HORMONES 2014, 13(1):65-73

*Research paper*

# Impact of dietary modification of advanced glycation end products (AGEs) on the hormonal and metabolic profile of women with polycystic ovary syndrome (PCOS)

Evangelia Tantalaki,[1] Christina Piperi,[2] Sarantis Livadas,[1] Anastasios Kollias,[1] Christos Adamopoulos,[2] Aikaterini Koulouri,[1] Charikleia Christakou,[1] Evanthia Diamanti-Kandarakis[1]

*[1]Endocrine Unit, Third Department of Internal Medicine, Medical School, National and Kapodistrian University of Athens; [2]Department of Biological Chemistry, University of Athens Medical School; Athens, Greece*

## ABSTRACT

**OBJECTIVE: To investigate the impact of dietary intervention on Advanced Glycation End products (AGEs) intake on the hormonal and metabolic profile in women with polycystic ovary syndrome (PCOS). METHODS: After baseline evaluation, 23 women with PCOS [mean±SD, age: 23.4±5.7 years; body mass index (BMI): 26±5.7 kg/m$^2$] underwent the following consecutive 2-month dietary regimens: a hypocaloric diet with ad-libitum AGEs content (Hypo), an isocaloric diet with high AGEs (HA) and an isocaloric diet with low AGEs (LA). Metabolic, hormonal and oxidative stress status was assessed and AGEs levels were determined in all subjects after the completion of each dietary intervention. RESULTS: Serum levels of AGEs, testosterone, oxidative stress, insulin and HOMA-IR index were significantly increased on the HA compared to the Hypo diet and subsequently decreased on the LA diet (compared to HA) ($p<0.05$ for all parameters). BMI remained unaltered throughout the HA and LA periods compared to the Hypo period. Serum AGEs were strongly correlated with insulin, as well as with HOMA, during the LA dietary period ($r=0.53$, $p=0.02$ and $r=0.51$, $p=0.03$, respectively). For the same period, dietary AGEs were correlated with insulin levels (rho=0.49, $p=0.04$). CONCLUSIONS: Modifications of dietary AGEs intake are associated with parallel changes in serum AGEs, metabolic, hormonal and oxidative stress biomarkers in women with PCOS. These novel findings support recommendations for a low AGEs dietary content along with lifestyle changes in women with PCOS.**

**Key words:** Dietary advanced glycation end products (AGEs), Insulin resistance, Oxidative stress, Polycystic ovary syndrome, Testosterone

*Address for correspondence:*
Evanthia Diamanti-Kandarakis, MD, Phd, Endocrine Unit, Third Department of Internal Medicine, Medical School, National and Kapodistrian University of Athens, Athens, Greece, e.diamanti.kandarakis@gmail.com

*Received 12-10-2012, Accepted 03-02-2014*

## INTRODUCTION

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrinopathy that affects 6-7% of premenopausal women.[1] The pathophysiology of the syndrome

appears to be multifactorial including metabolic and reproductive dysfunction.[2] Advanced Glycation End products (AGEs) emerge as a potential link between metabolic and reproductive aberrations in PCOS. AGEs are bioactive molecules with detrimental effects in tissues, which are ascribed to their chemical, pro-oxidant and inflammatory actions.[3-6] Among the most widely studied AGEs is carboxymethyl-lysine (CML), which has been used as a biomarker for in vivo formation of AGEs.[7] Interestingly, women with PCOS have elevated serum AGEs (CML) as compared to their healthy counterparts, independently of obesity and insulin resistance.[8,9] The positive association of serum AGEs levels with testosterone and anti-mullerian hormone levels in PCOS women[9,10] and the increased localization of these molecules in human polycystic ovaries[11] suggest a potential role of AGEs in ovarian tissue in PCOS, possibly beyond their metabolic actions.

Research interest in these molecules has been sparked by the fact that serum AGEs are partly exogenously derived, in addition to their endogenous production by non-enzymatic glycation of proteins, lipids and nucleic acids. Nutrition is an important source of exogenous AGEs and CML was one of the first to be characterized in foods. For this reason in most studies CML is chosen as a marker of AGEs in foods and in vivo.[12] Thermally processed foods, especially lipid- and protein-rich foods typical of Western diets, are a plentiful source of exogenous AGEs[4,5,13,14] and it was estimated that ≈10% of ingested immunoreactive AGEs are transported into the circulation, two thirds of which remain in the body and are incorporated covalently in tissues.[15] Therefore, it has been suggested that dietary AGEs are an important contributor to the body AGEs pool,[5] while they are also associated with elevation of serum inflammatory molecules and endothelial dysfunction as well as deterioration of renal function both in patients with type 2 diabetes mellitus and in non-diabetics.[4,16-18,26,27] Based on the above findings, AGEs have recently been considered as a potent endocrine disruptor.[19] Moreover, ingestion of a meal rich in AGEs has been shown to increase serum AGEs levels in normo-androgenic women with regular cycles (healthy women) as well as in women with PCOS.[20,21] A role of AGEs consumption in PCOS pathophysiology has been implied from the finding that animals fed an AGEs-enriched diet demonstrated a significant elevation of AGEs in serum and increased deposition in ovarian tissue in parallel with an increase of serum insulin and androgen levels.[22] However, the contribution of food-derived AGEs to glycooxidative stress, metabolic profile and androgen excess in women with PCOS has not been addressed.

Therefore, based on clinical and experimental data suggesting that dietary AGEs play a potential role in the pathophysiology of PCOS, we aimed to investigate whether high or low dietary intake of AGEs has an impact on the hormonal and metabolic profile of women with PCOS.

## RESEARCH DESIGN AND METHODS

### *Subjects*

We recruited 34 patients with PCOS (age range: 18-40 years) from the Outpatient Endocrinology Department of Evgenideio University Hospital in Athens and their data were collected prospectively in a computerized database. All women were invited to complete a 6-month period (3 phases of 2 months each) of dietary intervention. The local ethics board approved the study protocol and informed consent was obtained from all participants. The diagnosis of PCOS was based on the presence of irregular menstrual cycles (eight or fewer menses per year), elevated serum levels of testosterone and clinical signs of hyperandrogenism, according to the NIH criteria.[23] Non-classical congenital adrenal hyperplasia, androgen-secreting neoplasm, hyperprolactinemia and thyroid disease were excluded by appropriate tests in PCOS women. All the participants were in good health and non-smokers. None was taking any medication known to affect carbohydrate or sex hormone metabolism, including oral contraceptive pills, 3 months before the study.

### *Study protocol*

Blood samples were collected at 08:00 h after an overnight fast during the early follicular phase (days 2-5) of a spontaneous menstrual cycle, except in subjects with amenorrhoea in whom anovulation was confirmed via progesterone levels (<5ng/ml). Laboratory evaluations included serum AGEs (U/ml), oxidative stress, total and free testosterone (ng/

ml and pg/ml, respectively), sex hormone-binding globulin [SHBG (nmol/l)], serum fasting insulin (μIU/ml) and serum fasting glucose (mg/dl). Based on fasting values, HOMA-IR, an index of insulin resistance, was calculated as follows: HOMA-IR= (I0*G0)/22.5, where I0 is the fasting insulin (μU/ml) and G0 (mmol/l) is the fasting glucose. The samples were centrifuged immediately and serum was stored at -80°C until assayed.

### *Dietary protocol*

At recruitment, a 5-day weighed record assessed usual intake of calories and dietary AGEs, with information on the subjects' cooking practices and temperatures (Table 1). Energy requirements were calculated using the Harris-Benedict formula (Table 1). The AGEs content of free diet (baseline) was assessed to be moderate to high (Table 1) due to frequent consumption of candies as well as protein foods cooked or processed at high temperatures.

After baseline evaluation, all participants underwent the following dietary regimen: a hypocaloric diet with ad libitum AGEs content for 2 months, followed by an isocaloric diet with high AGEs content for another 2 months and afterwards an isocaloric diet with low AGEs content for the following 2 months. Laboratory measurements performed at baseline were repeated at the end of each 2-month dietary intervention.

Individual sessions were conducted every week during the whole study by the same registered dietitian in order to ensure dietary compliance (Table 1). During the first phase of dietary intervention (hypocaloric diet, Hypo) an energy deficit of 500 kcals was assigned (Table 1). Patients were advised to maintain their usual cooking temperatures and to weigh portion sizes. The prescribed diet was a multiple choice Mediterranean dietary regimen consisting of 20% of energy from protein, 50% from carbohydrate and 30% from fat, with six meals daily.

After the first biochemical re-assessment, at 2 months, patients were advised to increase energy intake to meet their personal requirements (isocaloric diet). They were also advised to increase cooking temperature to over 220° C in the oven and to consume more frequently roasted, grilled, broiled and baked foods, cooked at low humidity. In order to increase daily intake of dietary AGEs (isocaloric high AGEs diet, HA), consumption of protein foods (beef, pork, poultry, fish and baked cheese), nuts and sodas was encouraged at every meal, although fried foods and candies were not allowed.

In the last 2-month period, dietary modification consisted of lowering cooking temperature below 180° C in the oven, while preferring boiling, poaching, stewing or steaming. The purpose of this dietary modification was to decrease the participants' AGEs consumption while maintaining their energy intake at the level of their energy requirements (isocaloric low AGEs diet, LA). Patients were instructed to consume once a week red meat, poultry and fish, appropriately cooked as mentioned above, and to prefer pasta, boiled vegetables and legumes with unprocessed cheese twice a week. High-AGEs fast-food type foods and beverages were eliminated from the diet. The LA diet was estimated by food tables to offer less than

**Table 1.** Description of dietary intervention

| Period of dietary intervention | BASELINE | HYPO | HA | LA |
|---|---|---|---|---|
| Calorie intake /day (kcals/day) | 1578±477.2 | 1391.3±124 | 1869.6±114.6*† | 1869.6±114.6*† |
| AGEs intake/day (x10$^6$ U/day) | 10.9±4.3 | 9.6±4.3 | 16±1*† | 5.7±0.3*†‡ |
| Compliance (%) | | | | |
| High | - | 52.2 | 69.6 | 47.8 |
| Moderate | - | 47.8 | 30.4 | 43.5 |
| Poor | - | 0 | 0 | 8.7 |

No significant differences existed for compliance rates.
Values represent mean±SD.
AGEs: advanced glycation end products, HYPO: hypocaloric diet, HA: high AGEs isocaloric diet, LA: low AGEs isocaloric diet.
*$p<0.05$ vs. baseline, † $p<0.05$ vs. HYPO, ‡ $p<0.05$ vs. HA.

one third of the daily AGEs intake of the HA diet (Table 1). The study's dietary goals throughout the total 6-month period of dietary intervention were achieved while maintaining the required balance in macro- and micronutrients.

### *Chemicals, reagents and assays*

*Laboratory analysis*

Plasma glucose was determined by the glucose oxidase method (glucose analyzer, Beckman Coulter, Inc., Palo Alto, CA). Blood samples were centrifuged immediately and serum was stored at -80 C until assayed for insulin, total and free testosterone and SHBG. Serum insulin levels were measured using the RIA INSULIN-CT kits (CIS-Bio International, Gif-sur-Yvette, France). The assays employed for sex steroid levels have been reported elsewhere.[8] The competitive AGE-ELISA procedure was performed as described by Mitsuhashi et al[24,25] and has been reported previously in detail by our group.[9] Oxidative stress was determined by the commercially available PerOx-Assay (Immunodiagnostik AG, Stubenwald-Alee 8a, Bensheim, Germany) that measures total lipid peroxides concentration. Because of the direct correlation between oxygen radicals and lipid peroxides, it is possible to evaluate the oxidative status/oxidative stress in serum samples. Based on the manufacturer's suggestion, serum levels below 180 μmol/l indicate low oxidative stress, levels between 180 and 310 μmol/l indicate moderate and levels higher than 310 μmol/l indicate high oxidative stress. The intra-assay and inter- assay coefficient variations were 3.1% (221 μmol/l) and 5.1% (221 μmol/l), respectively. The detection limit for total lipid peroxides concentration was 7 μmol/l.

### *Data Analysis - Statistics*

Data were expressed as mean ± standard deviation. The Kolmogorov-Smirnov test was utilized for normality analysis of the parameters. Spearman (rho) or Pearson (r) correlations coefficients were determined for the assessment of the relationship between dietary and serum AGEs with hormonal and metabolic variables for each period separately. The comparison of variables between two consecutive diets, namely baseline and Hypo, Hypo and HA, HA and LA diet, respectively, was performed using the Wilcoxon or paired t-test. The chi-square test was applied for the comparison of the percentages of compliance during the dietary periods. To compare the effect of the HA diet in women previously receiving the Hypo diet and the effect of the LA diet in women previously receiving the HA diet, the mean percentage changes from baseline to Hypo, from Hypo to HA and from HA to LA diet are provided. These changes were compared between diets using the Friedman test. Pairwise comparisons were performed using the Wilcoxon signed rank test. All tests are two-sided; statistical significance was set at $p < 0.05$. All analyses were carried out using the statistical package SPSS vr 16.00 (Statistical Package for the Social Sciences, SPSS Inc., Chicago, Ill., USA).

## RESULTS

From the total population of the 34 PCOS women who entered the study, 23 patients [mean±SD: age: 23.4±5.7 years; body mass index (BMI): 26±5.7 kg/m$^2$] completed the study protocol and are included in the analysis. According to baseline BMI, 12 women had normal BMI, 6 were overweight and 5 were obese. Women who completed the study compared to those who did not presented no differences regarding age (23.4±5.7 vs. 26.3±6, respectively, p=0.08) and BMI (26±5.7 vs. 28.3±8.3, respectively, p=0.51). Concerning dietary compliance, no significant differences existed in terms of food choices and cooking temperature (Table 1).

Anthropometric, hormonal and metabolic parameters as well as serum levels of AGEs and oxidative stress at baseline and at the three following time points are shown in Table 2. In particular, serum levels of AGEs, testosterone, oxidative stress, insulin and HOMA-IR index were not significantly altered during the Hypo diet compared to baseline, although there was a decrease in BMI (Table 2), while these were significantly increased on the HA diet and decreased on the LA diet (Table 2). SHBG levels were significantly increased on the Hypo diet compared to baseline, while these were not significantly altered after the HA and LA diet (Table 2).

Regarding correlations, FAI were strongly correlated with insulin and HOMA during the baseline period (rho=0.47, p=0.03 and rho=0.42, p=0.05, respectively) and during the Hypo period (rho=0.61,

**Table 2.** Anthropometric, hormonal, metabolic parameters, serum levels of AGEs and oxidative stress at each dietary period

| | Baseline | HYPO | HA | LA |
|---|---|---|---|---|
| Age (years) | | 23.4 ± 5.7 | | |
| BMI (kg/m$^2$) | 26±5.7 | 24.6±5.2* | 24.6±5.5* | 24.2±5.2* |
| Serum AGEs (IU/ml) | 9.1±1.4 | 8.9±1.6 | 10.4±1.4*† | 8.2±1.6*†‡ |
| Testosterone (ng/ml) | 0.79±0.32 | 0.77±0.42 | 1.04±0.43*† | 0.77±0.32‡ |
| Free Testosterone (pg/ml) | 2.33±0.84 | 2.24±0.95 | 2.46±0.85 | 2.35±0.73 |
| SHBG (nmol/l) | 31.5±15.6 | 37.7±23.3* | 37.7±20.1* | 35.4 ± 18.7 |
| FAI | 13.2±11.7 | 12.8±14.9 | 15.4±16* | 11.1±10.7 |
| Androstendione (ng/ml) | 3.11±1.07 | 3.26±1.19 | 3.76±1.10* | 3.37±0.96 |
| Oxidative stress (μmol/l) | 216.7±125.6 | 201.8±120.8 | 341.7±243*† | 142.5±65.4*‡ |
| Glucose (mg/dl) | 82.8±9.1 | 86.4±5.3 | 87±5.7 | 83.3±7.9† |
| Insulin (μIU/ml) | 10.7±6.9 | 10.6±5.2 | 13.6±6.3*† | 9.2±2.8†‡ |
| HOMA-IR | 2.19±1.43 | 2.25±1.19 | 2.92±1.40*† | 1.86±0.59†‡ |

Data are expressed as mean value±SD.
BMI: body mass index, SHBG: sex hormone binding globulin, AGEs: advanced glycation end products, HYPO: hypocaloric diet, HA: high AGEs isocaloric diet, LA: low AGEs isocaloric diet.
*p<0.05 vs. baseline, † p<0.05 vs. HYPO, ‡ p<0.05 vs. HA.

p=0.006 and rho=0.57, p=0.01, respectively). Serum AGEs were strongly correlated with insulin, as well as with HOMA, during the LA diet (r=0.53, p=0.02 and r=0.51, p=0.03, respectively). For the same period, dietary AGEs were correlated with insulin levels (rho=0.49, p=0.04). Dietary AGEs tended to be correlated with serum AGEs for each period separately, although these associations did not reach the level of statistical significance (r ranged from 0.10 to 0.29, all p>0.05). The percent changes in anthropometric, metabolic and hormonal parameters between the examined dietary periods are presented in Figure 1.

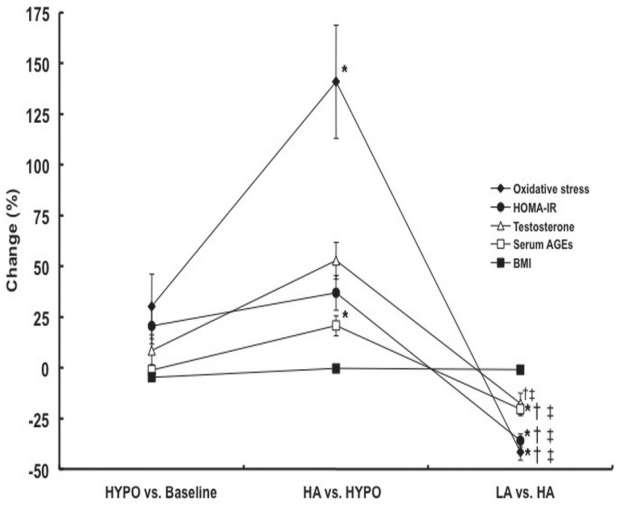


**Figure 1.** Changes (%) in anthropometric, hormonal and metabolic parameters between the consecutive dietary periods.
Data are presented as mean±SE. BMI: body mass index, AGEs: advanced glycation end products; HYPO: hypocaloric diet; HA: high AGEs isocaloric diet; LA: low AGEs isocaloric diet.
*p<0.05 vs. baseline-HYPO, † p<0.05 vs. HYPO-HA, ‡ p<0.05 for comparison of changes between all dietary periods.

## DISCUSSION

The main finding of the present study is that changes in dietary AGEs parallel changes in insulin sensitivity, oxidative stress and hormonal status. These findings are novel and have never been reported before in women with PCOS, to the best of our knowledge. AGEs result from nonenzymatic glycation reactions between reducing sugars and free amino groups of proteins, peptides or amino acids.

Recently, AGEs levels were found to be increased in lean women with PCOS, implicating them in the metabolic and reproductive abnormalities of the syndrome.[8] It should be noted that circulating levels of AGEs are mainly determined by the balance between dietary intake, endogenous formation, tissue utilization and renal clearance.[5,26,27] In particular, a correlation between dietary and serum AGEs has been shown in humans; available data exist in healthy

subjects, diabetics[28-31] and in patients with renal failure[27,29] as well as in several animal studies.[32,33] The role of the contribution of dietary AGEs to the body pool of AGEs has only recently been studied.[34,35]

In this study dietary AGEs tended to be correlated with serum AGEs, although these correlations did not reach the level of statistical significance due to the small sample size of each period examined separately. However, serum and dietary AGEs were strongly correlated with insulin levels during the LA period. Previous studies in healthy subjects and in patients with diabetes and renal failure have also shown an association between dietary AGEs and fasting insulin concentration, high sensitive C-reactive protein and mediators of vascular dysfunction.[5,13]

In support of the aforementioned associations, our study also showed that a HA diet increased serum levels of AGEs, insulin, testosterone, insulin resistance and oxidative stress in women with PCOS compared to preceding levels during the hypocaloric diet. Conversely, the LA diet seemed to improve the oxidative status aggravated by the HA diet without changes in BMI (Figure 1). This fact could imply that dietary AGEs contribute to PCOS abnormalities independently of BMI changes. Between baseline and hypocaloric dietary periods no changes were detected in the metabolic and hormonal profile. Although BMI decreased during the hypocaloric period, this change was less than 5%, considered necessary for measurable changes in the PCOS metabolic and hormonal profile, with no statistically significant alterations in WHR ($0.80 \pm 0.08$ vs. $0.83 \pm 0.07$ for baseline vs. hypo period, $p > 0.05$) and physical activity habits. These findings could suggest that the observed BMI changes during this period of the study were not sufficient to reveal the expected modifications in the measured parameters. Strikingly, it seems that 2-month-period interventions in dietary AGEs seem to effect changes in metabolic and hormonal status. A previous study in diabetic subjects showed that a single meal rich in AGEs induces acute endothelial and adipocyte dysfunction within hours post meal.[35]

Our findings may have important clinical implications for women with PCOS, as dietary habits seem to have a role in the pathophysiogy of the syndrome.[36] Dietary AGEs may modify both metabolic and hormonal parameters of affected women, without BMI changes. Regarding metabolic aspects, the diet-induced elevation of AGEs levels and the increased oxidative stress may directly induce insulin-signalling defects, as suggested by previous studies.[37] Oxidative stress is considered a major mediator of AGEs' action at tissue level[38] and is recognized as a major contributor to the metabolic syndrome, type 2 diabetes and atherosclerotic disease.[39] In addition, oxidative stress has been implicated in the pathogenesis of PCOS.[40,41] Moreover, AGEs may directly affect the hormonal profile of women with PCOS.[8,9] Although this study did not reveal a direct association of AGEs with testosterone, the levels of the latter were changed in parallel with those of AGEs, insulin and oxidative stress levels (Figure 1). Previous studies have shown such a relationship.[8,9] However, the fact that dietary AGEs were related to insulin resistance and the latter was in turn related to testosterone levels could instead imply an indirect association.

The study findings should be intepreted in light of some limitations, since there is as yet no agreement, among the different groups studying dietary AGEs, as to which molecule should be considered the most representative. However, in the recently published food database by Uribarri et al with approximately 500 food items, CML was used as the representative of AGEs detected by ELISA,[42] and this was also used in this study. One of the difficulties in diets with different dietary AGEs content is the content of other nutrients, such as heat-sensitive vitamins.[43] We tried to overcome this problem by advising our patients to take vitamin supplements, but we did not check their serum levels.

Also, the lack of a washout period between the consecutive dietary periods might have introduced a carry-over effect which could be pronounced in view of the long half-life of the plasma AGEs. Indeed, our conclusions should be interpreted in relation to this limitation. However, the main findings of this study can be considered as unaffected by a potential carry-over effect due to the following issues. First, the plasma half-life of the hormones evaluated ranges from 4-8 minutes (insulin) to less than 20 minutes (testosterone). Accordingly, the metabolic/hormonal profile documented in different phases cannot be attributed entirely to carry-over effect, since blood testing was

carried out at the end of each phase. Secondly, in this study the LA period followed the HA period. Thus, the changes observed during the LA period were initiated by a more adverse metabolic/hormonal status. Despite this, reduction in most metabolic/hormonal variables was noted (Figure 1).

Another limitation of the study is the number of women dropping out, though high dropout percentages are not uncommon in dietary intervention studies.[45] Of course, the high dropout rate could introduce a selection bias. The fact, however, that the characteristics of the subjects completing the study did not differ compared to those not completing is against a meaningful bias.

In conclusion, overexposure to exogenous AGEs, which are considered potent endocrine disruptors and are common in westernised diets, may exacerbate the metabolic and hormonal profile as well as oxidative stress in PCOS. Conversely, lowering the concentration of AGEs in food may improve these variables. The role of dietary AGEs in the clinical course of PCOS should be further explored. The forms of dietary AGEs, which can be absorbed, their mode of absorption and their pathway of cellular action upon target organs, including the ovary, have not been elucidated yet. Until then, it is advisable that assessment of dietary AGEs consumption be part of the evaluation of women with PCOS, and diets with low AGEs content along with other lifestyle modifications should be encouraged.

## DISCLOSURE STATEMENT

Nothing to disclose.

## FUNDING

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

## AUTHOR CONTRIBUTIONS

Tantalaki Evangelia, Koulouri Aikaterini: data collection and interpretation, conduct of the study.

Piperi Christina, Adamopoulos Christos: data analysis.

Livadas Sarantis: data analysis, data interpretation and manuscript writing.

Kollias Anastasios, Christakou Charikleia: data analysis, data interpretation and manuscript writing.

Diamanti-Kandarakis Evanthia: design and conduct of the study, data analysis, data interpretation and manuscript writing.

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