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HUMAN PRIMARY HEPATOCYTE TRANSCRIPTOME ANALYSIS UPON TREATMENT WITH ROSUVASTATIN AND IMPLICATION ON CARDIAC ATRIAL NATRIURETIC PEPTIDES LEVEL

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Abstract

The relationship between rosuvastatin and cardiac Atrial Natriuretic Peptides (ANP) levels has been explored in previous studies, while, the exact mechanisms underlying the modulation of ANP levels by rosuvastatin are not fully understood. Therefore, the aim of this study is to investigate the likely mechanism associated with changes in ANP levels following treatment with rosuvastatin. The study used 42 male Wistar rats, weighing 120-140 g, divided into six groups, to investigate the impact of Multidrug resistance 1 (MDR1) mutations and high-fat diet on serum lipid profiles. Rats were acclimatized for 2 weeks, given different diets, and treated with Rosuvastatin. The experiment lasted for 12 weeks, and serum NT-pro ANP was measured using ELISA. Microarray datasets from the GEO database were analyzed for differentially expressed genes using R software and enriched for gene ontology and KEGG pathways. There was a significant increase in ANP levels in the high-fat diet group treated with rosuvastatin in the evening compared to all other groups ($P < 0.05$). Similar trend was observed in the normal diet group treated with rosuvastatin either in the morning or evening (p-value < 0.05). After 48-hour treatment with rosuvastatin, the gene MVK displayed upregulation with a $\log_2$(fold change) of 1.291, CYB5B exhibited upregulation with a $\log_2$(fold change) of 2.477, FDFT1 showed upregulation with a $\log_2$(fold change) of 2.292, and SFN exhibited upregulation with a $\log_2$(fold change) of 3.8. While these findings provide insights into the potential mechanisms underlying the enhancement of cardiac ANP secretion, further research is necessary to confirm the functional relevance of these genes and the clinical significance of their effects on ANP levels in humans.

Keywords: ANP, serum NT-pro, Cardiac, KEGG pathways, MDR1 mutations

INTRODUCTION

Rosuvastatin is a widely prescribed medication used for managing hypercholesterolemia and preventing cardiovascular events (1). It belongs to the statin class of drugs, which are known for their cholesterol-lowering properties (2). One hormone of interest in cardiovascular regulation is atrial natriuretic peptide (ANP) (3), which is secreted by the heart and plays a role in maintaining blood pressure and fluid balance. ANP is a hormone that plays a crucial role in regulating cardiovascular homeostasis and fluid balance (4). It is primarily synthesized and secreted by the cardiac atria in response to various stimuli, including increased blood volume, atrial stretch, and sympathetic activation (5). ANP exerts its effects through

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binding to specific receptors, known as natriuretic peptide receptors (NPRs), located in various tissues throughout the body (6). Understanding the significance of ANP helps in elucidating its role in various physiological processes and its potential clinical applications.
The relationship between cardiac atrial natriuretic peptide and rosuvastatin therapy has been investigated in various studies (7, 8). The exact mechanisms underlying the modulation of ANP levels by rosuvastatin are not fully understood. Therefore, the aim of this study is to investigate the likely mechanism associated with changes in ANP levels following treatment with rosuvastatin.

METHODS

Experimental animals

Forty-two healthy, male Wistar rats (n=42) weighing 120-140 g, were purchased from McTemmy Farms, Ogbomosho Nigeria, for the study. The animals were housed in plastic cages at room temperature of 27±2°C in the University Central Laboratory animal house. They were acclimatized to the environment of the animal house before beginning of experiment for 2 weeks. The house has a 12-hour light/dark cycle and is well ventilated. The animals were free and had voluntary access to food and water. Ethical approval for the study was obtained from the Ethical Review Committee of the Faculty of Basic Clinical Sciences, University of Ilorin.

Grouping of Animals

The Wistar rats were divided by random sampling into six groups. Group A and group C were fed with normal rat diet and water, while groups B and D were fed with high fat diet and water. Group A and group B received rosuvastatin (RS) in the morning, while group C and group D received rosuvastatin treatment in the evening. Group E and group F served as the control groups which did not receive any.

The drug was given to groups A, B, C and D starting at 4 weeks of the experiment and continued till the end of the experiment (up to 12 weeks).
Group A (NDRM, n=7): Normal rat feed + RS 5 mg/kg morning dose
Group B (HDRM, n=7): High fat diet + RS 5 mg/kg morning dose
Group C (NDRE, n=7): Normal rat feed + RS 5 mg/kg evening dose
Group D (HDRE, n=7): High fat diet + RS 5 mg/kg evening dose
Group E (NDC, n=7): Normal rat feed control
Group F (HDC, n=7): High fat diet control

Experimental protocol

Animals were acclimatized for 2 weeks in the animal house while taking normal rat feed. Blood samples were collected at baseline from the lateral or dorsal veins of the tails of the rats, with the help of scalp vein, for the estimation of the serum lipid profile. Animals were weighed weekly using a digital weighing balance. Administration of different diets commenced at the end of acclimatization and continued until week 12. Blood samples were collected from the lateral or dorsal veins of the tails of the rats with the help of scalp vein for the estimation of all biochemical parameters (ANP) at the end of 4 weeks and after 12 weeks. Treatment with Rosuvastatin 5 mg/kg commenced at 6 weeks and continued till 12 weeks. At the end of the experiment, animals were sacrificed using ketamine 100 mg/kg (9) and organ of interest (thoracic aorta) was harvested.

Preparation of High fat diet

High fat diet was compounded by a commercial outfit (Ogo-Oluwa Feeds, Ilorin). The diet was prepared according to the method of Woods *et al*. (10) with some modifications by adding 3.1 kg of Beef tallow and 0.1 kg of groundnut oil to 1 kg of standard rat feed.

Assay of Wistar Rat Serum N-Terminal Pro-Atrial Natriuretic Peptide (NT-pro ANP)

The Rat serum NT-pro ANP was measured using Bioassay Technology Laboratory Elisa assay kit (Korain Biotech Co., Shanghai, China) which is based on the principle of immunoassay and uses sandwich ELISA method. The plate had been pre-coated with Rat NT-pro ANP antibody and NT-pro ANP present in the sample when added, binds to antibodies coated on the wells.

Microarray datasets

The GEO database (http://www.ncbi.nlm.nih.gov/geo) is a public functional genomics database that contains a variety of data, including data derived from microarrays and next-generation sequencing. The GEO database was searched using the following keywords: ("Rosuvastatin" [MeSH terms] OR ANP [all fields])) AND "Homo sapiens"[porgn] AND ("gse"[filter] AND "profile expression per matrix" [filter] AND "feature name texture" [filter]. A total of 33 datasets were identified for further analysis after a keyword-based meta-analysis. Following the selection criteria, one gene expression profile was collected, GSE24188.

Data pre-processing and identification of Differentially Expressed Genes (DEGs)

Table array files of GSE24188 was downloaded from the GEO database. Prior to analysis, probes in each data set were converted to standard gene symbols. Normalization of the datasets was applied based on robust multi-array averaging in R software, version 4.0.0 (www.R-project.org/), and normalization was performed separately on each gene expression dataset. The GEO2R platform is a comprehensive software for visualization and statistical analysis of microarray gene expression data (11). In the present study, DEGs from each dataset were pinpointed using GEO2R software. A p-value of 0.05 was set as the cut-off criterion. A volcano plot of DEGs was then generated from the dataset using the ggplo2 package in R software.

Functional and pathway enrichment analysis

A frequent method used for large-scale functional studies of transcriptomic data and genomic data analysis is Gene ontology (GO) analysis (12). The Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/pathway) is a database for the systematic analysis of gene functions (13). The Integrated Annotation and Discovery Visualization Database (DAVID; https://david.ncifcrf.gov) is a tool used to systematically determine biological significance in large lists of genes or proteins (14). In the present study, GO function and KEGG pathway enrichment analysis of the identified DEGs and genes in the significant sections were performed using DAVID. Terms with a p value <0.005, considered significant.

RESULTS AND DISCUSSION

Table 1 displays the results of ANP levels in ng/ml at different time points (Week 4 and Week 12) following treatment with rosuvastatin. The results are presented as means ± SEM (Standard Error of the Mean), and letters (a, b, ab) are used to indicate significant differences between the values within each row and column. There is significant increase in ANP levels in the high fat diet group treated with rosuvastatin in the evening ($p < 0.05$) when compared with all groups. This level decreases between week 4 and week 12. The same trend was observed in normal diet animals treated with rosuvastatin either in the morning or evening ($p < 0.05$).

Identification of DEGs

Understanding the therapeutic mechanisms upon treatment with rosuvastatin is essential to improve the survival rate of the patients and develop more potent and safer therapeutics. Recently, rapidly developed microarray technology has been widely applied to make an

analogy between gene expression levels and used to predict therapeutic mechanism (15). In the current study, a total of 91 DEGs from the GSE24188 (8 upregulated genes; 83 downregulated genes) (Figure 1a) dataset were identified in the hepatic tissues from patients after 24 hours of treatment with rosuvastatin compared to normal tissues, while 799 DEGs (146 upregulated genes; 653 downregulated genes) (Figure 1b) were identified from GSE24188 datasets in hepatic tissues from patients after 48 hours of treatment with rosuvastatin, when compared with the normal tissues.

In order to determine the DEGs associated in both therapeutic timepoints a Venn diagram is used (Figure 2). In this case, 77 genes (Figure 2) were found to be differentially expressed in both timepoints. Of these 77 DEGs (Supplementary file), only 3 are known to be associated with ANP secretion, which were subjected to further analysis.

After 24 hours of treatment with rosuvastatin, the following results were observed (Table 2): (a) the gene MVK, which encodes for mevalonate kinase, showed a downregulation with a $\log_2$(fold change) of -2.052, (b) CYB5B, which encodes for cytochrome b5 type B, also displayed downregulation with a $\log_2$(fold change) of -2.132, (c) FDFT1, which encodes for farnesyl-diphosphate farnesyltransferase 1, exhibited downregulation with a $\log_2$(fold change) of -2.283 and (d) SFN, which encodes for stratifin, showed downregulation with a $\log_2$(fold change) of -2.618. Overall, after 24 hours of treatment with rosuvastatin, these genes (MVK, CYB5B, FDFT1, and SFN) were downregulated, indicating a decrease in their expression levels compared to the control or baseline condition.

After 48 hours of treatment with rosuvastatin, the following results were observed (Table 3): (a) the gene MVK displayed upregulation with a log2(fold change) of 1.291, (b) CYB5B exhibited upregulation with a $\log_2$(fold change) of 2.477, (c) FDFT1 showed upregulation with a $\log_2$(fold change) of 2.292 and (d) SFN exhibited upregulation with a log2(fold change) of 3.8. Overall, after 48 hours of treatment with rosuvastatin, these genes (MVK, CYB5B, FDFT1, and SFN) were upregulated, indicating an increase in their expression levels compared to the control or baseline condition. The upregulation of these genes may have functional implications in cellular processes affected by rosuvastatin treatment.

Functional enrichment of the 72 Differentially Expressed Genes

Table 4 provides information on the gene ontology (GO) molecular function terms that were significantly associated with the treatment of rosuvastatin. The analysis indicates that after treatment with rosuvastatin, there is a statistically significant association with the activity of farnesyl-diphosphate farnesyltransferase, mevalonate kinase, and squalene synthase. The genes FDFT1 and MVK are specifically implicated in these molecular functions, suggesting that they may play important roles in the cellular processes affected by rosuvastatin treatment. The p-value suggests a statistically significant association between the treatment with rosuvastatin and the enzymes' activity.

Based on biological process, Table 4 shows statistically significant associations between rosuvastatin treatment and cholesterol biosynthetic, secondary alcohol biosynthetic, and sterol biosynthetic processes. The genes MVK and FDFT1 are implicated in these processes, suggesting their potential involvement in the effects of rosuvastatin on these biological processes. These results indicate that rosuvastatin treatment may have an impact on the expression or activity of genes related to cholesterol and sterol biosynthesis, potentially affecting lipid metabolism.

Also, based on Reactome pathway analysis, Table 4 shows statistically significant associations between rosuvastatin treatment and pathways related to cholesterol biosynthesis, gene expression activation by SREBF, and regulation of cholesterol biosynthesis by SREBP. The genes MVK and FDFT1 are implicated in these pathways, suggesting their potential involvement in the effects of rosuvastatin on these biological processes. These results indicate that rosuvastatin treatment may have an effect on the expression or activity of genes related to cholesterol metabolism and regulatory pathways. Furthermore, based on Wiki Pathways (WP) analysis, Table 4 shows statistically significant associations between rosuvastatin treatment and various pathways related to cholesterol biosynthesis, cholesterol metabolism, and cholesterol synthesis disorders. The genes - MVK and FDFT1 are implicated in these pathways, indicating their potential involvement in the effects of rosuvastatin on these biological processes. These results equally imply that rosuvastatin treatment may have influence on the expression or activity of genes involved in cholesterol metabolism and related pathways.

Rosuvastatin, a medication commonly prescribed for the management of hypercholesterolemia and prevention of cardiovascular events (16), has been found to have certain effects on cardiac atrial natriuretic peptide (ANP) levels (17). ANP is a hormone secreted by the heart that helps regulate blood pressure and fluid balance (18).

Studies have shown that rosuvastatin can influence ANP levels in the heart. One study published in the American Journal of Hypertension in 2019 found that rosuvastatin treatment significantly increased cardiac ANP levels in rats with hypertension (19), which coincided with our findings after 4 weeks of treatment with rosuvastatin. The researchers hypothesized that this increase in ANP levels might be attributed to the pleiotropic effects of rosuvastatin, beyond its cholesterol-lowering properties (20). It is believed that rosuvastatin's anti-inflammatory properties and its ability to improve endothelial function may contribute to the modulation of ANP levels (21). Additionally, rosuvastatin has been shown to enhance nitric oxide availability, which could further impact ANP synthesis and release (22). These findings suggest that rosuvastatin may have additional benefits beyond its lipid-lowering effects. By increasing cardiac ANP levels, rosuvastatin could potentially enhance the cardiovascular protective mechanisms and contribute to the prevention of heart disease (23).

Based on our analysis, the genes MVK, CYB5B, FDFT1, and SFN show upregulated expression with positive $\log_2$(fold change) values. These positive values suggest an increase in gene expression levels compared to the control or reference condition. In the context of enhancing the secretion of cardiac ANP, these upregulated genes may play a role in promoting ANP production and release.
Among these genes, MVK (mevalonate kinase), CYB5B (cytochrome b5 type B), and FDFT1 (Farnesyl-diphosphate farnesyltransferase 1) are involved in cholesterol biosynthesis and metabolism pathways (24). These genes are related to the mevalonate pathway, which is responsible for the synthesis of cholesterol and other important molecules. Enhancing the activity of these genes could potentially impact ANP secretion, as cholesterol and its derivatives have been implicated in the regulation of cardiac peptide synthesis (25).

Additionally, SFN (Stratifin) is a gene associated with various cellular processes, including cell signaling and apoptosis (26). While the specific role of SFN in ANP secretion is not well established, its

Table 1: Level of Wistar rat ANP following morning and evening exposure to Rosuvastatin at a constant dosage

ANP (ng/ml)	Week	
	Week 4	Week 12
High fat diet control	0.463 ± 0.0984^{b}	1.1 ± 0.0436^{ab}
High fat diet rosuvastatin evening	0.749 ± 0.168^{b}	0.0875 ± 0.0115^{b}
High fat diet rosuvastatin morning	2.77 ± 0.999^{a}	0.104 ± 0.0141^{b}
Normal diet control	0.566 ± 0.0961^{b}	1.87 ± 0.0333^{ab}
Normal diet rosuvastatin evening	1.29 ± 0.571^{ab}	0.079 ± 0.00743^{b}
Normal diet rosuvastatin morning	1.5 ± 0.481^{ab}	0.133 ± 0.0402^{b}

Values are means ± SEM. $^{a\text{-}d}$Means in a row without a common superscript letter differ ($P < 0.05$) as analyzed by two-way ANOVA and the TUKEY test.

Table 2: DEGs based on the three commonly expressed genes observed after 24 hours of rosuvastatin

ID	**Genesymbol**	**Genetitle**	**log2(fold change)**	**log10(Pvalue)**
215649_s_at	MVK	Mevalonate kinase	-2.052	4.304
227382_at	CYB5B	Cytochrome b5 type B	-2.132	5.883
210950_s_at	FDFT1	Farnesyl-diphosphate farnesyltransferase 1	-2.283	6.426
33323_r_at	SFN	Stratifin	-2.618	4.175

Table 3: DEGs based on the three commonly expressed genes observed after 48 hours of rosuvastatin

ID	**Genesymbol**	**Genetitle**	**log2(fold change)**	**log10(Pvalue)**
215649_s_at	MVK	Mevalonate kinase	1.291	3.165
227382_at	CYB5B	Cytochrome b5 type B	2.477	6.074
210950_s_at	FDFT1	Farnesyl-diphosphate farnesyltransferase 1	2.292	5.887
33323_r_at	SFN	Stratifin	3.8	5.345

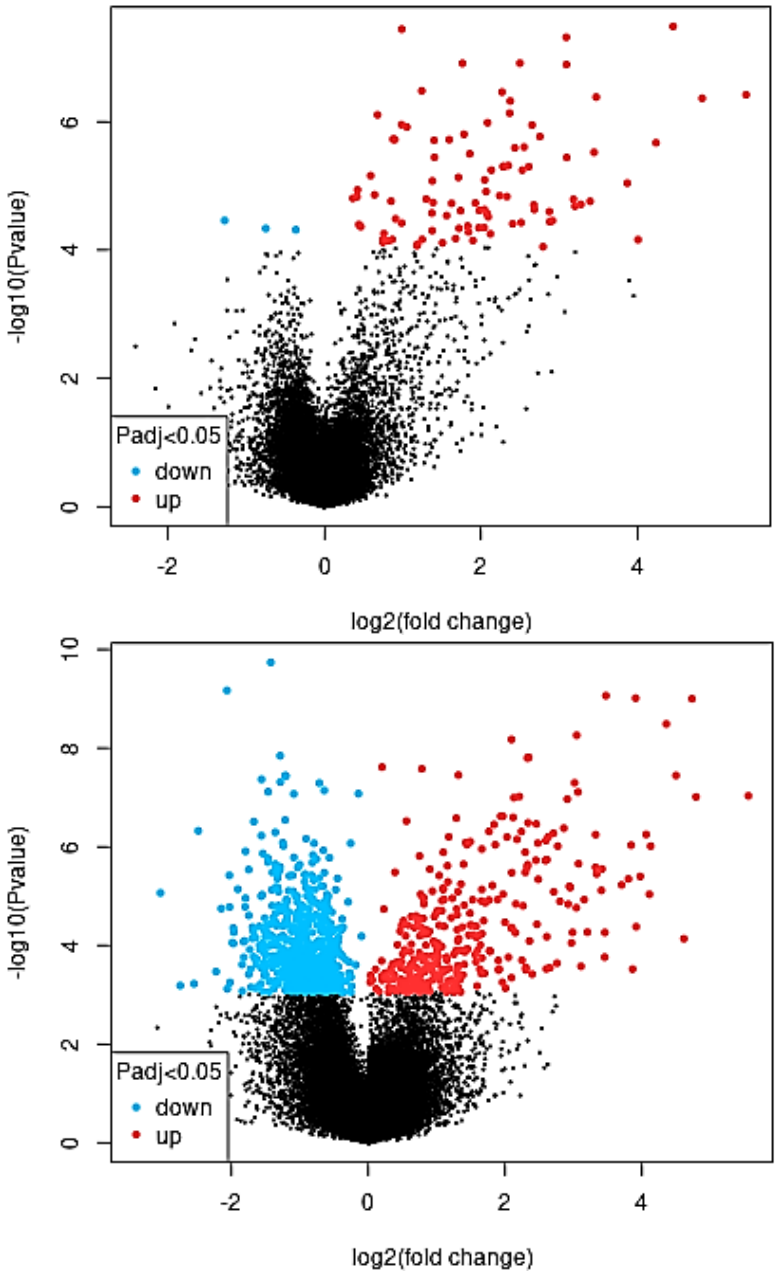

Figure 1: **Volcano plots created showing the distribution of DEGs in the data set**

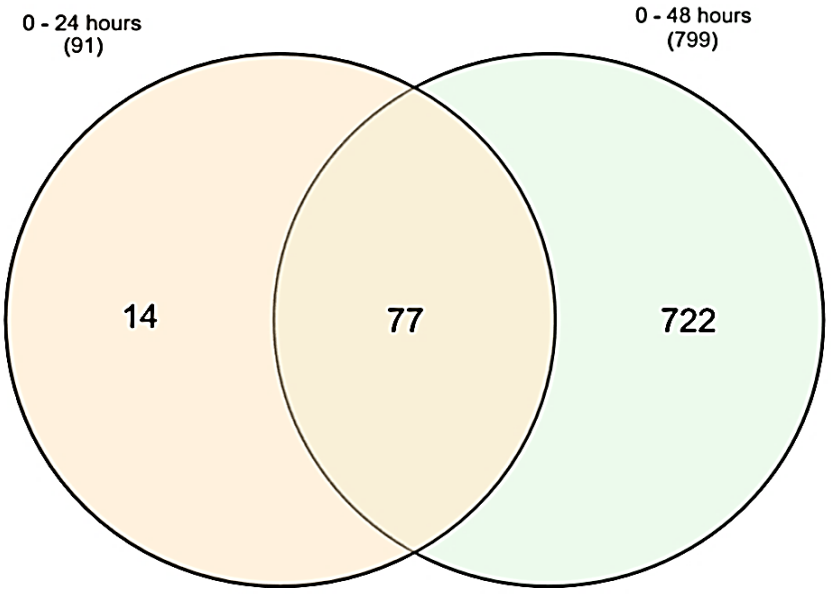

Figure 2: Commonly expressed genes associated in both therapeutic time points

Table 4: Functional enrichment computed/predicted profiles

Source	Term name	Term ID	p-Value	Term size	intersections
GO:MF	Farnesyl-diphosphate farnesyltransferase activity	GO:0004310	0.049766	1	FDFT1
GO:MF	Mevalonate kinase activity	GO:0004496	0.049766	1	MVK
GO:MF	Squalene synthase activity	GO:0051996	0.049766	1	FDFT1
GO:BP	Cholesterol biosynthetic process	GO:0006695	0.026308	54	MVK, FDFT1
GO:BP	Secondary alcohol biosynthetic process	GO:1902653	0.026308	54	MVK, FDFT1
GO:BP	Sterol biosynthetic process	GO:0016126	0.033628	61	MVK, FDFT1
REAC	Cholesterol biosynthesis	REAC:R-HSA-191273	0.00544	26	MVK,FDFT1
REAC	Activation of gene expression by SREBF (SREBP)	REAC:R-HSA-2426168	0.013034	40	MVK,FDFT1
REAC	Regulation of cholesterol biosynthesis by SREBP (SREBF)	REAC:R-HSA-1655829	0.022989	53	MVK,FDFT1
WP	Mevalonate arm of cholesterol biosynthesis pathway	WP:WP4190	0.000985	13	MVK,FDFT1
WP	Cholesterol biosynthesis pathway	WP:WP197	0.001326	15	MVK,FDFT1
WP	Cholesterol synthesis disorders	WP:WP5193	0.001931	18	MVK,FDFT1
WP	Cholesterol metabolism	WP:WP5333	0.007927	36	MVK,FDFT1
WP	Cholesterol metabolism with Bloch and Kandutsch-Russell pathways	WP:WP4718	0.013577	47	MVK,FDFT1
WP	Cholesterol metabolism	WP:WP5304	0.03197	72	MVK,FDFT1

upregulation could have indirect effects on ANP production through its influence on intracellular signaling pathways (26).

CONCLUSION

Rosuvastatin has been shown to influence cardiac atrial natriuretic peptide (ANP) levels, with studies demonstrating increased ANP levels in response to rosuvastatin treatment. This effect may be attributed to the pleiotropic properties of rosuvastatin, including its anti-inflammatory effects, improvement of endothelial function, and enhancement of nitric oxide availability. These findings suggest that rosuvastatin may have benefits beyond its lipid-lowering effects, potentially enhancing cardiovascular protective mechanisms and contributing to the prevention of heart disease.

Furthermore, the analysis of gene expression data upon treatment with rosuvastatin revealed the upregulation of genes involved in cholesterol biosynthesis and metabolism pathways, such as MVK, CYB5B, and FDFT1. These genes may play a role in promoting ANP production and release, as cholesterol and its derivatives have been implicated in the regulation of cardiac peptide synthesis. Additionally, the upregulation of SFN, a gene associated with cellular processes including cell signaling and apoptosis, may indirectly affect ANP production through its influence on intracellular signaling pathways. While these findings provide insights into the potential mechanisms underlying the enhancement of cardiac ANP secretion, further research is necessary to confirm the functional relevance of these genes and the clinical significance of their effects on ANP levels in humans.

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Conflicts of interest

We declare no conflicts of interest

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