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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Obesity increases allergic airway inflammation that can be successfully treated by oral tolerance

To the Editor,

The prevalence of obesity and allergy has increased in developed countries over the last decades,[1,2] leading to the hypothesis that these two noncommunicable diseases might be linked. It remains unclear whether obesity, characterized by chronic low-grade inflammation that affects host immunity, contributes to the exacerbation of respiratory allergies. Additionally, the efficacy of inducing mucosal tolerance as a prophylactic intervention against allergic airway disease in obese individuals is still a subject of inquiry. The impact of obesity on immune responses has been demonstrated in vaccinated individuals, revealing diminished vaccine efficacy and impaired duration of protection.[3] Our current study investigates whether obesity could impact respiratory allergy development and the induction of mucosal allergen-specific tolerance.

Male C57BL/6 mice fed with a high-fat diet (HFD) or standard chow diet (STD) for 9 weeks were immunized/sensitized and then challenged with ovalbumin (OVA) (Figure S1A). To induce allergen-specific tolerance, mice were orally treated with OVA before sensitization (Appendix S1).

We detected significant differences in body weight between STD and HFD-fed mice, whereby HFD-fed mice had twice the body weight and significantly higher serum leptin levels than STD-fed mice at the time of sacrifice (Figure 1A,B). Fourier-transform infrared spectroscopy (FTIR) of the cecal content demonstrated distinct spectra between obese and lean mice (Figure 1C–F). These findings were confirmed by 16S rRNA gene sequencing, showing that gut microbial profiles of the cecal content significantly differed between obese and lean mice, consistent with other studies.[4] Specifically, alpha (Figure 1G) and beta (Figure 1H) diversity results revealed that (i) the obese group exhibited significantly lower diversity levels than the lean group and (ii) microbial community structure was strongly affected by diet type (Figure 1I, S2, Table S1).

Obesity exacerbates the allergic response in mice, with sensitization and challenge with OVA leading to significantly heightened manifestations compared to lean mice. This is evidenced by elevated levels of allergen-specific IgE in bronchoalveolar lavage (BAL) and serum (Figure 2A,B), as well as increased levels of IL-5 and IL-13 in the lungs (Figure 2C,D). Eosinophils, B cells, and T cells were upregulated in the lungs of allergic obese compared to allergic lean (Figure 2E,H,I), which explains the significantly higher OVA-specific IL-13 levels (Figure 2D) and exacerbated lung inflammation in the allergic obese group (Figure 2F,G). The rise in Tregs (Figure 2J) was also evident, and this increase could potentially be attributed to the overall expansion of T cells in allergic obese animals.

Interestingly, obesity did not influence the capacity to develop oral tolerance (OT), and tolerized obese mice exhibited a nonallergic phenotype similar to that observed in lean animals. Our results demonstrate an efficient downregulation of allergic parameters upon tolerization (Figure 2A–J; *Allergy* vs. *OT groups*), including IgE levels locally and systemically (Figure 2A,B), OVA-specific IL-5/IL-13 (Figure 2C,D), and decreased lung inflammation (Figure 2F,G). Although significant differences were observed in microbial compounds, microbial diversity, and community structure between obese and lean mice (Figure 1C–I), no additional changes were evident based on the treatment (Allergy, OT, PBS) approach (Figure 2K–M).

We hypothesized that a pro-inflammatory state in the gut of obese mice influenced the microenvironment in the mucus layer around the pulmonary tissue affecting different immune cells, including macrophages. We observed a shift in distributions of activated M1 and M2 macrophages in obese PBS-treated control relative to its lean counterpart. Allergic obese showed a significant reduction of M1 compared to the obese PBS control.

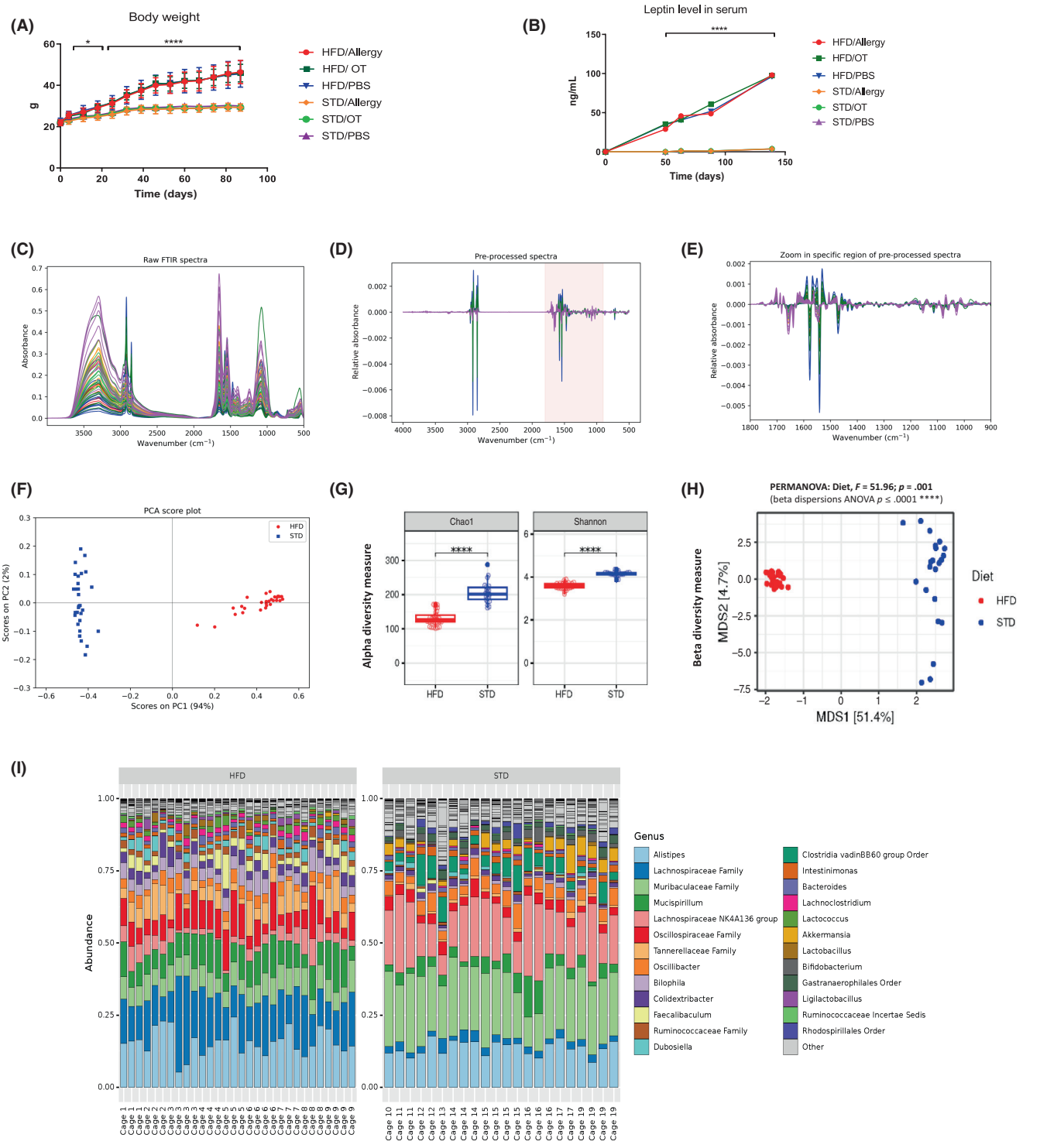

FIGURE 1 HFD-feeding in C57BL/6 mice induces an obese phenotype and leads to a change in the microbiota composition compared to STD-fed mice. (A) Body weight checked during HFD- and STD-feeding once a week. (B) Leptin concentration in sera was measured at five different time points. PCA of FTIR spectra from cecum content stratifies allergic, tolerized, and PBS-treated mice. FTIR spectra are shown as (C) unprocessed and (D) pre-processed with wavenumbers selected for comparison highlighted in red, (E) significant biomolecule regions of 1800–900 cm^{-1}. (F) PCA score plots for all experimental samples, colored based on the diet (red: HFD, blue: STD). (G) Alpha and (H) beta diversity plots are depicted per diet. (I) The relative abundance of taxa of the top 25 most abundant genera across the whole dataset is depicted in [barplot] agglomerated at the genus level. For groups with no genus-level assignment, the closest higher taxonomic level is shown. Test results with a *p*-value below 0.05 were considered significant. For illustrative purposes, *p*-values were indicated as: $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$, $^{****}p < 0.0001$.

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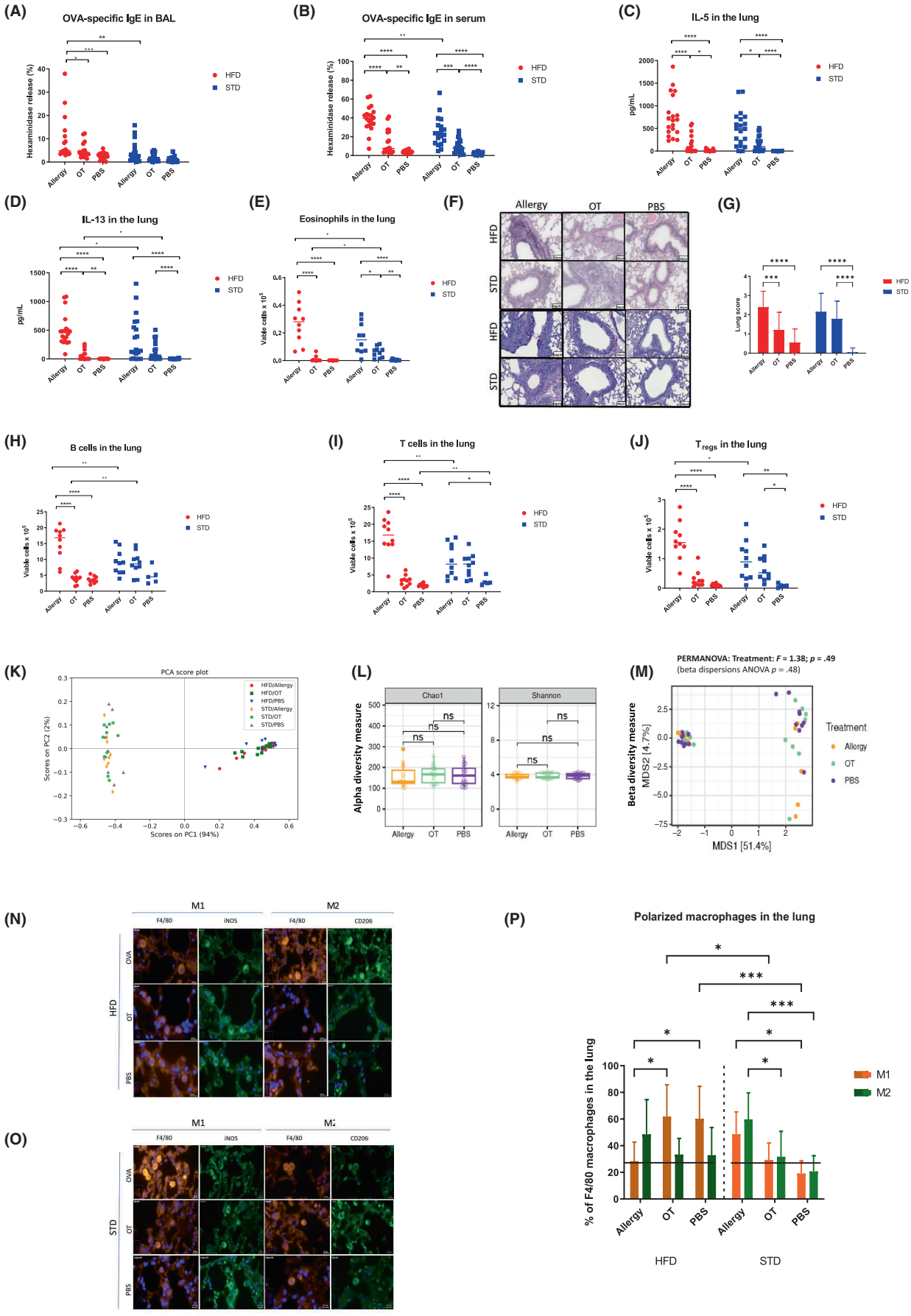

FIGURE 2 Legend on next page

FIGURE 2 Oral treatment with OVA does not affect the microbiota composition but maintains M1 polarized macrophages in obese C57BL/6 mice. Level of β-hexosaminidase released by RBL cells incubated with (A) BAL, and (B) serum samples. (C) The OVA-specific IL-5 and, (D) the OVA-specific IL-13 in OVA-stimulated lung supernatant. (E) The absolute number of eosinophils in the lung (F) H&E-stained and PAS-stained lung sections from one representative sample from each group. (G) Inflammation lung scores. The absolute number of (H) B cells, (I) T cells, and (J) Tregs in the lung. (K) PCA score plots for all experimental samples, colored based on the treatment (red: HFD/Allergy, dark green: HFD/OT, blue: HFD/PBS, orange: STD/Allergy, light green: STD/OT, purple: STD/PBS). (L) Alpha- and (M) beta diversity plots are depicted per treatment. One representative example from lung sections stained with anti-F4/80 + anti-iNOS antibodies and anti-F4/80 + anti-CD206 antibodies in (N) obese and (O) lean mice from allergic, OT and PBS groups. (P) The percentage of M1 and M2 macrophages in the lungs of obese and lean allergic, OT, and PBS-treated mice was analyzed by TissueQuest Software after acquiring images on a TissueFaxs microscope. Test results with a p-value below 0.05 were considered significant. For illustrative purposes, p-values were indicated as: $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$, $^{****}p < 0.0001$.

At the same time, oral tolerance led to an M1/M2 distribution similar to the PBS-treated control group, with significantly more M1 macrophages (Figure 2N–P). Higher allergen-specific IL-13 levels in the lung of the allergic obese shown in our study could be accountable for altered functions of macrophages in allergic obese, initiating macrophage polarization toward the M2 phenotype.

In conclusion, we show that oral tolerance induction could efficiently attenuate the increased allergic response and Th2-mediated lung inflammation associated with respiratory allergy in obese mice. Our results align with studies suggesting that M1 macrophages could be helpful in allergic diseases[5,6] and indicate that oral tolerance in obese and lean may be differently regulated due to a low-grade Th1 inflammatory state associated with increased leptin levels in obese. Therefore, we suggest that mucosal allergen-specific immunomodulation in obese mice functions mechanistically on two levels: (i) by efficiently reducing OVA-specific IL-5/IL-13 in the lungs and IgE in BAL and sera, and (ii) by maintaining and expanding the M1 macrophages in the lungs and thus protecting against Th2-mediated allergic responses.

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AUTHOR CONTRIBUTIONS

U.W., A.I.K., and I.S. conceived the study. A.I.K. and U.W. supervised the experiments. N.G., A.I.K., and U.W. designed the experiments and interpreted the data. N.G. performed the experiments. M.O., E.G.S., A.N., and T.S. helped to establish the obesity animal model. M.A., T.S., and M.E.S., analyzed FTIR results. J.S., performed and analyzed 16S sequencing results. M.O., E.K., and A.S. helped with in vivo experiments. M.K. did a statistical analysis. N.G., U.W., and A.I.K. wrote and reviewed the manuscript. All authors have read and agreed to the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Quantitative risk assessment of egg-white, milk and wheat in infants

To the Editor,

Several randomised controlled trials have revealed that early introduction of food allergens may reduce the risk of IgE-mediated food allergy (FA).[1] However, the optimal, safe and effective dose for prevention remains unknown. In infants with preexisting FA, the risk of an allergic reaction when ingesting a certain amount of the allergen must be considered.

To assess this risk, we analysed data on oral food challenges (OFC) in ≤12 month-old infants between 2014 and 2022 at the National Center for Child Health and Development and investigated the eliciting dose (ED) of egg-white, cow's milk and wheat, comparing it with those of 2 to 15-year-old children.

Children with objective symptoms as OFC-positive were included, whereas those with only subjective symptoms, no FA history with negative OFC, recent antihistamines use, or incomplete medical records were excluded. Figure S1 presents the flowchart of enrolled participants. Thus, 897 boiled egg-white, 646 fresh cow's milk, and 343 udon (Japanese boiled wheat noodles) OFCs, including 197 infants (≤ 12 months) for eggs, 109 for milk, and 91 for wheat were enrolled.

Table 1 summarises participant characteristics. Using interval-censoring survival analysis, we estimated a threshold distribution model and EDs for each food. Details of inclusion criteria, OFC methods[2] (Table S1) and data analysis are presented in the Supplementary Information.

In ≤12-month-old FA infants, the ED_{05} was 28.6 mg (95% confidence interval [CI]:16.6–49.4), 6.1 mg (2.3–16.3), and 27.7 mg (15.4–50.0) for egg-white protein, milk, and wheat, respectively, which were higher than those in older children, tending to decrease with increasing months of age. For example, the prevalence of children experiencing signs and symptoms on ingesting 30 mg of each food was estimated to be 5.2% for egg-white, 13.8% for milk, and 5.6% for