

CHAPTER SEVEN

Assessment of Neurochemical and Biochemical Safety of Levonorgestrel in Female Wistar Rats: Implications for Quality Assurance in Contraceptive Use and Supplement Co-administration

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Abstract

This study investigated the neurobiological effects of levonorgestrel (LNG), a widely used contraceptive medication, and the potential modulatory role of melatonin, a readily available supplement. Using a female Wistar rat model, the research examined the effects of varying dosages and exposure frequencies of LNG, alone and in combination with melatonin, on neuro-oxidative stress, lipid profiles, and bioenergetics. The findings of this study have significant implications for global health and well-being, particularly in ensuring medication safety and promoting sustainable health practices. The research contributed to a broader understanding of pharmaceutical impacts on diverse populations worldwide, addressing challenges in quality assurance and safe medication use. The study's results can inform educational initiatives regarding responsible medication use and supplement co-administration, ultimately promoting public health and well-being.

Keywords: Levonorgestrel, Melatonin, Neurobiology, Global Health.

Introduction

Levonorgestrel (LNG), a synthetic progestogen, is widely used in emergency contraceptive pills (ECPs) and hormonal contraceptives (Glasier et al., 2011). While its efficacy in preventing unintended pregnancies is well-established, concerns regarding its potential neurochemical and biochemical effects have emerged (Kallner and Danielsson, 2016). Understanding these effects is crucial for ensuring the safety and quality assurance of contraceptive use,

particularly in the light of the global prevalence of hormonal contraceptive usage (Rudzinski et al., 2023; UNPD, 2018). Furthermore, the co-administration of supplements like melatonin (MLT) with LNG warrants investigation to determine potential modulatory effects and ensure the safety of combined usage.

Neurochemical assessments are essential to evaluate the impact of LNG on brain function, as hormonal fluctuations can influence neurotransmitter systems and neuronal integrity (McEwen, 2012). Biochemical parameters, including lipid profiles and oxidative stress markers, provide insights into systemic effects and potential adverse outcomes. Disruptions in these parameters may indicate increased cardiovascular risk or compromised cellular function. This study aimed to assess the neurochemical and biochemical safety of LNG in female Wistar rats, simulating human dosages and frequencies of exposure. By evaluating various endpoints, including brain and serum lipid profiles, oxidative stress markers, and enzyme activities, the study sought to elucidate the potential risks associated with LNG use and the protective role of MLT co-administration. The findings would have significant implications for quality assurance in contraceptive use and supplement co-administration, informing clinical practice and public health guidelines.

Materials and Methods

Experimental Animals

Forty (40) mature female Wistar rats (average 200g) were used. They were housed under controlled conditions (temperature, humidity, light cycle). The animals were acclimatised for 15 days and had free access to food and water. All procedures were approved by the University of Ilorin Animal Care Committee and followed NIH guidelines.

Treatments

Levonorgestrel (LNG) was dissolved in 0.9% saline to simulate human doses in rats (4 µg/kg and 8 µg/kg). Melatonin was also dissolved in 0.9% saline (10mg/kg).

Experimental Design

The study looked at the effects of regular (once weekly) and frequent (three times weekly) LNG exposure at recommended dose (simulation, 4 µg/kg) and misused high dose (8 µg/kg) respectively. Rats were divided into five (5) main groups: control, low-dose LNG, high-dose LNG, low-dose LNG + melatonin, and high-dose LNG + melatonin. Each group was further divided into regular exposure (RE, once weekly) and frequent exposure (FE, three times weekly). Treatments were administered orally via gavage for eight weeks.

Sample Collection

Animals were euthanised, and blood samples were collected for serum analysis. Brain tissues were collected and homogenised for neurochemical analysis.

Biochemical Parameters

Lipid profiles (total cholesterol, triglycerides, HDL-C, LDL-C, VLDL-C, non-HDL-C, AIR, CRR) were measured. Very low density lipoproteins-cholesterol (VLDL-C) was assessed as one-fifth of TG and low density lipoproteins-cholesterol (LDL-C) was estimated using Friedewald's formula: $[LDL-C = TC - (HDL-C + VLDL)]$ mg/g (Friedewald et al., 1972). Also, Non-HDL-C was calculated by estimating the differences between total cholesterol and high density lipoprotein cholesterol $[Non-HDL-C = TC - HDL-C]$. The Atherogenic Index Risk (AIR) was evaluated by: $\log(TG/HDL-C)$ and Cardiovascular Risk Ratios (CRR) by: $CRR I = TC/HDL-C$ ratio; $CRR II = LDL-C/HDL-C$ ratio (Dobiasova, 2004).

Lipid peroxidation (MDA) and nitric oxide (NO) levels were determined. Antioxidant enzyme activities (SOD, GPx, CAT, GSH, G6PDH) were measured.

Data Analysis

GraphPad Prism 8.0 was used for statistical analysis. Data were presented as mean $\pm$ SEM. One-way ANOVA followed by Tukey's post hoc test was used to determine statistical significance at $p < 0.05$.

Results and interpretations

The available data analysis were presented in tables 1-3. These tables present the effects of Levonorgestrel (LNG) and Melatonin (MLT) on various

biochemical parameters in the brain and serum of female Wistar rats, with varying frequencies of LNG exposure (once or thrice weekly). The significance of these findings relates to the potential neurotoxicity of LNG and the protective role of MLT, which has implications for understanding the impact of pharmaceuticals on biological systems. In the context of globalised education, this type of research is crucial for ensuring quality assurance in scientific methodologies and the ethical use of experimental models.

Effect of LNG and/or MLT on brain Cholesterol and Lipoproteins:

In the brain, high-dose LNG administered once weekly significantly decreased total cholesterol and LDL levels compared to the control group (Table 1). This suggests a potential disruption of lipid metabolism within the central nervous system. Equally, frequent LNG exposure (thrice weekly) led to increased total cholesterol and LDL levels, highlighting the dosage-dependent effects of LNG on brain lipid profiles. Melatonin co-administration generally attenuated the adverse effects of LNG, particularly at the higher dose, suggesting a protective role against LNG-induced lipid dysregulation. This is important, because changes to lipid profiles within the brain, can profoundly affect neurological function (Alghamdi, 2018; Reiter et al., 2016).

Cardiovascular Risk Ratios (CRR) and Atherogenic Coefficient (AC):

The CRR I, CRR II and AC indices, which reflect cardiovascular risk, were significantly reduced in the LNG plus MLT groups (once weekly). This implies that melatonin exhibited a protective effect against potential cardiovascular complications induced by LNG (Table 1). Frequent LNG exposure, however, increased these risk ratios, suggesting a heightened cardiovascular risk with increased LNG frequency. These results provide important information relating to the systemic effects of LNG, and the potential protective properties of MLT (Table 1).

Implications for Assessment and Quality Assurance:

These findings emphasised the importance of rigorous assessment and quality assurance in pharmaceutical research, particularly when investigating potential neurotoxic effects and systemic impacts of drugs. The inclusion of multiple parameters and statistical analysis (ANOVA, Tukey's post hoc) ensured the reliability and validity of the results, aligning with best practices in scientific research.

Table 1: Effects of Levonorgestrel and Melatonin on lipid profiles and indices in the brain of female Wistar rats

Treatments /Parameters	NS 1 ml/kg	LNG 4 µg/kg	LNG 8 µg/kg	LNG 4 µg/kg /MLT 10 mg	LNG 8 µg/kg /MLT 10 mg
Brain Total Cholesterol mg/dL					
Once /week	405.12±17.85	421.44±18.33	313.68±11.96*	241.23±13.37*	310.59±1.54*
Thrice/week	197.30±17.42	235.84±15.39	296.72±13.70 [#]	322.16±8.16 [#]	379.86±15.52 [#]
Brain Triacylglyceride mg/dL					
Once /week	324.26±4.14	295.27±8.22*	283.78±10.60*	246.15±2.13*	265.68±12.98*
Thrice/week	235.51±4.61	258.70±8.45*	308.88±8.13*	291.71±4.84*	310.99±5.89*
Brain very low density lipoproteins (VLDL) mg/dL					
Once /week	64.85±0.83	59.05±1.64*	56.76±2.12*	49.23±0.43*	53.14±2.59*
Thrice/week	47.10±0.92	51.74±1.69*	61.78±1.63*	58.34±0.97*	62.19±1.18*
Brain high density lipoprotein (HDL) mg/dL					
Once /week	30.37±2.83	29.54±1.25	27.24±2.71	27.15±2.01	32.30±0.83
Thrice/week	24.02±2.63	25.77±1.81	28.07±1.53	28.89±1.99	29.08±2.17
Brain low density lipoprotein (LDL) mg/dL					
Once /week	309.89±15.23	332.84±15.60	229.68±13.28*	164.85±14.85*	225.16±3.18*
Thrice/week	126.18±14.28	158.33±16.89	206.88±13.98*	234.91±9.88*	288.58±13.18*
Brain non-HDL mg/dL					
Once /week	374.75±15.97	391.89±17.21	286.44±13.00*	214.08±15.26*	278.29±0.71*
Thrice/week	173.28±15.20	210.07±17.01	268.65±14.00*	293.26±8.96*	350.78±14.34*
Cardiovascular risk ratio I (CRR I)					
Once /week	13.51±1.04	14.27±0.28	11.75±1.24	9.06±1.20*	9.63±0.20*
Thrice/week	8.27±0.49	9.31±1.14	10.64±0.85	11.26±0.87*	13.15±0.76*
Cardiovascular risk ratio II (CRR II)					
Once /week	10.34±0.85	11.27±0.24	8.64±1.11	6.22±1.05*	6.97±0.11*
Thrice/week	5.27±0.39	6.27±0.99	7.42±0.72	8.23±0.78*	9.99±0.64*

Atherogenic coefficient (AC)

Once /week	12.51±1.04	13.27±0.28	10.75±1.24	8.06±1.21*	8.63±0.20*
Thrice/week	7.27±0.49	8.31±1.14	9.64±0.85	10.26±0.87*	12.15±0.76*

Data were expressed as mean ±SEM; once per week (RE-regular exposure)

**P<0.05 vs. NS (control group) and thrice per week (FE-frequent exposure)*

#P<0.05 vs. NS (control group)

Effect of LNG and/or MLT on serum lipid profiles and cardiovascular risk

Levonorgestrel (LNG) treatment, especially at 4 µg/kg, consistently lowered total cholesterol, triacylglycerides, VLDL, LDL, and non-HDL levels compared to the control (NS). LNG, particularly at 4ug/kg once a week, and 8ug/kg thrice a week, resulted in elevated HDL levels. The combination of LNG and melatonin generally showed a further improvement in lipid profiles, suggesting a synergistic effect. LNG and LNG+MLT treatments significantly reduced cardiovascular risk ratios (CRR I and CRR II) and the atherogenic coefficient (AC), indicating a lower risk of cardiovascular disease.

The frequency of administration (once vs. thrice weekly) influenced the magnitude of the effects, with some variations observed between the two schedules. These changes in serum lipid profiles may reflect systemic effects of LNG, impacting lipid metabolism beyond the brain. The use of serum lipid levels is a common practice when assessing the overall health of the test subject (Table 2).

Table 2: Effects of Levonorgestrel and Melatonin on lipid profiles and indices in the serum of female Wistar rats

Treatments/ Parameters	NS 1 ml/kg	LNG 4 µg/kg	LNG 8 µg/kg	LNG 4 µg/kg /MLT 10 mg	LNG 8 µg/kg /MLT 10 mg
Serum Total Cholesterol					
Once /week	220.42±5.05	176.49±8.58*	207.32±22.39	200.39±10.71	184.20±2.78
Thrice/week	219.65±18.49	170.32±5.05 #	185.74±12.11	185.74±13.50	191.99±3.14
Serum triacylglyceride					
Once /week	217.75±9.52	160.95±11.24*	200.59±15.51	185.21±16.34	165.09±5.12*

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Thrice/week	219.53±23.76	154.44±5.71*	161.54±9.78*	158.58±8.71*	156.22±5.12*
Very low density lipoproteins (VLDL)					
Once /week	43.55±1.90	32.19±2.25*	40.12±3.10	37.04±3.27	33.02±1.02*
Thrice/week	43.91±4.75	30.89±1.14*	32.31±1.96*	31.72±1.74*	31.25±1.02*
High density lipoprotein HDL					
Once /week	38.47±1.93	53.01±5.89*	33.50±3.13	40.31±3.92	47.31±2.18
Thrice/week	40.49±3.93	41.32±3.95	52.46±2.77*	55.49±4.25*	45.39±1.76
Low density lipoprotein (LDL)					
Once /week	138.40±2.94	91.29±8.91*	133.70±21.32	123.03±10.82	103.88±4.27
Thrice/week	135.25±17.90	98.11±6.69*	100.97±10.43	98.53±13.95	115.36±3.87
Serum Non-HDL					
Once /week	181.95±4.27	123.48±11.14*	173.82±24.13	160.07±14.09	136.89±4.66*
Thrice/week	179.16±22.29	129.00±7.79*	133.28±12.33*	130.24±15.47*	146.60±4.89
Cardiovascular risk ratio I (CRR I)					
Once /week	5.75±0.23	3.42±0.48*	6.34±1.05	5.12±0.81	3.91±0.22
Thrice/week	5.60±0.92	4.20±0.43	3.56±0.31*	3.39±0.39*	4.25±0.23
Cardiovascular risk ratio II (CRR II)					
Once /week	3.61±0.19	1.79±0.36*	4.13±0.89	3.17±0.62	2.21±0.18
Thrice/week	3.45±0.71	2.44±0.35	1.94±0.25*	1.82±0.33*	2.56±0.18
Atherogenic coefficient (AC)					
Once /week	4.75±0.23	2.42±0.48*	5.35±1.05	4.12±0.81	2.91±0.22
Thrice/week	4.60±0.92	3.20±0.43	2.56±0.31*	2.39±0.39*	3.25±0.23

Data were expressed as mean ±SEM; once per week (RE-regular exposure)

**P<0.05 vs. NS (control group) and thrice per week(FE-frequent exposure)*

#P<0.05 vs. NS (control group)

Effects of Levonorgestrel and Melatonin on redox status and bioenergetics in the brain of female Wistar rats

Frequent LNG and /or Melatonin exposure (FE) improves energy metabolism by activating the AST and LDH activities but co-administered LNG/MLT at RE decreases LDH activity when compared to control (Table 3).

Levonorgestrel (LNG) and /or Melatonin effects on oxidative stress markers

Levonorgestrel (LNG), used in hormonal contraceptives, may have neurobiological effects. This parameter explored the impact of LNG on brain enzyme activities and oxidative stress markers (Song et al., 2023). The result shows that LNG, especially when administered thrice weekly, significantly increases aspartate transferase (AST) and lactate dehydrogenase (LDH) levels. This suggests potential cellular damage or altered metabolic activity in the brain (Zhang et al., 2016). The effects of LNG on oxidative stress markers; malondialdehyde MDA, and nitric oxide (NO) and antioxidant enzymes; superoxide dismutase (SOD), glutathione peroxidase (GPx), Catalase (CAT) are variable and depend on the dosage and frequency of administration.

Malondialdehyde MDA is a marker of lipid peroxidation, indicating oxidative damage. The data shows that LNG, in some combinations with MLT, reduces MDA levels, suggesting a potential antioxidant effect of the combined treatment. Nitric oxide NO plays a complex role in the brain. LNG significantly reduced NO levels when administered once a week. This implies that the NO-signaling related pathway may be disrupted. SOD, GPx, and catalase are antioxidant enzymes. Their activities reflect the brain's ability to combat oxidative stress. The results are varied, showing both increases and decreases depending on the treatment (Rummel and Butterfield, 2022).

Melatonin, a neuroprotective hormone, is often used to counteract oxidative stress (Tarocco et al., 2019). The combination of LNG and MLT appears to modulate the effects of LNG. In some cases, MLT seems to mitigate the adverse effects of LNG, particularly on LDH and MDA levels (Hardeland, 2018). However, the results are complex and needs more research to understand the full interaction of these two substances.

Levonorgestrel (LNG) and /or Melatonin effects on LDH, AST, AChE and G6PDH activities for neuronal membrane integrity

Aspartate transferase (AST) and lactate dehydrogenase (LDH) are indicators of cellular damage. Elevated levels suggest potential neurotoxicity. These enzymes also serve as bioenergetics marker for neuronal metabolic function. AChE is involved in neurotransmission. Changes in AChE activity can affect cognitive function. The result shows that some treatments increased AChE activity. Alteration in the Ach/AChE ratio (acetylcholine/cholinesterase ratio) may influence cholinergic-like discharge and cognitive outflow. G6PDH is an enzyme in the pentose phosphate pathway, crucial for producing NADPH, which is essential for antioxidant defense, in the iodination of thyroglobulin during the synthesis of thyroid hormones and reductive biosynthesis like steroids. The results of this measurement are very varied. This may be attributable to frequency of administration; thrice-weekly administration generally leads to more pronounced changes in enzyme activities and oxidative stress markers.

Potential Implications:

This study revealed the interaction between levonorgestrel and/or melatonin supplement (LNG/MLT) in the brain of female animal model. Here, the generated dataset suggested that LNG may have neurobiological effects, and MLT can modulate these effects. The findings highlighted the importance of considering the frequency and dosage of LNG administration.

Further research is needed to elucidate the underlying mechanisms and clinical implications of these findings.

Table 3: Effects of Levonorgestrel and Melatonin on redox status and bioenergetics in the brain of female Wistar rats

Treatments /Parameters	NS 0.3 ml	LNG 4 µg/kg	LNG 8 µg/kg	LNG 4 µg/kg /MLT 10 mg	LNG 8 µg/kg /MLT 10 mg
Brain Aspartate Transferase (AST) U/L					
Once /week	35.38±1.29	41.26±4.92	38.71±3.69	41.97±0.91	43.67±3.14
Thrice/week	15.52±2.14	41.48±3.55*	43.74±4.97*	41.26±2.56*	46.72±1.73*
Brain Lactate dehydrogenase (LDH) U/L					

Once /week	1537.58±30.73	1468.22±16.69	1498.02±17.75	1365.78±61.55*	1333.51±62.11*
Thrice/week	191.59±5.97	1432.42±18.77*	1518.31±8.68*	1434.04±36.54*	1400.15±33.48*
Brain Malondialdehyde MDA μM					
Once /week	1.03±0.07	0.98±0.08	1.23±0.29	0.72±0.02	0.52±0.01*
Thrice/week	0.88±0.18	0.81±0.18	1.03±0.11	0.62±0.06	0.55±0.09
Brain Nitric oxide (NO) μM					
Once /week	16.59±1.70	10.54±0.51*	10.34±2.04*	7.30±0.95*	7.43±1.08*
Thrice/week	5.15±1.19	5.19±0.61	8.70±1.88	8.57±1.18	8.54±1.36
Brain SOD activity μ/ml					
Once /week	0.66±0.11	0.60±0.09	0.78±0.19	0.81±0.18	0.56±0.06
Thrice/week	0.53±0.10	0.91±0.39	0.56±0.07	0.65±0.07	0.55±0.09
Brain GPx activity U/L					
Once /week	27.34±3.37	18.55±1.50	15.38±3.00	15.00±1.50	21.00±8.65
Thrice/week	10.88±0.99	21.76±5.45	31.13±4.88*	24.38±7.61	14.25±0.99
Brain Catalase activity U/mg protein					
Once /week	17.62±3.59	15.89±3.65	14.62±2.73	21.69±1.79	24.10±3.45
Thrice/week	16.63±2.22	14.59±0.75	17.42±1.44	24.94±4.01	17.29±3.68
Brain GSH U/L					
Once /week	1.77±0.21	1.72±0.17	1.58±0.03	1.41±0.05	1.44±0.04
Thrice/week	1.73±0.16	1.69±0.09	1.75±0.25	1.62±0.35	1.56±0.43
Brain G6PDH U/L					
Once /week	42.84±14.24	6.59±1.47*	23.13±4.20	26.65±4.20	25.52±5.46
Thrice/week	9.00±3.72	12.61±4.78	26.85±17.23	42.64±10.96*	15.97±2.44
Brain AChE μmol					
Once /week	0.08±0.00	0.06±0.00	0.07±0.01	0.07±0.01	0.07±0.00
Thrice/week	0.04±0.00	0.06±0.00	0.06±0.01	0.07±0.01*	0.07±0.00*

*Data were expressed as mean $\pm$ SEM; once per week (RE-regular exposure) * $P < 0.05$ vs. NS (control group) and thrice per week (FE-frequent exposure) # $P < 0.05$ vs. NS (control group)*

Discussion

The present study investigated the neurochemical and biochemical effects of levonorgestrel (LNG) administration in female Wistar rats, with a focus on assessing potential adverse outcomes and the modulatory role of melatonin (MLT). The data revealed significant alterations in lipid profiles, oxidative stress markers, and enzyme activities, highlighting the importance of thorough safety assessments in contraceptive use. In the brain, high-dose LNG administered once weekly significantly decreased total cholesterol and LDL levels, while frequent exposure increased these parameters. This suggests that LNG's impact on brain lipid metabolism is dose- and frequency-dependent. The observed changes in lipid profiles may reflect disruptions in cholesterol homeostasis, which is crucial for neuronal function and membrane integrity (Yang et al., 2022; Pfrieger, 2003).

Serum lipid profiles also showed significant alterations, with LNG administration leading to decreased total cholesterol and triacylglyceride levels but increased HDL levels (Pfrieger, 2003). These systemic effects may indicate that LNG influences lipid metabolism beyond the central nervous system. The cardiovascular risk ratios (CRR) and atherogenic coefficient (AC) were significantly reduced in the LNG-MLT co-administrated groups, suggesting a protective role of melatonin against potential cardiovascular complications. Given that cardiovascular health is a major concern globally, this is an important finding.

These findings underscore the importance of lipid profile monitoring in individuals using LNG-based contraceptives, particularly those with pre-existing cardiovascular risk factors. The protective effects of MLT highlight the potential benefits of supplement co-administration in mitigating adverse lipid-related effects. Frequent LNG exposure significantly increased aspartate transferase (AST) and lactate dehydrogenase (LDH) levels in the brain, indicating potential neurotoxicity. These enzymes are markers of cellular damage, suggesting that LNG may compromise neuronal integrity (He et al., 2023).

Oxidative stress, as indicated by malondialdehyde (MDA) levels, was significantly reduced by MLT co-administration, demonstrating its antioxidant properties. LNG administration also reduced nitric oxide (NO) levels, which may have implications for neurotransmission and neuronal signaling. The observed changes in enzyme activities, such as G6PDH and AChE, further highlighted the potential neurochemical effects of LNG. These findings necessitate careful consideration of LNG's neurotoxic potential and the protective role of antioxidants like MLT. Comprehensive neurochemical assessments are crucial for ensuring the safety of contraceptive use, particularly in individuals with neurological vulnerabilities.

Quality Assurance in Contraceptive Use and Supplement Co-administration

The present study highlighted the importance of rigorous assessment and quality assurance in contraceptive use and supplement co-administration. The observed alterations in lipid profiles, oxidative stress markers, and enzyme activities, underscored the need for thorough safety evaluations.

Standardization of Assessment Protocols: The use of validated methods for biochemical and neurochemical assessments ensured the reliability and comparability of results. Standardised protocols are crucial for global research collaborations and evidence-based decision-making (Bolarinwa, 2015; OECD, 2005).

Ethical Considerations: The use of animal models necessitated adherence to ethical guidelines, ensuring humane treatment and responsible research practices. Ethical considerations are paramount in global research collaborations and knowledge dissemination.

Supplement Co-administration: The protective effects of MLT highlighted the potential benefits of supplement co-administration in mitigating adverse effects. However, further research is needed to determine optimal dosages and long-term effects.

Clinical Implications: The findings of this study have significant implications for clinical practice and public health guidelines. Healthcare providers should be aware of the potential neurochemical and biochemical effects of LNG and consider individualized risk assessments.

Limitations and Future Directions

The interpretation of these results was subject to certain limitations, including the utilisation of an animal model that may not entirely recapitulate human physiological responses. Subsequent research should prioritise the evaluation of the long-term effects of concurrent LNG and MLT administration, as well as their impact on reproductive function and behavioural endpoints. Clinical trials in human subjects are warranted to validate these preclinical observations.

Globalizing Education and Research Standards

These findings highlighted the need for standardised assessment protocols in global research, ensuring consistency and comparability of results across different laboratories and regions (OECD, 2005).

Conclusion

The present study provided valuable insights into the neurochemical and biochemical safety of LNG in female Wistar rats. The findings highlighted, the potential adverse effects of LNG and the protective role of MLT co-administration. Rigorous assessment and quality assurance are crucial for ensuring the safety of contraceptive use and supplement co-administration. Future research should focus on validating these findings in human populations and developing evidence-based guidelines for clinical practice.

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Conflict of Interest

Authors have declared that no potential conflicts of interest exist.

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