ORIGINAL ARTICLE

# The Dual Action of Vitamin D and Atorvastatin in Rebalancing Metabolic Functions and Adipocytokine Expression in Metabolic Syndrome Rats

**Rajesh A. Maheshwari, Snehal Joshi**

*Department of Pharmacy, Sumandeep Vidyapeeth Deemed to be University, Vadodara, Gujarat, India*

## Abstract

**Aims:** This research explored the impact of Vitamin D (Vit D) and Atorvastatin (AT) on metabolic syndrome (MS) triggered by a high-fat diet (HFD) in Wistar rats. **Materials and Methods:** Over the course of 8 weeks, the rats were placed on an HFD and divided into five groups: Normal control, HFD only, HFD + Vit D, HFD + AT, and HFD + Vit D + AT. The treatments were administered daily throughout the duration of the experiment. The study assessed changes in body weight, lipid profiles, liver function, and adipokine levels. **Results:** The HFD led to weight gain and metabolic disruptions, but the administration of Vit D, AT, or their combination helped mitigate these effects, with the combined treatment showing the most pronounced improvements. Both Vit D and AT improved lipid levels and liver enzyme activity, while also causing significant shifts in adipokine levels. Histopathological analysis revealed a reduction in liver damage. **Conclusion:** These outcomes propose that the co-administration of Vit D with AT may work synergistically to alleviate metabolic disturbances associated with MS.

**Key words:** Adipokines, atorvastatin, high-fat diet, liver enzyme, metabolic syndrome, vitamin D

## INTRODUCTION

Metabolic syndrome (MS) refers to a cluster of interconnected health issues, including dyslipidemia, insulin resistance, hypertension, and obesity, that significantly increase the likelihood of developing cardiovascular disease and diabetes.[1,2] The mechanisms driving MS involve disruptions in lipid profiles, liver function, and adipocytokine production, all of which lead to the syndrome's beginning and progression. In individuals with MS, lipid imbalances, such as elevated low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), total cholesterol (TC), and reduced high-density lipoprotein cholesterol (HDL-C), are common, raising the risk of cardiovascular diseases.[3] Indicators of liver function, comprising alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are often found at elevated levels in those with metabolic abnormalities, indicating possible liver damage or dysfunction. Increased AST and ALT levels are ordinarily allied with non-alcoholic fatty liver disease, a condition frequently observed in MS and often linked to insulin resistance.[4] Adipocytokines, such as adiponectin and resistin, play significant roles in regulating insulin sensitivity, inflammation, and lipid metabolism.[5,6] Adiponectin is typically present at higher levels in individuals with normal metabolic function and exerts anti-inflammatory and insulin-sensitizing effects. Conversely, resistin is elevated in obese individuals, contributing to insulin resistance and promoting inflammation. The balance of these adipocytokines is essential for metabolic regulation, and their imbalance is a hallmark of MS.[7]

Present reports have highlighted the role of Vitamin D (Vit D) not only in bone health but also in metabolic regulation, suggesting that deficiency may contribute to the pathophysiology of MS.[8] Besides its role in calcium regulation, Vit D also offers anti-inflammatory and insulin-sensitizing benefits, which may help improve lipid profiles.[9]

**Address for correspondence:**
Dr. Rajesh A. Maheshwari, Department of Pharmacy, Sumandeep Vidyapeeth Deemed to be University, Vadodara, Gujarat, India. Mobile: +91-2668-245279.
E-mail: rajpharma2007@gmail.com

**Received:** 11-04-2025
**Revised:** 04-06-2025
**Accepted:** 17-06-2025

Atorvastatin (AT), a widely prescribed medication for lowering LDL-C and reducing cardiovascular risk, has also been shown to influence broader metabolic processes, such as modulating adipocytokine levels and liver enzyme activity.[10,11] This study intentions to examine the combined impacts of Vit D and AT on lipid profiles, liver function, and adipocytokine expression in rats with MS. By investigating these interactions, the research seeks to uncover potential therapeutic strategies for managing MS and its related complications.

## MATERIALS AND METHODS

### Drugs and chemicals

AT was sourced from Bharat Parenteral Limited in Vadodara, India, while Vit D and a high-fat diet (HFD) were purchased from local suppliers. The kits used in the study were obtained from a reputable supplier, and all other chemicals and reagents were of analytical grade.

### Laboratory animals

Ethical clearance for the protocol was granted by the Institutional Animal Ethics Committee and followed the guidelines set by the Committee for Control and Supervision of Experiments on Animals. The study involved healthy adult Wistar rats, both male and female, weighing between 200 and 250 g. The rats were housed in polypropylene cages under a diurnal cycle of 12 h light and 12 h dark, temperature controlled at 24°C, with humidity maintained between 35% and 60% and were provided with HFD and purified drinking water.

### Experimental design

MS was induced in rats by feeding them a HFD for 8 weeks. In a randomized manner, the rats were assigned to five sets, each containing six rats. Group I served as the normal control (NC), while Group II was fed only the HFD.[3] Group III received both the HFD and Vit D (400 IU/kg/day),[12] Group IV was given the HFD and AT (10 mg/kg),[11] and Group V was treated with both Vit D and AT along with the HFD. These treatments were administered orally every day for 8 weeks, starting on the 1st day of the diet. Upon finalization of the experimental period, blood collection was carried out through the retro-orbital route under anesthetized conditions, employing glass capillaries. Samples were left to clot at room temperature for 15 min, then centrifuged (5000 rpm, 20 min) to obtain serum, which was stored at −20°C for subsequent evaluation. Biochemical parameters, such as glucose levels, lipid profiles, liver function tests (LFTs), calcium, and Vit D levels were assessed using standard kits. In addition, enzyme-linked immunosorbent assay kits were employed to assess serum levels of adiponectin and resistin.

### Histopathology

Post-euthanasia, hepatic tissues were rapidly harvested, washed in saline solution, and fixed in 10% phosphate-buffered formalin. The fixed tissues were embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin. Histological evaluation was carried out using light microscopy (Olympus BX10, Tokyo, Japan), and images were captured using an Olympus DP12 camera (Japan) to assess histopathological changes. The pathologist performing the analysis was unaware of the treatment group allocations.

### Statistical evaluation

Results are expressed as mean ± standard error of the mean (SEM). A one-way analysis of variance was used to evaluate differences between groups, with Bonferroni *post hoc* tests applied as necessary, using Prism software (GraphPad). A $P < 0.05$ was considered significant.

## RESULTS

### Effect of Vit D and AT on body weight in HFD-induced MS

In rats on a HFD, body weight was expressively higher related to the NC group, reflecting the negative impact of a HFD on overall health and metabolism. This increased body weight is commonly linked to various metabolic disorders and a higher risk of developing conditions, such as obesity and insulin resistance. However, treatment with Vit D, AT, and their combination resulted in significant reductions in body weight compared to the HFD group. This suggests that both Vit D and AT may play key roles in managing weight and improving metabolic health. In addition, rats receiving the combined treatment of Vit D and AT experienced even greater weight loss than those treated with Vit D alone. This suggests that combining these two treatments could have synergistic effects, offering a promising strategy for addressing weight-related issues associated with HFDs [Figure 1].

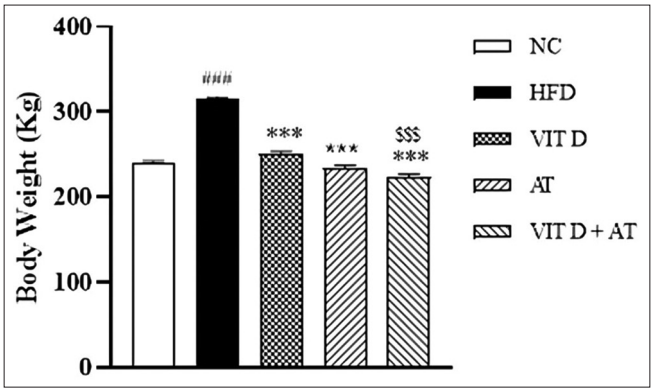


**Figure 1:** Impact of Vit D and AT on body weight in HFD-induced MS. Values are reported as mean ± standard error of the mean, with $n = 6$ for each group. $^{###}P < 0.001$, versus normal control, $^{***}P < 0.001$, versus HFD, $^{\$\$\$}P < 0.001$, versus Vit D.

### Effect of Vit D and AT on Vit D or calcium levels in HFD-induced MS

In rats on a HFD, Vit D and calcium levels were meaningfully lower related to the NC group, reflecting the detrimental effects of HFD on nutrient absorption and metabolism. This deficiency can contribute to various health problems. Treatment with Vit D, either alone or in combination with AT, led to a noteworthy surge in Vit D levels linked to the HFD group, proposing that supplementation can help restore these levels. The combination treatment resulted in greater improvements than Vit D alone, indicating potential synergistic effects. Similarly, Vit D, AT, and the combination of Vit D plus AT all elevated calcium levels in the treated rats, which is essential for normal physiological functions. However, the combination therapy did not provide any significant additional benefit over the individual treatments in terms of calcium levels [Figure 2].

### Effect of Vit D and AT on lipid profile in HFD-induced MS

In rats on a HFD, TC, TG, and LDL levels were markedly elevated, while HDL levels were considerably lower compared to the NC group. This imbalance in lipid levels underscores the harmful effects of a HFD on cardiovascular health. However, treatment with Vit D, AT, and their combination caused in substantial falls in TC, TG, and LDL levels, alongside an increase in HDL levels when compared to the HFD group. These results propose that both Vit D and AT, individually or in combination, may help correct lipid imbalances and improve cardiovascular health affected by a HFD [Figure 3].

### Effect of Vit D and AT on liver enzymes levels in HFD-induced MS

In rats on a HFD, the levels of ALT and AST were pointedly greater verses NC rats, showing liver stress and potential injury produced by the HFD. However, treatment with Vit D, AT, and their combination led to significant reductions in these liver enzyme levels compared to the HFD group, demonstrating their protective effects on liver function. The combination therapy resulted in particularly notable improvements in both AST and ALT levels compared to the Vit D-only group, highlighting the enhanced efficacy

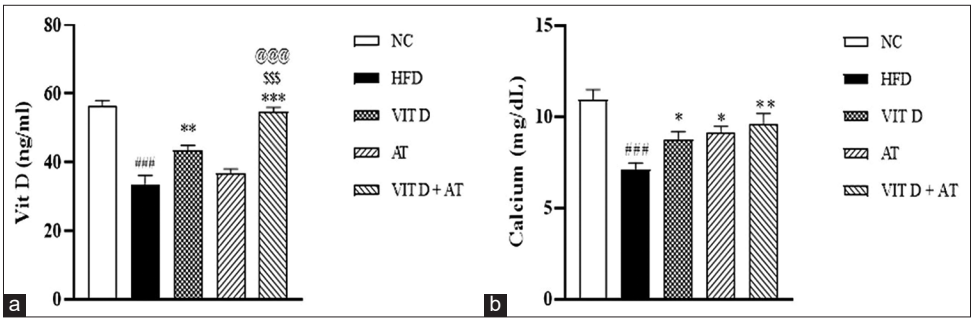


**Figure 2:** Impact of Vit D and AT on (a) Vit D and (b) Calcium levels in HFD-induced MS. Values are reported as mean ± standard error of the mean, with $n$ = 6 for each group. $^{###}P$ < 0.001, versus normal control, $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001, versus HFD, $^{\$\$\$}P$ < 0.001 versus Vit D, $^{@@@}P$ < 0.001 versus AT

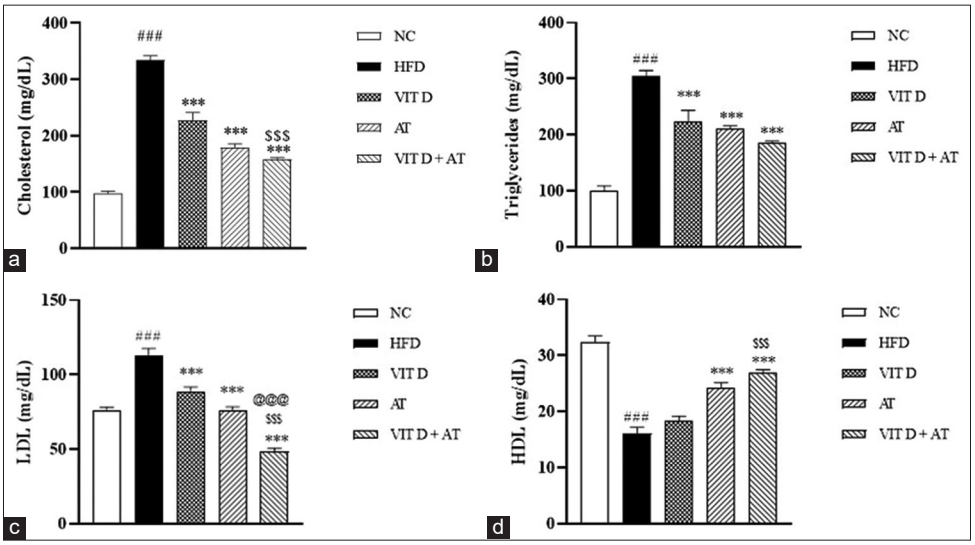


**Figure 3:** Impact of Vit D and AT on (a) Cholesterol, (b) Triglycerides, (c) low-density lipoprotein, and (d) high-density lipoprotein levels in HFD-induced MS. Values are reported as mean ± standard error of the mean, with $n$ = 6 for each group. $^{###}P$ < 0.001, versus normal control, $^{***}P$ < 0.001, versus HFD, $^{\$\$\$}P$ < 0.001 versus Vit D, $^{@@@}P$ < 0.001 versus AT

of combining these treatments. Interestingly, while the combination therapy also caused a significant reduction in AST levels, it showed a more pronounced effect on AST compared to AT alone. Overall, these results suggest that both individual treatments and their combination can effectively reduce liver enzyme elevations associated with a HFD [Figure 4].

### Effect of Vit D and AT on adiponectin and resistin levels in HFD-induced MS

In rats on a HFD, adiponectin levels were significantly reduced, while resistin levels were elevated compared to NC rats, indicating an imbalance in adipokine levels that may contribute to metabolic dysfunction. Treatment with Vit D, AT, or their combination resulted in significant changes in both adiponectin and resistin levels compared to the HFD group, suggesting that these treatments can effectively modulate these key biomarkers. The combination therapy notably caused a considerable rise in adiponectin levels verses the Vit D-only group, emphasizing its enhanced effectiveness. However, it is worth noting that no noteworthy alterations in adiponectin and resistin levels were noticed between the combination treatment and AT monotherapy, suggesting that while both treatments are beneficial, the combination may not offer additional advantages over AT alone in this context [Figure 5].

### Histopathology study

The histopathological analysis of liver tissue from NC rats revealed healthy hepatocyte structure, with intact nuclei and clear cell boundaries. The hepatocytes were organized in uniform strands within the hepatic parenchyma, and the blood vessels, including the central vein, hepatic artery, and portal vein in the portal triad, exhibited normal histological characteristics. The bile duct also showed normal cellular structure with intact epithelium. In contrast, the HFD group displayed mild to moderate degenerative changes in hepatocytes, especially around the central vein, with multiple areas of hepatic degeneration, cellular swelling, enlarged nuclei, and granular changes in the cytoplasm. Fatty droplets were present in the cytoplasm of hepatocytes. The Vit D group exhibited minimal, focal degenerative changes with granular alterations in the cytoplasm, while the hepatocytes maintained a uniform arrangement in strands. The blood vessels remained normal, with focal accumulation of fatty droplets. The AT group exhibited mild degenerative changes, including vacuolar changes in the cytoplasm, alongside multifocal areas of cellular swelling, enlarged nuclei, granular cytoplasm, and fatty droplet accumulation in hepatocytes. Focal vascular congestion in the hepatic parenchyma was also observed. Finally, the combined Vit D and AT group showed minimal and focal degenerative changes with granular cytoplasmic alterations, maintaining the normal arrangement of hepatocytes. The blood vessels and portal triad had normal histological features, with only minimal fatty droplet accumulation in the hepatocyte cytoplasm [Figure 6].

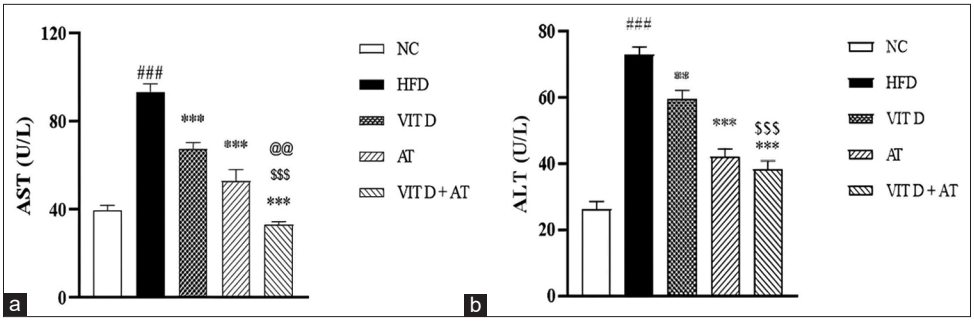


**Figure 4:** Impact of Vit D and AT on (a) AST and (b) ALT levels in HFD-induced MS. Values are reported as mean ± standard error of the mean, with $n = 6$ for each group. $^{###}P < 0.001$, versus normal control, $^{**}P < 0.01$, $^{***}P < 0.001$, versus HFD, $^{\$\$\$}P < 0.001$ versus Vit D, $^{@@}P<0.01$ versus AT

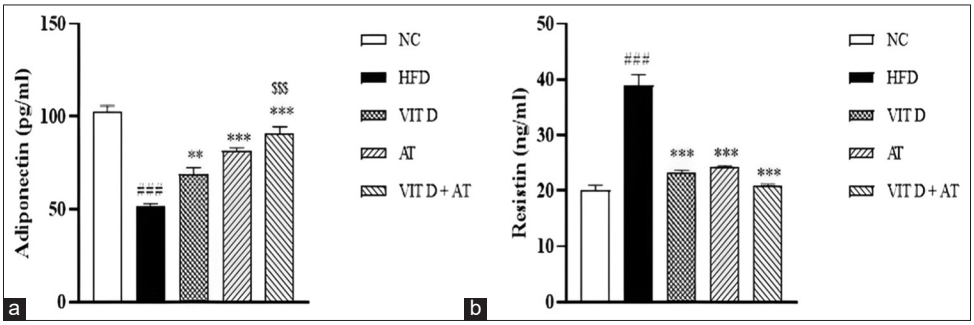


**Figure 5:** Impact of Vit D and AT on (a) Adiponectin and (b) Resistin levels in HFD-induced MS. Values are reported as mean ± standard error of the mean, with $n = 6$ for each group. $^{###}P < 0.001$, versus normal control, $^{**}P < 0.01$, $^{***}P < 0.001$ versus HFD, $^{\$\$\$}P < 0.001$ versus Vit D

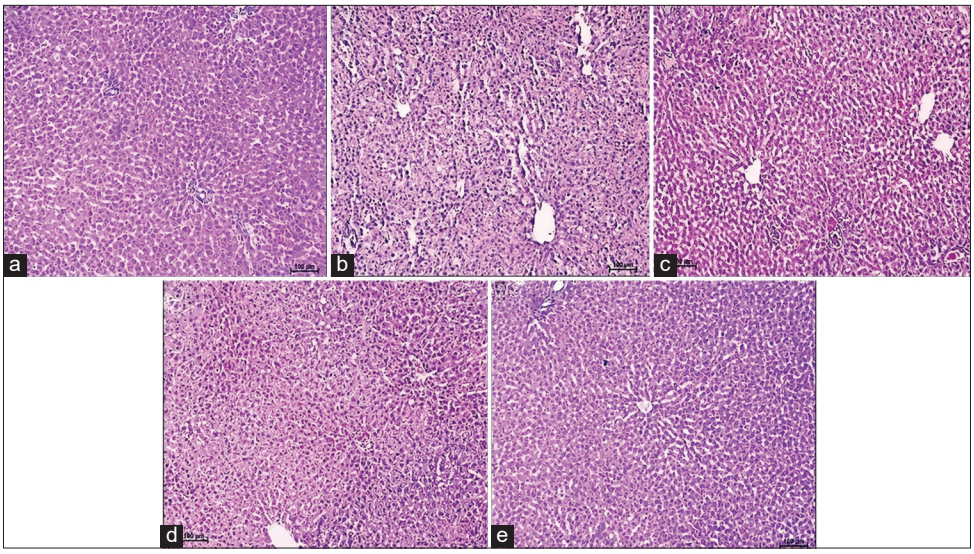


**Figure 6:** Light microscopy of liver tissue from rats (a) normal control, (b) HFD, (c) Vit D, (d) AT, and (e) Vit D + AT

## DISCUSSION

This study investigates the effects of Vit D alone and in combination with AT on parameters related to MS, including lipid profiles, liver function, and adipocytokines, along with calcium and Vit D levels. The results show significant improvements in these areas, demonstrating the effectiveness of these treatments in addressing the complexities of MS. These findings support previous research suggesting the potential benefits of Vit D and AT therapies for managing MS. Overall, the study highlights the value of combined therapeutic approaches to improve metabolic health and reduce MS-related risks.

In terms of lipid profiles, our study found noteworthy declines in TC, TG, and LDL levels, along with a rise in HDL in the treated animals. These outcomes are steady with conclusions from Firdous *et al.* (2021) and Mostafa *et al.* (2016) who showed that both Vit D and statin therapies effectively lower TC, TG, and LDL while potentially increasing HDL.[3,11] Notably, our combination therapy had a synergistic effect on lipid profiles, except for TG, compared to Vit D alone, suggesting that the combination offers better lipid management than either treatment on its own. This aligns with previous studies indicating that Vit D supplementation can improve serum lipid levels in patients with hypercholesterolemia on statin therapy, further highlighting its potential as an effective adjunct treatment.[13] However, it is important to note that the results of our study did not show any significant differences in lipid changes between the combination therapy and AT monotherapy groups, except for LDL levels. This suggests that while the combination therapy may provide additional benefits, AT alone is also effective in managing lipid profiles in this context. Overall, these findings emphasize the importance of investigating combination therapies for optimizing lipid management in patients at risk of MS.

LFTs are essential for evaluating the effects of HFD-induced MS on liver health. In individuals on a HFD, common changes in LFT parameters include elevated serum levels of ALT and AST, which are indicators of liver cell damage or inflammation.[14] In our study, we observed elevated AST and ALT levels in rats with HFD-induced MS, which aligns with previous research. Significantly, treatment with Vit D, AT, or their combination resulted in marked improvements in LFTs compared to the HFD control group, demonstrating the effectiveness of these treatments. Findings from Sneha *et al*. and Firdous *et al*. further support our results, emphasizing the protective roles of Vit D and AT against liver inflammation and damage.[11,15] These results highlight the potential benefits of combining these therapies, suggesting that this approach may improve liver health and reduce the negative effects of HFDs. Overall, our study emphasizes the importance of monitoring liver function in MS and the promising therapeutic strategies for improving liver health.

A HFD can significantly impact the levels of adiponectin and resistin, two important adipokines involved in metabolic regulation. Adiponectin typically has anti-inflammatory and insulin-sensitizing effects, but its levels often decrease in individuals on HFDs. On the other hand, resistin, which is allied with inflammation and insulin resistance, tends to increase under these conditions. This imbalance can lead to a diversity of metabolic issues, such as obesity and type 2 diabetes, highlighting the importance of diet in maintaining metabolic health.[16-20] In our study, rats on a HFD revealed a substantial decline in adiponectin levels and an increase in resistin levels, which is consistent with previous research. Treatment with Vit D, AT, or their combination led to a noteworthy surge in adiponectin levels verses the HFD group, with the combination therapy proving more effective than Vit D alone. Furthermore, these treatments resulted in significant reductions in resistin levels compared to the HFD group,

although no substantial alterations were observed between the combination treatment and the individual therapies. These findings are consistent with earlier studies that showed Vit D's ability to influence adiponectin and resistin levels.[21-24] However, one study showed that long-term AT treatment in MS patients did not affect the secretion of adiponectin, leptin, or resistin by adipose tissue.[25] The observed increase in adiponectin, which is known for its insulin-sensitizing effects, along with the reduction in resistin, is in line with the metabolic benefits seen in our study.

The histopathological findings also support the beneficial effects of treatment, as evidenced by the reduced liver damage observed in the Vit D and combination groups compared to the HFD group. While the HFD group showed significant hepatocyte degeneration, including cellular swelling, enlarged nuclei, and fatty droplet deposition, both the Vit D and its combination with AT exhibited only minimal, focal degenerative changes and maintained normal hepatocyte arrangement. These results suggest that Vit D and its combination with AT treatment may help mitigate liver damage and fatty accumulation persuaded by a HFD, supporting their potential therapeutic effects for liver health.

## CONCLUSION

The outcomes of this study emphasize the significant role that Vit D and AT play in influencing key metabolic parameters in Wistar rats fed a HFD, which induced MS. Both Vit D and AT, whether used individually or in combination, effectively mitigated the adverse effects of the HFD, improving body weight and various metabolic health markers, such as lipid profiles and liver enzyme function. Furthermore, the treatments had a positive impact on adipokine levels, which are crucial in the development of MS. These findings suggest that combining Vit D and AT could be an effective approach for managing weight and addressing metabolic disturbances linked to poor dietary habits, particularly HFDs. Future research might offer deeper insights into the mechanisms behind these synergistic effects and explore the potential for applying this strategy in clinical settings to treat metabolic disorders.

## REFERENCES


1. Huang PL. A comprehensive definition for metabolic syndrome. Dis Model Mech 2009;2:231-7.
2. Alberti KG, Zimmet P, Shaw J; IDF Epidemiology Task Force Consensus Group. The metabolic syndrome-a new worldwide definition. Lancet 2005;366:1059-62.
3. Mostafa DK, Nasra RA, Zahran N, Ghoneim MT. Pleiotropic protective effects of Vitamin D against high fat diet-induced metabolic syndrome in rats: One for all. Eur J Pharmacol 2016;792:38-47.
4. Muzurović E, Mikhailidis DP, Mantzoros C. Non-alcoholic fatty liver disease, insulin resistance, metabolic syndrome and their association with vascular risk. Metabolism 2021;119:154770.
5. Fadaei R. Adipokines as a link between adipose tissue with inflammation and insulin resistance in cardiometabolic diseases. Acta Biochim Iran 2023;1:112-8.
6. Tripathi D, Kant S, Pandey S, Ehtesham NZ. Resistin in metabolism, inflammation, and disease. FEBS J 2020;287:3141-9.
7. Kumari R, Kumar S, Kant R. An update on metabolic syndrome: Metabolic risk markers and adipokines in the development of metabolic syndrome. Diabetes Metab Syndr 2019;13:2409-17.
8. Melguizo-Rodríguez L, Costela-Ruiz VJ, García-Recio E, De Luna-Bertos E, Ruiz C, Illescas-Montes R. Role of vitamin D in the metabolic syndrome. Nutrients 2021;13:830.
9. Szymczak-Pajor I, Drzewoski J, Śliwińska A. The molecular mechanisms by which vitamin D prevents insulin resistance and associated disorders. Int J Mol Sci 2020;21:6644.
10. Yamada Y, Takeuchi S, Yoneda M, Ito S, Sano Y, Nagasawa K, *et al*. Atorvastatin reduces cardiac and adipose tissue inflammation in rats with metabolic syndrome. Int J Cardiol 2017;240:332-8.
11. Firdous SM, Hazra S, Gopinath SC, El-Desouky GE, Aboul-Soud MA. Antihyperlipidemic potential of diosmin in Swiss Albino mice with high-fat diet induced hyperlipidemia. Saudi J Biol Sci 2021;28:109-15.
12. Alsolami KM. Potential protective effect of vitamin D on cardiac apoptosis in obese rats. Genet Mol Res 2019;18:gmr16039950.
13. Qin XF, Zhao LS, Chen WR, Yin DW, Wang H. Effects of vitamin D on plasma lipid profiles in statin-treated patients with hypercholesterolemia: A randomized placebo-controlled trial. Clin Nutr 2015;34:201-6.
14. Efati M, Khorrami M, Zarei Mahmmudabadi A, Raouf Sarshoori J. Induction of an animal model of non-alcoholic fatty liver disease using a formulated high-fat diet. J Babol Univ Med Sci 2016;18:57-62.
15. Sneha M, Reddy GS, Rajesham VV, Ali PR, Rao TR, Raghavendra M. Effects of Vitamin D3 against sodium fluoride induced hepatotoxicity in male Wistar Albino rats. Int J Pharma Res Health Sci 2023;11:3674-9.
16. Catta-Preta M, Martins MA, Brunini TM, Mendes-Ribeiro AC, Mandarim-de-Lacerda CA, Aguila MB. Modulation of cytokines, resistin, and distribution of adipose tissue in C57BL/6 mice by different high-fat diets. Nutrition 2012;28:212-9.
17. Mahmoud AM, Abdel-Rahman MM, Bastawy NA, Eissa HM. Modulatory effect of berberine on adipose tissue PPARγ, adipocytokines and oxidative stress in high fat diet/streptozotocin-induced diabetic rats. J Appl Pharm Sci 2017;7:1-10.
18. Cano P, Cardinali DP, Ríos-Lugo MJ, Fernández-Mateos MP, Toso CF, Esquifino AI. Effect of a high-fat diet on 24-hour pattern of circulating adipocytokines in

rats. Obesity (Silver Spring) 2009;17:1866-71.
19. Szewczyk-Golec K, Woźniak A, Reiter RJ. Inter-relationships of the chronobiotic, melatonin, with leptin and adiponectin: Implications for obesity. J Pineal Res 2015;59:277-91.
20. Farias TD, Paixao RI, Cruz MM, de Sa RD, Simão JD, Antraco VJ, *et al*. Melatonin supplementation attenuates the pro-inflammatory adipokines expression in visceral fat from obese mice induced by a high-fat diet. Cells 2019;8:1041.
21. Ismail MM, Hamid TA, Ibrahim AA, Marzouk H. Serum adipokines and vitamin D levels in patients with type 1 diabetes mellitus. Arch Med Sci 2017;13:738-44.
22. Mousa A, Naderpoor N, Wilson K, Plebanski M, de Courten MP, Scragg R, *et al*. Vitamin D supplementation increases adipokine concentrations in overweight or obese adults. Eur J Nutr 2020;59:195-204.
23. Blanco-Colio LM, Martín-Ventura JL, Gómez-Guerrero C, Masramon X, de Teresa E, Farsang C, *et al*. Adiponectin plasma levels are increased by atorvastatin treatment in subjects at high cardiovascular risk. Eur J Pharmacol 2008;586:259-65.
24. Ohbayashi H. Pitavastatin improves serum resistin levels in patients with hypercholesterolemia. J Atheroscler Thromb 2008;15:87-93.
25. Szotowska M, Czerwienska B, Adamczak M, Chudek J, Wiecek A. Effect of low-dose atorvastatin on plasma concentrations of adipokines in patients with metabolic syndrome. Kidney Blood Press Res 2012;35:226-32.

**Source of Support:** Nil. **Conflicts of Interest:** None declared.