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Effects of Vitamin D on asprosin and meteorin-like protein in liver tissue of diabetic rats

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Abstract

Diabetes mellitus (DM), a considerable concern for public health, serves as a predisposing factor for numerous diseases. Asprosin and meteorin-like protein (metrnl) are two peptides that play essential roles in glucose and energy metabolism. This study investigated the possible therapeutic potential of vitamin D (Vit D) against diabetes-induced liver damage in terms of asprosin and metrnl proteins. The study comprised four distinct groups: control, DM (60 mg/kg streptozotocin), Vit D (200 IU/kg), and DM+Vit D. Asprosin and metrnl levels were determined using both ELISA and immunohistochemical methods. Additionally, Total Oxidant Status (TOS) and Total Antioxidant Status (TAS) levels were assessed using the ELISA method. Asprosin and TOS levels increased in the DM group, whereas TAS and metrnl levels decreased. In the DM+Vit D group, metrnl levels increased while asprosin and TOS levels decreased significantly. These findings suggest that Vit D had protective effects against diabetes-induced liver damage and improved asprosin and metrnl levels. It has been concluded that Vit D could be considered as an attractive therapeutic agent target for asprosin and metrnl, which have positive effects on diabetes.

Keywords: Asprosin, diabetes, meteorin-like factor, streptozotocin, Vitamin D

Introduction

The World Health Organization has stated that about 347 million people are afflicted with Diabetes mellitus (DM), with estimates indicating a rise to 439 million people by the year 2030 [1]. Insufficient secretion of insulin from pancreatic β cells and development of insulin resistance in peripheral metabolic organs such as the liver, skeleton muscle, and adipose tissue are the main mechanisms of DM pathogenesis [2]. Studies have shown the effects of peptides secreted from peripheral organs on lipid, amino acid, and carbohydrate metabolism in insulin-targeted tissues might play roles in the pathogenesis of the disease [3]. Adipose tissue plays a vital role in maintaining energy equilibrium and substrate metabolism by producing and releasing various substances that have endocrine or paracrine roles related to energy homeostasis as well as regulating glucose metabolism. Therefore, it has been linked to the emergence of cardiovascular disease and atherosclerosis [4]. Asprosin is a glucogenic adipokine manufactured and discharged by white adipose tissue in response to fasting as a product of the fibrillin-1 gene [5]. Asprosin which accelerates the release of glucose into the bloodstream, is involved in many metabolic processes such as insulin resistance, apoptosis, and appetite regulation besides it has been stated that it may have an antioxidant character due to its cytoprotective function [6,7].

Meteorin-like protein (metrnl) released by adipose tissue and skeletal muscle, is highly expressed in both rodents and humans. According to reports, elevated metrnl levels in the bloodstream enhance energy utilization and enhance glucose tolerance in mice [8,9]. Furthermore, reports are indicating that it triggers the thermogenesis-related gene expression in adipose tissue and

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regulates adipocyte differentiation, lipid-mediated inflammation, and insulin resistance [10-12]. Recent studies have reported that metrnl has beneficial effects in various scenarios, including chronic colitis, maintenance of cholesterol and triglyceride levels, insulin resistance, coronary artery disease, and chronic obstructive pulmonary disease in addition the anti-apoptotic activity [13-16]. The mechanisms for circulating metrnl concentrations in obesity and DM need to be explored [17-20].

The deficiency of Vitamin D (Vit D) has been observed commonly worldwide in recent years and is associated with an increased risk of some autoimmune diseases [21]. It also has properties such as anti-inflammatory, anti-apoptotic, and anti-fibrotic activity, so it can act in various physiological pathways [22-24].

The objective of this study was to investigate the effect of Vit D treatment on serum asprosin and metrnl levels against diabetes-induced liver damage in rats by using immunohistochemical and biochemical parameters.

Material and Methods

Experimental design

Following the approval of the Animal Experiments Ethics Committee at Munzur University, the investigation was carried out (Approval number: 2022/16-03, date: 20/07/2022). Experimental applications were started after 10 days of adaptation period and maintained at temperatures 22-25 °C with a 12-hour light/dark cycle, and free food and water. This study lasted 56 days and involved the measurement of animal weights and blood glucose levels at the beginning and end of the experiment. A total of twenty-eight male Wistar rats, weighing between 200 to 250 g, were randomly distributed into four groups, each comprised of seven rats, as listed below:

- Control group: No treatment.
- DM group: A single dose of 60 mg/kg streptozotocin (STZ; Sigma Chemical Co., St. Louis, MO, USA) was prepared by dissolving it in 0.1 M sodium citrate buffer (pH: 4.5) and then administered intraperitoneal (ip). Following 72 hours, blood glucose was measured from the tail vein, considering values exceeding 250 mg/dl as indicative of diabetes [25].
- DM+Vit D group: In addition to the DM group, 200 IU/kg/day Vit D (Devit-3, 50000 IU/15 ml, DEVA, Turkey) was administered orally every day [26].
- Vit D group: Vit D administered orally at 200 IU/kg/day every day.

Twenty-four hours after the last experimental application, rats were anesthetized by ip administration of 10% ketamine (Alfamine; Alfasan IBV, Woerden, the Netherlands) and 2% xylazine (Alfazine; Alfasan IBV, Woerden, the Netherlands). Blood samples were gathered in tubes and subsequently preserved at -80°C until subjected to analysis. The liver tissues were then fixed in 10% formalin in phosphate buffer for 48 hours and embedded in paraffin blocks after routine histologic follow-up series. Sections of 4–6 μm thickness from these blocks were deparaffinized for immunohistochemical examinations and stored under appropriate conditions.

Immunohistochemical analysis

The application of avidin-biotin-peroxidase complex (ABC) was performed with minor modifications. Primary antibodies; anti-metrnl (NBP1-91064PEP, Novus, USA) and anti-asprosin (FNab09797, Fine Test, China) were diluted 1/200 with the Termo Scientific™ TP-015-HA commercial kit used. Diaminobenzidine (DAB, ab64238; Abcam) was applied after that counterstaining was performed using Mayer's hematoxylin and examined under a Leica DM500 light microscope. The histoscore was determined by considering both the prevalence (0,1: <%25, 0.4:%26-50, 0.6:%51-75, 0.9:%76-100) and severity of immunoreactivity in staining (0: no, +0.5: very little, +1: little, +2: moderate, +3: severe). The histoscore was calculated as the product of the prevalence and severity values [27,28].

Biochemical analysis

Serum samples were analyzed for asprosin (Shanghai Sunred Biological) Technology Co., Ltd. China), metrnl (Shanghai Sunred Biological Technology Co., Ltd. China), total oxidant status (TOS; Rel Assay Diagnostics, Turkey), and total antioxidant status (TAS; Rel Assay Diagnostics, Turkey) using the ELISA method, following the manufacturer's instructions and guidelines

Statistical analysis

The statistical analysis of the data was conducted using GraphPad 8.01 software. One-way ANOVA and post-hoc tests were applied in group comparison. The results were represented as mean±standard deviation (SD) or as median (min-max) values. The criterion for statistical significance was established as $p < 0.05$.

Results

Asprosin and Metrnl expression

The expression of asprosin and metrnl were similar in the liver tissue of rats belonging to the control (Figure 1a, Figure 3a) and Vit D (Figure 1b, Figure 3b) groups ($p > 0.05$). Asprosin expression increased significantly in the DM (Figure 1c) group when compared to the control group ($p=0.047$) but lower in the DM+Vit D group (Figure 1d), which was statistically significant, compared to the DM group ($p<0.001$), (Figure 2).

Metrnl expression decreased significantly in the DM group (Figure 3c) when compared to the control group ($p=0.005$) but increased in the DM+Vit D group (Figure 3d), which was statistically significant, compared to the DM group ($p=0.0045$), (Figure 4).

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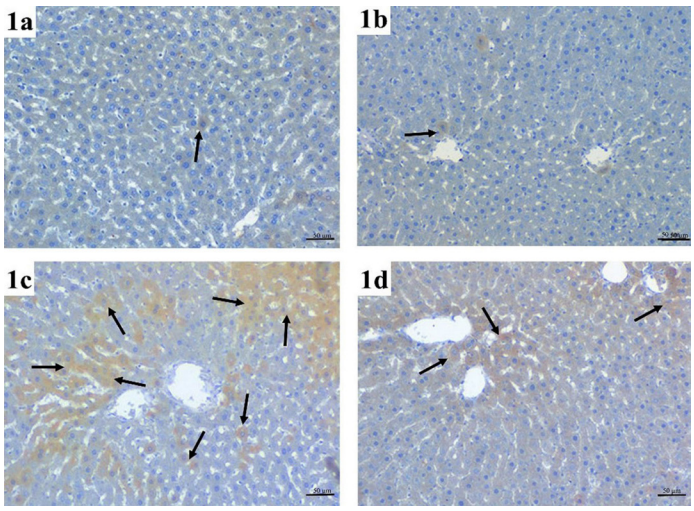

Figure 1. Photomicrographs of immunohistochemical staining for asprosin (black arrows) in liver tissue. (a) Control group, (b) Vit D group, (c) DM group, and (d) DM+Vit D group. Scala bar: 50 µm

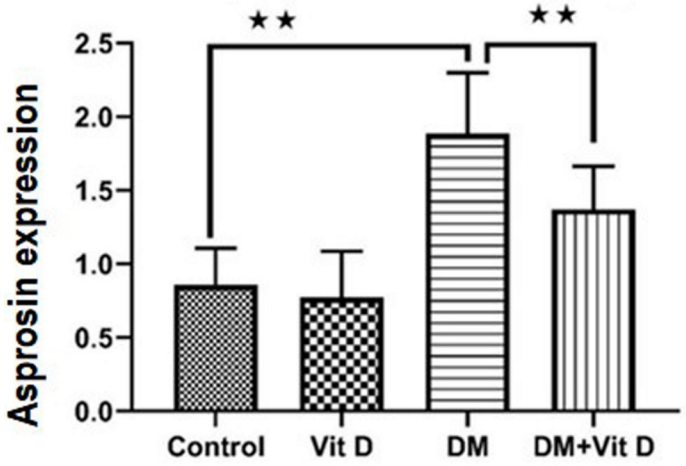

Figure 2. Comparison of asprosin immunoreactivity between groups

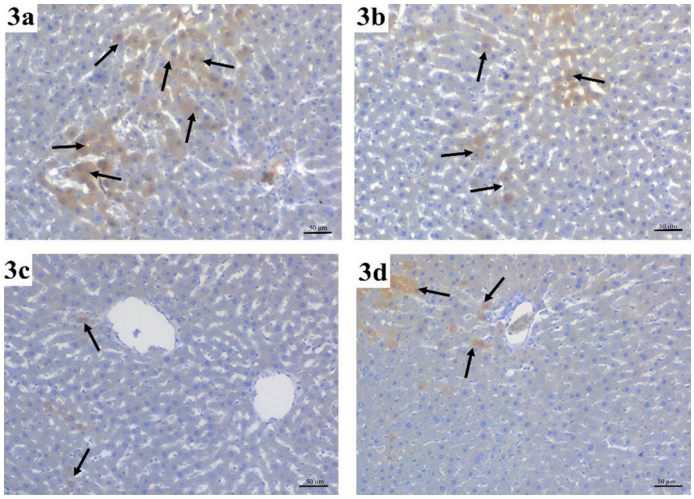

Figure 3. Photomicrographs of immunohistochemical staining for metrnl (black arrows) in liver tissue. (a) Control group, (b) Vit D group, (c) DM group, and (d) DM+Vit D group. Scala bar: 50 µm

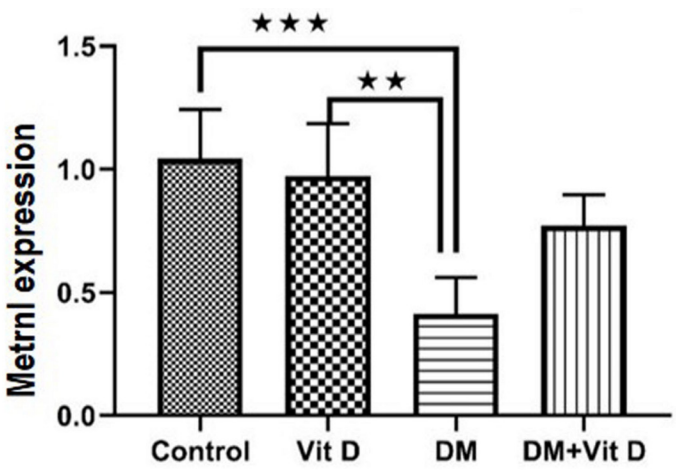

Figure 4. Comparison of metrnl immunoreactivity between groups. *The difference between the groups was significant

Body weights

Both the control and Vit D groups showed a highly significant increase in body weights compared to the baseline ($p<0.0001$). In contrast, the DM and DM+Vit D groups displayed a significant decrease ($p<0.0001$), (Table 1).

Table 1. Comparison initiation and end weights of the experimental animals in the groups

Weight	Control-post Control-pre	Vit D-post Vit D-pre	DM-post DM-pre	DM+Vit D-post DM+Vit D-pre
Paired t test				
P-value	<0.0001	<0.0001	<0.0001	<0.0001
Number of pairs	7	7	7	7
Mean of differences	40.18	32.31	-47.24	-43.12
SD of differences	5.914	7.617	13.58	8.502
Pre mean	226.1	242.1	241.6	244.9
Post mean	266.3	274.4	194.3	201.8

SD: standard deviation

Biochemical analysis findings

Blood glucose values

The blood glucose levels of the experimental animal are summarized in Table 2. It was observed that high glucose levels were detected in the experimental animals and this increase was ameliorated with the treatment of Vit D.

Table 2. Final blood glucose values of the experimental animals (mg/dl)

Blood glucose levels	Control	Vit D	DM	DM+Vit D
Minimum	105.0	108.0	335.0	255.0
Maximum	123.0	125.0	467.0	395.0
Range	18.00	17.00	132.0	140.0
Mean	113.3	116.4	401.7	316.4
SD	6.751	7.547	44.99	46.52
SEM	2.552	2.852	17.00	17.58

SD: standard deviation, SEM: standart error of mean

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TAS and TOS levels

As shown in Figure 5, the analysis comparing the control group with the Vit D group exhibited no statistically significant disparity in TOS and TAS levels (p>0.05 In the DM group, there was a notable elevation in TOS levels (p<0.0001), whereas TAS levels displayed a significant decrease (p<0.0001). Comparing the DM+Vit D group to the DM group revealed that Vit D treatment decreased TOS levels (p<0.0001) and increased TAS levels (p<0.0001), (Figure 5a and Figure 5b).

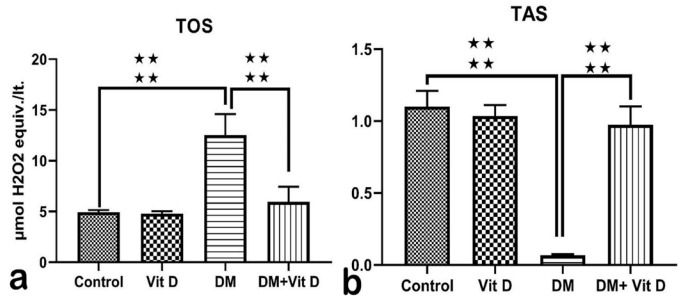
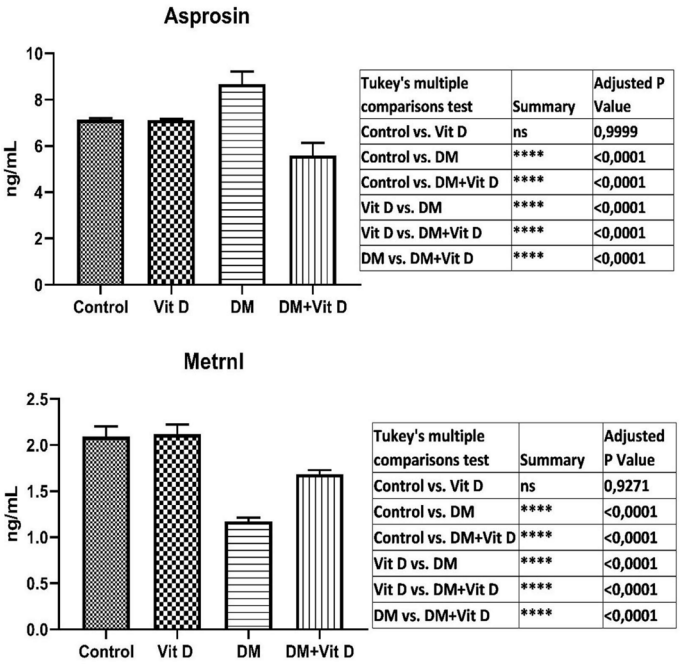


Figure 5. Comparison of serum TOS and TAS levels between the groups. *The difference between the groups was significant

Asprosin and Metrnl levels

Serum asprosin and metrnl levels were similar between the control and Vit D groups (p>0.05). In the DM group, there was a significant increase in asprosin levels compared to the control group (p<0.0001), whereas a significant decrease was determined in the DM+Vit D group (Figure 6). A significant reduction in metrnl levels was evident in the DM group (p<0.0001), while an increase was observed in the DM+Vit D group following Vit D treatment (p<0.0001), (Figure 6).



Tukey's multiple comparisons test	Summary	Adjusted P Value
Control vs. Vit D	ns	0,9999
Control vs. DM	****	<0,0001
Control vs. DM+Vit D	****	<0,0001
Vit D vs. DM	****	<0,0001
Vit D vs. DM+Vit D	****	<0,0001
DM vs. DM+Vit D	****	<0,0001

Tukey's multiple comparisons test	Summary	Adjusted P Value
Control vs. Vit D	ns	0,9271
Control vs. DM	****	<0,0001
Control vs. DM+Vit D	****	<0,0001
Vit D vs. DM	****	<0,0001
Vit D vs. DM+Vit D	****	<0,0001
DM vs. DM+Vit D	****	<0,0001

Figure 6. Comparison of serum asprosin and metrnl levels between the groups

Discussion

The liver has vital functions in the regulation of glucose homeostasis, and hyperglycemia increases reactive oxygen species (ROS), which can affect various organs such as the kidney, heart, and liver [28]. The main indicators for ROS are biomarkers such as malondialdehyde (MDA), TAS, and TOS which are useful in the assessment of diabetes prognosis [29]. The present study focused on exploring the possible effects of Vit D on asprosin and metrnl peptides, which we have limited information about their roles in insulin sensitivity in diabetic rat liver tissue. The present results of our study show that TAS and metrnl levels decreased, whereas TOS and asprosine levels increased in the DM group compared to the control group. In previous studies, consistent with our findings, it was reported that an increase in TOS levels and a decrease in TAS levels in DM and Vit D administration approached these parameters to normal levels [30,31].

Research findings indicate that circulating asprosin can cross the blood-brain barrier and influence appetite by triggering orexigenic AgRP+ neurons through the cAMP-dependent pathway [32]. Clinical research and experimental studies have shown that plasma and protein levels of asprosin are higher in Type 1 DM (T1DM), Type 2 DM (T2DM), and polycystic ovary syndrome, consistent with our findings [33,34]. It has also been stated that recombinant asprosine treatment significantly increases blood glucose levels [5] and that increased asprosine may lead to impaired hepatic glucose metabolism in diabetes [34]. As reported in the literature, there is a correlation between body mass index (BMI) and the release of asprosin [35]. In the current study, we observed that Vit D treatment decreased serum glucose levels and improved weight in rats. Previous studies have shown that Vit D contributes to the development of insulin resistance by affecting insulin sensitivity and β-cell function [36-38]. Our data revealed that Vit D suppresses serum asprosin and TOS levels, and liver damage, on the other hand, it increases serum TAS levels and metrnl expression.

While the exact mechanism of metrnl's action remains incompletely elucidated, it has demonstrated the capacity to induce macrophage activation, the expression of thermogenic anti-inflammatory genes, catecholamines, and interleukin (IL)-4 in adipose tissue [9]. Researchers indicated that metrnl inhibits the increase of tumor necrosis factor-alpha (TNF-α) levels in high-fat diet mice, suggesting its potential therapeutic utility in managing metabolic disorders and inflammation by modulating tissue energy balance [10]. Another study revealed that metrnl manages insulin sensitivity in adipocytes by upregulation of PPARγ [11]. In a clinical investigation, it was documented that patients with T2DM exhibited notably reduced levels of circulating metrnl, and this decline exhibited an inverse correlation with serum glucose levels and insulin resistance [20]. The administration of metrnl enhanced glucose homeostasis and led to a decrease in insulitis severity in mice with T1DM [39].

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In our study, we observed a significant reduction in both serum and tissue metrnl levels in the DM group when compared to the control group. This observation is in line with findings from other research studies [20,40].

Conclusion

In the current study, it was observed that increased asprosin levels in diabetic rats contributed to elevated blood glucose levels, and Vit D treatment improved asprosin and metrnl levels. Therefore, the results showed that Vit D may be given as dietary supportive therapy in diabetic patients and susceptible populations. Moreover, it was concluded that further comprehensive research is required to elucidate the functions of metrnl and to isolate conflicting findings.

Conflict of Interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

The authors declare that they have received no financial support for the study.

Ethical Approval

This experimental study received ethical approval from the Local Ethics Committee of Experimental Animals at Munzur University (Approval number: 2022/16-03, date: July 20, 2022).

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