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ORIGINAL ARTICLE

# Glutathione-dependent enzyme activities of peripheral blood mononuclear cells decrease during the winter season compared with the summer in normal-weight and severely obese adolescents

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## Abstract

Oxidative stress-related inflammation is known to play a vital role in obesity-associated cardiovascular disease, contributing to the early stages of the pathology as well as during its development. Therefore, it is of great interest to understand how obesity-induced stress modulates antioxidant enzyme activity during puberty. To this end, 27 severely obese adolescents (body mass index > 30, $z$-score > 3.7) were recruited from a paediatric weight management centre. Eighteen were recruited during the summer and nine in the winter. All underwent a 4-month weight loss programme consisting in diet and physical activity. Twenty normal-weight age-matched adolescents were recruited from the same geographical area to serve as controls. Blood samples were extracted, and antioxidant enzyme activities were determined in peripheral blood mononuclear cells (PBMCs) and erythrocytes. The enzymes studied included catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase. Severely obese adolescents presented lower PBMC-glutathione reductase activity than their corresponding normal-weight counterparts. In addition, glutathione-dependent activities tended to be lower in both groups during the winter compared with summer. These changes coincided with differences in circulating vitamin D levels. Results may suggest that season-dependent factors such as vitamin D could affect glutathione-dependent activities in severely obese as well as in normal-weight adolescents.

**Keywords** Antioxidants · Glutathione peroxidase · Glutathione reductase · Obesity · Seasonality

## Introduction

Obesity is characterised by an excessive accumulation of fat mass as a result of an imbalance between energy intake and expenditure. This results in metabolic alterations in many body compartments, where the cardiovascular system is particularly affected [5]. In fact, the risk of developing cardiovascular alterations strongly correlates with the prevalence of obesity in adults [21]. On the other hand, the study of childhood obesity is instrumental in the identification of the early events that can give rise to the development of cardiovascular disease during adulthood. Endothelial dysfunction is an early sign of cardiovascular alterations that can evolve to atherosclerosis [25, 27]. Oxidative stress, in close correlation with inflammation, is known to be an instrumental factor in obesity-related cardiovascular disease by contributing during the early stages and development of the pathology [26, 28]. In fact, these 3 components

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(endothelial dysfunction, increased inflammation and oxidative stress) are strongly interrelated in the pathology's onset.

Oxidative stress-related markers associated with cardiovascular disease have been described both in obese children and adults. These include elevated circulating levels of malondialdehyde (MDA), oxidized LDL, $F_2$-isoprostanes, 8-hydroxi-guanosine, asymmetric dimethylarginine (ADMA), oxidized prostaglandins and high oxidized/reduced glutathione ratio (GSSG/GSH) [20]. This variety of markers could reflect that cardiovascular dysfunction is very complex and possibly influenced by a number of factors that still need to be elucidated. Puberty seems to worsen the inflammatory status and increases oxidative stress, with higher levels of peroxides, accompanied by a decrease in insulin sensitivity and enhanced endothelial dysfunction [14, 23]. Nevertheless, the interactions between these inflammatory and oxidative stress factors leading to endothelial dysfunction during puberty are currently unknown.

One possible hypothesis to at least partially explain these changes could be a deficient adaptation of antioxidant defences to the new situation that puberty represents. In addition, an effective lifestyle intervention may encourage antioxidant response by decreasing oxidative stress markers and reversing the deleterious effects of obesity in children. In this context, our laboratory is interested in understanding how antioxidant enzymes can be modulated in obesity-related stress during puberty. To address this question, the aim of the present study was to determine the activities of the main antioxidant enzymes in circulating blood cells from severely obese adolescents (SOA) and compare with non-obese age-matched counterparts.

## Materials and methods

### Subjects and protocol

Twenty-seven SOA were recruited from a paediatric weight management centre under the supervision of an Endocrinology Service (Nimes Hospital, France). Individuals make a stage of 4 months in order to change their lifestyle, and after this period, they continue treatment at home under the supervision of the parents but controlled by the Endocrinology Service of the Hospital. Adolescents were included at stage 3 of pubertal development according to the Tanner scale. Obesity was defined according to the body mass index (BMI) in kg/m$^2$ and BMI $z$-scores. Values of BMI > 30 and BMI $z$-score > 3 were considered as severely obese [7] (Table 1). Eighteen SOA were recruited during the summer (SOA-SU) and nine in the winter (SOA-WI). Twenty normal-weight pubertal stage-matched adolescents (NW) were recruited from the same geographical area to serve as controls (Table 1). Fourteen participated in the winter (NW-WI) and six in the summer (NW-SU) intervention. All participants were normotensive (Table 1), nondiabetic and free from other known obesity-related pathologies. Exclusion criteria for all subjects included familial premature cardiovascular disease, intake of medication, pubertal status assessed by Tanner < 2, weight loss larger than 5% of the total body weight during the previous 3 months and non-sedentary (more than 3 h of physical activity/week) in order to discriminate the training effect as confounding factor. The informed consent was signed by parents and adolescents. The study was approved by the "Comité de Protection de Personnes Sud-Mediterranée-III (Committee for People Protection of the South-Mediterranean Area-III)" and performed in accordance with the principles outlined in the Declaration of Helsinki and registered in ISRCTN99414527 (www.ISRCTN.org). Informed consent was obtained from all individuals and legal representatives participating in the study.

### Weight loss programme

The SOA group underwent a 4-month weight loss programme consisting in diet and exercise indicated by the paediatric centre. Eighteen SOA started the programme in August and finished in December, a period of the year with predominant high temperatures and sun exposition. This group was called SOA-summer (SOA-SU), although it does not correspond exactly to year season. Two participants from SOA-SU were withdrawn from the study for personal reasons. Nine SOA started the programme in March and finished in July, a period of the year with predominant low temperatures and sun exposition. This group was called SOA-winter (SOA-WI), although it does not correspond exactly to year season. Clinical and circulating parameters of both SOA groups were assessed within the first week of intervention and 4 months later (Table 1). SOA groups received a hypocaloric diet with a reduction of 300–500 kcal/day based on a balanced distribution of macronutrients (55% carbohydrates, 15% proteins and 30% lipids with less than 10% saturated fat). Micronutrients and fibre intakes were into the recommended daily allowances. The physical activity programme consisted in four 90-min supervised sessions per week. Each session included a variety of exercises such as aerobic running, dancing, tennis and recreational games. Six normal-weight participants were recruited in July (NW-SU) and fourteen in December (NW-WI) as controls. Clinical and circulating parameters are indicated in Table 1.

### Blood sampling and analysis

Blood samples were obtained from the antecubital vein after overnight fasting in EDTA vacutainers. PBMCs were purified following an adaptation of the method described by Boyum [4]. Blood cell numbers were determined by an automatic haematology analyser (Roche Diagnostics, Meylan, France). Biochemical markers related to inflammatory response such

**Table 1** General characteristics and circulating cell values in the summer and winter groups of normal-weight adolescents and severely obese adolescents before and after a 4-month weight loss programme beginning in summer and ending in winter or reversely

| Variable | SOA-SU ($n = 16$) | | NWA-SU ($n = 6$) | SOA-WI ($n = 9$) | | NWA-WI ($n = 14$) |
|---|---|---|---|---|---|---|
| | Starting in summer | Finishing in winter | | Starting in winter | Finishing in summer | |
| Gender (boys/girls) | 5/11 | 5/11 | 3/3 | 2/7 | 2/7 | 6/8 |
| Age (years) | 13.5 ± 0.3 | 13.8 ± 0.3** | 14.6 ± 0.5 | 13.7 ± 0.3$^{\dagger}$ | 14.0 ± 0.3** | 15.4 ± 0.0 |
| Height (cm) | 162.6 ± 1.5 | 163.8 ± 1.6* | 164.8 ± 4.6 | 162.6 ± 2.1 | 163.7 ± 2.2* | 165.0 ± 2.2 |
| Weight (kg) | 89.2 ± 4.0$^{\dagger\dagger}$ | 82.4 ± 3.5** | 53.8 ± 3.8 | 82.6 ± 4.0$^{\dagger\dagger}$ | 76.7 ± 3.3** | 54.7 ± 3.1 |
| BMI (kg/m$^2$) | 33.6 ± 1.2$^{\dagger\dagger}$ | 30.7 ± 1.1** | 19.8 ± 1.2 | 31.2 ± 1.0$^{\dagger\dagger}$ | 28.6 ± 0.9** | 19.9 ± 0.8 |
| BMI *z*-score | 4.3 ± 0.2$^{\dagger\dagger}$ | 3.5 ± 0.2** | 0.3 ± 0.5 | 3.7 ± 0.2$^{\dagger\dagger}$ | 3.1 ± 0.2** | 0.1 ± 0.3 |
| SBP (mmHg) | 99.9 ± 2.5 | 102.8 ± 1.2 | 104.7 ± 6.9 | 103.8 ± 3.5 | 105.2 ± 3.8 | 109.0 ± 3.7 |
| DBP (mmHg) | 56.6 ± 2.5 | 57.9 ± 1.5 | 59.8 ± 2.5 | 58.0 ± 2.2 | 60.8 ± 1.6 | 62.2 ± 1.7 |
| Leukocytes (cells·mm$^{-3}$) | 6562 ± 319 | 6866 ± 334 | 6700 ± 838 | 7711 ± 704 | 8600 ± 415 | 6329 ± 586 |
| Erythrocytes (cells mm$^{-3}$) | 4,992,667 ± 72,635$^{\dagger}$ | 5,120,000 ± 80,747* | 4,636,667 ± 104,870 | 5,305,556 ± 144,896$^{\dagger}$ | 5,154,444 ± 120,198 | 4,635,000 ± 73,055 |
| PBMCs (cells mm$^{-3}$) | 2570 ± 114$^{\dagger}$ | 2532 ± 114 | 1960 ± 112 | 2747 ± 305$^{\dagger}$ | 3135 ± 217* | 1876 ± 106 |
| PBMCs (%) | 39.9 ± 1.2$^{\dagger}$ | 36.4 ± 2.0 | 31.2 ± 3.3 | 36.2 ± 3.6 | 36.8 ± 2.5 | 31.4 ± 2.2 |
| CRP (μg/ml) | 2.0 ± 0.7 | 1.8 ± 0.9 | ND | 2.3 ± 0.8 | 0.1 ± 0.0* | ND |
| MPO (ng/ml) | 45.5 ± 4.8 | 36.4 ± 3.1 | ND | 38.0 ± 4.9 | 28.1 ± 3.9 | ND |
| 25-Hydroxy vit D (ng/ml) | 20.4 ± 1.3 | 18.6 ± 1.1* | 23.2 ± 6.8 | 17.9 ± 1.6 | 21.5 ± 1.8* | 17.7 ± 1.3 |

Values are mean ± SEM

*BMI* body mass index, *CRP* C-reactive protein, *DBP* diastolic blood pressure, *MPO* myeloperoxidase, *ND* not determined, *NWA* normal-weight adolescents, *PBMCs* peripheral blood mononuclear cells, *SBP* systolic blood pressure, *SOA* severely obese adolescents, *SU* summer, *vit* vitamin, *WI* winter

*$p < 0.05$, **$p < 0.001$ post-intervention vs pre-intervention within the same SOA group

$^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.001$ SOA vs NWA within the same season

as C-reactive protein (CRP) and myeloperoxidase (MPO) were determined in plasma by multiplex immunoassay (FlowCytomix, eBioscience, San Diego, CA). 25-Hydroxy vitamin D levels were determined following clinical laboratory standard procedures.

## Enzymatic activities

All activities were determined on a microplate reader (SPECTROstar Omega, BMG LabTech GmbH, Offenburg, Germany) at 37 °C. Superoxide dismutase (SOD) activity measurements were adapted from McCord and Fridovich [17]. Catalase activity was determined according to Aebi [1]. GPX activity was determined according to Flohé and Gunzler [10], with certain modifications. GRD was determined according to Goldberg and Spooner [11].

## PBMC RNA extraction and qRT-PCR analysis

Total RNA was isolated from lymphocytes using the TriPure extraction kit (Roche Diagnostics, Germany). RNA (1 μg) was reverse transcribed using 50 U of Expand Reverse Transcriptase (Roche Diagnostics) and 20 pmol oligo-dT for 60 min at 37 °C in a 20 μl final volume, according to the manufacturer's instructions. The resulting cDNA (0.5 μl) was amplified using the LightCycler FastStart DNA Master$^{PLUS}$ SYBR Green 1 kit (Roche Diagnostics). Amplification (109 bp product size) was performed at 94 °C/0.5 min (denaturation), 58 °C and 65 °C/0.5 min (annealing for GPX and GRD respectively) and 72 °C/1.5 min (synthesis) for 40 cycles, using the following primers.

For GPX cDNA:
forward: 5′-GCCTGCAGCTGTGTAGTGCTGG-3′
reverse: 5′-GCTGGTTTTTCCTTTGGGTTTAGGTG-3′
For GRD cDNA:
forward: 5′-CAAGGAAGAAAAGGTGGTTGGGATC-3′
reverse: 5′-GTCAAAGTCTGCCTTCGTTGCTCC-3′

The relative quantification was performed by using the $2^{(-\Delta\Delta Ct)}$ method, normalized to 36B4 rRNA, as control:
forward: 5′-ATGTGAAGTCACTGTGCCAG-3′
reverse: 5′-GTGTAATCCGTCTCCACAGA-3′

### Statistical analysis

Statistical analyses were performed using SPSS 22.0 software (SPSS, Chicago, Ill). Data were tested for normal distribution with the Kolmogorov-Smirnov test and for homogeneity of variances with Levene's test. Variables in SOA vs normal weight subjects were compared by independent *t* tests and analysis of covariance (ANCOVA), including gender as a covariate. Paired student *t* tests were used to assess the effect of the weight loss programme on SOA variables. A two-tailed *p* value less than 0.05 was considered significant.

## Results

The weight loss programme included diet and physical activity interventions (see the "Materials and methods" section). After the 4-month period, significant changes were observed (Table 1). Both SOA groups significantly increased their height at the end of each intervention. At the same time, weight and BMI were significantly reduced, showing a tendency to normal weight values, although they still remained obese at the end of the intervention. Also, certain values indicated a tendency towards improvement, emphasizing that longer interventions including balanced hypocaloric diet and moderate-vigorous physical activity may be necessary to reach normal weight values. No changes in blood pressure were noticed; however, certain significant changes in circulating cells were observed at the end of the intervention in SOA-SU (increased erythrocytes) and SOA-WI (increased PBMCs) (Table 1). Regarding inflammatory markers (CRP and MPO), CRP was significantly lower in the SOA-WI group at the end of the intervention. MPO presented a tendency to decrease in both interventions (SOA-SU and SOA-WI), but differences between the beginning and the end of the intervention were not significant. Altogether, these results indicate that the weight reduction intervention seemed to improve the health status assessed by a significant weight reduction and a tendency to decrease certain inflammatory markers.

The NWA-SU and NWA-WI groups did not present significant differences in anthropometric parameters, blood pressure nor circulating cell figures (Table 1). Comparing NWA and SOA within the same season, significant anthropometric differences were observed in all cases, as expected due to the changes in weight and BMI (Table 1). Significant differences were also observed for circulating cell numbers, where the SOA group presented higher values for erythrocytes and PBMCs than NWA (Table 1).

Since oxidative stress in close correlation with inflammation is an instrumental factor in the development and progression of cardiovascular disease associated with obesity [26, 28], the status of the major antioxidant enzymes was analysed.

Firstly, the evolution of antioxidant activities (comparing beginning vs end of each intervention) was analysed in the SOA groups in different circulating cells (PBMCs and erythrocytes). Regarding the glutathione-dependent enzymes, significant differences were observed in GPX and GRD in PBMCs and erythrocytes. The activities of both enzymes significantly increased when the intervention finished in the summer (SOA-WI group) and significantly decreased when intervention finished in the winter (SOA-SU group) (Table 2). However, catalase and SOD did not follow this particular pattern, showing no significant changes (i.e. PBMC catalase at the end of each intervention) or changes in the opposite direction than observed for glutathione-dependent enzymes (i.e. erythrocyte catalase at the end of SOA-SU intervention) (Table 2). Another way to verify if antioxidant activities tend to display higher values in the summer, as opposed to the winter, is to compare SOA-SU vs SOA-WI groups at the beginning of each intervention. As it can be observed in Table 2, glutathione-dependent enzymes present significantly higher values in SOA-SU (intervention starting in summer) than in SOA-WI (starting in winter) in both erythrocytes and PBMCs (Table 2). This tendency was also observed for SOD in both cell types, but not for catalase (Table 2).

Regarding NWA, it was not possible to analyse the evolution of antioxidant activity within the same group, since no intervention was performed. However, it was possible to compare activities in blood samples from similar groups of individuals at different seasons (NWA-SU vs NWA-WI). As observed in SOA, glutathione-dependent enzymes presented significantly higher values in NWA-SU vs NWA-WI in both erythrocytes and PBMCs (Table 2). The remaining antioxidant activities presented a variable pattern following the same observation made for SOA groups (Table 2).

Finally, the antioxidant activities in SOA vs NWA within the same season were compared (Table 2). The only significant difference observed in PBMC was with GRD, presenting significantly higher values in NWA-SU and NWA-WI compared with SOA-SU and SOA-WI, respectively, at the beginning of each intervention (Table 2). In erythrocyte GRD, a similar pattern was observed when comparing SOA-WI vs NWA-WI, but the difference was not significant when comparing SOA-SU vs NWA-SU (Table 2). PBMC and erythrocyte GPX showed a similar tendency as previously described for GRD, but the differences were not significant (Table 2). For the remaining antioxidant enzymes, variable results were observed (Table 2).

Altogether, the data seem to indicate that glutathione-dependent activities (GPX and GRD) in PBMCs tend to present lower values in NWA and SOA individuals when blood samples were obtained during the winter period (Table 2). In

**Table 2** PBMC and erythrocyte antioxidant activities in the summer and winter groups of normal-weight adolescents and severely obese adolescents before and after a 4-month weight loss programme beginning in summer and ending in winter or reversely

| Variable | SOA-SU ($n = 16$) | | NWA-SU ($n = 6$) | SOA-WI ($n = 9$) | | NWA-WI ($n = 14$) |
|---|---|---|---|---|---|---|
| | Starting in summer | Finishing in winter | | Starting in winter | Finishing in summer | |
| Erythrocyte GPX (nKat/g Hb) | 3807.3 ± 144.2 | 464.6 ± 17.8$^{\&\&}$ | 4220.7 ± 242.1 | 486.9 ± 38.9** | 5279.0 ± 309.1$^{\&\&}$ | 551.8 ± 39.2** |
| PBMC GPX (nKat/$10^9$ cells) | 360.4 ± 51.1 | 70.7 ± 3.9$^{\&\&}$ | 638.7 ± 140.2 | 33.9 ± 6.6** | 500.8 ± 81.5$^{\&}$ | 36.4 ± 4.2* |
| Erythrocyte GRD (nKat/g Hb) | 4253.9 ± 404.1 | 1382.8 ± 85.9$^{\&\&}$ | 5749.8 ± 1576.2 | 1806.7 ± 241.5**$^{\dagger\dagger}$ | 5365.6 ± 1285.5$^{\&}$ | 544.1 ± 74.6* |
| PBMC GRD (nKat/$10^9$ cells) | 1539.8 ± 124.5$^{\dagger}$ | 473.7 ± 53.4$^{\&\&}$ | 2259.9 ± 264.2 | 160.2 ± 21.9**$^{\dagger}$ | 1082.8 ± 123.6$^{\&\&}$ | 305.6 ± 41.7** |
| Erythrocyte SOD (nKat/g Hb) | 74.2 ± 5.0$^{\dagger\dagger}$ | 54.7 ± 6.9$^{\&}$ | 13.5 ± 2.8 | 17.9 ± 4.0** | 39.0 ± 7.0$^{\&}$ | 36.0 ± 9.9* |
| PBMC SOD (nKat/$10^9$ cells) | 19.8 ± 4.4 | 22.2 ± 2.4 | 26.6 ± 5.1 | 7.3 ± 1.8$^{\dagger}$ | 23.4 ± 2.7$^{\&\&}$ | 18.6 ± 3.9 |
| Erythrocyte catalase (Kat/g Hb) | 76.2 ± 3.9 | 119.1 ± 6.5$^{\&\&}$ | 104.2 ± 10.8 | 142.3 ± 13.1* | 83.0 ± 14.8$^{\&}$ | 140.8 ± 7.5* |
| PBMC catalase (nKat/$10^9$ cells) | 168.6 ± 21.9$^{\dagger}$ | 99.5 ± 16.7 | 394.9 ± 72.7 | 248.5 ± 47.7*$^{\dagger}$ | 270.6 ± 64.2 | 137.8 ± 24.1* |

Values are mean ± SEM

*GPX* glutathione peroxidase, *GRD* glutathione reductase, *NWA* normal-weight adolescents, *PBMC* peripheral blood mononuclear cell, *SOA* severely obese adolescents, *SOD* superoxide dismutase, *SU* summer, *WI* winter

$^{\&}p < 0.05$, $^{\&\&}p < 0.001$ post-intervention vs pre-intervention within the same SOA group

$^{*}p < 0.05$, $^{**}p < 0.001$ NWA-WI vs NWA-SU and SOA-WI vs SOA-SU at the beginning of intervention

$^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.001$ SOA vs NWA within the same season

addition, only GRD activity was significantly higher in NWA when compared with SOA within the same season (Table 2). Therefore, environmental factors could be considered as modulating elements for GPX and GRD activities in both adolescent groups.

In order to determine if the observed changes in GPX and GRD activities could be due to a different pattern of gene expression, RNA from PBMCs was isolated. As a result, no significant differences were detected when comparing both SOA groups at the beginning vs the end of the intervention, except for a decreased GRD expression (approximately 50%) only in SOA-SU at the end of the intervention (1 arbitrary unit at the beginning vs 0.47 arbitrary units at the end). This result matches with the observation made at the level of enzymatic activity only for GRD in the SOA-SU group. However, the pattern of activities observed in erythrocytes (an anucleated cell) suggests that post-translational mechanisms could also be involved in the modulation of GPX and GRD activities. Future research is necessary to address this question.

In order to identify candidate seasonal factors regulating GPX and GRD activities, circulating levels of vitamin D were determined. These were significantly higher in both groups during the summer periods in SOA (Table 1). No significant differences for vitamin D values were observed when comparing NWA-SU vs NWA-WI, likely due to the low *n* in NWA-SU, although a tendency to increase during the summer was observed in this group (Table 1).

## Discussion

The key observation that arises from this study is that the antioxidant glutathione-dependent activities (GPX and GRD) tend to decrease during the winter compared with the summer period, regardless of the group analysed (SOA or NWA). Differences were significant when comparing the beginning vs the end of the intervention in both SOA groups, when comparing SOA-SU vs SOA-WI at the beginning of the intervention and when comparing NWA-SU vs NWA-WI. In all these situations, GPX and GRD enzymatic activities in PBMCs and erythrocytes during the winter were always significantly lower than during the summer. On the other hand, other antioxidant activities tend to increase. A likely interpretation of this observation is that catalase increases its activity in order to compensate the decrease observed in the other antioxidant enzymes during the winter. Nevertheless, additional determinations need to be performed to verify this hypothesis. These differences were not attributable to sample manipulation, since blood samples were extracted and manipulated in similar conditions. Therefore, we hypothesise that environmental factors could be responsible for the changes observed regarding the activities of glutathione-dependent enzymes.

In the list of factors, vitamin D was the most obvious candidate, since vitamin D is a main regulator of the antioxidant response. It seems that this is achieved at the level of gene expression through activation of the nuclear factor Nrf2 [13] which seems to be a target for vitamin D receptor. This

transcription factor is instrumental for antioxidant gene expression. Taking into account the results obtained in erythrocytes, an alternative mechanism for vitamin D modulation of glutathione-dependent enzymes is by acting directly on enzymatic activity and therefore increasing intracellular glutathione levels [12, 16]. Further experiments will be necessary to verify both hypothesis. Nevertheless, all individuals in this study presented vitamin D deficiency during the winter period (SOA-SU at the end of the intervention, SOA-WI at the beginning of the intervention and NWA-WI) and higher levels as expected in the summer period (SOA-SU at the beginning of the intervention, SOA-WI at the end of the intervention and NWA-SU). The higher levels of vitamin D during the summer coincided with the higher GPX and GRD activities in both populations (SOA and NWA). Nevertheless, it has to be noted that the levels of circulating vitamin D during the summer in both groups were below the optimal range. This key observation in a sunny area such as the South of France is out of the scope of this research, but must be considered for future investigations.

Therefore, the increase in vitamin D levels could explain the increase in glutathione-dependent enzyme activities. In addition, other modulators of this group of enzymes must be considered as well, including temperature variations [18] and non-nutrient compounds present in fresh fruits and vegetables [9]. Regarding temperature, glutathione-dependent enzymes, as well as other antioxidants, are capable of adapting to the temperature-related stress situations by increasing their activity [18]. However, the adaptation of antioxidant activities does not occur during the winter, presenting a low efficiency to mitigate oxidative stress, as reported previously [19]. In the present report, physical activity during the summer was always performed outside, increasing sun exposure that favours vitamin D biosynthesis and experiencing higher temperatures than during the winter, which induces GRD and GPX activation. Regarding non-nutrient compounds in diet, the activity of glutathione-dependent enzymes seemed to increase when diet contained abundant fresh fruits and vegetables [8]. The summer diets of the present study contained more servings of fresh fruits and vegetables in the form of salads, compared with winter diets where vegetables were cooked, mashed or souped, and the variety of fruits and vegetables was limited. Seasonal variations in diet are common in modern societies and need to be considered when making nutritional studies [2].

How vitamin D and other factors can modulate glutathione-dependent enzymes is still an open question. Changes in gene expression can partially explain the decrease of PBMC GRD activity in SOA-SU at the end of the intervention. However, the presence of similar changes in erythrocytes (a cell with no nucleus) supports the idea that post-translational mechanisms might be involved in the modulation of activities of glutathione-dependent enzymes.

The other key observation from this study is that SOA presented lower PBMC GRD activity than the corresponding NWA counterparts within the same season. GRD is a key antioxidant enzyme instrumental in recycling oxidized glutathione to the reduced form in order to drive GPX activity. The latter is involved in eliminating hydrogen peroxide with no production of molecular oxygen, representing an alternative and safer pathway to catalase activity that produces molecular oxygen. In this context, obesity is a pathology characterised by chronically low levels of oxidative stress and inflammation [29]. This explains the systemic presence of oxidative stress markers that have been found in many studies of obese individuals [24]. Since antioxidant enzymes are involved in defence mechanisms against reactive oxygen species (ROS), the lower activity in obesity could favour changes in the signalling pathways or oxidative damage to macromolecules, leading to obesity-related alterations. In this context, our results show a tendency coincident with previous observations that describe low SOD and GPX activities in erythrocytes isolated from obese adolescents [15, 22]. In any case, we need to increase the number of subjects to assess this particular point and analyse the specific role of gene expression modulation within this particular context.

The main working hypothesis for our future work is that the summer favours oxidative stress, as observed in animal models [3, 6]. In addition, obese individuals seem to have a poor adaptation to this environmental stress. The increase in antioxidant activities strengthened by vitamin D rises due to sun exposure, and diet polyphenols could help to mitigate this unfavourable situation. Obviously, we are open to other hypotheses in order to solve this complex question that we have grouped in the concept of "seasonality-related factors."

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## Compliance with ethical standards

The protocol was in accordance with national legal requirements and the Helsinki Declaration for research on human beings and approved by the Ethics Committee for People Protection of the South-Mediterranean Area-III (France).

**Conflict of interest** The authors declare that they have no conflict of interest.

**Disclosure statement** The authors alone are responsible for the contents and writing of the paper.

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