

# Coexistence of low testosterone and metabolic syndrome associated with increased arterial stiffness in male patients with type 2 diabetes

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## Keywords

metabolic syndrome, Pulse Wave Velocity, Arterial Stiffness, low testosterone, type 2 Diabetes

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## Abstract

### Introduction

Male patients with type 2 diabetes (T2D) have a high prevalence of low testosterone (LT) or metabolic syndrome (MetS). While LT or MetS are associated with arterial stiffness, few studies have investigated the effect of these conditions when manifested together. Our study was performed to explore the influence of coexistence of LT and MetS on arterial stiffness in male patients with T2DM.

### Material and methods

We recruited 332 male patients with T2D at the endocrine and metabolic clinic of the Taitung branch of the Mackay Memorial hospital. Subjects were divided according to the presence of LT or MetS as follows: normal (neither condition present), LT only, MetS only and the coexistence of LT and MetS. All enrolled subjects consecutively underwent brachial-ankle pulse wave velocity (PWV) to evaluate arterial stiffness.

### Results

Patients with LT have a higher prevalence of MetS than those without LT (80.1% vs. 64.2%;  $P=0.03$ ). Age, weight, triglycerides and PWV were significantly higher in patients with coexisting LT and MetS than in the groups with LT or MetS alone. Multiple linear regression analysis was performed to demonstrate that PWV was significantly positively associated with age ( $P < 0.001$ ), SBP ( $P=0.002$ ) and triglycerides ( $P=0.001$ ) and negatively associated with testosterone ( $P=0.039$ ). ANCOVA analysis revealed that PWV remained highest in patients with coexisting MetS and LT compared to the other groups, even after adjusting for confounding factors.

### Conclusions

In our study, the coexistence of MetS and LT was significantly associated with a high risk of increased arterial stiffness in male patients with T2D.

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## Explanation letter

### Response letter

Thank you very much for the reviewer's excellent and valuable comments. Our response as follows:

### Review 1:

Would it have been interesting to know if the patients with type 2 diabetes in the study were smokers and had diabetic neuropathy or not? These factors also affect the outcome of the PWV.

### #Response:

(1)  Thank you for your valuable comment.

(2)  Your suggestion was constructive. Due to our study being retrospective, our research focused on risk associated with metabolic syndrome and testosterone level on arterial stiffness. We did not collect data about smoking and diabetic neuropathy.

(3)  Page 11, Line 16: We have added a sentence to the limitations section as follows: "Relative risk factors for arterial stiffness such as smoking or diabetic neuropathy were not collected or analyzed".

## Review 2:

In this MS, the authors hypothesized that the coexistence of LT and MetS leads to increased arterial stiffness. From the results generated from this observational study, the authors concluded that the coexistence of MetS and LT was significantly associated with a high risk of increased arterial stiffness in male patients with T2D. I have a few comments listed below:

### Abstract:

1.  The aim of the study is missing

#### #Response:

(1)  Thank you for all of your valuable comments.

(2)  We added the aim of the study to the Abstract section as follows: "Our study was performed to explore the influence of the coexistence of LT and MetS on arterial stiffness in male patients with T2DM."

2. The results are given descriptively. At least the Pa values should be included.

#### #Response:

(1)  According to your recommendation, we added Pa values in the results of the abstract section.

#### Material and methods:

1.  The exclusion and inclusion criteria should be listed

#### #Response:

(1)  We have clarified our inclusion and exclusion criteria as follows in the methods section:

Page 2, Line 14: "Enrolled patients met the inclusion criteria as below: 1) male patients over 18 years-old and 2) T2DM patients."

Page 2, Line 16: "Exclusion criteria were as follows: history of coronary artery disease, stroke or peripheral artery disease, malignancy, or sepsis, or undergoing testosterone replacement therapy."

2.  The methods for measuring biochemical and clinical parameters are not included in the manuscript.

#### #Response:

(1)  I modified the paragraph "2.2 Laboratory measurements and clinical parameters" in the Methods section of the manuscript.

3.  A statement that the "Higher prevalence of MetS was closely associated with lower testosterone in our study (Figure 1)" should be supported by the Pa values.

#### #Response:

(1)  We added the Pa values and adjusted "Figure 1" as below:

### Results:

1. Why is the serum level of insulin and testosterone not measured? These results will significantly support the other results presented within this manuscript.

#### #Response:

(1)  I totally agree with your suggestion that insulin and testosterone measurements are very important to support our hypothesis. We did check serum testosterone levels but not serum insulin due to the study involving a chart review of retrospective studies. Therefore, we added serum testosterone levels to Table 1.

2.  What is the correlation between other parameters with the level of testosterone?

#### #Response:

(1)  Testosterone was significantly correlated with age, BMI, weight, waist circumference, diabetes duration, TG, HDL and ACR, as shown below.

Supplementary Table 1. Correlations between testosterone and different parameters (Pearson correlation model)

Testosterone

R  P value

Age (years)  -0.128  0.045

BMI (kg/m<sup>2</sup>)  -0.238  <0.001

Weight (kg)  -0.165  0.025

Waist (cm)  -0.236  <0.001

Diabetes duration (year)  $\square$ -0.209  $\square$ 0.002

FPG (mg/dL)  $\square$ -0.085  $\square$ NS

PPG (mg/dL)  $\square$ -0.048  $\square$ NS

HbA1c (%)  $\square$ -0.050  $\square$ NS

TG (mg/dL)  $\square$ -0.268  $\square$ <0.001

HDL (mg/dL)  $\square$ 0.321  $\square$ <0.001

eGFR (mL/min/1.73 m<sup>2</sup>)  $\square$ 0.074  $\square$ NS

ACR (mg/g)  $\square$ 0.156  $\square$ 0.03

BMI=body mass index; SBP=systolic blood pressure; FPG=fasting plasma glucose; PPG=post-meal plasma glucose; HbA1c=glycosylated hemoglobin; TG=triglyceride; HDL-C=high-density lipoprotein cholesterol; eGFR=estimated glomerular filtration rate; UACR=urine albumin-creatinine ratio; NS=not significant.

## Discussion

1.  $\square$ The discussion needs to focus more on the effects of the results obtained on CVD in the patients involved in this study. For example: "The results from our study suggest that the coexistence of MetS and LT was associated with higher PWV compared to subjects with LT or MetS alone, or with neither condition..."; how these findings affect CVD needs to be explained. Same comment for the other results.

#Response:

(1)  $\square$ We have modified our manuscript in the Discussion section on the effect of low testosterone and metabolic syndrome on CVD as per your recommendation.

2.  $\square$ A schematic presentation showing the relationship between low testosterone and metabolic syndrome, arterial stiffness and type 2 diabetes will help readers of this journal get a clear picture of the importance of the results presented.

#Response:

(1)  $\square$ Based on your suggestion, I tried to draw Figure 3, a schematic diagram to expression the relationship between low testosterone and metabolic syndrome and arterial stiffness in type 2 diabetes

Figure 3. A schematic diagram for pathogenesis of metabolic syndrome and low testosterone on arterial stiffness in T2DM

Other comments:

1.  $\square$ A list of abbreviations is recommended

#Response:

(1)  $\square$ I included a list of abbreviations in the manuscript as follow: "Abbreviations: T2D: type 2 diabetes; LT: low testosterone; MetS: metabolic syndrome; PWV: pulse wave velocity; ABI: ankle brachial index; SBP: systolic blood pressure; CVD: cardiovascular disease; eGFR: estimated glomerular filtration rate; ACR: urinary albumin-creatinine ratio."

2.  $\square$ English needs improvement

#Response:

(1)  $\square$ We are supported by a professional editing service and have improved the English.

Review 3:

Dear author. Apart from some minor comments about text quality, there is a very important dimension that you overlook in your paper and this is something very essential if you want to properly approach your results. This is the significance of advanced glycation end products (AGEs) in the determination of pulse wave velocity (PWV) and of testosterone levels. I am not proposing that you measure AGEs (it would be of great interest), but it is not acceptable to overlook this in your discussion. Only indicative I propose to look 2 publications (doi.org/10.3892/ijmm.2016.2645 and DOI: 10.1016/j.diabet.2012.04.004)

Advanced glycation end products inhibit testosterone secretion by rat Leydig cells by inducing oxidative stress and endoplasmic reticulum stress.

The relationship between tissue glycation measured by autofluorescence and pulse wave velocity in young and elderly non-diabetic populations.

So, please rewrite the introduction and the discussion considering this perspective.

#Response:

(1)  Thank you for your valuable comment.

(2)  Page 10, Line 17: We have added the interpretation of discussion section as follows: "Because all of the subjects selected had T2DM, advanced glycation end-products (AGEs) associated with insulin resistance should be considered. In a variety of metabolic diseases such as diabetes, obesity or metabolic syndrome, the formation and accumulation of AGEs are easily accelerated and subsequently trigger the pathogenesis of diabetes-related complications. Our research found that the coexistence with MetS and LT was associated with high PWV levels in T2DM patients. Additionally, AGEs readily accumulate in T2DM with metabolic syndrome and are also associated with suppressed testosterone secretion and even worsening arterial stiffness. Although AGEs were not measured in this study, we propose that these findings may be relevant to AGEs and suggest that their association is confirmed in future studies."

Review 4:

I was honored to review the manuscript entitled 'Coexistence of low testosterone and metabolic syndrome associated with increased arterial stiffness in male patients with type 2 diabetes' which describes a cross-sectional retrospective study.

The study presents good quality and deals with an important clinical issue. I have only a few comments that you should address properly listed in the attached document.

Comments to the Authors

The aim of the current study was to investigate a possible association between low testosterone and/or metabolic syndrome and high risk of increased arterial stiffness in male patients with type 2 diabetes mellitus.

The study reflects an important clinical issue and is a well-written manuscript as far as language, clarity and conciseness are concerned; there are only a few points to correct:

1.  Line 91: Please provide the relative reference.

#Response:

(1)  Thank you for all of your valuable comments.

(2)  I have provided reference in line 91.

2.  Table 1: Please illustrate in a more clarifying way between which variables there are the statistically significant differences.

#Response:

(1)  I have adjusted Table 1.

3.  Finally, please provide information on how your results will be translated into clinical practice.

#Response:

(1)  Page 9, Line 3: We add a sentence in the discussion section as follows: "We suggest that testosterone measurements, combined with traditional risk factors such as metabolic syndrome, could play an important role as a complementary method to screen for arterial stiffness in male subjects with T2DM."

Review 5:

I have read the article AMS-14336-2022-01. The article describes some medical mechanisms of the endocrine system which are many times ignored; changes which might influence the human organism functionality are more than expected, however it remains to be clarified how some hormonal components in daily foods might change the endocrine system, toward a visible increase in the masculinity of women and increased sensitivity and female behaviors for men. The article is very well written and can be accepted for publication.

#Response:

(1)  Thank you for your valuable comment.

(2)  Page 11, Line 12: I agree with your recommendation about the impact of dietary involvement on then endocrine system; therefore, I have added the following to the Discussion section of limitations: "Some of the confounding factors, such as diet, physical activity, drug use and genetics, have an impact on the development of metabolic disease and hypogonadism, but these were not recorded in our study."

#### Review 6:

The manuscript is well written, but my main concerns are the lack of data on free testosterone and SHBG and the cross-sectional design of the study. The authors should consider previous conclusions from cross-sectional data. For instance, cross-sectional data from the Framingham Heart Study cohort showed that both testosterone and SHBG levels are associated with MS. However, in a prospective study, only SHBG and not testosterone level was associated with MS (PMID: 21926281). Also, in a multivariate analysis, BMI rather than weight should be included as a covariate.

#Response:

(1) Thank you for your valuable comment.

(2) Page 11, Line 14: This is a very important perspective. In the future, if further relevant research is conducted, SHBG must be included. Our study was a preliminary study to investigate the relationship between low testosterone and MS on arterial stiffness. Since SHBG was not a common item in the hospital in Taiwan and was not provided by routine examination, we failed to collect and analyze this in the retrospective study. Therefore, I added the interpretation of limitation as follows: "Finally, serum insulin, SHBG and free testosterone measures were not available in our study because it was a retrospective study..... Despite the significant results of this cross-sectional study, additional, larger prospective clinical trials are needed to confirm these findings."

(3) I used BMI instead of weight as a covariate in the multivariate analysis.

#### Review 7:

This is an interesting observational study that analyzes the extent of arterial stiffness in patients with the coexistence of low testosterone and metabolic syndrome. The study is well written and the methods and results are clearly presented. The main concern is related to the study design. The authors analyzed differences in PWV values determined in subjects with the concomitant presence of metabolic syndrome and low testosterone in comparison with subjects with only low testosterone, only metabolic syndrome and without any of these conditions. However, all subjects had T2DM, therefore metabolic alterations inherent to metabolic syndrome and obesity-induced hypogonadism were present in each of them and no true control group was included in the analyses. Comparison with diabetes-free subjects with/without MetS and low testosterone would greatly improve the significance of the obtained results.

1. The aim of the study should be defined carefully. Namely, the authors stated that they aimed to explore the hypothesis that the coexistence of LT and MetS leads to increased arterial stiffness. However, this is an observational study, so no causal relationship can be revealed.

#Response:

(1) Thank you for all of your valuable comments.

(2) Page 2, Line 6: We have modified our sentence in the introduction section about hypothesis as follows: "We hypothesized that the coexistence of LT and MetS was associated with increased arterial stiffness".

2.

Subjects and methods

The criteria for low testosterone are not completely clear. Did the authors search for possible reasons for low testosterone, other than obesity and diabetes in their patients? What were the exclusion criteria?

#Response:

(1) Page 4, Line 9: We have clarified our definition of low testosterone as follows in the Methods section: "LT was defined as total testosterone level <300 ng/dl based on the American Urological Association (AUA) guidelines of 2018. We divided all of the included patients into those with LT or those without".

(John P Mulhall. Evaluation and Management of Testosterone Deficiency: AUA Guideline. J Urol. 2018;200: 423-432.)

(2) Page 10, Line 12: We have added the interpretation of discussion as follows: "Low endogenous testosterone was also attributed to hypothalamic suppression due to pro-inflammatory adipocytokines and dysregulated leptin signaling" as other possible reasons for low testosterone.

(3) Page 2, Line 16: We have clarified our exclusion criteria as follows in the Methods section: "Exclusion criteria were as follows: history of coronary artery disease, stroke or peripheral artery disease, malignancy, or sepsis, or undergoing testosterone replacement therapy."

3. Results

## Figure 2

The title of this figure is somewhat misleading: The figure represents the results of ANCOVA analysis for differences in PWV among subgroups of examinees, but not the interaction of low testosterone and metabolic syndrome. Similarly, in the Discussion, the authors stated that synergism between low testosterone and MetS was demonstrated, although such analysis was not conducted, given that synergism represents a greater combined effect of two or more factors on the analyzed variable than the sum of their particular effects.

#Response:

(1)  We adjusted the title of Figure 2 according to your recommendation as follows: "Differences in brachial-ankle pulse wave velocity among different subgroups of male patients with T2DM".

(2)  The use of "Synergistic effect" was not appropriate for our study, so we deleted this sentence from the Discussion section as below: "Our study was performed to explore whether the coexistence of LT and MetS has a synergistic effect on arterial stiffness".

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Preprint

**Title of the article:** Coexistence of low testosterone and metabolic syndrome associated with increased arterial stiffness in male patients with type 2 diabetes

## **Abstract**

**Background:** Male patients with type 2 diabetes (T2D) have a high prevalence of low testosterone (LT) or metabolic syndrome (MetS). While LT or MetS are associated with arterial stiffness, few studies have investigated the effect of these conditions when manifested together. **Our study was performed to explore the influence of coexistence of LT and MetS on arterial stiffness in male patients with T2DM.**

**Materials and Methods:** We recruited 332 male patients with T2D at the endocrine and metabolic clinic of the Taitung branch of the Mackay Memorial hospital. Subjects were divided according to the presence of LT or MetS as follows: normal (neither condition present), LT only, MetS only and the coexistence of LT and MetS. All enrolled subjects consecutively underwent brachial-ankle pulse wave velocity (PWV) to evaluate arterial stiffness.

**Results:** Patients with LT have a higher prevalence of MetS than those without LT (80.1% vs. 64.2%;  $P=0.03$ ). Age, weight, triglycerides and PWV were significantly higher in patients with coexisting LT and MetS than in the groups with LT or MetS alone. Multiple linear regression analysis was performed to demonstrate that PWV was significantly positively associated with age ( $P < 0.001$ ), SBP ( $P=0.002$ ) and triglycerides ( $P=0.001$ ) and negatively associated with testosterone ( $P=0.039$ ). ANCOVA analysis revealed that PWV remained

highest in patients with coexisting MetS and LT compared to the other groups, even after adjusting for confounding factors.

**Conclusion:** In our study, the coexistence of MetS and LT was significantly associated with a high risk of increased arterial stiffness in male patients with T2D.

**Keywords:** Pulse Wave Velocity, Arterial Stiffness, low testosterone, metabolic syndrome, type 2 diabetes.

**Abbreviations:** T2D: type 2 diabetes; LT: low testosterone; MetS: metabolic syndrome; PWV: pulse wave velocity; ABI: ankle brachial index; SBP: systolic blood pressure; CVD: cardiovascular disease; eGFR: estimated glomerular filtration rate; ACR: urinary albumin-creatinine ratio.

## 1 **1. Introduction**

2 Male patients with type 2 diabetes (T2D) have a high prevalence of low total testosterone  
3 (LT).<sup>1</sup> Low testosterone is closely associated with some cardiovascular risk factors such as  
4 dyslipidemia, hypertension, obesity, insulin resistance and metabolic syndrome (MetS).<sup>2</sup>  
5 Clinically, male patients with T2D often present with combined with LT and MetS due to the  
6 same core pathophysiology: insulin resistance and obesity.<sup>3</sup> Although T2D has not yet been  
7 identified as a risk factor for LT, it has been shown to be correlated in many studies.<sup>4,5</sup> Low  
8 testosterone is also associated with major cardiovascular disease (CVD), such as coronary  
9 heart disease, stroke and peripheral arterial disease.<sup>6</sup> In addition to LT, MetS was associated  
10 with several clustering risk factors of CVD such as central obesity, hyperglycemia,  
11 hypertension and dyslipidemia.<sup>7</sup> Some studies have also demonstrated that MetS increased the  
12 incidence of CVD and related mortality.<sup>8</sup> The presence of MetS has been shown to be a key  
13 phenotype leading to atherosclerosis and diabetes.<sup>9</sup> However, the interaction between LT and  
14 MetS and their combined effects on the risks of CVD has not been investigated in detail.  
15 Atherosclerosis resulting in early abnormal cardiometabolic diseases is mainly related to  
16 endothelial and vascular smooth muscle dysfunction. Branchial-ankle pulse wave velocity  
17 (PWV) is one of the most convenient parameters and non-invasive techniques used to  
18 determine arterial stiffness.<sup>10</sup> Some studies have demonstrated that PWV-confirmed  
19 atherosclerosis is correlated with lower testosterone levels.<sup>11,12</sup> In addition, MetS is thought to

1 be associated with high PWV.<sup>13,14</sup> Arterial stiffness is not only recognized as a cardiovascular  
2 risk factor, but is also an independent predictor of all-cause CV morbidity and mortality.<sup>15</sup>  
3 Although both LT and MetS are associated with arterial stiffness, whether the interaction  
4 between LT and MetS further increases arterial stiffness, as indicated by PWV, has not been  
5 determined.

6 We hypothesized that the coexistence of LT and MetS was associated with increased  
7 arterial stiffness. We conducted this observational study to highlight the relationship between  
8 MetS, LT and PWV in male patients with T2D.

## 10 2. Methods

### 11 2.1 Study design and participants

12 The present study was retrospective and cross-sectional. The study was carried out at the  
13 endocrine and metabolic clinic of the Taitung branch of the Mackay Memorial Hospital from  
14 September 2014 to February 2015. Enrolled patients met the inclusion criteria as follows: 1)  
15 male patients over 18 years-old and 2) T2DM patients. A total of 370 patients were recruited  
16 during this time. Exclusion criteria were as follows: history of coronary artery disease, stroke  
17 or peripheral artery disease, malignancy, or sepsis, or undergoing testosterone replacement  
18 therapy. Thirty-eight patients were excluded due to hospitalization for acute illness,  
19 established CVD, malignancy, or sepsis, or were already receiving testosterone replacement

1 therapy.

2 Participants were treated with intensive management for diabetes and hypertension for at  
3 least six months and received lifestyle control recommendations from diabetes educators. This  
4 study was approved by the Institutional Ethics Committee at Mackay Memorial Hospital  
5 (16MMHIS088e).

6 The study sample was divided into four groups as follows: normal (no LT or MetS) (n=63)  
7 and presenting with LT only (n=31), MetS only (n=113), or with both LT and MetS (n=125).

## 8 **2.2 Laboratory measurements and clinical parameters**

9 Blood pressure was measured in both arms at rest for at least 5 minutes, with the average  
10 being taken for analysis. The weight, height and waist circumference of participants were  
11 measured while wearing light clothing without shoes. The body mass index was calculated as  
12  $\text{kg/m}^2$  (body weight [kg] / height [m]<sup>2</sup>). The following laboratory results were obtained for  
13 each patient: plasma glucose, glycated hemoglobin (HbA1c), lipid profiles (total cholesterol,  
14 low density lipoprotein cholesterol, high density lipoprotein cholesterol and triglycerides),  
15 liver enzymes, serum creatinine, estimated glomerular filtration rate (eGFR,) and urinary  
16 albumin-creatinine ratio (ACR). The calculation of eGFR (expressed in ml/min/1.73m<sup>2</sup>) was  
17 modified by the Diet in Renal Disease Study formula and further adjusted for the Taiwan  
18 ethnicity.<sup>16</sup>

## 19 **2.3 Definition of metabolic syndrome**

1 The diagnosis of MetS was conducted on the basis of the National Cholesterol Education  
2 Program Adult Treatment Panel III (NCEP-ATPIII) and modified criteria including the waist  
3 circumference cutoff for Asian populations.<sup>7</sup> Since the patients in this study all had T2D, a  
4 diagnosis of MetS was established as long as two of the following four criteria were met:  
5 waist circumference >90 cm, fasting plasma triglyceride >150 mg/dL, fasting HDL-C  
6 <40mg/dL and blood pressure >130/85 mmHg.

#### 7 **2.4 Definition of low testosterone**

8 All subjects were assessed for serum total testosterone using radioimmunoassay. Serum  
9 testosterone was measured between 8:00 am and 10:00 am during fasting. **LT was defined as**  
10 **total testosterone level <300 ng/dl base on the American Urological Association (AUA)**  
11 **guidelines of 2018. We divided all of the patients into those with LT or those without.**<sup>17</sup>

#### 12 **2.5 Arterial stiffness measurements**

13 Colin VP-1000 (Model BP203RPE III, Omron Corporation, Japan) is an arteriosclerosis  
14 test device using an ankle brachial index (ABI) and brachial-ankle PWV to assess subclinical  
15 atherosclerosis. Subjects need to rest for at least 5 minutes in the supine position before taking  
16 the test. ABI is calculated by the higher systolic blood pressure (SBP) reading as measured at  
17 the dorsal foot or posterior tibial artery, divided by the higher brachial artery systolic blood  
18 pressure.<sup>18</sup>

#### 19 **2.6 Statistical analysis**

1 We compared baseline characteristics between four groups using one-way ANOVA for  
2 continuous variables. Baseline characteristics of the study participants are expressed as the  
3 mean  $\pm$  SD or median with IQR. Correlations between PWV and clinical variables were  
4 assessed using the Pearson correlation coefficient. The average PWV between four groups  
5 was analyzed using an ANCOVA test after adjusting for confounding factors as a covariate.  
6 Multivariate stepwise linear analyses were then performed to obtain predictive factors of the  
7 clinical outcomes in order to analyze the relationship; all significant variables were  
8 incorporated in the Pearson correlation analysis. Results were considered significant when the  
9 probability (p-value) was less than 0.05% (two-sided). All of the statistical analyses and  
10 graphs were made using IBM SPSS release 21.0 (IBM, Armonk, New York).

11

### 12 **3. Results**

13 Three hundred and thirty-two male patients with T2D were enrolled, with ages ranging  
14 from 19 to 90 years. Comparisons between subjects' clinical and biochemical characteristics  
15 for those with LT and/or MetS and normal individuals are shown in Table 1. BMI, waist,  
16 triglyceride, HDL, eGFR and PWV values in subjects with LT and/or MetS were significantly  
17 higher than in normal subjects. In this study, the prevalence of MetS was 71% and LT was  
18 47%. Subjects with LT demonstrated a higher prevalence of MetS than those without LT  
19 (80.1% vs. 64.2%; **P=0.03**) (Figure 1).

1 In the Pearson correlations, PWV was significantly positively correlated with age, SBP and  
2 TG and PWV level was negatively correlated with testosterone, weight and eGFR (Table 2).  
3 As shown in Table 3, in the univariate analysis, PWV was significantly positively associated  
4 with age, SBP and hypertriglyceridemia and negatively associated with weight, eGFR and  
5 testosterone. After multiple linear regression analysis, PWV remained significantly positively  
6 associated with age, SBP and triglyceride and negatively associated with testosterone but not  
7 with weight or eGFR.

8 The mean PWV value of the group with both MetS and LT was highest among the other  
9 groups. The mean PWV in patients with MetS only was higher than in those with LT only or  
10 normal individuals. After adjusting for age, systolic blood pressure, weight, triglyceride and  
11 eGFR, the mean PWV in subjects with both LT and MetS was significantly higher than that in  
12 other groups, as determined by ANCOVA analysis (Figure 2).

#### 14 **4. Discussion**

15 To the best of our knowledge, this is the first study to investigate the association between  
16 MetS and/or LT with PWV in male patients with T2D. Significant differences in age, BMI,  
17 waist circumference, TG, HDL, eGFR and PWV were observed among the four patient  
18 groups (normal, LT only, MetS only and LT/MetS). For male patients with T2D, LT was often  
19 accompanied by MetS due to obesity and insulin resistance. Some studies have demonstrated

1 that low testosterone is an independent risk factor for high PWV.<sup>12</sup> This result was also  
2 observed in subjects with T2D,<sup>11</sup> including in our study. Many studies have suggested that  
3 MetS and increasing components of MetS were associated with high PWV.<sup>13,14</sup> While  
4 previous studies have shown that either LT or MetS are associated with arterial stiffness, we  
5 wonder whether coexistence of MetS and LT may be associated with aggravated arterial  
6 stiffness in male patients with T2D. The results from our study suggest that the coexistence of  
7 MetS and LT was associated with higher PWV compared to in subjects with LT or MetS  
8 alone, or in those with neither condition.

9 Arterial stiffness is thought to be a risk factor for CVD. Brachial-ankle and carotid-femoral  
10 PWV, primarily used in Asian countries, were established to indicate arterial stiffness and  
11 demonstrate its associations with CVD risk factors and clinical events.<sup>19,20</sup> Arterial stiffness is  
12 mainly attributable to the cumulative effect of related risk factors on the vessel wall.<sup>21</sup> Obese  
13 male patients with T2D often present with combined MetS and LT. Although MetS and LT  
14 both prompt arterial stiffness, few studies have investigated whether MetS or LT have a  
15 cumulative effect on PWV. Our study showed that coexistence with MetS and LT was  
16 associated with high PWV levels, possibly due to different pathogenic mechanisms. (Figure 3)  
17 The cluster of cardiovascular risk factors associated with arterial stiffness, including  
18 abdominal obesity, hyperglycemia and dyslipidemia, were also components of MetS. It is  
19 unclear how MetS may cause a deterioration in arterial stiffness, but current evidence

1 supports a vital role for inflammation in atherosclerosis progression in individuals with  
2 MetS.<sup>22</sup> Relative markers of inflammation, such as C-reactive protein, tumor necrosis factor-  
3  $\alpha$ , interleukin-6 and interleukin-18, were associated with the promotion of atherosclerosis and  
4 increased cardiovascular risk.<sup>23,24</sup> Insulin resistance was also strongly associated with MetS in  
5 T2D and an increased risk of high PWV. Hyperinsulinemia promotes the accumulation of  
6 endothelium collagen, enhances the hypertrophy of arterial smooth muscle and stimulates the  
7 sympathetic nervous system.<sup>25</sup> Different component clusters of MetS have variable  
8 associations with PWV. Subjects with increased components of MetS demonstrated higher  
9 PWV compared to those without MS.<sup>26</sup>

10 Generally, LT predicts the development of MetS.<sup>27</sup> The higher prevalence of MetS was  
11 closely associated with lower testosterone in our study (Figure 1). Visceral adiposity, which  
12 was present in all subjects with LT, MetS and T2D, caused vascular dysfunction with adverse  
13 sequelae of increasing CVD risk through pro-inflammatory factors.<sup>28</sup> In middle-aged men  
14 with diabetes, low testosterone also aggravated endothelial dysfunctions and atherosclerotic  
15 plaques and increased highly sensitive C-reactive protein compared to in men with normal  
16 testosterone.<sup>29</sup> Some studies suggest that arterial stiffness related to testosterone deficiency  
17 may be a possible mechanism that causes CVD and increased all-cause mortality.<sup>30</sup> In some  
18 studies, LT was associated with premature death due to CVD.<sup>31</sup> It is believed that sufficient  
19 testosterone had positive effects on coronary vasculatures, myocardium and the cardiac renin-

1 angiotensin system.<sup>32</sup> One longitudinal study revealed that men with LT have a higher  
2 prevalence of cardiovascular-renal disease and MetS than those with normal testosterone.<sup>33</sup>  
3 We suggest that testosterone measurements, combined with traditional risk factors such as  
4 metabolic syndrome, could play an important role as a complementary method to screen for  
5 arterial stiffness in male subjects with T2DM.

6 In male patients with T2D, if presenting with symptoms such as lower sexual desire,  
7 impotence, weakness or fatigue, morning serum total testosterone should be measured. The  
8 need for routine testosterone examination in male patients with T2D is still uncertain. Due to  
9 the frequent relationship between LT and T2D, it is necessary to explore the significance of  
10 LT in T2D. Endogenous testosterone levels associated with acute myocardial infarction or all-  
11 cause mortality have been established in the T2D population.<sup>34,35</sup> Lower testosterone  
12 concentrations were also correlated with a higher incidence and severity of coronary heart  
13 disease.<sup>36</sup>

14 In the present study, the prevalence of MetS, LT, or both was 69.9%, 43.6% and 33.7%,  
15 respectively. Patients with both LT and MetS had higher waist circumferences, BMI, TG,  
16 eGFR, ACR and PWV compared with the other three groups. Although LT is associated with  
17 MetS and T2D, the degree of association is variable. In our study, the prevalence of MetS  
18 increased in patients with lower testosterone levels (Figure 1). Serum testosterone is mainly  
19 released due to luteinizing hormone (LH) stimulation, which releases after the stimulation of

1 gonadotropin-releasing hormone (GnRH) from the hypothalamus. Total testosterone levels  
2 consist of sex hormone-binding globulin (SHBG) and albumin bound by testosterone and free  
3 testosterone. Although both circulating testosterone bound to albumin and free testosterone  
4 are biologically measurable, about half of total testosterone is underestimated due to less  
5 SHBG being bound by testosterone. For male patients with T2D, the correlation between  
6 MetS and LT was close, but a definite mechanism or causality was not determined. The effect  
7 of age or obesity could affect serum total testosterone through their respective mechanisms.  
8 Although SHBG levels increase with age, lower testosterone levels are found in the elderly  
9 due to bioavailable testosterone levels decreasing to a greater extent. Obesity can cause a  
10 decrease in SHBG, leading to a subsequent decrease in total testosterone levels.<sup>37</sup> In an animal  
11 model, LH concentrations reduced by 60–90% were attributed to the inactivation of insulin  
12 receptors due to insulin resistance, resulting in decreased testosterone levels.<sup>38</sup> Low  
13 endogenous testosterone was also attributed to hypothalamic suppression due to pro-  
14 inflammatory adipocytokines and dysregulated leptin signaling.<sup>39</sup> Some studies have  
15 suggested that obesity can also lead to androgen deficiency in men with T2D by enhancing  
16 aromatase activity.<sup>40</sup>

17 Because all of the subjects selected had T2DM, advanced glycation end-products (AGEs)  
18 associated with insulin resistance should be considered. In a variety of metabolic diseases  
19 such as diabetes, obesity or metabolic syndrome, the formation and accumulation of AGEs are

1 easily accelerated and subsequently trigger the pathogenesis of diabetes-related  
2 complications.<sup>41</sup> Our research found that the coexistence of MetS and LT was associated with  
3 high PWV levels in T2DM patients. Additionally, AGEs readily accumulate in patients with  
4 T2DM and metabolic syndrome and are also associated with suppressed testosterone secretion  
5 <sup>42</sup> and even worsening arterial stiffness.<sup>43</sup> Although AGEs were not measured in this study, we  
6 propose that these findings may be relevant to AGEs and that their association should be  
7 confirmed in future studies.

8 Several limitations need to be considered when interpreting these findings. First, as this  
9 study was cross-sectional and data were collected in a single hospital, it was difficult to  
10 establish the exact effect of LT and MetS on PWV due to the small sample size and  
11 retrospective design of the study. While there is a risk of overestimating effects in subgroup  
12 analyses, these findings were significant across the strata. Some of the confounding factors,  
13 such as diet, physical activity, drugs use and genetics, have an impact on the development of  
14 metabolic disease and hypogonadism, but these were not recorded in our study.<sup>44</sup> Finally,  
15 serum insulin, SHBG and free testosterone measures were not available in our study because  
16 it was retrospective, so relative risk factors for arterial stiffness such as smoking or diabetic  
17 neuropathy were not also collected or analyzed and we could not explain the influence of  
18 these values on PWV. Despite the significant results of this cross-sectional study, additional,  
19 larger prospective clinical trials are needed to confirm these findings.

1

2 **5. Conclusion**

3 Arterial stiffness, as evaluated by PWV, was attributed to multiple cardiovascular risk factors

4 including MetS or LT. In our study, the coexistence of MetS and LT was significantly

5 associated with high PWV in male patients with T2D.

6

Preprint

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### **Contributions**

S.C. and K.N: Conception and design of study. Y.H., C.L. and S.L: Acquisition of data (laboratory or clinical). S.C, C.L, M.C., H.S. and S.L.:Data analysis and/or interpretation. S.C. and K.N: Drafting of manuscript and/or critical revision. C.L.,and S.L.:Approval of final version of manuscript

**Competing interests.** No potential conflicts of interest relevant to this article were reported.

**Funding.** This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

**Acknowledgments.** The authors thank Ms. Fang-Ju Sun (from Department of Medical Research, MacKay Memorial Hospital, Taipei, Taiwan) for their excellent assistance in data analysis. The authors also thank all of the involved clinicians, nurses, and technicians for dedicating their time and skill to this study.

**Data Availability Statement:** All relevant data are within the paper

Table 1. Baseline characteristics of the analysis population

	normal	LT only	MetS only	MetS and LT	P value
N	63 (18.9%)	31 (9.3%)	113 (34.0%)	125 (37.6%)	
Age (year)	55.4±8.8	56.9±10.8	56.5±12.0	61.7±10.7*†	0.017
Diabetes duration(year)	6.5±5.3	8.0±4.4	7.4±10.3	8.1±6.1	0.792
BMI (kg/m <sup>2</sup> )	25.0±3.7	26.4±4.5	28.4±3.9*	28.5±3.9*	<0.001
Waist (cm)	87.2±9.1	92.0±9.4	99.6±8.6*†	101.4±8.8*†	<0.001
Weight <sup>1</sup>	69.0±10.7	75.0±16.7	79.8±13.4*	81.1±12.3*	<0.001
SBP (mmHg)	124.3±16.8	126.8±11.4	131.4±15.5	131.4±13.9	0.097
DBP (mmHg)	77.18±10.7	81.08±14.6	80.53±11.2	80.92±10.0	0.272
FPG (mg/dL)	129.7±35.9	127.2±32.7	133.6±33.9	136.7±48.8	0.762
PPG (mg/dL)	182.9±61.0	160.6±34.9	174.9±60.9	187.3±60.6	0.553
HbA1c (%)	7.5±1.9	7.4±0.6	7.2±1.2	7.3±1.2	0.590
TC (mg/dL)	157.5±36.0	149.5±38.0	159.4±33.2	149.8±30.08	0.342
TG (mg/dL)	92.6±39.8	103.2±61.0	150.6±69.9*†	186.0±87.9*†	<0.001
LDL (mg/dL)	86.66±33.67	89.5±25.3	85.9±26.7	83.2±28.7	0.914
HDL (mg/dL)	48.9±14.0	47.3±8.1	40.7±10.0*†	37.8±8.9*†	<0.001
GPT (mg/dL)	25.0±14.5	25.0±11.0	27.0±18.0	24.0±15.0	0.486
Cr (mg/dL)	1.2±1.6	0.8±0.2	1.2±1.1	1.3±1.5	0.626
eGFR (mL/min/1.73 m <sup>2</sup> )	87.6±26.9	98.5±26.0	79.4±30.4†	70.4±24.4*†	0.002
ACR (mg/g)	78.3±198.9	52.5±125.4	145.5±394.2	118.2±372.1	0.648
<b>Testosterone (ng/dL)</b>	<b>495.3±144.2</b>	<b>237.3±30.1*</b>	<b>413.3±109.1*†</b>	<b>219.5±66.0*†</b>	<b>&lt;0.001</b>
PWV (cm/s)	1570.7±226.3	1619.6±501.0	1698.1±396.4	1936.0±372.1*†	<0.001

Data are presented as the mean value or median ± standard deviation or median (IQR)

BMI=body mass index; SBP=systolic blood pressure; DBP=dystolic blood pressure; FPG=fasting plasma glucose; PPG=post-meal plasma glucose; HbA1c=glycosylated hemoglobin; TC=total cholesterol; TG=triglyceride; LDL=low-density lipoprotein cholesterol; HDL-C=high-density lipoprotein cholesterol; GPT=glutamic-pyruvic transaminase; Cr=creatinine; eGFR=estimated glomerular filtration rate; UACR=urine albumin-creatinine ratio.

\*P <0.05 for the significant difference from normal subjects.

†P <0.05 for the significant difference from LT only subjects.

‡P <0.05 for the significant difference from MetS only subjects.

Table 2. Correlations between brachial-ankle pulse wave velocity and different parameters (Pearson correlation model)

	Pulse Wave Velocity	
	R	<i>P value</i>
Testosterone (ng/dl)	-0.182	0.013
Age (years)	0.545	<0.001
BMI (kg/m <sup>2</sup> )	-0.094	NS
Weight <sup>1</sup>	-0.165	0.025
Waist (cm)	0.266	0.428
Diabetes duration (year)	0.058	NS
SBP (mmHg)	0.204	0.005
FPG (mg/dL)	0.061	NS
PPG (mg/dL)	-0.048	NS
HbA1c (%)	0.021	NS
TG (mg/dL)	0.144	0.047
HDL (mg/dL)	-0.053	NS
eGFR (mL/min/1.73 m <sup>2</sup> )	-0.166	0.027
ACR (mg/g)	-0.028	NS

BMI=body mass index; SBP=systolic blood pressure; FPG=fasting plasma glucose; PPG=post-meal plasma glucose; HbA1c=glycosylated hemoglobin; TG=triglyceride; HDL-C=high-density lipoprotein cholesterol; eGFR=estimated glomerular filtration rate; UACR=urine albumin-creatinine ratio; NS=not significant.

Table 3 Multiple linear regression analyses conducted to assess independent relationships between pulse wave velocity and clinical variables

Variable	Unadjusted		Adjusted	
	Regression coefficient (95% CI)	P	Regression coefficient (95% CI)	P
BMI (kg/m <sup>2</sup> )	-8.86 (-22.68, 4.96)	0.207	-3.43 (-16.42, 9.56)	0.603
SBP (mmHg)	4.19 (1.29, 7.11)	0.005	4.79 (1.87, 7.72)	0.001
TG (mg/dL)	0.55 (0.01, 1.09)	0.047	0.87 (0.18, 1.56)	0.014
eGFR (mL/min/1.73 m <sup>2</sup> )	-2.28 (-4.29, -0.27)	0.027	0.20 (-2.28, 2.32)	0.986
Age (year)	18.97 (14.79, 23.15)	<0.001	20.74 (15.58, 25.90)	<0.001
Testosterone (ng/dl)	-0.54 (-0.90, -0.18)	0.004	-0.45 (-0.81, -0.009)	0.015

SBP=systolic blood pressure, TG=triglyceride, eGFR=estimated Glomerular filtration rate; NS=not significant

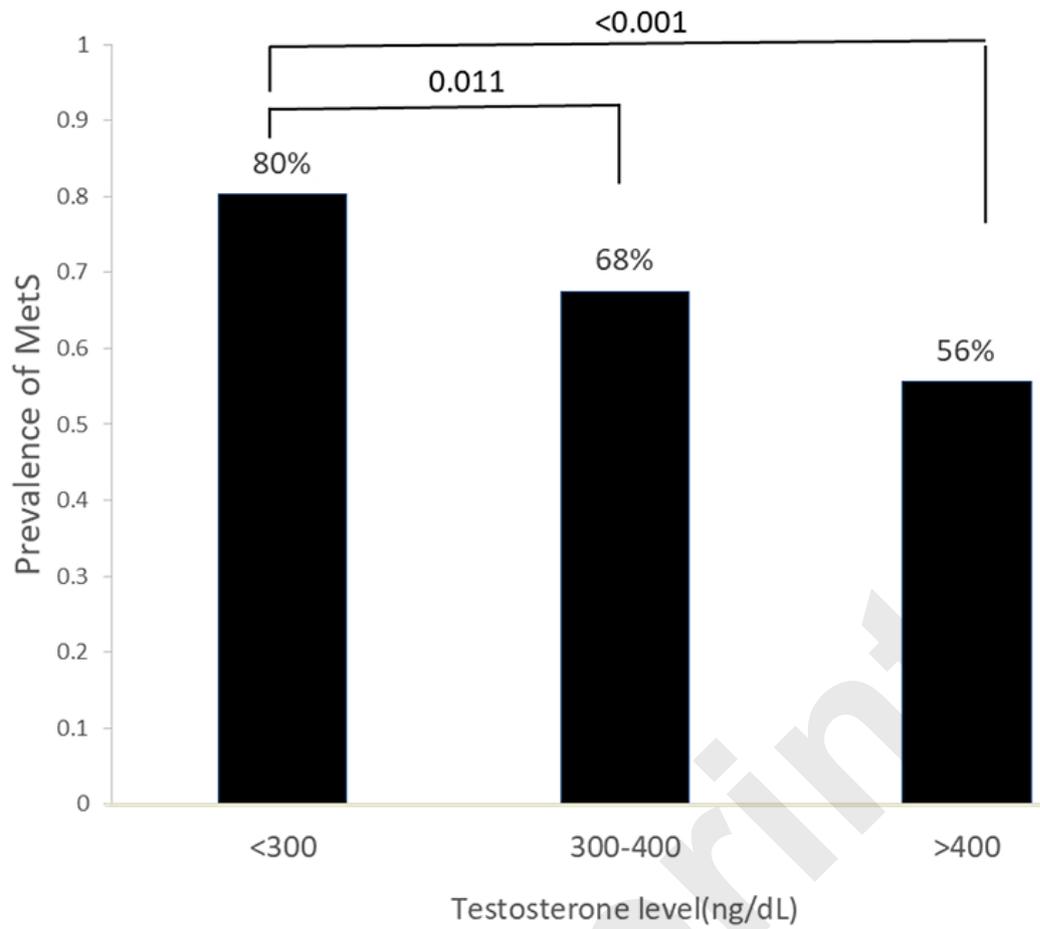


Figure 1. The prevalence of metabolic syndrome according to variable degrees of testosterone levels.

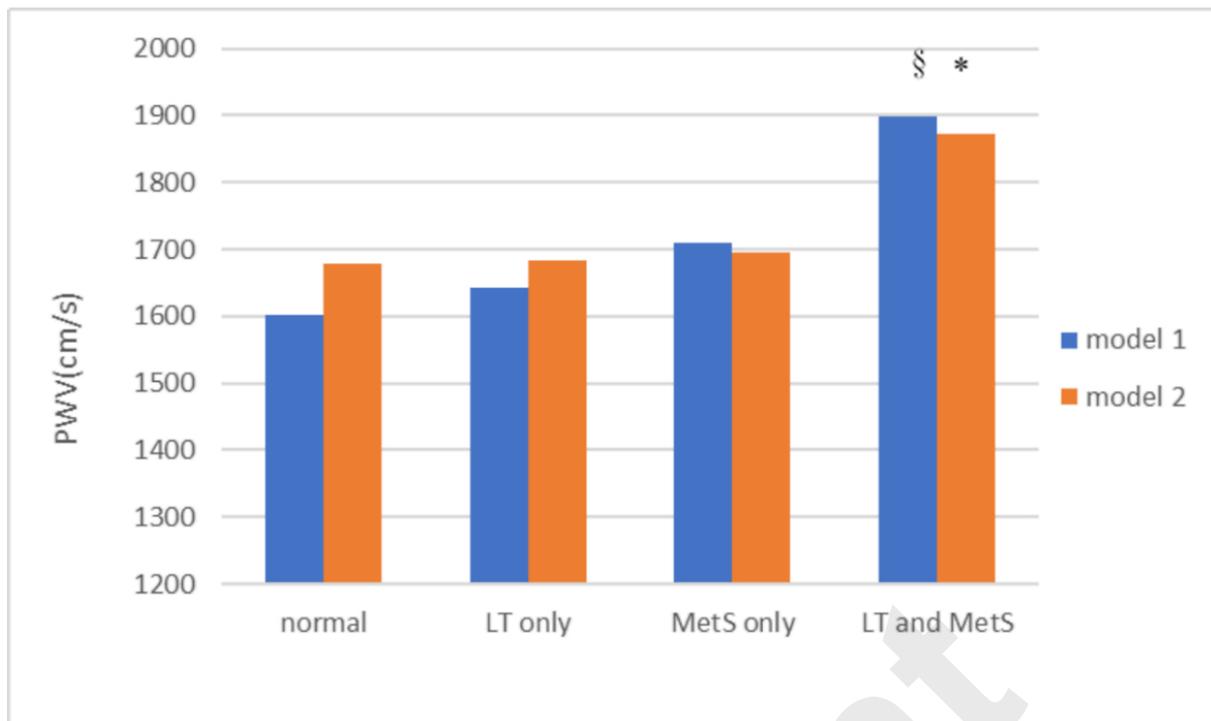


Figure 2. Differences in brachial-ankle pulse wave velocity among different subgroups in male patients with T2DM.

\* $P < 0.05$  vs. normal, LT only and MetS, §  $P < 0.01$  vs. normal, LT only and MetS only.

Model 1; adjusted for age

Model 2; adjusted for age, systolic blood pressure, weight, triglyceride and eGFR.

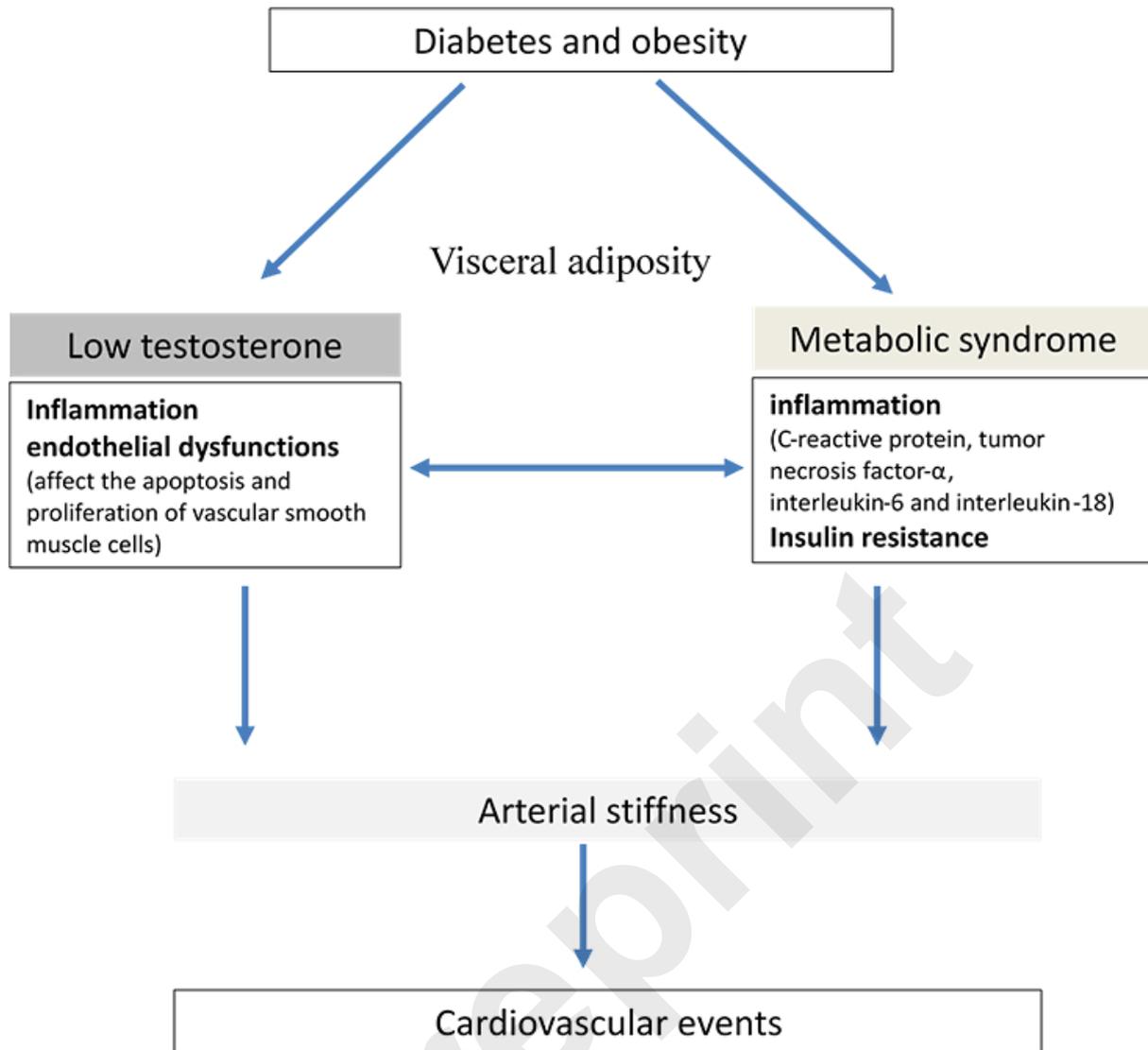


Figure 3. A schematic diagram of the pathogenesis of metabolic syndrome and low testosterone on arterial stiffness in T2DM