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RESEARCH ARTICLE

# Type 2 diabetes mellitus but not overweight/obesity reduces testicular volume and endocrine function in men

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**Background** Type 2 diabetes mellitus (T2DM) is commonly associated with decreased serum testosterone levels and hypogonadotropic hypogonadism in affected men. Testicular volume is an important indicator of serum testosterone levels. However, there are limited data on testicular volume among men with T2DM compared with their healthy counterparts. This study aimed to investigate the effect of T2DM and overweight/obesity on testicular volume as well as the factors associated with low testicular volume in T2DM and healthy men.
**Methods** This cross-sectional study involved 358 T2DM men and 179 age-matched healthy controls, categorised into four groups: control non-obese, control overweight/obese, T2DM non-obese, and T2DM overweight/obese. Testicular volume was measured using a Prader orchidometer, with normal volume defined as 15–25 ml. Serum samples were analysed for total testosterone, luteinising hormone (LH), follicle-stimulating hormone (FSH), and sex hormone-binding globulin (SHBG).
**Results** Total testosterone and free testosterone concentrations were significantly lower in T2DM patients independent of obesity ($P_{T2DM} < 0.0001$, $P_{T2DM} < 0.0001$, respectively). T2DM was associated with reduced average testicular volume, with significantly lower volumes observed in non-obese and overweight/obese T2DM compared with their respective healthy control ($p = 0.0194$; $p = 0.0492$, respectively). Among obese T2DM men, there was a significant positive correlation between average testicular volume and bodyweight ($p = 0.0426$). Additionally, average testicular volume was positively correlated with waist circumference in obese T2DM men ($p = 0.0269$).
**Conclusion** Our study reveals that men with T2DM exhibit reduced testicular volume regardless of their BMI, potentially indicating implications for low testosterone and impaired reproductive function in this population. Moreover, average testicular volume demonstrates positive associations with select clinical markers of adiposity and adverse lipid profiles, suggesting heightened cardiovascular risk among men with increased testicular volume. Further investigations are required to elucidate the precise mechanisms underlying these observed associations.

**Keywords:** men, overweight/obesity, Type 2 diabetes mellitus, testicular volume, testosterone

## Background

Diabetes mellitus (DM) is a group of metabolic disorders characterised by chronic hyperglycaemia resulting from defects in insulin secretion, insulin action, or both, affecting carbohydrate, fat, and protein metabolism.[1] Type 2 diabetes mellitus (T2DM) represents the predominant form, accounting for over 90% of cases globally. Its prevalence is increasing, particularly in low- and middle-income countries (LMIC), where approximately 80% of the cases are based.[2] According to the International Diabetes Federation (IDF) 10th edition atlas, there were 537 million adults living with DM worldwide in 2021, with projections nearing 783 million by 2045.[3] In Nigeria, the prevalence of DM has doubled from 2.2% in 1992 to 4.99% in 2013.[4]

Hypogonadism is highly prevalent among men with T2DM in Nigeria, reaching approximately 80%.[5–7] Cross-sectional studies have reported decreased serum testosterone in these individuals.[5–11] Studies from Ghana and Zaria, Nigeria, have highlighted hypergonadotropic hypogonadism (primary hypogonadism) as the predominant form in T2DM males.[7,8] However, other studies have reported that hypogonadotropic hypogonadism (secondary hypogonadism) may be more common.[9,10,12,13]

The testis comprises the interstitial tissues that produce testosterone, other androgens, and the seminiferous tubules that generate spermatozoa.[14] Primarily, testosterone regulates puberty, sexual behaviour, libido, and testicular volume.[15] Studies have consistently shown a significant decrease in testicular volume associated with a decline in endocrine function.[16,17] Studies have reported that testicular volume is an important correlate of serum testosterone levels.[16,18] Ruiz-Olvera *et al.*[19] conducted a study on men with sexual dysfunction or infertility, revealing that total testicular volume was lower in those with hypergonadotropic hypogonadism compared with those with hypogonadotropic hypogonadism.[19] They further identified testicular volume as a predictor of testosterone levels.[19] Similarly, Sakamoto *et al.*[20] found that testicular volume measured by Prader orchidometer or ultrasound positively correlated with testicular function among infertile men.[20] T2DM is linked to testicular damage with subsequent male infertility. Long *et al.*[21] demonstrated testicular morphological damage in T2DM rats due to impaired microcirculation and reduced vascular endothelial growth factor (VEGF) expression.[21] Conversely, Barsiah *et al.*[22] observed a slight increase in cell number, cell layer thickness, seminiferous tubule diameter and testicular weight in T2DM rats compared with those with T1DM.[22] Tang Fui *et al.*[23] reported that testicular volume inversely correlates with body mass index (BMI) and total fat mass, independent of age and testosterone levels.[23] These findings suggest that decreased testicular volume may

be the underlying mechanism by which obesity causes low testosterone levels, similar to the effects in T2DM.[24–26]

Limited data exist on testicular volume in men with T2DM compared with healthy men. This study aimed to investigate the effects of T2DM and overweight/obesity on testicular volume and identify the correlates of testicular volume in T2DM and healthy Nigerian men.

## Methods

### *Study participants and ethics*

This cross-sectional study involved 358 men with T2DM and 179 age-matched healthy controls. Participants were further subcategorised into four groups: control non-obese, control overweight/obese, T2DM non-obese, and T2DM overweight/obese men (Figure 1). The details of the study, including the study participant enrolment, exclusion, and inclusion criteria, have been reported previously.[7, 27] Ethical clearance was obtained from the Ahmadu Bello University Teaching Hospital (ABUTH) ethical committee (ABUTHZ/HREC/NP18/2015). Eligible participants were requested to give written informed consent at recruitment, and patients who declined consent received no compromised standard care. Confidentiality was observed throughout the study.

The well-characterised clinical parameters and anthropometric measurements have been previously published.[7] Testicular volume was measured using a Prader orchidometer, with normal testicular volume defined as 15–25 ml. Non-obese was defined as a body mass index (BMI) of 18.5–24.9 kg/m$^2$, while overweight/obese was defined as a BMI of > 25 kg/m$^2$.

### *Collection and analysis of blood samples*

Fasting plasma samples (10 ml) were collected from antecubital veins between 8:00 am and 10:00 am into plain tubes and centrifuged for 10 minutes to separate the serum from the cells. The serum samples were collected into sample bottles and stored at −20°C for the analysis of total testosterone, luteinising hormone (LH), follicle-stimulating hormone (FSH), and sex hormone binding globulin (SHBG), fasting plasma glucose (FPG), and albumin. Vermeulen's method was used to calculate free testosterone.[28] Additionally, 5 ml of blood was collected into EDTA bottles for the glycated haemoglobin (HbA1c) assay. Enzyme-linked immunosorbent assay (ELISA) was used to measure serum total testosterone, LH, FSH (Monobind Inc, Lake Forest, CA, USA), and SHBG (Diagnostic Automation Inc, Woodland Hills, CA, USA).

### *Statistical analysis*

Data were analysed using GraphPad Prism software version 10 for Windows (San Diego, California, USA), and results were presented as mean ± standard error of mean (SEM). The distribution of continuous variables was assessed using the Shapiro–Wilk test. A two-way analysis of variance (ANOVA) was used to determine the effect of T2DM and overweight/obesity, as well as their interaction, on clinical and biochemical parameters and hormones. $P_{BMI}$ represents the *p*-value for the effect of BMI, while $P_{T2DM}$ represents the *p*-value for the effect of T2DM, and $P_{interaction}$ is the *p*-value for the interaction between T2DM and BMI. Significant differences among groups were determined using Bonferroni post-hoc tests. Pearson's correlation coefficient (*r*) analyses were used to determine the relationships between average testicular volume and clinical and biochemical parameters and hormones. *P*-values < 0.05 were considered statistically significant.

## Results

### *Effect of T2DM and overweight/obesity on clinical and biochemical parameters of the study population*

The sociodemographic characteristics of our study cohorts have been published previously.[7] Both T2DM and obesity independently significantly increased systolic blood pressure ($P_{T2DM}$ < 0.0001, $P_{BMI}$ = 0.0443). Bodyweight, waist circumference, and hip circumference appear to be higher in T2DM and overweight/obese men ($P_{T2DM}$ = 0.0026, $P_{BMI}$ < 0.0001; $P_{T2DM}$ < 0.0001, $P_{BMI}$ < 0.0001; $P_{T2DM}$ < 0.0001, $P_{BMI}$ < 0.0001, respectively). As expected, BMI was higher in overweight and obese men ($P_{BMI}$ < 0.0001). FPG and HbA1c were significantly higher in T2DM men. Additionally, the interaction between T2DM and overweight/obesity appeared to increase HbA1c levels ($P_{T2DM}$ < 0.0001, $P_{T2DM}$ < 0.0001, $P_{interaction}$ < 0.0095, respectively). Interestingly, T2DM independently increased total cholesterol, triglycerides, and LDL cholesterol levels ($P_{T2DM}$ = 0.0003, $P_{T2DM}$ < 0.0001, $P_{T2DM}$ < 0.0001, respectively) but did not significantly affect HDL cholesterol ($P_{T2DM}$ = 0.0875). Serum total testosterone and free testosterone concentrations were

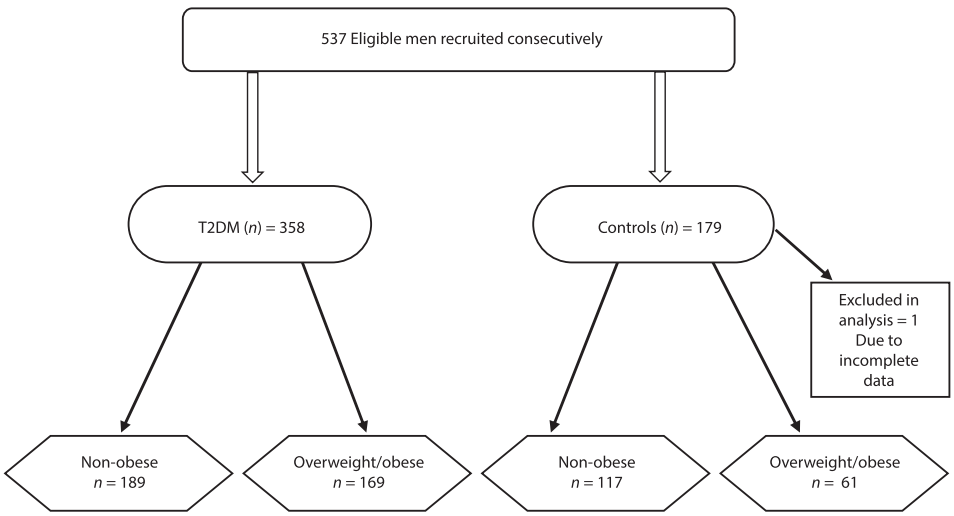


Figure 1: Flowchart of the study participants.

**Table 1.** Effect of T2DM and overweight/obesity on clinical and biochemical parameters of the study population

| Item | Control Non-obese ($n = 117$) | Control Overweight/ obese ($n = 61$) | T2DM Non-obese ($n = 189$) | T2DM Overweight/ obese ($n = 169$) | *p*-value Interaction | *p*-value T2DM | *p*-value BMI |
|---|---|---|---|---|---|---|---|
| Clinical data | | | | | | | |
| Age (years) | 6.98 ± 12.75 | 43.67 ± 14.32* | 56.38 ± 11.29# | 57.59 ± 9.746# | 0.0130 | < 0.0001 | 0.0004 |
| Systolic blood pressure (mmHg) | 127.4 ± 22.8 | 131.4 ± 22.3 | 140.8 ± 26.5# | 146.2 ± 24.4# | 0.7896 | < 0.0001 | 0.0443 |
| Diastolic blood pressure (mmHg) | 79.20 ± 13.63 | 81.16 ± 14.89 | 81.30 ± 13.75 | 83.92 ± 12.11 | 0.7954 | 0.0564 | 0.0712 |
| Weight (kg) | 61.66 ± 7.51 | 79.51 ± 11.02* | 63.69 ± 9.245 | 83.06 ± 11.01* | 0.4104 | 0.0026 | < 0.0001 |
| Body mass index (kg/m$^2$) | 22.10 ± 1.93 | 28.36 ± 3.18* | 22.08 ± 2.76 | 28.84 ± 3.07* | 0.2610 | 0.4849 | < 0.0001 |
| Waist circumference (cm) | 74.94 ± 5.89 | 88.16 ± 10.15* | 85.00 ± 8.59# | 100.7 ± 9.56*# | 0.1311 | < 0.0001 | < 0.0001 |
| Hip circumference (cm) | 83.54 ± 7.05 | 95.39 ± 8.19* | 87.71 ± 6.91# | 98.61 ± 6.86*# | 0.4785 | < 0.0001 | < 0.0001 |
| Biochemical data | | | | | | | |
| FBG | 4.358 ± 1.759 | 4.575 ± 1.872 | 8.760 ± 5.048 | 7.817 ± 4.398 | 0.1300 | < 0.0001 | 0.3434 |
| HbA1C | 5.015 ± 0.695 | 5.166 ± 0.825 | 9.467 ± 2.344 | 8.746 ± 1.762 | 0.0095 | < 0.0001 | 0.0886 |
| Total cholesterol | 3.937 ± 0.677 | 4.039 ± 0.739 | 4.234 ± 0.924 | 4.336 ± 0.925 | 0.9953 | 0.0003 | 0.2104 |
| HDL cholesterol | 1.104 ± 0.345 | 1.093 ± 0.326 | 1.053 ± 0.345 | 1.036 ± 0.309 | 0.9179 | 0.0875 | 0.6559 |
| Triglycerides | 1.13 ± 0.370 | 1.087 ± 0.356 | 1.324 ± 0.472 | 1.328 ± 0.500 | 0.5776 | < 0.0001 | 0.6531 |
| LDL cholesterol | 2.315 ± 0.515 | 2.448 ± 0.622 | 2.579 ± 0.731 | 2.695 ± 0.752 | 0.9055 | < 0.0001 | 0.0567 |
| Total testosterone | 15.67 ± 3.982 | 14.85 ± 3.378 | 8.866 ± 3.367 | 8.711 ± 3.345 | 0.3247 | < 0.0001 | 0.1475 |
| LH | 8.115 ± 5.782 | 8.341 ± 6.129 | 10.13 ± 6.847 | 9.047 ± 6.772 | 0.2913 | 0.0287 | 0.4888 |
| FSH | 5.363 ± 4.044 | 4.738 ± 3.589 | 9.031 ± 8.033 | 7.914 ± 8.294 | 0.7135 | < 0.0001 | 0.1934 |
| SHBG | 99.38 ± 69.85 | 88.81 ± 87.89 | 87.69 ± 60.84 | 83.24 ± 67.37 | 0.6383 | 0.1849 | 0.2484 |
| Albumin | 41.51 ± 3.428 | 41.54 ± 3.462 | 39.34 ± 3.320 | 40.07 ± 3.163 | 0.2639 | < 0.0001 | 0.2277 |
| Free testosterone | 0.209 ± 0.155 | 0.245 ± 0.168 | 0.109 ± 0.0674 | 0.110 ± 0.066 | 0.0858 | < 0.0001 | 0.0674 |

$P < 0.05$, pairwise comparisons. *Significantly different from non-obese. #Significantly different from control. FBG: fasting blood glucose, FSH: follicle stimulating hormone, HbA1c: glycated haemoglobin, HDL: high-density lipoprotein, LDL: low-density lipoprotein, LH: luteinising hormone, SHBG: sex hormone binding globulin.

significantly lowered by T2DM ($P_{T2DM} < 0.0001$, $P_{T2DM} < 0.0001$, respectively). Conversely, T2DM significantly increased serum LH and FSH levels ($P_{T2DM} = 0.0287$, $P_{T2DM} < 0.0001$) (Table 1).

Study participants were divided into four groups: non-obese control, overweight/obese control (≥ 25 kg/m$^2$), non-obese T2DM, overweight/obese T2DM (≥ 25 kg/m$^2$). Data are means ± SEM. Differences between groups were determined using 2-way ANOVA and Bonferroni post hoc test.

### *Testicular volume in men with T2DM and controls with or without overweight/obesity*

T2DM reduced the average testicular volume, with significantly lower average volumes observed in non-obese and overweight/obese T2DM men than in their control counterparts ($p = 0.0194$; $p = 0.0492$, respectively). Similarly, T2DM was associated with a reduction in right testicular volume; non-obese and overweight/obese T2DM men had significantly lower right testicular volumes than their non-obese and overweight/obese control

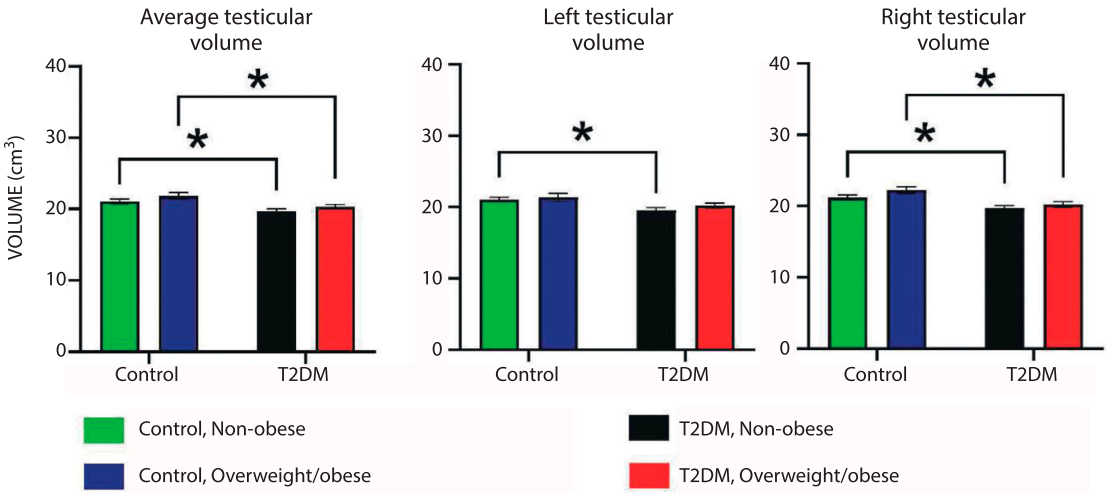


**Figure 2:** Differences in average left and right testicular volumes in men with T2DM and controls with or without overweight/obesity. Study participants were divided into four groups: non-obese and overweight/obese patients with or without T2DM. Data are represented as means ± SEM. Differences between groups were determined using 2-way ANOVA and Bonferroni post hoc test. *$p < 0.05$, pairwise comparisons.

counterparts ($p = 0.0237$; $p = 0.0123$, respectively). However, there was no significant difference in left testicular volume between controls and T2DM men overall. Notably, non-obese T2DM men had lower left testicular volume than non-obese controls ($p = 0.0330$) (Figure 2).

### *Correlation analysis between average testicular volume and clinical parameters*

A correlation analysis between average testicular volume and clinical parameters was conducted. We found a significant positive correlation between average testicular volume and bodyweight in obese men with T2DM ($p = 0.0426$). Additionally, the average testicular volume was significantly positively correlated with waist circumference in obese T2DM men ($p = 0.0269$). However, no significant relationship was found between average testicular volume and blood pressure, height, and other markers of adiposity in men with T2DM (Figure 3).

### *Correlation analysis between average testicular volume and biochemical and male reproductive hormones*

We identified significant positive correlations between average testicular volume and LDL cholesterol levels in overweight/obese T2DM men ($p = 0.0134$). Furthermore, an inverse correlation was observed between average testicular volume and FSH levels in obese men with T2DM ($p = 0.0083$); However, average testicular volume correlated negatively with LH levels in overweight/obese controls ($p = 0.0327$) (Figure 4).

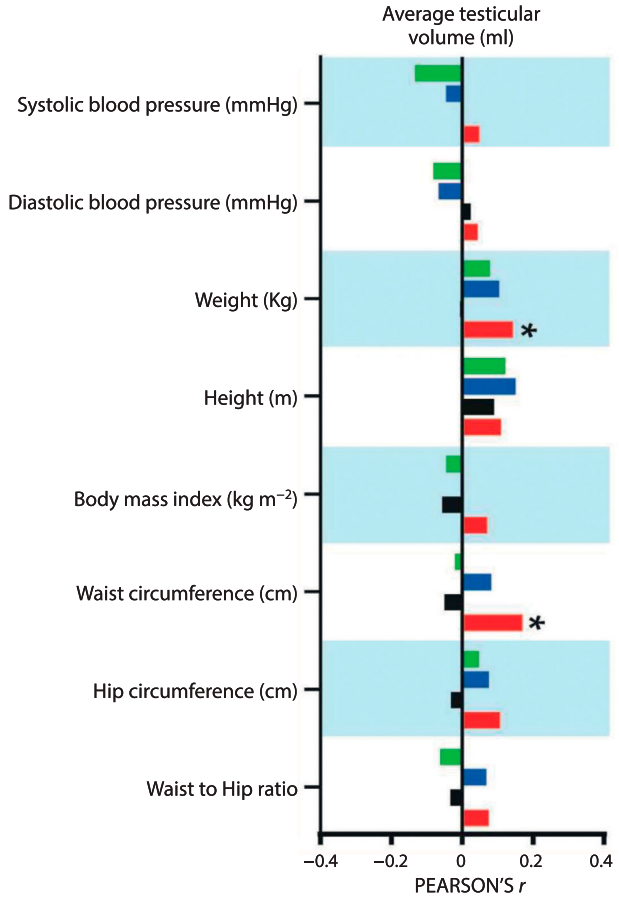


**Figure 3:** Correlation analysis between average testicular volume and clinical parameters. Study participants were divided into four groups: non-obese control (green), overweight/obese control (blue), non-obese T2DM (black), and overweight/obese T2DM (red). To indicate the strength of the relationship between data, the Spearman *r* coefficient was plotted, and analysis was performed on each group separately; *correlation is statistically significant, $p < 0.05$.

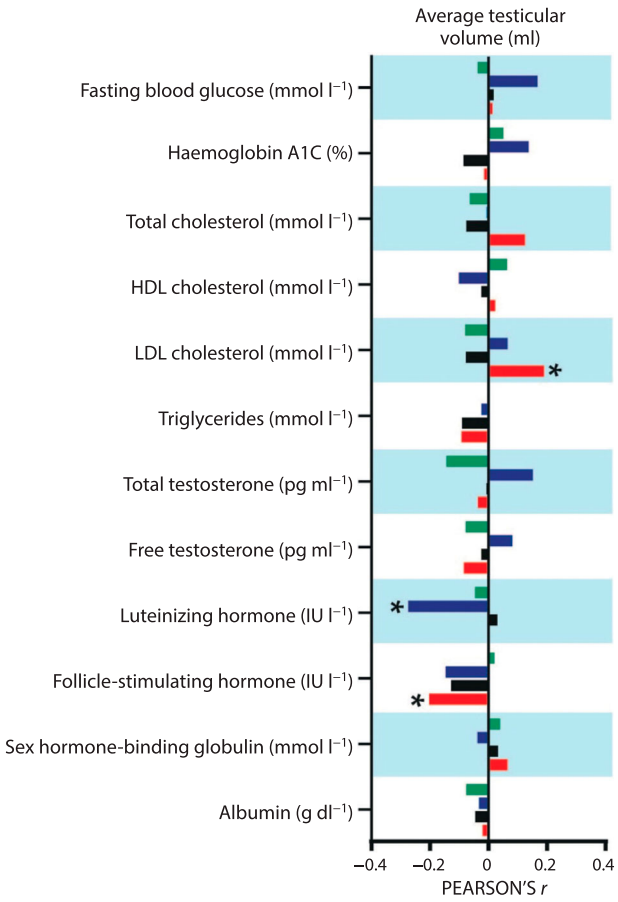


**Figure 4:** Correlation analysis between average testicular volume and biochemical parameters and male reproductive hormones. Study participants were divided into four groups: non-obese control (green), overweight/obese control (blue), non-obese T2DM (black), and overweight/obese T2DM (red). To indicate the strength of the relationship between data, the Spearman *r* coefficient was plotted, and analysis was performed on each group separately; *correlation is statistically significant, $p < 0.05$.

## Discussion

This study investigated the effect of T2DM and overweight/obesity on testicular volume and serum testosterone concentrations in control and T2DM men. To our knowledge, this is the first study to explore the impact of T2DM and overweight/obesity on the testicular volume and serum testosterone concentrations in men with T2DM. Limited studies have assessed the testicular volume in men with T2DM. Our study found that T2DM was associated with reduced average testicular volume, regardless of BMI. This finding is consistent with the results of Long *et al.*,[21] who reported lower testicular volume in type 2 diabetic rats compared with controls.[21] However, this contradicts the findings of Inih *et al.*,[29] who reported no significant difference in mean testicular volume between men with T2DM and control subjects.[29] The precise mechanisms underlying the reduced average testicular volume in men with T2DM, regardless of obesity status, remain unclear. However, several potential pathophysiological mechanisms may play a crucial role in this phenomenon.

First, hyperglycaemia can disrupt testicular microcirculation due to reduced expression of VEGF, as observed in T2DM rats. This disruption may lead to morphological changes in the testis,

increased cell apoptosis, and sperm failure via the PI3 K/Akt pathway. Such morphological changes could contribute to the observed reduction in testicular volume in men with T2DM.[21]

Second, our findings revealed that men with T2DM had lower levels of total and free testosterone, which may reduce the formation of seminiferous tubules and Leydig cells, further decreasing testicular volume.[15] Additionally, robust evidence demonstrates the role of cytokines and chemokines in regulating testicular function in both disease and physiological states.[30] T2DM is associated with hyperglycaemia-induced chronic inflammation mediated by TNFα and IL-6, leading to testicular interstitial fibrosis and reduced testicular volume.[31] These findings imply that men with T2DM are at risk of both testicular endocrine dysfunction and reproductive disruption.

Our study revealed that T2DM significantly decreases right testicular volume in men, independent of BMI, when compared with control subjects. The biological mechanisms underlying these findings remain unclear and warrant further investigation. In paediatric populations, Atalabi *et al*.[32] and Kolade-Yunusa *et al*.[33] observed that right testicular volume is greater than left testicular volume.[32,33] This difference may be attributed to a more prominent pampiniform plexus and the relatively sluggish venous drainage on the left side, which could contribute to a smaller left testis.[34] Moreover, non-obese men with T2DM displayed lower left testicular volumes compared with their non-obese normoglycaemic counterparts. The precise mechanisms explaining this observation are not well understood and require additional research.

To the best of our knowledge, this is the first study to investigate the relationship between average testicular volume and clinical parameters in men with T2DM. Interestingly, we found a positive correlation between average testicular volume and both weight and waist circumference in overweight/obese T2DM men, implying that as weight and waist circumference increase, the average testicular volume also increases. These findings contradict those of Tang *et al*.,[23] who reported that testicular volume inversely correlates with BMI and total fat mass, independent of age and testosterone levels.[23] The exact mechanism underpinning the positive correlation between average testicular volume and weight and waist circumference requires further studies. Additionally, we found that average testicular volume significantly positively correlates with LDL cholesterol levels in overweight/obese men with T2DM. This novel finding warrants further investigation to determine the pathobiological mechanisms underlying the relationship between average testicular volume and lipid profiles in overweight/obese men with T2DM. Our study also found an inverse correlation between average testicular volume and FSH and LH levels in overweight/obese men with T2DM and overweight/obese controls, respectively. The precise mechanisms to explain these findings are unknown and require further investigation.

Our study further found that T2DM, but not obesity, independently lowered serum total testosterone and free testosterone concentrations. This aligns with previous studies that have reported lower testosterone levels in men with T2DM.[6,8–11] In contrast, obesity has been widely reported to have an inverse relationship with testosterone levels.[35,36] For example, Wu *et al*.[36] found lower total and free testosterone levels in overweight and obese men compared with their lean counterparts and with greater severity in obese men.[36] The pathophysiological pathways by which T2DM causes lower testosterone concentrations include high oestrogen levels, hyperleptinaemia, inflammatory cytokines (TNFα, IL-6), increased peripheral aromatisation and persistent hyperglycaemia.[31] More recently, the peptide kisspeptin, secreted by the hypothalamus, has been shown to bind KISS1R on GnRH neurons to increase plasma luteinising hormone and testosterone; this suggests that lower serum kisspeptin concentrations may result in hypogonadotropic hypogonadism in obese and T2DM men.[37]

## Conclusion

Our study shows that men with T2DM have lower testicular volume irrespective of their BMI and this may have clinical implications for low testosterone and reproductive failure in T2DM men. Additionally, average testicular volume is directly associated with certain clinical markers of adiposity and harmful cholesterol levels, suggesting a high risk of cardiovascular disease in men with increased testicular volume. Based on these findings, routine assessment of testicular volume and serum testosterone levels should be considered in men with T2DM, particularly those presenting with symptoms of hypogonadism, to facilitate early detection of endocrine dysfunction and timely therapeutic intervention. However, the underlying pathophysiological mechanisms related to these findings remain unclear and require further investigation.

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