ORIGINAL RESEARCH

Can Low SHBG Serum Concentration Be A Good Early Marker Of Male Hypogonadism In Metabolic Syndrome?

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Introduction: In men suffering from metabolic syndrome, accompanying insulin resistance may result in a lowering of sex hormone–binding globulin (SHBG) plasma levels and cause changes in their androgenic status.

Aim: The objective of the research was to assess selected androgens and SHBG plasma levels in males meeting diagnostic criteria for MS compared to healthy males.

Patients and methods: The group consisted of 65 men aged between 40 and 70 years old fitting IDF metabolic syndrome criteria and 84 controls. Dehydroepiandrosterone (DHEA) and its sulphate (DHEA–S), total and free testosterone and SHBG serum levels were evaluated. Calculated free and bioavailable testosterone were estimated using an algorithm proposed by the International Society for the Study of the Aging Male.

Results: Men diagnosed with MS showed a statistically significant decrease in plasma levels of DHEA in comparison to healthy ones: 11.579 (8.39–15.56) vs 14.014 (9.611–17.125) ng/mL; p = 0.0350, SHBG: 47.46 (35.78–62.83) vs 71.965 (54.45–91.56) nM/L; p<0.0001 and total testosterone: 5.2 (3.8–6.5) vs 6.3 (5.4–8.25) ng/mL; p = 0.0001 (values presented as a median with Q1–Q3).

Conclusion: The results suggest that SHBG is a good early marker for metabolic dysregulation in MS, considering its strength of association and significance is comparable to, or better than, those of MS criteria.

Keywords: MS, DHEA, testosterone, CRP, cholesterol, androgens

Introduction

The changes in the biology of an aging male are complicated and not yet fully understood. A growing number of publications show a link between age-related hormonal changes and prevalence of metabolic syndrome (MS). The decrease in androgens (independent of the cause: e.g. through aging or usage of gonadotropin-releasing analogs)[1] as well as in SHBG has been linked with visceral obesity and exacerbation of aspects of MS.[2–4]

With age, the natural production of adrenal androgens and testosterone decreases, and this, as a consequence, negatively affects the risk of developing not only MS but also atherosclerosis.[5]

Aim Of Study

The intention of this study was to explore the changes in serum androgen balance, both adrenal and gonadal (DHEA [dehydroepiandrosterone], DHEA–S

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[dehydroepiandrosterone-sulfate], SHBG [sex hormone–binding globulin], FT [free testosterone], TT [total testosterone], c–FT [calculated free testosterone] c–BAT [calculated bioavailable testosterone]), in a given age group among patients with metabolic syndrome and comparing them to healthy individuals.

Patients And Methods

Recruitment for the study was carried out in an outpatient endocrinology clinic in rural regions of southwestern Poland, presented in Figure 1, from a city of less than 15,000 inhabitants plus neighboring villages. Controls were acquired from the same population and the same age groups. The patients were chosen in such a way that they were exactly 40, 50, 60 or 70 years old. The group consisted of 65 patients (11 forty-year-olds, 19 fifty-year-olds, 18 sixty-year-olds and 17 seventy-year-olds). The control group was comprised of 84 (23 forty-year-olds, 21 fifty-year-olds, 20 sixty-year-olds and 20 seventy-year-olds) healthy participants matching the test group, recruited from the same population. Controls following any particular diets like gluten-free diet, vegetarians, vegans or suffering from any preexisting diseases or known genetic disorders were excluded.

The inclusion criteria were meeting the IDF metabolic syndrome criteria, which means central obesity understood as waist circumference > 94cm plus meeting 2 out of 4 MS criteria, namely triglycerides ≥150mg/dL or treatment for this lipid abnormality, glucose ≥100mg/dL or previously

Figure 1 Wschowa county, Lubuskie province, Poland.

diagnosed type 2 diabetes, HDL cholesterol <40mg/dL or treatment for this lipid abnormality, systolic blood pressure (SBP) ≥130 mmHg or diastolic blood pressure (DBP) ≥85 mmHg or treatment of previous hypertension.

Exclusion criteria included orchitis (present or past), prostate cancer treated with GnRH-analogues, pituitary dysfunction, cryptorchidism (present or past) – a total of 11 men.

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The baseline characteristics are presented in Table 1.

Total testosterone (TT) serum levels were measured using the ELISA test (DRG International Inc, USA) with a reference range of 2.0–6.9 ng/mL and a sensitivity of 0.083 ng/mL.

Measurements of free testosterone (FT) plasma concentrations were assessed using DSL-4900 ACTIVE Free Testosterone RIA created by Diagnostic Systems Laboratories, Inc, USA marked with J^{125} with a reference range of 1.7–62.58 pg/mL and a sensitivity of 0.18 pg/mL.

DHEA (dehydroepiandrosterone) and its sulfate (DHEA–S) serum levels were evaluated using the ELISA test (Biosource – Belgium) with a reference range of 1.8–12.5 ng/mL for DHEA and 1.0–4.2 μg/mL for DHEA–S and a sensitivity of ≤0.1 ng/mL for DHEA and ≤0.02 μg/mL for DHEAS.

SHBG (sex hormone–binding globulin) serum levels were measured using immunoradiometric assay (IRMA) produced by Beckman Coulter Immunotech, USA/Czech Republic, with a reference range of 20–70 nM/L and sensitivity of 0.1nM/L.

The homocysteine plasma level analysis was carried out in column chromatography by means of HPLC/EC (P580, Dionex, Germany) coupled to an electrochemical detector (CoulArray 5600, ESA, USA). The reference values of homocysteine concentrations were determined in the 5–15 μM/L range.

The serum hs–CRP levels were determined using high-sensitivity immunoassays with specific antibodies (ELISA) produced by BioCheck (USA). The normal ranges for hs–CRP were below 5 mg/L.

All tests were done twice and an average was taken from both measurements.

We also analyzed calculations of free testosterone (c–FT) and bioavailable testosterone (c–BAT) using a mathematical algorithm proposed by the International Society for the Study of the Aging Male.

Statistical analysis was conducted using Statsoft Statistica 12.1 software. Normal distribution was tested

Table 1 Baseline Characteristics Of All Study Participants (Variables Are Shown As Medians With Q_1 And Q_3 Values)

Variable	Studied Group (Diagnosed With MS) N=65	Controls (Healthy) N=84	p
Age	60 (50–70)	50 (40–60)	0.2992
Brinkmann Index	300 (0–700)	300 (0–500)	0.4713
Waist circumference	106 (101–113)	92 (86–98.5)	*<0.0001*
WHR	1.01 (1.0–1.03)	1 (0.97–1.01)	*0.0004*
BMI	30 (27.7–32.5)	25.15 (22.8–28.05)	*<0.0001*
Systolic BP	150 (130–160)	140 (125–150)	*0.0038*
Diastolic BP	90 (80–95)	80 (80–90)	*0.0027*
Glucose	105 (98–112)	93 (86–96)	*<0.0001*
Total cholesterol	250 (217–296)	239.5 (208–282.5)	0.1511
Non-HDL cholesterol	208 (176–249)	184 (148.5–219.5)	*0.0025*
LDL cholesterol	172 (147–200)	163.5 (132.5–199)	0.3122
HDL cholesterol	43 (38–50)	55.5 (47–68.5)	*<0.0001*
Triglycerides	183 (135–229)	100 (75.5–124)	*<0.0001*

Note: Statistically significant p<0.05 shown in Italics.

with the Lillierfors test. Most data lacked normal distribution and was thus analyzed using non-parametric tests, namely Spearman's rank-order correlation and Mann–Whitney *U*-tests. The data were presented as medians with 25th and 75th quartiles.

To assess factors which may affect MS occurrence, we performed univariate and multivariate logistic regression analysis. In the univariate logistic regression model, the dependent variable was diagnosis of MS and independent factors were age, smoking (yes/no), Brinkmann Index, waist circumference, BMI, systolic BP, diastolic BP, glucose, homocysteine, hs-CRP, total cholesterol, HDL, LDL, non-HDL, triglycerides, FT (free testosterone) – measured, FT (free testosterone) – calculated, bioavailable testosterone, DHEA-S, DHEA, total Testosterone and SHBG.

For multivariate logistic regression, model variables with a p-value <0.1 in the aforementioned univariate regression analysis were chosen.

Differences with p-value <0.05 were considered statistically significant.

The study was accepted by the local medical ethics committee.

The study was approved by "Komisja Bioetyczna przy Uniwersytecie Medycznym im. Karola Marcinkowskiego w Poznaniu".

Written consent in accordance with Declaration of Helsinki has been obtained from each patient or subject after full explanation of the purpose and nature of all procedures used.

Results

The values of selected investigated parameters are shown in Table 2.

Groups were analyzed for smoking using the Brinkmann Index (daily cigarettes multiplied by years of smoking), and there was no statistical significance between

Table 2 Selected Investigated Parameters Of All Study Participants (Variables Are Shown As Medians With Q_1 And Q_3 Values)

Variable	Studied Group (Diagnosed With MS) N=65	Controls (Healthy) N=84	p
Homocysteine	10 (8–12.2)	9.1 (7.4–11.95)	0.4371
hs–CRP	1.65 (0.94–4.56)	1.02 (0.44–2.60)	*0.0062*
Free testosterone – measured	11.53 (9.56–14.43)	12.57 (10.71–15.18)	0.1340
DHEA–S	0.97 (0.55–1.41)	1.115 (0.6568–1.65)	0.2556
DHEA	11.58 (8.39–15.56)	14.01 (9.61–17.13)	*0.0350*
Total testosterone	5.2 (3.8–6.5)	6.3 (5.4–8.25)	*0.0001*
SHBG	47.46 (35.78–62.83)	71.965 (54.45–91.56)	*<0.0001*
Free testosterone – calculated	85.2 (58.7–108)	80 (63.1–105.5)	0.9969
BAT	2.01 (1.46–2.66)	1.88 (1.48–2.495)	0.7305

Note: Statistically significant p<0.05 shown in italics.

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the control and study groups regarding exposure to smoking. The groups did not differ statistically significant in baseline characteristics with reference to total and LDL cholesterol as well as to homocysteine serum levels. Likewise, there was no statistical significance for the difference in plasma levels of FT and parameters calculated: c–FT, c–BAT.

In contrast, statistical significance was achieved for DHEA, SHBG, hs-CRP and total testosterone plasma concentrations.

Total testosterone plasma levels showed positive associations with HDL cholesterol (p = 0.0256, R = 0.182), and negative ones with waist circumference (p = 0.0000003, R = –0.4), WHR (p = 0.000046, R = –0.327), BMI (p = 0.000025, R = –0.338), fasting glucose level (p = 0.0138, R = −0.21) and triglycerides (p = 0.0388, R = –0.17). No association was shown with age in our study, neither in both groups together nor in either of the groups separately.

SHBG serum levels demonstrated positive association with total testosterone (p<0.0000001, R = 0.564), HDL cholesterol (p = 0.000533, R = 0.28) and age (p = 0.0105, R = 0.209) while negative with waist circumference (p = 0.000002, R = –0.375), WHR (p = 0.0013, R = −0.261), BMI (p = 0.000003, R = –0.372), TGA (p = 0.000004, R = –0.366) and fasting glucose level (p = 0.000251, R = –0.308).

Further analysis, namely Kruskal–Wallis ANOVA, shows the difference in SHBG between patients with MS in different age groups (p = 0.0415), but does not show any statistical difference among the controls in different age groups. Close analysis of each age group with the Mann–Whitney *U*-test shows a statistical difference in SHBG plasma levels among patients with metabolic syndrome aged 40 and 60 (p = 0.0328), as well as aged 50 and 60 (p = 0.04) years, showing, with statistical significance, lower levels of SHBG in younger groups than in older groups.

Changes in the concentrations of SHBG, DHEA and total testosterone in particular age groups are shown in Figure 2.

DHEA plasma levels showed a negative association with age (p = 0.0003, R = –0.292), systolic blood pressure (p = 0.032, R = –0.176) and metabolic syndrome (p = 0.034, R = –0.174). DHEA–S serum concentrations showed a negative association with age (p = <0.0001, R = −0.495) and systolic blood pressure (p = 0.0009, R = –0.269), but showed no association with metabolic syndrome (p = 0.2562).

Serum hs–CRP levels correlated positively with age (p = 0.0403, R = 0.168), waist circumference (p = 0.000062, R = 0.322), WHR (p = 0.009521, R = 0.212), BMI (p = 0.00106, R
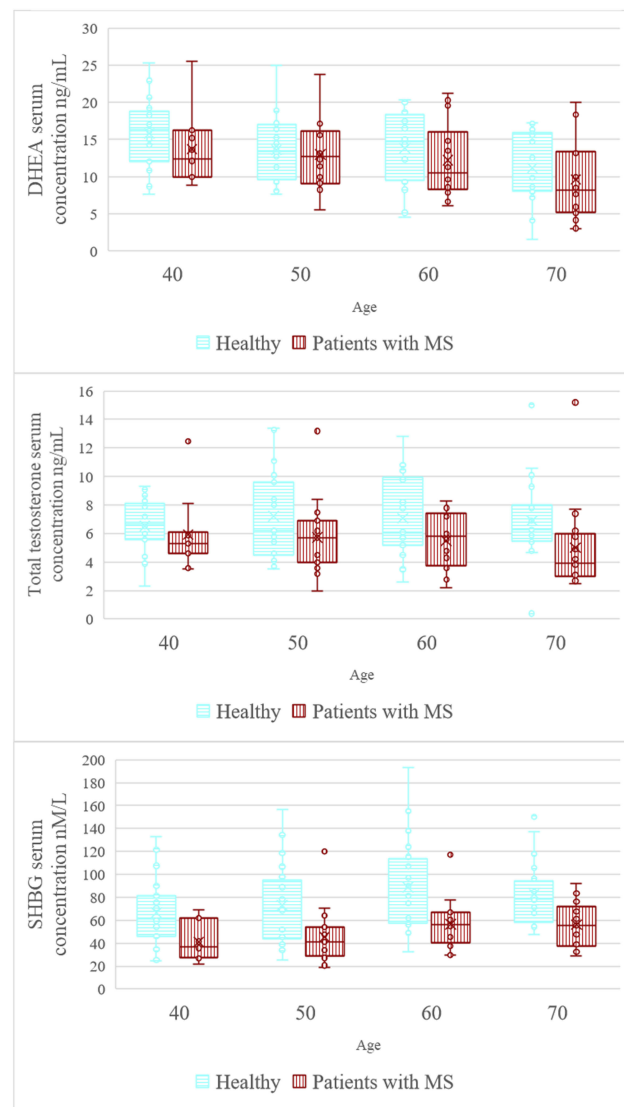

Figure 2 Serum concentrations of selected parameters in age group among patients with metabolic syndrome.

= 0.266), systolic blood pressure (p = 0.02, R = 0.190), diastolic blood pressure (p = 0.02, R = 0.191), metabolic syndrome (p = 0.006, R = 0.225) and negatively with SHBG (p = 0.0166, R = –0.196) and HDL (p = 0.00915, R = –0.213).

In the study, positive associations were found between age and BMI (p = 0.01 R = 0.2) as well as between age and waist circumference (among healthy p = 0.047 R = 0.217 among patients with MS p = 0.0046, R = 0.35 and both groups together p = 0.001 R = 0.266).

Comparison of the Spearman's rank correlation coefficient of particular MS diagnostic criteria and SHBG with MS prevalence is shown in Table 3.

Furthermore, all of the abovementioned parameters differ with statistical significance in each age group,

Table 3 Comparison Of Association Between Particular MS Elements Plus SHBG And MS Prevalence (Spearman's Rank Correlation Coefficient (r_s) And P–Value Are Presented)

	r_s	p
Waist circumference	0.635	<0.0001
Fasting plasma glucose	0.621	<0.0001
Triglycerides	0.602	<0.0001
Cholesterol HDL	−0.464	<0.0001
SHBG	−0.435	<0.0001
Diastolic blood pressure	0.247	0.0024
Systolic blood pressure	0.238	0.0035

Note: Statistically significant p<0.05 shown in italics.

except for blood pressure. Systolic and diastolic blood pressure only differ statistically in the eldest group.

Univariate and multivariate regression models are presented in Table 4 and Figure 3.

Discussion

Andropause is a condition associated with a decrease in testosterone. This decrease can lead to many problems, namely changes in mood, sex drive, and attitude to life, but can also lead to an increased risk of a multitude of health complications, like osteopenia and osteoporosis or heart disease.

The criteria for metabolic syndrome differ depending on the source, but in 2009, the criteria were unified,[6] requiring 3 out of 5 criteria to be met, with increased waist circumference being one of them, but not the mandatory one. We decided to choose International Diabetes Federation criteria (established in 2005), which differ by requiring central obesity as a major criterion plus two out of four other criteria, as we believe in the importance of central obesity in metabolic syndrome, affecting cytokines and whole inflammatory homeostasis.

SHBG increases with age at a rate of roughly 1.6%/year, while total testosterone sees a decrease of roughly 1.6%/year.[7] DHEA and DHEA-S showed a sharp decline of 2–3%/year.[8] Those results are generally in line with our research, namely an increase in SHBG and a decrease in DHEA and DHEA-S with age.

When it comes to total testosterone decline, the results are conflicting. There are those showing a decline,[9–11] as well as those showing no change,[12,13] as was the case in our study, especially among exceptionally healthy elderly males.[14] Those conflicting results, together with differences between cross-sectional and longitudinal results and disappearance of changes in the decline of testosterone

when analyzed for a lack of "apparent health" in this particular study of 1709 men, suggest that incidental poor health has a major impact on androgen decline.[7] We imply that an increase in weight with age, as shown in our study, and incidents of poor health cause changes in TT, not age alone. Findings suggesting the negative influence of fat and obesity on TT and SHBG are found in many publications.[2,13,15–17] Our suggestion is that the increase of SHBG with age is insignificant in terms of its change related to MS and obesity among patients both young and old, and is thus a good early marker of metabolic dysregulation affecting androgen status in patients with MS.

In our results, both BMI and waist circumference increase with age. In the case of waist circumference, the increase is present for controls, the study group and both together, but the Spearman's rank correlation coefficient for a waist circumference increase with age is higher in patients with metabolic syndrome. This suggests that metabolic syndrome is associated with even higher age-related central adiposity.

There are a number of publications that link metabolic syndrome, insulin resistance and low TT, as well as adrenal C12 steroids and decreased SHBG concentrations.[18,19] These findings correlate with our own. Findings generally link a decrease in androgens with age, namely testosterone, as an underlying cause for MS in elderly men.[20]

We found positive associations between testosterone and HDL cholesterol and negative with waist circumference, fasting glucose level, WHR, BMI and TGA, which are comparable to the results of another publication.[21]

In our study, we show that free indexes (both measured and calculated) are not good methods for assessment of decrease in testosterone as a consequence of metabolic syndrome. In our analysis, free-T showed no association with age nor MS neither in both groups together nor in healthy and MS groups separately, suggesting its low diagnostic usefulness. This may be caused by the imperfection of free-testosterone assays and interference of the age- and weight-dependent changes in plasma SHBG concentrations.

In terms of calculated bioavailable testosterone and free testosterone, they both show no differences between healthy and MS patients (Table 1), which is also found in another study.[17] There is also no association of calculated BAT and FT with MS, but both have a negative and statistically significant association with age. This association can be attributed to a general increase in SHBG with

Table 4 Univariate And Multivariate Regression Analysis

Parameters	Section A: Univariate Regression Analysis:				Section B: Multivariate Regression Analysis:											
					Model 1				Model 2				Model 3			
	OR	95% CI-	95% CI+	p	OR	95% CI -	95% CI +	p	OR	95% CI -	95% CI +	p	OR	95% CI -	95% CI +	p
Age	1.02	0.99	1.05	0.2945												
Smoking	1.32	0.67	2.60	0.4228												
Brinkmann Index	1.00	1.00	1.00	0.1818												
Waist circumference[a]	1.19	1.13	1.26	<0.0001*												
BMI	1.46	1.28	1.66	<0.0001*	1.51	1.28	1.78	<0.0001*	1.56	1.32	1.84	<0.0001*	1.51	1.28	1.78	<0.0001*
Systolic BP[a]	1.02	1.00	1.04	0.0164*												
Diastolic BP[a]	1.05	1.01	1.09	0.0163*												
Glucose[a]	1.10	1.05	1.15	<0.0001*												
Homocysteine	1.00	0.94	1.06	0.8650												
hs-CRP	1.00	0.97	1.04	0.9179												
Total cholesterol	1.01	1.00	1.01	0.0690[b]												
Non-HDL	1.01	1.00	1.02	0.0013*	1.01	1.00	1.03	0.0063*	1.02	1.01	1.03	0.0011*	1.01	1.00	1.02	0.0073*
LDL	1.01	1.00	1.01	0.1452												
HDL[a]	0.92	0.89	0.95	<0.0001*												
Triglycerides[a]	1.02	1.02	1.03	<0.0001*												
FT – measured	0.94	0.86	1.03	0.2052												
FT – calculated	1.00	0.99	1.01	0.7936												
Bioavailable T – calculated	1.04	0.78	1.37	0.8033												
DHEA–S	0.86	0.57	1.30	0.4774												
DHEA	0.93	0.87	1.00	0.0468*	0.83	0.75	0.92	0.0006*	0.85	0.77	0.94	0.0015*	0.83	0.75	0.93	0.0007*
Total testosterone	0.79	0.68	0.92	0.0020*	0.94	0.76	1.15	0.5445	0.81	0.68	0.98	0.0272*				
SHBG	0.96	0.95	0.98	<0.0001*	0.97	0.94	0.99	0.0053*					0.96	0.94	0.99	0.0012*

Notes: *Statistically significant p<0.05. [a]Not included in the analysis due to being diagnostic criteria of MS. [b]Not included in the analysis due to total cholesterol including HDL and thus having significance. For univariate regression significance cutoff point is p<0.1 not 0.05, as stated in Methods.
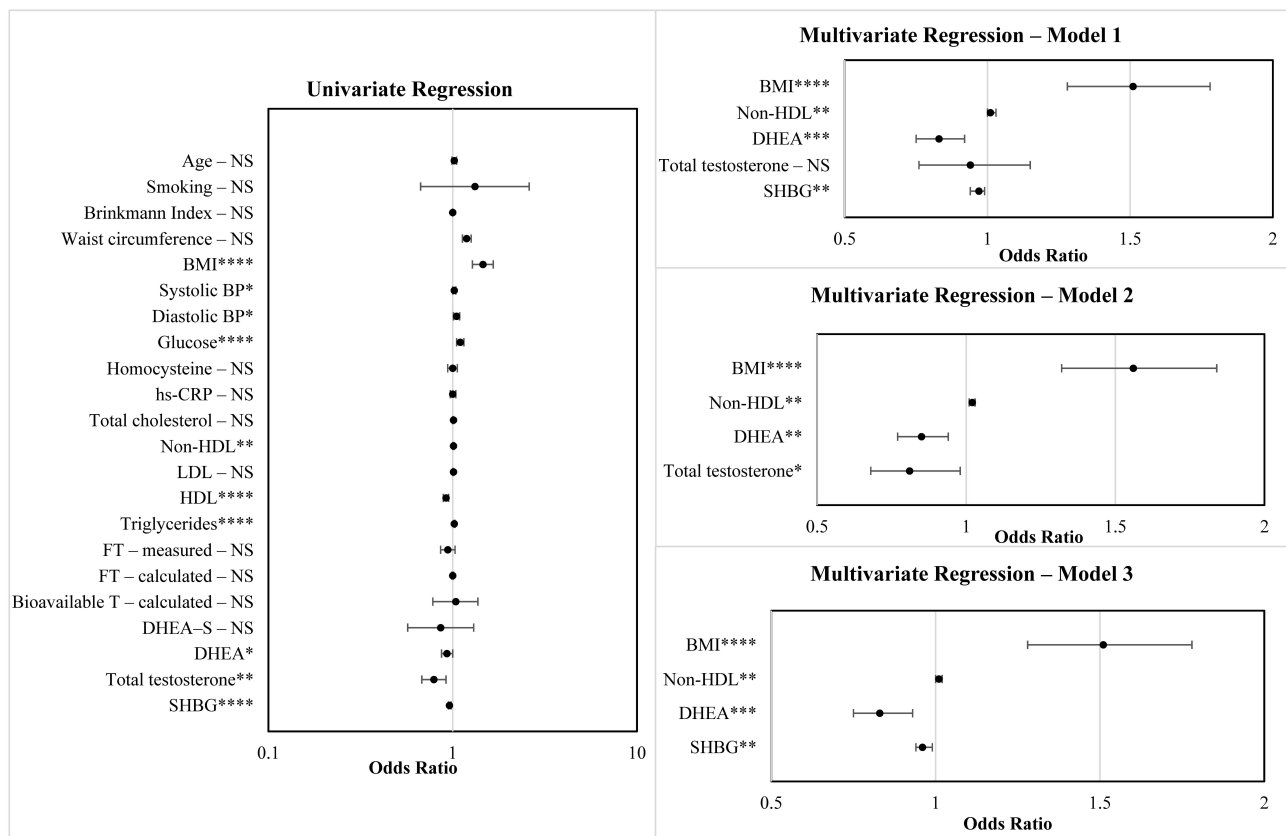
Figure 3 Univariate regression and three models for multivariate regression – forest plots.
Notes: NS – non-significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.
Abbreviations: BMI, body mass index; BP, blood pressure; CRP, c-reactive protein; non-HDL, total cholesterol minus HDL; LDL, low-density lipoprotein; HDL, high-density lipoprotein; FT, free testosterone; DHEA, dehydroepiandrosterone; SHBG, sex hormone–binding globulin.

age, thus falsely showing a negative association between c-FT and c-BAT with age due to the way it is calculated. Thus, in our opinion, the parameters calculated can give false information to clinicians about the androgenic status of patients and should not be used.

There is still a great deal of inconsistency in the results showing the impact of DHEA and DHEA–S plasma concentrations on the development of MS and cardiovascular disease, as well as possible causation and underlying mechanisms. DHEA and DHEA–S focused review article suggests that DHEA and DHEA–S levels decrease because of glucose intolerance and insulin resistance increasing with age.[22]

It is especially important to note that the role of changing of levels of DHEA–S in the development of MS is not evident. Some publications show no association between DHEA–S and MS, which is consistent with our results.[20,23–25] However, Chinese authors show DHEA–S elevation to be a risk factor for the development of metabolic syndrome in elderly men.[26] The authors suggest that

one of the plausible mechanisms is DHEA conversion to testosterone, explaining both elevated DHEA and a decrease in testosterone. Insulin also further increases the production of DHEA–S, as shown in an in-vitro study, but the authors suggest that the effects are not uniform between subjects.[27] However, other articles completely debunk those claims, proving the complete opposite by stating that insulin has no impact on DHEA or DHEA-S production and regulation,[28,29] or even by showing that the decrease in insulinemia through benfluorex treatment raises DHEA and DHEA-S.[30] There is also evidence that replacement of DHEA in aging humans can positively affect insulin resistance,[31] while another publication showed no effect except an increase of DHEA-S.[32] The only thing all of those articles agree on is that further research warranting a bigger study group is definitely needed, preferably in a long-term prospective study.

Studies also find that high insulin levels increase the metabolic clearance of DHEA and DHEA–S, thus explaining its serum decrease in MS.[33,34] The unknown is

whether DHEA is only decreased because of hyperinsulinemia in MS or whether it actually plays some underlying cause in the patomechanisms of MS.

DHEA increases the expression of glucose transporters within adipose tissue and increases oxidation of lipids and overall glucose usage. DHEA shows an insulin-like effect, thus decreasing insulin concentrations. What is also worth mentioning is the positive effect of both T and DHEA on visceral adipose tissue, namely decreasing visceral fat accumulation in men and visceral fat oxidation.[35,36] Recent publications on the topic suggest that both DHEA and its sulfate might exert an effect by plasma membrane receptors, neuroreceptors, steroid receptors and being metabolized into more potent steroids.[37]

Although the p-value of difference in plasma levels of DHEA–S between MS group and controls in our study was not of statistical significance, it was close to it, warranting a bigger studied group and further research, especially after considering conflicting results from previous studies.

Another avenue worth exploration and consideration are diseases that affect androgen metabolism. Some of them might be greatly underdiagnosed, like Kallmann syndrome, Klinefelter syndrome and rare forms of congenital adrenal hyperplasia. Patients with Klinefelter syndrome have a higher risk of cardiovascular disease and develop T2DM, MS and insulin resistance more often than the general population.[38]

In case of Kallmann syndrome, the risk for is also worth considering, in one study 9 out of 23 participants suffered from obesity.[39] There is a possibility that a part of the patients suffering from both hypogonadism and MS actually suffer from some underlying, undiagnosed condition. This implies that clinicians dealing with patients suffering from MS and hypogonadism should always have those rarer diseases in mind.

Low SHBG is associated with hyperinsulinemia and insulin resistance, showing the negative impact of high insulin on hepatic SHBG production.[40–42] This indicates that men with isolated hyperinsulinemia or insulin resistance may have hypogonadism and/or be on the path to metabolic syndrome. This would be suggestive of SHBG being a good early marker for metabolic dysregulation and potentially hypogonadism. The same study on in-vitro cell line found an inhibitory effect of high levels of prolactin on hepatic production of SHBG.

On the other hand, low concentrations of SHBG affect the ectopic location of adipose tissue (mainly in the liver, skeletal muscle and pancreas). Recently, it has been shown that a reduction in SHBG concentration in young men is a predictor of nonalcoholic fatty liver disease development in middle age.[43]

In one particular study, SHBG presented with a strong and inverse association not only with MS, but also its components alone, namely waist circumference, HDL-C and triglycerides, and the authors suggest that its association with MS is stronger than testosterone, which is in accordance with our results. They also took into consideration the negative impact of insulin on levels of SHBG, and even after taking it as confounder in analysis, the relation between SHBG and MS remained statistically significant, thus further increasing its value as a predictor of MS.[24] Comparable results of a negative association between SHBG and metabolic syndrome were also found among monozygotic twins in another study.[44] It is suggested in some research papers that SHBG might not be only a transport protein, but also interact with tissue-specific membrane receptors in different tissues, namely breast, prostate, testes, liver and possibly muscles.[45,46]

One publication shows an increase in SHBG, testosterone and HDL-C, along with a decrease in insulin and leptin levels following a 10-week regimen of very-low-energy diet in obese men losing weight.[47] This further shows a link between the aforementioned serum markers and metabolic syndrome, showing an improvement in testosterone, SHBG and HDL-C, and a decrease in factors of MS, namely insulin levels, leptin and weight. The significant reduction in SHBG levels is worth noting in the youngest men suffering from MS, whereas the levels of testosterone, and especially DHEA, were not much different from each other. Our analysis of differences of SHBG among patients with metabolic syndrome in different age groups showed differences between younger and older patients, which is not present in the healthy controls. This shows that the decrease of SHBG is most pronounced in younger patients (namely mostly 40 and 50 years old), suggesting the early diagnostic role of SHBG.

The strength of association and the significance of SHBG plasma concentrations with MS diagnosis is at least the same or higher (blood pressure) as the associated strength of its particular MS diagnostic elements (Table 2)

In our study, CRP shows a positive association with age, which was also found in a previous study.[48] It also displays a positive association with many components or

consequences of MS and negative with SHBG and HDL, which suggests that inflammation plays an important role in the development of MS[49] and is connected to insulin resistance.[50–52] SHBG also affects inflammatory pathways by interacting with lymphocytes via membrane receptors.[53] CRP shows no direct association with androgens, both gonadal and adrenal, implying that SHBG changes might happen prior to changes in androgens, suggesting even more the important role of early SHBG imbalance in MS. Our current and earlier research suggests that the consequence of chronic inflammation in patients with metabolic syndrome might be more complicated, affecting hormones and their carrier proteins as well.[54]

The evidence from an older study on HepG2 cell production of SHBG being inhibited by insulin was questioned, suggesting that the action of insulin is not specific and instead that TNF-α is the main factor by which SHBG is downregulated in obesity,[55] further supporting the bigger impact of the inflammatory process. This warrants further research into the avenue of inflammation in MS, considering that treatment with anti-TNFα in patients with autoimmune diseases has beneficial effects on insulin sensitivity.[56] A second article debunking the aspect of insulin action on downregulation of hepatic production SHBG is actually associated with the consumption of sugars and its metabolic consequences, rather than insulin itself.[57]

In our regression analysis, we first conducted an univariate analysis to find good variables for multivariate analysis. We did not include variables associated with MS, namely its diagnostic criteria and total cholesterol, because it includes HDL and thus we decided to include non-HDL cholesterol. We decided to present 3 models. The first one shows all 5 variables fitting the criteria for inclusion in multivariate analysis. The second one without SHBG and the third one without total testosterone. The reason for the two models with exclusion of either SHBG and TT was their strong association and through these models we wanted to show which one has a bigger impact on MS. Our results show that changes in TT associated with MS are linked to underlying changes in SHBG and indirectly to TT. Based on the results in model 2 vs 1, we show that TT only is significant as long as SHBG is kept out of the analysis, while SHBG results are not affected greatly by TT (model 1 vs 3). This implies that the more important variable in MS prediction is SHBG, TT changes are secondary to changes in SHBG and in line with

previous research on the subject both in a cross-sectional analysis of 3294 men and in a longitudinal analysis of 618 men.[58] Furthermore, this would imply that people with high SHBG are less inclined to suffer from MS and diabetes throughout their life, suggesting genetic predisposition to different levels of SHBG having an impact on the chance of developing MS or T2DM throughout life,[59,60] while another study debunks previous claims.[61] The small amount of research on the genetic predisposition of SHBG regulation and expression warrants further research into the topic.

People with low SHBG are at higher risk of MS (shown in the results of our multivariate analysis model 1 and 3). This relates to both men and women, according to many studies,[2,4,15,16,58] as well as meta-analysis consisting of 2500 men and 4765 women.[62]

Our study is cross-sectional research. The role of SHBG in the early diagnostics of metabolic syndrome is greatly undervalued. For this reason, we began a follow-up prospective study of individual age groups to confirm the diagnostic value of SHBG concentrations for early MS diagnosis in young men.

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Disclosure

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