# Stress Induced Cortisol Release Depresses The Secretion of Testosterone in Patients With Type 2 Diabetes Mellitus

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**ABSTRACT**

**BACKGROUND:** Both hormonal and genetic data reveal that the stress hormone cortisol and its regulating genes may affect the level of testosterone in humans. It is uncertain whether type 2 diabetes mellitus would manifest similarly. Furthermore, a genetic strategy to screen out the stress system genes that may contribute to testosterone decline in humans is less understood.

**OBJECTIVES:** In this study, we aimed to elucidate the link between stress and testosterone levels, both hormonally and genetically.

**METHOD:** This study comprised 37 individuals with type 2 diabetes mellitus and 50 healthy individuals. For the analysis of hormones and the targeted genes, we used the RIA system and bioinformatics expertise.

**RESULTS:** The patients had significantly elevated cortisol and lower testosterone readings, according to data from hormonal analyses. The bioinformatics approach reveals that SHBG was intracellularly suppressed by 2 defined stress system genes: FKB5 and CYP17. TCF4/TCF8, ATRX, and AR in skeletal muscle were inversely related to stress system genes. Furthermore, all testosterone regulated genes were positively linked with SHBG in the current study. A strong relationship between GNAS and PKA with CYP17 and FKBP5 reveals that the Gαs-cAMP/PKA signaling pathway may be one of the regulatory pathways through which the suppression of testosterone system genes happens. In conclusion, this study demonstrated that beyond stress, the key stress system genes might affect cortisol levels, which in turn affect testosterone figures via the Gαs-cAMP/PKA signaling pathway.

**KEYWORDS:** Type 2 diabetes mellitus, stress, cortisol, testosterone, bioinformatics

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## Introduction

The group of metabolic complications type 2 diabetes mellitus (T2DM) is a chronic hyperglycemic condition caused by insufficient insulin action. The involvement of the major organs in this disease state is a worldwide health problem affecting any age group, causing high illness and mortality rates.[1] Diabetes prevalence estimates for any age group worldwide were 2.8% in 2000, and it is expected to be around 4.4% in 2030, according to the World Health Organization (WHO). Diabetes patients worldwide are set to rise from 171 million in 2000 to 366 million in 2030. Diabetes accounts for about 5% of all deaths worldwide each year. T2DM is the most common type of diabetes, affecting 85 to 90% of people with the disease.[2] Adults mostly have T2DM, but this ratio rises faster among teenagers and young adults than in other age groups.[2] T2DM is characterized by insulin insensitivity, which results from insulin resistance, which decreases insulin synthesis and the letdown of pancreatic beta cells. Environmental factors like stress can contribute to the development of T2DM.[3]

Stress is an Internal process when a person is faced with a question, i.e., Considered to be available to respond effectively but continuously let down in pulseless. Chronic Stress has been shown to produce a physiological response mediated by stimulation of the hypothalamic-pituitary-adrenal (HPA) axis.[4] The stimulation axis of HPA results in the release of cortisol, a primary biological marker for stress reactivity in response to a challenge from the environment. Stimulation of HPA axis directs adrenocorticotropic hormone (ACTH) secretion from the pituitary through corticotrophin-releasing factor (CRF) from the hypothalamus. ACTH stimulates the adrenal cortex to release cortisol. The peripheral release of cortisol and the central release of CRF in various areas of the brain initiates a

cascade of biological responses to modify the homeostatic balance of organisms in response to stress.[5] Previous studies have shown that stress with or without type 2 diabetes is associated with increased cortisol levels and lower testosterone.[6,7,8,9] CRHR1, CYP17A1, and FKBP5 are defined as stress system genes mainly expressed in the following tissues: the pituitary gland, adrenal gland, and skeletal muscle. The gene CYP17 is responsible for making an enzyme that converts cholesterol into cortisol. Cortisol turns genes on or off to regulate our physical responses to stressful situations.[10] Several molecular feedback loops that regulate adaptation require FKBP51. FKBP51 affects the stress hormone axis (HPA axis) and physiological stress response through its regulatory role on glucocorticoid receptor (GR).[11] The FKBP5 gene, which codes for FKBP51, is triggered by stress and binds to glucocorticoid-sensitive regions to raise the amount of FKBP51 inside cells, creating an intracellular ultrashort feedback loop. By supporting their regulatory hetero complexes, protein kinase and phosphatase activity is further controlled by FKBP51.[11] A study that analyzed the genomes of 8938 males discovered mutations in just one gene, SHBG, that were connected with low blood levels of testosterone. These genetic indicators significantly impact testosterone levels, outweighing recognized risk factors for low testosterone by a wide margin.[12] Other genes, including AR, TCF8, ATRX, NCOA3, RBL2, and TCF4, that are involved in the regulation of Testosterone have been identified in skeletal muscle.[13] Patients with diabetes have increased HPA axis activation.[14] According to,[15] there might be a link between stress, diabetes, and plasma cortisol levels. Cortisol and testosterone levels in T2DM patients and controls were to be compared as part of this investigation. We investigated after finding that elevated cortisol reduces plasma testosterone levels. We became interested in comparing our hormonal findings with certain targeted genes. Then, we incorporated the TCGA dataset analysis for healthy individuals with significant genes that control human cortisol and testosterone. There is no available database to evaluate the same data on people who have T2DM. Our current findings provide a strong indicator for future investigations, including omitting T2DM patients in light of prior studies utilizing the analyzed gene set.

## Materials and Methods

### *Study population*

The study was conducted in D.I.Khan, Divisional hospital, Khyber Pakhtunkhwa (KP), Pakistan. In the present study, 37 men aged 20 to 60 years who were diagnosed as T2DM patients and confirmed by the estimation of fasting plasma glucose (⩾125 mg/dl) and postprandial blood glucose (⩾200 mg/dl) were selected, 50 healthy age and BMI matched individuals, were selected as controls. Written informed consent was obtained from all patients/controls or guardians before the trial. The PMAS Arid Agriculture University Rawalpindi Research Ethical Committee approved the current work.

### *Exclusion criteria*

Neurological and cerebrovascular conditions, including chronic renal impairment, asthma, alcoholism, renal insufficiency, advanced hepatic disease, and any other endocrinological disorders in any of the participants, were excluded from the study.

### *Inclusion criteria*

Males with high blood glucose levels, moderate or severe stress, and healthy persons. The research comprised people who visited the DHQ hospital of the D.I. Khan Division, Khyber Pakhtunkhwa, Pakistan.

### *Clinical assessment*

Each participant had a small amount of blood taken from an antecubital vein while attending DHQ-D.I.K (about 1.5 mL). After collection, the samples were centrifuged at 3000 rpm for 5 minutes to separate the sera. The serum samples were frozen at −20°C as soon as possible until they were analyzed. To minimize the impact of the diurnal variation in the circulating levels of cortisol and Testosterone, blood samples were taken between 9:00 am and 12:00 pm. Serum measurements were used to estimate testosterone levels, and radioimmunoassay (RIA) kits were used to evaluate cortisol (IMMUNOTECH s. r. o. - Radiova 1 102 27 Prague 10 - Czech Republic). The assay's detection range for cortisol was 5 to 2000 nmol/L, and for Testosterone, it was 0.02 to 20 ng/ml. The normal reference range for plasma cortisol was 49 to 724 nmol/L and for testosterone was 03 to 12 nmol/L.

### *GEPIA platform*

A program based on TCGA and GTEx data, GEPIA (Gene Expression Profiling Interactive Analysis; https://gepia.cancer-pku.cn),[16] provides correlation data for 165 testes, 128 for the adrenal gland and 396 skeletal muscle tissue. Using this database, we conducted association analyses for genes involved in the stress response and testosterone regulation for normal tissue, with their specific enriched tissue as the focus.

### *Statistical analysis*

Unpaired 't-test', and Pearson's Correlation Coefficient 'r' were used to examine the data, expressed as SME. The $P$-value ⩽ .05 was adjusted as the level of significance.[17] We used a log scale to show the mRNA expression level for a specific gene.[18]

## Results

### *Cortisol versus testosterone levels in T2DM patients*

We examined the cortisol levels in patients with T2DM and compared them with control subjects. As expected cortisol levels in patients were substantially higher than in the control

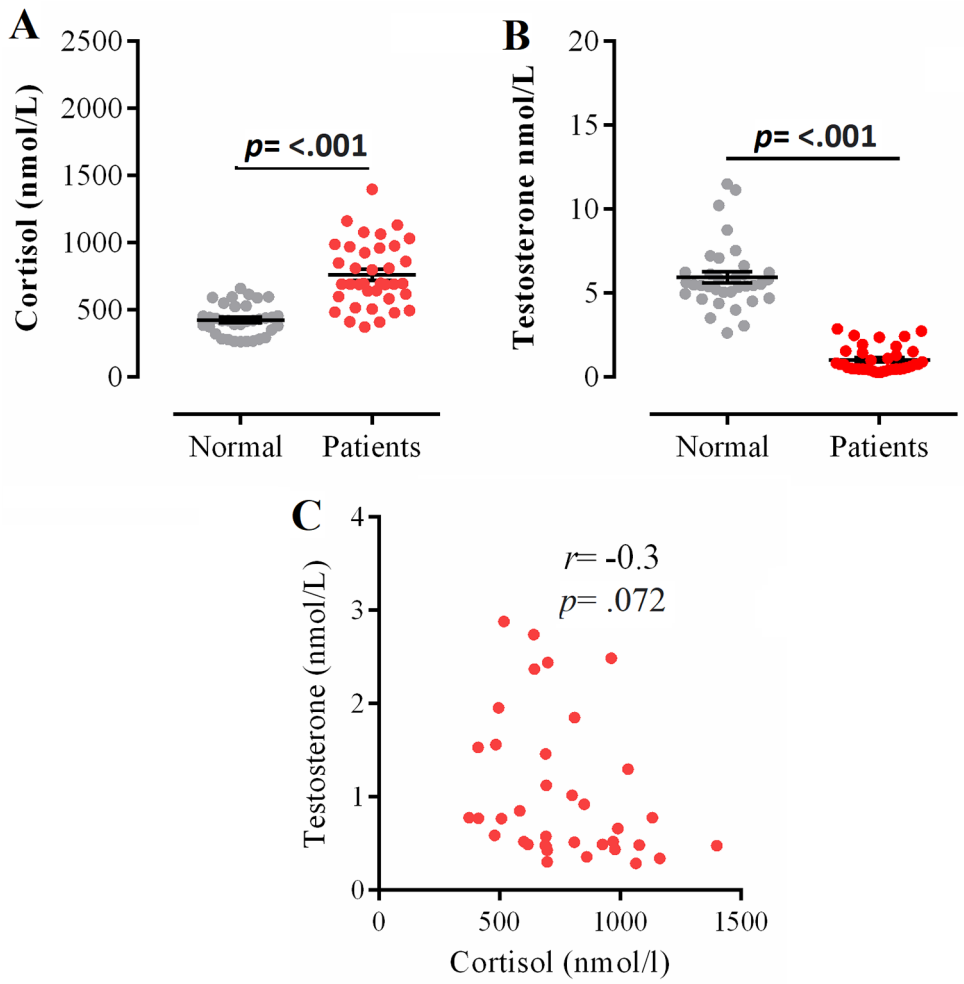


**Figure 1.** (A) Left, the representative graph shows healthy control vs. the patient's cortisol levels. (B) Graph showing testosterone readings across patients and control group. (C) Graph showing a correlation between testosterone and cortisol across patients. *P*-values were calculated by Student t-test or ANOVA two-tailed test with recommended option. Error bars indicate S.E.M. *$P < .05$ for the stated comparison.

group (Figure 1A). In contrast, the mean serum testosterone level in T2DM participants was considerably lower ($P = <.001$) compared to healthy subjects (Figure 1B). Furthermore, we observed a negative correlation between cortisol and testosterone as depicted in (Figure 1C). These data imply that a rise in cortisol may drop the level of testosterone in these patients.

### *New approach and collection*

The above findings provide a way for cortisol and testosterone levels to be compared genetically via their regulatory genes. As yet, no research has shown that type of investigation beyond the 2 hormones. Stress may alter testosterone levels in T2DM and normal individuals via stress-induced cortisol release in circulation, as previously reported that elevated cortisol levels decreased testosterone levels,[17] but whether or not this system follows some genetic roots is vital. PUBMED and other sites and search engines did not yield data that addressed the issue at the molecular level. Our strategy is the first struggles to uncover the potential molecular mechanisms and pathway via which we can find the underlying cause of why a person has low testosterone due to elevated cortisol. We carefully analyzed all data in order to produce a good report for future research. We employed hormonal analysis in our data to recall the fact that high cortisol levels decreased testosterone levels. As we and others[17] demonstrated the negative association between cortisol and testosterone, our main goal here was to illustrate the interaction between stress system genes and testosterone-regulated genes in the testes and skeletal muscles. This association makes us investigate whether the genes beyond them will behave similarly. The basic and hypothetical concept of testosterone repression by cortisol or its system genes in circulation and skeletal muscle is depicted in (Figure 2A and B).

### *SHBG is intracellularly suppressed by CYP17 and FKBP5*

Using bioinformatics expertise, we examined the expression and relationships between SHBG and stress system genes (Figure 3). We found a substantial difference between SHBG and 2 stress system genes (CYP17, FKBP5). According to 361 subjects, CYP17 and FKBP5 were more highly expressed than

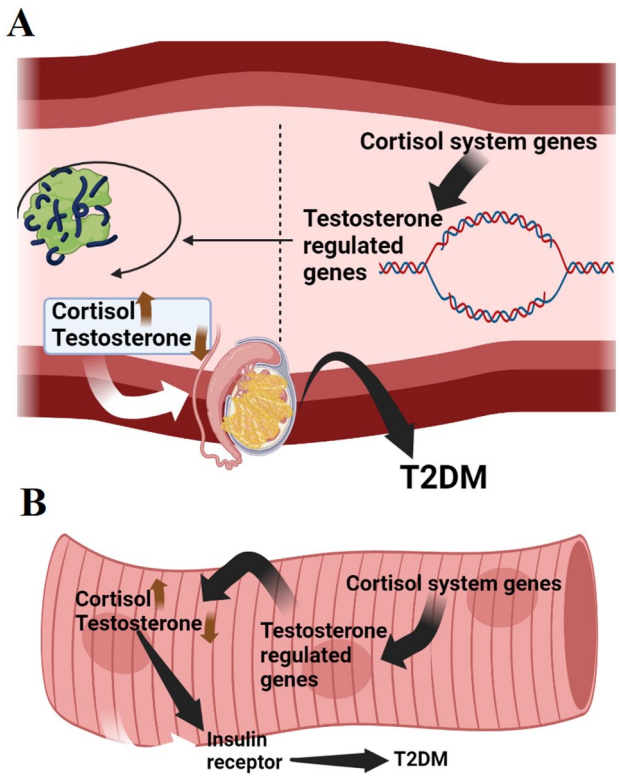


**Figure 2.** A theoretical model of testosterone suppression via the cortisol release mechanism. (A) Higher expression of cortisol system genes may suppress testosterone levels. This modulation may result in hypogonadism and, eventually, T2DM. The green bubble signifies testosterone, and the black drawing 65 symbolizes cortisol (B), An illustration of testosterone suppression in skeletal muscle cells via cortisol system genes. This modulation may result in a faulty insulin receptor in the muscle, resulting in T2DM.

SHBG (Figure 3A). However, according to correlation data, all stress system genes were strongly inversely linked to SHBG (Figure 3B–D). These findings imply that increased CYP17 and FKBP5 expression may cause SHBG suppression in the testes, which results in a drop in testosterone levels. Other evidence from these data suggests that stress genes negatively correlated with SHBG may adversely influence SHBG, which in turn affects testosterone levels. Stress system genes negatively impact testosterone-regulated genes in skeletal muscle. Next, we looked at whether the stress system genes identified as being controlled by Testosterone in skeletal muscle were negatively linked to each other. Our findings showed that TCF4 and AR were significantly inversely connected with FKBP5 and CRHR1, TCF8, but ATRX, and CRHRI were not significantly inversely associated (Figure 4A–D). The second question addressed here is that if these genes are connected negatively with stress system genes, they must be correlated favorably with SHBG. As depicted in Figure 4E, all genes had a substantial positive correlation with SHBG. These findings imply that CRHR1 and FKB5 genes may repress testosterone-regulated genes in skeletal muscles, which may further lead to a decrease in testosterone.

### *Stress system genes suppress testosterone-regulated genes through the Gαs-cAMP/PKA signaling pathway*

We also analyzed the potential route taken by the stress system genes. The stress system genes utilized the Gαs-cAMP/PKA pathway, according to the current analysis. We discovered a favorable connection between Gas-PKA and CYP17/FKBP5 (Figure 5A–F). Since Gαs is the first to contact adenylate cyclase (AC) and protein kinase A (PKA) is the last kinase to receive signals from cAMP, our strategy was limited to the first and last factor in this pathway with our target genes. Following ligand attachment, AC is activated, and cAMP is produced, activating PKA. The phosphorylation of CREB starts the expression of CYP17 and FKBP5 by activated PKA (Figure 6).

### *Transcription factor target analysis of stress and testosterone-regulated genes*

Since there is a significant difference in the expression of stress and testosterone-regulated genes in normal tissues, we explored possible transcription factor targets of the stress and testosterone-regulated genes using the TRRUST database in (Table 1). All genes of interest were included in TRRUST. Thirty-nine transcription factors were significantly related to stress and testosterone-regulated gene regulation.

## Discussion

We found lower testosterone and higher cortisol readings in T2DM patients than in non-diabetic subjects. The cortisol levels were significantly higher in patients where the testosterone levels were significantly lower. These results are consistent with some other investigators.[19,20] Elevated cortisol is associated with increased hepatic gluconeogenesis and glycogenolysis and, consequently, hyperglycemia.[21] The value discovered in this study may contribute to the development of T2DM. The increased cortisol concentrations in our study may agree with the finding that individuals with T2DM may be under minor stress.[22] In our study, the patients with T2DM exhibited elevated cortisol levels and lower concentrations of Testosterone, indicating that these patients were experiencing a type of stress, which caused a reduction in the circulating concentrations of testosterone. Previous studies have suggested that dexamethasone, a synthetic glucocorticoid, lowers testosterone levels in the blood. In terms of the mechanism of glucocorticoids' action in decreasing testosterone levels, it has been documented that cortisol inhibits testosterone secretion at the level of the testes.

In contrast, glucocorticoids decrease testosterone secretion from the testes. In addition, glucocorticoids have been reported to decrease GnRH, FSH, LH, and gonadal steroids. In contrast, corticotropin-releasing hormone (CRH) has been shown to inhibit GnRH release from the hypothalamus.[23]

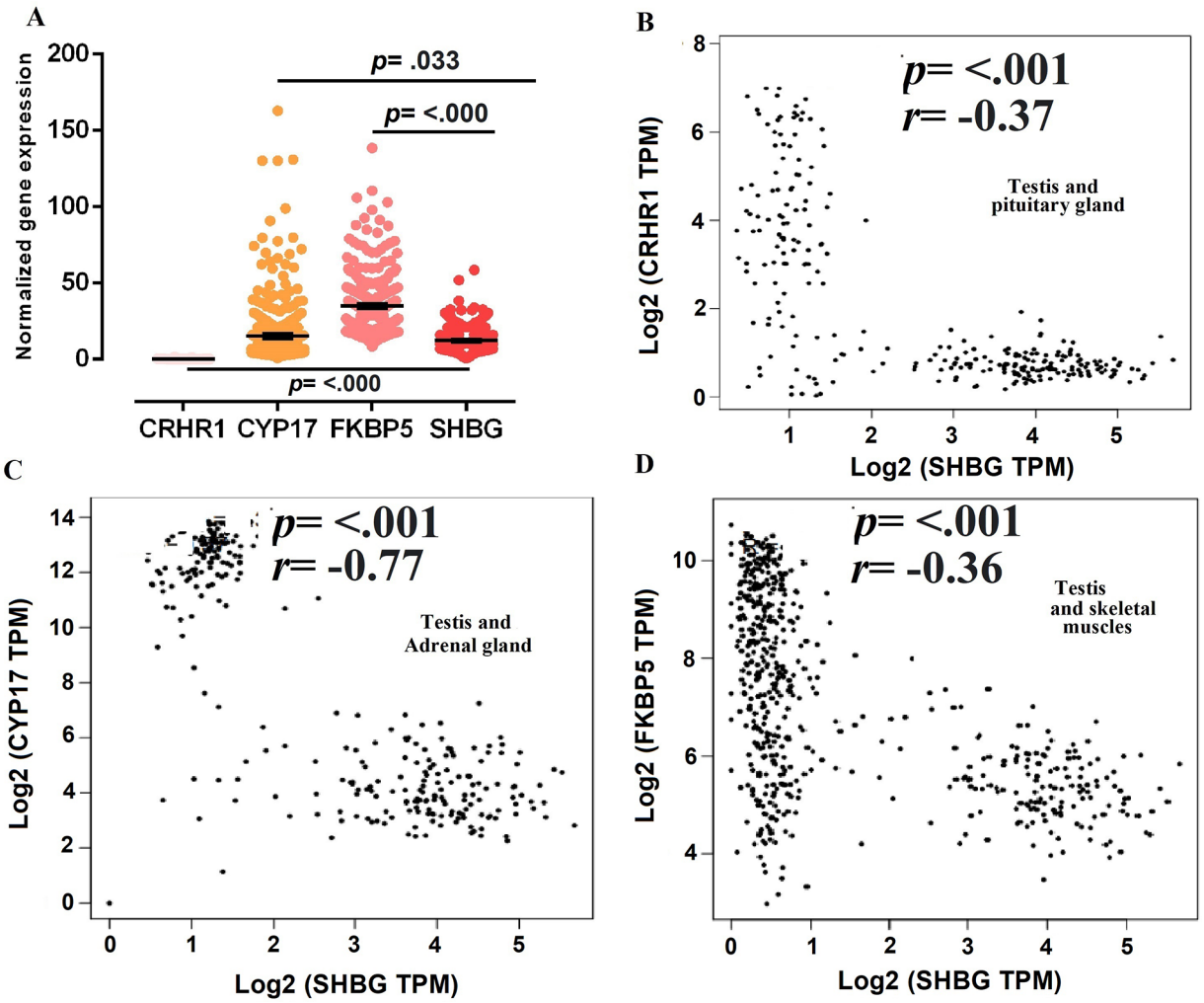


**Figure 3.** Expresión and Correlation between stress system genes vs SHBG. (A) The mRNA expression of stress system genes versus SHBG. *$P < .05$ and |Log2 (fold-change)| cutof = 1. We used a log scale to show mRNA expression level. (B–D) Correlation between SHBG and stress system genes. Pearson correlation r was done for indicated comparison. *$P < .05$ for the indicated comparisons.

Elevated cortisol levels in patients with T2DM are related to low testosterone levels.[24,25] An investigation into the suppression of testosterone concentrations by increasing cortisol concentrations revealed that chronic stress is associated with a decline in serum concentrations of testosterone. In contrast, acute stress induces a rapid increase in serum concentrations of cortisol followed by a decrease in the serum concentration of Testosterone. In the present study, insulin resistance in T2DM may cause alterations in the hypothalamic-pituitary-gonadal axis. This could lead to a decrease in the production of luteinizing hormone and follicle-stimulating hormone, leading to a drop in testosterone production from the testis' Leydig cells. Furthermore, it has been found that dexamethasone, a synthetic glucocorticoid, lowers testosterone levels in the blood.

Here we used bioinformatics skills to reveal the data from the TCGA platform for normal individuals. We included critical genes that were reported earlier by other researchers with established roles in causing or playing a role in cortisol production or having a role in testosterone regulation. For instance, CRHR1, CYP17, and FKBP5 are defined as stress system genes,[26] CRHR1 mediates active defensive behavior caused by controlled stress.[27] CYP17A1 makes an enzyme that creates cortisol from cholesterol.[12] The FK506-binding protein 51 (FKBP5/FKBP51) is an essential stress response modulator. FKBP5 functions as a co-chaperone, modulating glucocorticoid receptor activity in response to stressors and a slew of other cellular processes in the brain and the periphery.[28]

No data are available on comparing these genes with testosterone-regulated genes in the literature. Therefore, our second goal was to expose it through bioinformatics initially and compare it with our hormonal data. The bioinformatics approach revealed that CYP17 and FKBP5 intracellularly suppressed SHBG. As earlier mentioned, CYP17 and FKBP5 are defined as stress system genes. Highly expressed CYP17 and FKBP5 may suppress SHBG intracellularly and affect testosterone production in the testes. Their negative correlation suggests they were not linked, and their function would be antagonistic. This antagonism resulted in a higher level of cortisol, which further caused low testosterone. FKBP51 is involved in several molecular feedback loops mediating adaptation. Through its

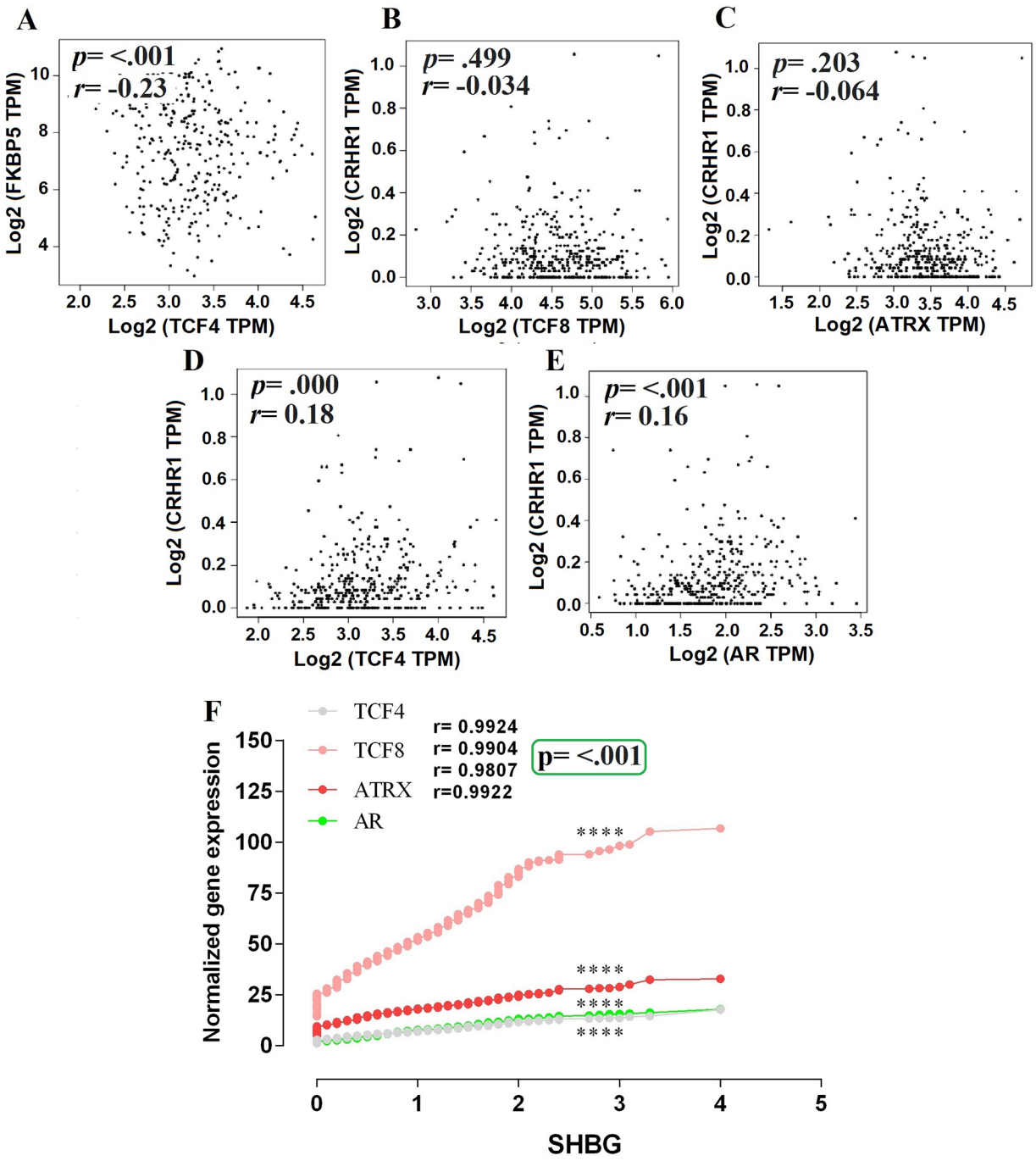


**Figure 4.** Correlation analysis between stress system genes vs testosterone-regulated genes in skeletal muscle. (A) TCF4 versus FKBP5. (B) TCF8 versus CRHR1. (C) ATRX1 versus CRHR1. (D) TCF4 versus CRHR1. (E) AR versus CRHR1. (F) Correlation between testosterone-regulated genes in skeletal muscles and SHBG. Pearson correlation r was done for indicated comparison. *$P < .05$ for indicated comparisons.

regulatory function on GR, FKBP51 influences the stress hormone axis (HPA axis) and physiological stress response.[29] Likewise, stress increases the levels of FKBP51 through activated GR that binds to glucocorticoid-responsive elements in the FKBP5 gene. Through scaffolding, FKBP51 further controls the activity of several protein kinases and phosphatases through its regulatory heterocomplexes.[11]

We found a strong positive correlation between testosterone-regulated genes in skeletal muscle and testosterone. We did not find any direct research on SHBG's correlation with other testosterone-regulated genes, although the concept can be taken from the following research. SHBG is a homodimeric glycoprotein, and both monomers can bind to sex steroids.[30,31] The binding affinity of SHBG for testosterone is high and has been reported to vary across total testosterone levels,[32] demonstrating an allosteric interaction between the 2 binding sites.[33] Thus, it follows that the level of SHBG, which binds testosterone with high affinity and transports testosterone in circulation, is strongly positively correlated with the level of testosterone in plasma.[34,35]

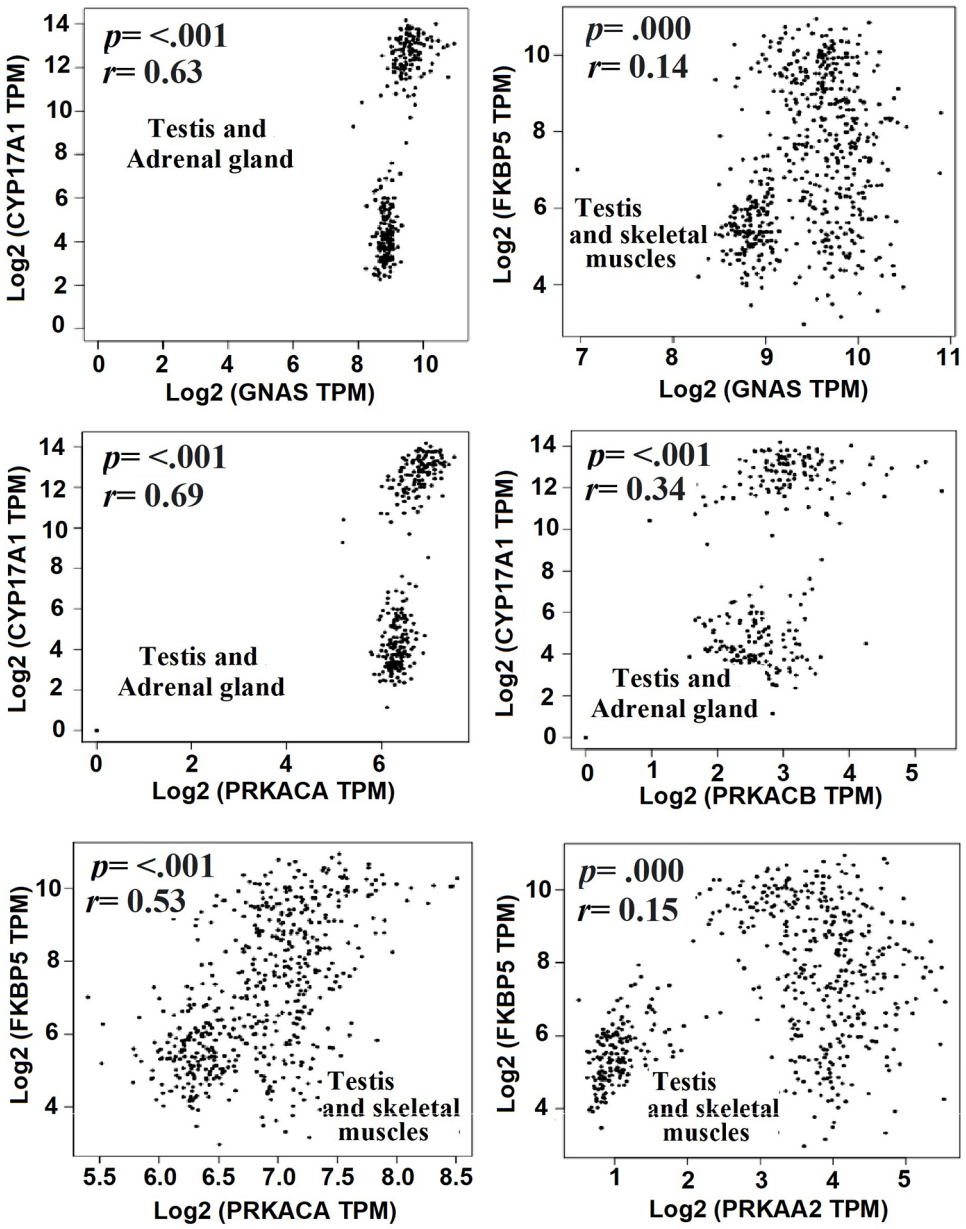


**Figure 5.** Correlation analysis between stress system genes versus GNAS and PKA. (A) *$P < .05$ for indicated comparisons.

On the other hand, TCF4/8, ATRX, and AR have been demonstrated very well to regulate testosterone levels in skeletal muscle.[36] We discovered that CRHR1 and FKB5 might repress TCF4 and AR in skeletal muscles, as defined by testosterone-regulated genes, which may lead to a decrease in testosterone. On the one hand, we found that all testosterone regulated genes in skeletal muscle significantly correlated with SHBG levels, whereas TCF4 and AR showed a negatively significant correlation with FKB5 and CRHR1. More research will be conducted to determine whether these skeletal muscle genes directly affect testosterone-regulated genes or whether they first approach SHBG. A study that examined the genomes of 8938 men found changes within a single gene, SHBG, that were associated with low levels of testosterone in the blood. The effect of these genetic markers on testosterone levels is substantial and too far from the impact of known risk factors for low testosterone.[37] Stimulation of CRHR1/ACTH by CRH activates adenylate cyclase, and subsequent cAMP production leads to the activation of PKA. Activated PKA phosphorylates CREB, which initiates the expression of CYP17 and FKBP5.[38,39] Our analysis also revealed a significantly positive correlation between Gαs, FKBP5, CYP17, and protein kinase A (PKA), further phosphorylated the CREB and thereby transcriptional factor activation and protein synthesis. This process will lead to higher expression of FKBP5 and CYP17. This high expression may suppress TCF4/8, ATRX, and AR.

The current study's limitations included its small sample size and single-location setting. Other proteins, for example, corticosteroid-binding globulin and sex-hormone-binding

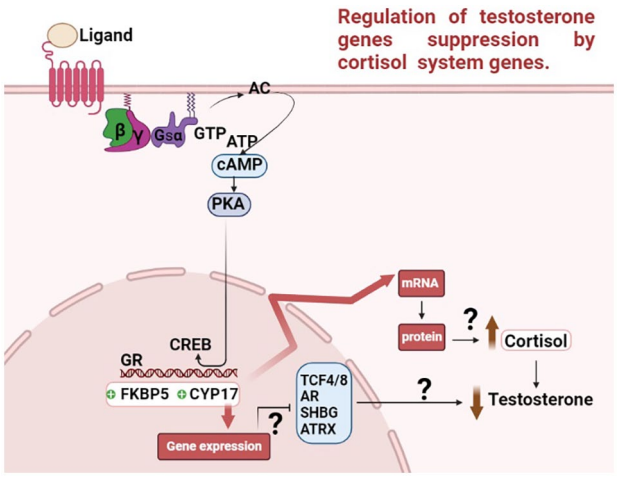


**Figure 6.** A schematic representation of the intracellular pathways that may mediate the interaction between the genes for testosterone and those involved in the stress system, leading to the suppression of the genes for testosterone regulation. When the receptor is stimulated, AC is triggered, which leads to the production of cAMP, which then activates PKA. PKA activation leads to the phosphorylation of CREB, which initiates the expression of CYP17 and FKBP5. On the basis of these representations as interpreted by bioinformatics analysis, the additional studies should concentrate on in vitro or in vivo where the question mark is highlighted.

globulin, may cause changes in serum cortisol and testosterone levels, which were not considered in our study. Second, we investigated a considerable sample of data on mRNA expression for all gene sets except the data pertaining to healthy subjects. No platform exists to examine the same data in T2DM patients under the same settings. Low testosterone levels are now prevalent in T2DM patients and healthy individuals due to stress-induced cortisol release. In this regard, our approach provides a solid clue for T2DM patients and healthy individuals. This concept is the first by which we or others may address the problem, attracting researchers to its genetic relationship, not just hormonally. It may be constructive to recruit a larger population with more T2DM or healthy individuals and increase the observation time. The interesting associations observed in the current study can still provide valuable clues for future investigations.

## Conclusions

High cortisol and low testosterone values were observed in T2DM individuals. Beyond these results, a genetic role exists, which we examined using bioinformatics skills. CYP17 and FKBP5 may suppress SHBG, thereby resulting in a decrease in the level of Testosterone. CRHR1 and FKBP5 may repress

**Table 1.** Transcriptional factors for stress and testosterone system genes in humans (TRRUST).

| TF | *P*-VALUE | ODDS RATIO | COMBINED SCORE | GENES |
|---|---|---|---|---|
| VHL human | .000 | 369.8888889 | 3897.964407 | AR;TCF4 |
| LEF1 human | .000 | 246.4814815 | 2410.753549 | AR;TCF4 |
| EGR1 human | .000 | 77.15503876 | 582.421461 | AR;TCF4 |
| CREB1 human | .000 | 75.39393939 | 565.749705 | AR;CYP17A1 |
| PIAS1 human | .002 | 571.0571429 | 3445.289983 | CYP17A1 |
| NCOR1 human | .002 | 475.8571429 | 2797.6605 | AR |
| GATA6 human | .003 | 356.8571429 | 2008.476466 | CYP17A1 |
| NR2F1 human | .003 | 356.8571429 | 2008.476466 | CYP17A1 |
| NKX3-1 human | .003 | 317.1904762 | 1751.859055 | AR |
| NR0B1 human | .003 | 317.1904762 | 1751.859055 | CYP17A1 |
| ZEB1 human | .005 | 237.8571429 | 1251.41637 | AR |
| RELA human | .005 | 21.95429208 | 112.4885743 | AR;CRHR1 |
| TP63 human | .005 | 203.8571429 | 1043.434298 | TCF4 |
| NR4A1 human | .005 | 203.8571429 | 1043.434298 | CYP17A1 |
| NFKB1 human | .006 | 21.80620155 | 111.4492434 | AR;CRHR1 |
| FOXM1 human | .006 | 178.3571429 | 890.6521752 | AR |
| FOXO3 human | .007 | 167.8571429 | 828.6537823 | AR |
| GATA2 human | .007 | 158.5238095 | 774.0350338 | AR |

*(Continued)*

**Table 1.** (Continued)

| TF | *P*-VALUE | ODDS RATIO | COMBINED SCORE | GENES |
|---|---|---|---|---|
| GATA4 human | .007 | 158.5238095 | 774.0350338 | CYP17A1 |
| RUNX2 human | .007 | 158.5238095 | 774.0350338 | AR |
| CTNNB1 human | .008 | 135.8571429 | 643.5130349 | AR |
| NR5A1 human | .008 | 135.8571429 | 643.5130349 | CYP17A1 |
| E2F4 human | .009 | 129.6753247 | 608.4900471 | AR |
| SOX9 human | .009 | 129.6753247 | 608.4900471 | AR |
| FOXO1 human | .009 | 124.0310559 | 576.7477724 | AR |
| NFIC human | .010 | 109.7032967 | 497.2596291 | CYP17A1 |
| SREBF1 human | .010 | 109.7032967 | 497.2596291 | AR |
| YBX1 human | .011 | 98.33990148 | 435.4424062 | AR |
| RUNX3 human | .011 | 98.33990148 | 435.4424062 | TCF4 |
| SMAD3 human | .012 | 95.05714286 | 417.8062797 | CYP17A1 |
| REST human | .012 | 91.98617512 | 401.4040374 | AR |
| SP1 human | .014 | 13.84539007 | 58.94229858 | AR;CYP17A1 |
| POU2F1 human | .015 | 77.04633205 | 323.0507581 | AR |
| HNF4A human | .017 | 64.76623377 | 260.689817 | SHBG |
| WT1 human | .022 | 50.85714286 | 192.7890777 | AR |
| HDAC1 human | .028 | 40.65714286 | 145.2929193 | AR |
| ESR1 human | .030 | 37.93714286 | 133.0240675 | AR |
| HIF1A human | .032 | 34.68641115 | 118.6118717 | AR |
| SP3 human | .044 | 25.35714286 | 79.0189046 | CYP17A1 |

skeletal muscle genes designed to regulate testosterone levels. Due to the increased expression of stress system genes over SHBG, this gene can be severely suppressed during its premature processing. Future research should use in vitro or in vivo models to reveal our findings. SHBG in testes, whereas ATRX, AR, and TCF4/8 in the skeletal muscles may influence the production of Testosterone, which can be affected by CYP1, CRHR1 and FKBP5. Stress system genes were identified as having the potential to either directly or indirectly alter testosterone synthesis. Still, it will be fascinating to explore whether their primary targets are skeletal muscle testosterone-regulated genes or SHBG.

## Declarations

### *Ethics approval and consent to participate*

This study was approved by the institutional review board (IRB) of Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan (20-2(9)/ASIP/R&D/HEC/17/000448 (PIEAS-Ibd)). Consent was waived due to the retrospective nature of the study and the lack of active intervention.

### *Consent for publication*

Informed consent was received for the current study.

### *Author contributions*

Safir Ullah Khan: Conceptualization; Data curation; Formal analysis; Software; Writing – review & editing. Saba Jannat: Conceptualization; Data curation; Methodology. Hadia Shaukat: Methodology; Writing – review & editing. Shiza Unab: Conceptualization; Data curation; Methodology. Tanzeela Tanzeela: Writing – review & editing. Maleeha Akram: Formal analysis; Methodology. Amir Ali: Validation; Visualization. Muhammad Nasir Khan Khattak: Conceptualization; Data curation. Monica Vizcara Soto: Writing – review & editing. Muhammad Fiaz Khan: Software; Supervision; Validation. Syed Shakeel Raza Rizvi: Supervision; Validation; Visualization.

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### *Availability of data and materials*

The datasets generated and analyzed during the current study are available to the corresponding author upon reasonable request.


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