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Original article

Vitamin D improves hepatic alterations in *ACE1* and *ACE2* expression in experimentally induced metabolic syndrome

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ABSTRACT

Metabolic Syndrome (MetS) is a term used to describe a cluster of pathophysiological, biochemical, and metabolic criteria; including high Blood Pressure (BP), high cholesterol, dyslipidaemia, central obesity and Insulin Resistance (IR). The Renin Angiotensin System (RAS) has a regulatory function in BP, hydroelectrolyte balance, and cardiovascular function. RAS is composed of angiotensinogen (AGT), (Ang I), (Ang II), (ACE1), (ACE2), (AT1R), (AT2R), and (Ang 1–7). Vitamin D had been proved to act as a protective factor against MetS. Therefore, the study is pursued to explore vitamin D supplementation roles on hepatic RAS in MetS experimental model. At first, 36 males Albino rats were separated into 4 groups and induced to MetS under controlled circumstances for 3 months. Then, data were collected from blood samples, whereas RNA extracted from liver were analyzed using biochemical and statistical analysis tests. As a result, the major finding was proving that vitamin D can balance the expression of *ACE1* and *ACE2*. Also, confirming that it can improve MetS components by elevating HDL and insulin levels while reducing the levels of BP, cholesterol, LDL, TG, GLU, ALT, AST, and IR. These outcomes may give a new insight into the RAS pathways associated with MetS.

1. Introduction

Metabolic Syndrome (MetS) is a term that describes a cluster of biochemical, pathophysiological, clinical and metabolic criteria; including high Blood Pressure (BP), dyslipidaemia, hypercholesterolaemia, Insulin Resistance (IR), and central obesity. Insulin resistance caused by abundant free fatty acids (FFAs) can increase blood glucose level which lead to prediabetes. An excessive influx of FFAs into liver increases triglyceride content. Increased fat in liver will cause insulin resistance as well as atherogenic dyslipidemia; which resulted in high serum triglycerides and reduced HDL cholesterol concentrations. High FFAs levels may contribute to higher blood pressure; hence the imbalance of this component will increase the incidence of Diabetes Mellitus (DM), cardiovascular disease, nonalcoholic fatty liver disease (NAFLD), chronic kidney disease and mortality (Milici, 2010; Kaur, 2014; Lee and Jeong, 2017). Many factors are involved in the prevalence of MetS globally such as geographical location; sociodemographic factors and composition of the population studied (e.g., race, sex, age, and ethnicity); and the definition used for the syndrome. Worldwide pervasiveness of MetS ranges from <10% to as much as 84% (Mabry et al., 2010; McCracken, et al., 2018).

Renin Angiotensin System (RAS) has a regulatory effect in BP, hydroelectrolyte balance, and cardiovascular function (Milici, 2010; Santos & Andrade, 2014; e Silva et al., 2017). Classic RAS includes angiotensinogen (AGT) which is secreted from liver and cleaved into angiotensin I (Ang I) by renin. Ang I in turn is processed by angiotensin converting enzyme 1 (ACE1) to produce angiotensin II (Ang II) that act via two type of receptors; Ang Type 1 Receptor (AT1R) and Ang Type 2 Receptor (AT2R).

ACE1 is a glycoprotein ectoenzyme and it is considered as the fundamental component of the RAS which regulates PB. *ACE 1* is expressed initially in endothelial cells of lung, kidney, intestine, placenta, and adipose tissue. Moreover, the main function of ACE1 is synthesizing active Ang II from Ang I and degrading bradykinin (vasodilator peptide) (Luhtala, et al., 2009). Ang II is a factor

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involved in regulating vasoconstriction, fibrosis, cell proliferation, sodium homoeostasis, and inflammation in numerous diseases; including liver cirrhosis (Shim et. al., 2018).

ACE 2 - a homologue of ACE1 - has been discovered by Tipnis, et al., (2000) and it was initially identified by collecting cDNA libraries from human heart failure and lymphoma tissues (Bindom, 2009). Currently, it is found that *ACE 2* is expressed on the cell membrane of cardiac myocytes, kidney, and testis (Hilal-Dandan, 2015). As previously stated, ACE 2 is a peptide with vasodilatory and cardioprotective effects mediated through binding to AT2Rs. ACE 2 mainly converts Ang I to Ang (1–9) and decreases Ang II level to limit its effects by metabolizing it to Ang (1–7). Ang (1–7) is a peptide with vasodilatory activity, antifibrotic, and anti-inflammatory effects, which in turn binds to the Mas receptor (Mak et al., 2015).

On the other hand, RAS components can be produced in multiple tissues and act locally, thus they are called local RAS (Lubel et al., 2008). This experiment focused on hepatic RAS since many RAS components seem to be upregulated in many liver diseases (Cheng & Leung, 2011). Hepatic RAS affects liver fatty acid metabolism via the activation of AT1R or AT2R (de Kloet et al., 2010). Ang II increases FFAs and stimulates triglyceride (TG) production via AT2R. This change results in increasing FFA flux to the liver and rising TGs levels which contributes to IR (Ran et al., 2004).

Vitamin D is a fat-soluble steroid pro-hormone circulating hormone producing in the skin following ultraviolet exposure (Ke et al., 2015). Vitamin D plays essential roles in calcium homeostasis and bone metabolism (Kulie et al., 2009). The vital form of vitamin D is 1,25-dihydroxyvitamin D ($1,25(OH)_2$ D) and mediated via Vitamin D Receptor (VDR) (Judd & Tangpricha, 2009). Vitamin D and VDR are involved in all body systems such as immune, cardiovascular and reproductive system (Li et al., 2002). Li et al., (2002), showed that vit D regulates RAS in animals as a negative endocrine regulator and keeps serum $1,25(OH)_2$ D standard at a normal level which is crucial for electrolytes, BP and extracellular fluid volume.

The target of the current study is to examine the effect of vitamin D supplementation on hepatic *ACE1* and *ACE2* expression in MetS models.

2. Material and methods

2.1. Animals and diet

All experimental procedures were performed in accordance of King Abdul-Aziz University (KAU) guidelines for animal care experimentation. The experiment was approved by Research Ethics Committee (Ref. No. PH-1442–80), Faculty of Pharmacy, KAU, Jeddah, according to Implementing Regulations of the Law of Ethics of Research on living Creatures in the Saudi Arabia. Thirty-six male albino rats with body weight (215 ± 25 g) were obtained from the Animal House Unit in Prince Naif bin Abdulaziz Health Research Center. They were housed in plastic cages (3 rats/cage) at the standard temperature 20 ± 2 °C with a 12 light/dark cycle. Rats were kept two-weeks for acclimatization to the laboratory condition earlier to the experiment. They were randomly divided into four groups (n = 9) rats and caged individually (three rats/cage). Rats of the first group (the control group) were fed on normal laboratory animal feed pellets and water (pellets are from First Mills Company with 15% crude protein, 6.50% crude fiber, 3.50% crude fat and 800 kcal energy). For the second group (vitamin D group), 500 IU/Kg/day of vitamin D_3 supplement were given daily by oral gavage (Salum et al., 2012). The rats at third group (MetS group) were induced to have MetS by giving 20% fructose solution in drinking water daily (Mamikutty et al., 2014). Rats in fourth group (MetS + vitamin D group), which were supplemented with vitamin D_3 (MetS + Vit D), received 20 percent of fructose solution and vitamin D (NOVARTIS, Switzerland) in a daily dose of 500 IU/Kg/day, orally via gavage (Salum et al., 2012). The experiment ended after twelve weeks, while food and water intake were observed on daily.

2.2. Body weight and body mass index

Rats' weight and length (nose-to-anus) were measured via portable electronic digital scale weekly. BMI for each rat was calculated using the following equation: BMI = the body weight (g) / the height (cm^2) (Garrow & Webster, 1985).

2.3. Blood pressure measurement

BP in rats was measured via Tail-Cuff Plethysmography (TC) by PowerLab 2/26 BP Controller every two weeks. BP was calculated as the mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) value of three consecutive readings obtained from each rat in the morning (Garrow & Webster, 1985).

2.4. Biochemical analysis

ELISA kits for the assays of lipid profile, glucose homeostasis and liver enzymes were named as rat total Cholesterol (Cat. No. MBS846775), rat triglyceride (TG) (Cat. No. MBS726298), rat high-density lipoprotein (HDL) (Cat. No. MBS2505961), rat low-density lipoprotein (LDL) (Cat. No. MBS702165), rat glucose (Cat. No. MBS7233226) rat Insulin (Cat. No. MBS824729), rat Aspartate Aminotransferase (AST) (Cat. No. MBS264975) and rat Alanine Aminotransferase (ALT) (Cat. No. MBS269614); and were obtained from MyBioSource, USA.

2.5. Blood samples and serum collecting

Blood samples were assembled through retroorbital route twice; once in the beginning of the study after fasting for overnight and the other in the end of nine weeks before sacrificing animal. Serum samples were centrifuged (15 min, 3000 xg) and kept at −80 °C to screen lipid profile, glucose homeostasis and liver enzymes. At the last step, rats' livers were carefully dissected out and saved at −80 °C in RNA later solution (RNA stabilization reagent- QIAGEN) to estimate the expression levels of *ACE1* and *ACE2* by Quantitative Polymerase Chain Reaction (qPCR).

2.6. Insulin resistance calculation

IR was measured from insulin concentrations and fasting plasma glucose by homeostasis model assessment (HOMA) index. It was measured via the equation (Matthews et al., 1985):

$$\text{HOME index} = \text{Fasting serum insulin}(\text{mU/ml}) \times \text{fasting plasma glucose}(\text{mmol/L})/22.5.$$

2.7. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis

Total RNA was collected and purified randomly from liver lobes' cells of all rats using QIAGEN RNeasy® Mini Kit (50). This kit depends on purifying RNA strands using silica-membrane RNeasy spin columns. cDNA is generated by reverse transcribing the total RNA via ImProm-|| reverse transcription system kit – Promega protocol. The primers of transcript (mRNA) that encodes the (ACE1) and (ACE2) were designated by National Center for Biotechnology information (NCBI) (Table 1). qPCR was applied using the SYBR @

Table 1
ACE1 and *ACE2* designed primers.

Genes	Primer	Sequence	Temperature (c)	GC%
ACE1	Forward	5′-ACGGAAGCATCACCAAGGAG-3′	60.5	55
	Reverse	5′-GCTTCCCTGCTTCCTTGGAT-3′	60.5	55
ACE2	Forward	5′-AACAGCCTGGAGTTTCTGGG-3′	60.5	55
	Reverse	5′-ACAATGCCAACCACTACCGT-3′	58.5	50

Green master mix (MACROGEN). All reactions were run on PCR Thermal Cyclers from Applied biosystems and the conditions were 1 cycle of 5 min at 95 °C, 40 cycles of 30 s at 95 °C, 60 cycles at 58 °C, and 20 cycles at 72 °C. The relative expression level was determined by $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) using housekeeping gene *GAPDH* as a reference. The two types of primers used are as follows:

ACE1, 5′-ACGGAAGCATCACCAAGGAG-3′ (forward) and 5′-GCTT CCCTGCTTCCTTGGAT-3′ (reverse); ACE 2, 5′-AACAGCCTG GAGTTTCTGGG-3′ (forward) and 5′-ACAATGCCAACCACTACCGT-3′ (reverse).

2.8. Statistical analysis

Data were statistically analyzed using SPSS for windows package version 20 (SPSS Inc., Chicago, IL, USA). Results are expressed as the mean ± Standard Error (SE). The difference between measured parameters in different experimental groups was made using OneWay ANOVA test followed by Multivariate analysis (Tukey's) test. Correlations between measured parameters were made using Pearson correlation. Significance level at $P < 0.05$ was considered.

3. Results

3.1. Body weight and body mass index (BMI)

Body weight and BMI did not show any significant change between all studied groups both at weeks 6 and 12 (Figs. 1 and 2).

3.2. Systolic and diastolic blood pressure

As can be displayed from (Figs. 3 and 4), SBP and DBP showed persistent pattern through week 6 and 12 when compared MetS group to control and MetS + Vit D group to untreated MetS which was mitigated by treatment of vitamin D. Vitamin D treatment alone did not alter DBP or SBP when compared to control group in week 6 and 12.

3.3. Lipid profile

From (Table 2) below, the levels of serum TG, cholesterol, and LDL in MetS group were significantly higher ($P < 0.05$) than the levels in rats of control group. In contrast, serum cholesterol, TG, and LDL were minimized ($P < 0.05$) by Vit D in comparison with

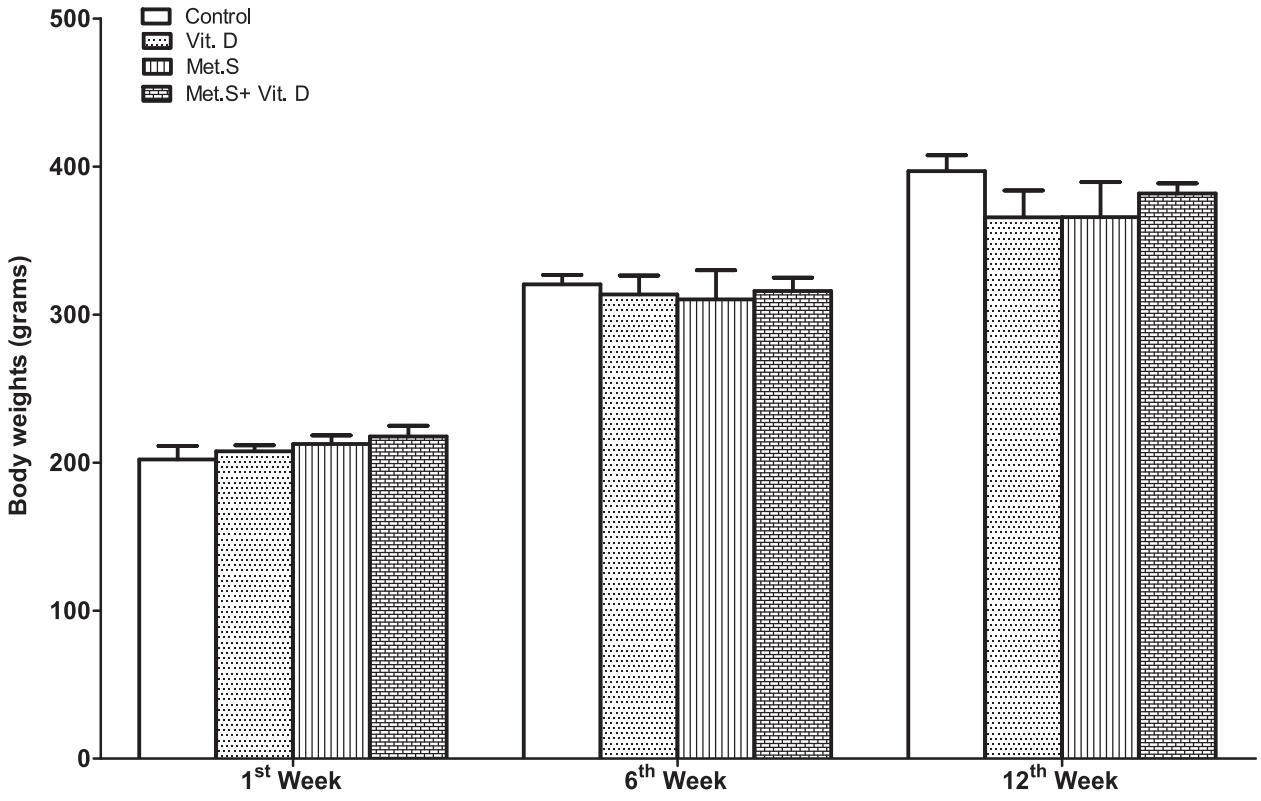

Fig. 1. Comparison of body weight (grams) in different studied groups at 1st, 6th and 12th weeks. *: significance versus control.

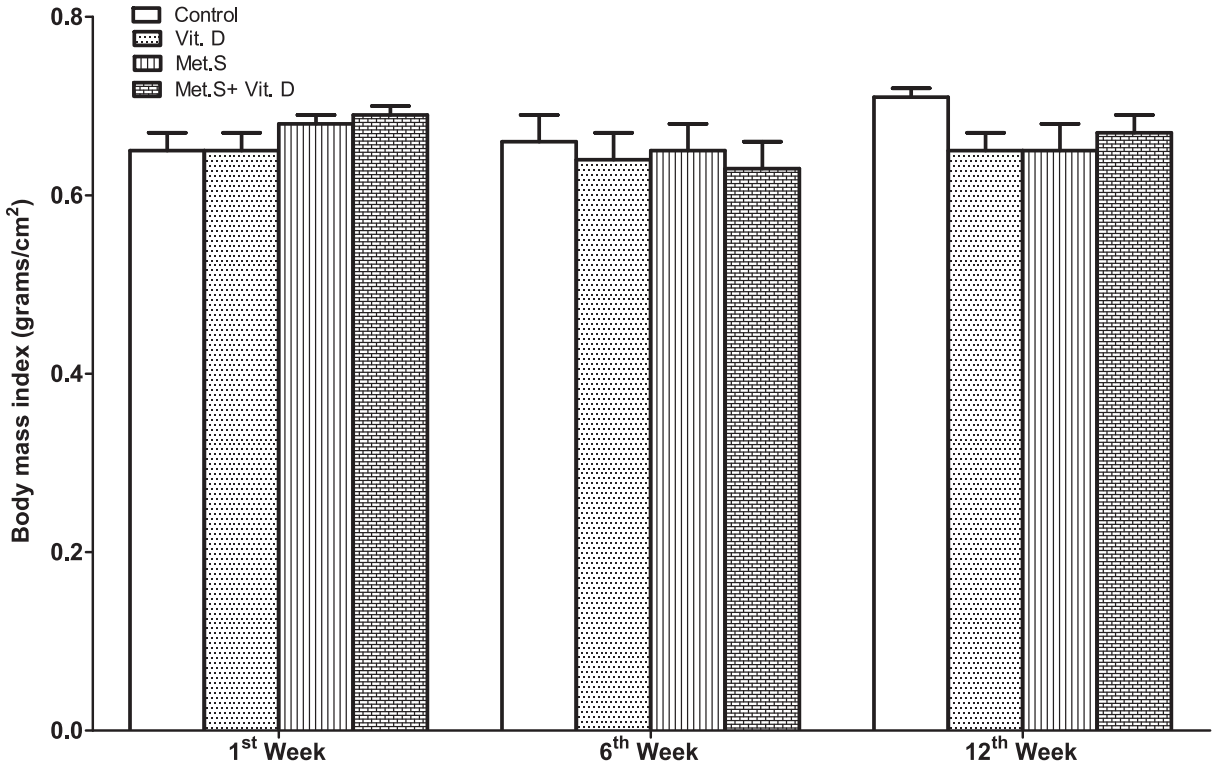

Fig. 2. Comparison of body mass index (grams/cm^2) in different studied groups at 1st, 6th and 12th weeks. *: significance versus control.

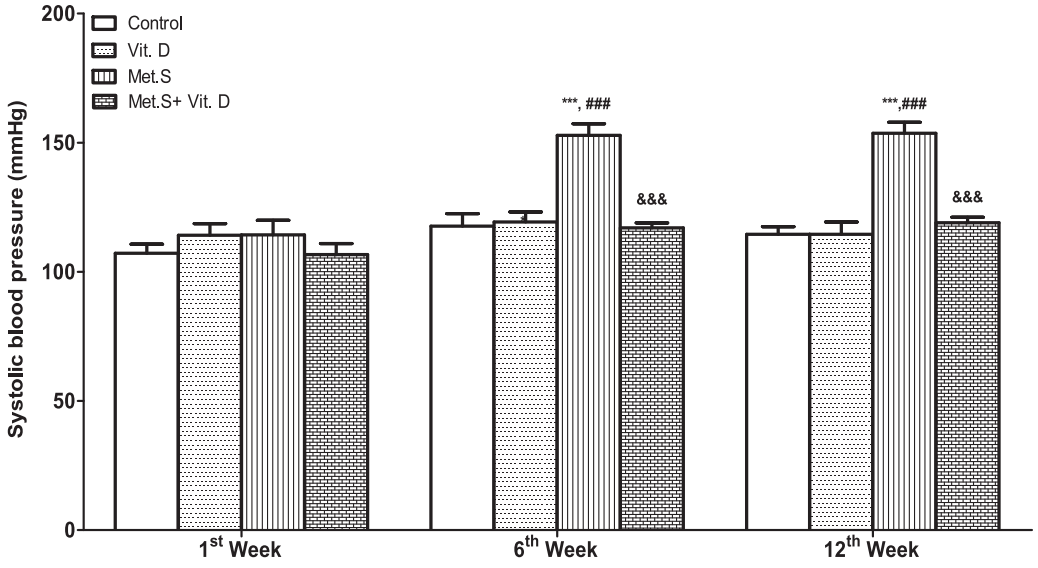

Fig. 3. Comparison of SPB (mmHg) in different studied groups at 1st, 6th and 12th weeks. *: significance versus control. #: significance versus Vit D $^{\&}$: significance versus MetS. *: $P < 0.050$, **: $P < 0.010$, ***: $P < 0.001$.

MetS group. HDL was significantly reduced ($P < 0.05$) in MetS group comparing to control while was significantly risen ($P < 0.05$) in MetS + vit D compared with MetS rats.

3.4. Glucose homeostasis

Data shown in (Figs. 5, 6, and 7) demonstrated a significant rise ($P < 0.05$) in the level of blood glucose and HOMA-IR in MetS rats when compared with control. However, group of treated MetS showed a significant reduction ($P < 0.05$) when compared to untreated MetS. In addition, level of serum insulin decreased significantly ($P < 0.05$) in MetS group comparing with the levels of control rats. Otherwise, fourth group of MetS animal supplemented with vitamin D_3 demonstrated significant rise ($P < 0.05$) in insulin level comparing with MetS animal.

3.5. Liver function effect of vitamin D

Data in (Table 3), (Figs. 8 and 9) presented a significant rise ($P < 0.05$) in the levels of both AST and ALT in rats of MetS rats

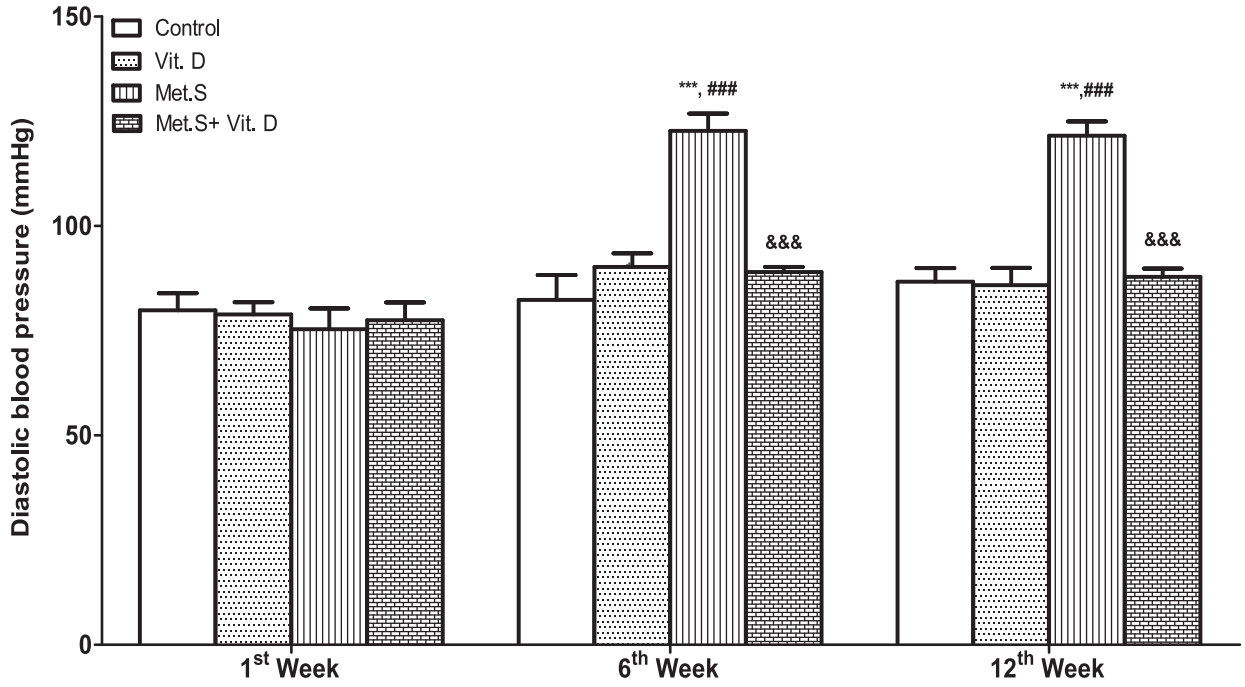

Fig. 4. Comparison of DBP (mmHg) in different studied groups at 1st, 6th and 12th weeks. *: significance versus control. #: significance versus Vit D &: significance versus MetS. *: $P < 0.050$, **: $P < 0.010$, ***: $P < 0.001$.

Table 2
Lipid profile in different studied groups at 1st and 12th weeks.

Parameters	Control	Vit D	MetS	MetS + VitD
Triglyceride (mg/dl)				
1st week	73.83 ± 0.87	76.17 ± 1.47	76.50 ± 2.26	75.50 ± 1.48
12th week	72.33 ± 1.05	76.17 ± 3.18	138.17 ± 20.28$^{***,\#\#\#}$	75.00 ± 1.86$^{\&\&\&}$
Total cholesterol (mg/dl)				
1st week	119.67 ± 1.31	124.67 ± 2.54	119.17 ± 3.61	120.67 ± 2.57
12th week	122.00 ± 5.05	135.83 ± 8.83	248.67 ± 18.35$^{***,\#\#\#}$	118.33 ± 4.40$^{\&\&\&}$
HDL-C (mg/dl)				
1st week	46.33 ± 1.28	45.50 ± 1.50	46.50 ± 1.29	45.83 ± 1.45
12th week	42.33 ± 0.76	45.00 ± 1.21	33.50 ± 3.33$^{*,\#\#}$	45.67 ± 1.91$^{\&}$
LDL-C (mg/dl)				
1st week	64.67 ± 4.92	64.67 ± 1.54	72.83 ± 2.75	65.67 ± 4.04
12th week	70.00 ± 4.95	78.00 ± 6.50	196.67 ± 21.02$^{***,\#\#\#}$	68.00 ± 3.42$^{\&\&\&}$

Data expressed as mean +/- SEM. *: significance versus control. #: significance versus Vit D &: significance versus MetS. *: $P < 0.050$, **: $P < 0.010$, ***: $P < 0.001$.

compared with control. However, the levels of both enzymes in MetS with vitamin D supplementation group were significantly reduced ($P < 0.05$) in comparison with levels of MetS rats.

3.6. Angiotensin converting enzyme 1 and Angiotensin converting enzyme 2 expression and effect of vitamin D

3.6.1. Angiotensin converting enzyme 1 (ACE1)

The relative expression levels data of *ACE1* (Table 4) and (Fig. 10) showed a significant upregulation in MetS rats compared with the expression in control rats. However, the expression was downregulated in MetS + Vit D_3 supplementation group and vit D group when compared to MetS group, although did not reach significance.

3.6.2. Angiotensin converting enzyme 2 (ACE2)

The relative expression of *ACE2* was downregulated significantly in MetS rats compared to the control. While it was upregulated compared with MetS group in MetS + vit D and vit D groups. The results obtained from qPCR analysis are showed in (Table 4) and (Fig. 10).

3.7. Correlation of ACE1 and ACE2 with blood pressure and insulin resistance

There was a significant positive correlation $P < 0.0001$ in *ACE1* expression levels with SBP, DBP and IR levels in the samples (Figs. 11, 12, and 13). On other hand *ACE2* expression levels had a negative correlation with SBP and DBP ($P = 0.012$, $P = 0.020$) (Figs. 14 and 15), while Insignificant negative correlation $P = 0.058$ with IR (Fig. 16).

4. Discussion

MetS is considered as one of the serious public health problems that affect a great percentage of the population and finding a new modality for the treatment is essential.

In this thesis, 12 weeks of daily administration of 20% fructose was used to induce MetS. It was successfully developed as evidenced by significant changes in lipid profile, increased IR and elevated BP. While the BMI was not significantly altered in response to fructose, the current findings are consistent with that of Korkmaz et al., (2019). They observed insignificant difference in

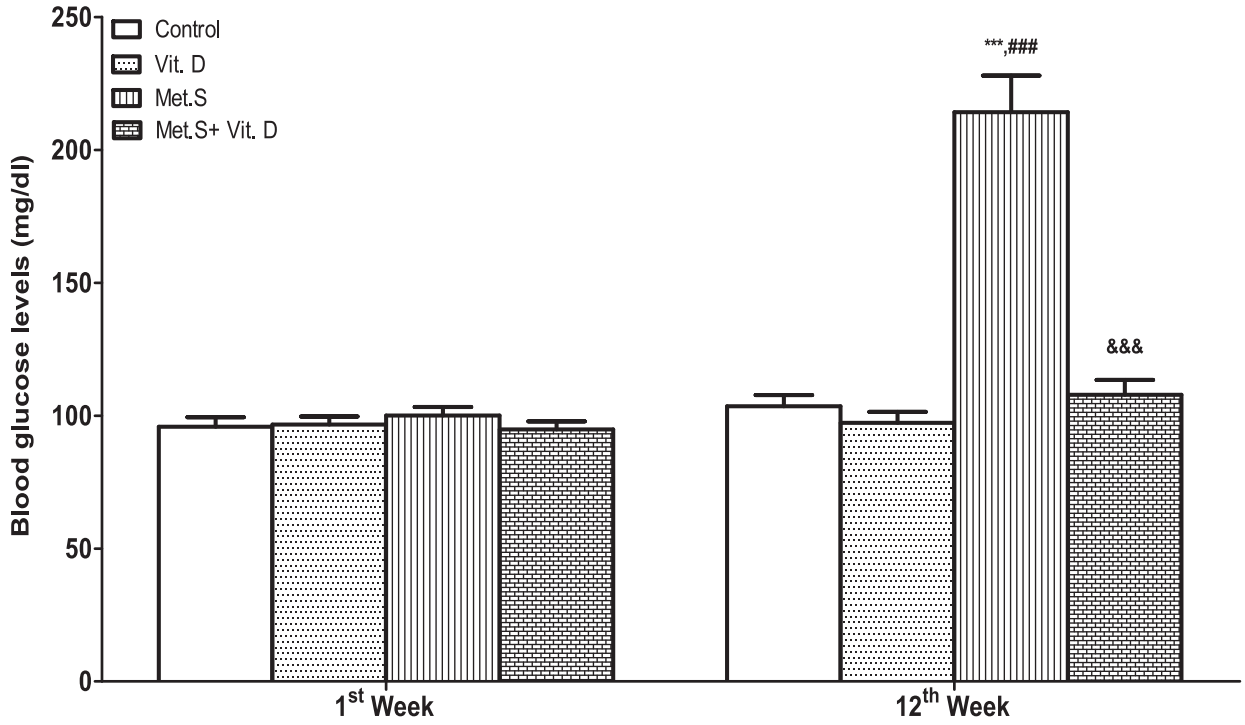

Fig. 5. Comparison of blood glucose levels (mg/dl) in different studied groups at 1st and 12th weeks. *: significance versus control. #: significance versus Vit D &: significance versus MetS. *: $P < 0.050$, **: $P < 0.010$, ***: $P < 0.001$.

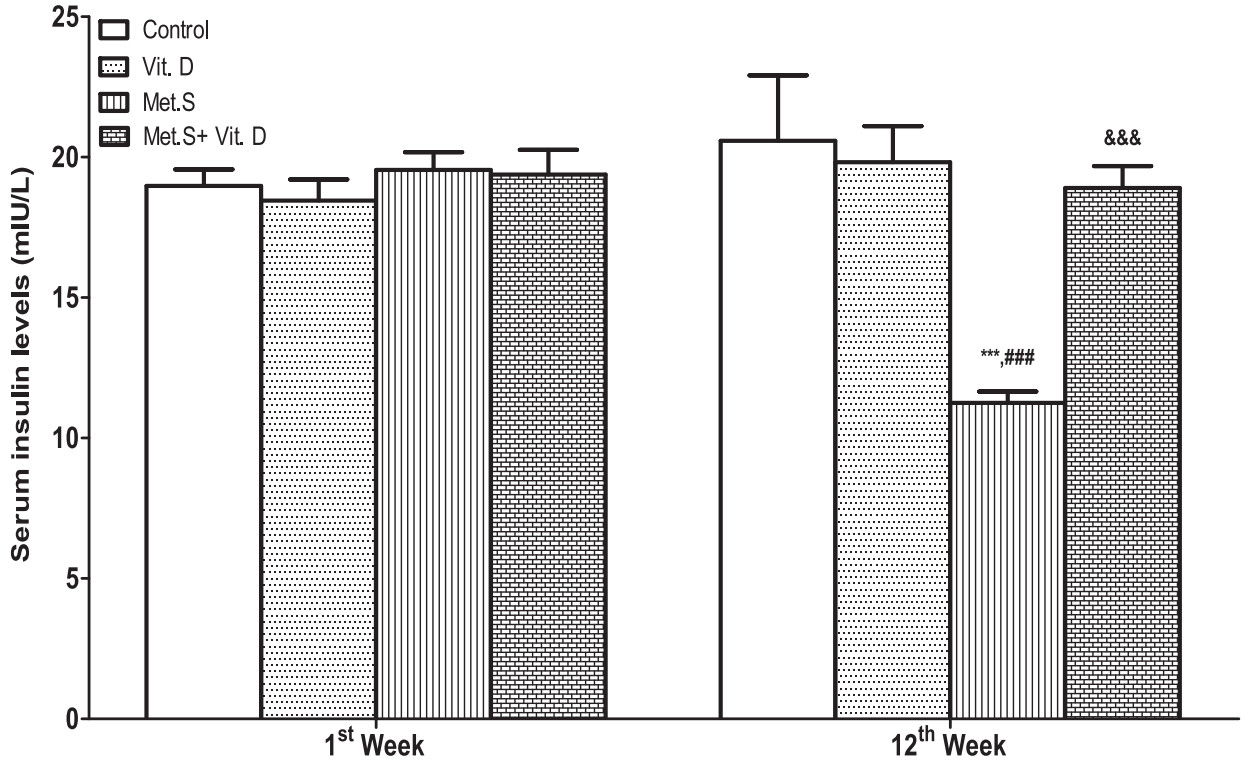

Fig. 6. Comparison of serum insulin levels (mIU/L) in different studied groups at 1st and 12th weeks. *: significance versus control. #: significance versus Vit D &: significance versus MetS. *: $P < 0.050$, **: $P < 0.010$, ***: $P < 0.001$.

body weight of Wistar rats given 20% fructose in drinking water for 15 weeks when compared to control rats; although the fructose-administered rats showed significant increase in plasma glucose, TGs, VLDL, and insulin. Furthermore, Belhadj et al., (2020) showed no notable difference between rats' body weight receiving either normal diet or high fat diet with 25% fructose in drinking water.

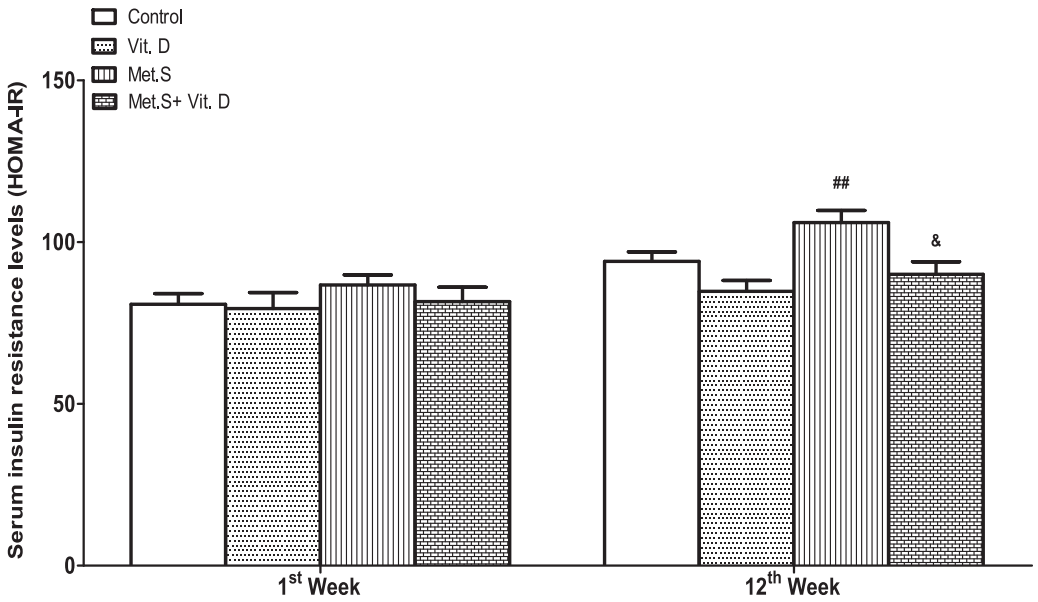

Fig. 7. Comparison of HOMA-IR in different studied groups at 1st and 12th weeks. *: significance versus control. #: significance versus Vit D &: significance versus MetS. *: $P < 0.050$, **: $P < 0.010$, ***: $P < 0.001$.

Table 3
Liver functions effect of vitamin D in different studied groups at 1st and 12th weeks.

Parameters	Control	Vit D	MetS	MetS + VitD
AST (IU/L)				
1st week	26.00 ± 1.06	26.67 ± 2.35	26.83 ± 1.74	29.17 ± 2.46
12th week	24.30 ± 1.02	25.00 ± 1.86	107.42 ± 3.50$^{***, \#\#\#}$	31.63 ± 3.83$^{\&\&\&}$
ALT (IU/L)				
1st week	15.17 ± 1.49	18.50 ± 1.71	21.33 ± 1.65	23.00 ± 2.82
12th week	16.15 ± 1.67	22.17 ± 2.52	76.00 ± 5.57$^{***, \#\#\#}$	22.52 ± 4.29$^{\&\&\&}$

Data expressed as mean +/- SEM. *: significance versus control. #: significance versus Vit D &: significance versus MetS. *: $P < 0.050$, **: $P < 0.010$, ***: $P < 0.001$.

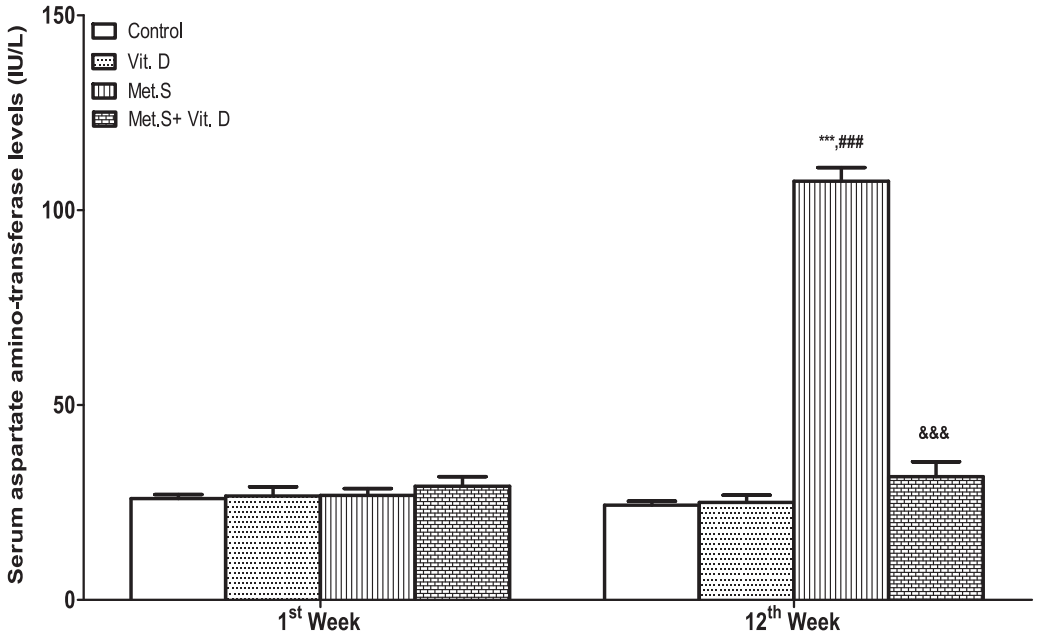

Fig. 8. Comparison of AST (IU/L) in different studied groups at 1st and 12th weeks. *: significance versus control. #: significance versus Vit D &: significance versus MetS. *: $P < 0.050$, **: $P < 0.010$, ***: $P < 0.001$.

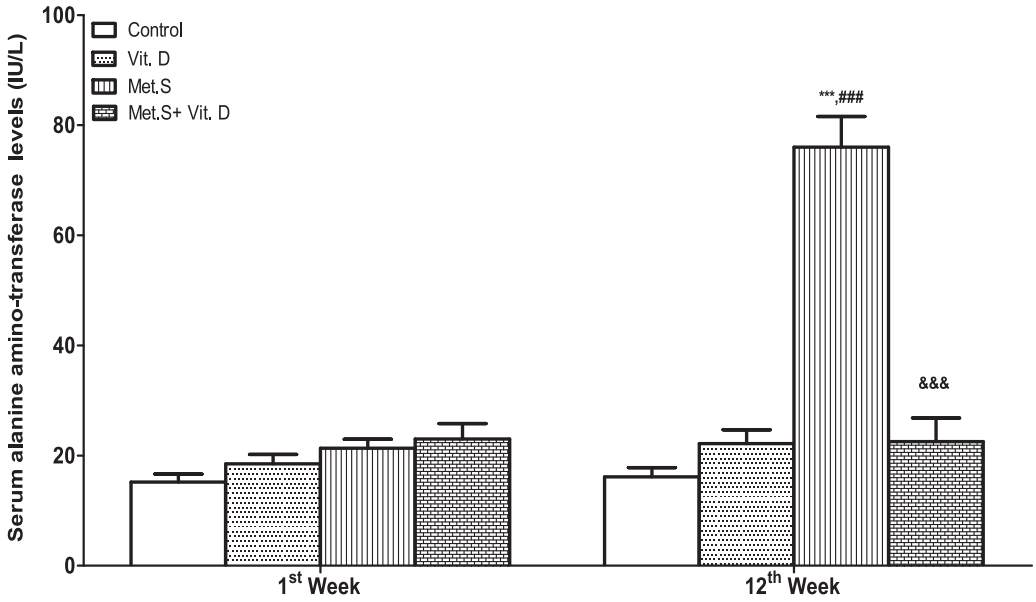

Fig. 9. Comparison of ALT (IU/L) in different studied groups at 1st and 12th weeks. *: significance versus control. #: significance versus Vit D &: significance versus MetS. *: $P < 0.050$, **: $P < 0.010$, ***: $P < 0.001$.

Table 4
ACE1 and *ACE2* expression in different studied groups at 12th week.

Parameters	Control	Vit D	MetS	MetS + VitD
ACE1 (ng/ml)	1.088 ± 0.056	1.286 ± 0.405	4.338 ± 0.707$^{***,\#\#\#}$	0.797 ± 0.355$^{\&\&\&}$
ACE2 (ng/ml)	1.306 ± 0.118	3.342 ± 0.989	0.177 ± 0.123$^{\#\#}$	3.289 ± 0.623$^{\&\&}$

Data expressed as mean +/- SEM. *: significance versus control. #: significance versus Vit D &: significance versus MetS. *: $P < 0.050$, **: $P < 0.010$, ***: $P < 0.001$.

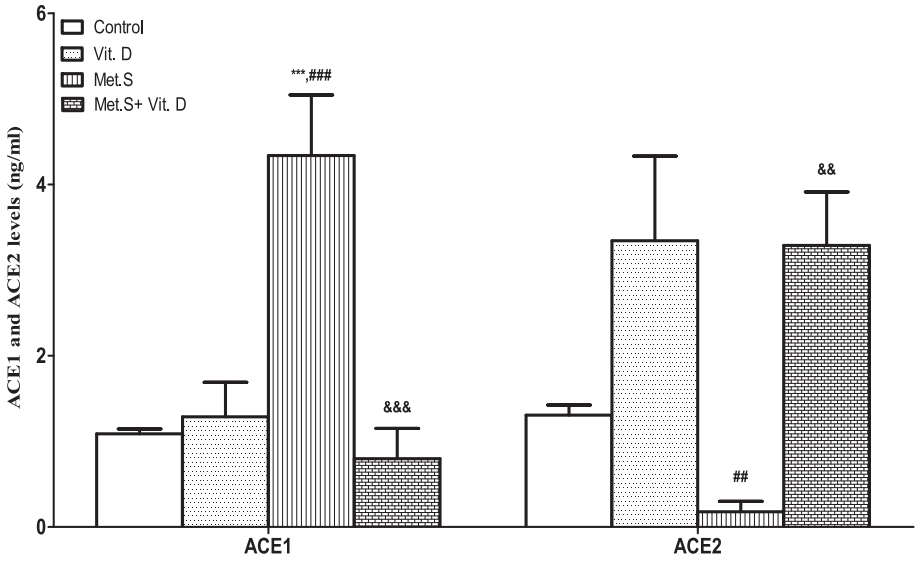

Fig. 10. Comparison of *ACE1* (ng/ml) and *ACE2* (ng/ml) in different studied groups at 12th week *: significance versus control. #: significance versus Vit D &: significance versus MetS. *: $P < 0.050$, **: $P < 0.010$, ***: $P < 0.001$.

Despite that; the high fat diet with fructose induced IR, hyperglycemia, and significant difference on fasting glycemia. On the contrary, Kim et al., (2020) reported an increase in body weight following 2 weeks of drinking 20% fructose together with increased SBP, TGs, and cholesterol. Moreover, Gambaro et al., (2018) demonstrated increased body weight in Swiss mice which were subjected to 6–10 weeks of 20% fructose in water. The differences between current finding regarding body weight and that of Kim *et al.* and Gambaro *et al.* could be explained by the different duration implemented in their studies.

The present work revealed that co-administration of vitamin D with fructose significantly mitigated the alterations produced by

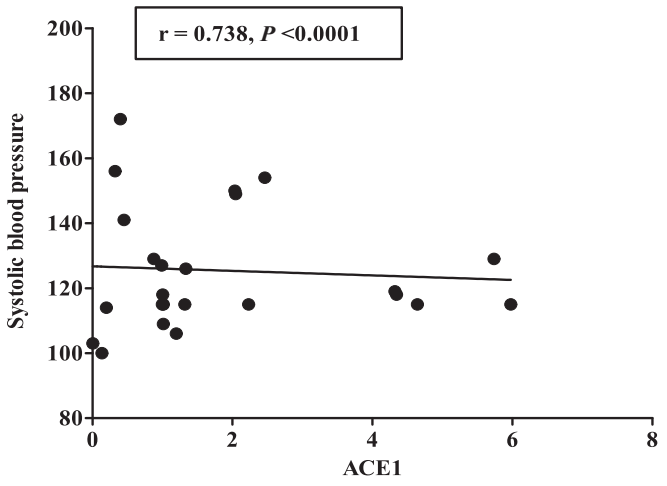

Fig. 11. Correlation between *ACE1* concentrations and SBP. Significant positive correlation (r = 0.738, P < 0.0001).

fructose ingestion in lipid profile, BP and IR. In agreement with the present observation, vitamin D treatment to obese rat model in the experiment of Jin et al., (2018) corrected IR and lipid profile alteration as well as reduced body weight. Recently, Wahba et al., (2021) induced MetS by giving 10% fructose and 3% salt in drinking water to Wistar rats for 6 weeks. This has resulted in weight gain, hyperuricemia, IR, dyslipidemia, hyperinsulinemia and impaired glucose tolerance. MetS rats were further supplemented orally with vitamin D_3 (10 μg/kg/day) for 6 weeks. After 12 weeks, the group of MetS with vitamin D_3 supplementation have shown an improvement in MetS risk factors.

Regarding studies on humans; Schmitt et al., (2018) noticed that postmenopausal women with vitamin D deficiency had high total cholesterol, TGs, insulin, and HOMA-IR levels. Authors clarified that low level of vitamin D (<30 ng/mL) was significantly correlated with MetS components. Gagnon et al., (2012) also reported that lower vitamin D concentrations was related to increased risk factors of MetS; including serum TG, fasting glucose, and IR in Australian adults. In addition, it was shown that vitamin D deficiency in the Korean adolescents was associated with increasing the risk of high fasting blood glucose levels and diabetes (Kim et al., 2018).

Tang et al., (2018) showed that vitamin D_3 supplementations notably reduced fasting levels of glucose and IR. Additionally, Lemieux et al., (2019) have demonstrated that daily 5000 IU of vitamin D_3 supplementation for 6 months had elevated pancreatic β-cell activity and peripheral insulin in people at-risk of diabetes. Likewise, Barbalho et al., (2018) showed that patients with impaired glucose tolerance present lower values of vitamin D when compared with those having normoglycemia. Vitamin D reduces the excessive insulin release in response to increase blood sugar; hence, it reduces IR and increases the insulin sensitivity (Durmaz et al., 2017). Furthermore, it may enhance insulin sensitivity by rising the expression of insulin receptors and activating transcription factors important in glucose homeostasis (Hoseini et al., 2013).

The outcomes of current work support the existing data concerning vitamin D impact on lipid profile in patients with MetS. In human; Chaudhuri et al., (2013) observed that vitamin D deficiency was linked to dyslipidemia in Indian and Chinese people. Besides, vitamin D_3 supplementation was suggested to decrease hepatic TG accumulation (Cheng et al., 2016) and increase the activity of lipoprotein in adipocytes (Querfeld et al., 1999). Another postulated mechanism of the vitamin D is that it can inhibit the secretion of parathyroid hormone (PTH) which increases peripheral uptake of TG (Kohno *et al.*, 1997). Also, vitamin D increases intestinal calcium absorption which reduces the formation and secretion of serum TGs in the liver (Cho et al., 2005) and reduced cholesterol level by converting cholesterol into bile acids (Vaskonen et al., 2002). Some studies tried to clarify the relation between vitamin D and HDL cholesterol level. Filgueiras et al. (2018) reported that diminished vitamin D intake was associated with increasing prevalence of low HDL level in Brazilian children. A previous *meta*-analysis in 2019 reported that supplementation of vitamin D3 minimizes the levels of serum total cholesterol, TG, and LDL cholesterol but not HDL cholesterol level.

Along with previous studies, current work illustrates that vitamin D_3 supplementation lowers SBP and DBP. Scragg et al., (2007) observed that vitamin D was inversely associated with BP in a large sample representative of US population. In addition,

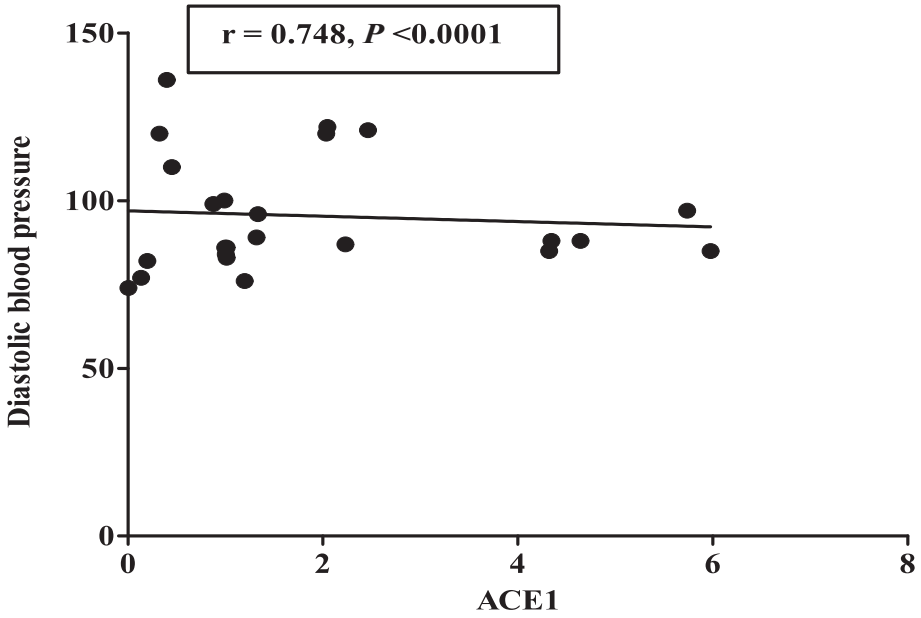

Fig. 12. Correlation between *ACE1* concentrations and DBP. Significant positive correlation (r = 0.748, P < 0.0001).

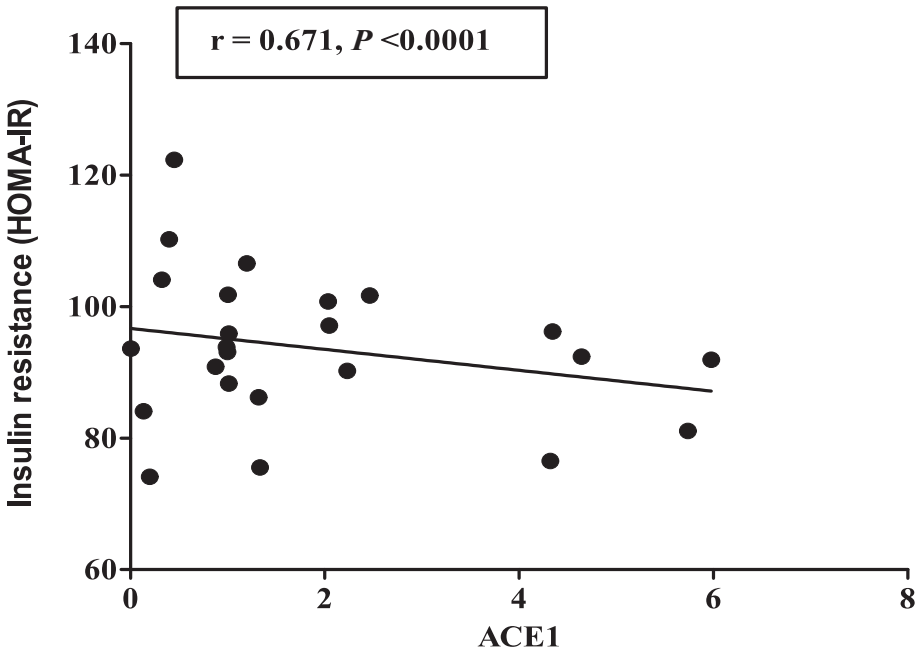

Fig. 13. Correlation between *ACE1* concentrations and IR. Significant positive correlation (r = 0.671, P < 0.0001).

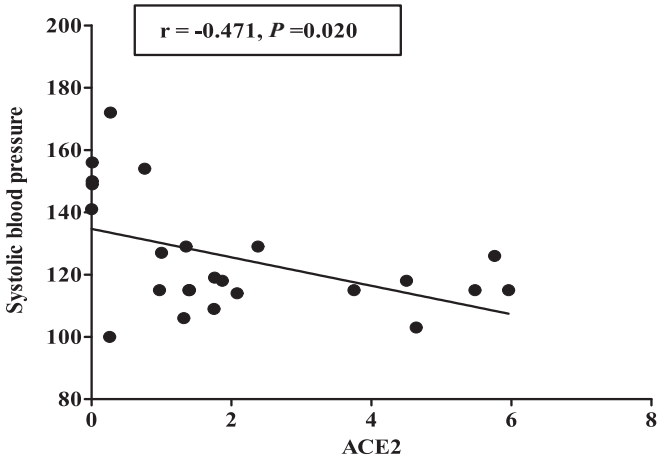

Fig. 14. Correlation between *ACE2* concentrations and SBP. Significant negative correlation (r = -0.471, P = 0.020).

Song & Park, (2013) reported that low serum level of 25(OH)D was significantly associated with the MetS components; especially BP and high TG level in post-menopausal women. The impact of vitamin D on RAS was illustrated by Li et al., (2002), who showed that VDR in null mice had significant rise in renin activity and circulating plasma Ang II concentrations that developed hypertension.

It is important to highlight the fact that chronic liver diseases are quiet common problem in the world. More importantly, RAS and local hepatic RAS are participated in most chronic liver diseases such as hepatitis B and C virus infections; and alcoholic and NAFLD (Ahmadian et al., 2016). NAFLD is described as a TG accumulation and a different degree of hepatic injury, inflammation, and repair. While steatosis is characterized by estimating the proportion of hepatocytes containing fat droplets (Paschos & Paletas, 2009). MetS increases the risk of NAFLD (Lee & Jeong, 2017); and according to the previous *meta*-analysis from 1980 to 2017, it was proven that MetS can increase the risk factor for liver diseases, especially NAFLD. Thus, understanding the mechanisms underlying MetS control is important to reduce liver diseases (Ren et al., 2019). Lee & Jeong, (Lee & Jeong, 2017) clarified that hypertriglyceridemia and high TG/HDL-C ratio may rise the risk of NAFLD. The hepatic ACE1/ANG II axis have a major impact on chronic liver disease, portal hypertension and development of liver fibrosis (Yoshiji et al., 2001; Shim et al., 2018). Since it was not previously investigated in MetS models, the present work aimed to assess the intercorrelation between MetS, hepatic RAS and vitamin D_3 supplementation.

According to the results demonstrated in the present work, MetS impairs liver function and enzymes, as both ALT and AST enzymes were significantly raised in MetS group rats when compared to the control. It was demonstrated that vitamin D is protective against effect of MetS on the liver and can lower liver enzymes levels. These findings are consistent with former reports. For instance, Wang et al. (2017) showed that ALT level was significantly higher in male patient with MetS than females and related to liver function with MetS risks. Another study by Perera et al., (2008) observed that the elevation of MetS components lead to increased concentrations of AST and ALT in Thai adults. In addition, Chen et al., (2019) noticed a decrease in vitamin D level while AST and ALT were elevated in MetS model. Furthermore, Tavakoli et al., (2019) illustrated that vitamin D_3 supplementation may improve liver function in adolescents with abnormal liver function tests.

An interesting and novel finding of this study was evident by the PCR results which indicate the expression of hepatic *ACE1* was upregulated in MetS group, however vitamin D supplementation downregulated *ACE1* expression while upregulated the expression of hepatic *ACE2*. This finding is further proved by the significant positive correlation between *ACE1* expression levels and SBP (r = 0.738, P < 0.0001), DBP (r = 0.748, P < 0.0001), and IR (r = 0.671, P < 0.0001). On other hand, *ACE2* expression levels showed negative correlation with SBP (r = -0.471, P = 0.020) and DBP (r = -0.506, P = 0.012) with insignificant negative correlation with IR (r = -0.392, P = 0.058). ACE1 converts Ang I into Ang II which is a proinflammatory, prooxidant, and prothrombotic agent that also interferes with intracellular insulin signaling. In contrast; ACE2 cleaves Ang II to produce Ang (1–7) which enhances glucose tolerance, insulin sensitivity, insulin stimulated glucose uptake

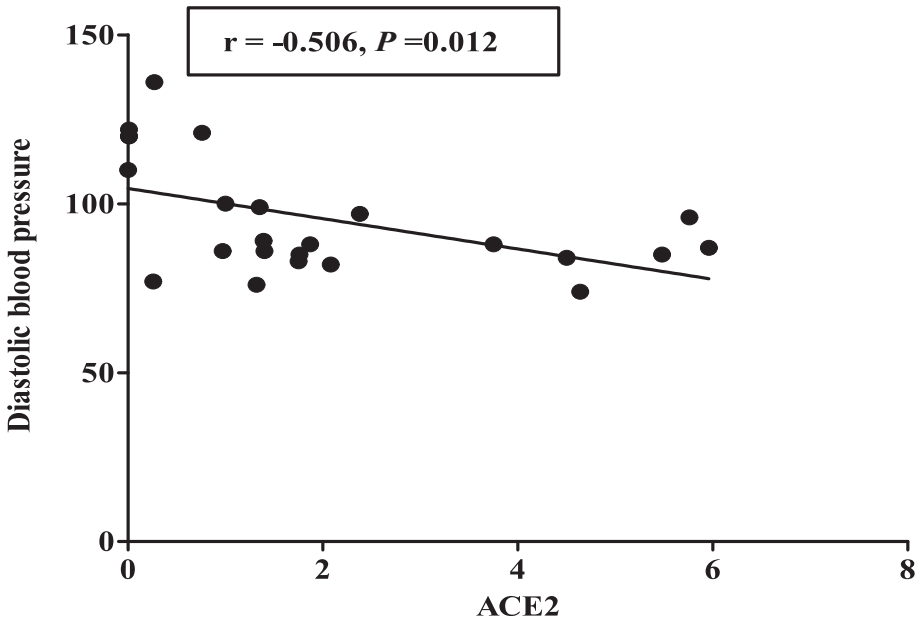

Fig. 15. Correlation between *ACE2* concentrations and DBP. Significant negative correlation (r = -0.506, P = 0.012).

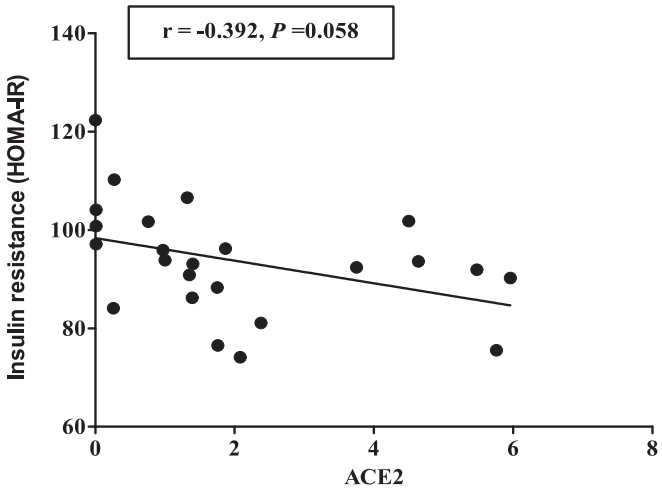

Fig. 16. Correlation between *ACE2* concentrations and IR. Insignificant negative correlation (r = -0.392, P = 0.058).

while decreases TG and cholesterol levels; and furthermore reduces abdominal fat mass. In addition, Ang (1–7) has been demonstrated to decrease liver gluconeogenesis and the Mas receptor for Ang (1–7) is an essential component of the insulin receptor signaling pathway (de Macêdo et al., 2014). It is noteworthy that the current work is the first to clarify the effect of vitamin D_3 supplementation on the expression of hepatic *ACE1* and *ACE2* in MetS model.

Vitamin D regulates the expression of more than 200 genes. There are two mechanisms of the molecular action of vitamin D: (1) genomic actions of vitamin D produced by the interaction of nuclear VDR with the retinoid × receptor (RXR) inside cell nucleus to form a heterodimer. Then, it binds to vitamin D response elements (VDREs) in specific target genes to promote their expression which require time to take effect (Lv et al., 2020). (2) mechanism generated rapid effect called nongenomic actions which produced by receptors in plasma membrane (Bikle, 2014; Trochoutsou et al., 2015). This may explain the effect of vitamin D to enhance the regulation of hepatic *ACE1* and *ACE2*.

Various earlier works have investigated the relevance of local RAS perturbations to liver function. Nabeshima et al., (2009) showed that *AT1*-deficient mice were resistant to steatohepatitis and had reduced liver TG. *AT1* knockout mice had less hepatic fibrosis in the study of Goh et al., (2015). According to de Kloet et al., (2010), Ang II infusion catalyzed TG production in the liver via AT2R, thus secondarily contributed to IR. Moreover, Jayasooriya et al., (2008) suggested a close link between RAS and hepatic lipid metabolism,. The authors further showed an increase in the expression of liver *ACE2* in both human and rat in response to increasing hepatocellular hypoxia, which can modulate RAS activity in cirrhosis (Paizis et al., 2005). Previous studies on human and animal models showed that some RAS component, particularly Ang II, are overexpressed in fibrotic liver tissues (Speca et al., 2012; Goh et al., 2015) These earlier findings together with the current observations point to the important possible role for vitamin D in balancing hepatic *ACE1* and *ACE2* expression levels, thus improving liver functions and minimizing hepatic fibrosis and steatosis.

5. Conclusion

The current findings pointed to the alteration in hepatic gene expression of *ACE1* and *ACE2* in induced MetS model and the possible mitigation of such alteration by vitamin D supplementation. It's a novel observation that provides the basis for better understanding the pathophysiology of hepatic function impairment in MetS and the mechanisms underlying protective role of vitamin D in such model. It is recommended that future studies should further investigate and consider the potential of vitamin D as a protective effect and further roles of hepatic RAS in MetS models.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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