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Sex Hormone-Binding Globulin and Its Association to Cardiovascular Risk Factors in an Italian Adult Population Cohort

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Abstract: Abnormal sex hormone-binding globulin (SHBG) and sex hormone concentrations are the cause or the consequence of cardiometabolic diseases, however, the clinical correlates of SHBG is clearly less understood. In our study we investigate sex- and age-specific serum SHBG levels and their association with cardiovascular risk (CVR) factors and high-risk conditions in an adult cohort of Italian population. Data from 1176 men and 2236 women, aged 20–81 were analyzed and serum SHBG determined in stored samples using an immunoassay. SHBG concentrations, higher in women than in men in the younger age groups, exhibited a curvilinear increase with age in men and a U-shaped curve across the lifespan in women, with a decrease from the 2nd to the 6th decade of age and an increase after the 6th decade when SHBG concentrations were similar in both sexes. Low SHBG serum levels correlated with the traditional CVR factors diabetes, obesity, and hypertension, whereas high level of SHBG correlated with cholesterol HDL. These associations were more numerous in women than in men, in whom decreased with age. The sex- and age specific differences observed in our population-based cohort should be considered in establishing reference ranges and clinical cut-off points to improve CVR score charts and therapeutic approaches.

Keywords: sex hormone-binding globulin; cardiovascular risk biomarker; epidemiology; age; sex

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1. Introduction

Sex hormone-binding globulin (SHBG) is a glycoprotein that binds sex steroid hormones (testosterone, progesterone and estradiol) and it is produced mainly in the liver. Although the affinity of androgens for SHBG is stronger than that of estrogens [1], SHBG regulates the bioavailability of both hormones modulating the transport of testosterone to target tissues [2]. SHBG synthesis are regulated by hormonal and metabolic factors. Several clinical studies have demonstrated the metabolic function of SHBG. Interest in SHBG has escalated in recent years because of its inverse association with metabolic syndrome and type 2 diabetes in women and men [3–6]. Moreover, low SHBG serum were linked with cardiovascular disease in men [7]. Although low SHBG serum level in women was associated with poorer cardiovascular health, such endothelial dysfunction, carotid atherosclerosis, and vascular remodeling, greater prevalence of metabolic syndrome, type 2 diabetes, hypertension, the clinical correlates of SHBG in women is less clearly understood [8–10].

Further sex-specific research is needed to understand the role of SHBG in the etiology of diabetes type II and in establishing new approaches to target prevention and therapeutic strategies for the improvement of overall cardiovascular health [11,12].

In future research, special attention should be paid in regards to the role of abnormal SHBG and sex hormone levels in women's cardiovascular health and to elucidate whether the reproductive hormones are the cause or the consequence of cardiometabolic diseases. [10]. Studies with women from the general population are rare and previous studies are mainly conducted in post-menopausal women or in selected community or in patient-based study samples, or in small sample sizes. Furthermore, to our knowledge no studies evaluated SHBG differences across decades of the male and female lifespan in a large Italian population-based cohort.

To overcome these limitations, the distribution of SHBG in the adult Italian population was investigated using data from a cohort of adult general population, Malattie Aterosclerotiche Istituto Superiore di Sanità (MATISS), to assess the sex- and age-specific profile of circulating SHBG across the lifespan and to assess the association of SHBG with cardiovascular risk factors and high risk conditions.

2. Materials and Methods

2.1. Study Population

Within the MATISS study, men and women aged 20–81 years were extracted randomly from a geographical area located in Central Italy about 100 km south of Rome and comprised 4 adjacent rural towns (Sezze, Roccagorga, Bassiano, and Priverno) and examined in 1993–1996 collecting socio-demographic characteristics, lifestyles (smoking, diet, physical activity), risk factors (blood pressure, heart rate, ECG read by Minnesota Code, blood lipids, glycemia, body mass index—BMI, pulmonary function), using standardized procedures and methods [13–15]. During the screening, serum, plasma, buffy coat, red cells were collected and were stored in liquid nitrogen in the population Biological Bank of the Italian National Institute of Health (ISS). The MATISS study was approved as part of the CUORE Project by Ethical Committee of ISS on 15 March 2006; the Ethical Committee approved the use of pooled samples for research activity in epidemiological and genetic/genomic studies. Personal data were stored and managed according to the European personal data regulation (GDPR 679/2016). Personal data were excluded from the database used for statistical analyses that, instead, includes a unique code for each person. The same codes were used to label the serum samples stored in the biological bank.

2.2. Measurements SHBG and Testosterone

The MATISS cohort was part of MORGAM (MOnica Risk, Genetics, Archiving and Monograph) and BIOMARCARE (Biomarker for Cardiovascular Risk Assessment in Europe) international projects. The BIOMARCARE consortium is an EU-funded consortium including over 30 partners from academia and industry [16]. BiomarCaRE aims to determine the value of established and emerging biomarkers to improve risk estimation of cardiovascular disease in Europe. Within the BIOMARCARE consortium, a large panel of biomarkers was assessed in the MATISS Study to improve disease prediction among different European populations. Among the panel, SHBG and testosterone were assessed in 2016 at the BiomarCaRE Laboratory (University Heart Center Hamburg, Germany) using immunoassays. Briefly, SHBG was measured on the Architect i2000 system using the Abbott Architect SHBG assay and total testosterone on the Abbott ARCHITECT 2nd Generation Testosterone (Abbott Diagnostics, Abbott Park, IL, USA). The tests used chemiluminescent microparticle immunoassay on the ARCHITECT system (Abbott Diagnostics, Abbott Park, IL, USA). The assay ranges were as follows: SHBG, 0–250 nmol/L; Testosterone, 0.45–35 nmol/L. The coefficients of variability (CV): SHBG, intra assay CV 3.79% and inter assay CV 8.2%; Testosterone, intra assay CV 4.3% and inter assay CV 8.38%.

2.3. Statistical Analysis

With the aim to describe age-specific trends of SHBG across the lifespan, the study population was stratified by sex and age classes (20–29, 30–39, 40–49, 50–59, 60–69, 70–81 years-old). Mean, standard deviation, confidence interval of mean, median, minimum, and maximum values were calculated for SHBG in each group. After testing SHBG distribution, Mann–Whitney non-parametric test was used to evaluate statistically significant differences among groups; differences associated to p-values ≤ 0.05 were considered statistically significant.

To study the association between SHBG and cardiovascular diseases risk (CVR) factors and conditions population sample, SHBG was stratified by sex, age classes, and SHBG tertiles. Due to the combination of strata, to avoid excessive fragmentation of the sample size, age groups were aggregated into 20–39, 40–59, and 60–81 years, representing young adults, adults, and the elderly. Within each tertile, cardiovascular risk factors and high-risk conditions were evaluated: body mass index (BMI), diastolic and systolic blood pressure, glycemia, triglyceridemia, cholesterol, testosterone, smoking habits, hypertension, hypercholesterolemia, type 2 diabetes mellitus. In female population, the presence of menopause and use of oral contraceptives were also reported. Mean and standard deviation were assessed for continuous variables, percentages for categorical variables; 95% confidence intervals were used to compare tertiles within age classes and to assess the association between SHBG and risk factors/high risk conditions as well as ANOVA models and chi-squared tests for continuous variables, percentages for categorical variables respectively. Statistical analyses were performed by the use of the software IBM SPSS (version 25) (IBM, New York, NY, USA) and SAS software, release 9.4, (SAS Institute Inc., Cary, North Carolina, NC, USA).

3. Results

3.1. Sex Hormone-Binding Globulin Trends in Males and Females across the Adult Lifespan

Table 1 reports descriptive statistics of circulating SHBG in randomly selected 1176 adult men and 2236 adult women) divided in six age groups. Results demonstrated that SHBG concentrations in men ranged from a value of 8.5 nm/L to a value of 203.2 nm/L and the mean value increased with age from approximately 32 nm/L in the age groups 20–29 and 30–39 years to 57.1 nm/L in the oldest persons (age group 70–81 years) (Table 1 and Figure 1).

Table 1. Sex Hormone-Binding Globulin (SHBG) distribution by age groups and sex. The Malattie Aterosclerotiche Istituto Superiore di Sanità (MATISS) study, men and women aged 20–81 years [13–15].

Age Groups (Years)	SHBG (nm/L)—Men								SHBG (nm/L)—Women							
	n	Mean	Std Dev	IC 95% of Mean		Median	Min	Max	*n*	Mean	Std Dev	IC 95% of Mean		Median	Min	Max
20–29	116	32.4	12.6	30.1	34.7	29.8	12.2	75.5	216	87.6	55.9	80.1	95.1	69.0	13.2	246.4
30–39	229	32.8	13.0	31.2	34.6	30.7	9.2	77.4	360	77.1	51.5	71.7	82.4	60.1	11.4	249.4
40–49	264	37.8	18.3	35.6	40.0	35.1	12.7	203.2	465	63.7	37.2	60.3	67.1	56.3	7.0	246.0
50–59	257	43.1	17.3	41.0	45.3	41.4	10.0	112.4	475	50.1	25.9	47.8	52.4	45.7	10.6	220.5
60–69	232	50.2	20.0	47.6	52.8	48.0	8.5	197.3	489	52.9	24.4	50.8	55.1	48.9	9.4	184.0
70–81	78	57.1	21.9	52.1	62.0	54.2	22.9	148.7	231	60.4	25.1	57.1	63.6	54.5	17.7	145.6
20–81	1176	41.2	18.9	40.1	42.3	37.9	8.5	203.2	2236	62.6	38.7	61.0	64.2	53.4	7.0	249.4

Data collected between 1993 and 1996 in Central Italy and serum SHBG levels analyzed in 2016. CI: confidence interval. Mean men age (standard deviation): 48.6 nm/L (14.3); mean women age (standard deviation): 50.61 nm/L (14.4).

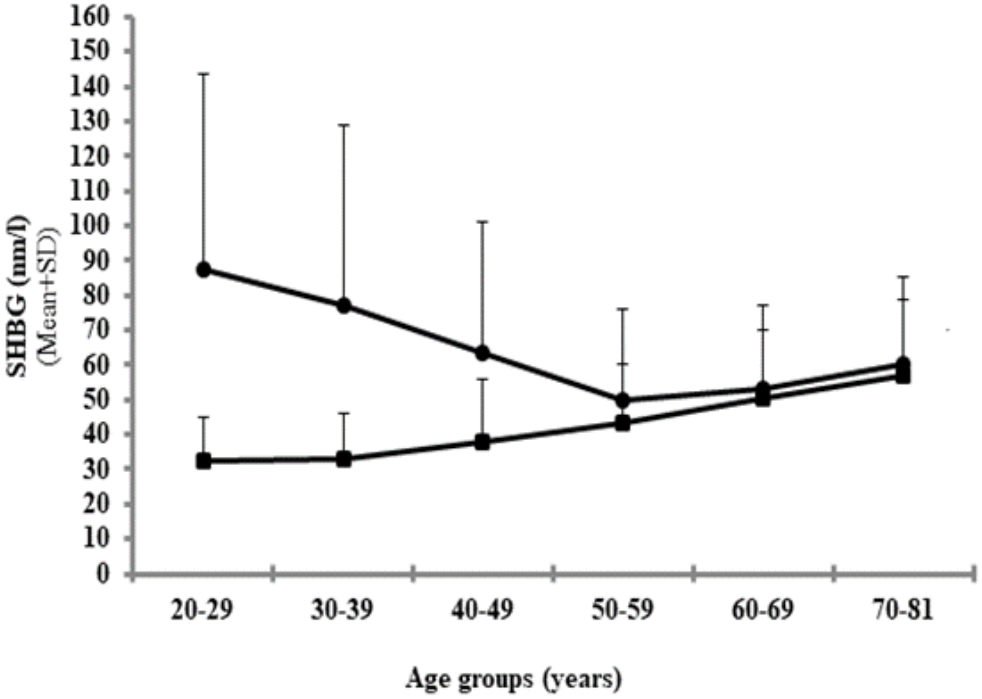

Figure 1. The linear plots indicate the geometric means of serum sex hormone-binding globulin (SHBG) levels in men (■) and women (●) in decades of age 20–29 years, 30–39 years, 40–49 years, 50–59 years, 60–69 years, and 70–81 years. The Malattie Aterosclerotiche Istituto Superiore di Sanità (MATISS) study, data collected between 1993 and 1996 in Central Italy and serum SHBG levels analysed in 2016 [13–15].

The mean value of SHBG in men was 41.2 ± 18.9 nm/L. Considering the results in women we observed that concentrations ranged from a value of 7 nm/L to a value of 249.4 nm/L (Table 1 and Figure 1). In contrast to that observed in males the highest mean value of SHBG (87.6 nm/L) was detected in the younger group of persons (20–29 years). Then the SHBG value decreased gradually with age until 59 years, afterward it started to increase again. The mean value of SHBG in women was 62.6 ± 38.7 nm/L.

Comparison of SHBG distribution by the use of Mann–Whitney non-parametric test showed that SHBG concentrations significantly differed among age groups in both sexes ($p < 0.018$) with the exception of the first two groups in men and of the groups 40–49 and 70–81 years in women whose values of protein concentration were almost the same.

The comparison between results in men and women showed that SHBG concentrations were higher in women than in men in the lower age groups ($p < 0.0001$), especially in the age group 20–29 years (Table 1 and Figure 1). From the group 50–59 years values tended to be similar in both sexes ($p < 0.002$). The differences in SHBG concentrations between men and women were not statistically significant in persons over 60 years of age (Table 1 and Figure 1).

3.2. Sex Hormone-Binding Globulin Levels and Cardiovascular Risk Factors and Conditions

In Table 2, distribution of persons in each group of sex, age (20–39, 40–59, 60–81 years-old), and SHBG tertiles were reported as well as SHBG minimum and maximum. Of note, only in the age group 60–81 years, SHBG values were almost overlapping in men and women (Table 2).

Table 2. Sex Hormone-Binding Globulin (SHBG) tertiles by age groups and sex. The Malattie Aterosclerotiche Istituto Superiore di Sanità (MATISS) study, men and women aged 20–81 years [13–15].

	Men										
	SHBG (nm/L)										
	Total		**First Tertile**			**Second Tertile**			**Third Tertile**		
Age Class (Years)	*n*	%	***n***	**Min**	**Max**	*n*	**Min**	**Max**	*n*	**Min**	**Max**
20–39	345	29.3	115	9.2	26.1	116	26.2	36.5	114	36.6	77.4
40–59	521	44.3	174	10.0	31.1	174	31.2	45.2	173	45.3	203.2
60–81	310	26.4	103	8.5	41.3	103	41.5	56.5	104	56.9	197.3
20–81	1176		392			393			391		
	Women										
	SHBG (nm/L)										
	Total		**First Tertile**			**Second Tertile**			**Third Tertile**		
Age Class (Years)	***n***	%	***n***	**Min**	**Max**	***n***	**Min**	**Max**	***n***	**Min**	**Max**
20–39	576	25.8	192	11.4	50.6	192	50.7	83.6	192	84.3	249.4
40–59	940	42.0	315	7.0	40.3	312	40.4	61.1	313	61.7	246.0
60–81	720	32.2	240	9.4	41.7	240	42.0	61.6	240	61.7	184.0
20–81	2236		747			744			745		

Data collected between 1993 and 1996 in Central Italy and serum SHBG levels analyzed in 2016.

Cardiovascular risk factors and high-risk conditions were reported in Tables 3 and 4 by age class and SHBG tertile, respectively for men and women. In men, testosterone was highly significant ($p < 0.0001$) across SHBG tertiles in all of the three age groups, whereas BMI significantly decreased in the third tertile in all of the three age groups with particular relevance to the age group 20–39 years. Of note, the prevalence of smoking habits was high in all groups. Prevalence of smoking habit in men was higher in the third tertile than in the first tertile. In the age group 20–39 years, men within the highest tertile had significantly lower blood pressure (BP) (systolic and diastolic) in comparison to those within the lowest tertile. In addition, prevalence of hypertension tended to be lower when concentration of SHBG increased. In the older age group (60–81 years), glycemia and triglyceride values resulted significantly lower in the third than in the first tertile. Of note, in the same older age group prevalence of diabetes was lower in comparison to other tertiles when concentration of SHBG increased.

In women, in all the age groups, SHBG was associated with several risk factors and high-risk conditions. Analysis across SHBG tertiles determined that all the three age groups shared the presence of an inverse association of SHBG serum concentration (lower level of SHBG) with diastolic BP, BMI, glycemia, diabetes, and a direct association (higher level of SHBG) with cholesterol HDL. According to the World Health Organization (WHO) (2000) standards [17] the analysis of the BMI showed that overweight (BMI < 30) and obese (BMI > 30.0) conditions tended to be lower in women when concentration of SHBG increased across tertiles. The age groups 20–39 and 40–59 years shared the presence of a negative association of SHBG with the prevalence of hypertension. Of note, across tertiles, women taking contraceptive drugs shared the presence of a positive association with SHBG. Unexpectedly, in the age group 20–39 years, cholesterol resulted lower in the SHBG first and second tertile than in the third tertile, the prevalence of hypercholesterolemia was lower in the second tertile than in the first and third tertile and the prevalence of hypertension was lower in the second than in the third tertile. In the age group 30–49 years, we observed that higher SHBG concentrations were associated to lower systolic BP and as well as to higher number of smokers and of women with menstrual cycles. In the age group 60–81 years, we found a positive association of SHBG concentration with low systolic BP.

Table 3. Baseline characteristics by Sex Hormone-Binding Globulin (SHBG) tertiles and age groups. The Malattie Aterosclerotiche Istituto Superiore di Sanità (MATISS) study, men aged 20–81 years [13–15].

Men 20–39 Years Old	**SHBG First Tertile**					**SHBG Second Tertile**					**SHBG Third Tertile**					**ANOVA *p*-Value**
Risk Factors	***n***	**Mean**	**std**	**95% CI**		***n***	**Mean**	**std**	**95% CI**		***n***	**Mean**	**std**	**95% CI**		
SHBG (nm/L)	115	20	3.7	19.4	20.7	116	30.7	3.1	30.1	31.3	114	47.6	9.6	45.8	49.4	<0.0001
Age (years)	115	31.1	5	30.2	32	116	30.7	5.4	29.7	31.7	114	31.8	4.8	30.9	32.7	0.2084
Systolic BP(mmHg)	115	132.1	10.5	130	134	116	130.3	16	127	133	114	125.5	12.6	123	128	0.0006
Diastolic BP(mmHg)	115	82.9	10.4	80.9	84.8	116	80.3	14	77.8	82.9	114	78.4	10.8	76.4	80.4	0.0189
BMI (Kg/m^2)	115	26.9	3.1	26.3	27.4	116	26	3.3	25.4	26.7	114	24.6	3.6	23.9	25.3	<0.0001
Glycemia (mg/dL)	115	85.1	11	83	87.1	116	84.7	19	81.3	88.1	114	81.4	8.8	79.8	83	0.0767
Cholesterol (mg/dL)	115	211.6	45.3	203	220	116	202.5	43	195	210	114	204.1	57.7	193	215	0.3269
HDL(mg/dL)	115	43	10.5	41.1	45	116	45.8	11	43.8	47.8	114	45.9	11.4	43.8	48	0.0767
Triglycerides (mg/dL)	115	192	129	168	216	116	155.6	165	125	186	114	169.8	215	130	210	0.2745
Testosterone (nm/L)	115	16.8	4.7	15.9	17.7	114	21.5	5.1	20.5	22.4	90	26	5.2	24.9	27.1	<0.0001
Risk Conditions	***n***	**%**		**95% CI**		***n***	**%**		**95% CI**		***n***	**%**		**95% CI**		**Chi-Squared *p*-Value**
Smoking	51	44.3		35.1	53.6	52	44.8		35.6	54	71	62.3		53.2	71.3	0.0084
Hypertension	47	40.9		31.7	50	32	27.6		19.3	35.8	21	18.4		11.2	25.6	0.0008
Hypercholesterolemia	29	25.2		17.2	33.3	21	18.1		11	25.2	15	13.2		6.9	19.5	0.0637
Diabetes	1	0.9		0	2.6	2	1.7		0	4.1	0	0		-	-	0.3711
Men 40–59 Years Old	**SHBG First Tertile**					**SHBG Second Tertile**					**SHBG Third Tertile**					**ANOVA *p*-Value**
Risk Factors	***n***	**Mean**	**std**	**95% CI**		***n***	**Mean**	**std**	**95% CI**		***n***	**Mean**	**std**	**95% CI**		
SHBG (nm/L)	174	24	4.7	23.3	24.7	174	37.9	4	37.3	38.5	173	59.5	17.1	57	62.1	<0.0001
Age (years)	174	48	5.6	47.2	48.9	174	49.5	5.5	48.7	50.4	173	51.2	5.3	50.4	52	<0.0001
Systolic BP(mmHg)	174	138.1	19	135	141	174	136.5	19	134	139	173	136.8	18.9	134	140	0.7089
Diastolic BP(mmHg)	174	88.9	12	87.2	90.7	174	87.2	12	85.5	88.9	173	86.4	12.2	84.6	88.3	0.136
BMI (Kg/m^2)	173	28.1	3.1	27.6	28.5	174	27.6	3.1	27.1	28	172	27.1	3.8	26.5	27.7	0.0291
Glycemia (mg/dL)	174	94	20	91.1	97	172	91.6	18	88.9	94.3	173	93.1	24.8	89.4	96.9	0.5631
Cholesterol (mg/dL)	174	216.5	39	211	222	172	222.7	44	216	229	173	226	45.9	219	233	0.1125
HDL(mg/dL)	174	43.8	11	42.2	45.4	172	45.1	12	43.3	46.8	173	46.7	11.7	44.9	48.4	0.0604
Triglycerides (mg/dL)	174	194.3	131	175	214	172	176.8	131	157	197	173	165.6	115	148	183	0.102

Table 3. *Cont.*

Men 40–59 Years Old	**SHBG First Tertile**					**SHBG Second Tertile**					**SHBG Third Tertile**					**ANOVA *p*-Value**
Risk Factors	*n*	**Mean**	**std**	**95% CI**		*n*	**Mean**	**std**	**95% CI**		*n*	**Mean**	**std**	**95% CI**		
Testosterone (nm/L)	173	15.3	3.9	14.7	15.9	174	20.1	4.3	19.5	20.8	140	25.2	5	24.3	26	<0.0001
Risk Conditions	***n***	**%**		**95% CI**		***n***	**%**		**95% CI**		***n***	**%**		**95% CI**		**Chi-Squared *p*-Value**
Smoking	50	28.7		21.9	35.5	71	40.8		33.4	48.2	81	46.8	3.8	39.3	54.3	0.002
Hypertension	98	56.3		48.9	63.8	91	52.3		44.8	59.8	83	48	3.8	40.5	55.5	0.2979
Hypercholesterolemia	52	29.9		23	36.8	58	33.3		26.3	40.4	67	38.7	3.7	31.4	46.1	0.2152
Diabetes	9	5.2		1.8	8.5	8	4.7		1.5	7.8	9	5.2	1.7	1.9	8.5	0.9658
Men 60–81 Years Old	**SHBG First Tertile**					**SHBG Second Tertile**					**SHBG Third Tertile**					**ANOVA *p*-Value**
Risk Factors	***n***	**Mean**	**std**	**95% CI**		***n***	**Mean**	**std**	**95% CI**		***n***	**Mean**	**std**	**95% CI**		
SHBG (nm/L)	103	33.1	6.2	31.9	34.3	103	48.8	4.2	48	49.7	104	73.6	19.7	69.8	77.5	<0.0001
Age (years)	103	65.5	4.7	64.6	66.4	103	66	4.4	65.1	66.9	104	67.5	4.6	66.6	68.4	0.0042
Systolic BP(mmHg)	103	156.2	25.3	151	161	103	152.9	24	148	158	104	154.9	25.4	150	160	0.6327
Diastolic BP(mmHg)	103	88.4	13.8	85.7	91.1	103	84.5	13	81.9	87.1	104	86.3	14.7	83.5	89.2	0.1366
BMI (Kg/m^2)	102	28.8	3.9	28	29.6	102	27.6	3.3	26.9	28.2	103	27.5	3.6	26.8	28.2	0.0139
Glycemia (mg/dL)	103	102	30.5	96	108	103	100.1	23	95.6	105	104	92.5	15.8	89.4	95.6	0.0114
Cholesterol (mg/dL)	103	234.7	41.5	227	243	103	225.9	47	217	235	104	225.8	39.3	218	233	0.2272
HDL(mg/dL)	103	48.1	15.4	45.1	51.1	103	47.5	14	44.8	50.2	104	49.8	14.6	47	52.7	0.4805
Triglycerides (mg/dL)	103	205	126	180	230	103	169.1	95	151	188	104	163.8	118	141	187	0.0189
Testosterone (nm/L)	103	16.6	3.9	15.8	17.4	99	21.3	5.1	20.2	22.3	87	26	5.8	24.8	27.2	<0.0001
Risk conditions	***n***	**%**		**95% CI**		***n***	**%**		**95% CI**		***n***	**%**		**95% CI**		**Chi-Squared *p*-Value**
Smoking	25	24.3		15.9	32.7	35	34	4.7	24.7	43.3	42	40.4	4.8	30.8	50	0.0458
Hypertension	82	79.6		71.7	87.5	79	76.7	4.2	68.4	85	73	70.2	4.5	61.3	79.1	0.272

Table 3. *Cont.*

Men 60–81 Years Old	SHBG First Tertile					SHBG Second Tertile					SHBG Third Tertile					ANOVA *p*-Value
Risk Factors	*n*	**Mean**	**std**	**95% CI**		*n*	**Mean**	**std**	**95% CI**		*n*	**Mean**	**std**	**95% CI**		
Hypercholesterolemia	47	45.6		35.8	55.4	45	43.7	4.9	33.9	53.4	37	35.6	4.7	26.2	44.9	0.2972
Diabetes	12	11.7		5.3	18	16	15.5	3.6	8.4	22.6	5	4.8	2.1	0.6	9	0.0403

Data collected between 1993 and 1996 in Central Italy. std: standard deviation; CI: confidence interval; BMI, body mass index; BP, blood pressure. ANOVA model and chi-squared test compare respectively mean values and prevalence among tertiles. Hypertension: mean of two consecutive blood pressure values of systolic $\geq$ 140 mmHg or diastolic $\geq$ 90 mmHg or under pharmacological treatment. Hypercholesterolemia: total cholesterol $\geq$ 240 mg/dL (6.2 mmol/L) or under regular lipid lowering treatment. Diabetics: persons with blood glucose $\geq$ 126 mg/dL (7 mmol/L) or under antidiabetic treatment (oral hypoglycemic medication and/or insulin) or with self-reported diabetes at the time of the screening).

Table 4. Baseline characteristics by Sex Hormone-Binding Globulin (SHBG) tertiles and age groups. The Malattie Aterosclerotiche Istituto Superiore di Sanità (MATISS) study, women aged 20–81 years [13–15].

Women 20–39 Years Old	SHBG First Tertile					SHBG Second Tertile					SHBG Third Tertile					ANOVA *p*-Value
Risk Factors	*n*	**Mean**	**std**	**95% CI**		*n*	**Mean**	**std**	**95% CI**		*n*	**Mean**	**std**	**95% CI**		
SHBG (nm/L)	192	36.1	9.7	34.7	37.5	192	64.6	9.4	63.3	65.9	192	142.4	48	136	149	<0.0001
Age (years)	192	32.3	5.2	31.6	33.1	192	31.3	5.2	30.5	32	192	31.3	5.2	30.6	32.1	0.0858
Systolic BP(mmHg)	192	123.2	14	121	125	192	119.6	12	118	121	192	121.7	14	120	124	0.0322
Diastolic BP(mmHg)	192	78.3	11	76.7	79.9	192	73.2	9.8	71.8	74.6	192	76.1	11	74.5	77.6	<0.0001
BMI (Kg/m^2)	192	26.7	4.6	26	27.3	192	23.8	3.6	23.3	24.3	192	24.1	3.6	23.6	24.6	<0.0001
Glycemia (mg/dL)	192	79.2	14	77.2	81.3	192	75.6	8.5	74.3	76.8	192	74.8	8.9	73.6	76.1	0.0001
Cholesterol (mg/dL)	192	192.7	36	188	198	192	184.2	31	180	189	192	204.6	42	199	211	<0.0001
HDL(mg/dL)	192	50.3	10	48.8	51.8	192	55.2	9.8	53.8	56.6	192	58.8	13	56.9	60.6	<0.0001
Triglycerides (mg/dL)	192	102.6	56	94.6	111	192	78.6	33	73.9	83.3	192	114.8	77	104	126	<0.0001
Testosterone (nm/L)	188	1.2	0.4	1.1	1.2	190	1.1	0.4	1.1	1.2	183	1.1	0.4	1	1.1	0.0588
Risk Conditions	***n***	**%**		**95% CI**		***n***	**%**		**95% CI**		***n***	**%**		**95% CI**		**Chi-Squared *p*-Value**
Smoking	63	32.8		26.1	39.5	60	31.3		24.6	37.9	58	30.2		23.7	36.8	0.8581
Hypertension	42	21.9		16	27.8	18	9.4		5.2	13.5	29	15.1		10	20.2	0.0032
Hypercholesterolemia	21	10.9		6.5	15.4	6	3.1		0.6	5.6	40	20.8		15	26.6	<0.0001

Table 4. *Cont.*

Women 20–39 Years Old	**SHBG First Tertile**					**SHBG Second Tertile**					**SHBG Third Tertile**					**ANOVA *p*-Value**
Risk Factors	*n*	**Mean**	**std**	**95% CI**		*n*	**Mean**	**std**	**95% CI**		*n*	**Mean**	**std**	**95% CI**		
Diabetes	3	1.6		0	3.3	0	0		-	-	0	0		-	-	0.049
Menstrual cycles	184	97.9		95.8	100	186	98.4		96.6	100	178	97.8		95.7	100	0.55
Estrogen therapy *	0	0		-	-	0	0		-	-	0	0		-	-	-
Contracceptive drugs **	10	5.6		2.2	9	29	16.1		10.7	21.5	103	58.9		51.5	66.2	<0.0001
Women 40–59 Years Old	**SHBG First Tertile**					**SHBG Second Tertile**					**SHBG Third Tertile**					**ANOVA *p*-Value**
Risk Factors	***n***	**Mean**	**std**	**95% CI**		***n***	**Mean**	**std**	**95% CI**		***n***	**Mean**	**std**	**95% CI**		
SHBG (nm/L)	315	29.6	7.4	28.7	30.4	312	50.5	6	49.8	51.2	313	90.6	34	86.8	94.5	<0.0001
Age (years)	315	51	5.1	50.4	51.6	312	49.9	5.8	49.3	50.6	313	48.2	5.6	47.6	48.8	<0.0001
Systolic BP(mmHg)	314	146	22	144	148	312	139.9	21	138	142	313	133.6	19	132	136	<0.0001
Diastolic BP(mmHg)	314	89.8	13	88.4	91.2	312	86.5	13	85.1	88	313	82.7	13	81.3	84.1	<0.0001
BMI (Kg/m^2)	314	31.4	4.9	30.8	31.9	312	29.4	4.5	28.9	29.9	313	26.7	4.1	26.3	27.2	<0.0001
Glycemia (mg/dL)	315	92.2	32	88.6	95.7	312	84.1	20	81.9	86.4	311	83.2	18	81.2	85.1	<0.0001
Cholesterol (mg/dL)	315	227.1	40	223	232	312	224.5	40	220	229	311	220.2	41	216	225	0.1041
HDL(mg/dL)	315	50.6	12	49.3	52	312	53.9	13	52.4	55.4	311	57.3	12	56	58.6	<0.0001
Triglycerides (mg/dL)	315	148.3	85	139	158	312	126.1	69	118	134	311	108.7	55	103	115	<0.0001
Testosterone (nm/L)	289	1.1	1.8	0.9	1.3	305	1.1	1.2	0.9	1.2	297	1	0.6	1	1.1	0.9529
Risk Conditions	***n***	**%**		**95% CI**		***n***	**%**		**95% CI**		***n***	**%**		**95% CI**		**Chi-Squared *p*-Value**
Smoking	19	6		3.4	8.7	41	13.1		9.4	16.9	53	16.9		12.8	21.1	0.0001
Hypertension	223	71		66	76.1	180	57.7		52.2	63.2	132	42.2		36.7	47.7	<0.0001
Hypercholesterolemia	108	34.3		29	39.6	105	33.7		28.4	38.9	92	29.4		24.3	34.5	0.3633
Diabetes	21	6.7		3.9	9.4	9	2.9		1	4.8	6	1.9		0.4	3.5	0.0048
Menstrual cycles	77	24.6		19.8	29.4	106	34		28.7	39.3	161	51.4		45.9	57	<0.0001
Estrogen therapy *	13	5.6		2.6	8.6	12	5.9		2.6	9.1	10	6.6		2.6	10.6	0.9161
Contracceptive drugs **	3	2.8		0	5.9	3	2.4		0	5.1	24	13.1		8.2	18.1	0.0002

Table 4. *Cont.*

Women 60–81 Years Old	**SHBG First Tertile**					**SHBG Second Tertile**					**SHBG Third Tertile**					**ANOVA *p*-Value**
Risk Factors	*n*	**Mean**	**std**	**95% CI**		*n*	**Mean**	**std**	**95% CI**		*n*	**Mean**	**std**	**95% CI**		
SHBG (nm/L)	240	31.7	6.9	30.8	32.5	240	51.1	5	50.5	51.7	240	83.3	20	80.7	85.9	<0.0001
Age (years)	240	66.1	4.7	65.5	66.7	240	67.2	4.9	66.6	67.9	240	67.6	4.8	67	68.2	0.002
Systolic BP(mmHg)	240	161	25	158	164	240	159.7	25	157	163	239	155.1	23	152	158	0.0192
Diastolic BP(mmHg)	240	91.7	14	89.9	93.4	240	88.8	13	87.2	90.3	239	86.9	12	85.3	88.4	0.0002
BMI (Kg/m^2)	239	32.3	4.8	31.7	32.9	240	30.6	4.4	30	31.1	238	28.3	4.7	27.7	28.9	<0.0001
Glycemia (mg/dL)	240	101.6	37	96.9	106	240	91.8	25	88.6	94.9	240	87.5	16	85.4	89.6	<0.0001
Cholesterol (mg/dL)	240	237.6	40	233	243	240	234.2	40	229	239	240	234.1	39	229	239	0.5391
HDL(mg/dL)	240	49.6	12	48.1	51.2	240	53.1	12	51.5	54.6	240	55.3	13	53.7	56.9	<0.0001
Triglycerides (mg/dL)	240	178.2	92	167	190	240	149.9	71	141	159	240	132.6	53	126	139	<0.0001
Testosterone (nm/L)	227	1.2	2	0.9	1.4	231	1.1	0.6	1	1.2	230	1.1	1	1	1.3	0.7892
Risk Conditions	***n***	**%**		**95% CI**		***n***	**%**		**95% CI**		***n***	**%**		**95% CI**		**Chi-Squared *p*-Value**
Smoking	9	3.8		1.3	6.2	7	2.9		0.8	5.1	5	2.1		0.3	3.9	0.5546
Hypertension	216	90		86.2	93.8	206	85.8		81.4	90.3	201	84.1		79.4	88.8	0.1489
Hypercholesterolemia	114	47.5		41.1	53.9	106	44.2		37.8	50.5	112	46.7		40.3	53	0.7478
Diabetes	40	16.7		11.9	21.4	16	6.7		3.5	9.8	17	7.1		3.8	10.4	0.0002
Menstrual cycles	0	0		-	-	0	0		-	-	1	0.4		0	1.2	0.3658
Estrogen therapy *	9	3.8		1.4	6.3	9	3.8		1.4	6.3	10	4.3		1.7	7	0.944
Contracceptive drugs **	0	0		-	-	0	0		-	-	0	0		-	-	-

Data collected between 1993 and 1996 in Central Italy. std: standard deviation; CI: confidence interval; BMI, body mass index; BP, blood pressure. ANOVA model and chi-squared test compare respectively mean values and prevalence among tertiles. Hypertension: mean of two consecutive blood pressure values of systolic $\geq$ 140 mmHg or diastolic $\geq$ 90 mmHg or under pharmacological treatment. Hypercholesterolemia: total cholesterol $\geq$ 240 mg/dL (6.2 mmol/L) or under regular lipid lowering treatment. Diabetics: persons with blood glucose $\geq$ 126 mg/dL (7 mmol/L) or under antidiabetic treatment (oral hypoglycemic medication and/or insulin) or with self-reported diabetes at the time of the screening). * Only for those without regular cycles or with not regular cycles. ** Only for those with regular cycles.

4. Discussion

Sex hormone-binding globulin is a major carrier of sex hormones in human plasma [18] and its concentration changes with age [18,19]. Evidence indicates that a low serum level of SHBG may be associated with increased risk of cardiovascular disease in both women and men.

To date, epidemiological studies reporting significant associations of SHBG with CVR factors and high-risk conditions were predominantly sex-specific or conducted in small, selected study samples. The present study determined the distribution of serum SHBG across decades of adult male and female lifespan in a large prospective cohort of central Italy population at enrollment in 1993–1996 and investigated the relationship of SHBG with the distribution of cardiovascular risk factors and prevalence of high-risk conditions. The results of this study demonstrate the presence of gender differences as concern blood SHBG concentration (mean value and trend during the lifespan) and its associations with risk factors and conditions, more numerous in women than in men. To our knowledge this is the first study evaluating SHBG differences across decades of the male and female lifespan in a large Italian population-based cohort. The relationship of SHBG with age is well documented and clear in men [18,20,21] whereas data in women are few and controversial and are referred mainly in peri-menopausal women [22–27].

The MATISS data in men confirm in a larger Caucasian population previous findings from Harman et al. who determined on a population of 890 men from the Baltimore Longitudinal Study on Aging that SHBG exhibits a curvilinear increase with age, rising at a slightly greater rate in the older than in younger men [18]. Our data in men are also in accordance with recent findings on a population-based sample of 6296 men aged 40 years–79 years old from China, who describe the same positive correlation between SHBG level and age [21]. In contrast, data from an Australian population of more than 110,000 persons with an age range between 10 and 90 years showed that SHBG declines to a nadir in males at the age of 20 years and remains stable till the 6th decade with a gradual progressive rise thereafter [20]. As concerning women, our results here agree with most studies describing a U-shaped curve for SHBG across the lifespan of women, with a decrease from the 2nd to the 6th decade and an increase after the 6th decade [28]. Interestingly, Maggio et al., in their study [28], observed in the group of women between 20 and 60 years that BMI and insulin had opposite trajectories to SHBG, thus suggesting that in part insulin levels and BMI negatively influenced SHBG concentration. A previous study from Davison et al., showed no substantial change in SHBG levels across the lifespan of Australian women, apart from a slight increase in the oldest [24]. In the more recent Australian population study by Handelsman et al., SHBG reached a nadir early, in late adolescence, with median levels rising progressively with age and with an acceleration after the age of 70 years [20]. In the same study, women exhibited a serum SHBG peak during the early half of reproductive life due to pregnancy or to the use of oral contraceptives. It was reported that at the menopausal transition, women experience a drastic decrease in the level of SHBG as well as a concomitant but more gradual decrease in total testosterone thus acquiring a more androgenic profile of sex hormones (higher free testosterone and lower SGBG levels) [29]. This profile is associated with adverse CVR factors levels. Our data showing that testosterone levels do not change in women over the course of life suggests that other key risk factors may be involved in the occurrence of cardiovascular diseases in Italian female population.

Current literature reports that among CVR factors and high risk conditions, components of metabolic syndrome are associated to SHBG levels in both sexes with a threefold increase in cardiovascular disease risk and a fivefold increase to develop type 2 diabetes [30]. Low SHBG levels are reported as an independent risk factor for insulin resistance and diabetes. The correlation between SHBG and insulin resistance was demonstrated in many cross-sectional studies, suggesting that the association between SHBG and type 2 diabetes was due to insulin resistance [4,31]. Moreover, this inverse association is stronger in women than in men [11]. In a later study, Ding et al., demonstrated that in post-menopausal women,

higher levels of SHBG were strongly associated with decreased risk of type 2 diabetes, as observed in men (4). In our female sample population, we found that glycemia was inversely associated to SHBG in all the three age groups and that low concentration of SHBG were associated with a higher prevalence of diabetes. Genetic studies have also strengthened the evidence that SHBG and sex hormones are involved in the etiology of type 2 diabetes [4,32].

Low SHBG levels have also been associated to an adverse lipid profile. Many studies have shown that SHBG levels correlate positively with HDL-cholesterol concentration. A cross-sectional study on 79 morbidly obese Italian persons, 27 men and 52 women (age 30–45 years) demonstrated that an increase in SHBG levels might be related to an increment of HDL-cholesterol [33]. According with this study, the increment of HDL was observed among SHBG tertiles in women whereas it was not observed in men. A possible explanation may be ascribed to the different BMI showed by men and women (normal-weight vs. overweight) from MATISS population.

An unexpected and apparently contrasting result, which deserves further investigation, is that in the younger group of women (20–39 years) cholesterol concentration and the prevalence of hypercholesterolemia are higher in the highest SHBG tertile than in the first tertile. A possible explanation may be ascribed to hormonal contraceptive use that is reported positively associated with lipoprotein subclasses, including HDL and total cholesterol, although the metabolic perturbations are reversed upon discontinuation [34].

It is well documented that in women CVR presents a rapid increase with the onset of menopause. In the perimenopausal period, a large number of women suffer from metabolic syndrome, that conveys a fourfold higher risk of cardiovascular events in women than in men [35]. A very recent cross-sectional study [36] investigating associations of sex hormones and anthropometric markers in the general population in Pomerania, demonstrated the presence of inverse associations of serum SHBG with multiple body parameters, such as subcutaneous adipose tissue, BMI and waist-circumference, in both women and men, thus supporting a role for low SHBG in incremented obesity risk in men and widening this result also to women [10]. In another very recently published paper, in 270 men (29% blacks) and 304 women (34% blacks) from the Heritage Family study, low SHBG and testosterone were associated with higher adiposity and abdominal and visceral fat in men and a similar adiposity profile was observed in women with low SHBG [37]. In our study, we confirmed the presence of the previously reported inverse association between SHBG and BMI in women, association that we found independent of age because it was present in all the three age groups. In men, many previous studies with cross-sectional and longitudinal design report that SHBG, as well as testosterone, was inversely associated with metabolic syndrome and many of its components, although the inverse associations with the sex hormones were not always found across the different component of metabolic syndrome. Associations with metabolic syndrome were primarily mediated by hyperglycemia, hypertriglyceridemia, abdominal obesity and were weaker for hypertension [30,38–40]. In a meta-analysis of 20 observational studies, Brand et al. observed that SHBG concentrations decreased with increasing BMI [38]. Ding et al. demonstrated in two prospective studies that higher levels of SHBG in men were strongly associated with decreased risk of type 2 diabetes [4,11]. An epidemiological study conducted on a representative sample of the population living in the Tuscany region of Italy (InCHIANTI study) to investigate the association between hormones and metabolic syndrome in older Italian men (mean age: 75 years; age range: 65–96) demonstrated that total testosterone and SHBG were negatively associated with metabolic syndrome [41] and independent of age, SHBG was positively associated with HDL and negatively associated with abdominal obesity and triglycerides. In contrast to the InCHIANTI study, a cross-sectional study by Xiao et al. determined that in elderly men, lower SHBG levels but not testosterone may be an independent predictor for the prevalence of metabolic syndrome [36]. In a recent international collaborative study reporting statistical analyses of individual participant data from 12,330 male controls aged 25–85 years from 25 published studies on prostate cancer risk SHBG results inversely associ-

ated with BMI and directly associated with age, testosterone, and current smoking [42]. The MATISS Study findings here reported agree with previous studies reporting the presence of an inverse association between SHBG and testosterone and BMI, association that we found in all age groups both men and women. Our results in the group of old-aged men were in line with findings by Brand et al. on the presence of an inverse association between SHBG and glycemia [38], whereas results in the all groups of men confirm the direct association of SHBG with current smoking and with age reported by Watts et al. [42]. Indeed, we observed that in all age groups, men within the third tertile were older than those in the first tertile. Finally, our observations that men in the age group 20–39 years within the highest SHBG tertile have significantly lower BP (systolic and diastolic) in comparison to those within the lowest tertile and that in the all age groups the prevalence of hypertension tend to be lower when concentration of SHBG increases, were in line with results from Siddiqui et al., who found that low SHBG levels were predictive of high risk for developing not only metabolic syndrome but also hypertension [39].

Hypertension is a major CVR factor possibly explaining the increase of cardiovascular disease occurrence in postmenopausal women. In line with this hypothesis, in our study of general population, we found that, blood pressure was inversely associated to SHBG in women, particularly in the group of middle- and old-aged women where we found that both diastolic and systolic blood pressure as well as the prevalence of hypertension significantly decreased across SHBG tertiles. In the younger group of women blood pressure and the prevalence of hypertension seem to be inversely associated to SHBG only in the second SHBG tertile.

Similarly, to what observed in men, the middle age group of women presents a direct association of SHBG with current smoking. This point should be studied further to understand the nature of this association.

In female population we confirm previous findings on the positive association of SHBG with the use of contraceptive drugs or the presence of menstrual cycles in the middle age population [43]. Of note, in our study, the group of the middle-aged women with menstrual cycles was more numerous (51%) in third SHBG tertile, association that we found dependent on age thus confirming previous observation that in premenopausal women estradiol levels take a part in the fine tuning of SHBG level besides age [44].

Even though, several studies have demonstrated the role of endogenous estrogens on the cardiovascular protection [45], information is lacking about the role played by SHBG in the regulation of estradiol. It is important to note that circulating SHBG has functions in addition to the binding and transport of sex steroids. Indeed, recent evidence suggest that SHBG exerts a direct action through its internalization, as well as by influencing estradiol uptake and signaling [46,47]. By interacting with its putative receptors such as low-density lipoprotein-related protein 2 receptor (Megalin receptor) and matrix-associated proteins [48,49], SHBG activates the non-genomic sex hormone intracellular pathways in reproductive tissues/cells. There is also evidence indicating that the SHBG-SHBG receptor-membrane estradiol receptor (ER) complex participates in the estradiol signaling in non-reproductive cells such as lymphocytes and neurons cells [47,50]. On the contrary, SHBG acts as negative modulator of estradiol action in breast cancer thus playing a protective role in the exposure of breast cells to estrogens [51]. Of note, this effect is highly selective, depending on SHBG interaction with cells, and restricted to genes associated to cell growth and estrogen-sensitivity. Another aspect to be considered to explain the biological role played by SHBG is its binding affinity for exogenous chemical compounds that protectively mitigate target tissue exposure thus avoiding the endocrine disrupting potential of these compounds [52]. The displacement of the endogenous ligand by chemicals binding to SHBG could expose target tissues to higher endogenous hormone levels thus potentially resulting in adverse effects. Therefore, studies are needed in non-reproductive cells to establish whether the possible cardiometabolic benefits associated to SHBG are estrogen independent effects or reflect the complex crosstalk between hormonal and metabolic factors.

5. Conclusions

The sex- and age specific differences we found in our population-based cohort should be considered in establishing reference ranges and clinical cut-off points. Further studies regarding SHBG as biomarker for CVD-related risk factor burden are necessary to evaluate individual CVR profile and to consider SHBG as part of CVR score charts. Future research from prospective cohort studies, as well as interventional trials, are also needed to investigate the molecular mechanisms of these associations and to assess their causal direction. For example, an important question that needs to be addressed is whether a low concentration of SHBG causes insulin resistance or whether insulin resistance causes a low SHBG concentration [31]. The elucidation of causality direction could bring to therapeutic perspectives.

Author Contributions: The authors' responsibilities were as follows—B.B. and R.R. interpreted the results and drafted the manuscript; C.D. prepared the data sent to laboratory, cooperated to the management of serum samples sent to laboratory, coordinate the management of data and statistical analyses, interpreted the results, and revised the manuscript; B.B., R.R. and C.D. performed the statistical analyses, interpreted the results, and revised the manuscript; L.P. cooperated with the management of serum samples sent to laboratory, and revised the manuscript; C.L.N. collected data and biological samples, cooperated with the management of serum samples sent to laboratory, and revised the manuscript; A.D.L. and S.V. handled the biological samples storage and revised the manuscript; S.B. coordinated the funds and the laboratory for analysis of serum samples and revised the manuscript; T.Z. headed the laboratory for the analysis of serum samples and revised the manuscript; M.G. interpreted the results and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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