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Metabolic shift favoring C18:0 ceramide accumulation in obese asthma

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Running title: Alteration of ceramide accumulation in asthma

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Abstract

Background: Obesity associated with various complications has increased worldwide. Body weight gain alters lipid metabolites (especially sphingolipids) contributing to obesity-induced inflammation. However, the significance of the metabolites in the development of obese asthma is not yet clear.

Methods: The serum levels of sphingolipids were measured using liquid chromatography-tandem mass spectrometry in obese controls (n = 7) and patients with asthma: the obese group (BMI > 25 kg/m³, n = 13) vs the non-obese (n = 28) group. To examine the relationship between metabolic changes in sphingolipids and macrophage polarization, public microarray data were analyzed. In addition, the alteration in sphingolipid metabolism was investigated in wild-type BALB/c mice fed a high-fat diet.

Results: The obese asthma had higher levels of serum C18:0 and C20:0 ceramides than the non-obese asthma group ($P = .028$ and $P = .040$, respectively). The value of the serum C18:0 ceramide (184.3 ng/mL) for discriminating the obese asthma from the non-obese asthma group showed 53.9% sensitivity and 85.7% specificity (AUC = 0.721, $P = .024$). The microarray data showed significantly increased ceramide synthesis and metabolic shift to ceramide accumulation during M1 macrophage polarization in humans. Increased airway hyperresponsiveness, M1 macrophage polarization, and C18:0 ceramide levels were noted in obese mice, but not in non-obese mice. Increased expression of ceramide synthase (CerS) 1 and CerS6 (not CerS2) was noted in lung tissues of obese mice.

Conclusion: Alteration in sphingolipid metabolism favoring ceramide accumulation (especially long-chain ceramides) may contribute to developing obese asthma.

Key words: Asthma, obesity, ceramide, sphingolipid, macrophage

Introduction

Obesity is becoming prevalent worldwide (especially Western countries) with increasing rates in adults and children.¹ The epidemic of obesity predisposes individuals to various complications, such as type 2 diabetes and cancer.² Recent studies have suggested that obesity is a risk factor associated with the development of asthma.³⁻⁵ The incidence of asthma symptoms increases with increasing body mass index (BMI).⁶ In addition, obese patients with asthma do not fully respond to conventional treatment.⁷ The lack of specific therapies for obese asthma indicates poor understanding of immunological and metabolic mechanisms affected by fat body weight gain.

In obesity, chronic low-grade inflammation induces immune cell recruitment and activation, resulting in a stronger pro-inflammatory response.⁸ Obesity is related to an increase in CD8⁺ T cells and a polarization in macrophages from M2 to M1.^{9,10} High levels of circulating tumor necrosis factor (TNF)- α , interleukin (IL)-6, and adipokines are associated with obese asthma, but not with allergic asthma.¹¹ Some previous studies demonstrated that sputum neutrophils, but not eosinophils, were elevated according to BMI.^{12,13} As obese asthma is a distinct phenotype from that of allergic asthma, a specific pathogenic mechanism needs to be clarified.

Energy oversupply has been known to alter sphingolipid metabolism in obesity.¹⁴ This cellular metabolism is a complex pathway that generates the metabolites sphingolipids which are implicated as potent mediators in multiple diseases.¹⁵ Moreover, a recent study has shown that sphingolipids are associated with airway inflammation and allergic responses.¹⁶ Especially, it has been revealed that sphingosine-1-phosphate (S1P) is involved in airway hyperresponsiveness (AHR).^{17,18} Ceramides (a precursor of S1P) and sphingomyelins have also been reported to consistently increase by fat accumulation^{19,20}; however, the role of sphingolipids in the pathogenesis of asthma is still controversial.

To clarify whether sphingolipids are associated with the development of obese-related asthma phenotype, this study showed (1) higher levels of serum C18:0 ceramide in overweight individuals with asthma than normal individuals with asthma; (2) differentially expressed genes in sphingolipid metabolism related to M1 macrophage polarization in human; and (3) changes in sphingolipid accumulation and ceramide synthase expression in high-fat diet (HFD)-fed obese mice.

Methods

Study Subjects

This study was approved by the Institutional Review Board of Ajou University Hospital (AJIRB-GEN-SMP-13–108). The clinical investigation was conducted in adults including obese controls ($n = 7$) and patients with asthma ($n = 41$). The obese group ($> 25 \text{ kg/m}^3$) and the non-obese group were defined according to the BMI.²¹ A diagnosis of asthma was based on typical symptoms. Spirometry was performed to evaluate the degree of airway obstruction. Methacholine bronchial challenge test was conducted to examine the degree of AHR. Atopy status was defined when patients had positive responses on skin prick test to $> one$ inhalant allergens including two house dust mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*), cat, dog, cockroach, *Aspergillus*, and *Alternaria*, mugwort and ragweed pollens, tree and grass pollen mixtures (Bencard, Bradford, UK).

Measurement of serum levels of the metabolites sphingolipids using liquid chromatography-tandem mass spectrometry

Sera were separated using a BD Vacutainer® SST tube according to the manufacturer's instructions and stored at -20°C until analysis. Human serum was well mixed and internal standard solution (500 nM of C17 ceramide) was added to the samples before extraction. Sphingolipids were extracted using the Bligh and Dayer method.²² Sphingomyelin and ceramide were evaluated using a liquid chromatography-tandem mass spectrometry system equipped with 1290 HPLC (Agilent, Waldbronn, Germany) and QTRAP 5500 (AB Sciex, Toronto, Canada). Multiple reaction monitoring was performed in the positive ion mode and the extracted ion chromatogram corresponding to the specific transition for each lipid was used for quantification. The calibration range for each lipid was 0.1-1,000 nM ($r \geq 0.99$). Data analysis was performed using either Analyst 1.5.2 or Xcalibur software. Sphingosine was quantified using the Mass Hunter Quantitative Analysis B.07.00 system (Agilent Technologies, Santa Clara, CA, USA).

Gene expression microarray analysis for sphingolipid metabolisms during macrophage polarization in humans

The expression monitoring array raw data were downloaded from the gene expression omnibus (GEO) database with accession numbers GSE85346.^{23,24} GSE85346 included human

monocyte-derived macrophages, classically activated macrophages and control macrophages without any stimulation analyzed with Human Whole Genome ver. 2.0 arrays (Agilent Technologies, Santa Clara, CA, USA). Statistical significance of the expression data was determined using fold change and local-pooled-error test, in which the null hypothesis showed no differences among the 2 groups.²⁵ Functional annotation analysis for significant probe list was performed using KEGG (www.genome.jp/kegg/). All statistical analyses and the visualization of differentially expressed genes was conducted using R 3.1.2 (www.r-project.org).

High fat diet (HFD)-fed mice with asthma phenotype

Female 6-week-old BALB/c mice (Jackson Laboratory, Bar Harbor, USA) were maintained under specific pathogen-free conditions. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Ajou University (IACUC-2016-0022). To induce obese asthma, mice were fed a HFD (Research Diets, New Brunswick, NJ, USA) for 6 months which provided 60% of energy in the form of fat. The airway resistance to inhaled methacholine (Sigma-Aldrich, St. Louis, MO, USA) was measured using the flexiVent System (SCIREQ, Montreal, Canada) as previously described. Each mouse was injected with aerosol methacholine at increasing concentrations (0, 1.56, 3.12, 6.25, 12.5, and 25 mg/mL), and the peak airway responses to the inhaled methacholine were recorded. Bronchoalveolar lavage fluid (BALF) was centrifuged to isolate the cells, and then stained with haematoxylin and eosin (Sigma-Aldrich). These cells were counted and classified as macrophages, eosinophils, or neutrophils.

To analyze macrophage polarization, antibodies were used as follows: fluorochrome-conjugated anti-CD45, anti-F4/80, anti-CD11c, and anti-CD206. Stained cells were analyzed with BD FACSCanto™ II (BD Bioscience, San Jose, CA, USA), and graphs were produced using FlowJo software (Tree Star, Ashland, KY, USA). To evaluate the expression of ceramide synthase (CerS), lungs tissues were fixed, embedded, and cut into 5-μm sections. Antibodies used for detecting ceramide synthases are as follows: anti- CerS1, anti-CerS2, and anti-CerS6 (Abcam). The tissues were examined under a Zeiss LSM710 confocal microscope with a 63 × oil objective lens (Carl Zeiss, Oberkochen, Germany). Images were analyzed using ZEN 2009 software (Carl Zeiss).

Statistical analysis

P values were obtained by Pearson's chi-squared test for categorical variables and Student's *t* test for continuous variables. $P < 0.05$ was considered to be statistically significant. Statistical analyses were performed using SPSS software version 20.0 (IBM Corp., Armonk, NY, USA). GraphPad Prism 5.0 software (GraphPad Inc., San Diego, CA, USA) was used to produce graphs.

Results

Comparison of clinical characteristics in the study subjects

Demographic data from the study subjects are shown in Table 1. When we classified the study subjects into 2 groups based on BMI, the obese asthma group ($> 25 \text{ kg/m}^3$) vs the non-obese asthma group, no significant differences were observed in asthma-related parameters, such as atopy rate, asthma severity, and FEV_1 % and PC_{20} methacholine values ($P > .05$ for all). In addition, blood eosinophil ($P = .484$) and sputum eosinophil/neutrophil counts (were not significantly different between the 2 groups. $P = .713$ and $P = .554$, respectively)

Changes in serum levels of the metabolite sphingolipids

When serum levels of sphingolipids including sphingomyelin, ceramide, and sphingosine were compared between the 2 groups, the levels of C18:0 and C20:0 ceramides were significantly higher in the obese asthma than in the non-obese asthma group ($P = .028$ and $P = .040$, respectively), while no differences were found in other sphingolipids (Table 2) with a positive correlation between levels of serum C18:0/C20:0 ceramide and BMI (Figure S1). However, levels of serum SM (d18:0/16:0) and C24:1 ceramide were significantly elevated in the obese asthma group compared with obese controls ($P = .023$ and $P = .003$, respectively). Using the receiver operating characteristic analysis, the optimal cutoff value (C18:0 ceramide: 184.3 ng/mL) for the highest levels of sensitivity and specificity was decided. This mediator showed 53.9% sensitivity and 85.7% specificity ($\text{AUC} = 0.721$, $P = .024$; Figure 1) for discriminating the obese asthma from the non-obese asthma group among adult patients.

A metabolic shift favoring ceramide accumulation during M1 polarization

To examine a metabolic change in sphingolipids during macrophage polarization, public microarray data from GEO database (GEO accession number: GSE85346, human monocyte-derived macrophages) were downloaded and analyzed to identify differentially expressed genes in the sphingolipid metabolism between classically activated macrophages and control macrophages without any stimulation in humans (Table S1, Figure 2). Differentially expressed genes in the sphingolipid metabolism during M1 macrophage polarization are shown in Table S1 and depicted in Figure 2. Increased ceramide synthesis (*CERS6* and *UGCG*) and metabolic shift to ceramide accumulation (*PPAP2A/B*, *SGMS2*, *CERK*) were significantly noted ($P < .001$ for all).

Induction of AHR and airway inflammation in mice fed a HFD

As an *in vivo* model of obese asthma, mice were fed a HFD for 6 months. Body weights significantly increased in mice on a HFD compared to mice on a regular diet (Figure 3A). These obese mice developed AHR after methacholine inhalation, whereas non-obese mice did not (Figure 3B). In BALF, macrophage counts (neither eosinophils nor neutrophils) were significantly elevated (Figure 3C); when macrophage polarization was noted, the population of M1 macrophages (CD45⁺F4/80⁺CD11c⁺ cells) were enhanced in obese mice compared to non-obese mice (Figure 3D). In addition, lung tissues stained with hematoxylin and eosin showed increased macrophage infiltration (Figure 3E). These suggest that obese asthma was well established in mice fed an HFD.

Elevated levels of the metabolites sphingolipids in obese mice with asthma phenotype

The present study further analyzed various sphingolipid metabolites in serum. As a result, levels of long-chain (C16 and C18) sphingomyelin (SM) and ceramides were significantly increased in the sera of obese mice, while those of very long-chain (C24) sphingolipids were not (Figure 4A); levels of SM (d18:0/16:0), SM (d18:0/18:0), SM (d18:0/18:1), C16:0, C18:0, C18:1 and C20:0 ceramides were markedly elevated in obese mice. In addition to serum sphingolipids, higher levels of SM (d18:0/18:0), SM (d18:0/18:1), and C18:0 ceramide were also noted in BALF of obese mice (Figure 4B). When ceramide synthase (CerS) expression was investigated, CerS1 and CerS6 (Figure 4C) were highly expressed in the lungs of obese mice, whereas CerS2 did not (Figure S2). Taken together, fat accumulation may affect CerS expression, leading to altered levels of sphingolipid metabolites in obese mice.

Discussion

To date, the phenotype of obese asthma is not easy to distinguish from other asthma phenotypes using various clinical parameters except BMI. A previous paper reported altered sphingolipid metabolism (generation of ceramides) in obese subjects with type 2 diabetes.²⁶ However, the clinical significance of sphingolipid metabolism in obese asthma has not been fully understood. They proposed that the C18:0 ceramide may be a potential biomarker for discriminating the obese asthma from the non-obese asthma group among adult patients. Furthermore, we demonstrated that highly expressed CerS1 and CerS6 (not CerS2) with M1 macrophage polarization in the lungs of obese mice caused the metabolic disturbance of sphingolipids, contributing to developing airway hyperresponsiveness and inflammation.

Obesity has emerged as a significant public health concern increasing medical costs. For this reason, extensive studies have been performed to find the pathophysiology of obesity-associated diseases. In Korea, interest in the development of asthma in an increased obese population has also emerged.²⁷ Although the mechanism of obesity involved in various complications was not completely determined, it is partially explained by chronic low-grade inflammation-mediated free fatty acids.²⁸ These fatty acids are the source of sphingolipid synthesis in response to energy oversupply.²⁹ Sphingolipids are bioactive lipids involving cellular signaling pathways.¹⁵ As fat accumulation significantly alters sphingolipid metabolism, we identified a specific molecule involved in the pathogenesis of obese asthma.

Ceramides have been already suggested to be a novel biomarker in several diseases, such as cancer, multiple sclerosis, and type 2 diabetes.³⁰ Moreover, a recent study has demonstrated that C18:0 ceramide induces obesity-associated diseases.³¹ Although the aberrantly high level of sphingosine-1-phosphate in the lungs contributes to airway inflammation and airway remodeling,³²⁻³⁴ the role of ceramides in the pathogenesis of asthma is still unclear. Nevertheless, ceramides have been known to enhance the expression of the transcription factor NF- κ B, which can up-regulate many genes involved in inflammation.^{35,36} In addition, ceramides are associated with accumulation of neutrophils and macrophages in the lungs and production of pro-inflammatory cytokines.³⁷ In this study, we showed that significantly higher levels of serum C18:0 ceramide in the obese asthma than in the non-obese asthma group. This long-chain ceramide is expected to be used for the diagnosis of obese asthma as BMI has limitation to explain asthma severity in obese adults.³⁸

In the current study, obese mice showed enhanced M1 macrophage polarization in the lungs as previously reported.⁵ Moreover, significantly increased AHR in response to inhaled methacholine was noted in obese mice. Although the precise mechanism that links obesity to airway inflammation has not been completely determined, many studies suggest that the metabolites sphingolipids may be an important factor for obesity-induced asthma.¹⁶⁻¹⁸ Elevated serum levels of sphingomyelins (one of the most abundant sphingolipids) in obese mice were reported.³⁹ In addition, increased levels of circulating ceramides were also demonstrated in obese mice.^{40,41} In our study, significantly elevated levels of long-chain (C16 and C18) sphingolipid metabolites, but not very long-chain (C24) sphingolipid metabolites were noted in obese mice. In addition, our study further measured sphingolipids in BALF and found higher levels of C18:0 ceramide in obese mice, indicating that long-chain sphingolipid metabolites may be helpful in differentiating the obese asthma from non-obese asthma group among adult patients.

Ceramides are produced by multiple ceramide synthases (CerS1-6) by transferring a fatty acyl-coenzyme A to the amine group of a sphingolipid base.⁴² Especially, CerS1 forms C18 ceramide, CerS2 forms C24 ceramide, and CerS6 forms C16 ceramide.⁴³⁻⁴⁵ Although many studies have revealed the significance of ceramide synthase and their products in the regulation of metabolic processes,^{46,47} studies about lung diseases associated with sphingolipid metabolism are lacking. Nevertheless, a previous study has suggested that dysfunction of CerS2 is implicated in the pathogenesis of acute or chronic lung injury,⁴⁸ increased airway inflammation, and airflow resistance along with C16:0 ceramide accumulation in the lungs were noted in CerS2-null mice. In the present study, increased expression of CerS1 and CerS6 in the lung of obese mice indicates the important roles of long-chain ceramide in the pathogenesis of obese asthma. Gene expression microarray analysis for sphingolipid metabolisms during macrophage polarization also supported a metabolic shift favoring ceramide accumulation leading to polarization. Considering a central role of CerS6 in sphingolipid metabolism, CerS6 inhibition could be a novel therapeutic target in obese asthma. Recently, CerS6 antisense oligonucleotide treatment *in vivo* (CerS6 knock-down) has been shown to selectively down-regulate CerS6 expression and to reduce C16:0 ceramide in plasma, leading to decreased body weight gain.⁴⁹ Taken together, these results indicate that blockage of C16:0 ceramide production may have a potential benefit for obese asthma.

This study has some limitations. First, levels of serum ceramides discriminating phenotypes of obese asthma need to be validated in another larger cohort as this is a small study.

Secondly, we did not evaluate whether long-chain ceramides directly induce obese asthma. Overexpression of CerS involved in long-chain ceramide production in *in vitro* and *in vivo* models may help prove the exact function of long-chain ceramides in airway hyperresponsiveness and inflammation. Further studies based on clinical applications are needed to manage asthma symptoms through modulation of sphingolipid metabolism in patients with obese asthma.

In conclusion, the results of this study suggest that long-chain ceramides particularly C16:0 ceramide in M1 macrophage polarization may play a crucial role in the pathogenesis of obese asthma and that C18:0 ceramide can be used to discriminate the phenotypes of obese asthma.

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Author contribution

Y. Choi designed experiments, analyzed the data and wrote the paper; M. Kim performed experiments; S.J. Kim and H.J. Yoo analyzed sphingolipid metabolites using LC-MS/MS; H.S. Park collected samples and supervised all the processes. S.H. Kim designed the study, analyzed the data, revised the manuscript, and supervised all the processes.

Conflict of interest

The authors declare no conflict of interest.

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Table 1. Comparison of clinical characteristics in the study subjects

Variables	NOA (n = 28)	OA (n = 13)	OC (n = 7)	<i>P</i> value		
				OA vs. NOA	OA vs. OC	NOA vs. OC
Age (y)	48.3 ± 15.6	52.0 ± 14.3	42.6 ± 15.8	.466	.191	.395
BMI (kg/m ²)	21.9 ± 1.9	27.5 ± 2.3	27.4 ± 1.3	.001	.928	.001
Female sex (%)	67.9	61.5	42.9	.691	.423	.221
Atopy (%)	60.7	69.2	NA	.596	NA	NA
Current/ex-smoker (%)	53.6	30.8	NA	.141	NA	NA
Severe asthma (%)	14.8	18.2	NA	.796	NA	NA
Type 2 diabetes mellitus (%)	7.1	15.4	NA	.408	NA	NA
FEV ₁ (%)	89.1 ± 13.4	93.3 ± 13.2	102.9 ± 7.2	.348	.092	.013
PC ₂₀ (mg/mL)	2.6 ± 2.2	1.5 ± 0.8	NA	.188	NA	NA
TEC (cells/μL)	307.4 ± 253.8	376.9 ± 370.0	200.0 ± 100.0	.484	.236	.285
Sputum Eos (%)	32.9 ± 40.0	28.3 ± 24.2	NA	.713	NA	NA
Sputum Neu (%)	56.1 ± 32.7	63.5 ± 27.1	NA	.554	NA	NA

Values are given as n (%) for categorical variables and as mean ± SD for continuous variables.

P values were obtained by Pearson's chi-squared test for categorical variables and Student's *t* test for continuous variables. NOA, non-obese asthma; OA, obese asthma; OC, obese controls. BMI, body mass index; FEV₁, forced exhaled volume at 1 second; PC₂₀, the provocative concentration of methacholine required to cause a 20% fall in FEV₁; Tec, total eosinophil count; Eos, eosinophils; Neu, neutrophils; NA, not applicable.

Table 2. Levels of sphingolipids in the sera of the study subjects

Sphingolipids		NOA (n = 28)	OA (n = 13)	OC (n = 7)	P value		
					OA vs. NOA	OA vs. OC	NOA vs. OC
Sphingomyelin (μg/mL)							
	SM (d18:0/16:0)	82.6 ± 16.7	80.0 ± 16.1	61.4 ± 15.5	.636	.023	.005
	SM (d18:0/18:0)	18.6 ± 3.4	19.8 ± 4.6	16.2 ± 3.2	.356	.088	.106
	SM (d18:0/18:1)	11.5 ± 2.7	12.0 ± 3.7	9.8 ± 2.1	.624	.165	.126
	SM (d18:0/24:0)	23.3 ± 4.2	23.8 ± 4.2	23.7 ± 5.7	.695	.952	.826
	SM (d18:0/24:1)	46.2 ± 7.3	43.3 ± 7.7	40.5 ± 7.3	.262	.430	.073
Ceramide (ng/ml)							
	C14:0 Ceramide	13.6 ± 3.3	15.1 ± 4.9	13.9 ± 4.3	.235	.579	.835
	C16:0 Ceramide	286.9 ± 55.9	302.6 ± 55.8	268.4 ± 54.7	.407	.204	.439
	C18:0 Ceramide	145.7 ± 46.2	180.8 ± 45.2	136.9 ± 52.5	.028	.066	.663
	C18:1 Ceramide	23.2 ± 6.8	26.3 ± 7.7	21.4 ± 8.2	.137	.163	.553
	C20:0 Ceramide	125.1 ± 38.9	153.8 ± 43.7	125.3 ± 59.5	.040	.235	.987
	C24:0 Ceramide	2590.4 ± 670.0	2845.9 ± 466.6	2510.4 ± 604.6	.223	.183	.775
	C24:1 Ceramide	1019.5 ± 319.4	1081.1 ± 272.8	670.6 ± 215.0	.552	.003	.010

Values are given as mean ± SD. *P* values were obtained by Student's *t* test for continuous variables.

Figures

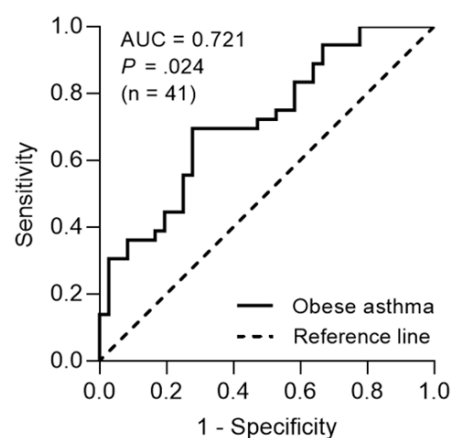


Figure 1. Receiver operating characteristic curve of levels of C18:0 ceramide in sera for discriminating the obese asthma from non-obese asthma group

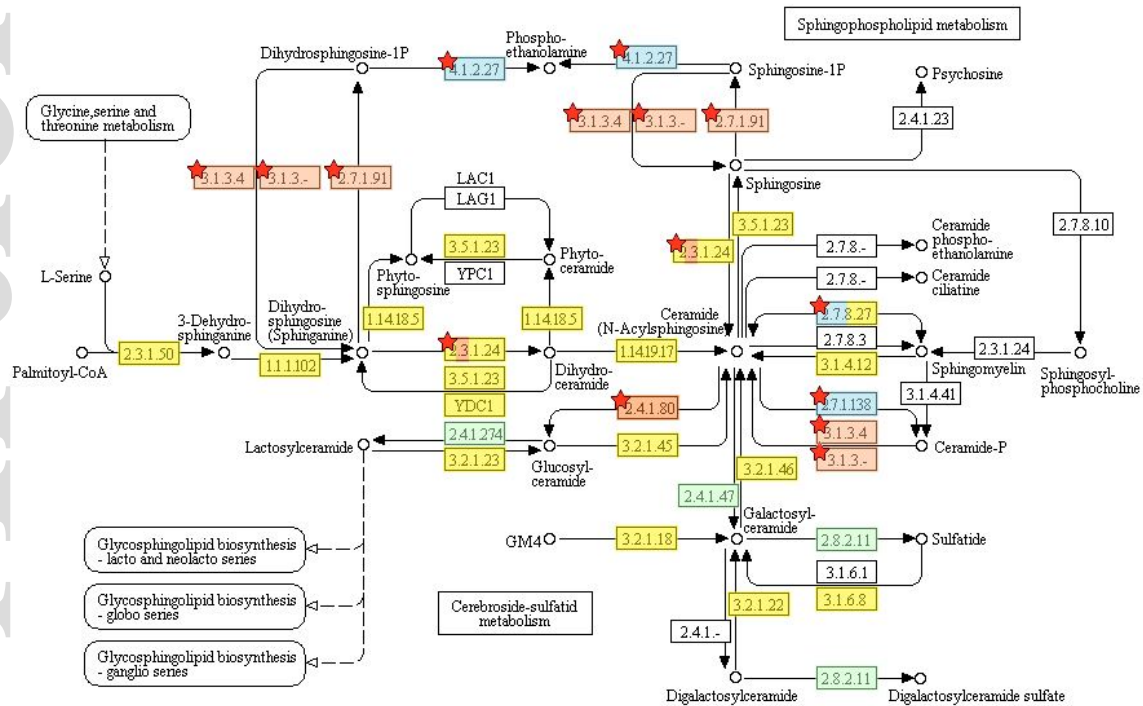


Figure 2. Schematic of gene expressions in sphingolipid metabolisms during M1 macrophage polarization in humans. Boxes in yellow represent up-regulated genes and boxes in blue represent down-regulated genes. Stars indicate significant differences between M1 and M0 control macrophages.

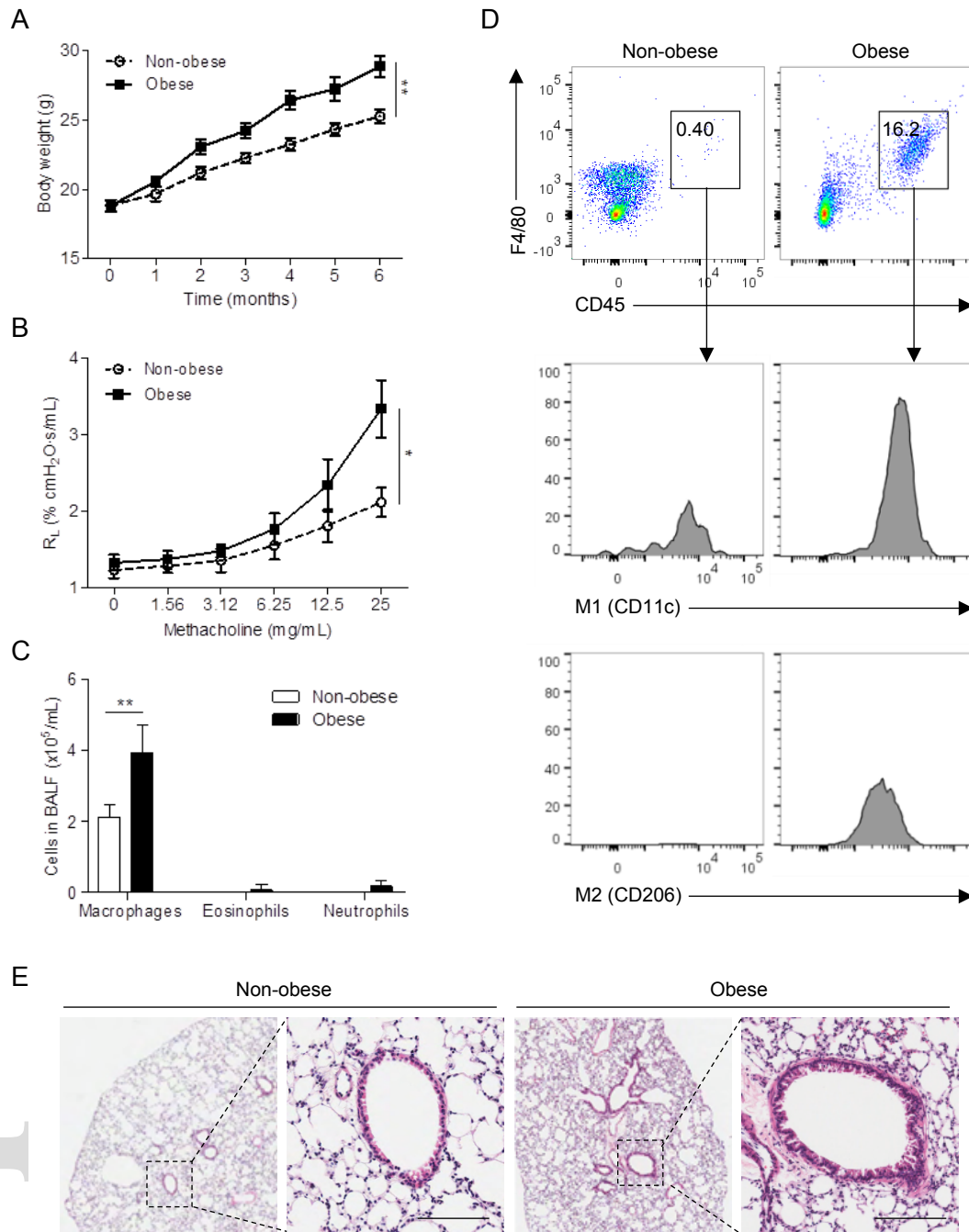


Figure 3. Increased airway hyperresponsiveness in obese mice. (A) Changes in body weight. (B) Airway hyperresponsiveness. (C) Differential cell counts. Data are presented as means $\pm$ SD, $n = 5$. $*P < .05$ and $**P < .01$ obtained by unpaired t test. (D) Macrophage polarization in the lungs. M1 (CD11c) and M2 (CD206) macrophages were determined by gating on CD45⁺F4/80⁺ cells. (E) Lung tissues stained with hematoxylin and eosin. Scale bar, 100 μ m.

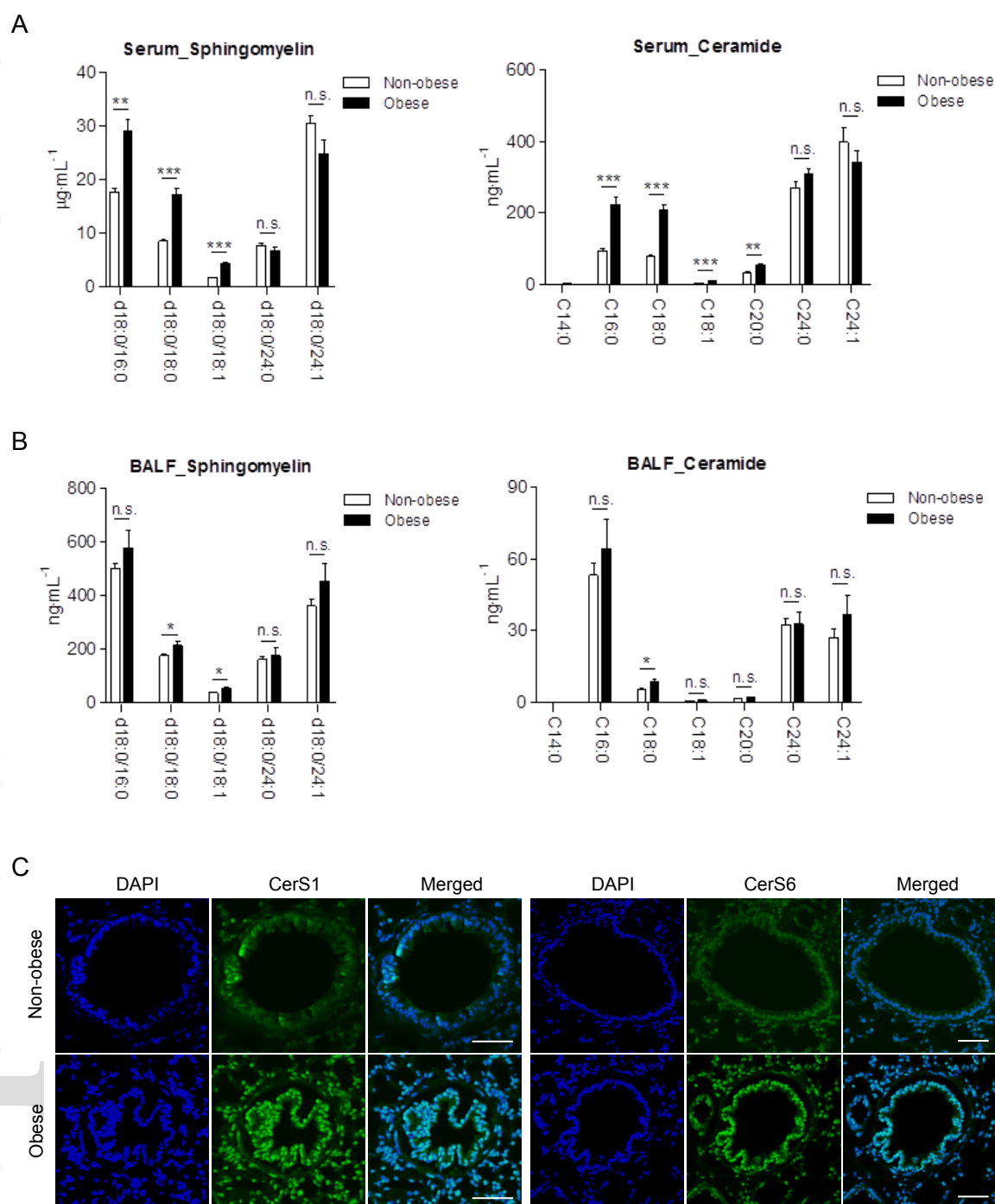


Figure 4. Changes in sphingolipid levels in obese mice. Levels of sphingomyelin and ceramide in (A) serum and (B) BALF analyzed using LC-MS/MS. Data are presented as means $\pm$ SEM, n = approximately 4-5 mice per group. * P < .05, ** P < .01, and *** P < .001 obtained by Unpaired t test. n.s., not significant. (C) Altered expression of CerS1 (left panel) and CerS6 (right panel) in lung tissues. Scale bar, 25 μ m. DAPI, 4',6-diamidino-2-phenylindole; CerS1, ceramide synthase 1; CerS6, ceramide synthase 6.

Supporting Information

Metabolic shift favoring C18:0 ceramide accumulation in obese asthma

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Running title: Alteration of ceramide accumulation in asthma

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Table S1. Differentially expressed genes in sphingolipid metabolism during M1 macrophage polarization in human.

Gene symbol*	Gene name	Fold change M1/M0	Function	KEGG EC number	P value
CERS6	ceramide synthase 6	5.24	Cer synthesis	2.3.1.24	<.001
UGCG	UDP-glucose ceramide glucosyltransferase	4.12	Cer->Glu-Cer	2.4.1.80	<.001
PPAP2B	phosphatidic acid phosphatase type 2B	3.7	C1P->Cer	3.1.3.4	<.001
SPHK1	sphingosine kinase 1	2.82	SO->S1P	2.7.1.91	<.001
SPHK2	phosphatidic acid phosphatase type 2A	2.62	SO->S1P	2.7.1.91	<.001
PPAP2A	sphingosine kinase 2	2.2	C1P->Cer	3.1.3.4	<.001
SGPP1	sphingosine-1-phosphate phosphatase 1	2.12	S1P->SO	3.1.3.-	<.001
SGMS2	sphingomyelin synthase 2	-2.35	Cer->SM	2.7.8.27	<.001
CERK	ceramide kinase	-2.43	Cer->C1P	2.7.1.138	<.001
SGPL1	sphingosine-1-phosphate lyase 1	-3.19	S1P degradation	4.1.2.27	<.001

SO, sphingosine; Cer, ceramide; Glu-Cer, Glucosylceramide; S1P, sphingosine-1-phosphate; C1P, ceramide-1-phosphate.

Supplementary figures

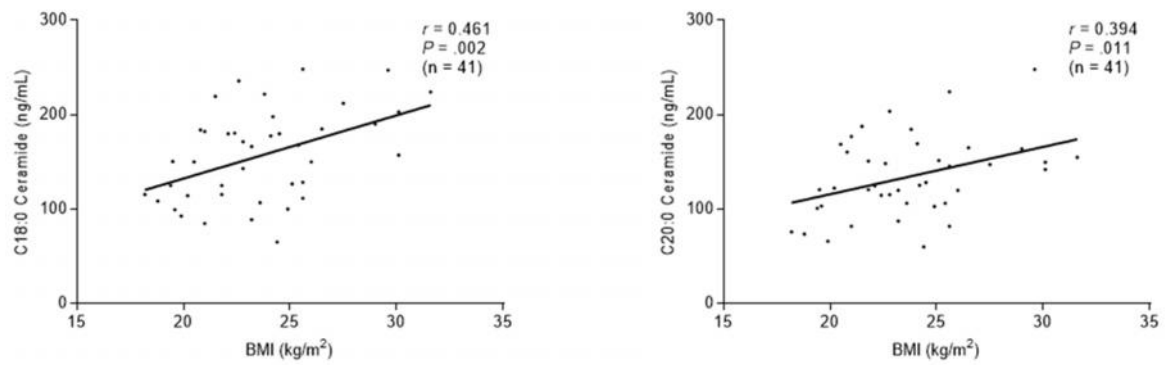


Figure S1. Correlations between BMI and levels of serum ceramides. The data are presented as Spearman correlation coefficient r (P value). BMI, body mass index.

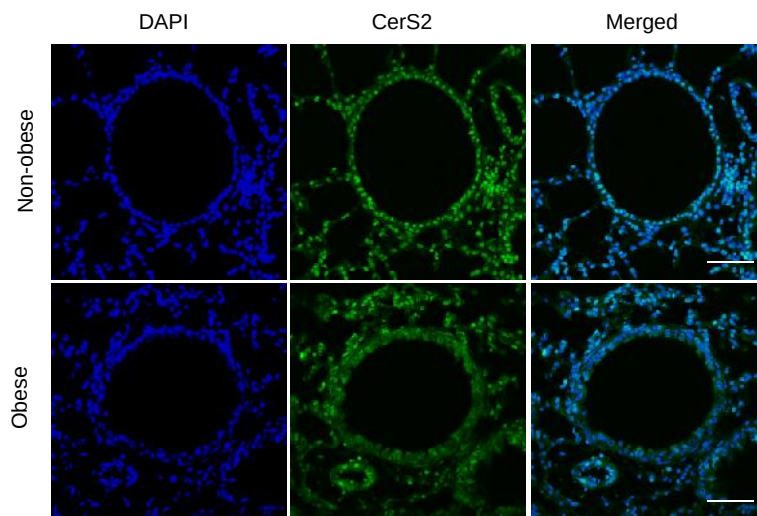


Figure S2. Expression of CerS2 in lung tissues. Scale bar, 25 μm . DAPI, 4',6-diamidino-2-phenylindole; CerS2, ceramide synthase 2.