A Meta-analysis for analyzing changes in serum-free T4 levels with Metformin treatment in patients with or without thyroid disease and/or diabetes

Context: Previous studies suggest the effect of metformin on TSH levels, but its impact on free T4 (fT4) levels has not been understood clearly.

Objective: This Meta-analysis aims to study the effects of metformin on serum-free T4 (fT4) levels in patients with or without underlying thyroid disease and/or diabetes.

Data Sources: We reviewed articles from the Cochrane Library based on the systematic protocol.

Data Extraction: Demographic, clinical, and relevant data was extracted. Data were analyzed according to the changes in fT4 levels with metformin administration.

Data Synthesis: A total of 5 datasets (475 patients) were included in our analysis. There was a reduction in free thyroxine levels evident by the statistically significant mean difference in this meta-analysis in the treatment group between the baseline levels and post-follow-up (Metformin MD= 1.0 pg/ml, CI= 0.3423 to 1.657, P value=0.005).

Conclusion: While this meta-analysis found no significant overall effect of metformin on fT4 levels in patients with or without underlying diabetes or thyroid disease, the results from individual studies like Oleandri et al. (1999) suggest that metformin may cause a slight decrease in fT4 levels. However, given the small effect size and minimal clinical significance, the impact of metformin on fT4 levels remains uncertain and warrants further investigation to better understand its clinical relevance. Clinically, anthropometric and metabolic characteristics can alter these levels differently in each case. Dose adjustment for Levothyroxine (LT4) replacement in patients is the main concern when metformin is given concomitantly.

Keywords: Metformin, FreeT4, Diabetes, Thyroid Disease, Levothyroxine

Introduction:

Metformin is a drug used for various medical conditions such as Diabetes, and polycystic ovarian syndrome (PCOS). These patients often have some other underlying endocrine abnormalities including thyroid disorders. It has been suggested in randomized controlled trials and observational studies in the past, that metformin causes a reduction in TSH levels but the effect on fT4 levels remained controversial. The reduction in TSH levels would suggest successful treatment of hypothyroidism in patients. These patients may either have overt or subclinical hypothyroidism (SCH). In SCH, the serum fT4 levels remain normal but TSH levels reduce, thus lacking clinical manifestation of symptoms in these patients. In such scenarios, TSH correlation with free thyroxine levels in all the patients receiving metformin becomes crucial and a key parameter to indicate the subsequent management plan.

Studies have shown that metformin affects deiodinase enzyme activity which causes peripheral conversion of fT4 to T3, which affects their serum levels and could be indicative of the function of thyroid in patients with underlying thyroid disorder. This would impact the interpretation of the state of thyroid function in patients with euthyroid, subclinical, or overt hypothyroidism who may or may not even have underlying diabetes. Since metformin is also prescribed in patients with PCOS who have insulin resistance, pre-diabetes, and gestational diabetes, it is essential to rule out any thyroid disorders in such patients correlating their fT4 levels to the effect of metformin. Previous studies have evaluated serum TSH levels that have been affected by metformin use. We have assessed that fT4 levels can increase, decrease, or remain the same, similar to TSH levels, and therefore change the interpretation of thyroid profile in general. We have combined studies that have reported different dynamics of fT4 levels with Metformin therapy in this meta-analysis.

Materials and Methods:

Data Search: We conducted a systematic search for relevant articles on two databases: Cochrane Library and PubMed Library. There was no limitation for the publishing year. All the articles written in the English language were considered. We used relevant keywords, synonyms, and acronyms to evaluate the articles such as Metformin, thyroxine, fT4 levels, and Metf. The search engine revealed 26 articles, out of which we found 11 articles that discussed the impact of metformin on thyroid profile but 5 studies mentioned mean fT4 levels before and after metformin treatment. One post hoc analysis discussed fT4 levels at baseline and following treatment, however did not compare the levels to placebo and hence, it was excluded. We evaluated the findings in those 5 studies based on their study population which included patients diagnosed with subclinical hypothyroidism, overt hypothyroidism, diabetes, PCOS, and obesity.

Inclusion criteria: studies evaluating baseline fT4 levels and fT4 levels after Metformin therapy, irrespective of underlying comorbidity which could be underlying diabetes, PCOS, obesity, or thyroid disease in the adult human patient population.

Exclusion criteria: Studies that did not report fT4 levels both before and after Metformin therapy. Any case reports, literature reviews, and post hoc analysis were also excluded. We also excluded studies that did not compare the metformin group to a placebo group or included patients given additional supplementation with Levothyroxine or any other drug to all patients receiving metformin as well. Data analysis of these subgroups in the included studies was not considered.

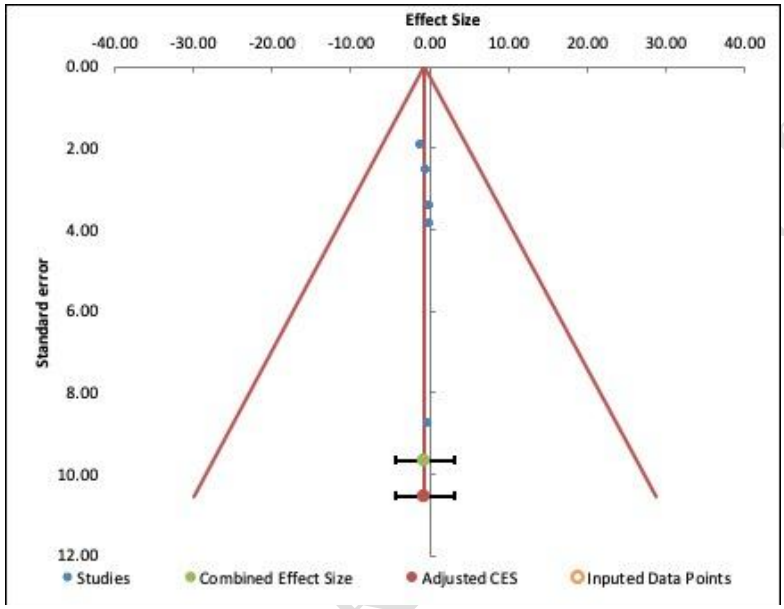
Data collection and assessment of risk of bias: The articles were based on original studies, which utilized randomized controlled trial methods to primarily discuss the effects of metformin on TSH. Our focus was to extract data specifically about mean thyroxine levels and the standard deviations. Serum thyroxine levels of individual subjects were not mentioned in the studies. Serum T3 levels were not evaluated as it was beyond the scope of our study. Irrespective of the underlying pathology, whether present or absent, we analyzed data from two groups, the treatment arm and the placebo arm. We did not consider the data for patients receiving LT4 treatment which could have altered the serum thyroxine levels. One independent researcher analyzed and formulated the data, which was cross-checked by other researchers in the study.

We inspected the baseline characteristics of the studied populations and looked for allocation bias in those studies. A randomized, blinded protocol was followed by investigators in all the studies. Strict criteria were followed during the process of data extraction and analysis. The risk of bias for randomized controlled trials was analyzed with the Cochrane Risk of Bias tool [1] and compiled in Table 1 given below. Egger's test p-value was 0.181 which indicated there was no publication bias.

Table 1: Cochrane Risk of Bias tool results to evaluate included Randomized Controlled trials

Study ID	Reference	Randomization process	Deviations from intended interventions	Missing outcome data	Measurement of the outcome	Selection of the reported result	Overall Bias
Severo et al 2017	Dornelles Severo, Mateus et al. "Metformin effect on TSH in subclinical hypothyroidism: randomized, double-blind, placebo-controlled clinical trial." *Endocrine* vol. 59,1 (2018): 66-71. doi:10.1007/s12020-017-1462-7	Low	High	Low	Low	Low	Some concerns
Taghavi et al 2011	Morteza Taghavi, S et al. "Metformin decreases thyrotropin in overweight women with polycystic ovarian syndrome and hypothyroidism." *Diabetes & vascular disease research* vol. 8,1 (2011): 47-8. doi:10.1177/1479164110391917	Some concerns	High	Low	Low	Low	Some concerns
Oleandri et al 1999	Oleandri, S E et al. "Three-month treatment with metformin or dexfenfluramine does not modify the effects of diet on anthropometric and endocrine-metabolic parameters in abdominal obesity." *Journal of endocrinological investigation* vol. 22,2 (1999): 134-40. doi:10.1007/BF03350893	Low	Low	Low	Some concerns	High	High
Palui et al 2019	Palui, R et al. "Effect of metformin on thyroid function tests in patients with subclinical hypothyroidism: an open-label randomised controlled trial." *Journal of endocrinological investigation* vol. 42,12 (2019): 1451-1458. doi:10.1007/s40618-019-01059-w	Low	Some concerns	Some concerns	Low	Some concerns	Some concerns

Statistical analysis: We calculated effect sizes and respective confidence intervals using a software tool-Meta-essentials [2]. The calculation for heterogeneity and publication bias plot was created using this tool.



Results: We included 5 articles in our study after carefully excluding duplicate articles, and those with insufficient data to finally evaluate 475 patients in this study. This meta-analysis found no significant difference in fT4 levels between the metformin and placebo groups (p=0.647), suggesting no overall effect of metformin on fT4 levels. However, the study by Oleandri et al. (1999) reported a significant reduction in fT4 levels following metformin use (mean difference = 1.0, p=0.005). Although the effect size was negative (ES = -1.12), it was small, indicating minimal clinical significance. These findings point to a possible decrease in fT4 levels with metformin use, though the overall effect appears to be minor.

Study characteristics:

Table 1 shows five studies with varying patient populations, metformin dosage, and duration of treatment. The population differed in two studies and was similar in three studies. Study 1 with 27 sample population provides information about the effect of metformin on thyroxine levels in PCOS patients. Three studies selected patients diagnosed with subclinical hypothyroidism. Euthyroid diabetic patients were selected in one trial who were not sub-classified according to their type of diabetes and one trial studied 18 obese patients taking a hypo-caloric diet. The duration of follow-up was usually 3 or 6 months except for one study with 322 patients, which showed continued trial for 12 months. This study also had a contrasting metformin dosage of

1735 mg once daily, compared to other studies with 1500mg or 1700mg once daily and with 1500 mg twice daily. In all the studies, women were higher in number with average age in 40s. There was no significant difference in the baseline fT4 levels between the metformin and the placebo group. Only one study reported a p-value less than 0.05. All were randomized controlled trials except for one retrospective study.

No subgroup analysis was done due to fewer number of studies included in this meta-analysis.

The test for heterogeneity revealed non-significant results (I^2 0.00% and p-0.997), meaning a sampling error could have been present in the studies. Egger test showed intercept p value 0.181 (>0.05), indicating no publication bias. Begg and Mazumdar's rank correlation test also showed no publication bias (p-value - 0.071). Ideally, funnel plot should be done with at least 10 studies, however, an average Cochrane analysis includes fewer than 10 studies, resulting in low power. The scatter seen in the plot can be interpreted as symmetrical and no significant difference between the combined effect sizes of the studies. The combined effect size was similar in both observed and adjusted calculations (ES= 0.60, CI=-4.25 to 3.05, SE=1.32).

We used the statistical tool, Meta-essentials, to calculate and run regression analysis. However, it is programmed to conduct single-variate analysis only. A fixed effect model was used and with a confidence interval of 95%, the regression coefficient was non-significant for all variables. Effect size was not associated with any study characteristic; duration, size, dose, age, BMI, and baseline fT4 levels. The regression coefficient for BMI had a p-value of 0.709 and a Z-value of -0.37. Interestingly, the mean square model showed significant results for BMI (p-value 0.014, F-value 27.10) and accounted for 90% R^2. BMI accounted the most for the variation in different effect sizes and the associated variance was small (Mean square = 0.14). BMI did not contribute significantly to this explained variance. There could be other unaccounted moderators that were driving significance.

Study name	Year	Duration	Sample size	Study population	Metformin Dose	Men	Women	Age /yrs.	BMI/ Kg/m^2	Baseline fT4 levels/ pmol/l
Taghavi	2011	6 months	27	Overweight, PCOS, subclinical hypothyroidism	1500mg OD	0	27	NR*	26.0-32.2	24.6(2.26)
Severo	2017	3 months	48	Subclinical hypothyroidism	1700mg OD	11	37	18-65	22.5-33.4	13.4 (2.0)
Palui	2019	6 months	60	Subclinical hypothyroidism with autoimmune thyroiditis	1500mg OD	6	54	18-50	20.9-30.8	15.36(NR)
Capelli	2012	12 months	322	Euthyroid Diabetic	1735 mg OD	157	175	45-62	27.2-38	19.04(1.7)
Oleandri	1999	3	18	Obese patients	1500mg	3	15	46-49	34.3-36.5	19.2(0.7)

		months		on a hypo-caloric diet	BD					

*Table 2: Study characteristics. Abbreviations: NR-Not recorded, OD-Once daily, BD- Twice daily. Baseline fT4 levels are mentioned as mean and their standard deviation. *Age used for regression analysis was 32 years, i.e. the mean reproductive age*

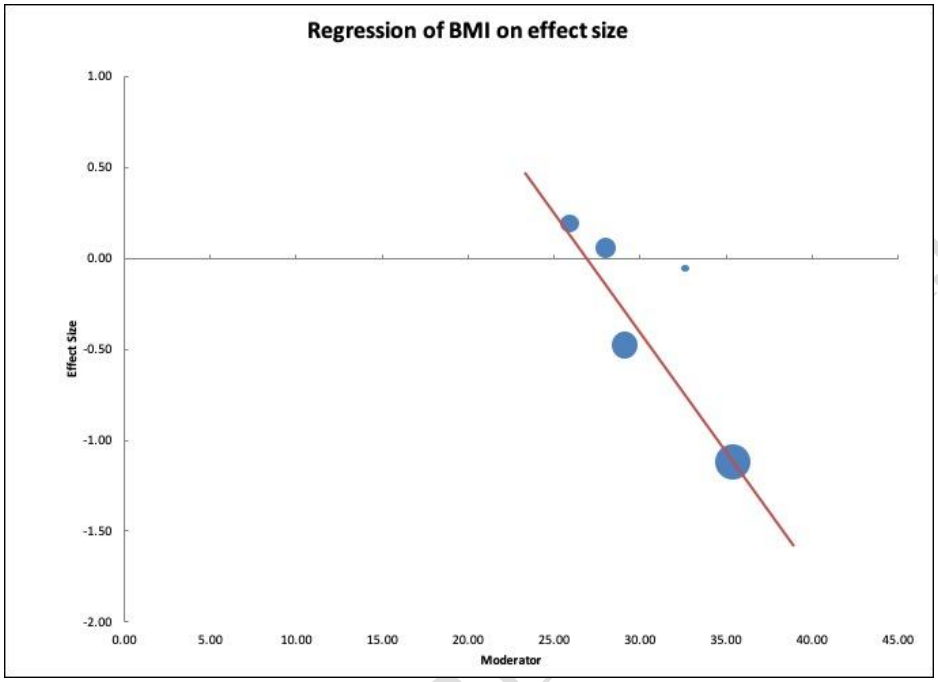


Figure 1 Moderator was BMI calculated as the mid-point of the range. The size of the circle indicates weight of each study. Z-score= -0.37, p> 0.05.

Discussion:

This meta-analysis was performed to evaluate changes in thyroxine levels in patients taking metformin regardless of the indication of its use. The aim was to establish any existing correlational factor associated between metformin and fT4 levels based on previous studies that have largely focused on TSH as the main component of thyroid functionality. We found that the combined effect size of the metformin group versus the placebo group (Table 3b) was non-significant (two-tailed p- value= 0.647) but interestingly the mean difference between levels before Metformin and after the drug was significant (p=0.005) in the study conducted by Oleandri et al [3] [MD=1.0, CI=0.3423-1.657, CI≠0]. This shows that fT4 levels decreased due to Metformin use which was also seen as the negative effect size (ES= -1.12, CI= -5.26 to 3.02] of the metformin group compared to the placebo group. The effect size was small, meaning little or no practical implications for this data exist according to the data considered for the included studies. Theoretically, this gives us some explanation for the decrease in thyroxine levels after metformin administration when evaluating the thyroid profile.

The results for the different effect sizes of the studies are shown in Table 3a where discrepancies in the results can be seen. Two studies show positive values and have smaller study weights when compared to the studies that show negative values (Figures 1 and 2). Their mean differences have been insignificant (Table 4) except for one study as mentioned above, that also

showed a non-zero confidence interval (figure 3). These non-significant results were due to the small sampling size, the smallest number was 18 patients in one study. The study published by Cappelli et al (2012) [4] was conducted in 3 patient cohorts, including those who received both metformin and levothyroxine. Since supplemental levothyroxine would affect the mean serum fT4 levels, we did not consider this group for analysis.

Table 3a:

#	Study name	Effect size (Metformin versus placebo group)	CI Lower limit (95%)	CI Upper limit (95%)	Weight
1	Taghavi	-0.48	-5.72	4.76	26.64%
2	Severo	0.06	-6.89	7.00	14.53%
3	Palui	0.19	-7.54	7.92	11.59%
4	Capelli	-0.06	-17.31	17.20	2.25%
5	Oleandri	-1.12	-5.26	3.02	44.98%

Figure 1:

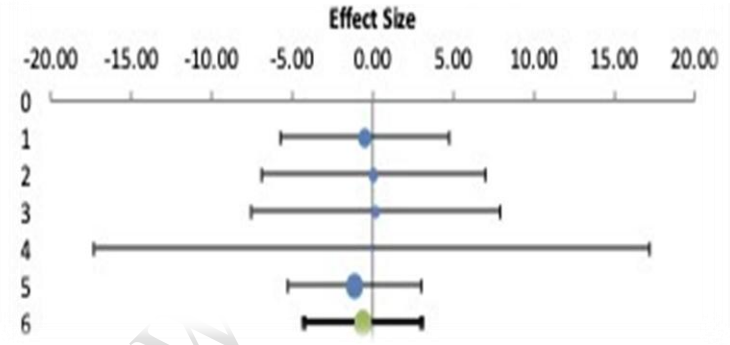


Figure 2:

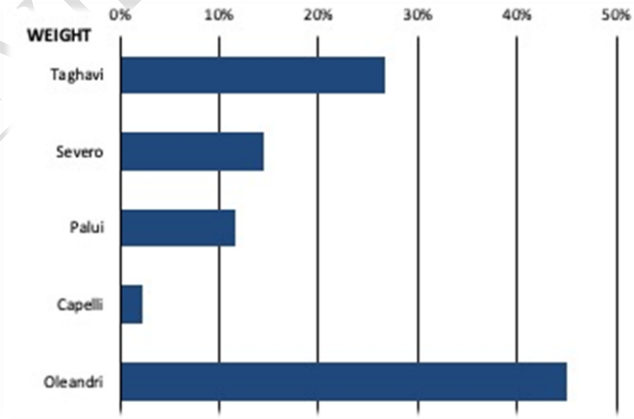


Table 3b:

Combined Effect Size	Values
Effect Size	-0.60
Standard error	1.32
CI Lower limit	-4.25
CI Upper limit	3.05
Two-tailed p-value	0.647

Table 3c:

Heterogeneity	
Q	0.15
p_q	0.997
I^2	0.00%
T^2	0.00
T	0.00

Table 4:

Study Name	Mean (Before Metformin)	SD	Mean (After Metformin)	SD	Mean Difference (Metformin Group)	upper CI (95%)	lower CI (95%)	P value
Taghavi 2011	16	2.26	15.6	2.45	-0.4	1.3629	-2.1629	0.64
Severo 2017	13.4	2	13.9	2.2	0.5	1.7216	-0.7216	0.41
Palui 2019	12.81	2.68	12.84	2.7	0.03	1.4203	-1.3603	0.97
Capelli 2012	12.4	1.7	12.6	1.9	0.2	0.5518	-0.1518	0.26
Oleandri 1999	12.5	0.7	13.5	0.7	1	1.657	0.3423	0.005

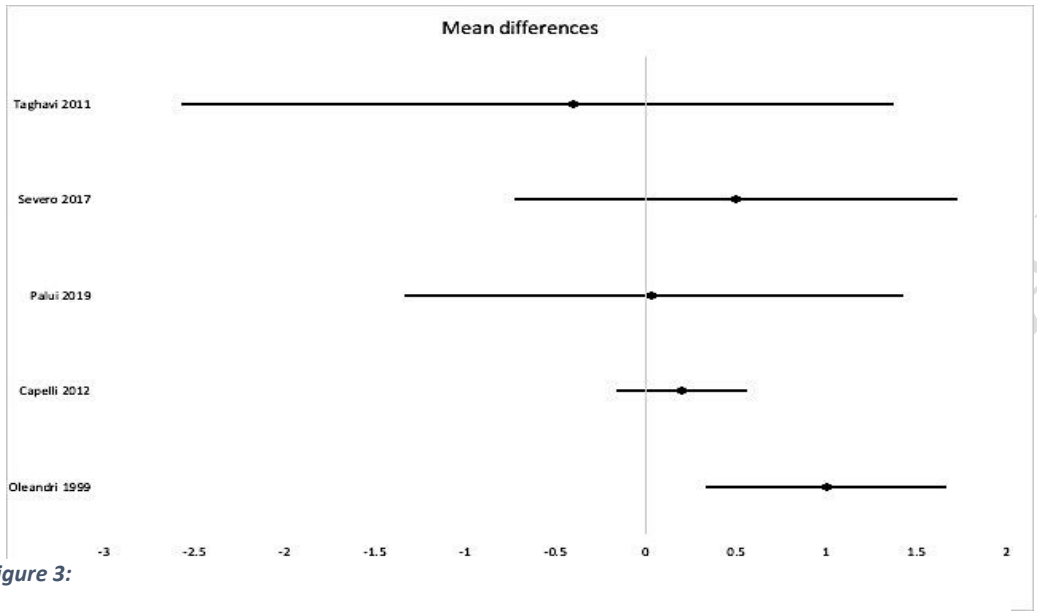


Figure 3:

Our study is contrary to the suggested hypothesis of metformin causing increased gastrointestinal absorption of LT4, as we suggest that there is a reduction seen in serum thyroxine levels with metformin. This would correlate with the clinical aspect of regulating Levothyroxine dose in patients with metabolic and thyroid disorders.

Previously, it was found in various studies that there was no associated change in thyroxine levels with a suppression of TSH caused by Metformin. This concept was stated by Vigersky et al [5] who studied four patients, three of whom were receiving Levothyroxine supplementation and did not have baseline thyroxine levels available. On the contrary, Isidro et al [6] observed that mean fT4 levels increased following metformin administration and decreased with its withdrawal. However, there was a non-significant difference between the basal fT4 levels and post-withdrawal levels in addition to a larger thyroxine replacement dose relative to body weight which potentially contributed towards the higher mean fT4 values. Capelli et al (2009) [7] presented non-significant results for change in serum fT4 levels in their two-phase study, pilot and long term; which showed SCH patients not receiving LT4 replacement had a slight decrease in mean fT4 levels from baseline however, statistically insignificant. Rotondi et al [8] recruited PCOS patients who were either hypothyroid or euthyroid and found insignificant changes in fT4 levels within the overall cohort. Similarly, Krysiak et al [9] established a non-significant increase in thyroxine levels, probably affected by the interaction with bromocriptine that was administered to some of the PCOS patients in their study. Dimic et al [10] emphasized again upon TSH-lowering effect of metformin being not related to serum thyroxine changes. Interestingly, Sloot et al [11] concluded a significant decrease in serum T3 levels without significant differences in TSH and fT4 levels with either metformin or a hypocaloric diet. Recently, Trouva et al [12] studied thyroxine levels in majorly euthyroid, pregnant patients with

PCOS taking metformin in a post hoc analysis based on two randomized controlled trials. They concluded that serum thyroxine levels have a smaller decline in the metformin group versus placebo group throughout the gestation possibly because of suppression of peripheral deiodinase activity by metformin. According to some papers, changes in thyroxine levels during pregnancy have been considered controversial [13]. This indicates that metformin may or may not have been the causal factor for their observation. In a meta-analysis study by Lupoli et al [14] to study the effect of metformin on TSH levels, two of the studies included were common to our meta-analysis as well. They stated no significant change in thyroxine levels but only a reduction in TSH levels in overt and subclinical hypothyroid patients with metformin.
Metformin was administered to male rats to predict the impact over thyroid profile. There was an increase in serum fT4 and fT3, irrespective of their induction to the diabetic model [15].

Given the discrepancy between different studies and non-significant results reported previously in many trials, we suggest that there should be further trials to know the relation between metformin and serum fT4, which may help us in calculating the correct dose of levothyroxine replacement.
No subgroup analysis was done due to the small number of studies and no heterogeneity (Table 3c). Limitations of our analysis include small sample size, confounding underlying patient characteristics, and concomitant medication given for diabetic management that caused probable interactions with metformin.
In conclusion, these data suggest that metformin has a significant effect over thyroxine levels that need to be studied in large-scale randomized controlled trials.

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Conflict of Interest:

We confirm that the manuscript has not been previously published and is not under consideration for publication elsewhere & has been approved by all co-authors. The authors also declare that they have no conflict of interest regarding the publication of this research. No financial, personal, or professional affiliations influenced the content or conclusions of this work.