ORIGINAL ARTICLE

Weight Loss and Vitamin D Improve Hyporesponsiveness to Corticosteroids in Obese Asthma

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■ **Abstract**

Background: Obesity negatively impacts on the response of asthma patients to inhaled corticosteroids. The mechanisms underlying this impact are unknown.

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

Methods: The study population comprised 23 obese asthma patients (OA) (18 females; median (IQR) age 56 [51-59] years), 14 nonobese asthma patients (NOA) (11 females; 53 [43-60] years), 15 obese patients (OP) (13 females; 47 [45-60] years), and 19 healthy controls (HC) (14 females; 43 [34-56] years). Ten OA and 11 OP were evaluated at baseline (V1) and 6 months after bariatric surgery (V2). Corticosteroid response was measured using dexamethasone-induced inhibition of peripheral blood mononuclear cell (PBMC) proliferation. Lung function and 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 OP and OA with respect to NOA and HC; this inversely correlated with the adiponectin/leptin ratio and vitamin D levels. Bariatric surgery improved corticosteroid responses in OP and OA and normalized the 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 *MKP1* expression in OP and OA.

Conclusions: The efficacy of weight loss to improve symptoms and lung function in OA may be due, at least in part, 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.

Summary box

- **What do we know about this topic?**

  Obese asthma patients present a reduced response to corticosteroids and poor asthma control compared with their nonobese counterparts. Vitamin D deficiency is related to asthma exacerbations and obesity. Weight loss induced by bariatric surgery improves systemic inflammation and asthma symptoms.

- **How does this study impact our current understanding and/or clinical management of this topic?**

  Obese participants with and without asthma showed a reduced response to corticosteroid treatment that inversely correlated with vitamin D serum levels. Weight loss through bariatric surgery and vitamin D supplementation significantly improve corticosteroid response in these patients.

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 2 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, contrasting 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, adherence to the prescribed ICS regimen is often poor in asthma patients, and the response to treatment is highly variable among adherent patients, ranging from those who respond to low doses of ICS to those who are resistant to even very high ICS doses. In both cases, the result is similar: increased risk of exacerbations, hospitalization, and mortality [1,3].

Multiple molecular mechanisms have been involved in hyporesponsiveness to corticosteroids. Obesity has been recognized as a risk factor for hyporesponsiveness to ICS in asthma patients, and asthma patients with obesity (OA) have reduced odds of achieving asthma control, higher risk of asthma hospitalizations, and poorer quality of life than asthma patients with a normal body mass index (BMI) [4-6]. A positive correlation has been found between high BMI and residual asthma symptoms that remained present in OA 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 airway inflammation have been proposed as potential mechanisms to explain hyporesponsiveness to ICS in OA [7,8]. Defective induction by corticosteroids of anti-inflammatory genes such as mitogen-activated protein kinase phosphatase-1 (*MKP1*) has also been reported to be involved [9].

In adults, increased weight is associated with a relatively small reduction in forced expiratory volume in 1 second (FEV_1) and in forced vital capacity (FVC), with the FEV_1/FVC ratio usually remaining unaltered or slightly increased and associated with a reduction in lung volumes, such as functional residual capacity. These alterations characterize the so-called restrictive ventilatory defect [7].

Concerning inflammation, the excessive accumulation of adipose tissue in individuals with obesity results in increased production of proinflammatory cytokines (IL-1β, IL-6, IFN-γ, TNF-α) and adipokines such as leptin, whereas release of adiponectin, a cytokine with anti-inflammatory properties, is reduced [8]. Very little is known about possible links between obesity and asthma-associated inflammation, as the high systemic production of adipokines produced in visceral adipose tissue is capable of affecting airway reactivity without apparently modifying airway inflammation in OA [10]. Similarly, the mechanisms responsible for the loss of anti-inflammatory efficacy of corticosteroids in OA remain to be elucidated. One study showed that the ability of dexamethasone to induce *MKP1* gene expression in peripheral blood mononuclear cells (PBMCs) is reduced in patients 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 that act beyond bone health [12]. The active form 1,25-dihydroxyvitamin D (1,25[OH]2D) binds the VitD receptor, which is present in all tissues including T and B lymphocytes, dendritic cells, and bronchial epithelial cells [12]. VitD inhibits the expression of IL-2, suppresses the T helper 1 cytokines IL-12, IL-6, and TNF, and increases release of IL-4, IL-5, and IL-10 [12,13].

The serum level of 25-hydroxyvitamin D (25[OH]D) that defines VitD deficiency remains somewhat controversial. Previous guidelines defined VitD deficiency as 25(OH)D values below 20 ng/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 decreased VitD levels appear to be mainly due to its dilution in increased adipose tissue mass [19].

Low serum 25(OH)D levels are associated with asthma exacerbations [21]. Moreover, an inverse correlation between serum 25(OH)D concentrations and FEV_1 and BMI has been reported [22]. Other reports described an association between VitD deficiency and the risk of resistance to corticosteroids [23]. In PBMCs from corticosteroid-resistant patients, the active form of VitD, $1,25(OH)_2D$, enhances inhibition of cell proliferation by dexamethasone [24], thus increasing the expression of *MKP1* [25]. Interestingly, leptin levels inversely correlated with the ability of $1,25(OH)_2D$ to inhibit in vitro production of inflammatory cytokines [26].

Weight reduction significantly improves systemic and adipose tissue inflammatory activity levels [26,27]. Bariatric surgery 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 bariatric surgery [28-33].

We hypothesized that understanding the links between asthma, obesity, and VitD can help elucidate the mechanisms underlying hyporesponsiveness to ICS in patients with OA. This approach may also contribute to the development of VitD-based therapies with VitD to target this often difficult-to-manage asthma phenotype.

The objective of this study was to demonstrate that the poor response to ICS in obese asthma is due to impaired anti-inflammatory activity of corticosteroids associated with VitD deficiency and that both deficiencies are corrected through weight loss following bariatric surgery combined with VitD supplementation.

Patients and Methods

Patients

We recruited 37 asthma patients: 23 obese asthma patients (all OA [BMI ≥30 kg/m²]) and 14 nonobese asthma [NOA] patients [BMI <25 kg/m²]. Fifteen obese patients (OP) and 19 age- and sex-matched healthy controls (HC) were also recruited. Twenty-one OP (10 OA and 11 OP) were evaluated at baseline (V1) and 6 months after undergoing bariatric surgery (V2). Obese patients were recruited from the Obesity Unit of Hospital Clínic, Barcelona, Spain. According to the standard procedure, the patients received VitD supplementation after bariatric surgery.

The criteria used to select asthma patients were as follows: (*1*) a clinical history of asthma; and (*2*) either bronchodilator responsiveness (>12 % and 200-mL improvement in FEV_1 after 180 µg of salbutamol via a metered-dose inhaler) or airway hyperresponsiveness (PC_{20} methacholine <8 mg/mL). Forced spirometry and a methacholine test were performed according to the European Respiratory Society/American Thoracic Society standards [34,35]. Reference values were those of Roca et al [36,37]. None of the participants had received systemic corticosteroids for 1 month or longer prior to evaluation. None of the participants were current smokers. All participants were nonsmokers or ex-smokers for more than 1 year with a smoking history of <10 pack-years.

Serum was obtained by peripheral venipuncture followed by centrifugation and stored at –80°C until analysis. Serum adiponectin and leptin levels were quantified using the Luminex multiplex immunoassay with Human ProcartaPlex Mix&Match kits (Thermo Fisher Scientific) on a Luminex 200 analyzer. Serum 25(OH)D concentrations were determined using a chemiluminescent immunoassay (Atellica, Siemens).

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

Blood Collection and Isolation of PBMCs

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

Proliferation Assay

CFSE dilution

Five hundred microliters of cell suspension was incubated with 5 µM of carboxyfluorescein diacetate succinimidyl ester (CFSE) for 5 minutes in the dark at room temperature. PBMCs were resuspended in X-Vivo15 medium (Cultek) containing gentamicin and supplemented with 10% charcoal stripped fetal bovine serum (Sigma-Aldrich) at a final concentration of 2×10^6 cells/mL.

Cell culture conditions

To assess suppression of cell proliferation by dexamethasone, 500 µL of labeled PBMCs was stimulated with 1 µg/mL of phytohemagglutinin-L (Sigma-Aldrich) in the presence of a range of dexamethasone (Kern Pharma) concentrations (10^{-11}-10^{-5}M), as well as in the presence or absence of $1,25(OH)_2D$ at 10^{-7} M. Cultures were incubated at 37°C and 5% CO_2 for 4 days, and cell proliferation was assessed based on the CFSE dilution.

Flow cytometry analysis

PBMCs were transferred to round-bottom flow cytometry tubes and stained with Zombie Violet Fixable Viability Kit (BioLegend) and anti-CD4 APC antibody (BioLegend). Cells were then analyzed on a FACSCanto II flow cytometer (BD

Biosciences), and the percentage of proliferation was recorded for PBMCs and CD4+ T cells, which were strategically gated. Sensitivity to dexamethasone was expressed as the half maximal inhibitory concentration (DEX IC_{50}).

Analysis of Gene Expression by Reverse Transcription Quantitative Polymerase Chain Reaction

Total RNA was isolated from PBMCs cultured for 24 hours in the presence or absence of dexamethasone at 10-9 M and with or without $1,25(OH)_2D$ at 10^{-7} M using the TRIzol reagent (Life Technologies) according to the manufacturer's protocol. RNA was converted into cDNA using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher).

MKP1 mRNA expression was analyzed using real-time quantitative polymerase chain reaction (PCR) with TaqMan probes in the Viia7 Real-Time PCR system (Applied Biosystems). The thermal cycler was set to 95°C for 20 minutes followed by 40 reaction cycles of 1 second at 95°C and 20 seconds at 60°C. All PCR assays were run in duplicate for both target genes and 2 control genes (*B2M*, *HPRT1*).

Statistical Analysis

Clinical and experimental data were reported as median (IQR). Differences between the 2 groups were analyzed using nonparametric tests (Mann-Whitney test [unpaired data], the Wilcoxon rank test [paired data], or the Kruskal-Wallis H test [multiple comparisons]). Correlation coefficients were calculated using the Spearman rank method. All analyses were performed using GraphPad Prism version 8.4 for Windows (GraphPad Software). Statistical significance was set at $P<.05$.

Results

Baseline Characteristics

The participants' demographic and clinical characteristics are shown in Table 1. The severity of asthma (mild, moderate, severe) was established according to the pharmacological treatment used to control the disease [1,3]. Control was assessed using the asthma control test (ACT) [1,3]. FVC and FEV_1 were lower in OA than in HC, whereas the FEV_1/FVC ratio was significantly lower in NOA and OA than in OP and HC. Serum 25(OH)D levels tended to be lower in OP than in HC, although the difference was not statistically significant. The adiponectin/leptin ratio was significantly lower in OP and OA than in HC and NOA.

Relationship Between Obesity and Sensitivity to Corticosteroids

DEX IC_{50} values were calculated in PBMCs and CD4+ T cells to examine the link between obesity and response to corticosteroids. IC_{50} values were significantly higher in OA than in NOA when both PBMCs and CD4+ T cells were

Table 1. Baseline Demographic and Clinical Data of the Study Population.[a]

|	HC (n=19)	NOA (n=14)	OA (n=23)	OP (n=15)
Age, y	43 (34-56)	53 (43-60)	56 (51-59)	47 (45-60)
Female, No. (%)	14 (74)	11 (79)	18 (78)	13 (87)
BMI, kg/m²	23.7 (22.2-25-4)	23.2 (22.3-25.3)	37.6 (34.5-45)[b,c]	42.7 (39.5-48.5)[b,c]
Mild asthma, No. (%)	-	0 (0)	4 (17.4)	-
Moderate asthma, No. (%)	-	5 (35.7)	5 (21.7)	-
Severe asthma, No. (%)	-	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)[b,c]	93.0 (82.7-96.2)
FEV_1, % pred	102.0 (95.0-109.0)	80.0 (75.2-100.3)	80.0 (62.0-95.0)[b]	90.0 (86.2-101.0)
FEV_1/FVC	81.0 (76.0-84.0)	65.5 (57.0-75.0)[b]	77.0 (69.0-82.0)	81.5 (75.0-83.0)[c]
ICS, No. (%)[d]	-	13 (92.9)	19 (82.6)	-
Eosinophils, %	2.3 (1.6-3.3)[c]	4.7 (3.6-7.1)	3.5 (2.4-5)	2.3 (3.4-1.2)[c]
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)[c]	4.3 (4.0-9.0)[c]
Leptin, ng/mL	1.7 (1.0-2.5)	3.4 (2.2-4.8)	3.6 (1.8-6.1)[b]	4.1 (2.7-6.3)[b]
Adipo/Lept	6.7 (2.1-8.6)	4.6 (1.9-6.5)	1.3 (0.9-2.9)[b,c]	1.1 (0.7-2.7)[b,c]

Abbreviations: Adipo/Lept, adiponectin/leptin ratio; BMI, body mass index; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; HC, healthy controls; ICS, inhaled corticosteroids; IgE, immunoglobulin E; NOA, nonobese asthma patients; OA, obese asthma patients; OP, obese patients; 25(OH)D, 25-hydroxyvitamin D.
[a]Data are presented as median (IQR).
[b]$P<.05$, compared with HC.
[c]$P<.05$, compared with NOA; Kruskal-Wallis followed by Dunn multiple comparisons test.
[d]For NOA and OA who received ICS, the mean (SD) of the ICS dose in budesonide equivalents was 557.1 (256.6) and 1222.1 (862.9) µg/d, respectively.
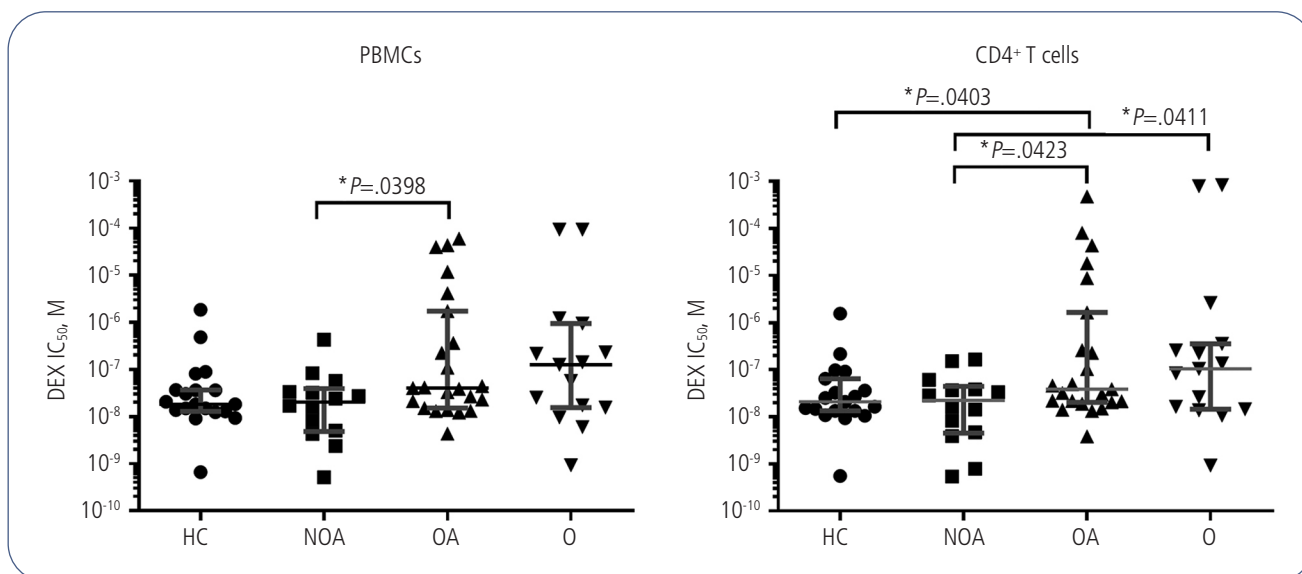
Figure 1. DEX IC$_{50}$ values from HC, NOA, OA, and OP in both PBMCs and CD4+ T cells. Data are presented as median (IQR). PBMCs indicates peripheral blood mononuclear cells; DEX IC$_{50}$, dexamethasone half maximal inhibitory concentration; HC, healthy controls; NOA, nonobese asthma patients; OA, obese asthma patients; OP, obese patients. *P<.05 (Mann-Whitney test).

analyzed (Figure 1). There were no differences in sensitivity to corticosteroids between OA and OP. DEX IC$_{50}$ values were higher in OP than in HC, although not all differences were statistically significant, owing to differences in the types of cells selected for the analysis (PBMCs vs CD4+ T cells) (Figure 1).

Relationship Between Response to Corticosteroids and Adipokines and VitD

Analysis of the adiponectin/leptin ratio and CD4+ T-cell sensitivity to corticosteroids in vitro showed a negative correlation between the adiponectin/leptin ratio and the DEX IC$_{50}$ value (r=–0.4113, P=.0008), ie, the higher the ratio, the greater the antiproliferative effect of dexamethasone.

Moreover, we studied the association between serum 25(OH)D concentrations and response to dexamethasone in vitro. We observed a negative correlation between 25(OH)D concentrations and DEX IC$_{50}$ values (r=–0.2512, P=.0346). The higher the VitD concentration, the greater the antiproliferative effects of dexamethasone.

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 DEX IC$_{50}$ values was observed only in those with VitD deficiency (<12 ng/mL) (n=8, 4 OA and 4 OP patients), compared to patients with VitD sufficiency (>20 ng/mL) and insufficiency (12-20 ng/mL) (Figure 2) [15].

Effects of Weight Loss After Bariatric Surgery

Bariatric surgery was performed in 21 patients (10 OA and 11 OP): 8 (38%) underwent sleeve gastrectomy, while the remaining 13 (62%) underwent Roux-en-Y gastric bypass. Table 2 presents changes in clinical, functional, and
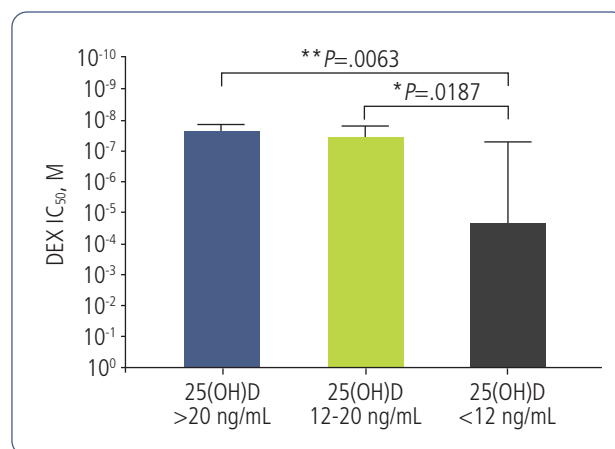
Figure 2. Patients classified according to 25(OH)D serum levels and in vitro response to corticosteroids. DEX IC$_{50}$ indicates dexamethasone half maximal inhibitory concentration; 25(OH)D, 25-hydroxyvitamin D. *P<.05, **P<.01(Mann-Whitney test).

inflammatory markers after surgery. BMI was significantly reduced in both groups. Weight loss was associated with a significant improvement in the ACT scores and a reduction in the dose of therapy needed to control the disease. Lung function tests improved after surgery and the adiponectin/leptin ratio increased significantly after surgery in OP and OA. Serum 25(OH)D levels increased in OA and OP, although no deficiency (<12 ng/mL) was recorded, reflecting the effect of both bariatric surgery and VitD supplementation.

Weight Loss and Sensitivity to Corticosteroids

To evaluate the impact of weight loss on sensitivity to corticosteroids, we compared DEX IC$_{50}$ in both groups with

Table 2. Demographic and Clinical Data of Obese Asthma and Nonasthma Patients Before (V1) and 6 Months After (V2) Bariatric Surgery.[a]

|	Obese asthma patients (n=10)		Obese patients (n=11)	
	V1	**V2**	**V1**	**V2**
Female, No. (%)	10 (100)	10 (100)	10 (91)	10 (91)
BMI, kg/m^2	44.1 (38.7-47.1)	30.0 (26.4-34.9)[b]	41.9 (37.3-45.7)	29.7 (27.4-34.6)[c]
Mild asthma, No. (%)	4 (40)	9 (90)	-	-
Moderate asthma, No. (%)	4 (40)	1 (10)	-	-
Severe asthma, No. (%)	2 (20)	0 (0)	-	-
ACT	18 (18-24)	25 (25-25)[b]	-	-
FVC, % pred	85.5 (77.5-96.0)	95.0 (88.3-106.0)	94.5 (85.3-98.0)	99.0 (91.0-106.0)
FEV$_1$, % pred	89.0 (79.5-97.3)	96.5 (92.5-104.8)	93.5 (87.0-101.0)	101.0 (89.0-109.0)[c]
FEV$_1$/FVC	80.5 (76.5-82.5)	81.5 (77.5-83.0)	81.0 (75.0-83.0)	79.0 (76.0-83.0)
ICS, No. (%)[d]	6 (60)	2 (20)	-	-
Eosinophils, %	3.0 (2.0-3.7)	2.6 (1.4-3.3)	2.8 (1.6-3.6)	2.5 (1.8-2.8)
IgE, kU/L	32.3 (12.4-113.3)	33.8 (7.6-52.1)	51.7 (16.8-115)	28.3 (14.4-62.8)
25(OH)D, ng/mL	25.2 (11.9-43.2)	31.7 (20.6-38.3)	18.8 (10.1-30.6)	37.7 (23.2-41.8)[c]
Adiponectin, µg/mL	7.0 (3.5-9.5)	9.4 (4.8-20.6)[b]	5.6 (4.0-10.6)	9.8 (6.0-12.1)
Leptin, ng/mL	5.0 (3.2-8.3)	2.3 (0.8-4.7)	4.3 (3.3-7.2)	2.5 (1.3-3.4)[c]
Adipo/Lept	1.2 (0.7-2.0)	4.7 (1.2-43.2)[b]	1.1 (0.7-2.7)	4.2 (2.7-6.9)[c]

Abbreviations: ACT, asthma control test; Adipo/Lept, adiponectin/leptin ratio; BMI, body mass index; FEV$_1$, forced expiratory volume in 1 second; FVC, forced vital capacity; ICS, inhaled corticosteroids; IgE, immunoglobulin E; 25(OH)D, 25-hydroxyvitamin D3.
[a]Data are presented as median (IQR).
[b]$P<.05$, compared between V1 and V2 in the obese asthma group.
[c]$P<.05$ compared between V1 and V2 in the obese group (Wilcoxon test).
[d]For obese asthma 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/d, respectively.
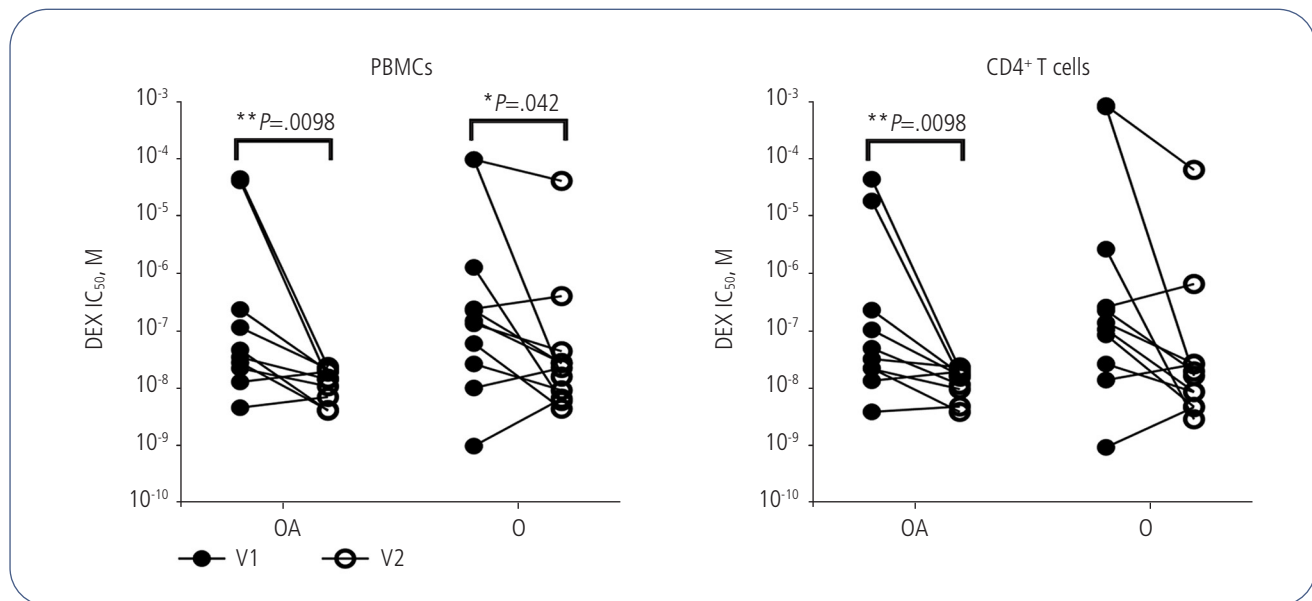
Figure 3. Comparison between DEX IC$_{50}$ values from OA and OP before (V1) and after (V2) bariatric surgery in both PBMCs and CD4$^+$ T cells. PBMCs indicates peripheral blood mononuclear cells; DEX IC$_{50}$, dexamethasone half maximal inhibitory concentration; OA, obese asthma patients; OP, obese asthma patients. *$P<.05$, **$P<.01$ (Wilcoxon test between V1 and V2).
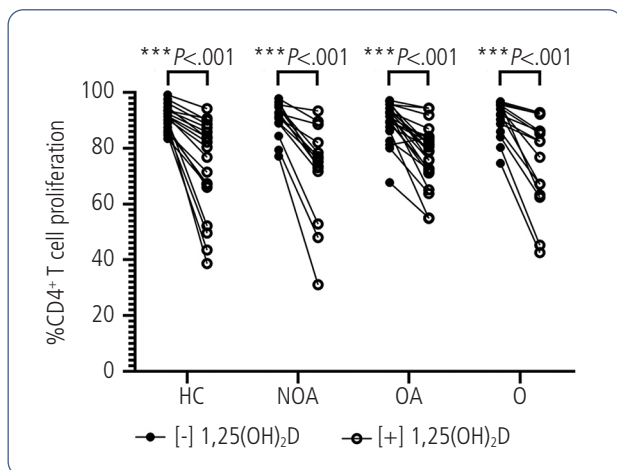
Figure 4. Inhibition of CD4+ T-cell proliferation by 1,25(OH)₂D in HC, NOA, OA, and OP. Solid circles represent cells without treatment and open circles represent cells treated in vitro with 1,25(OH)₂D 1x10⁻⁷ M. 1,25(OH)₂D indicates 1,25-dihydroxyvitamin D; HC, healthy controls; NOA, nonobese asthma patients; OA, obese asthma patients; OP, obese patients. ***$P<.001$ (Wilcoxon test).

obesity before (V1) and after bariatric surgery (V2) (Figure 3). Values diminished in both PBMCs and CD4+ T cells after surgery in OA and OP. However, the reduction was not statistically significant in CD4+ T cells from OP.

Effect of 1,25(OH)₂D on CD4+ T-Cell Proliferation In Vitro

The addition of 1,25(OH)₂D to culture media resulted in a significant reduction in phytohemagglutinin-stimulated CD4+ T-cell proliferation, with no statistically significant differences between the 4 groups. We found similar results in PBMCs (data not shown) (Figure 4).

### Impact of 1,25(OH)₂D on Sensitivity to Corticosteroids

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

MKP1 Gene Expression

We analyzed the expression of *MKP1* in PBMCs after stimulation with dexamethasone for 24 hours. As expected, dexamethasone induced *MKP1* mRNA expression in PBMCs, which correlated with sensitivity to dexamethasone in PBMCs expressed as the IC₅₀ value (r=0.4860, $P=.0075$). However, no differences were found between the groups. We also examined the expression of *MKP1* in response to VitD and dexamethasone. *MKP1* expression increased similarly in the 4 groups (data not shown). However, there were statistically
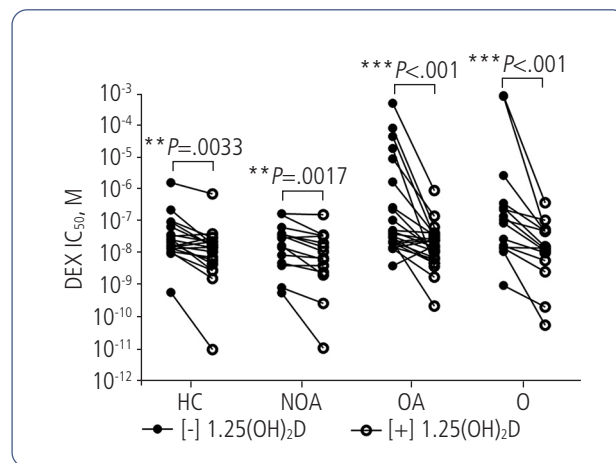
Figure 5. Comparison between sensitivity to corticosteroids in CD4+ T cells with the absence (solid circles) or presence (open circles) of 1,25(OH)₂D. 1,25(OH)₂D indicates 1,25-dihydroxyvitamin D; HC, healthy controls; NOA, nonobese asthma patients; OA, obese asthma patients; OP, obese patients. **$P<.01$, ***$P<.001$ (Wilcoxon test).
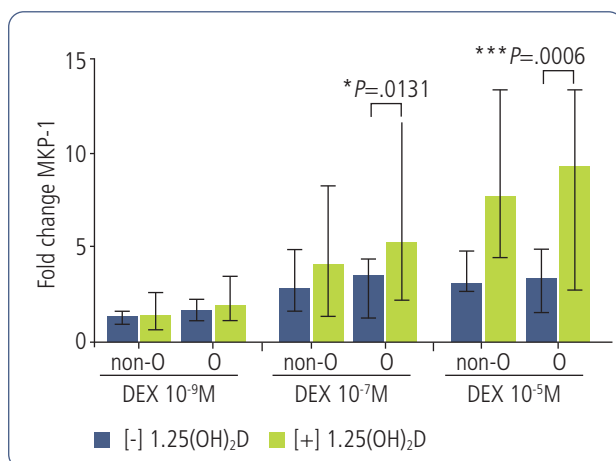
Figure 6. DEX-induced *MKP1* expression with (chequered columns) and without (solid columns) 1,25(OH)₂D comparison in nonobese and obese groups. Data are presented as median (IQR). DEX indicates dexamethasone; 1,25(OH)₂D, 1,25-dihydroxyvitamin D; non-O, nonobese group (healthy controls and nonobese asthma patients); O, obese group (obese asthma patients and obese patients). *$P<.05$; ***$P<.001$ (Wilcoxon test).

significant differences in the induced expression of *MKP1* when 1,25(OH)₂D was added to dexamethasone in obese patients (Figure 6).

Discussion

Our study demonstrates that obese individuals with and without asthma are characterized by a reduced response to corticosteroids, as assessed by means of a PBMC proliferation assay. The reduced response negatively correlates with the serum adiponectin/leptin ratio, a marker associated with the level of inflammation in obese persons. A similar negative

correlation was observed between the antiproliferative effects of corticosteroids and VitD serum levels. Obese patients with VitD deficiency (<12 ng/mL) were those with the poorest antiproliferative response to corticosteroids.

Weight loss after bariatric surgery was associated with a marked improvement in the reduced antiproliferative effects of corticosteroids in obese individuals. Weight loss was also associated with an increase in both the adiponectin/leptin ratio and serum VitD levels. No obesity or VitD deficiency was recorded after bariatric surgery and VitD supplementation. These observations suggest that reduced obesity-related systemic inflammation and increased levels of serum VitD can account for the recovered ability of corticosteroids to inhibit proliferation of PBMCs.

However, it is well known that association does not necessarily imply a causal relationship. To further investigate the potential causal relationship between VitD deficiency and hyporesponsiveness to corticosteroids, 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 corticosteroids. Collectively, these findings support the notion that VitD can play a role in the complex interaction between obesity, inflammation, and hyporesponsiveness to corticosteroids.

In order to establish the role of VitD deficiency in hyporesponsiveness to corticosteroids in asthma, randomized clinical trials (RCTs) should be performed to evaluate the clinical efficacy of VitD in OA. Numerous RCTs, mostly in children, have been carried out to evaluate the efficacy of VitD 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 pediatric asthma [39]; others, however, could not find significant evidence favoring the use of this complementary therapy in children or adults [40].

Analysis of VitD supplementation in asthma faces many unresolved issues. Thus, for example, studies on the anti-inflammatory and antioxidant 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, owing to the effect of excessive accumulation of fat on the distribution of VitD, OA would require a supplemental VitD dose higher than that required by NOA with VitD deficiency [41]. RCTs tailored to 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 hyporesponsiveness to corticosteroids in OA. Despite progress in the field, the mechanisms involved in insensitivity to corticosteroids in patients with asthma are not fully understood [9]. The anti-inflammatory actions of corticosteroids are mediated by glucocorticoid receptor α (GRα), which suppresses inflammation through transactivation and transrepression mechanisms [9]. In the presence of corticosteroids, GRα translocates into the nucleus to regulate the expression of various anti-inflammatory genes (transactivation) such as $MKP1$ [9]. We decided to examine this gene, as previous studies found that the expression of $MKP1$ induced by dexamethasone is blunted in overweight/obese asthma patients compared with lean asthma patients [11]. Interestingly, obesity in nonasthmatic individuals had no effect on $MKP1$ expression, suggesting that, for reasons that are not altogether clear, the effect of obesity only impacts on $MKP1$ expression in asthma patients [11]. In our study, we did not find any significant differences in the dexamethasone-dependent induction of $MKP1$ in OA compared with NOA. This finding suggests that obesity does not affect the capacity of corticosteroids 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 $MKP1$ was analyzed against a single dose of dexamethasone (10^{-6} M), while in our study it was analyzed by means of a dose-dependent response, which is considered more suitable for evaluating pharmacologically induced responses in vitro. Moreover, asthma severity has been shown to be closely related to the level of dexamethasone-induced $MKP1$ expression [38], and most patients enrolled in our study had mild/moderate disease. Sutherland et al found pulmonary function to be associated with more pronounced airway obstruction (FEV_1) in OA than in the present study (70% vs. 77%).

The anti-inflammatory effects of corticosteroids can be explained by mechanisms other than activation of $MKP1$, such as alterations in the nuclear translocation of GRα, increased expression of the GRβ isoform—a dominant negative regulator of active GRα—and inhibition of proinflammatory gene expression through blockade of proinflammatory transcription factors [9]. Interestingly, a very recent study found that dysregulation of the GRα/GRβ isoform ratio may contribute to hyporesponsiveness to corticosteroids in OA [42].

We also examined the effects of VitD on the induction of $MKP1$ in patients with and without obesity, finding that the hormone also increases expression of $MKP1$, with some differences between obese and nonobese individuals, a finding that suggests that the efficacy of VitD to increase dexamethasone-induced expression of anti-inflammatory genes is more pronounced in persons with obesity.

As a complementary methodological contribution, we used PBMCs and CD4+ T-cell proliferation to assess sensitivity to dexamethasone. Both methods have been used elsewhere to examine sensitivity to corticosteroids [21,38,43]. We found differences in the results obtained with PHA-induced PBMCs and CD4+ T-cell proliferation in the response to corticosteroids, 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 of patients examined and the short follow-up time of 6 months after bariatric surgery. Patients were enrolled from the obesity program at our center, and, in all cases, bariatric surgery was indicated to treat obesity, not asthma.

In summary, our study makes several novel contributions that may help to better understand the links between obesity and poor response to ICS in OA. Our findings support the role of obesity in ICS hyporesponsiveness and demonstrate that

the efficacy of weight loss for improving asthma symptoms and lung function can be, at least in part, due to the recovered anti-inflammatory response to corticosteroids. Finally, our results suggest that VitD supplementation in asthma patients with obesity and VitD deficiency may contribute to achieving asthma control by improving the efficacy of ICS.

Funding

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

Conflicts 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. The remaining authors declare that they have no conflicts of interest.

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