestradiol (pmol/L)	81.25 ± 3.95	66.03 ± 3.55	**< 0.001**
Free oestrogen index (10^{-2})	21.08 ± 0,18	16.30 ± 0,12	**0,02**
Oestradiol/testosterone ratio (10^{-4})	53.30 ± 3.23	47.87 ± 2.87	0.21
FAI/FEI ratio	289.0 ± 42.63	267.80 ± 15,55	0.22

Data are expressed as mean ± standard deviation. FAI, free androgen index; FEI, free oestrogen index.

Table 3. Clinical and metabolic characteristics according to terciles of oestradiol in men submitted to coronary angiography

	1º Tercile (n=53)	**2º Tercile (n=41)**	**3º Tercile (n=46)**	**p**
Oestradiol (pmol/L)	50.0 (50.0 – 50.0)	65.33 ± 5.05 (60.0 – 70.0)	108.97 ± 35.45 (80.0 – 250.0)	**<0.001**
Age (years)	57.08 ± 7.28	58.45 ± 7.43	57.51 ± 7.37	0.67
BMI (kg/m^2)	26.66 ± 4.05	27.42 ± 3.69	27.46 ± 3.73	0.50
Waist circumference (cm)	96.10 ± 10.98	101.10 ± 10.85	97.45 ± 10.0	0.13
Family history of CAD (%)	34.62	68.29	55.56	0.003
Diabetes mellitus (%)	16.98	26.83	26.09	0.10
Hypertension (%)	84.91	97.56	84.78	0.28
Hyperlipidemia (%)	62.26	63.41	67.39	0.86
Total cholesterol (nmol/L)	4.62 ± 1.11	4.61 ± 1.30	4.65 ± 1.00	0.98
HDL-cholesterol (nmol/L)	1.12 ± 0.31	1.09 ± 0.21	1.10 ± 0.26	0.91
LDL-cholesterol (nmol/L)	2.72 ± 1.01	2.93 ± 1.09	2.73 ± 0.88	0.51
Triglycerides (nmo/L)	1.75 ± 0.85	2.16 ± 1.55	1.63 ± 0.85	0.07
FSH (UI/L)	6.49 ± 4.82	5.80 ± 3.91	6.41 ± 8.43	0.84
LH (UI/L)	4.09 ± 3.66	4.28 ± 2.83	4.42 ± 4.01	0.90
SHBG (nmol/L)	50.12 ± 27.42	43.80 ± 19.21	48.54 ± 22.03	0.31

Data are expressed as mean ± standard deviation. BMI, body mass index; CAD, coronary artery disease; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Table 4. Coronary Artery Disease according to terciles of oestradiol in men submitted to coronary angiography.

	1º Tercile	**2º Tercile**	**3º Tercile**
Coronary artery disease (%)	35.85	48.78	71.74
Odds ratio	1.0	1.29	2.50
Confidence interval (95 %)		0.835 – 1.998	1.429 – 4.406

4.3 SHBG, UM NOVO MARCADOR DA SÍNDROME METABÓLICA?

A síndrome metabólica consiste em um conjunto de fatores que implicam risco elevado para doenças cardiovasculares, tais como obesidade (especialmente abdominal), resistência insulínica com ou sem diabetes mellito tipo 2, dislipidemia (elevação dos triglicerídeos e redução do HDL colesterol) e hipertensão. A anormalidade central associada à síndrome metabólica parece ser a resistência dos tecidos periféricos à insulina (1). SHBG é a proteína ligadora de esteróides sexuais produzida pelo fígado que se liga com elevada afinidade com testosterona e com menor ao estradiol. A insulina é um importante regulador da produção de SHBG pelo fígado. Estudos *in vitro* têm mostrado que a insulina em concentrações fisiológicas inibe a produção de SHBG por células de cultura de hepatomas (2). Pasquali *et al* (3) demonstraram que a inibição da secreção de insulina pelo diazóxido em homens com peso normal e em obesos promoveu elevação dos níveis de SHBG. Além disso, homens com reduzida concentração de SHBG tem um risco aumentado de desenvolver síndrome metabólica (4), sugerindo que resistência insulínica talvez seja um determinante dos níveis séricos de SHBG.

Esse estudo verificou a associação entre tercis de SHBG com os componentes e a prevalência de síndrome metabólica em uma amostra de pacientes do sexo masculino adultos atendidos em um serviço de Cardiologia Invasiva de referência na cidade de São Paulo (São Paulo – Brasil).

Material e Métodos

Desenho do estudo

O presente estudo trata-se de uma análise retrospectiva de dados do estudo “Estradiol, mas não Testosterona se correlaciona com doença arterial coronariana” (5).

População do estudo

Foram selecionados pacientes do sexo masculino com idade entre 40 e 70 anos que foram submetidos a angiografia eletiva de artérias coronárias para investigação e/ou estadiamento de doença cardíaca isquêmica no Instituto Dante Pazzanese de Cardiologia. Foram excluídos os pacientes tabagistas, que utilizassem fármacos com atividade antiandrogênica (cetoconazol, cimetidina, espironolactona, ciproterona e finasterida), com infarto agudo do miocárdio, acidente vascular cerebral e/ou cirurgias de grande porte nos últimos 6 meses, com índice de massa corporal [(IMC) peso (Kg)/ altura (m^2)] ≥ 40 kg/m^2, com creatinina sérica superior a 2,0 mg/dL ou evidência ao exame clínico de doença hepática importante.

Consentimento informado foi obtido de todos os participantes e o projeto foi aprovado pelo Comitê de Ética e Pesquisa das duas instituições envolvidas (Intituto Dante Pazzanese de Cardiologia e Escola Paulista de Medicina / UNIFESP).

Avaliação

A idade, a história dos componentes da síndrome metabólica (dislipidemia, hipertensão e diabetes mellitus) e os dados antropométricos como o peso, a altura, cintura e o IMC, foram coletados na entrevista de contato, na ante-sala do exame, mediante preenchimento de protocolo padrão, imediatamente após o esclarecimento e aceitação de participação na pesquisa.

A definição de síndrome metabólica seguiu os critérios de NCEP/ATP III (6).

Análise laboratorial

Amostras séricas foram coletadas entre 08:00 e 10:00, com o paciente em jejum, previamente à realização do cateterismo das artérias coronárias. Análises bioquímicas da glicose, colesterol total, colesterol-HDL, triglicerídeos e albumina foram realizadas no laboratório de análises clínicas do Hospital Dante Pazzanese de Cardiologia. O colesterol LDL foi obtido através da fórmula de Friedewald (7).

Amostras séricas para avaliação da SHBG foram levadas logo após a coleta para o laboratório de esteróides da Escola Paulista de Medicina/UNIFESP, sendo imediatamente centrifugadas e congeladas a -21°C para análise posterior, as quais foram realizadas em duplicata. A análise da SHBG foi realizada pelo ensaio IFMA [Delfia PerkinElmer, sensibilidade do método 0,5 nmol/L, coeficiente de variação (CV) intra-ensaio 3.9, 4.9 e 3.3 % e CV inter-ensaio 2.3, 3.0 e 2.4 % para 25.5, 63.9 e 138.0 nmol/L respectivamente).

Análise estatística

O tratamento estatístico utilizado no estudo para comparação entre os tercis de SHBG foi realizado com a utilização da análise de variância (ANOVA). Para cálculo da diferença entre a prevalência de síndrome metabólica entre os tercis de SHBG foi utilizado risco relativo. Os dados foram analisados no GraphPad Prism 5.0. Significância estatística foi considerada quando $p < 0,05$.

Resultados

Foram considerados elegíveis para o estudo 141 pacientes. Níveis elevados de SHBG foram negativamente correlacionados com IMC, circunferência abdominal e prevalência de diabetes mellitus tipo 2 (tabela 1). Foi verificada uma correlação negativa, porém não significativa, entre os níveis de SHBG e os níveis de triglicerídeos em jejum ($p = 0,06$) e prevalência de Hipertensão arterial sistêmica ($p = 0,08$) (tabela 1). Houve diferença estatística entre a prevalência de síndrome metabólica e os tercis de SHBG, com a maior prevalência da síndrome observada no menor tercil de SHBG (tabela 2).

Discussão

Em uma amostra de 141 pacientes atendidos no Setor de Hemodinâmica e Cardiologia Invasiva do Instituto Dante Pazzanese de Cardiologia, verificou-se que

SHBG apresentou uma associação inversa e significativa com IMC, medida de cintura abdominal e prevalência de *diabetes mellitus.* Houve uma associação negativa, porém não significativa com níveis séricos de triglicerídeos em jejum (p=0,06) e com prevalência de HAS (p=0,08). Não houve associação entre níveis séricos de SHBG e colesterol-HDL. Osbservou-se uma associação negativa e significativa entre os tercis de SHBG e prevalência de síndrome metabólica.

Vem aumentando as evidências que correlacionam baixos níveis de SHBG aos componentes da síndrome metabólica. Em artigo recentemente publicado, Ding et al (8) chegaram à conclusão que baixos níveis de SHBG são um forte preditor de risco de diabete melito tipo 2. Muller et al (9) também encontraram uma associação negativa entre níveis de SHBG e fatores de risco da síndrome metabólica.

A anormalidade central da síndrome metabólica parece ser a resistência tecidual à insulina. Tendo em vista que a insulina é um potente inibidor da produção hepática de SHBG, pode-se supor que reduzidos níveis desta globulina poderiam ser um marcador precoce do desenvolvimento da síndrome metabólica. Em concordância com essa linha de pensamento, Heald et al (10) em estudo com populações européia, paquistanesa e afro-caribenhos e Chubb et al (11) em estudo de base populacional em pacientes idosos verificaram que a SHBG é um potencial marcador para síndrome metabólica. Em recente estudo observacional com 80 pacientes portadores de síndrome metabólica foi observado que o aumento de uma unidade nos níveis de insulina resultava em queda de 0,25 unidade nos níveis de SHBG (12).

Conclusão

Baixos níveis séricos de SHBG estiveram associados aos componentes da síndrome metabólica e com sua prevalência, em uma população homens brasileiros adultos com idade entre 40 a 70 anos de idade.

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Tabela 1. Características Clínicas dos pacientes de acordo com os tercis da Globulina Ligadora de Esteróides Sexuais.

	SHBG tercil			**p**
	1 (menor)	**2**	**3** (maior)	
Média SHBG (nmol/L)	27,20	43,11	72,01	< 0,001
Variação SHBG (nmol/L)	12,2 - 35,5	36,1 – 49,9	50,5 - 159	
Idade (anos)*	57,0	56,73	58,88	0,33
IMC** (kg/m^2)*	28,76	27,46	25,13	**< 0,001***
Cintura abdominal (cm)*	103,49	97,66	93,53	**< 0,001***
Diabetes (%)	38,30	21,28	8,51	**0,002***
Dislipidemia (%)	70,21	63,83	57,45	0,44
Hipertensão (%)	95,74	80,85	87,23	0,08
Glicemia ***(mg/dl)*	111,04	107,49	99,07	0,42
Colesterol total *** (mg/dl)*	183,72	180,30	168,38	0,22
C-LDL** (mg/dl)*	107,24	105,34	98,70	0,51
C-HDL ***(mg/dl)*	40,30	41,98	43,34	0,35
C-VLDL** (mg/dl)*	33,95	28,97	26,40	**0,04***
Triglicerídeos** (mg/dl)*	174,38	171,28	131,79	0,06

*Valores dispostos em média
**IMC – índice de massa corporal
***Exames coletados em jejum

Tabela 2. Prevalência da Síndrome metabólica de acordo com os tercis da Globulina Ligadora de Esteróides Sexuais.

	1º Tercil	**2º Tercil**	**3º Tercil**
Média de SHBG* (nmol/L)	80,85	50,32	38,89
Síndrome metabólica (%)	19,15	44,68	70,21
Risco Relativo	1,0	0,60	0,29
Intervalo de confiança (95%)		0,40 – 0,90	0,16 – 0,54

*SHBG – globulina ligadora de esteróides sexuais

CONCLUSÕES

5 CONCLUSÕES

5.1 Revisão da literatura

A Testosterona endógena exerce um efeito neutro e/ou benéfico no sistema cardiovascular masculino. Não há dados suficientes na literatura para permitir definir o papel da testosterona exógena na saúde cardiovascular dos homens.

5.2 Dados experimentais

Ao contrário da testosterona, dos outros marcadores de androgenicidade (testosterona livre, testosterona biodisponível e índice de andrógenos livres), relação estradiol / testosterona, relação IEL / IAL, SHBG, LH e FSH observou-se associação positiva entre os níveis séricos de estradiol e índice de estrógenos livres e doença arterial coronariana severa em homens com idade entre 40 a 70 anos.

5.3 Análise retrospectivados dados

A globulina ligadora de esteróide sexuais associou-se negativamente com os componentes e com a prevalência da Síndrome Metabólica, reforçando a sua importância como um novo constituinte e marcador dessa síndrome.

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ANEXOS

7 - ANEXOS

7.1 Termo de consentimento livre e esclarecido

"NÍVEIS DE TESTOSTERONA E RISCO CARDIO-VASCULAR EM HOMENS ADULTOS SUBMETIDOS A ANGIOGRAFIA CORONARIANA"

As Doenças cardiovasculares (DCV) representam atualmente o principal grupo de causa de morte nas regiões sudeste, sul e centro-oeste, sendo responsável por 27,88% do total de mortes no Brasil (DATASUS 2004). As doenças cardíacas isquêmicas (Infarto do coração e angina do peito) foram responsáveis por 30,39% do total dessas mortes por DCV no país (DATASUS 2004). Diversos fatores de risco para doença arterial coronariana são conhecidos e comprovados, como hipertensão arterial sistêmica (pressão alta), hábito de fumar, dislipidemias (níveis de altos de colesterol), obesidade, falta de exercício físico, diabetes mellitus (DM) e antecedentes na família de infarto.

Os homens morrem duas vezes mais de doença arterial coronariana, possuem maior chance infarto agudo do miocárdio e níveis mais elevados de testosterona (hormônio sexual) que mulheres. Dessa forma, foi sugerido que os elevados níveis desse hormônio encontrados nos homens poderiam causar danos ao coração.

No entanto, têm aumentado na literatura (estudos científicos) informações que mostram que testosterona seja um fator de proteção ao coração, possivelmente por inibir efeitos pró – coagulantes (que provocam angina, infarto e trombose). Apesar dos resultados desses estudos a maioria deles apresenta pequeno número de pacientes, fazendo-se necessário que mais trabalhos analisem a relação entre testosterona e doenças do coração como infarto ou angina.

Serão coletadas amostras de 30 ml de seu sangue para as dosagens de testosterona total e biodisponível, globulina ligadora de esteróides sexuais (SHBG), LH, FSH, estradiol, glicose, insulina, colesterol total e frações (LDL, HDL e VLDL), triglicerídeos, fibrinogênio, fator VII e inibidor de ativador do plasminogênio (PAI-1).

A coleta de sangue é feita respeitando sempre as normas de segurança utilizando materiais descartáveis e seguros da mesma forma como uma coleta de sangue do dia a dia que por ventura o Sr. já possa ter realizado, sem risco para sua saúde.

O benefício é que o Sr. estará prestando uma grande ajuda ao conhecimento científico e poder dar mais informações à medicina para que no futuro possamos desenvolver novas terapias, medicações e condutas para prevenir ou mesmo tratar doenças ajudando ao nosso próximo.

Em qualquer etapa do estudo o participante terá acesso aos profissionais relacionados com a pesquisa para o esclarecimento de dúvidas. Os principais investigadores são Dra. Emmanuela Quental Callou de Sá, Profª Dra. Ieda T N Verreschi, da disciplina de Endocrinologia – Departamento de Medicina da UNIFESP, rua Pedro de Toledo, 781, 13º andar, telefone 11 5574 6502. Se houver alguma consideração a fazer ou dúvida sobre a ética da pesquisa, entrar em contato com o Comitê de Ética em Pesquisa (CEP) na Escola Paulista de Medicina – rua Botucatu, 572, 1º andar, cj 4 São Paulo-SP – telefone (11)-5571 1062, FAX 5539 7162 ou no Comitê de Ética em Pesquisa (CEP) do Hospital Dante Pazzanese - Av. Dr. Dante Pazzanese, 500 prédio I, São Paulo-SP telefone (11)-5085-6040.

Todos os pacientes terão total liberdade de retirar seu consentimento a qualquer momento, deixando imediatamente de participar do estudo, sem qualquer prejuízo de seu tratamento na Instituição.

Não há despesas pessoais para o participante do estudo. Também não há compensação financeira relacionada à participação.

Todas as informações coletadas ao longo do estudo serão protegidas pelo sigilo médico, garantindo a não identificação das pacientes e mantendo o caráter confidencial das informações relacionadas com a sua privacidade.

Eu_____
_____, fui esclarecido sobre os objetivos do presente estudo. Concedo meu acordo de participação de livre e espontânea vontade.

_____ Data____/_____/_____

Assinatura do participante/ responsável legal

Declaro que obtive de forma apropriada e voluntário Consentimento Livre e Esclarecido deste paciente ou seu representante legal para a participação neste estudo.

_____

Assinatura do responsável pelo estudo

7.2 Carta de Aprovação do Comitê de Ética em Pesquisa (Escola Paulista de Medicina / UNIFESP)

UNIFESP
PAULISTA
Universidade Federal de São Paulo
Escola Paulista de Medicina
Comitê de Ética em Pesquisa
Hospital São Paulo

São Paulo, 24 de agosto de 2007.
CEP 1305/07

Ilmo(a). Sr(a).
Pesquisador(a) EMMANUELA QUENTAL CALLOU DE SÁ
Co-Investigadores: Francisco Carleial Feijó de Sá, Fausto Feres, Ivone Martins Ferreira, Ieda Therezinha do Nascimento Verreschi (orientadora)
Disciplina/Departamento: Endocrinologia/Medicina da Universidade Federal de São Paulo/Hospital São Paulo
Patrocinador: CNPq/FAPESP.

PARECER DO COMITÊ DE ÉTICA INSTITUCIONAL

Ref: Projeto de pesquisa intitulado: "**Niveis de testosterona e risco cardiovascular em homens adultos submetidos à angiografia coronariana**".

CARACTERÍSTICA PRINCIPAL DO ESTUDO: Observacional, caso controle.
RISCOS ADICIONAIS PARA O PACIENTE: Risco mínimo, desconforto leve, envolvendo coleta de sangue.
OBJETIVOS: Avaliar a relação existente entre testosterona total, testosterona biodisponível e índice de andrógenos livres com DAC em pacientes submetidos à angiografia coronariana.
RESUMO: Estudo caso controle, com uma população de pacientes atendida no Hospital Dante Pazzanese de Cardiologia de São Paulo. Participarão do estudo pacientes do sexo masculino entre 40 e 70 anos de idade, que serão submetidos a cateterismo de artérias coronárias. Pacientes submetidos a cateterismo de artérias coronárias com obstrução >=70% com pelo menos uma das principais artérias coronárias serão considerados como portadores de DAC significativa; aqueles com obstrução inferior a 70% constituirão o grupo controle. Serão obtidos dados da história de fatores de risco para DAC, dados antropométricos. Serão realizadas dosagens laboratoriais séricas..
FUNDAMENTOS E RACIONAL: Há evidências controversas sobre o papel dos andrógenos no sistema cardiovascular. Este estudo visa avaliar a relação entre testosterona e fatores de risco para DAC em pacientes submetidos a cateterismo coronariano..
MATERIAL E MÉTODO: Estão descritos os procedimentos. Apresenta parecer do núcleo de proteção radiológica para utilização de material radiativo, bem como a carta de autorização do local onde será realizada a pesquisa.
TCLE: Adequado, contemplando a resolução 196/96.
DETALHAMENTO FINANCEIRO: CNPq / FAPESP - R$8642,00 por paciente (160 pacientes0.
CRONOGRAMA: 18 meses.
OBJETIVO ACADÊMICO: Mestrado.
ENTREGA DE RELATÓRIOS PARCIAIS AO CEP PREVISTOS PARA: **23/8/2008** e **23/8/2009**.

Rua Botucatu, 572 - 1º andar – conj. 14 - CEP 04023-062 - São Paulo / Brasil
Tel.: (011) 5571-1062 - 5539.7162

O Comitê de Ética em Pesquisa da Universidade Federal de São Paulo/Hospital São Paulo **ANALISOU** e **APROVOU** o projeto de pesquisa referenciado.

1. Comunicar toda e qualquer alteração do projeto e termo de consentimento livre e esclarecido. Nestas circunstâncias a inclusão de pacientes deve ser temporariamente interrompida até a resposta do Comitê, após análise das mudanças propostas.
2. Comunicar imediatamente ao Comitê qualquer evento adverso ocorrido durante o desenvolvimento do estudo.
3. Os dados individuais de todas as etapas da pesquisa devem ser mantidos em local seguro por 5 anos para possível auditoria dos órgãos competentes.

Atenciosamente,

Prof. Dr. José Osmar Medina Pestana
Coordenador do Comitê de Ética em Pesquisa da
Universidade Federal de São Paulo/ Hospital São Paulo

Rua Botucatu, 572 - 1º andar – conj. 14 - CEP 04023-062 - São Paulo / Brasil
Tel.: (011) 5571-1062 - 5539.7162

7.3 Carta de Aprovação do Comitê de Ética em Pesquisa – Instituto Dante Pazzanese de Cardiologia

Instituto Dante Pazzanese de Cardiologia
Fundação Adib Jatene

Comitê de Ética em Pesquisa

DATA DA ENTRADA: 27 de agosto de 2007.

DATA DA AVALIAÇÃO: 04 de setembro de 2007.

CAAE: 0077.0.131.174-07
N. º DO PROTOCOLO NO CEP: 3559.
(ESTE N. º DEVERÁ CITAR NAS CORRESPONDÊNCIAS REFERENTES A ESTE PROJETO)
INVESTIGADOR PRINCIPAL: Emmanuela Quental Callou de Sá

PROJETO DE PESQUISA: "Níveis de Testosterona e Risco cardiovascular em Homens Adultos submetidos à Angiografia Coronariana".

DURAÇÃO DA PESQUISA: 18 meses

Nº de Sujeitos no Centro: 160

Considerações: Avaliar a relação existente entre testosterona total, testosterona biodisponível e índice de andrógenos livres com DAC em pacientes submetidos à angiografia coronariana.

Ao se proceder à análise ao projeto em questão, considera-se que:

a) O projeto preenche os requisitos fundamentais das resoluções CNS 196/96, 251/97 e 292/99, sobre as Diretrizes e Normas Regulamentadoras de Pesquisa Envolvendo Seres Humanos, do Conselho Nacional de Saúde / Conselho Nacional de Ética em Pesquisa / Agência Nacional de Vigilância Sanitária e as Boas Práticas de Pesquisa Clínica do ICH-GCP.

b) O Comitê de Ética em Pesquisa avaliou o Protocolo de Estudo e o Termo de Consentimento Livre e Esclarecido.

c) O Comitê de Ética em Pesquisa segue os preceitos das resoluções CNS196/96, 251/97 e 292/99, sobre as Diretrizes e Normas Regulamentadoras de Pesquisa Envolvendo Seres Humanos, do Conselho Nacional de Saúde / Conselho Nacional de Ética em Pesquisa / Agência Nacional de Vigilância Sanitária e as Boas Práticas de Pesquisa Clínica do ICH-GCP.

AV DR DANTE PAZZANESE,500-PRÉDIO I- -IBIRAPUERA-04012-180-S.P
Telefax:(11) 5085-6040 cepidpc@terra.com.br

Instituto Dante Pazzanese de Cardiologia
Fundação Adib Jatene

Comitê de Ética em Pesquisa

Diante do exposto, O Comitê de Ética em Pesquisa, manifesta-se pela:

- Protocolo de Estudo: Aprovado.
- Termo de Consentimento Livre e Esclarecido – Versão – 09/09/2007 – Aprovado.

O Comitê de Ética em Pesquisa, solicita que:

a. **Informar imediatamente relatório sobre qualquer evento adverso ocorrido**
b. **Comunicar qualquer alteração no projeto e no TCLE.**

Situação: projeto avaliado e aprovado em reunião do dia 04 de setembro de 2007.

São Paulo, 18 de setembro de 2007.

Pedro Silvio Farsky
Coordenador CEP
CRM 55073

AV.DR.DANTE PAZZANESE,500-PRÉDIO I- -IBIRAPUERA-04012-180-S.P
Telefax:(11) 5085-6040 cepidpc@terra.com.br

7.4 **Protocolo do estudo**

Nome:_____

Data de Nascimento:___/___/___ Data atual:___/___/___

História Clínica

História de dislipidemia: sim ( ) não ( ),

Fármacos hipolipemiante: sim ( ) não ( )

Quais_____

História de tabagismo: sim ( ) não ( )

História de DM tipo 2: sim ( ) não ( ),

Fármacos hipoglicemiantes: sim ( ) não ( )

Quais_____

História de HAS: sim ( ) não ( ),

Fármacos anti-hipertensivos: sim ( ) não ( )

Quais_____

Uso de outras medicações sim ( ) não ( )

Quais_____

História familiar de doença arterial coronariana em parentes de primeiro grau: sim ( ) não ( )

Uso de fármacos com ação anti-androgênicas: sim ( ) não ( )

Insuficiência renal: sim ( ) não ( )

Doença hepática: sim ( ) não ( )

Dados Antropométricos

Peso: _____ Alt:_____ IMC:_____ Cintura:_____

Laboratório

Glicemia:_____ Colesterol total:_____LDL:_____ HDL:_____

VLDL:_____ TG:_____LH:_____ FSH:_____ Estradiol:_____

SHBG:_____Testo:_____ Índice de andrógenos livres:_____

Cateterismo

DAC significativa sim ( ) não ( )