

# Weight loss and vitamin D improve hyporesponsiveness to corticosteroids in obese asthma

**Short Title: Weight loss improves steroid response in obese asthma**

Bantulà M<sup>1</sup>, Tubita V<sup>1</sup>, Roca-Ferrer J<sup>1,2</sup>, Mullol J<sup>1,2,3</sup>, Valero A<sup>1,2,4</sup>, Bobolea I<sup>1,2,4</sup>, Pascal M<sup>5</sup>, de Hollanda A<sup>1,6,7</sup>, Vidal J<sup>1,6,8</sup>, Picado C<sup>\*1,2,4</sup>, Arismendi E<sup>\*1,2,4</sup>

<sup>1</sup>Institut d'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS), Barcelona, Spain.

<sup>2</sup>Centro de Investigaciones Biomédicas en Red de Enfermedades Respiratorias (CIBERES), Madrid, Spain.

<sup>3</sup>Rhinology Unit & Smell Clinic, ENT Department, Hospital Clinic, Barcelona, Spain.

<sup>4</sup>Pulmonology and Allergy Department, Hospital Clinic, University of Barcelona, Barcelona, Spain.

<sup>5</sup>Immunology Department, CDB, Hospital Clinic, University of Barcelona, Barcelona, Spain.

<sup>6</sup>Obesity Unit, Endocrinology and Nutrition Department, Hospital Clínic, Barcelona, Spain.

<sup>7</sup>Centro de Investigaciones Biomédicas en Red de Fisopatología de la Obesidad y Nutrición (CIBEROBN), Madrid, Spain.

<sup>8</sup>Centro de Investigaciones Biomédicas en Red en Diabetes y Enfermedades Metabólicas (CIBERDEM), Madrid, Spain.

\* Both authors contributed equally to this work with senior responsibilities.

## Corresponding author:

Marina Bantulà

Institut d'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS)  
Barcelona, Spain.

E-mail: [bantula@clinic.cat](mailto:bantula@clinic.cat)

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.18176/jiaci.0861

**Abstract**

**Background:** Obesity negatively impacts on asthma response to inhaled corticosteroids by unknown mechanisms.

**Objective:** To demonstrate that the poor response to inhaled corticosteroids in obese asthma is associated with an impaired anti-inflammatory activity of corticosteroids and vitamin D deficiency, both improved by weight loss.

**Methods:** 23 obese asthmatics (OA) [18 females; median age (interquartile range) 56 (51-59) years], 14 non-obese asthmatics (NOA) [11 females; 53 (43-60) years], 15 obese (O) [13 females; 47 (45-60) years], and 19 healthy controls (HC) [14 females; 43 (34-56) years] were enrolled. 10 OA and 11 O patients were evaluated at baseline (V1) and six months after (V2) bariatric surgery. Corticosteroid response was measured by dexamethasone inhibition of peripheral blood mononuclear cell (PBMC) proliferation. Lung function, serum levels of leptin, adiponectin, and vitamin D were measured at V1 and V2.

**Results:** We found a reduced response to dexamethasone in PBMCs of O and OA patients with respect to NOA and HC subjects, that inversely correlated with the adiponectin/leptin ratio and vitamin D levels. Bariatric surgery improved corticosteroid responses in O and OA patients and normalized adiponectin/leptin ratio and vitamin D levels. Exposure of PBMCs to vitamin D potentiated the antiproliferative effects of corticosteroids. Dexamethasone and vitamin D induced similar MKP-1 expression in O and OA patients.

**Conclusion:** The efficacy of weight loss to improve symptoms and lung function in OA patients can be, at least in part, due to the recovered anti-inflammatory effects of corticosteroids. Vitamin D deficiency may contribute to corticosteroid hyporesponsiveness in OA.

**Key words:** Asthma. Bariatric surgery. Corticosteroid. Obesity. Vitamin D

## Resumen

**Antecedentes:** La obesidad tiene un impacto negativo en la respuesta del asma a los corticosteroides inhalados por mecanismos desconocidos.

**Objetivo:** Demostrar que la mala respuesta a los corticosteroides inhalados en pacientes obesos asmáticos se asocia con una actividad antiinflamatoria alterada de los corticosteroides, así como también a la deficiencia de vitamina D, ambos mejorados por la pérdida de peso.

**Métodos:** 23 obesos asmáticos (OA) [18 mujeres; mediana de edad (rango intercuartílico) 56 (51-59) años], 14 asmáticos no obesos (NOA) [11 mujeres; 53 (43-60) años], 15 obesos (O) [13 mujeres; 47 (45-60) años], y 19 controles sanos (HC) [14 mujeres; 43 (34-56) años] fueron incluidos. Se evaluaron 10 pacientes OA y 11 O al inicio (V1) y seis meses después (V2) de cirugía bariátrica. La respuesta a los corticosteroides se midió mediante la inhibición con dexametasona de la proliferación de células mononucleares de sangre periférica (PBMC). La función pulmonar, los niveles séricos de leptina, adiponectina y vitamina D se midieron en V1 y V2.

**Resultados:** Encontramos una respuesta reducida a la dexametasona en PBMC de pacientes O y OA con respecto a los NOA y HC, que se correlacionó de forma inversamente proporcional con la relación adiponectina/leptina y los niveles de vitamina D. La cirugía bariátrica mejoró las respuestas de los corticosteroides en los grupos de pacientes O y OA y normalizó la relación adiponectina/leptina y los niveles de vitamina D. La exposición de las PBMC a la vitamina D potenció los efectos antiproliferativos de los corticosteroides. La dexametasona y la vitamina D indujeron una expresión similar de MKP-1 en los pacientes O y OA.

**Conclusiones:** La eficacia de la pérdida de peso para mejorar los síntomas y la función pulmonar en pacientes OA puede deberse, al menos en parte, a los efectos antiinflamatorios restablecidos de los corticosteroides. La deficiencia de vitamina D puede contribuir a la baja respuesta a los corticosteroides en los OA.

**Palabras clave:** Asma. Cirugía bariátrica. Corticosteroides. Obesidad. Vitamina D

## Introduction

Asthma is a chronic respiratory illness characterized by chronic airway inflammation, chronic airflow obstruction, and airway hyperresponsiveness [1]. Based on the inflammatory pathway involved, chronic inflammation in asthma is broadly divided into two predominant endotypes: type 2 (T2-high) and non-type 2 (T2-low) [2].

Inhaled corticosteroids (ICS) are considered the cornerstone of controller therapy for T2-high asthma, which contrasts with their poor therapeutic efficacy in T2-low asthma [1,2]. ICS have the ability to effectively control asthma by reducing airway inflammation, airway obstruction, airway hyperresponsiveness, and asthma symptoms [1,3]. However, asthma patients often do not follow the prescribed ICS regimen and, among the compliant patients, the response to treatment is highly variable, from those who respond to low doses of ICS to those who are resistant to even very high ICS doses. In both cases the results are similar: increased risk of exacerbations, hospitalization, and mortality [1,3]. Multiple molecular mechanisms have been involved in corticosteroids (CS) hyporesponsiveness. Obesity has been recognized as a risk factor for ICS hyporesponsiveness in asthma patients. Asthmatic patients with obesity have reduced odds of achieving asthma control, higher risk of asthma hospitalizations, and lower quality of life compared with asthmatics with a normal body mass index (BMI) [4–6]. Positive correlation has been found between high BMI with residual asthma symptoms that remained present in OA patients despite intensive treatment with high doses of ICS [6]. The mechanism(s) by which obesity negatively impacts on asthma control by ICS remains to be fully elucidated. Detrimental effects of obesity on lung function and additive or synergistic effects of obese systemic inflammation on airways inflammation, have been proposed as potential mechanisms to explain ICS hyporesponsiveness in OA [7,8]. On the other hand, a defective induction by CS of anti-inflammatory genes such as mitogen-activated protein kinase phosphatase-1 (MKP-1) has been involved [9].

In adults, the increased weight is associated with a relatively small reduction of forced expiratory volume in 1s (FEV<sub>1</sub>) and of forced vital capacity (FVC), with the FEV<sub>1</sub>/FVC ratio usually remaining unaltered or slightly increased and associated with a reduction of lung volumes, such as the functional residual capacity, alterations that characterize the so-called restrictive ventilatory defect [7].

Concerning inflammation, the excessive accumulation of adipose tissue in subjects with obesity results in an increased production of pro-inflammatory cytokines (IL-1 $\beta$ , IL-6, IFN- $\gamma$ , TNF- $\alpha$ ) and adipokines such as leptin, while there is a reduced release of adiponectin, a cytokine with anti-inflammatory properties [8]. Very little is known about the links that may exist between obesity and asthma inflammations, as it has been observed that the high systemic production of adipokines produced in visceral adipose tissue is capable of affecting the reactivity of the airways, without apparently modifying airway inflammation in OA patients [10]. Similarly, the mechanisms responsible for the loss of anti-inflammatory efficacy of CS in OA remain to be elucidated. A single study showed that the ability of dexamethasone (DEX) to induce MKP-1 gene expression in peripheral blood mononuclear cells (PBMCs) is reduced in subjects with obesity and severe asthma compared with normal-weight asthma patients [11].

Vitamin D (VitD) is a hormone with pleiotropic effects and numerous regulatory mechanisms beyond bone health [12]. The active form 1,25-dihydroxyvitamin D (1,25(OH) $_2$ D) binds the VitD receptor, present in all tissues including T and B lymphocytes, dendritic cells, and bronchial epithelial cells [12]. VitD inhibits the expression of IL-2, and suppresses T helper 1 cytokines IL-12, IL-6, and TNF, but increases IL-4, IL-5, and IL-10 release [12,13].

The serum level of 25-hydroxyvitamin D (25(OH)D) that is defined as VitD deficiency remains somewhat controversial. Previous guidelines defined VitD deficiency as 25(OH)D values below 20ng/ml [14]. However, in a more recent consensus statement, VitD deficiency is defined as 25(OH)D values below 12 ng/ml [15,16]. Evidence from observational studies suggests that obesity is associated with VitD deficiency [17,18]. Moreover, serum 25(OH)D levels decline with increasing BMI and body fat mass [19,20], and the decreased level of VitD appears to be mainly due to its dilution in the increased adipose tissue mass [19].

Low serum 25(OH)D levels have been found associated with asthma exacerbations [21]. Moreover, an inverse correlation between serum 25(OH)D concentrations with FEV $_1$  and BMI has been reported [22]. Other reports described an association between VitD deficiency and the risk of CS resistance [23]. In PBMCs from steroid resistant patients, the active form of VitD, 1,25(OH) $_2$ D, enhances DEX inhibition of cell proliferation [24], increasing the expression of MKP-1 [25]. Interestingly, leptin levels inversely correlated with the ability of 1,25(OH) $_2$ D, to inhibit *in vitro* production of inflammatory cytokines [26].

Weight reduction significantly improves systemic and adipose tissue inflammatory activity levels [26,27]. Bariatric surgery (BS) is considered the most effective and sustained long-term treatment of severe obesity. Several studies have shown an improvement in asthma control, medication use, hospitalization rate, lung function, systemic inflammatory markers, and decreased mast cell numbers in the airways of OA after weight loss via BS [28–33].

We hypothesized that understanding the links between asthma, obesity, and VitD can help elucidate the mechanisms underlying ICS hyporesponsiveness in OA. A step that can also contribute to the development of therapies with VitD specifically designed for this often difficult to manage asthma phenotype.

The objective of this study is to demonstrate that the poor response to ICS in obese asthma is due to an impaired anti-inflammatory activity of CS associated with a VitD deficiency and that both deficiencies are corrected following BS-associated weight loss with VitD supplementation.

## **Subjects and methods**

### **Subjects**

We recruited thirty-seven asthma patients: 23 obese (all of them(OA) [BMI  $\geq$  30 kg/m<sup>2</sup>] and 14 non-obese asthma (NOA) patients [BMI < 25 kg/m<sup>2</sup>]. Fifteen obese (O) patients and 19 age- and gender-matched healthy control (HC) individuals were also recruited. Twenty-one O (10 OA and 11 O) were evaluated at baseline (V1) and six months after undergoing BS (V2). Obese patients were recruited from the Obesity Unit of our Institution. According to the standard procedure the patients were supplemented with VitD after BS.

The following criteria were used to select asthmatic patients: (1) a clinical history of asthma; and 2) either bronchodilator responsiveness (>12 % and 200 ml improvement in FEV<sub>1</sub> after 180 µg metered-dose inhaler salbutamol) or airway hyperresponsiveness (PC20 methacholine < 8 mg/ml). Forced spirometry and methacholine test were performed according to the ERS/ATS standards [34,35]. Reference values were those of Roca et al. [36,37]. None of the subjects had received systemic CS for one month or longer prior to evaluation. None of the subjects were current smokers. All of them were nonsmokers or ex-smokers for more than one year with tobacco history <10 packs/years.

Serum was obtained by peripheral venipuncture followed by centrifugation and stored at  $-80^{\circ}\text{C}$  until analysis. Serum adiponectin and leptin levels were quantified by Luminex® multiplex immunoassay using Human ProcartaPlex Mix&Match kits (Thermo Fisher Scientific, Vienna, Austria) on a Luminex 200 analyser. Serum 25(OH)D concentrations were determined by a chemiluminescent immunoassay (Atellica™, Siemens).

The study was performed with written informed consent from participating subjects and approved by the Ethics Committee of Hospital Clínic of Barcelona (2018/4015).

### **Blood collection and PBMC isolation**

Based on previous studies, we used PBMC proliferation to investigate CS response [38]. Ten ml of whole blood was collected from each participant into a vacutainer tube containing heparin. PBMCs were isolated using Lymphoprep™ (Palex Medical) following manufacturer's instructions. Cell viability was determined using Trypan blue staining and final cell concentration was  $10 \times 10^6$  cells/ml.

### **Proliferation assay**

#### *CFSE dilution*

Five hundred  $\mu\text{l}$  of cell suspension were incubated with  $5 \mu\text{M}$  carboxyfluorescein diacetate succinimidyl ester (CFSE) for 5 min in the dark at room temperature. PBMCs were resuspended in X-Vivo15 medium (Cultek) containing gentamycin and supplemented with 10% charcoal stripped foetal bovine serum (FBS) (Sigma-Aldrich) at a final concentration of  $2 \times 10^6$  cells/ml.

#### *Cell culture conditions*

To assess cell proliferation suppression by DEX,  $500 \mu\text{l}$  of labelled PBMCs were stimulated with  $1 \mu\text{g/ml}$  of phytohemagglutinin-L (PHA-L) (Sigma-Aldrich) in the presence of a range of DEX (Kern Pharma) concentrations ( $10^{-11}$  –  $10^{-9}\text{M}$ ), as well as in the presence or absence of  $1,25(\text{OH})_2\text{D}$  at  $10^{-7}\text{M}$ . Cultures were incubated at  $37^{\circ}\text{C}$  and 5%  $\text{CO}_2$  for four days and the cell proliferation assessed based on CFSE dilution.

#### *Flow cytometer analysis*

PBMCs were transferred to flow cytometry round-bottom tubes and stained with Zombie Violet™ TM Fixable Viability Kit (BioLegend) and anti-CD4 APC antibody (BioLegend). Cells were analysed on a FACSCanto II flow cytometer (BD Biosciences) and % proliferation was recorded for PBMCs and CD4+

T cells, strategically gated. Sensitivity to DEX was expressed as the half maximal inhibitory concentration (IC<sub>50</sub>).

### **Analysis of gene expression by reverse transcription quantitative PCR (RT-qPCR)**

Total RNA was isolated from PBMCs cultured (24 h) in the presence or absence of DEX at 10<sup>-9</sup> M and with or without 1,25(OH)<sub>2</sub>D at 10<sup>-7</sup> M, using the TRIzol (Life Technologies) reagent according to the manufacturer's protocol. RNA was converted into cDNA using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher).

MKP-1 mRNA expression was analysed by real-time qPCR using TaqMan probes in the Viiia7 Real-Time PCR system (Applied Biosystems). The thermal cycler was set to 95°C for 20 min followed by 40 reaction cycles of 1 s at 95°C and 20 s at 60°C. All PCRs were run in duplicate for both target genes and two control genes (B2M, HPRT1).

### **Statistical analysis**

Clinical and experimental data were reported as median and interquartile range. Differences between two groups were analysed using non-parametric tests: Mann-Whitney U test (unpaired data), Wilcoxon rank test (paired data), or Kruskal-Wallis H test for multiple comparisons. Correlation coefficients were calculated using Spearman's Rank method. All analyses were performed using GraphPad Prism version 8.4 for Windows, (GraphPad Software, La Jolla California, USA). Statistical significance was defined as P-value < 0.05.

## **Results**

### **Subjects' characteristics at baseline**

The participants' demographic and clinical characteristics are shown in Table 1. The level of severity of asthma (mild, moderate, severe) was established according to the pharmacological treatment used to control the disease [1,3]. The level of control was assessed using the asthma control test (ACT) [1,3]. OA patients presented reduced FVC and FEV<sub>1</sub>, compared with HC, whereas the FEV<sub>1</sub>/FVC ratio was significantly lower in NOA and OA participants in comparison with O and HC individuals. Serum 25(OH)D levels showed a tendency to be lower in O subjects than in HC subjects, however the difference did not reach statistical significance. The adiponectin/leptin ratio was significantly reduced in O and OA patients, compared with both HC and NOA individuals.

### **Relationship between obesity and CS sensitivity**

To examine the link between obesity and CS response, DEX IC<sub>50</sub> values were calculated in PBMCs and CD4+ T cells. IC<sub>50</sub> values were significantly higher in OA patients compared with NOA patients when analysing both PBMCs and CD4+ T cells (Figure 1).

There were no differences in CS sensitivity between OA and O subjects. IC<sub>50</sub> values were higher in O subjects with respect to the HC group. However, not all differences were statistically significant, which in part was dependent on the type of cells selected for the analysis (PBMCs vs. CD4+T cells) (Figure 1).

### **Relationship of adipokines and VitD with CS response**

Analysis of the adiponectin/leptin ratio and CD4+ T cell sensitivity to CS *in vitro* showed a negative correlation between the adiponectin/leptin ratio and the DEX IC<sub>50</sub> value ( $r = -0.4113$ ,  $p = 0.0008$ ). The higher the ratio, the greater the antiproliferative effect of DEX.

Moreover, we studied the association between serum 25(OH)D concentrations and DEX response *in vitro*. We observed a negative correlation between 25(OH)D concentrations and DEX IC<sub>50</sub> values ( $r = -0.2512$ ,  $p = 0.0346$ ). The higher the VitD concentration, the greater the antiproliferative effects of DEX. There was no correlation between adiponectin/leptin and 25(OH)D (data not shown). Similar results were found in PBMCs (data not shown).

If patients were separated according to the reference values established for serum 25(OH)D levels, a significant decrease in IC<sub>50</sub> values was observed only in patients who presented VitD deficiency levels (< 12 ng/ml) ( $n = 8$ , 4 OA and 4 O patients), compared with patients with sufficiency (>20 ng/ml) and, also with insufficiency levels (12 – 20 ng/ml) (Figure 2) [15].

### **Effects of weight loss after bariatric surgery**

BS was performed in 21 subjects (10 OA and 11 O), in 8 of whom a sleeve gastrectomy was performed (38%), while in the remaining 13 (62%) a Roux-en-Y gastric bypass was carried out. Table 2 presents the clinical, functional, and inflammatory marker changes after BS. BMI was significantly reduced in both groups of subjects with obesity. Weight loss was associated with a significant improvement in the ACT scores and a reduction in the amount of therapy needed to control the disease. Lung function tests improved after BS and the adiponectin/leptin ratio increased significantly after BS in O and OA patients.

Serum 25(OH)D levels increased in both groups with obesity, with no subjects with deficient (<12 ng/ml) reference values, reflecting the effect of both BS and VitD supplementation.

### **Weight loss and CS sensitivity**

To evaluate the impact of weight loss on CS sensitivity we compared the DEX IC<sub>50</sub> in both groups with obesity before (V1) and after BS (V2) (Figure 3). DEX IC<sub>50</sub> values diminished in both PBMCs and CD4+ T cells after BS in OA and O patients. However, the reduction was not statistically significant in CD4+ T cells from O subjects.

### **Effect of 1,25(OH)<sub>2</sub>D on CD4+ T cell proliferation *in vitro***

The addition of 1,25(OH)<sub>2</sub>D to culture media resulted in a significant reduction of PHA-stimulated CD4+ T cell proliferation without any statistically significant differences between the four groups. We found similar results in PBMCs (data not shown) (Figure 4).

### **Impact of 1,25(OH)<sub>2</sub>D on CS sensitivity**

To further assess the effects of VitD on the inhibitory ability of DEX, PBMCs were incubated in the presence of both DEX and 1,25(OH)<sub>2</sub>D. 1,25(OH)<sub>2</sub>D significantly increases the antiproliferative effects of DEX in CD4+ T cells. Although the effect appears to be greater in OA and O patients than in HC and NOA subjects, the difference was not statistically significant (Figure 5). We found the same results in PBMCs (data not shown).

### **MKP-1 gene expression**

We analysed the expression of MKP-1 in PBMCs after stimulation with DEX for 24 h. As expected, DEX induced MKP-1 mRNA expression in PBMCs, which correlated with DEX sensitivity in PBMCs expressed as IC<sub>50</sub> value ( $r=0.4860$ ,  $p=0.0075$ ). However, no differences were found between groups. We also examined the expression of MKP-1 in response to VitD and DEX. MKP-1 expression increased similarly in the four groups (data not shown). However, there were statistically significant differences in the induced expression of MKP-1 when 1,25(OH)<sub>2</sub>D was added to DEX in subjects with obesity (Figure 6).

### **Discussion**

Our study demonstrates that obese subjects with and without asthma are characterized by a reduced response to CS treatment, assessed by means of a PBMC proliferative assay.

The reduced response negatively correlates with serum adiponectin/leptin ratio, a marker associated with the level of inflammation in subjects with obesity. A similar negative correlation was observed between the antiproliferative effects of CS with VitD serum levels. Obese patients with VitD deficiency levels (<12 ng/ml) were those with the poorest antiproliferative response to CS.

Weight loss after BS was associated with a marked improvement in the reduced antiproliferative effects of CS in subjects with obesity. Weight loss was also associated with an increase in both the adiponectin/leptin ratio and serum VitD levels. After BS and VitD supplementation, there were no patients with obesity with VitD deficiency levels (<12 ng/ml). These observations suggest that reduction of obesity-related systemic inflammation and increased levels of serum VitD can account for the recovered ability of CS to inhibit PBMCs proliferation.

However, it is well known that association does not necessarily mean a causal relationship. To further investigate the potential causal relationship between VitD deficiency and CS hyporesponsiveness, we conducted an *in vitro* study to assess the effects of VitD on cell proliferation and found that the hormone exerts antiproliferative effects on cells by itself and that it is also capable of potentiating the antiproliferative effects of CS. Collectively these findings support the notion that VitD may play a role in the complex interaction between obesity, inflammation, and CS hyporesponsiveness.

In order to definitely establish the role of VitD deficiency in CS hyporesponsiveness in asthma, randomized clinical trials (RCTs) should be performed to evaluate the clinical efficacy of VitD administration in OA. Numerous RCTs studies, mostly in children, have been carried out to evaluate the efficacy of VitD administration in asthma patients. The trials enrolled patients with significant differences in the selection of participants, baseline VitD levels and dosage of VitD supplementation. Some systematic reviews of RCTs concluded that high-dose VitD may be effective in paediatric asthma [39]; however, others could not find significant evidence favouring the use of this complementary therapy in children and adults [40].

VitD supplementation studies in asthma face many unresolved issues. Thus, for example, studies on the anti-inflammatory and anti-oxidant effects of VitD suggest that the serum level of VitD that should be reached to be effective in asthma may be higher than that generally accepted for bone health [41]. In addition, it is unknown whether, due to the effect of excessive accumulation of fat on the distribution

of VitD, OA patients should require a supplemental VitD dose higher than that required by NOA patients with VitD deficiency [41]. RCT studies tailored for this specific group of overweight/obese asthma patients are needed to elucidate the potential therapeutic effect of VitD supplementation that our findings suggest.

The present study also aimed to investigate the mechanisms responsible for CS hyporesponsiveness in asthmatic patients with obesity. Despite some progress made in the field, the mechanisms involved in CS insensitivity in patients with asthma remain to be fully understood [9]. Anti-inflammatory actions of CS are mediated by the glucocorticoid receptor alpha (GR $\alpha$ ), which suppresses inflammation through transactivation and transrepression mechanisms [9]. In the presence of CS, GR $\alpha$  translocates into the nucleus to regulate the expression of various anti-inflammatory genes (transactivation) such as MKP-1 [9]. In our study, we examined this gene as previous studies found that the induced expression of MKP-1 by DEX is blunted in overweight/obese asthma patients compared with lean asthma subjects [11]. Interestingly, obesity in non-asthmatic subjects had no effect on MKP-1 expression, suggesting that, due to unclear reasons, the effect of obesity only impacts on MKP-1 expression in asthma patients [11]. In our study we did not find any significant difference in the DEX-dependent induction of MKP-1 in OA versus NOA. This finding suggests that obesity does not affect the capacity of CS to transactivate anti-inflammatory genes in PBMCs. Differences in methods and the characteristics of the populations studied might explain discrepancies between our study and that of Sutherland *et al.* [11]. In that study, the induction of MKP-1 was analysed against a single dose of DEX ( $10^{-6}$  M), while in our study it was analysed by means of a dose-dependent response, which is considered most suitable for evaluating pharmacologically-induced responses *in vitro*. Moreover, asthma severity has been shown to be closely related to the level of DEX-induced MKP-1 expression [38], and most patients enrolled in our study suffered from mild/moderate disease. In the Sutherland study [11], pulmonary function showed a more pronounced airway obstruction (FEV<sub>1</sub>) in OA than in the present study (70% vs. 77%).

CS anti-inflammatory effects are explained by mechanisms other than activation of MKP-1, such as alterations in the nuclear translocation of GR $\alpha$ , increased expression of the GR $\beta$  isoform—a dominant negative regulator of active GR $\alpha$ —or inhibition of proinflammatory gene expression through blockade of proinflammatory transcription factors [9]. Interestingly, a very recent study found that dysregulation of the GR $\alpha$ /GR $\beta$  isoform ratio may contribute to CS hyporesponsiveness in OA [42].

We also examined the effects of VitD on the induction of MKP-1 in subjects with and without obesity, finding that the hormone similarly increases the expression of MKP-1, with some differences between obese and non-obese subjects, a finding that suggests that the efficacy of VitD to increase DEX-induction of anti-inflammatory genes is more pronounced in subjects with obesity.

As a complementary methodological contribution, we used both PBMCs and CD4+T cell proliferation to assess sensitivity to DEX treatment. The two methods have been used to examine CS sensitivity in previous studies [21,38,43]. We found some differences in the results obtained with PHA-induced PBMC and CD4+T cell proliferation in the response to CS, a finding that should be taken into account when results from different studies are compared.

Some of the limitations of the study include the relatively small sample size of patients examined and the short follow-up time of six months after BS. Patients were enrolled from the obesity program of our centre and, in all cases, BS was indicated to treat obesity, not asthma.

In summary, our study has several novel contributions that may help to better understand the links between obesity and poor response to ICS in adults with OA. Our findings support the role of obesity in ICS hyporesponsiveness and demonstrate that the efficacy of weight loss to improve asthma symptoms and lung function can be, at least in part, due to the recovered anti-inflammatory response to CS. Finally, our results suggest that VitD supplementation in asthma patients with obesity and VitD deficiency may contribute to achieving asthma control by improving ICS efficacy.

**Funding**

The study was supported by grants from the Fundació Respira (SEPAR) 167/2016 and 736/2018, Fundació Catalana de Pneumologia (FUCAP) and by an unrestricted grant from Menarini.

**Conflict of interest**

J. Mullol is a member of national or international advisory boards and has received speaker fees or funding for clinical trials and research projects from Allakos, AstraZeneca, Genentech, GSK, Glenmark, Menarini, Mitsubishi-Tanabe, MSD, Mylan-MEDA Pharma (Viatris), Novartis, Procter & Gamble, Regeneron Pharmaceuticals, Inc., Sanofi-Genzyme, UCB Pharma, and Uriach Group. Other authors declare that they have no conflicts of interest.

## References

1. Papi A, Brightling C, Pedersen SE, Reddel HK. Asthma. *Lancet*. 2018;391:783–800.
2. Hammad H, Lambrecht BN. The basic immunology of asthma. *Cell*. 2021;184:1469–85.
3. Reddel HK, Bacharier LB, Bateman ED, Brightling CE, Brusselle GG, Buhl R, et al. Global Initiative for Asthma Strategy 2021 Executive Summary and Rationale for Key Changes. *Am J Respir Crit Care Med*. 2022;205:17–35.
4. Boulet LP, Franssen E. Influence of obesity on response to fluticasone with or without salmeterol in moderate asthma. *Respir Med*. 2007;101:2240–7.
5. Forno E, Lescher R, Strunk R, Weiss S, Fuhlbrigge A, Celedón JC. Decreased response to inhaled steroids in overweight and obese asthmatic children. *J Allergy Clin Immunol*. 2011;127:741–9.
6. Farah CS, Kermode JA, Downie SR, Brown NJ, Hardaker KM, Berend N, et al. Obesity is a determinant of asthma control independent of inflammation and lung mechanics. *Chest*. 2011;140:659–66.
7. Arismendi E, Bantulà M, Perpiñá M, Picado C. Effects of Obesity and Asthma on Lung Function and Airway Dysanapsis in Adults and Children. *J Clin Med*. 2020;9:3762.
8. Bantulà Fonts M, Roca-Ferrer J, Arismendi E, Picado C. Asthma and Obesity: Two Diseases on the Rise and Bridged by Inflammation. *J Clin Med*. 2021;10:1–21.
9. Barnes PJ. Corticosteroid resistance in patients with asthma and chronic obstructive pulmonary disease. *J Allergy Clin Immunol*. 2013;131:636–45.
10. Sideleva O, Suratt BT, Black KE, Tharp WG, Pratley RE, Forgione P, et al. Obesity and asthma: An inflammatory disease of adipose tissue not the airway. *Am J Respir Crit Care Med*. 2012;186:598–605.
11. Sutherland ER, Goleva E, Strand M, Beuther DA, Leung DYM. Body mass and glucocorticoid response in asthma. *Am J Respir Crit Care Med*. 2008;178:682–7.
12. Bouillon R, Marcocci C, Carmeliet G, Bikle D, White JH, Dawson-Hughes B, et al. Skeletal and Extraskkeletal Actions of Vitamin D: Current Evidence and Outstanding Questions. *Endocr Rev*. 2019;40:1109.

13. Dankers W, Colin EM, van Hamburg JP, Lubberts E. Vitamin D in Autoimmunity: Molecular Mechanisms and Therapeutic Potential. *Front Immunol.* 2017;7:697.
14. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: An endocrine society clinical practice guideline. *J Clin Endocrinol Metab.* 2011;96:1911–30.
15. Giustina A, Adler RA, Binkley N, Bollerslev J, Bouillon R, Dawson-Hughes B, et al. Consensus statement from 2nd International Conference on Controversies in Vitamin D. *Rev Endocr Metab Disord.* 2020;21:89–116.
16. Cashman KD. Vitamin D Deficiency: Defining, Prevalence, Causes, and Strategies of Addressing. *Calcif Tissue Int.* 2020;106:14–29.
17. Afzal S, Brøndum-Jacobsen P, Bojesen SE, Nordestgaard BG. Vitamin D concentration, obesity, and risk of diabetes: a mendelian randomisation study. *Lancet Diabetes Endocrinol.* 2014;2:298–306.
18. Vimalaswaran KS, Berry DJ, Lu C, Tikkanen E, Pilz S, Hiraki LT, et al. Causal relationship between obesity and vitamin D status: bi-directional Mendelian randomization analysis of multiple cohorts. *PLoS Med.* 2013;10:e1001383.
19. Drincic AT, Armas LAG, Van Diest EE, Heaney RP. Volumetric dilution, rather than sequestration best explains the low vitamin D status of obesity. *Obesity (Silver Spring).* 2012;20:1444–8.
20. González-Molero I, Rojo-Martínez G, Morcillo S, Gutierrez C, Rubio E, Pérez-Valero V, et al. Hypovitaminosis D and incidence of obesity: a prospective study. *Eur J Clin Nutr.* 2013;67:680–2.
21. Szentpetery SE, Han YY, Brehm JM, Acosta-Pérez E, Forno E, Boutaoui N, et al. Vitamin D insufficiency, plasma cytokines, and severe asthma exacerbations in school-aged children. *J Allergy Clin Immunol Pract.* 2018;6:289-91.
22. Sutherland ER, Goleva E, Jackson LP, Stevens AD, Leung DYM. Vitamin D Levels, Lung Function, and Steroid Response in Adult Asthma. *Am J Respir Crit Care Med.* 2010;181:699–704.

23. Devereux G, Wilson A, Avenell A, McNeill G, Fraser WD. A case-control study of vitamin D status and asthma in adults. *Allergy*. 2010;65:666–7.
24. Searing DA, Zhang Y, Murphy JR, Hauk PJ, Goleva E, Leung DYM. Decreased Serum Vitamin D Levels in Children with Asthma are Associated with Increased Corticosteroid Usage. *J Allergy Clin Immunol*. 2010;125:995.
25. Zhang Y, Leung DYM, Goleva E. Anti-inflammatory and Corticosteroid Enhancing Actions of Vitamin D in the Monocytes of Steroid Resistant and Steroid Sensitive Asthmatics. *J Allergy Clin Immunol*. 2014;133:1744.
26. Dias ASO, Santos ICL, Delphim L, Fernandes G, Endlich LR, Cafasso MOSD, et al. Serum leptin levels correlate negatively with the capacity of vitamin D to modulate the in vitro cytokines production by CD4 + T cells in asthmatic patients. *Clin Immunol*. 2019;205:93–105.
27. Arismendi E, Rivas E, Agustí A, Ríos J, Barreiro E, Vidal J, et al. The Systemic Inflammome of Severe Obesity before and after Bariatric Surgery. Thatcher TH, editor. *PLoS One*. 2014;9:e107859.
28. Van Huisstede A, Rudolphus A, Cabezas MC, Biter LU, Van De Geijn GJ, Taube C, et al. Effect of bariatric surgery on asthma control, lung function and bronchial and systemic inflammation in morbidly obese subjects with asthma. *Thorax*. 2015;70:659–67.
29. Maniscalco M, Zedda A, Faraone S, Cerbone MR, Cristiano S, Giardiello C, et al. Weight loss and asthma control in severely obese asthmatic females. *Respir Med*. 2008;102:102–8.
30. Reddy RC, Baptist AP, Fan Z, Carlin AM, Birkmeyer NJO. The effects of bariatric surgery on asthma severity. *Obes Surg*. 2011;21:200–6.
31. van Huisstede A, Rudolphus A, van Schadewijk A, Cabezas MC, Mannaerts GHH, Taube C, et al. Bronchial and Systemic Inflammation in Morbidly Obese Subjects with Asthma: A Biopsy Study. *Am J Respir Crit Care Med*. 2014;190:951–4.
32. Dixon AE, Pratley RE, Forgione PM, Kaminsky DA, Whittaker-Leclair LA, Griffes LA, et al. Effects of obesity and bariatric surgery on airway hyperresponsiveness, asthma control, and inflammation. *J Allergy Clin Immunol*. 2011;128:508-15.
33. Boulet LP, Turcotte H, Martin J, Poirier P. Effect of bariatric surgery on airway response and lung function in obese subjects with asthma. *Respir Med*. 2012;106:651–60.

34. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. *Eur Respir J*. 2005;26:319–38.
35. Coates AL, Wanger J, Cockcroft DW, Culver BH, Carlsen KH, Diamant Z, et al. ERS technical standard on bronchial challenge testing: General considerations and performance of methacholine challenge tests. *Eur Respir J*. 2017;49:1–17.
36. Roca J, Burgos F, Barberà JA, Sunyer J, Rodriguez-Roisin R, Castellsagué J, et al. Prediction equations for plethysmographic lung volumes. *Respir Med*. 1998;92:454–60.
37. Roca J, Burgos F, Sunyer J, Saez M, Chinn S, Antó JM, et al. Reference values for forced spirometry. *Eur Respir J*. 1998;11:1354–62.
38. Goleva E, Jackson LP, Gleason M, Leung DYM. Usefulness of PBMcs to predict clinical response to corticosteroids in asthmatic patients. *J Allergy Clin Immunol*. 2012;129:687-93.
39. Martineau AR, Cates CJ, Urashima M, Jensen M, Griffiths AP, Nurmatov U, et al. Vitamin D for the management of asthma. *Cochrane Database Syst Rev*. 2016;9:CD11511.
40. Kumar J, Kumar P, Goyal JP, Thakur C, Choudhary P, Meena J, et al. Vitamin D supplementation in childhood asthma: a systematic review and meta-analysis of randomised controlled trials. *ERJ Open Res*. 2022;8:662.
41. O'Sullivan BP, James L, Majure JM, Bickel S, Phan LT, Serrano Gonzalez M, et al. Obesity-related asthma in children: A role for vitamin D. *Pediatr Pulmonol*. 2021;56:354–61.
42. Al Heialy S, Gaudet M, Ramakrishnan RK, Mogas A, Salameh L, Mahboub B, et al. Contribution of IL-17 in Steroid Hyporesponsiveness in Obese Asthmatics Through Dysregulation of Glucocorticoid Receptors  $\alpha$  and  $\beta$ . *Front Immunol*. 2020;11:1724.
43. Koetzier SC, Van Langelaar J, Blok KM, Van Den Bosch TPP, Wierenga-Wolf AF, Melief M-J, et al. Brain-homing CD4 + T cells display glucocorticoid-resistant features in MS. *Neurol Neuroimmunol Neuroinflamm*. 2020;7:894.

## TABLES

Table 1. Baseline demographic and clinical data of the study population.

	HC (n, 19)	NOA (n, 14)	OA (n, 23)	O (n, 15)
Age, years	43 (34-56)	53 (43-60)	56 (51-59)	47 (45-60)
Female, n (%)	14 (74)	11 (79)	18 (78)	13 (87)
BMI, kg/m <sup>2</sup>	23.7 (22.2-25.4)	23.2 (22.3-25.3)	37.6 (34.5-45) **	42.7 (39.5-48.5) **
Mild asthma, n (%)	-	0 (0)	4 (17.4)	-
Moderate asthma, n (%)	-	5 (35.7)	5 (21.7)	-
Severe asthma, n (%)	-	9 (64.3)	14 (60.9)	-
FVC, % pred	101.0 (95.0-107.0)	97.5 (89.7-107.3)	84.0 (73.0-93.0) **	93.0 (82.7-96.2)
FEV <sub>1</sub> , % pred	102.0 (95.0-109.0)	80.0 (75.2-100.3)	80.0 (62.0-95.0) *	90.0 (86.2-101.0)
FEV <sub>1</sub> /FVC	81.0 (76.0-84.0)	65.5 (57.0-75.0) *	77.0 (69.0-82.0)	81.5 (75.0-83.0) #
ICS §, n (%)	-	13 (92.9)	19 (82.6)	-
Eosinophils, %	2.3 (1.6-3.3) #	4.7 (3.6-7.1)	3.5 (2.4-5)	2.3 (3.4-1.2) #
IgE, kU/L	26.9 (16.5-80.2)	122 (34.8-391)	67.1 (19.43-278.8)	38 (15.43-107.5)
25(OH)D, ng/ml	20.8 (17.5-28.8)	20.1 (14.1-24.9)	20.0 (14.7-31.9)	16.4 (11.7-27.5)
Adiponectin, µg/ml	11.9 (5.3-16.0)	12.3 (7.7-21.8)	5.8 (3.6-8.4) #	4.3 (4.0-9.0) #
Leptin, ng/ml	1.7 (1.0-2.5)	3.4 (2.2-4.8)	3.6 (1.8-6.1) *	4.1 (2.7-6.3) *
Adipo/Lept	6.7 (2.1-8.6)	4.6 (1.9-6.5)	1.3 (0.9-2.9) **	1.1 (0.7-2.7) **

Data are presented as median (interquartile range). HC, healthy controls; NOA, non-obese asthmatic patients; OA, obese asthmatic patients; O, obese patients; BMI, body mass index; FVC, forced vital capacity; FEV<sub>1</sub>, forced expiratory volume in 1 s; ICS, inhaled corticosteroids; IgE, immunoglobulin E; 25(OH)D, 25-hydroxyvitamin D; Adipo/Lept, adiponectin/leptin ratio. \*P < 0.05, compared with HC; # P < 0.05, compared with NOA subjects; Kruskal-Wallis followed by Dunn's multiple comparisons test. § For NOA and OA patients who received ICS, the mean ± SD of the ICS dose in budesonide equivalents was 557.1 ± 256.6 and 1222.1 ± 862.9 µg/day, respectively.

**Table 2. Demographic and clinical data of asthmatic and non-asthmatic obese patients before (V1) and six months after (V2) BS.**

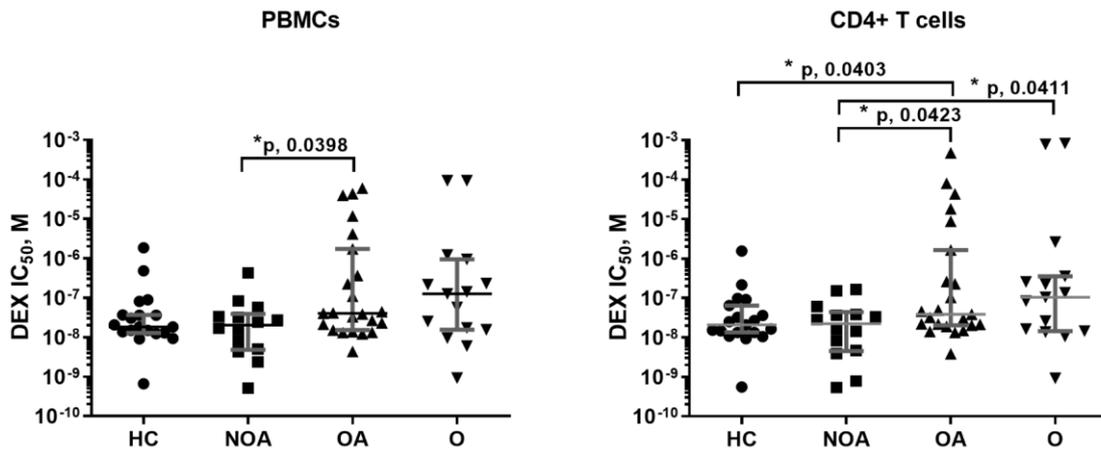
	Obese asthmatic patients (n, 10)		Obese patients (n, 11)	
	V1	V2	V1	V2
<b>Female, n (%)</b>	10 (100)	10 (100)	10 (91)	10 (91)
<b>BMI, kg/m<sup>2</sup></b>	44.1 (38.7-47.1)	30.0 (26.4-34.9) *	41.9 (37.3-45.7)	29.7 (27.4-34.6) #
<b>Mild asthma, n (%)</b>	4 (40)	9 (90)	-	-
<b>Moderate asthma, n (%)</b>	4 (40)	1 (10)	-	-
<b>Severe asthma, n (%)</b>	2 (20)	0 (0)	-	-
<b>ACT</b>	18 (18-24)	25 (25-25) *	-	-
<b>FVC, % pred</b>	85.5 (77.5-96.0)	95.0 (88.3-106.0)	94.5 (85.3-98.0)	99.0 (91.0-106.0)
<b>FEV<sub>1</sub>, % pred</b>	89.0 (79.5-97.3)	96.5 (92.5-104.8)	93.5 (87.0-101.0)	101.0 (89.0-109.0) #
<b>FEV<sub>1</sub>/FVC</b>	80.5 (76.5-82.5)	81.5 (77.5-83.0)	81.0 (75.0-83.0)	79.0 (76.0-83.0)
<b>ICS §, n (%)</b>	6 (60)	2 (20)	-	-
<b>Eosinophils, %</b>	3.0 (2.0-3.7)	2.6 (1.4-3.3)	2.8 (1.6-3.6)	2.5 (1.8-2.8)
<b>IgE, kU/L</b>	32.3 (12.4-113.3)	33.8 (7.6-52.1)	51.7 (16.8-115)	28.3 (14.4-62.8)
<b>25(OH)D, ng/ml</b>	25.2 (11.9-43.2)	31.7 (20.6-38.3)	18.8 (10.1-30.6)	37.7 (23.2-41.8) #
<b>Adiponectin, µg/ml</b>	7.0 (3.5-9.5)	9.4 (4.8-20.6) *	5.6 (4.0-10.6)	9.8 (6.0-12.1)
<b>Leptin, ng/ml</b>	5.0 (3.2-8.3)	2.3 (0.8-4.7)	4.3 (3.3-7.2)	2.5 (1.3-3.4) #
<b>Adipo/Lept</b>	1.2 (0.7-2.0)	4.7 (1.2-43.2) *	1.1 (0.7-2.7)	4.2 (2.7-6.9) #

Data are presented as median (interquartile range). ACT, asthma control test; BMI, body mass index; FVC, forced vital capacity; FEV<sub>1</sub>, forced expiratory volume in 1 s; ICS, inhaled corticosteroids; IgE, immunoglobulin E; 25(OH)D, 25-hydroxyvitamin D<sub>3</sub>; Adipo/Lept, adiponectin/leptin ratio. \*P < 0.05, compared between V1 and V2 in obese asthmatic group, # P < 0.05 compared between V1 and V2 in obese group; Wilcoxon test. § For OA patients before and after bariatric surgery who received ICS, the mean ± SD of the ICS dose in budesonide equivalents was 643.3 ± 496.1 and 150.0 ± 70.7 µg/day, respectively.

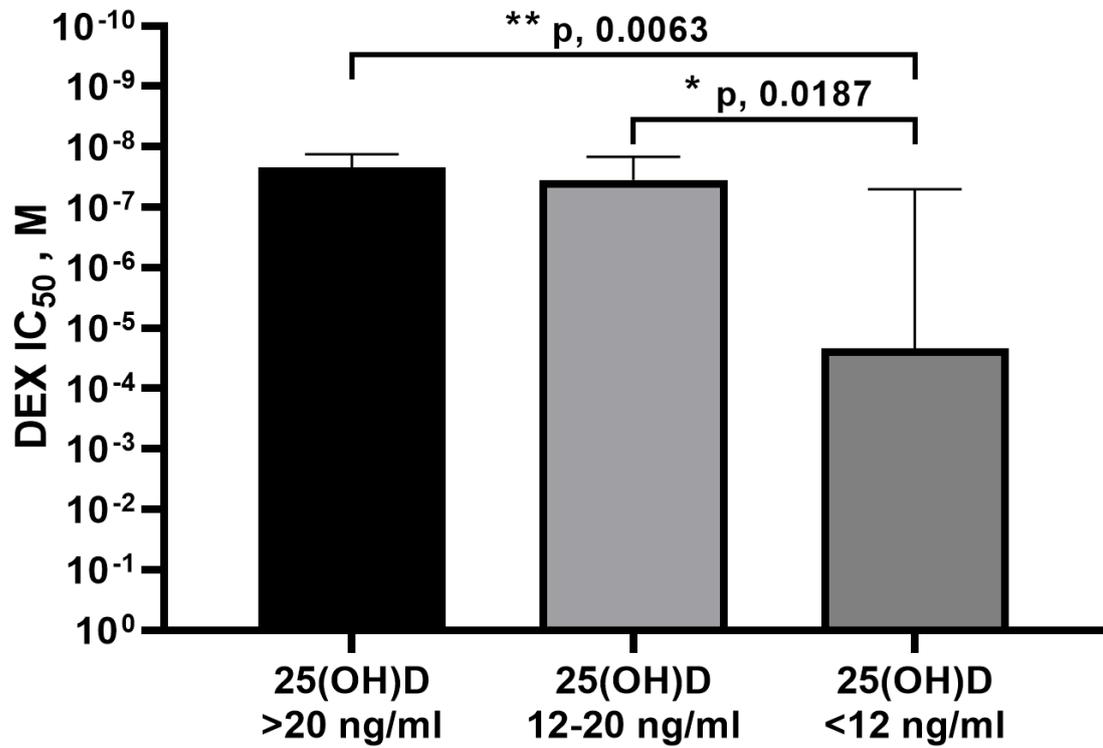
## FIGURES

**Figure 1. DEX IC<sub>50</sub> values from HC, NOA, OA, and O participants in both PBMCs and CD4+ T cells.**

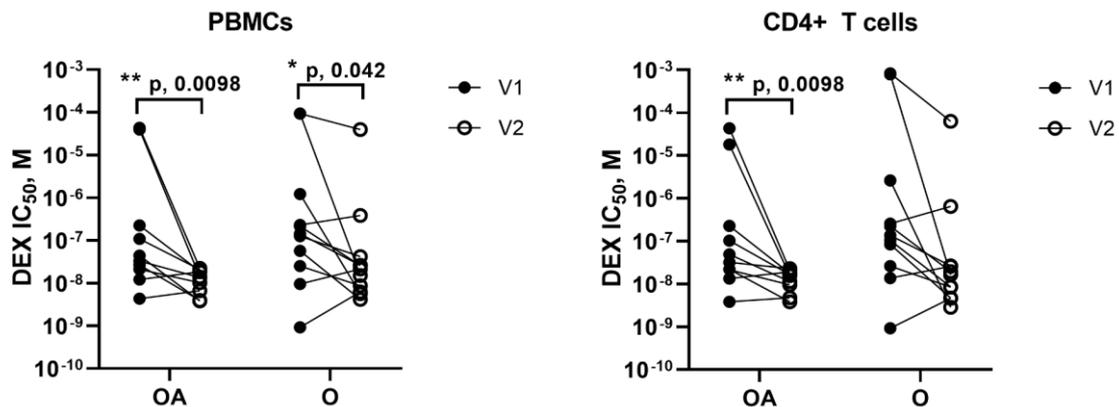
Data are presented as median with interquartile range. PBMCs, peripheral blood mononuclear cells; DEX IC<sub>50</sub>, dexamethasone half maximal inhibitory concentration; HC, healthy controls; NOA, non-obese asthmatics; OA, obese asthmatics; O, obese. \*P < 0.05, Mann-Whitney test.



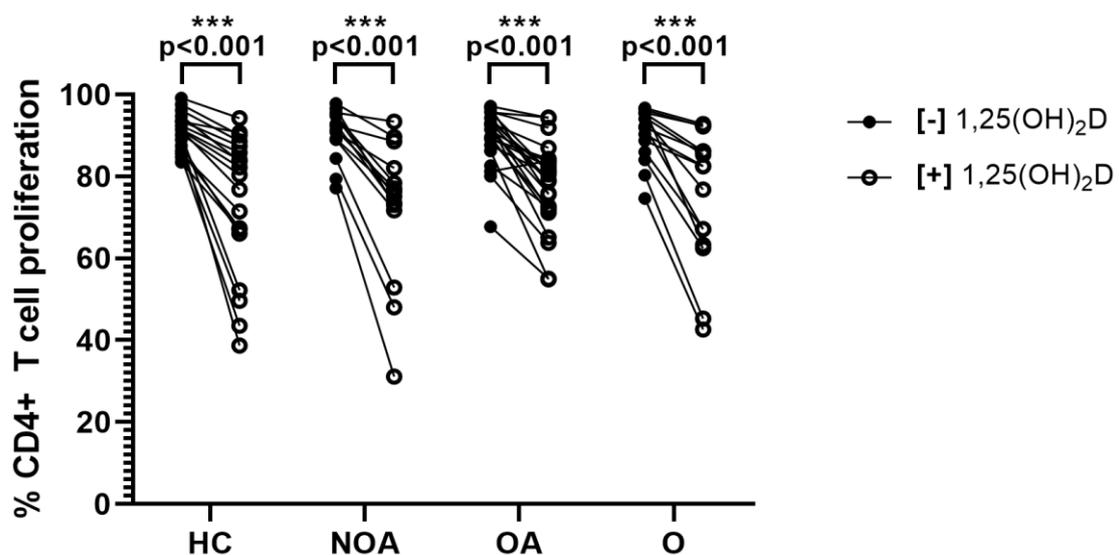
**Figure 2. Patients classified accordingly to 25(OH)D serum levels and *in vitro* CS response.** DEX IC<sub>50</sub>, dexamethasone half maximal inhibitory concentration; 25(OH)D, 25-hydroxyvitamin D. \*\*\*P < 0.001; Mann-Whitney test.



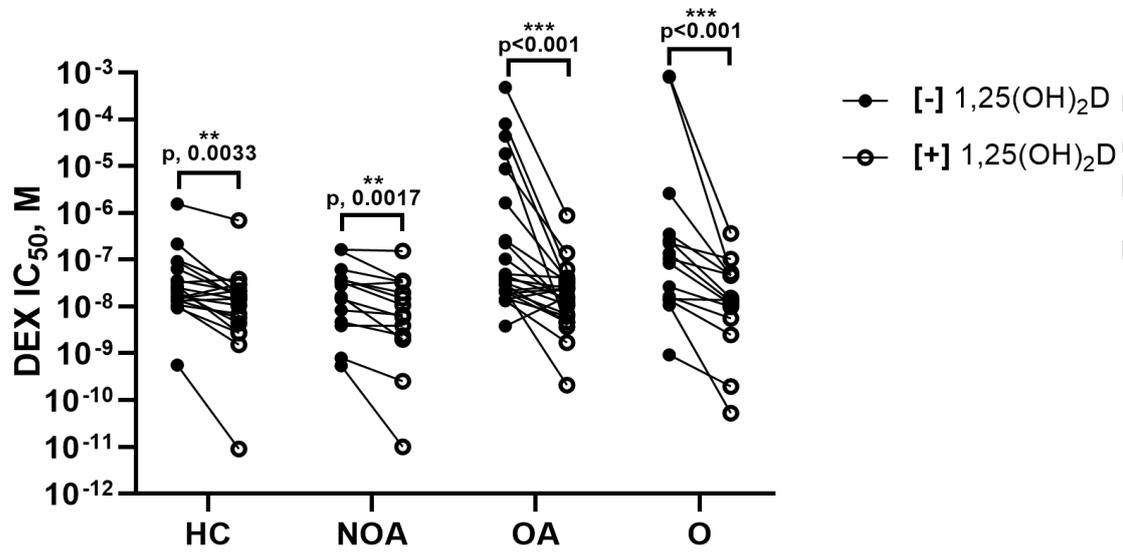
**Figure 3. Comparison between DEX IC<sub>50</sub> values from OA and O groups before (V1) and after (V2) BS in both PBMCs and CD4+ T cells.** PBMCs, peripheral blood mononuclear cells; DEX IC<sub>50</sub>, dexamethasone half maximal inhibitory concentration; OA, obese asthmatic; O, obese. \*P < 0.05, \*\*P < 0.01; Wilcoxon test between V1 and V2.



**Figure 4. Inhibition of CD4+ T cell proliferation by 1,25(OH)<sub>2</sub>D in HC, NOA, OA, and O participants.** Solid circles represent cells without treatment and open circles represent cells treated *in vitro* with 1,25(OH)<sub>2</sub>D 1x10<sup>-7</sup> M. 1,25(OH)<sub>2</sub>D, 1,25-dihydroxyvitamin D; HC, healthy controls; NOA, non-obese asthmatics; OA, obese asthmatics; O, obese. \*\*\*P < 0.001; Wilcoxon test.



**Figure 5. Comparison between CS sensitivity in CD4+ T cells with the absence (solid circles) or presence (open circles) of 1,25(OH)<sub>2</sub>D.** 1,25(OH)<sub>2</sub>D, 1,25-dihydroxyvitamin D; HC, healthy controls; NOA, non-obese asthmatics; OA, obese asthmatics; O, obese. \*\*P < 0.01, \*\*\*P < 0.001; Wilcoxon test.



**Figure 6. DEX-induced MKP-1 expression with (chequered columns) and without (solid columns) 1,25(OH)<sub>2</sub>D comparison in non-obese and obese groups.** Data are presented as median with interquartile range. DEX, dexamethasone; 1,25(OH)<sub>2</sub>D, 1,25-dihydroxyvitamin D; non-O, non-obese group (healthy controls and non-obese asthmatic subjects); O, obese group (obese asthmatic and obese subjects). \*P < 0.05; \*\*\*P < 0.001; Wilcoxon test.

