Journal of Affective Disorders 356 (2024) 167–176

Contents lists available at ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Research paper

Disturbed sex hormone milieu in males and females with major depressive disorder and low-grade inflammation

Giulia Lombardo [a,*], Valeria Mondelli [a,i], Courtney Worrell [a], Luca Sforzini [a,i], Nicole Mariani [a], Naghmeh Nikkheslat [a], Maria A. Nettis [a,j], Melisa Kose [a], Zuzanna Zajkowska [a], Annamaria Cattaneo [b,c], Linda Pointon [d], Lorinda Turner [d], Philip J. Cowen [e], Wayne C. Drevets [f], Jonathan Cavanagh [g], Neil A. Harrison [h], Edward T. Bullmore [d], the Neuroimmunology of Mood Disorders and Alzheimer's Disease (NIMA) Consortium, Paola Dazzan [a,i], Carmine M. Pariante [a,i]

[a] Institute of Psychiatry, Psychology and Neuroscience, Department of Psychological Medicine, Maurice Wohl Clinical Neuroscience Institute, King's College London, SE5 9RT, UK
[b] Biological Psychiatric Unit, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, 25125 Brescia, Italy
[c] Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy.
[d] Department of Psychiatry, School of Clinical Medicine, University of Cambridge, Cambridge CB2 0SZ, UK
[e] University of Oxford Department of Psychiatry, Warneford Hospital, Oxford OX3 7JX, UK
[f] Janssen Research & Development, Neuroscience Therapeutic Area, 3210 Merryfield Row, San Diego, CA 92121, USA
[g] Centre for Immunobiology, University of Glasgow and Sackler Institute of Psychobiological Research, Queen Elizabeth University Hospital, Glasgow G51 4TF, UK
[h] School of Medicine, School of Psychology, Cardiff University Brain Research Imaging Centre, Maindy Road, Cardiff CF24 4HQ, UK
[i] National Institute for Health Research (NIHR) Maudsley Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King's College London
[j] South London and Maudsley NHS Foundation Trust, UK

ARTICLE INFO

Keywords:
Major depressive disorder
Sex hormones
Inflammation
C-reactive protein
Sex differences

ABSTRACT

Sex hormones have biological effects on inflammation, and these might contribute to the sex-specific features of depression. C-reactive protein (CRP) is the most widely used inflammatory biomarker and consistent evidence shows a significant proportion (20–30 %) of patients with major depressive disorder (MDD) have CRP levels above 3 mg/L, a threshold indicating at least low-grade inflammation. Here, we investigate the interplay between sex hormones and CRP in the cross-sectional, observational Biomarkers in Depression Study.

We measured serum high-sensitivity (hs-)CRP, in 64 healthy controls and 178 MDD patients, subdivided into those with hs-CRP below 3 mg/L (low-CRP; 53 males, 72 females) and with hs-CRP above 3 mg/L (high-CRP; 19 males, 34 females). We also measured interleukin-6, testosterone, 17-β-estradiol (E2), progesterone, sex-hormone binding globulin (SHBG), follicle-stimulating and luteinising hormones, and calculated testosterone-to-E2 ratio (T/E2), free androgen and estradiol indexes (FAI, FEI), and testosterone secretion index.

In males, high-CRP patients had lower testosterone than controls ($p = 0.001$), and lower testosterone ($p = 0.013$), T/E2 ($p < 0.001$), and higher FEI ($p = 0.015$) than low-CRP patients. In females, high-CRP patients showed lower SHGB levels than controls ($p = 0.033$) and low-CRP patients ($p = 0.034$). The differences in testosterone, T/E2 ratio, and FEI levels in males survived the Benjamini-Hochberg FDR correction. In linear regression analyses, testosterone ($\beta = -1.069$ $p = 0.033$) predicted CRP concentrations ($R^2 = 0.252$ $p = 0.002$) in male patients, and SHBG predicted CRP levels ($\beta = -0.628$ $p = 0.009$, $R^2 = 0.172$ $p = 0.003$) in female patients.

These findings may guide future research investigating interactions between gonadal and immune systems in depression, and the potential of hormonal therapies in MDD with inflammation.

1. Introduction

Elevated levels of immune biomarkers, such as C-reactive protein (CRP) and interleukin (IL)-6, have been detected in patients with major depressive disorder (MDD), in particular in those with treatment-resistant depression (Valkanova et al., 2013; Strawbridge et al., 2017;

* Corresponding author.
E-mail address: giulia.lombardo@kcl.ac.uk (G. Lombardo).

https://doi.org/10.1016/j.jad.2024.03.018
Received 30 September 2023; Received in revised form 6 February 2024; Accepted 7 March 2024
Available online 15 March 2024

Chamberlain et al., 2019; Chin Fatt et al., 2023; Medina-Rodriguez et al., 2023). Moreover, about 20–30 % of MDD patients manifest CRP levels above 3 mg/L, a threshold that indicates the presence of at least low-grade inflammation (Osimo et al., 2019; Pitharouli et al., 2021) and is associated with an increased risk of cardiovascular diseases (Ridker, 2003), although our recent work has shown that even MDD patients with CRP less than 3 mg/L have evidence of immune activation at a molecular level (Sforzini et al., 2023). Of note, Zajkowska et al. (2023) detected sex-specific inflammatory biomarkers associated with the severity and the risk of depression in adolescence, with, specifically, elevated IL-2 for boys and elevated IL-6 for girls (Zajkowska et al., 2023).

Interestingly, sex hormones can affect both innate and adaptive immune responses (Beagley and Gockel, 2003; Taneja, 2018), as testosterone exhibits mainly anti-inflammatory proprieties and estrogen shows both anti- and pro-inflammatory proprieties (Straub, 2007; Gilliver, 2010). For instance, in the general population, testosterone levels correlate negatively with CRP in males, while 17-β-estradiol (E2) levels correlate positively with CRP in males (Tsilidis et al., 2013) but negatively in premenopausal females (Park and Lee, 2020). Even though there are clinical studies measuring both sets of markers in individuals with mood disorders, the evidence is quite limited, and there is a lack of studies specifically investigating interactions between inflammation and sex hormones in males and females with MDD. Interestingly, one study found that male depressed patients show lower testosterone and higher CRP than controls (Peng and Li, 2021), supporting the anti-inflammatory action of testosterone, but they did not look at the correlation between these two systems. The putative pro-inflammatory action of estrogens might also explain why oral contraceptives, which comprise combinations of estrogens and progesterone, are associated with increased depressive symptoms and increased CRP levels, as Masama et al. (2022) recently found with exogenous estradiol in young females.

Additionally, corroborating evidence from meta-analyses supports a sex-specific association between sex hormones and depression. Male MDD individuals exhibit decreased testosterone and increased E2 (Fischer et al., 2019), while premenopausal females with depression show increased testosterone (Maharjan et al., 2021).

Of note, also the immune system shows a certain degree of sex dimorphism (Markle and Fish, 2014), with females showing a more robust immune response than males, and higher rates of autoimmune diseases (Schmidt et al., 2006). Thus, sex hormones may be involved in the immune abnormalities described in depression, and abnormalities in these two interacting systems may contribute to the sex-specific differences in the prevalence and clinical phenotypes of this disorder. Nevertheless, to our knowledge, no previous study has examined the relationship between endogenous sex hormones and immune biomarkers in MDD individuals (Lombardo et al., 2021).

In the present study, we investigate the association between sex hormones and peripheral inflammation as measured with serum CRP in the cross-sectional, observational Biomarkers in Depression (BIODEP) study. We aim to understand whether MDD patients, males and females separately, exhibit sex-specific differences in sex hormone levels compared with healthy controls and whether MDD patients with low-grade inflammation (CRP above 3 mg/L) have different levels of sex hormones compared with both controls and MDD patients with CRP below 3 mg/L. To our knowledge, this is the first study to investigate endogenous sex hormones in patients with MDD stratified according to levels of peripheral inflammation (serum high-sensitivity (hs)-CRP).

2. Materials and methods

2.1. Study design and sample characteristics

The current sample was selected from the larger primary and secondary cohorts of the BIODEP study. The study overview has been described previously (Chamberlain et al., 2019). Briefly, the BIODEP study is a cross-sectional, observational, non-interventional study that is part of a multi-centre study investigating immune markers in depression, through the Wellcome Trust Consortium for Neuroimmunology of Mood Disorders and Alzheimer's disease (NIMA Consortium), approved by the National Research Ethics Service East of England, Cambridge Central, UK (15/EE/0092) and conducted according to the Declaration of Helsinki.

All participants (aged between 25 and 50 years) gave written informed consent prior to data collection, were capable and willing to fast for 8 hours prior to blood draw and to abstain from strenuous exercise for 72 hours prior to assessment. They were recruited and assessed in 5 clinical centres in the UK: Brighton, Cambridge, Glasgow, London (King's College London), and Oxford. Patients were selected if they met the MDD diagnosis as defined by structured clinical interview (SCID) in accordance with DSM-5 criteria (Spitzer et al., 1992).

Healthy subjects were selected from the general population to match the patient group in terms of age, sex, and BMI. We investigated the severity of depressive symptoms with the HAMD-17 clinical scale (Hamilton, 1960).

As previously described (Chamberlain et al., 2019; Nikkheslat et al., 2020; Cattaneo et al., 2020; McLaughlin et al., 2021), exclusion criteria were the following: 1) use of any medication (e.g., statins, corticosteroids, antihistamines, anti-inflammatory medications), or lifetime history of any serious medical disorder or recent infection/illness which could have compromised the interpretation of immunological data; 2) pregnancy or breastfeeding; 3) active alcohol or drug abuse or dependence in the last 6 months; 4) participation in clinical trial of an investigational drug within the last 12 months; 5) history of bipolar disorder or non-affective psychosis; 6) for controls, history of MDD, antidepressant treatment for depressive symptoms or any other indication of any major psychiatric disorder as defined by DSM-5.

We excluded from the secondary cohort 23 participants as "repeated participants" in both the primary and secondary cohorts, so that their data are only presented once. For the purpose of our analyses, we further excluded subjects: 1) who were taking any hormonal therapies (e.g., oral contraceptives, contraceptive injections, hormone replacement therapy (HRT), intrauterine devices); 2) who declared having medical conditions associated with disruption of the reproductive system (e.g., erectile dysfunction, recent abortion, hysterectomy, endometriosis, polycystic ovary syndrome (PCOS), menopause, perimenopause); and 3) who had follicle-stimulating hormone (FSH) levels above 20 IU/L ($n = 12$, age mean 47.09 ± 2.21), a threshold indicating a possible ovarian insufficiency (Luisi et al., 2015; Tariq et al., 2022). After having excluded one subject with a missing value for hs-CRP, we obtained a study sample of 242 subjects (males, $n = 97$; females, $n = 145$), with 25 male controls, 72 male patients, 39 female controls, and 106 female patients. We further stratified our depressed sample according to levels of hs-CRP, using a threshold above and below 3 mg/L: in males, 53 patients had a CRP below 3 mg/L (low-CRP) and 19 patients had a CRP equal to or greater than 3 mg/L (high-CRP), while in females, 72 patients had low-CRP and 34 patients had high-CRP. The full breakdown of the screening process is displayed in Fig. 1.

2.2. Depressive symptoms

The clinical and sociodemographic characteristics of the BIODEP sample have been extensively described by (Chamberlain et al., 2019; Nikkheslat et al., 2020; Cattaneo et al., 2020; McLaughlin et al., 2021), and overall, the sub-sample used for this study showed similar clinical and immune profiles as those in the larger sample, even when stratified for males and females and low/high CRP (see Table 1). The average HAMD-17 score was around 17, and the sample also included patients on antidepressants and in remission. Specifically, 124 individuals were taking antidepressants (around 70 % of the sample, for both males and females), and of these, 26.5 % of males and 20 % of females were in

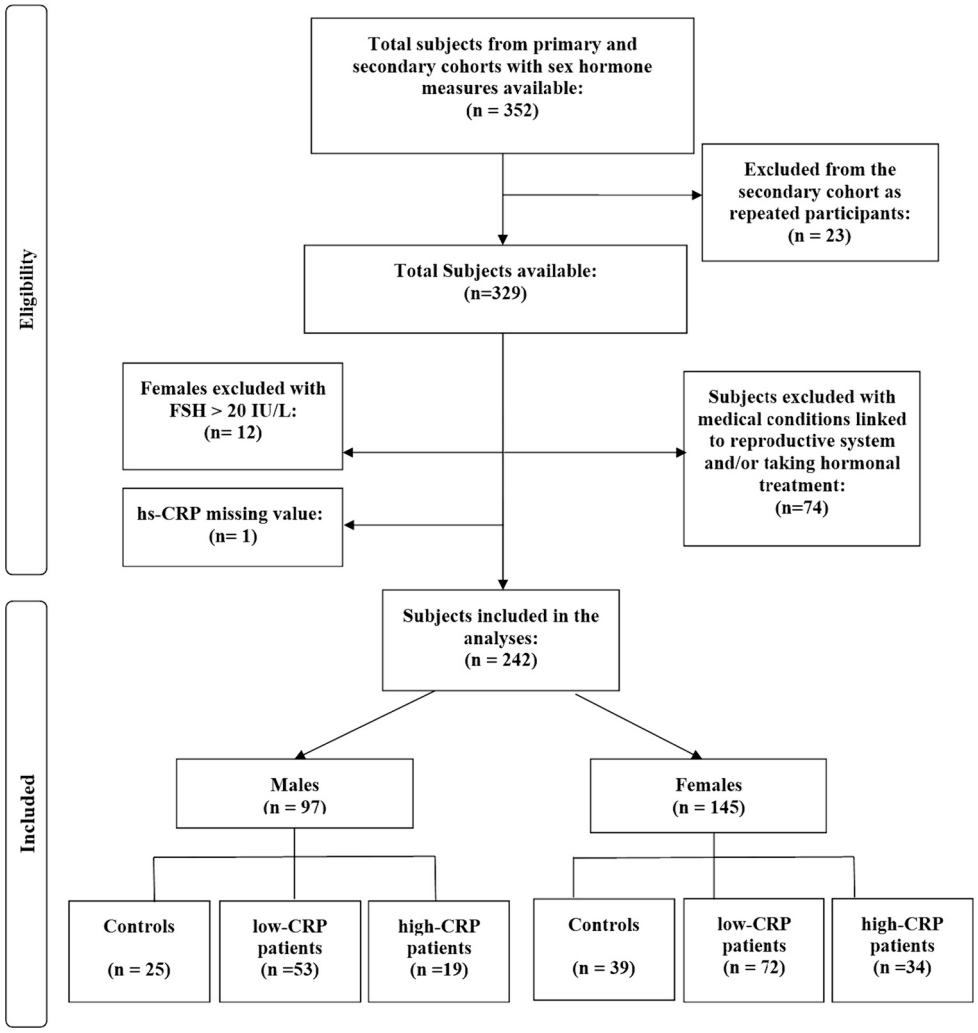

Fig. 1. Flowchart of the selection process.
Note: CRP (C-reactive protein); hs (high sensitivity); high-CRP (hs-CRP above 3 mg/L); low-CRP (hs-CRP below 3 mg/L); n (number of subjects).

remission, as showed by a HAMD-17 < 7. As expected, high-CRP patients showed significantly larger BMI than controls and patients with low-CRP, in both sexes, and controls showed lower depressive symptom scores in HAMD-17 compared with MDD patients (both low- and high-CRP groups) in both sexes, but we did not detect significant differences in depression scores between low- and high-CRP patient groups. The majority of MDD individuals on antidepressants (65 % for males and 76 % for females) were taking SSRIs, alone or in combination with other antidepressants, while the remaining were taking a mixture of other classes (tricyclics, serotonin and noradrenaline reuptake inhibitors, mood stabilizers, and others). There was no difference in the distribution of antidepressant (SSRIs vs. others) classes between the low- and high-CRP groups ($\chi2 = 1.643$, $p = 0.200$ in males, and $\chi2 = 0.019$, $p = 0.889$ in females).

2.3. Biomarkers

As described by Aruldass et al. (2021), at the time of the clinical assessment, fasting venous blood was collected between 8:00 and 10:30 in the morning from participants who abstained from exercise for 72 hours prior blood sampling. The tubes were centrifuged at 1600 Relative Centrifugal Force for 15 minutes. Blood samples were transferred to Q2 Solutions Laboratory to be analysed within the same day of the collection day. Samples were exposed to anti-CRP-antibodies on latex particles, and the increase in light absorption due to complex formation was used to quantify serum hs-CRP levels (Turbidimetry on Beckman Coulter AU analysers). Inter and intra-assay coefficient of variations were < 10 %. Serum IL-6 levels were measured in peripheral blood. Tubes were centrifuged at 1600 *g* for 15 minutes at room temperature and serum supernatant was frozen at −80 °C. Serum IL-6 levels were measured using Meso Scale Discovery (MSD; Rockville, M, USA). Blood (8.5 mL) was collected into BD Vacutainer® Serum Separator Tubes (SST II Advance) Silica tubes and incubated for 30 minutes at room temperature. The tubes were then centrifuged at 1600 Relative Centrifugal Force for 15 minutes and serum was collected in aliquots. All serum samples were stored at −70 °C. We were specifically interested in investigating the sex-specific relationship between CRP and IL-6, so our analyses focussed on this cytokine.

Sex hormones: Serum samples kept at −80 °C were transferred to VIApath laboratories (a collaboration between SYNLAB UK & Ireland, Guy's & St Thomas' NHS Foundation Trust and King's College Hospital NHS Foundation Trust) for the analyses. As illustrated by the assays' descriptions provided by the laboratory, electrochemiluminescence immunoassay "ECLIA" was used on COBAS e801 (the Roche) immunoassay analysers to measure sex hormone levels in serum samples. The following assays were performed: Elecsys Estradiol III, the Elecsys Testosterone II, the Elecsys Progesterone III, the Elecsys Prolactin II, the Elecsys SHBG, the Elecsys FSH and the Elecsys luteinizing hormone (LH) to detect levels of E2, testosterone, progesterone, prolactin, SHBG, FSH, and LH, respectively. The frequency of missing values is specified in

Table 1
Clinical and sociodemographic characteristics in males and females separately.

Sex		Controls	Low-CRP MDD	High-CRP MDD	Statistics
Males	Number of individuals	$n = 25$	$n = 53$	$n = 19$	/
	BMI (kg/m), mean (SD)	n = 25, 25.95 (3.71)	n = 53, 25.71 (3.80)	n = 19, 30.32 (5.88)	**F = 8.726 $p < 0.001$[a]**
	Age (years), mean (SD)	n = 25, 33.36 (6.93)	n = 53, 36.81 (8.01)	n = 19, 37.32 (7.67)	F = 2.050 $p = 0.134$
	Ethnicity (n) (%)	n = 25, White 21 (84.0 %)	n = 53, White 50 (94.3 %)	n = 19, White 18 (94.7 %)	$\chi^2 = 2.678$ $p = 0.262$
	Antidepressant use (%)	n = 25, 0 (0 %)	n = 53, 37 (69.8 %)	n = 19, 12 (63.2 %)	**$\chi^2 = 34.630$ $p < 0.001$**
	HAMD-17 mean (SD)	n = 25, 0.84 (1.34)	n = 53, 16.79 (8.28)	n = 19, 16.89 (7.59)	**F = 47.957 p < 0.001[b]**
	Serum hs-CRP (mg/L)	n = 25, 0.91 (0.64)	n = 53, 0.94 (0.71)	n = 19, 5.84 (3.17)	**F = 64.710 p < 0.001[a]**
	Serum IL-6 (pg/mL)	$n = 15$, 0.55 (0.34)	$n = 33$, 1.68 (5.98)	$n = 13$, 2.10 (2.78)	**F = 5.446 p = 0.007 [a]**
Females	Number of individuals	$n = 39$	$n = 72$	$n = 34$	/
	BMI (kg/m), mean (SD)	$n = 38$, 23.31 (4.69)	n = 72, 25.63 (5.07)	$n = 32$, 30.83 (5.53)	**F = 19.12 p < 0.001[a]**
	Age (years), mean (SD)	n = 39, 35.21 (7.55)	n = 72, 33.71 (7.17)	n = 34, 35.21 (7.12)	F = 0.770 $p = 0.465$
	Pre-ovulatory/ovulatory (%)	n = 39, 71.8 %	$n = 71$, 67.6 %	n = 32, 87.5 %	$\chi^2 = 4.512$ $p = 0.105$
	Ethnicity (n) (%)	n = 39, White 28 (71.8 %)	n = 72, White 60 (83.3 %)	n = 34, White 30 (88.2 %)	$\chi^2 = 3.600$ $p = 0.165$
	Antidepressant use (%)	n = 39, 0 (0 %)	n = 72, 51 (70.8 %)	n = 34, 24 (70.6 %)	**$\chi^2 = 57.160$ p < 0.001**
	HAMD-17 mean (SD)	n = 39, 0.67 (1.11)	n = 72, 16.15 (5.75)	n = 34, 16.71 (6.96)	**F = 124.377 p < 0.001 [b]**
	Serum hs-CRP (mg/L)	n = 39, 1.16 (1.53)	n = 72, 0.98 (0.75)	n = 34, 7.51 (6.46)	**F = 91.969 p < 0.001[a]**
	Serum IL-6 (pg/mL)	$n = 24$, 0.61 (0.23)	$n = 58$, 1.30 (4.86)	$n = 21$, 0.94 (0.51)	F = 1.637 p = 0.200

Note: BMI (body mass index); CRP (C-reactive protein); HAMD-17 (Hamilton Rating Scale for Depression 17-item); hs- (high sensitivity); IL (interleukin); SD (standard deviation). Significant tests ($p < 0.05$) are in bold. In post-hoc analyses with Bonferroni correction we reported only statistically significant differences $p < 0.05$; with "a" indicating a difference at statistical significance between the high-CRP group vs. others; and with "b" indicating a difference at statistical significance between Controls vs. others.

Table 2
Differences in sex hormone levels between controls and patients in the male group.

Male group			
	Controls	MDD	Statistics
T (nmol/L) mean (SD)	20.12 (5.74)	17.01 (5.50)	**$t = 2.242$ p = 0.027**
E2 (pmol/L) mean (SD)	102.18 (55.56)	82.99 (54.17)	$t = 1.458$ $p = 0.152$
FSH (IU/L) mean (SD)	3.77 (1.81)	4.79 (4.09)	$t = 1.139$ $p = 0.258$
LH (IU/L) mean (SD)	5.47 (2.63)	5.63 (2.50)	$t = 0.323$ $p = 0.747$
SHBG (nmol/L) mean (SD)	31.34 (11.40)	31.65 (13.76)	$t = 0.121$ $p = 0.904$
P4 (nmol/L) mean (SD)	0.49 (0.39)	0.40 (0.44)	$t = 1.294$ $p = 0.199$
PRL (μIU/L) mean (SD)	239.61 (94.03)	221.72 (118.76)	$t = 1.074$ $p = 0.286$
T/E2 mean (SD)	0.033 (0.034)	0.040 (0.035)	$t = 0.838$ $p = 0.407$
FAI mean (SD)	0.71 (0.31)	0.59 (0.22)	*$t = 1.945$ $p = 0.055$*
FEI mean (SD)	4.01 (2.64)	3.20 (2.75)	$t = 1.334$ $p = 0.186$
TSI mean (SD)	4.10 (1.46)	3.39 (1.46)	**$t = 2.203$ p = 0.030**

*Note: E2 (17-β-estradiol); FAI (Free androgen index); FEI (Free estradiol index); FSH (follicle stimulating hormone); LH (Luteinizing hormone); P4 (progesterone); PRL (prolactin); SHBG (sex hormone binding globulin); T (testosterone); T/E2 (testosterone to estradiol ratio); TSI (testosterone secretion index). * Significant tests ($p < 0.05$) are in bold.*

Supplementary Table S1 in the Supplementary Material. Values below the lowest Limit of Quantitation (LoQ) were substituted with the Limit of Detection (LoD) values specific for each sex hormone.

Bioactive sex steroids are those which are not bound to specific proteins such as SHBG (i.e., free androgen index (FAI) and free estradiol index (FEI) as they are capable of binding to their associated receptors (Krakowsky and Grober, 2015). To determine the bioavailable ratios of testosterone and E2, we calculated the FAI as the molar ratio of total testosterone/SHBG, and the FEI as the molar ratio of estradiol/SHBG (Rexrode et al., 2003). We calculated the testosterone to E2 ratio (T/E2) using the following formula: testosterone/(10*E2) (van Koeverden et al., 2019), and the testosterone secreting index (TSI) as testosterone (nmol/L)/LH (IU/L) (Chen et al., 2020).

2.4. Menstrual cycle

We examined the ovulatory phase in the female sample using the progesterone threshold of 5 ng/mL, as pre-ovulatory/ovulatory (progesterone ≤5 ng/mL) and post-ovulatory (progesterone >5 ng/mL). Leiva and colleagues used this cut-off and could predict that ovulation had occurred with a specificity of 98.4 % (Leiva et al., 2015).

2.5. Statistical analyses

All the statistical analyses were performed within the sexes. The data were graphically assessed for normality (Q-Q plots) and analysed as appropriate. We applied the natural logarithmic transformation to normalise sex hormones, hs-CRP and IL-6 values. Chi-square tests and one-way ANOVA were used to identify significant differences in the demographic variables between study groups. These comprised independent samples *t*-test and one-way ANOVA with post-hoc comparisons with Bonferroni correction to investigate differences in sex hormone between controls and MDD patients, overall and stratified according to CRP threshold. In addition, we used the Benjamini-Hochberg False Discovery Rate (FDR) approach ($p < 0.05$) to control for multiple comparisons for sex hormone measures in the independent samples t-test and in the ANOVA analyses (Benjamini and Hochberg, 1995).

We performed Pearson's bivariate correlational analyses to identify relevant correlations between serum hs-CRP, sex hormones, and sociodemographic data (i.e., BMI, age, and ethnicity). We then conducted ANCOVA analyses to investigate the effect of study groups (controls, low- and high-CRP MDD) in modulating levels of relevant sex hormones while controlling for the effects of ovulatory phases (pre-ovulatory/ovulatory and post-ovulatory) and the relevant sociodemographic data associated with sex hormone measures, i.e., BMI and age. Finally, we

Table 3
Differences in sex hormone levels between controls and patients in the female group.

Female group			
	Controls	Patients	Statistics
T (nmol/L) mean (SD)	1.01 (0.54)	1.05 (0.53)	$t = 0.389$ $p = 0.691$
E2 (pmol/L) mean (SD)	523.88 (381.56)	458.27 (333.26)	$t = 1.091$ $p = 0.277$
FSH (IU/L) mean (SD)	5.69 (3.46)	5.88 (3.45)	$t = 0.357$ $p = 0.721$
LH (IU/L) mean (SD)	9.56 (11.74)	8.38 (6.43)	$t = 0.085$ $p = 0.932$
SHBG (nmol/L) mean (SD)	60.47 (22.17)	57.84 (33.49)	$t = 1.245$ $p = 0.215$
P4 (nmol/L) mean (SD)	12.16 (16.52)	11.62 (17.77)	$t = 1.057$ $p = 0.292$
PRL (μIU/L) mean (SD)	343.85 (171.10)	345.47 (212.08)	$t = 0.381$ $p = 0.704$
T/E2 mean (SD)	0.00042 (0.00086)	0.00055 (0.00100)	$t = 1.279$ $p = 0.203$
FAI mean (SD)	0.0200 (0.0143)	0.0230 (0.0189)	$t = 0.995$ $p = 0.322$
FEI mean (SD)	9.79 (6.66)	9.88 (8.87)	$t = 0.733$ p = 0.465
TSI mean (SD)	0.191 (0.293)	0.185 (0.218)	$t = 0.314$ $p = 0.754$

Note: E2 (17-β-estradiol); FAI (Free androgen index); FEI (Free estradiol index); FSH (follicle stimulating hormone); LH (Luteinizing hormone); P4 (progesterone); PRL (prolactin); SHBG (sex hormone binding globulin); T (testosterone); T/E2 (testosterone to estradiol ratio); TSI (testosterone secretion index).

Table 4
Differences in sex hormone levels between controls, low-CRP patients and high-CRP patients in the male group.

Male group				
	Controls	Low-CRP MDD	High-CRP MDD	Statistics
T (nmol/L) mean (SD)	20.12 (5.74)	17.92 (5.19)	14.34 (5.67)	**$F = 6.96$ $p = 0.002$** [a]
E2 (pmol/L) mean (SD)	102.18 (55.56)	73.62 (52.08)	109.14 (52.53)	**$F = 3.47$ $p = 0.035$** [b]
FSH (IU/L) mean (SD)	3.77 (1.81)	5.29 (4.61)	3.41 (1.28)	*$F = 2.81$ $p = 0.065$*
LH (IU/L) mean (SD)	5.47 (2.63)	5.86 (2.67)	4.97 (1.88)	$F = 0.94$ $p = 0.394$
SHBG (nmol/L) mean (SD)	31.34 (11.40)	32.60 (13.45)	28.85 (14.69)	$F = 0.86$ $p = 0.425$
P4 (nmol/L) mean (SD)	0.487 (0.388)	0.460 (0.486)	0.237 (0.239)	*$F = 2.81$ $p = 0.066$*
PRL (μIU/L) mean (SD)	239.61 (94.03)	225.19 (129.52)	211.50 (81.32)	$F = 0.573$ $p = 0.566$
T/E2 mean (SD)	0.033 (0.034)	0.048 (0.038)	0.018 (0.016)	**$F = 8.52$ $p < 0.001$** [c]
FAI mean (SD)	0.71 (0.31)	0.60 (0.20)	0.56 (0.27)	*$F = 2.60$ $p = 0.080$*
FEI mean (SD)	4.01 (2.64)	2.70(2.51)	4.65 (3.00)	**$F = 5.12$ $p = 0.008$** [c]
TSI mean (SD)	4.09 (1.46)	3.51 (1.51)	3.04 (1.22)	*$F = 2.96$ $p = 0.057$*

*Note: E2 (17-β-estradiol); FAI (Free androgen index); FEI (Free estradiol index); FSH (follicle stimulating hormone); LH (Luteinizing hormone); P4 (progesterone); PRL (prolactin); SHBG (sex hormone binding globulin); T (testosterone); T/E2 (testosterone to estradiol ratio); TSI (testosterone secretion index). * Significant tests ($p < 0.05$) are in bold. In post-hoc analyses with Bonferroni correction we reported only statistically significant differences $p < 0.05$; with "a" indicating a difference at statistical significance between high-CRP group vs. others; with "b" indicating a difference at statistical trend between high-CRP group and low-CRP group; with "c" indicating a difference at statistical significance between high-CRP group vs. low-CRP group.*

performed linear regression analyses to test whether the sex hormones correlating with hs-CRP (including predictors with $p < 0.1$) as well as BMI and age significantly predicted peripheral inflammation in male and female patients. All statistical analyses were performed using IBM SPSS Statistics Version 28 (IBM Ltd., UK).

3. Results

3.1. Sex hormones in MDD patients

3.1.1. MDD males show significantly lower levels of testosterone and lower TSI compared with controls

In the male group, patients had lower levels of testosterone ($p = 0.027$) and TSI levels ($p = 0.030$), than healthy controls, and FAI levels showed a nonsignificant trend ($p = 0.055$) toward being lower in patients compared with controls. We did not identify other differences. Results are displayed in Table 2. The differences in testosterone and TSI did not survive the additional control with the Benjamini-Hochberg FDR adjusted *p*-value.

3.1.2. MDD females do not show significant differences in sex hormone levels compared with controls

In the female group, we did not detect significant differences in sex hormone levels between patients and controls. Results are displayed in Table 3. We did not find any difference in ovulatory phase between female patients (73.8 % pre-ovulatory/ovulatory phase, 26.2 % post-ovulatory phase) and controls (71.8 % pre-ovulatory/ovulatory phase, 28.2 % post-ovulatory phase; $\chi^2 = 0.057$ $p = 0.811$).

3.2. Sex hormones in MDD individuals with high-CRP

3.2.1. In males, high-CRP MDD individuals have significantly lower levels of testosterone compared with both controls and low-CRP MDD individuals

ANOVA analyses showed that testosterone ($p < 0.005$), E2 ($p < 0.05$), T/E2 ($p < 0.001$), and FEI ($p < 0.010$) significantly differed between study groups (controls, low-CRP patients, and high-CRP patients) (see Table 4). The post-hoc analyses with Bonferroni correction showed that high-CRP patients had lower testosterone than controls ($p = 0.001$), and lower testosterone ($p = 0.013$) and T/E2 ratio ($p < 0.001$) and higher FEI levels than low-CRP patients ($p = 0.015$). The differences in testosterone, T/E2 ratio, and FEI levels between groups survived the additional control with the Benjamini-Hochberg adjusted FDR *p*-value.

As expected, the high-CRP group had higher BMI than controls and the low-CRP groups; and given the correlations between sex hormones, BMI, and age in males (see Supplementary Material Table S4), we performed ANCOVA analysis adjusting for BMI and age to explore whether these differences remain significant. In BMI-adjusted analyses, the difference in T/E2 ratio remained significant ($F = 4.343$ $p = 0.016$), with post-hoc analyses showing a significant difference between low-CRP and high-CRP groups ($p = 0.034$), while the other differences maintained the same direction even though they only reached statistical trend (testosterone, $F = 2.570$ $p = 0.082$; FEI, $F = 3.071$ $p = 0.051$). In age-adjusted analyses, the differences between groups in FEI remained significant ($F = 5.228$ $p = 0.007$).

Table 5
Differences in sex hormone levels between controls, low-CRP patients and high-CRP patients in the female group.

Female group				
	Controls	Low-CRP MDD	High-CRP MDD	Statistics
T (nmol/L) mean (SD)	1.01 (0.54)	1.10 (0.51)	0.96 (0.55)	F = 1.20 p = 0.30
E2 (pmol/L) mean (SD)	523.88 (381.56)	465.92 (313.24)	441.07 (379.28)	F = 0.82 p = 0.44
FSH (IU/L) mean (SD)	5.69 (3.46)	5.86 (4.51)	5.92 (3.35)	F = 0.07 p = 0.93
LH (IU/L) mean (SD)	9.56 (11.74)	8.29 (6.85)	8.57 (5.46)	F = 0.004 p = 1.00
SHBG (nmol/L) mean (SD)	60.47 (22.17)	61.98 (33.23)	48.77 (32.77)	**F = 4.11 p = 0.02**[a]
P4 (nmol/L) mean (SD)	12.16 (16.52)	13.54 (19.17)	7.34 (13.48)	F = 1.13 p = 0.33
PRL (μIU/L) mean (SD)	343.85 (171.10)	342.20 (221.40)	352.72 (192.94)	F = 0.22 p = 0.81
T/E2 mean (SD)	0.0004 (0.0009)	0.0005 (0.0010)	0.0006 (0.0011)	F = 0.90 p = 0.41
FAI mean (SD)	0.0200 (0.0143)	0.0216 (0.0169)	0.0263 (0.0227)	F = 0.50 p = 0.61
FEI mean (SD)	9.79 (6.66)	9.39 (9.01)	10.98 (8.58)	F = 0.32 p = 0.73
TSI mean (SD)	0.191 (0.293)	0.186 (0.156)	0.183 (0.321)	F = 0.01 p = 0.99

*Note: E2 (17-β-estradiol); FAI (Free androgen index); FEI (Free estradiol index); FSH (follicle stimulating hormone); LH (Luteinizing hormone); P4 (progesterone); PRL (prolactin); SHBG (sex hormone binding globulin); T (testosterone); T/E2 (testosterone to estradiol ratio); TSI (testosterone secretion index). * Significant tests ($p < 0.05$) are in bold. In post-hoc analyses with Bonferroni correction we reported only statistically significant differences $p < 0.05$; with "a" indicating a difference at statistical significance between high-CRP group vs. others.*

3.2.2. In females, high-CRP patients have significantly lower levels of SHBG compared with both controls and low-CRP MDD individuals

ANOVA analyses showed that only SHBG levels were different between groups (controls, low-CRP patients, and high-CRP patients) (p = 0.02; see Table 5). The post-hoc analyses with Bonferroni correction showed that high-CRP patients had lower SHBG levels than both healthy controls ($p = 0.033$) and low-CRP patients ($p = 0.034$). The difference in SHBG levels between groups did not survive the additional control with the Benjamini-Hochberg FDR adjusted p-value.

As mentioned above (Table 1), there was no difference in the ovulatory phase between the high−/low-CRP groups and control; nevertheless, we conducted secondary ANCOVA analyses co-variating for the ovulatory phase, and all differences remained in the same direction but were no longer significant (F = 3.043, high-CRP group vs. controls $p = 0.075$; vs. low-CRP group $p = 0.092$). Again, given the correlations between sex hormones and BMI in females (see Supplementary Material Table S5), we performed BMI-adjusted analyses. The difference between groups in SHBG was no longer significant after this adjustment (F = 0.441 $p = 0.644$).

3.3. Correlations between peripheral inflammation and sex hormones

3.3.1. Testosterone predicts hs-CRP concentration in MDD males

In male depressed individuals, but not in controls, hs-CRP was negatively correlated with testosterone ($r = -0.328$ $p = 0.005$), progesterone ($r = -0.287$ p = 0.017), and T/E2 ($r = -0.282$ $p = 0.017$), and, with at statistical trend, with SHBG ($r = -0.223$ $p = 0.062$), LH ($r = -0.219$ p = 0.065), and (positively) with FEI ($r = 0.225$ $p = 0.060$). See Supplementary Table S2 in the Supplementary Material.

We further investigated these correlations by performing linear regression analysis to test whether testosterone, progesterone, T/E2, SHBG, LH, and FEI were able to predict hs-CRP levels in MDD male patients. The overall linear regression model was significant ($R^2 = 0.252$ F (5, 62) = 4.175 $p = 0.002$), with testosterone (β = −1.069 $p = 0.033$), significantly predicting hs-CRP (and LH at statistical trend, β = −0.544 $p = 0.094$). In adjusted analysis by covarying for BMI and age, the linear regression model including these variables was also significant ($R^2 = 0.369$ F (7, 60) = 5.005 $p < 0.001$) with only BMI significantly predicting hs-CRP in male patients (β = 0.090 $p = 0.003$).

3.3.2. SHBG predicts hs-CRP concentration in MDD females

In female depressed patients, hs-CRP was negatively correlated with testosterone ($r = -0.285$ p = 0.003), SHBG ($r = -0.292$ p = 0.003), TSI ($r = -0.225$ $p = 0.023$), and with a statistical trend with E2 ($r = -0.177$ $p = 0.072$), and progesterone ($r = -0.192$ $p = 0.052$). See Supplementary Table S3 in the Supplementary Material.

Again, we investigated the correlations by performing linear regression analysis to test whether testosterone, SHBG, TSI, E2, and progesterone significantly predicted hs-CRP levels in MDD female patients. The overall linear regression model was statistically significant ($R^2 = 0.172$ F (5, 92) = 3.819 p = 0.003), and SHBG alone (β = −0.628 $p = 0.009$) significantly predicted hs-CRP levels. In adjusted analysis by covarying for BMI and age, the linear regression model including these variables was significant ($R^2 = 0.519$ F (7, 89) = 13.708 p < 0.001), with E2 (β = −0.223 $p = 0.043$) and BMI (β = 0.144 p < 0.001) significantly predicting hs-CRP levels in female patients.

3.4. Sex differences in the association between hs-CRP and IL-6

We recently highlighted a significant correlation between hs-CRP and IL-6 in female but not in male patients with treatment-resistant depression who were currently receiving antidepressants (Lombardo et al., 2022), and we wanted to replicate this association also in the current study. Details of the analyses are presented in Supplementary Material (Paragraph S1), and we did replicate here that female but not male MDD individuals on antidepressants showed a significant correlation between hs-CRP and IL-6.

4. Discussion

To our knowledge, this is the first study investigating endogenous sex hormones in MDD patients stratified based on inflammatory levels, as measured by serum CRP. The present study suggests a possible interaction between sex hormones and inflammation in MDD patients. Specifically, we found that low levels of testosterone and SHBG are present in, respectively, male and female patients with depression and low-grade inflammation, possibly because of an anti-inflammatory role of these two hormonal biomarkers.

There is evidence of low testosterone in MDD (Zito et al., 2023); our findings of low testosterone and TSI in male depressed patients, irrespective of inflammation are consistent with Wainberg et al. (2021) which showed, in the UK Biobank, that low testosterone levels in males predict a higher 5-year incidence of MDD (Wainberg et al., 2021). The relationship between a lack of androgens and depression has been shown also in medical conditions such as males with hypogonadism (characterised by low levels of testosterone) who exhibit comorbidity with depressive symptoms (Hauger et al., 2022).

Interestingly, low testosterone concentrations predict high hs-CRP levels in male MDD patients in our study. Again, this is consistent with previous evidence in healthy males that those with low testosterone levels (below 11.44 nmol/L) are more likely to have CRP levels equal to or above 3 mg/L than males with normal/high testosterone (Tsilidis

et al., 2013), suggesting a protective role of testosterone against clinically meaningful inflammation. In our study, male MDD patients with hs-CRP above 3 mg/L have testosterone ~14 nmol/L, and similar levels of testosterone levels (<15 nmol/L) are associated with increased prevalence of specific symptoms, such as loss of libido, in older male depressed patients (Zitzmann et al., 2006). Indeed, high and low levels of this hormone can be associated with particular symptom profiles; the National Health and Nutrition Examination Studies (NHANES) found that decreased testosterone is associated with appetite problems (poor appetite or overeating as assessed by the Patient Health Questionnaire (PHQ-9)), and high levels of this hormone are associated with sleep problems and fatigue (Määttänen et al., 2021). Hence, it is possible that testosterone interacts with inflammation in producing different profiles of somatic symptoms.

Our data also suggest a relevant role of the combined action of testosterone and estradiol in male, inflamed depressed patients. In our study, a decreased T/E2 ratio in high-CRP patients was due to both low testosterone and increased E2, suggesting that elevated estrogen levels may have a pro-inflammatory effect associated with the absence of the testosterone-mediated anti-inflammatory action, as also shown in individuals with rheumatoid arthritis (RA) (Straub, 2007). Notably, the differences in testosterone and T/E2 ratio (and FEI) between the three study groups survived the adjusted FDR *p*-value, and the differences in T/E2 ratio and testosterone levels remained substantially unchanged even when controlling for BMI.

We also demonstrate that high-CRP female patients exhibited lowered SHBG levels. Despite the absence of evidence linking reduced sex hormone-binding globulin (SHBG) levels to depressive symptoms in women (Asselmann et al., 2019), our findings are consistent with the existing literature that SHBG possesses an anti-inflammatory function. In fact, SHBG determines the bioavailability of gonadal hormones, as testosterone and E2 are biologically inactive when bound to SHBG (Westphal, 1971). In an in-vitro study, SHBG inhibited inflammatory biomarkers mRNA expression, such as TNFα and IL-6 in macrophages and adipocytes (Yamazaki et al., 2018). This protective action of SHBG has been seen also in human studies, where serum SHBG negatively correlates with serum CRP in both sexes in the general population (Liao et al., 2012), and in post-menopausal women (Joffe et al., 2006). It is important to highlight, however, that this finding does not survive adjustment for multiple testing and also for BMI, and thus should be considered a preliminary finding requiring further investigation. Interestingly, the linear regression shows a relationship between low E2 levels and increased hs-CRP levels in MDD female individuals. These findings might confirm the previous evidence of pro-inflammatory proprieties of low estrogenic activity in premenopausal women in the general population, as suggested by Park and Lee (2020) and previously mentioned in the introduction.

As we expected, in both sexes high-CRP patients show higher BMI compared with both controls and low-CRP patients. This observation suggests a potential mechanism that may account for the variations in sex hormone levels between these groups, as adipose tissue could play a role in influencing both sex hormone levels and peripheral inflammation. For example, Grandys et al. (2021) reported that the negative correlation between low levels of testosterone and CRP is influenced by BMI (Grandys et al., 2021). Fat cells are also an important source of E2 in men, thus the increased E2 levels (and lowered T/E2 ratio) in the high CRP male patients could be due to the increased BMI causing both biological changes (inflammation and increased E2), although it is of note that the directions of the differences in hormones do not change in males by adjusting for BMI levels in the statistical analysis. In another study, increased BMI is associated with decreased testosterone and decreased SHBG levels (the same finding of our study) (Stárka et al., 2020). Indeed, contrary to the effects in males, the association between increased inflammation and low SHGB in females disappears when BMI is included in the statistical analysis, indicating that adiposity plays a particular role in this association. This notion is supported by studies showing that low SHGB is driven by hyperinsulinemia and decreased cortisol secretion associated with abdominal obesity (Hautanen, 2000). Evaluating visceral fat in males and subcutaneous fat in females may yield more informative results in terms of understanding the biological mechanisms underpinning the relationship between inflammation and sex differences, as also suggested by Gavin and Bessesen (2020).

We detect sex differences in the correlation between hs-CRP and IL-6. In the patient group taking antidepressants, only female participants showed a significant correlation between these two immune biomarkers, as we did not observe this association in male participants. These results confirm our previous findings of a significant correlation between hs-CRP and IL-6 in females but not in male patients with treatment-resistant depression who were all receiving antidepressant pharmacotherapies (Lombardo et al., 2022). However, here we also found the opposite scenario in patients not taking antidepressants, with a significant correlation in males but not in females. Due to the limited available evidence on sex differences in the relationship between these inflammatory biomarkers, further studies with larger sample sizes are warranted to investigate this sex-specific association. Even though previous studies supported a correlation between IL-6 and CRP (Panichi et al., 2002; Kamińska et al., 2023), our findings suggest that the use of antidepressants may impact this association. Antidepressants show anti-inflammatory properties (Hashioka et al., 2009). However, the efficacy of antidepressants exhibits sex differences, possibly due to variations in pharmacokinetics/pharmacodynamics, such as gastric emptying, rates of liver metabolism, and interactions between estrogen and serotonin systems (Sramek et al., 2016). Meta-analytical evidence highlighted decreased levels of inflammatory biomarkers (including IL-6) after antidepressant treatments (Köhler et al., 2018), however, the authors did not perform within-sexes analyses. Nevertheless, Mosiołek detected inconsistencies and equivocal results in the anti-inflammatory action of antidepressants across studies (Mosiołek et al., 2021), and it is tempting to speculate that part of these inconsistencies might derive from sex differences in the immunomodulatory proprieties of antidepressants.

From a translational point of view, restoring testosterone levels and its equilibrium with estradiol to their normal levels may exert protective effects against inflammation, and could be tested as a future add-on hormonal-based treatment in depression. Several studies have assessed adding testosterone in depressed patients, and indeed meta-analytical evidence reports an antidepressant effect of exogenous testosterone vs. placebo in males (Zarrouf et al., 2009). As highlighted by Nead, add-on testosterone therapy might be beneficial in ameliorating depressive symptoms in males with low levels of sex hormones (Nead, 2019). However, it is important to understand whether the clinical benefit of testosterone might be due to anti-inflammatory action and thus would particularly benefit those male patients with high levels of inflammation. Furthermore, a balanced T/E2 ratio may exert an antidepressant effect through its role in increasing neuroplasticity, stimulating the serotonin system, and inhibiting the hypothalamic-pituitary-adrenal axis and immune system (Fabian et al., 2023; Morssinkhof et al., 2020).

The main limitation of our study is the availability of one-time point single blood samples, as the secretion of sex hormones varies throughout the day. Nevertheless, testosterone and progesterone peak in the morning (Brambilla et al., 2009; Rezanezhad et al., 2018; Rahman et al., 2019), thus, the standardised, fasting morning collection in our study has reduced the variability introduced by this limitation. Second, given the nature of this cross-sectional study, we were not able to assess the causality of the relationship between hs-CRP and sex hormones on depression severity or risk. Indeed, reverse causality can also explain our findings; for example, a pro-inflammatory environment can increase the aromatase activity (of cytochrome P450) and thus can promote the conversion of testosterone to E2 (Capellino et al., 2014). Longitudinal or mechanistic studies are needed to unequivocally dissect the causal relationship between inflammation and sex hormone changes. Third, the results for the female sample are limited by the lack of information about

specific subphases of the menstrual cycle, and about mood alterations associated with the menstrual cycle, which have been associated with disturbances in sex hormone levels and increased sensitivity to fluctuation of gonadal hormones (Thys-Jacobs et al., 2008; Kuehner and Nayman, 2021), although reassuringly there was no difference between groups in the ovulatory phase. Finally, the multiple testing of this study may have limited the generalisability of our findings, in particular in females. In fact, the difference in SHBG concentrations between controls, low-CRP and high-CRP groups did not survive the Benjamini-Hochberg FDR approach. Notably, our findings in males survived the FDR correction.

In conclusion, our study highlights an imbalance of sex hormones in MDD patients with at least low-grade inflammation (low T and T/E2 in MDD male patients, and low SHBG in MDD female patients). Our study may guide future research investigating interactions between sex hormones and immune function in the risk and severity of depression, and, if replicated in larger sample sizes and in longitudinal studies, in developing tailored add-on hormonal-based treatments for MDD patients with depression and low-grade inflammation.

CRediT authorship contribution statement

Giulia Lombardo: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. **Valeria Mondelli:** Conceptualization, Supervision, Writing – review & editing. **Courtney Worrell:** Conceptualization, Investigation, Writing – review & editing. **Luca Sforzini:** Conceptualization, Investigation, Writing – review & editing. **Nicole Mariani:** Conceptualization, Investigation, Writing – review & editing. **Naghmeh Nikkheslat:** Conceptualization, Investigation, Writing – review & editing. **Maria A. Nettis:** Conceptualization, Investigation, Writing – review & editing. **Melisa Kose:** Conceptualization, Investigation, Writing – review & editing. **Zuzanna Zajkowska:** Conceptualization, Investigation, Writing – review & editing. **Annamaria Cattaneo:** Conceptualization, Investigation, Writing – review & editing. **Linda Pointon:** Conceptualization, Supervision, Writing – review & editing. **Lorinda Turner:** Conceptualization, Supervision, Writing – review & editing. **Philip J. Cowen:** Conceptualization, Supervision, Writing – review & editing. **Wayne C. Drevets:** Conceptualization, Supervision, Writing – review & editing. **Jonathan Cavanagh:** Conceptualization, Supervision, Writing – review & editing. **Neil A. Harrison:** Conceptualization, Supervision, Writing – review & editing. **Edward T. Bullmore:** Conceptualization, Supervision, Writing – review & editing. **Paola Dazzan:** Conceptualization, Supervision, Writing – review & editing. **Carmine M. Pariante:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known conflict of interest that could have appeared to influence the work reported in this paper. Dr. Lombardo, Dr. Sforzini, Ms. Worrell, Ms. Kose and Prof. Pariante have received research funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 853966–2, as part of the EU-PEARL project. This Joint Undertaking received support from the European Union's Horizon 2020 research and innovation programme and EFPIA. Prof. Pariante is also funded by a Senior Investigator award from the National Institute for Health Research (NIHR); the Medical Research Council (grants MR/L014815/1, MR/J002739/1 and MR/N029488/1); the European Commission (EARLYCAUSE grant SC1-BHC-01-2019); the NARSAD; the Psychiatry Research Trust; and the Wellcome Trust (SHAPER, Scaling-up Health-Arts Programme to scale up arts interventions, grant 219,425/Z/19/Z). <10 % of his support in the last 10 years derives from commercial collaborations, including consultation and speakers fees from Boehringer Ingelheim, Eli Lilly, Compass, Eleusis, GH Research, Lundbeck, and Värde Partners. Prof. Mondelli is also funded by MQ: Transforming Mental Health (Grant: MQBF/1 and MQBF/4), by the National Institute for Health and Care Research (NIHR) Maudsley Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King's College London, and the Medical Research Foundation (Grant: MRF-160-0005-ELP-MONDE). Prof. Dazzan has received speaker's fees from Lundbeck and Janssen and is supported by the Medical Research Council (MR/S003444/1). Dr. Nettis has received an honorarium for speaking for Janssen on one occasion.

Acknowledgements

This work was funded by the Psychiatry Research Trust through funding collected in memory of Claire Nacamuli. This research was funded by the Wellcome Trust strategy award to the Neuroimmunology of Mood Disorders and Alzheimer's Disease (NIMA) Consortium [104025/Z/14/Z], which is also funded by Janssen, GlaxoSmithKline, Lundbeck and Pfizer. This work was also supported by the National Institute for Health Research (NIHR) Biomedical Research Centre (BRC) at the South London and Maudsley NHS Foundation Trust and King's College London, the NIHR Cambridge Biomedical Research Centre (Mental Health) and the Cambridge NIHR BRC Cell Phenotyping Hub. All visits took place at the Clinical Research Facility of King's College Hospital. The team of nurses has to be thanked for providing their valuable expertise to the study, and we thank all the participants who have taken part in this study. We would like to gratefully thank all study participants, research teams and laboratory staff, without whom this research would not have been possible. We thank and acknowledge all members of the NIMA Consortium at the time of data collection (see Supplementary Material). The views expressed are those of the authors and not necessarily those of the Nacamuli family and the Psychiatry Research Trust, the NHS, the NIHR, or the Department of Health and Social Care.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jad.2024.03.018.

References

Aruldass, A.R., Kitzbichler, M.G., Morgan, S.E., Lim, S., Lynall, M.E., Turner, L., Vertes, P., Wellcome Trust Consortium for Neuroimmunology of Mood Disorders and Alzheimer's Disease (NIMA), Cavanagh, J., Cowen, P., Pariante, C.M., Harrison, N. A., Bullmore, E.T., 2021. Dysconnectivity of a brain functional network was associated with blood inflammatory markers in depression. Brain Behav. Immun. 98, 299–309. https://doi.org/10.1016/j.bbi.2021.08.226.

Asselmann, E., Kische, H., Haring, R., Hertel, J., Schmidt, C.O., Nauck, M., Beesdo-Baum, K., Grabe, H.J., Pané-Farré, C.A., 2019. Prospective associations of androgens and sex hormone-binding globulin with 12-month, lifetime and incident anxiety and depressive disorders in men and women from the general population. J. Affect. Disord. 245, 905–911. https://doi.org/10.1016/j.jad.2018.11.052.

Beagley, K.W., Gockel, C.M., 2003. Regulation of innate and adaptive immunity by the female sex hormones oestradiol and progesterone. FEMS Immunol. Med. Microbiol. 38 (1), 13–22. https://doi.org/10.1016/S0928-8244(03)00202-5.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. B. Methodol. 57 (1), 289–300.

Brambilla, D.J., Matsumoto, A.M., Araujo, A.B., McKinlay, J.B., 2009. The effect of diurnal variation on clinical measurement of serum testosterone and other sex hormone levels in men. J. Clin. Endocrinol. Metab. 94 (3), 907–913. https://doi.org/10.1210/jc.2008-1902.

Capellino, S., Straub, R.H., Cutolo, M., 2014. Aromatase and regulation of the estrogen-to-androgen ratio in synovial tissue inflammation: common pathway in both sexes. Ann. N. Y. Acad. Sci. 1317, 24–31. https://doi.org/10.1111/nyas.12398.

Cattaneo, A., Ferrari, C., Turner, L., Mariani, N., Enache, D., Hastings, C., Kose, M., Lombardo, G., McLaughlin, A.P., Nettis, M.A., Nikkheslat, N., Sforzini, L., Worrell, C., Zajkowska, Z., Cattane, N., Lopizzo, N., Mazzelli, M., Pointon, L., Cowen, P.J., Cavanagh, J., Pariante, C.M., 2020. Whole-blood expression of inflammasome- and glucocorticoid-related mRNAs correctly separates treatment-resistant depressed patients from drug-free and responsive patients in the BIODEP study. Transl. Psychiatry 10 (1), 232. https://doi.org/10.1038/s41398-020-00874-7.

Chamberlain, S.R., Cavanagh, J., de Boer, P., Mondelli, V., Jones, D., Drevets, W.C., Cowen, P.J., Harrison, N.A., Pointon, L., Pariante, C.M., Bullmore, E.T., 2019. Treatment-resistant depression and peripheral C-reactive protein. Br. J. Psychiatry J. Ment. Sci. 214 (1), 11–19. https://doi.org/10.1192/bjp.2018.66.

Chen, Z., Shen, X., Tian, K., Liu, Y., Xiong, S., Yu, Q., Dai, L., Shi, Y., Zhang, R., Zeng, R., Wan, Q., Xiong, C., Zhou, Y., 2020. Bioavailable testosterone is associated with symptoms of depression in adult men. J. Int. Med. Res. 48 (8), 300060520941715 https://doi.org/10.1177/0300060520941715.

Chin Fatt, C.R., Mayes, T.L., Trivedi, M.H., 2023. Immune dysregulation in treatment-resistant depression: precision approaches to treatment selection and development of novel treatments. Psychiatr. Clin. North Am. 46 (2), 403–413. https://doi.org/10.1016/j.psc.2023.02.010.

Fabian, C.B., Seney, M.L., Joffe, M.E., 2023. Sex differences and hormonal regulation of metabotropic glutamate receptor synaptic plasticity. Int. Rev. Neurobiol. 168, 311–347. https://doi.org/10.1016/bs.irn.2022.10.002.

Fischer, S., Ehlert, U., Amiel Castro, R., 2019. Hormones of the hypothalamicpituitary-gonadal (HPG) axis in male depressive disorders - a systematic review and meta-analysis. Front. Neuroendocrinol. 55, 100792 https://doi.org/10.1016/j.yfrne.2019.100792.

Gavin, K.M., Bessesen, D.H., 2020. Sex differences in adipose tissue function. Endocrinol. Metab. Clin. N. Am. 49 (2), 215–228. https://doi.org/10.1016/j.ecl.2020.02.008.

Gilliver, S.C., 2010. Sex steroids as inflammatory regulators. J. Steroid Biochem. Mol. Biol. 120 (2–3), 105–115.

Grandys, M., Majerczak, J., Zapart-Bukowska, J., Duda, K., Kulpa, J.K., Zoladz, J.A., 2021. Lowered serum testosterone concentration is associated with enhanced inflammation and worsened lipid profile in men. Front. Endocrinol. 12, 735638 https://doi.org/10.3389/fendo.2021.735638.

Hamilton, M., 1960. A rating scale for depression. J. Neurol. Neurosurg. Psychiatry 23 (1), 56–62. https://doi.org/10.1136/jnnp.23.1.56.

Hashioka, S., McGeer, P.L., Monji, A., Kanba, S., 2009 Mar. Anti-inflammatory effects of antidepressants: possibilities for preventives against Alzheimer's disease. Cent. Nerv. Syst. Agents Med. Chem. 9 (1), 12–19. https://doi.org/10.2174/187152409787601897 (PMID: 20021334).

Hauger, R.L., Saelzler, U.G., Pagadala, M.S., Panizzon, M.S., 2022. The role of testosterone, the androgen receptor, and hypothalamic-pituitary-gonadal axis in depression in ageing men. Rev. Endocr. Metab. Disord. 23 (6), 1259–1273. https://doi.org/10.1007/s11154-022-09767-0.

Hautanen, A., 2000. Synthesis and regulation of sex hormone-binding globulin in obesity. Int. J. Obes. Relat. Metab. Disord. 24 (Suppl. 2), S64–S70. https://doi.org/10.1038/sj.ijo.0801281.

Joffe, H.V., Ridker, P.M., Manson, J.E., Cook, N.R., Buring, J.E., Rexrode, K.M., 2006. Sex hormone-binding globulin and serum testosterone are inversely associated with C-reactive protein levels in postmenopausal women at high risk for cardiovascular disease. Ann. Epidemiol. 16 (2), 105–112. https://doi.org/10.1016/j.annepidem.2005.07.055.

Kamińska, M.S., Lubkowska, A., Panczyk, M., Walaszek, I., Grochans, S., Grochans, E., Cybulska, A.M., 2023 Jun 18. Relationships of body mass index, relative fat mass index, and waist circumference with serum concentrations of parameters of chronic inflammation. Nutrients 15 (12), 2789. https://doi.org/10.3390/nu15122789. PMID: 37375693; PMCID: PMC10304469.

Köhler, C.A., Freitas, T.H., Stubbs, B., Maes, M., Solmi, M., Veronese, N., de Andrade, N. Q., Morris, G., Fernandes, B.S., Brunoni, A.R., Herrmann, N., Raison, C.L., Miller, B. J., Lanctôt, K.L., Carvalho, A.F., 2018. Peripheral alterations in cytokine and chemokine levels after antidepressant drug treatment for major depressive disorder: systematic review and Meta-analysis. Mol. Neurobiol. 55 (5), 4195–4206. https://doi.org/10.1007/s12035-017-0632-1.

Krakowsky, Y., Grober, E.D., 2015. Testosterone deficiency - establishing a biochemical diagnosis. EJIFCC 26 (2), 105–113.

Kuehner, C., Nayman, S., 2021. Premenstrual exacerbations of mood disorders: findings and knowledge gaps. Curr. Psychiatry Rep. 23 (11), 78. https://doi.org/10.1007/s11920-021-01286-0.

Leiva, R., Bouchard, T., Boehringer, H., Abulla, S., Ecochard, R., 2015. Random serum progesterone threshold to confirm ovulation. Steroids 101, 125–129. https://doi.org/10.1016/j.steroids.2015.06.013.

Liao, C.H., Li, H.Y., Yu, H.J., Chiang, H.S., Lin, M.S., Hua, C.H., Ma, W.Y., 2012. Low serum sex hormone-binding globulin: marker of inflammation? Clin. Chim. Acta 413 (7–8), 803–807. https://doi.org/10.1016/j.cca.2012.01.021.

Lombardo, G., Mondelli, V., Dazzan, P., Pariante, C.M., 2021. Sex hormones and immune system: a possible interplay in affective disorders? A systematic review. J. Affect. Disord. 290, 1–14. https://doi.org/10.1016/j.jad.2021.04.035.

Lombardo, G., Nettis, M.A., Hastings, C., Zajkowska, Z., Mariani, N., Nikkheslat, N., Worrell, C., Enache, D., McLaughlin, A., Kose, M., Bogdanova, A., Sforzini, L., Cleare, A.J., Young, A.H., Dazzan, P., Mondelli, V., Pariante, C.M., 2022. Sex differences in a double-blind randomized clinical trial with minocycline in treatment-resistant depressed patients: CRP and IL-6 as sex-specific predictors of treatment response. Brain Behav. Immun. Health 26, 100561. https://doi.org/10.1016/j.bbih.2022.100561.

Luisi, S., Orlandini, C., Regini, C., Pizzo, A., Vellucci, F., Petraglia, F., 2015. Premature ovarian insufficiency: from pathogenesis to clinical management. J. Endocrinol. Investig. 38 (6), 597–603. https://doi.org/10.1007/s40618-014-0231-1.

Määttänen, I., Gluschkoff, K., Komulainen, K., Airaksinen, J., Savelieva, K., García-Velázquez, R., Jokela, M., 2021. Testosterone and specific symptoms of depression: evidence from NHANES 2011-2016. Compre. Psychoneuroendocrinol. 6, 100044 https://doi.org/10.1016/j.cpnec.2021.100044.

Maharjan, D.T., Syed, A., Lin, G.N., Ying, W., 2021. Testosterone in female depression: a Meta-analysis and Mendelian randomization study. Biomolecules 11 (3), 409. https://doi.org/10.3390/biom11030409.

Markle, J.G., Fish, E.N., 2014. SeXX matters in immunity. Trends Immunol. 35 (3), 97–104. https://doi.org/10.1016/j.it.2013.10.006.

Masama, C., Jarkas, D.A., Thaw, E., Daneshmend, A.Z.B., Franklyn, S.I., Beaurepaire, C., McQuaid, R.J., 2022. Hormone contraceptive use in young women: altered mood states, neuroendocrine and inflammatory biomarkers. Horm. Behav. 144, 105229 https://doi.org/10.1016/j.yhbeh.2022.105229.

McLaughlin, A.P., Nikkheslat, N., Hastings, C., Nettis, M.A., Kose, M., Worrell, C., Zajkowska, Z., Mariani, N., Enache, D., Lombardo, G., Pointon, L., NIMA Consortium, Cowen, P., Cavanagh, J., Harrison, N., Bullmore, E., Pariante, C.M., Mondelli, V., 2021. The influence of comorbid depression and overweight status on peripheral inflammation and cortisol levels. Psychol. Med. 1–8. Advance online publication. https://doi.org/10.1017/S0033291721000088.

Medina-Rodriguez, E.M., Cruz, A.A., De Abreu, J.C., Beurel, E., 2023. Stress, inflammation, microbiome and depression. Pharmacol. Biochem. Behav. 173561 https://doi.org/10.1016/j.pbb.2023.173561. Advance online publication.

Morssinkhof, M., van Wylick, D.W., Priester-Vink, S., van der Werf, Y.D., den Heijer, M., van den Heuvel, O.A., Broekman, B., 2020. Associations between sex hormones, sleep problems and depression: a systematic review. Neurosci. Biobehav. Rev. 118, 669–680. https://doi.org/10.1016/j.neubiorev.2020.08.006.

Mosiołek, A., Pięta, A., Jakima, S., Zborowska, N., Mosiołek, J., Szulc, A., 2021. Effects of antidepressant treatment on peripheral biomarkers in patients with major depressive disorder (MDD). J. Clin. Med. 10 (8), 1706. https://doi.org/10.3390/jcm10081706.

Nead, K.T., 2019. Androgens and depression: a review and update. Curr. Opin. Endocrinol. Diabetes Obes. 26 (3), 175–179. https://doi.org/10.1097/MED.0000000000000477.

Nikkheslat, N., McLaughlin, A.P., Hastings, C., Zajkowska, Z., Nettis, M.A., Mariani, N., Enache, D., Lombardo, G., Pointon, L., Cowen, P.J., Cavanagh, J., Harrison, N.A., Bullmore, E.T., NIMA Consortium, Pariante, C.M., Mondelli, V., 2020. Childhood trauma, HPA axis activity and antidepressant response in patients with depression. Brain Behav. Immun. 87, 229–237. https://doi.org/10.1016/j.bbi.2019.11.024.

Osimo, E.F., Baxter, L.J., Lewis, G., Jones, P.B., Khandaker, G.M., 2019. Prevalence of low-grade inflammation in depression: a systematic review and meta-analysis of CRP levels. Psychol. Med. 49 (12), 1958–1970. https://doi.org/10.1017/S0033291719001454.

Panichi, V., Migliori, M., De Pietro, S., Taccola, D., Bianchi, A.M., Giovannini, L., Norpoth, M., Metelli, M.R., Cristofani, R., Bertelli, A.A., Sbragia, G., Tetta, C., Palla, R., Colombo, R., 2002 Aug. C-reactive protein and interleukin-6 levels are related to renal function in predialytic chronic renal failure. Nephron 91 (4), 594–600. https://doi.org/10.1159/000065018. Erratum in: Nephron. 2002 Oct;92 (2):498. PMID: 12138260.

Park, J.M., Lee, Y.J., 2020. Serum oestradiol levels are inversely associated with C reactive protein levels in premenopausal women, but not postmenopausal women. J. Int. Med. Res. 48 (10), 300060520961228 https://doi.org/10.1177/0300060520961228.

Peng, R., Li, Y., 2021. Associations between tenascin-C and testosterone deficiency in men with major depressive disorder: a cross-sectional retrospective study. J. Inflamm. Res. 14, 897–905. https://doi.org/10.2147/JIR.S298270.

Pitharouli, M.C., Hagenaars, S.P., Glanville, K.P., Coleman, J.R.I., Hotopf, M., Lewis, C. M., Pariante, C.M., 2021. Elevated C-reactive protein in patients with depression, independent of genetic, health, and psychosocial factors: results from the UK biobank. Am. J. Psychiatry 178 (6), 522–529. https://doi.org/10.1176/appi.ajp.2020.20060947.

Rahman, S.A., Grant, L.K., Gooley, J.J., Rajaratnam, S., Czeisler, C.A., Lockley, S.W., 2019. Endogenous circadian regulation of female reproductive hormones. J. Clin. Endocrinol. Metab. 104 (12), 6049–6059. https://doi.org/10.1210/jc.2019-00803.

Rexrode, K.M., Manson, J.E., Lee, I.M., Ridker, P.M., Sluss, P.M., Cook, N.R., Buring, J.E., 2003. Sex hormone levels and risk of cardiovascular events in postmenopausal women. Circulation 108 (14), 1688–1693. https://doi.org/10.1161/01.CIR.0000091114.36254.F3.

Rezanezhad, B., Borgquist, R., Willenheimer, R., Elzanaty, S., 2018. Association between serum levels of testosterone and biomarkers of subclinical atherosclerosis. The Aging Male 21 (3), 182–186. https://doi.org/10.1080/13685538.2017.1412422.

Ridker, P.M., 2003. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation 107 (3), 363–369. https://doi.org/10.1161/01.cir.0000053730.47739.3c.

Schmidt, M., Naumann, H., Weidler, C., Schellenberg, M., Anders, S., Straub, R.H., 2006. Inflammation and sex hormone metabolism. Ann. N. Y. Acad. Sci. 1069, 236–246. https://doi.org/10.1196/annals.1351.021.

Sforzini, L., Cattaneo, A., Ferrari, C., Turner, L., Mariani, N., Enache, D., Hastings, C., Lombardo, G., Nettis, M.A., Nikkheslat, N., Worrell, C., Zajkowska, Z., Kose, M., Cattane, N., Lopizzo, N., Mazzelli, M., Pointon, L., Cowen, P.J., Cavanagh, J., Harrison, N.A., Pariante, C.M., 2023. Higher immune-related gene expression in major depression is independent of CRP levels: results from the BIODEP study. Transl. Psychiatry 13 (1), 185. https://doi.org/10.1038/s41398-023-02438-x.

Spitzer, R.L., Williams, J.B., Gibbon, M., First, M.B., 1992. The structured clinical interview for DSM-III-R (SCID). I: history, rationale, and description. Arch. Gen. Psychiatry 49 (8), 624–629. https://doi.org/10.1001/archpsyc.1992.01820080032005.

Sramek, J.J., Murphy, M.F., Cutler, N.R., 2016. Sex differences in the psychopharmacological treatment of depression. Dialogues Clin. Neurosci. 18 (4), 447–457. https://doi.org/10.31887/DCNS.2016.18.4/ncutler.

Stárka, L., Hill, M., Pospíšilová, H., Dušková, M., 2020. Estradiol, obesity and hypogonadism. Physiol. Res. 69 (Suppl. 2), S273–S278. https://doi.org/10.33549/physiolres.934510.

Straub, R.H., 2007. The complex role of estrogens in inflammation. Endocr. Rev. 28 (5), 521–574.

Strawbridge, R., Young, A.H., Cleare, A.J., 2017. Biomarkers for depression: recent insights, current challenges and future prospects. Neuropsychiatr. Dis. Treat. 13, 1245–1262. https://doi.org/10.2147/NDT.S114542.

Taneja, V., 2018. Sex Hormones Determine Immune Response. Front. Immunol. 9, 1931. https://doi.org/10.3389/fimmu.2018.01931.

Tariq, S., Okhai, H., Severn, A., Sabin, C.A., Burns, F., Gilson, R., Fox, J., Gilleece, Y., Mackie, N.E., Post, F.A., Reeves, I., Rosenvinge, M., Sullivan, A., Ustianowski, A., Miller, R.F., 2022. Follicle-stimulating hormone in postmenopausal women living with HIV: a prevalence study. HIV Med. 23 (4), 434–440. https://doi.org/10.1111/hiv.13205.

Thys-Jacobs, S., McMahon, D., Bilezikian, J.P., 2008. Differences in free estradiol and sex hormone-binding globulin in women with and without premenstrual dysphoric disorder. J. Clin. Endocrinol. Metab. 93 (1), 96–102. https://doi.org/10.1210/jc.2007-1726.

Tsilidis, K.K., Rohrmann, S., McGlynn, K.A., Nyante, S.J., Lopez, D.S., Bradwin, G., Feinleib, M., Joshu, C.E., Kanarek, N., Nelson, W.G., Selvin, E., Platz, E.A., 2013. Association between endogenous sex steroid hormones and inflammatory biomarkers in US men. Andrology 1 (6), 919–928. https://doi.org/10.1111/j.2047-2927.2013.00129.x.

Valkanova, V., Ebmeier, K.P., Allan, C.L., 2013. CRP, IL-6 and depression: a systematic review and meta-analysis of longitudinal studies. J. Affect. Disord. 150 (3), 736–744. https://doi.org/10.1016/j.jad.2013.06.004.

van Koeverden, I.D., de Bakker, M., Haitjema, S., van der Laan, S.W., de Vries, J., Hoefer, I.E., de Borst, G.J., Pasterkamp, G., den Ruijter, H.M., 2019. Testosterone to oestradiol ratio reflects systemic and plaque inflammation and predicts future cardiovascular events in men with severe atherosclerosis. Cardiovasc. Res. 115 (2), 453–462. https://doi.org/10.1093/cvr/cvy188.

Wainberg, M., Kloiber, S., Diniz, B., McIntyre, R.S., Felsky, D., Tripathy, S.J., 2021. Clinical laboratory tests and five-year incidence of major depressive disorder: a prospective cohort study of 433,890 participants from the UK biobank. Transl. Psychiatry 11 (1), 380. https://doi.org/10.1038/s41398-021-01505-5.

Westphal, U., 1971. Monographs on Endocrinology, 4. Springer-Verlag.

Yamazaki, H., Kushiyama, A., Sakoda, H., Fujishiro, M., Yamamotoya, T., Nakatsu, Y., Kikuchi, T., Kaneko, S., Tanaka, H., Asano, T., 2018. Protective effect of sex hormone-binding globulin against metabolic syndrome: in vitro evidence showing anti-inflammatory and Lipolytic effects on adipocytes and macrophages. Mediat. Inflamm. 2018, 3062319. https://doi.org/10.1155/2018/3062319.

Zajkowska, Z., Nikkheslat, N., Manfro, P.H., Souza, L., Rohrsetzer, F., Viduani, A., Pereira, R., Piccin, J., Zonca, V., Walsh, A.E.L., Gullett, N., Fisher, H.L., Swartz, J.R., Kohrt, B.A., Kieling, C., Mondelli, V., 2023. Sex-specific inflammatory markers of risk and presence of depression in adolescents. J. Affect. Disord. S0165-0327(23)00890-X https://doi.org/10.1016/j.jad.2023.07.055. Advance online publication.

Zarrouf, F.A., Artz, S., Griffith, J., Sirbu, C., Kommor, M., 2009. Testosterone and depression: systematic review and meta-analysis. J. Psychiatr. Pract. 15 (4), 289–305. https://doi.org/10.1097/01.pra.0000358315.88931.fc.

Zito, S., Nosari, G., Pigoni, A., Moltrasio, C., Delvecchio, G., 2023. Association between testosterone levels and mood disorders: a minireview. J. Affect. Disord. 330, 48–56. https://doi.org/10.1016/j.jad.2023.02.108.

Zitzmann, M., Faber, S., Nieschlag, E., 2006. Association of specific symptoms and metabolic risks with serum testosterone in older men. J. Clin. Endocrinol. Metab. 91 (11), 4335–4343. https://doi.org/10.1210/jc.2006-0401.