Comparative Effect of Vitamin D3 and Carbenoxolone Treatments in Metabolic Syndrome Rats

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<th>Journal:</th>
<th>Canadian Journal of Physiology and Pharmacology</th>
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<tr>
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<td>cjpp-2021-0400.R3</td>
</tr>
<tr>
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</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>21-Nov-2021</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Saleh, Nermine; Ain Shams University Faculty of Medicine, Physiology Department Seif, Ansam; Ain Shams University Faculty of Medicine, Physiology Department Bahaa, Ienass; Ain Shams University Faculty of Medicine, Physiology Department Abdel-Hady, Enas; Ain Shams University Faculty of Medicine, Physiology Department</td>
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<tr>
<td>Is the invited manuscript for consideration in a Special Issue:</td>
<td>Not applicable (regular submission)</td>
</tr>
<tr>
<td>Keyword:</td>
<td>Vitamin D3, carbenoxolone, obesity, insulin resistance, metabolic syndrome</td>
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Comparative Effect of Vitamin D3 and Carbenoxolone Treatments in Metabolic Syndrome Rats

Nermine Saleh, Ansam Aly Seif, Ienass Bahaa, Enas A. Abdel-Hady*

Physiology Department, Faculty of medicine, Ain Shams University, Cairo, Egypt

* Corresponding Author:
Enas A. Abdel-Hady, MD
Associate Professor of Medical Physiology
Physiology Department, Faculty of Medicine, Ain Shams University
- Address: 38 Abbasia, Cairo, Egypt
- Postal code: 11566
- Tel.: +2-01001597280
- E-mail: Dr_enas.hady@med.asu.edu.eg
- ORCID iD: https://orcid.org/0000-0002-0717-6805
ABSTRACT

Metabolic syndrome (MetS) is a cluster of cardiovascular risk factors including central obesity, hypertension, insulin resistance, dyslipidemia, and hyperglyemia. MetS is found to be a positive predictor of cardiovascular morbidity and mortality. The present study was planned to test the efficacy of vitamin D3 supplementation as compared to cortisol inhibition on MetS parameters. Wistar rats were allocated into four groups: controls, untreated MetS, and MetS treated with either vitamin D3 (10 \( \mu \text{g/kg} \)), or carbenoxolone (50 mg/kg). MetS was induced by combination of high fat diet and oral fructose. After the induction period (8 weeks), MetS was confirmed and treatment modalities started for a further 4 weeks. Compared to untreated MetS, vitamin D3 and carbenoxolone treated rats showed significant reduction in blood pressure, body mass index, lee index, waist circumference, retroperitoneal fat, and improvement of dyslipidemia. Meanwhile, treatment with carbenoxolone significantly lowered the elevated liver enzymes, vitamin D3 resulted in improved insulin sensitivity, enhanced glucose uptake by muscles and replenished glycogen content in the liver and muscles near control levels. In conclusion, although treatment with vitamin D3 or carbenoxolone reduced the risk factors associated with MetS, vitamin D3 was effective in ameliorating insulin resistance which is the hallmark of MetS.

Keywords:
Vitamin D3, carbenoxolone, obesity, insulin resistance, metabolic syndrome, cortisol, dyslipidemia
INTRODUCTION

Metabolic syndrome (MetS) is a crucial contributor to type2 diabetes, cardiovascular diseases (CVD), stroke, hepatic steatosis, and other disabilities (Saklayen 2018). In co-occurrence, the rising rates of CVD and diabetes, which are the two leading causes of death, simultaneously exist. The prevalence of MetS is higher in women than in men (Aboulghate et al. 2021), mainly driven by the constant rise in obesity in women. Therefore, to control the prevalence of MetS and prevent development into diabetes and CVD, we have to understand how MetS occurs and how it progresses. Basically, MetS appears to be due to dysregulated cellular metabolism, driven by underlying processes that lead to insulin resistance, including cellular dysfunction in adipocytes, myocytes, and hepatocytes; oxidative stress; and cellular inflammation (Swarup and Zeltser 2020).

The hypothalamic pituitary adrenal (HPA) axis is an important pathway by which perceived stress influences cardiovascular and metabolic processes (Rodriguez et al. 2015). It was documented that stress is positively associated with MetS using various criteria, even after accounting for physical activity, nutrition, smoking status, and alcohol intake (Cardel et al. 2018). Stress stimulates cortisol hormone secretion, which in turn directs target tissues to mobilize resources to address the threat. Chronic stress may result in malfunctioning HPA axis and greater overall cortisol exposure. Failure to decrease cortisol secretion across the day is associated with elevated blood pressure, hyperglycemia, and dyslipidemia (Almeida et al. 2021). In addition, all MetS symptoms occur in pathological, endogenous hypercortisolism (Cushing’s syndrome), and most of these features subside when excess cortisol is removed (Prasad Sakamuri et al. 2012). Thus, evidence supports an association between altered HPA axis
function and clinical manifestations of MetS; and that reducing cortisol action may provide a novel therapeutic approach in MetS.

The studies have shown that increased bioavailability of active glucocorticoids within tissues is more critical than the circulating pool for the development of MetS (Almeida et al. 2021). Tissue-specific metabolism of glucocorticoids is catalyzed by two enzymes, 11β-hydroxysteroid dehydrogenases type1 (11β-HSD1) and type2 (11β-HSD2). 11β-HSD1 converts inactive cortisone to physiologically active cortisol, while 11β-HSD2 inactivates cortisol to cortisone (Almeida et al. 2021). Interestingly, 11β-HSD1 is highly expressed in adipose tissue which explains the elevated glucocorticoid levels in patients with MetS (Nixon et al. 2012). Thus, pharmacological inhibition of 11β-HSD1 is believed to be an important target for the treatment of MetS.

Vitamin D3 deficiency is a worldwide phenomenon, which affects approximately 30%–50% of the world’s population (Hadjadj et al. 2018). It was documented that serum vitamin D3 levels correlate inversely with adiposity and is implicated in the pathogenesis of insulin resistance (Ganji et al. 2019). Actually, vitamin D3 deficiency is associated with higher incidence of metabolic syndrome and related risk factors of cardiovascular diseases (Melguizo-Rodríguez et al. 2021).

The diseases associated with MetS are chronic, debilitating, and lethal, and there is no single remedy can be prescribed for its eradication or even curtailment. Consequently, there is a need to find pathways or mechanisms for new therapeutic targets. The current study was conducted to evaluate the efficacy of vitamin D3 supplementation, as compared to 11β-HSD1 inhibitor in the setting of MetS.
MATERIALS AND METHODS

Experimental Animals

The study was carried on adult female Wistar rats (initially weighing 130–180 grams), that were purchased from Holding Company For Biological Products & Vaccines (VACSERA, Giza, Egypt). They were maintained at the Physiology Department Animal House, under standard conditions of boarding (temperature, 23 ± 2 °C; humidity, 55 ± 10%; and lighting, 07:00 to 19:00 h) with free access to water. All procedures involving experimental animals were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publications No. 85-23, revised 2011) and approved by the Research Ethics Committee (REC), Faculty of Medicine, Ain Shams University.

Drugs and Chemicals

Vitamin D3 was supplied in the form of a 1 ml vial containing 100 μg cholecalciferol, and dissolved in sesame oil at a concentration of 0.1 ml/ml. Both vitamin D3 and sesame oil were obtained from Arab Perfumes, Chemicals and Pharmaceutical Co., Egypt. Carbenoxolone, which is 11β-HSD1 inhibitor, was purchased as a powder from CHEMOS GmbH & Co. KG, Germany, and dissolved in distilled water at a concentration of 50 mg/ml.

Study Protocol

At first, rats were randomly divided into two groups: control rats (n=12) that were fed a regular diet (50% carbohydrates, 5% fat, 15% protein), and rats that were subjected to induction of MetS (n=36). MetS was induced by a combination of a high fat diet and oral fructose administration for 8 weeks according to Panchal and Brown (2011). Both regular and high fat diets were custom made, and sourced from the National Nutrition Institute (NNI, Cairo, Egypt). The high fat diet was butter enriched to increase the fat content of the regular diet up to 16–17 % (Holland and
Welch 1992). In MetS group, the average daily dietary requirement (≈ 5g/100g BW) was recorded, and then 30% of it was calculated and replaced with equivalent amount of fructose in grams. Fructose was supplied as a powder (Unifarma, Egypt), dissolved in distilled water at a concentration of 1.5 g/ml, and it was given daily by gavage at a dose of 1 ml/100g BW.

After 8 weeks of induction, MetS was confirmed by high levels of fasting blood glucose and triglycerides (through retro-orbital blood sampling) as well as significant increase in arterial blood pressure and BMI. Once MetS was diagnosed, this group of rats (n=36) was further subdivided into:

1. Untreated MetS rats (n=12): which continued on high fat diet and fructose for another 4 weeks.
2. Vitamin D3-treated MetS rats (n=12): which continued on MetS diet regimen and received intra-peritoneal injection of vitamin D3 at a dose of 10 μg/kg every other day for 4 weeks (Liu et al. 2011).
3. Carbenoxolone-treated MetS rats (n=12): which continued on MetS diet regimen and received subcutaneous injection of carbenoxolone at a dose of 50 mg/kg daily for 4 weeks (Prasad Sakamuri et al. 2012).

Weekly, animals were weighed and both the body mass index (BMI = Body weight / Length²) and lee index (LI = \sqrt{\text{Body weight}} / \text{Length}) were calculated according to Bernardis (1970). Arterial blood pressure (ABP) was also measured in conscious rats, using non invasive animal tail blood pressure system (NIBP200A) supplied by BIOPAC system, Inc., USA. Waist circumference was measured on the day of study termination.
**Blood and Tissue Sampling**

At the end of the experimental period (12 weeks), overnight fasted rats were weighed and anaesthetized with intra-peritoneal injection of sodium pentobarbital (40 mg/kg, Abbott Laboratories, Cairo, Egypt). A blood drop from the tail was taken for assessment of fasting blood glucose (FBG) using glucometer with specific blood glucose test strips. Then, a midline abdominal incision was made and the abdominal aorta was exposed and cannulated. Blood sample was collected into a heparinized tube and centrifuged at 3000 rpm for 15 minutes. The separated plasma was stored frozen at –20°C for subsequent determination of lipid profile, liver enzymes and hormonal assay.

Shortly after blood collection, the abdominal incision was extended upward to open the thoracic cage; the diaphragm was exposed, carefully excised, and immediately placed in ice-cold Krebs solution for 10 minutes. The diaphragm was then freed from any fat; the thick posterior portion was removed, divided into two halves and transferred to the incubation medium for studying glucose uptake. Finally, retroperitoneal (visceral) fat was dissected, dried by filter paper and weighed in a 5–Digit–Metler balance (AE 163). Liver, kidney and soleus muscle were also dissected and placed in 10 % formalin (Al-Gomhoria Company for medicines and medical supplies, Cairo, Egypt) for further histological examination.

**Biochemical Studies**

a) Glucose uptake by the diaphragm:

Diaphragm was used to measure in vitro glucose uptake by the muscles *(Kumar et al. 2016)*, under both basal condition and following insulin stimulation according to the method described by *Saleh and Saleh (2010)*. Each hemi-diaphragm was placed in a flask containing 2 ml of Krebs solution and aerated for 5 minutes with a mixture of O₂ (95%) and CO₂ (5%).
Insulin was added to only one of the hemi-diaphragm in a concentration of 20 mU/ml. The flasks were then incubated in a metabolic shaker (Kottermann D3156 Hanigsen, Germany) at 37°C for 90 minutes (shaking rate: 100 cycle/minute), together with a third flask containing 2 ml of Krebs solution without diaphragmatic tissue. At the end of the incubation period, diaphragm was removed, blotted with filter paper and weighed. Glucose concentration was measured in the incubation medium by quantitative enzymatic colorimetric technique described by Trinder (1969); using kits supplied by Stanbio, USA. Glucose uptake was calculated from the rate of decrease of glucose concentration in the media during incubation and it was expressed as mg/dl/g diaphragm/90 minute.

\[
\text{Rate of glucose uptake} = \frac{(\text{Glucose concentration before incubation} - \text{Glucose concentration after incubation})}{\text{Weight of diaphragm}}
\]

b) Lipid profile, liver enzymes and hormonal assay:

Plasma triglycerides (TG), total cholesterol (TC) and high density lipoprotein-cholesterol (HDL-C) were measured by quantitative enzymatic colorimetric technique using kits supplied by Greiner, Germany. Plasma low density lipoprotein-cholesterol (LDL-C) was calculated according to Friedewald et al. (1972) as follows: LDL-C = TC – (HDL-C + 1/5 TG). Enzyme activity of both alanin aminotransferase (ALT) and alkaline phosphatase (ALP) were measured by colorimetric optimized kinetic technique using kits supplied by Greiner, Germany. Both plasma insulin and corticosterone hormones were measured by enzyme immunoassay technique (ELISA) using kits supplied by DRG instruments GmbH, Germany. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated to reflect the whole-body insulin sensitivity from fasting glucose and insulin values, according to
Ehrampoush et al. (2021) using the following formula: HOMA-IR = Glucose (mg/dl) x Insulin (µIU/ml) / 405

**Histological Study**

Liver, kidney and soleus muscle were fixed in 10 % formalin solution immediately after dissection. The specimens were dehydrated, processed, embedded in paraffin and then serial sections of 5 µm thickness were cut and stained. Hematoxylin and eosin (H & E) was used to stain liver sections, periodic acid Schiff (PAS) stain was used to detect glycogen content in the liver and soleus muscle and Mallory stain was used to detect collagen fibers in the kidney (Bancroft et al. 1995).

**Statistical Analysis**

Results were expressed as mean ± SEM. After testing for normality using the Shapiro-Wilk test, comparisons between groups were performed using one way analysis of variance (ANOVA) followed by Tukey's *Post Hoc* Test. Student's "t" test for paired data was used to detect the significance from pre-treatment value in the same group. A probability of $p<0.05$ is considered statistically significant. All data and statistical significances were analyzed using Statistical Package for Social Science (SPSS) software (SPSS Inc., Chicago, Illinois, USA), version 16.0.

**RESULTS**

**Anthropometric measures and Blood pressure**

Before starting treatment, BMI and LI values were significantly higher in all MetS rats compared to the control group. By the end of the study, while BMI and LI continued to increase in the untreated rats, treatment with vitamin D3 significantly reduced both parameters, but they were still higher than that of the control group. Treatment with carbenoxolone produced the same
effects with a significant reduction in BMI to reach control values (Table 1). The treatment modalities also significantly reduced the increased visceral fat, which was observed in the untreated MetS group, as well as the waist circumference that reached the level of the control rats (Fig. 1).

The pre-treatment (at 8th week) values of SBP, DBP and MAP were significantly higher in MetS rats than in the control group, and continued to increase until the end of the study. In contrast, after 4 weeks of treatment with either vitamin D3 or carbenoxolone, blood pressure values were normalized (Table 1, Fig. 2).

**Lipids profile and liver enzymes**

Plasma levels of triglycerides and LDL-C were significantly elevated in untreated MetS rats, while they were normalized with vitamin D3 and carbenoxolone treatment compared to control rats. The same was observed regarding total cholesterol, unless its level was still higher in the treated rats compared to the control group. The significant reduction in HDL-C level in untreated MetS rats was reversed by both vitamin D3 and carbenoxolone treatment although it did not reach the level of significance (Fig. 3).

The significantly elevated plasma ALT level in untreated MetS rats was completely normalized upon treatment with either vitamin D3 or carbenoxolone. However, plasma ALP was significantly increased in all MetS-induced groups, whether treated or not compared to control rats. Carbenoxolone treatment revealed significant reduction in ALP level compared to both untreated and vitamin D3-treated rats (Fig. 3).

**Blood glucose, glucose uptake and hormonal assay**

MetS rats had significantly increased FBG, plasma insulin and HOMA-IR accompanied with diminished glucose uptake by the diaphragm (basal and insulin-stimulated) compared to normal
controls. Treatment with vitamin D3 significantly reduced FBG and insulin level and enhanced glucose uptake by the diaphragm compared to untreated rats. Meanwhile, carbenoxolone treatment had no effect on insulin level and glucose uptake, although it significantly reduced FBG. Insulin resistance as measured by HOMA-IR was significantly decreased by both treatments in MetS rats as compared with their respective untreated rats. Plasma corticosterone level was significantly elevated in all MetS-induced groups compared to control rats, and then decreased only by carbenoxolone treatment; however, it did not reach the control value (Fig. 4).

**Histological examination**

Liver sections from untreated MetS rats stained with H & E showed marked congestion of liver sinusoids and central vein. The hepatocytes appeared greatly distorted with many pyknotic and irregular nuclei. The cytoplasm revealed marked vaculation and areas of disintegration. Treatment with vitamin D3 resulted in minimal improvement as compared to untreated MetS rats. Although mild congestion was apparent, many of the nuclei appeared pyknotic and irregular with vaculated cytoplasm. Interestingly, carbenoxolone treatment revealed marked improvement of liver architecture and most of cells showed vesicular nuclei. Very minimal congestion could be detected and few cells showed vacuolated cytoplasm (Fig. 5(a)). PAS-stained sections revealed marked depletion of glycogen content in hepatocytes of untreated MetS group compared to control rats. Vitamin D3 treatment showed restoration of glycogen content near control levels, whereas in carbenoxolone-treated group, the glycogen content was still below that of control rats (Fig. 5(b)).

Soleus muscle sections stained with PAS stain showed intense positive reaction for glycogen in the control group that was markedly decreased in untreated MetS rats. The glycogen
content was increased by treatment compared to untreated MetS rats; however it reached the
control group with vitamin D3 treatment only (Fig. 6).

In the kidney sections stained with Mallory stain, there was apparent increase in collagen
content in the glomeruli of untreated MetS group compared to control rats, which was decreased
by treatment of either vitamin D3 or carbenoxolone (Fig. 7).

**DISCUSSION**

The present study aimed at investigating the impact of vitamin D3 and carbenoxolone treatment
on metabolic dysregulations accompanied MetS. Rats with untreated MetS revealed significantly
elevated blood pressure associated with increased BMI & LI, impaired lipid profile, elevated liver
enzymes, and fasting hyperglycemia. The hyperglycemia was accompanied by elevated plasma
insulin and HOMA-IR score and decreased glucose uptake by the diaphragm, both basal and
insulin-stimulated, denoting insulin resistance. The results also demonstrated expansion of
visceral fat depot, which is one of the characteristic features of MetS and gives explanation for
the increased BMI and waist circumference as well as the disturbed lipid profile. This
unfavorable lipid profile was evident histologically in the liver that showed marked congestion of
the sinusoids and central veins, and the hepatocytes appeared greatly distorted with cytoplasmic
vacuolation and areas disintegration. These findings most probably caused by hepatic lipid
infiltration resulting in liver steatosis with hepatocytes injury. The significantly elevated liver
enzymes also confirm the presence of hepatic damage in untreated MetS group.

Vitamin D3-treated rats showed significant reduction in their BMI & LI associated with
improved insulin sensitivity. This improvement was indicated by the significant decrease in FBG,
plasma insulin level, HOMA-IR score and enhancement of glucose uptake by the diaphragm,
both basally as well as insulin-stimulated (that reached control levels). Histological examination revealed that the depleted glycogen content of hepatocytes and soleus muscle observed in untreated MetS group was restored by vitamin D3 treatment to become indifferent from control rats. This enhanced insulin sensitivity with subsequent improvement in glycemic control is consistent with studies that reporting an inverse association between vitamin D3 concentration and the prevalence of MetS (Ganji et al. 2019; Melguizo-Rodríguez et al. 2021). Vitamin D3 is essential for both insulin secretion and its peripheral action (Leung 2016). It improves the action of insulin on target tissues either directly, by stimulating the expression of insulin receptors and thus increasing the response (Elseweidy et al. 2017), or indirectly by regulating the level of calcium required for insulin-mediated intracellular processes (Wallace et al. 2016). Changes in intracellular calcium level contribute to peripheral insulin resistance via impaired signal transduction leading to decreased glucose transporter's activity (Ojuka 2004).

The reduction in BMI exerted by vitamin D3 supplementation represents a crucial factor to preserve insulin sensitivity, being obesity a main causative factor for the development of insulin resistance. Treatment with vitamin D3 resulted in a significant reduction in visceral adipose tissue, which was evident compared to the carbenoxolone-treated rats, although it did not reach the level of significance. This reduction also contributes to the enhanced insulin sensitivity observed in these rats. It is well known that enlargement of adipose tissue is associated with chronic low-grade inflammation, another important factor that closely links obesity with insulin resistance (Crewe et al. 2017). Enlarged adipocytes overproduce pro-inflammatory cytokines that ultimately suppress the downstream insulin signaling molecules in target tissues, resulting in dysregulation of glucose and lipid metabolism (Desai et al. 2017). Numerous data indicate that vitamin D3 is a potential negative modulator of pro-inflammatory cytokines release and
significantly reduces inflammation in adipose tissue (Landrier et al. 2016; Szymczak-Pajor and Sliwinska 2019).

Notably, experimental evidence suggested that obesity is not per se the driver of insulin resistance, but rather the accumulation of intracellular lipid metabolites is the key trigger leading to insulin resistance (Engin 2017; Swarup and Zeltser 2020). Abnormal lipid metabolism has been documented to be accompanied by increased oxidative stress that impairs various points in insulin receptor signal transduction and activates cellular apoptosis (Wimalawansa 2018; Wenclewska et al. 2019). Therefore, the diminution in visceral fat mass and correction of the atherogenic lipid profile after treatment with vitamin D3 indicate an improvement in adipose tissue metabolism and increased insulin sensitivity (Sergeev 2020). In addition, Jiang et al. (2019) reported that vitamin D3 activates peroxisome proliferator activator receptor-delta (PPAR-δ), a transcription factor involved in the mobilization and metabolism of fatty acids (FAs) in skeletal muscle and adipose tissue, which in turn reduces free FAs-mediated insulin resistance and improves sensitivity.

Information about the effect of vitamin D3 supplementation on BP in MetS revealed heterogeneous results; whereas some authors found no significant effect (Swart et al. 2018; Abboud 2020) others observed a decrease in SBP and DBP values (Shu and Huang 2018; Rajakumar et al. 2020). Herein, the results obtained add to the efficacy of vitamin D3 in reducing BP figures in MetS. We considered this reduction to be a normal consequence of the weight loss, increased insulin sensitivity and improved glucose and lipid metabolism offered by vitamin D3 supplementation. The decline in BP was histologically evident as the collagen content was lower in the glomeruli of the vitamin D3-treated rats compared to the untreated rats, indicating a regression of renal fibrosis and subsequent kidney injury. Hypertension as a fundamental component of MetS, in addition to insulin resistance and dyslipidemia are risk
factors that can elicit various kidney injurious events and foster development of chronic kidney
disease. Keeping in mind, visceral adipose tissue is a rich source of the precursor protein of
angiotensin II (AngII) as well as aldosterone synthase. Hence, the expansion of adipose tissue in
cases of MetS would be accompanied by excessive production of AngII and in turn activation of
renin-angiotensin-aldosterone system, which would eventually elevate the BP and induce kidney
damage (Zhang and Lerman 2017).

Although ALT was significantly decreased with vitamin D3 treatment, alkaline phosphatase was still significantly elevated. Histological examination of the liver revealed also minimal improvement as compared to untreated MetS group, whereas many cells showed pyknotic nuclei with vacuolated cytoplasm and apparent mild congestion indicating little hepatoprotective effect of vitamin D3. This could be explained by the persistent elevation of plasma corticosterone with its known hepatotoxic effect. Other possible explanations could be the delayed onset of treatment, short duration of treatment and/or the dose of vitamin D3 used in this study.

Since glucocorticoids (GC) are involved in the regulation of almost all metabolic pathways, any change in their level will lead to metabolic disorders. The level of GC in target tissues depends on the concentration of cortisol in the plasma (controlled by the HPA axis) as well as on the activity of 11β-HSD1, which determines the amount of cortisol at the cellular level (Almeida et al. 2021). Thus, it is likely that reducing the concentration of intracellular GC by inhibiting 11β-HSD1 is effective in treating MetS. In this context, the current study revealed that treatment with carbenoxolone (an 11β-HSD1 inhibitor) produced a significant reduction in plasma corticosterone level compared to untreated MetS rats, yet it was significantly higher than the controls. This was in agreement with Prasad Sakamuri et al. (2012) who reported 32.5% decrease in plasma corticosterone levels by carbenoxolone treatment in obese rats compared to
their matching controls. In contrast, Livingstone and Walker (2003) demonstrated that the inhibition of 11β-HSD1 activity by carbenoxolone in obese insulin-resistant rats had no significant effect on plasma corticosterone levels.

Our results demonstrated that carbenoxolone treatment was effective in reducing visceral fat mass and waist circumference with normalizing BMI and attenuating the dyslipidemia, indicating improved adipose tissue metabolism as a result of lowered GC levels. This improvement was also evident on the liver architecture that was seen by histological examination associated with the significantly lower levels of liver enzymes points to a remarkable hepatoprotective effect of carbenoxolone in MetS rats. The results were in accordance with Prasad Sakamuri et al. (2012) who documented that 11β-HSD1 inhibition by carbenoxolone decreases body fat percentage, ameliorates dyslipidemia and reduces hepatic steatosis in obese rats. Along with reduced visceral fat and improved dyslipidemia, decreased intracellular GC have lowered BP in carbenoxolone-treated rats. GC are known to increase hepatic synthesis of angiotensinogen and AngII receptor in peripheral tissues (Schnackenberg et al. 2013). Therefore, it is also possible that inhibition of hepatic 11β-HSD1 activity would reduce high blood pressure through modulating the renin-angiotensin system by decreasing the level of GC.

Although, treatment with either vitamin D3 or carbenoloxone significantly reduced FBG to the same degree, carbenoloxone showed mild glycemic control as indicated by persistence hyperinsulinemia and impaired glucose uptake by the diaphragm (basal and insulin-stimulated). In addition, histological examination revealed that the glycogen content in hepatocytes and soleus muscle was apparently increased compared to untreated MetS rats, but it did not yet reach the control level. Meanwhile, Chen et al. (2019) reported that carbenoloxone reduces FBG, plasma insulin and dramatically improves insulin resistance in the liver of obese mice induced by a high-fat diet. This discrepancy sparked the important observation that there are tissue-specific changes
in enzyme activity and that 11β-HSD1 may serve to potentiate local concentrations of active GC in a tissue-specific manner (Stimson and Walker 2013). The lack of improvement in glycemic control was further illustrated by the suggestion of Livingstone and Walker (2003) that failure of carbenoxolone to inhibit 11β-HSD1 in skeletal muscle, a major site of glucose disposal, could be behind the absence of glucose tolerance. For these reasons, carbenoxolone had positive effects on carbohydrate and lipid metabolism but little effect on peripheral glucose uptake.

CONCLUSION

The current study provided clear evidence that vitamin D3 supplementation in MetS rats improves insulin sensitivity and induces better glycemic control as indicated by decreased FBG and plasma insulin, increased glucose uptake by the diaphragm and replenishment of liver and muscle glycogen content. Whereas, the beneficial effect of carbenoxolone was mainly evident in lowering elevated liver enzymes, with no significant improvement in glycemic control. Further study is needed to evaluate different doses of vitamin D3 and/or combined treatment with vitamin D3 and carbenoxolone.

ACKNOWLEDGEMENTS

The authors express their gratitude to Dr/ Hanan H. Saleh, Professor of Histology, Faculty of medicine, Ain Shams University for her kind help in processing and examining tissue samples.

CONFLICT OF INTEREST

The authors have no conflict of interest.
REFERENCES


### Table 1. Body measures and blood pressure values of control, metabolic syndrome and treated groups.

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<td>Pre-treatment</td>
<td>0.48 ± 0.01</td>
<td>0.67 ± 0.02†</td>
<td>0.68 ± 0.02†</td>
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<td>Final</td>
<td>0.52 ± 0.02</td>
<td>0.74 ± 0.02†²</td>
<td>0.63 ± 0.02†²</td>
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<td><strong>LI (g/cm)</strong></td>
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<td>Pre-treatment</td>
<td>0.283 ± 0.004</td>
<td>0.322 ± 0.004†</td>
<td>0.327 ± 0.006†</td>
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<tr>
<td>Final</td>
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<td>0.308 ± 0.004†²</td>
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<td><strong>SBP (mmHg)</strong></td>
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<td>Pre-treatment</td>
<td>121.92 ± 1.92</td>
<td>174.58 ± 2.82†</td>
<td>176.40 ± 3.22†</td>
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<td>Final</td>
<td>125.75 ± 1.48</td>
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<td>69.00 ± 2.88</td>
<td>103.42 ± 4.10†</td>
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<td>Final</td>
<td>69.67 ± 3.08</td>
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<td><strong>MAP (mmHg)</strong></td>
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<td>Pre-treatment</td>
<td>74.67 ± 2.60</td>
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<td>75.33 ± 2.83</td>
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<td>102.42 ± 9.53†²</td>
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Note: BMI, body mass index; LI, lee index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure.

Values are presented as mean ± SEM, n=12/group. Significance of differences at p<0.05, *: from pre-treatment value of the same group, †: from control group, ‡: from metabolic syndrome group, §: between vitamin D3-treated and carbenoxolone-treated groups.
FIGURE LEGENDS

Fig. 1. Body measures of control, metabolic syndrome (MetS) and treated groups.
Values are presented as mean ± SEM, n=12/group. Significance of differences at \( p<0.05 \), *: from pre-treatment value of the same group, †: from control group, ‡: from metabolic syndrome group, §: between vitamin D3-treated and carbenoxolone-treated groups.

Fig. 2. Blood pressure values of control, metabolic syndrome (MetS) and treated groups.
Values are presented as mean ± SEM, n=12/group. Significance of differences at \( p<0.05 \), *: from pre-treatment value of the same group, †: from control group, ‡: from metabolic syndrome group.

Fig. 3. Lipid profile and liver enzymes of control, metabolic syndrome (MetS) and treated groups.
Values are presented as mean ± SEM, n=12/group. Significance of differences at \( p<0.05 \), †: from control group, ‡: from metabolic syndrome group, §: between vitamin D3-treated and carbenoxolone-treated groups.

Fig. 4. Fasting blood glucose, glucose uptake by diaphragm, HOMA-IR score and plasma insulin and corticosterone levels of control, metabolic syndrome (MetS) and treated groups.
Values are presented as mean ± SEM, n=12/group. Significance of differences at \( p<0.05 \), †: from control group, ‡: from metabolic syndrome group.
Fig. 5. (a) Light photomicrographs of the liver stained with H & E. Metabolic syndrome showed marked congestion of the liver sinusoids and central veins, hepatocytes are greatly distorted with vaculated cytoplasm. Vitamin D3 showed mild improvement, while carbenoxolone treatment restored almost normal liver architecture with very minimal congestion. (b) Liver sections stained with PAS stain showed marked depletion of glycogen content in the hepatocytes of metabolic syndrome group that was restored near control levels with vitamin D3 treatment. It was still below that of the control group in carbenoxolone-treated rats.

Fig. 6. Light photomicrographs of soleus muscle stained with PAS stain showed marked reduction of glycogen content in the metabolic syndrome group that became similar to control rats with vitamin D3 treatment. It was apparently increased by carbenoxolone treatment but still not reaching the control level.

Fig. 7. Light photomicrographs of the kidney stained with Mallory stain showed apparent increase of collagen content in the glomeruli of metabolic syndrome group, which was decreased by both vitamin D3 and carbenoxolone treatment.
**Fasting Blood Glucose**

- Control: 60 mg/dl
- MetS: 120 mg/dl
- Vitamin D3: 90 mg/dl
- Carbenoxolone: 150 mg/dl

**Glucose Uptake by Diaphragm**

- Basal: Control: 0 mg/dl, MetS: 60 mg/dl, Vitamin D3: 120 mg/dl, Carbenoxolone: 180 mg/dl
- Insulin-stimulated: Control: 60 mg/dl, MetS: 120 mg/dl, Vitamin D3: 180 mg/dl, Carbenoxolone: 240 mg/dl

**Plasma Insulin**

- Control: 0 µU/ml, MetS: 1 µU/ml, Vitamin D3: 0.8 µU/ml, Carbenoxolone: 0.6 µU/ml

**HOMA-IR**

- Control: 1.0, MetS: 1.2, Vitamin D3: 0.8, Carbenoxolone: 0.6

**Plasma Corticosterone**

Controls

Metabolic Syndrome

Vitamin D-treated

Carbenoxolone-treated
Controls

Metabolic Syndrome

Vitamin D-treated

Carbenoxolone-treated

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