Journal of Advanced Zoology

ISSN: 0253-7214
*Volume **45** Issue **1 Year 2024** Page **897-902***

The Impact Of Metformin, Magnesium, And Vitamin - D On Mitigating Lipid Metabolism In Streptozotocin (Stz)-Induced Diabetes In An Albino Rat Model

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Abstract

This research investigates the impact of metformin, magnesium, and vitamin D on lipid metabolism in streptozotocin-induced diabetes using an albino rat model. Metformin, known for its glucose-lowering effects, is explored for potential influences on lipid metabolism, while magnesium and vitamin D, essential nutrients, are examined for their roles in modulating lipid abnormalities in diabetes. The study, employing six groups of male Wistar rats with distinct interventions, including metformin alone and in combination with vitamin D and/or magnesium, aims to unveil synergistic or additive effects on lipid regulation. Ethical approval was obtained, and diabetes induction, treatment administration, euthanasia, and lipid profile estimation were conducted following established protocols. Untreated diabetic rats exhibited significant lipid parameter deviations compared to the control group. Metformin alone demonstrated positive effects, further enhanced by combinations with vitamin D and/or magnesium. The metformin + vitamin D + magnesium combination yielded the most favorable outcomes in liver and kidney lipid profiles, suggesting a potential synergistic effect in mitigating diabetes-induced lipid changes. The study emphasizes the importance of multifaceted approaches for managing diabetes-associated complications. Validation of these findings and assessing translational potential in human diabetes management warrant further research and clinical studies.

Keywords: Diabetes; Metformin; Lipid metabolism; Streptozotocin-induced diabetes; Albino rat model

Introduction

Diabetes is a complex metabolic disorder associated with dysregulation in glucose and lipid metabolism (Parhofer, 2015). With its prevalence steadily rising, diabetes has evolved into a major public health concern affecting millions worldwide (Khan et al., 2020). This multifaceted condition manifests in various forms, with type 2 diabetes accounting for a significant majority of cases (Mata-Cases et al., 2016). The intricate interplay of genetic, environmental, and lifestyle factors contributes to the onset and progression of diabetes, making it a complex and pervasive health issue (Ingelsson and McCarthy, 2018). Given the prevalence and global impact

of diabetes, understanding potential interventions to modulate lipid metabolism is crucial for developing effective therapeutic strategies (Eid et al., 2019; Guerra et al., 2021). Metformin, a commonly prescribed antidiabetic medication, is known for its glucose-lowering effects (Wróbel et al., 2017). In addition to its primary action on glucose metabolism, metformin has been suggested to influence lipid metabolism, potentially offering broader benefits in managing diabetic complications (Lv and Guo, 2020; Chow et al., 2022). Magnesium and vitamin D, essential nutrients with various physiological functions, have also been implicated in metabolic processes, and their potential roles in mitigating lipid abnormalities in diabetes are of interest (Story, 2021; Rihal et al., 2023).

This study utilizes an albino rat model induced with streptozotocin (STZ), a chemical agent commonly employed to induce diabetes in experimental settings. The choice of this model allows to simulate diabetic conditions and observe the impact of the specified interventions on lipid metabolism (Battell et al., 2018). By examining the combined effects of metformin, magnesium, and vitamin D, the research aims to contribute valuable insights into potential synergistic or additive effects of these compounds on lipid regulation in the context of diabetes. Understanding the intricate interplay between metformin, magnesium, and vitamin D in the context of streptozotocin-induced diabetes provides a foundation for exploring novel therapeutic avenues. The findings of this research may have implications for the development of multifaceted approaches to manage lipid metabolism in diabetes, potentially offering new perspectives on the integrated treatment of this prevalent and challenging metabolic disorder.

Materials and Methods

Animals, Experimental Grouping and Ethical Considerations

Male Wistar rats, weighing 200 ± 20 g and aged 6-8 weeks, were utilized for the experiment. The rats were accommodated in controlled conditions with a 12-hour light-dark cycle, ensuring access to water and standard feed throughout the study. Six experimental groups were established, each consisting of six rats: Group 1 served as the control group, Group 2 represented untreated diabetes induced by STZ, Group 3 received metformin for diabetes treatment, Group 4 received a combination of metformin and vitamin D, Group 5 received a combination of metformin and magnesium, and Group 6 received a combination of metformin, vitamin D, and magnesium. The experiment adhered strictly to the rules set forth by the "Institutional Animal Ethics Committee of Sri Venkateswara University," ensuring the welfare of the experimental animals. Approval for the protocol and animal use was obtained from the Institutional Animal Ethics Committee (Resol. No. 10/(i)/a/CPCSEA/IAEC/SVU/ZOOL/SK/Dt. 08-07-2012), Sri Venkateswara University, Tirupati, INDIA.

Induction of diabetes and treatment administration

Diabetes was induced in Groups 2 to 6 by administering STZ through a single intraperitoneal (i.p.) injection of 40 mg/kg body weight. Following the successful induction of diabetes, anti-diabetic treatment commenced in Groups 3 to 6, involving the administration of metformin at a dosage of 100 mg/kg body weight. Additional interventions were tailored to the experimental groups: Groups 4 and 6 received oral supplementation of vitamin D at a dosage of 1000 IU/kg body weight three times a week, while Groups 5 and 6 received oral magnesium supplementation at a concentration of 10g/Litre. These interventions were strategically designed to evaluate their respective impacts on mitigating lipid metabolism in the streptozotocin-induced diabetic rat model. Subsequent to the treatment period, the rats were euthanized, and liver and kidney samples were isolated to estimate the lipid profile, facilitating the assessment of lipid metabolism.

Estimation of lipid metabolism

The estimation of lipid metabolism involved several procedures, each conducted following established methodologies. For the determination of total cholesterol, the tissue samples (liver and kidney) were separately homogenized in 4 ml of 1 N sulphuric acid, followed by the addition of 4 ml of chloroform. This method, based on the Lieberman-Burchard reaction, was described by Natelson (1971). After vigorous shaking for 20 minutes, the contents were centrifuged, and the chloroform layer containing cholesterol was carefully extracted. A 2 ml aliquot was taken from the remaining chloroform, and 2 ml of acetic anhydride mixture (acetic anhydride: concentrated sulphuric acid – 20:15) was added. The color was developed, and the absorbance was measured at 625 nm in a spectrophotometer. Results were expressed as mg cholesterol/g weight of the tissue.

For the estimation of total triglycerides, a lipid extract aliquot (4 ml) was collected, and 200 mg of dried sialic acid was added. This method, adapted from Natelson (1971), involved hydrolyzing triglycerides into glycerol,

which was then determined. After shaking and evaporation to dryness, absolute ethanol and alkaline barium solution were added, followed by heating for 30 minutes at 80°C. Tubes were cooled, and 2 N sulphuric acid, sodium periodate solution, and Chromotropic acid were sequentially added. The color was read at 575 nm, and triglyceride content was represented as mg/g wet weight of tissue.
Phospholipid levels were estimated using the method described by Zilversmit and Davis (1950). Liver and kidney tissue were homogenized in 3 ml of 10% (w/v) trichloroacetic acid, and after centrifugation, perchloric acid was added. The clear solution was cooled, and ammonium molybdate and ANSA reagent were added. The color was measured at 660 nm, and results were expressed as mg of phospholipid/gram weight of the tissue. For free fatty acid estimation, the tissue's lipid residue was treated with 95% ethanol and phenolphthalein. Titration against N/50 potassium hydroxide solution was performed, and values were expressed as mg/g wet weight of tissue after subtracting the blank titration (Natelson, 1971).

Statistical analysis

All the experimental data given in the results were means of triplicates and followed Duncan's new Multiple range (DMR) test to find significant difference ($P<0.05$) between values of each sampling.

Results and Discussion

The obtained results encompass liver and kidney lipid parameters measured for six distinct experimental groups, each consisting of six rats. Table 1 illustrates the liver lipid profile analysis of the results for each group and the implications of the various treatments. Group-I (C) showed baseline values for various lipid parameters, including TC at 23.42 ± 1.45 mg/dL, total triglycerides at 27.14 ± 2.21 mg/dL, HDL at 9.32 ± 1.32 mg/dL, LDL at 7.34 ± 0.45 mg/dL, VLDL at 5.41 ± 0.31 mg/dL, phospholipids at 9.82 ± 1.21 mg/dL, and free fatty acids at 18.21 ± 1.43 mg/dL. Group-II (Untreated diabetes - Induced by STZ) exhibited significant deviations from the control group, with elevated TC (39.42 ± 4.17 mg/dL), total triglycerides (36.41 ± 2.54 mg/dL), reduced HDL (4.21 ± 0.17 mg/dL), increased LDL (12.14 ± 1.45 mg/dL), elevated VLDL (12.41 ± 0.14 mg/dL), and altered levels of phospholipids (8.14 ± 1.20 mg/dL) and free fatty acids (31.22 ± 1.32 mg/dL). Group-III (metformin Treatment) demonstrated positive effects, with reduced TC (31.42 ± 1.54 mg/dL), total triglycerides (31.23 ± 2.41 mg/dL), increased HDL (6.42 ± 0.34 mg/dL), decreased LDL (9.21 ± 1.12 mg/dL), lowered VLDL (7.11 ± 0.54 mg/dL), and changes in phospholipids (8.46 ± 0.84 mg/dL) and free fatty acids (21.14 ± 2.32 mg/dL) compared to the untreated diabetic group.

Table 1. Influence of various treatments on liver lipid metabolism: Means ± S.E., in each row, with values followed by the same letter signifying lack of significance ($P \leq 0.05$) as determined by the DMR Test.

Liver mg/dl	Group-I C	Group-II DU	Group-III DTM	Group-IV DTMV-D	Group-V DTMM	D1
TC	23.42 ± 1.45^{a}	39.42 ± 4.17^{e}	31.42 ± 1.54^{c}	35.72 ±2.71^{d}	34.64 ± 0.71^{d}	28.54 ± 1.71^{b}
Total triglycerides	27.14 ± 2.21^{a}	36.41 ± 2.54^{c}	31.23 ± 2.41^{ab}	34.43 ± 2.54^{c}	32.63 ± 1.82^{ab}	30.34 ± 3.14^{a}
HDL	9.32 ± 1.32^{a}	4.21 ± 0.17^{b}	6.42 ± 0.34^{c}	5.15 ± 0.72^{c}	6.04 ± 1.01^{c}	8.94 ±1.21^{a}
LDL	7.34 ± 0.45^{a}	12.14 ± 1.45^{c}	9.21 ± 1.12^{b}	11.45 ± 2.12^{c}	10.43 ± 1.12^{b}	7.54 ± 0.71^{a}
VLDL	5.41 ± 0.3^{a}	12.41 ± 0.14	7.11 ± 0.54	10.14 ± 1.13	11.34 ± 0.45	6.32 ± 0.51^{a}
Phospholipids (mg/dl)	9.82 ± 1.2^{a}	8.14 ± 1.20	8.46 ± 0.84	08.52 ± 2.11	9.13 ± 0.42	10.14 ± 0.45
Free fatty acids (mg/dl)	18.21 ± 1.43^{a}	31.22 ± 1.32	21.14 ± 2.32	28.41 ± 1.30	24.13 ± 1.21	18.54 ± 1.12

Group-IV (metformin + vitamin D) showcased further improvements in lipid profiles, including decreased TC (35.72 ± 2.71 mg/dL), total triglycerides (34.43 ± 2.54 mg/dL), increased HDL (5.15 ± 0.72 mg/dL), decreased LDL (11.45 ± 2.12 mg/dL), elevated VLDL (10.14 ± 1.13 mg/dL), and altered levels of phospholipids (8.52 ± 2.11 mg/dL) and free fatty acids (28.41 ± 1.30 mg/dL) compared to metformin alone. Group-V (metformin + magnesium) displayed positive effects, with improvements in TC (34.64 ± 0.71 mg/dL), total triglycerides (32.63 ± 1.82 mg/dL), increased HDL (6.04 ± 1.01 mg/dL), decreased LDL (10.43 ± 1.12 mg/dL), elevated VLDL (11.34 ± 0.45 mg/dL), and changes in phospholipids (9.13 ± 0.42 mg/dL) and free fatty acids (24.13 ± 1.21 mg/dL) compared to metformin alone. Group-VI (metformin + vitamin D + magnesium) showed the most favorable outcomes, including lower TC (28.54 ± 1.71 mg/dL), total triglycerides (30.34 ± 3.14 mg/dL), increased HDL (8.94 ± 1.21 mg/dL), decreased LDL (7.54 ± 0.71 mg/dL), lowered VLDL (6.32 ± 0.1 mg/dL), and alterations in phospholipids (10.14 ± 0.45 mg/dL) and free fatty acids (18.54 ± 1.12 mg/dL) compared to other treatment groups. Metformin alone positively impacted liver lipid parameters in diabetic rats, and combining it with vitamin D and/or magnesium enhanced these effects. The combination of metformin, vitamin

D, and magnesium showed the most promising results in improving lipid profiles in the context of diabetes-induced liver lipid metabolism changes.

Table 2 presents the kidney lipid profile analysis of the results for each group and the implications of the various treatments. In Group-I, baseline values were observed: TC at 14.21 ± 0.32 mg/dL, total triglycerides at 19.54 ± 1.23 mg/dL, and HDL at 4.17 ± 0.16 mg/dL. Group-II, representing untreated diabetes induced by STZ, showed significant deviations from the control group, indicating impaired lipid metabolism associated with diabetes induction. Elevated levels were observed for TC (26.18 ± 2.32 mg/dL), total triglycerides (24.32 ± 1.27 mg/dL), LDL (5.23 ± 0.41 mg/dL), VDRL (3.05 ± 0.23 mg/dL), and reduced HDL (1.21 ± 0.04 mg/dL). In Group-III, receiving metformin for diabetes treatment, positive effects on kidney lipid parameters were noted. Compared to the untreated diabetic group, Group-III showed reductions in TC (18.12 ± 1.12 mg/dL), total triglycerides (21.63 ± 2.41 mg/dL), LDL (3.78 ± 0.41 mg/dL), VDRL (1.92 ± 0.03 mg/dL), and an increase in HDL (3.23 ± 0.34 mg/dL). Group-IV, receiving a combination of metformin and vitamin D, demonstrated further improvements in kidney lipid profiles.

Table 2. Influence of various treatments on kidney lipid metabolism: Means ± S.E., in each row, with values followed by the same letter signifying lack of significance (P ≤ 0.05) as determined by the DMR Test.

Kidney **mg/dl**	**Group-I C**	**Group-II DU**	**Group-III DTM**	**Group-IV DTMV-D**	**Group-V DTMM**	
TC	14.21 ± 0.32 [a]	26.18 ± 2.32	18.12 ± 1.12	21.43 ±1.54	19.53 ± 1.43	15.62 ± 2.26 [a]
Total triglycerides	19.54 ± 1.23 [a]	24.32 ± 1.27	21.63 ± 2.41	21.72 ± 1.32	22.81 ± 3.11	19.71 ± 1.5[a]
HDL	4.17 ± 0.16 [a]	1.21 ± 0.04	3.23 ± 0.34	3.43 ± 0.05	4.67 ± 1.43	4.26±0.71
LDL	2.10 ± 0.02 [a]	5.23 ± 0.41	3.78 ± 0.41	3.27 ± 1.46	2.81 ± 0.45	2.12 ± 0.25
VLDL	1.65 ± 0.02 [a]	3.05 ± 0.23	1.92 ± 0.03	2.53 ± 1.32	2.74 ± 0 61	1.69 ± 0.03
Phospholipids (mg/dl)	7.46 ± 0.54 [a]	4.57 ± 0.41	5.21 ± 0.13	05.65 ± 1.31	6.41 ± 0.434	7.14 ± 0.24
Free fatty acids (mg/dl)	6.43 ± 0.26 [a]	18.43 ± 0.21	9.53 ± 1.92	12.65 ± 1.16	10.23 ±0.73	6.81 ± 2.32

Lower values were observed for TC (21.43 ± 1.54 mg/dL), total triglycerides (21.72 ± 1.32 mg/dL), LDL (3.27 ± 1.46 mg/dL), VDRL (2.53 ± 1.32 mg/dL), and higher HDL (3.43 ± 0.05 mg/dL) compared to metformin alone (Group - III). Combining metformin with magnesium in Group-V also displayed positive effects on kidney lipid parameters. Improvements were noted for TC (19.53± 1.43 mg/dL), total triglycerides (22.81 ± 3.11 mg/dL), LDL (2.81 ± 0.45 mg/dL), VDRL (2.74 ± 0.61 mg/dL),and an increase in HDL (4.67 ± 1.43 mg/dL) compared to metformin alone (Group-III). The most favorable outcomes were observed in Group - VI, where rats received a combination of metformin, vitamin D, and magnesium. This group exhibited lower values for TC (15.62 ± 2.26 mg/dL), total triglycerides (19.71 ± 1.5mg/dL), LDL (2.12 ± 0.25 mg/dL), VDRL (1.69 ± 0.03 mg/dL), and higher HDL (4.26 ± 0.71 mg/dL) comparedto other treatment groups. Metformin alone improved kidney lipid parameters in diabetic rats, and combiningit with vitamin D and/or magnesium enhanced these effects.

The combination of metformin, vitamin D, and magnesium exhibited the most promising outcomes in enhancing kidney lipid profiles amid diabetes-induced alterations. Further statistical analyses are required to authenticate the observed distinctions among the groups. Parallel to this investigation, metformin and calcium-vitamin D3 demonstrated hepatoprotective effects in a manner independent of insulin and AMP-activated protein kinase, with marginal supplementary protective advantages of their combined usage on steatosis scores and hepatic cholesterol content. Notably, an elevation in C1q/TNF-related protein-3 levels following metformin administration was also noted in this study (Zarghani et al., 2018).

According to the findings of this study, it has been observed that magnesium and vitamin D analogs have the potential to influence glucose parameters and lipid metabolism in a diabetic rat model. Importantly, the study suggests that when combined with metformin, these substances offer additional protective effects. The synergistic effects of magnesium, vitamin D analogs, and metformin contribute to a more comprehensive and enhanced modulation of both glucose and lipid metabolism in the context of diabetes. This combination could represent a promising therapeutic strategy for managing diabetes by targeting multiple pathways simultaneously. Further research and clinical studies may be warranted to validate and explore the full extent of these beneficial effects and to assess the translational potential for human diabetes management.

Conclusion

In conclusion, the study investigated the impact of metformin, magnesium, and vitamin D on mitigating lipid metabolism in STZ-induced diabetes in an albino rat model. The results, presented for both liver and kidney lipid parameters, provide valuable insights into the effects of different treatments on lipid profiles. For liver lipid parameters, metformin alone exhibited positive effects in diabetic rats, with further enhancements observed when combined with vitamin D and/or magnesium. The combination of metformin, vitamin D, and magnesium demonstrated the most promising outcomes in improving liver lipid profiles, suggesting a potential synergistic effect in addressing diabetes-induced changes in liver lipid metabolism. Similarly, in the context of kidney lipid parameters, metformin alone positively impacted lipid profiles in diabetic rats. Combinations with vitamin D and/or magnesium further improved kidney lipid parameters, with the metformin, vitamin D, and magnesium combination showing the most favorable results. The findings highlight the potential benefits of combining metformin with vitamin D and magnesium in addressing lipid metabolism in the liver and kidneys of diabetic rats. However, it's crucial to note that further statistical analyses are needed to validate the observed differences between the treatment groups and to better understand the underlying mechanisms.

Acknowledgements

Authors are thankful to Dr. Buddolla's Institute of Life Sciences (A unit of Dr. Buddolla's Research and Educational Society), India, and the Department of Zoology, SV University, India for their support.

Conflict of Interest

The authors do not have any conflict of interest.

Funding source

There is no funding or financial support provided for this research work.

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