

ORIGINAL PAPER

High-fat feeding redirects cytokine responses and decreases allergic airway eosinophilia

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Summary

Background Dietary fat intake has been associated with obesity and obesity in its turn with attenuated airway function and asthma, but it is unclear whether or how high-fat intake *per se* alters immune function relevant to development of allergic asthma.

Objective To use a non-obese mouse model of mild to moderate allergic asthma to compare effects of high-fat with isocaloric control-diet on allergic immune responses.

Methods C57BL/6 mice weaned and maintained on control (11% fat calories) or isocaloric high-fat diet (58% fat calories) were systemically sensitized with ovalbumin and challenged in the lungs. Allergic airway inflammation was assessed by measuring lung inflammation; serum antibodies; and, cytokines in serum, bronchoalveolar lavage (BAL) fluid and in supernatants of *in vitro* stimulated lung draining lymph node and spleen lymphocytes.

Results There was a significant reduction in lung eosinophilia and IL-5 in high-fat fed mice. Lung draining lymph node cells from these mice showed reduced pro-inflammatory cytokine (MCP-1 and TNF- α) release after ovalbumin re-stimulation and reduced release of IL-13 after concanavalin-A stimulation, indicating a general rather than just an antigen-specific change. There was no difference in IFN- γ release. In contrast, pro-inflammatory cytokine release was increased from splenocytes. Decreased eosinophilia was not due to increased regulatory T cell or IL-10 induction in draining lymph nodes or spleen, nor to changes in antibody response to ovalbumin. However, decreased levels of serum and BAL eotaxin were found in high-fat fed animals.

Conclusions The data indicate that high-fat dietary content redirects local immune responses to allergen in the lungs and systemic responses in the spleen and serum. These effects are not due to changes in regulatory T cell populations but may reflect a failure to mobilize eosinophils in response to allergic challenge.

Keywords allergen, cytokine, diet, eosinophilia, eotaxin, fat, immunity, inflammation, lung

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Introduction

Over the last few decades, incidence of allergic asthma in the developed world has 'exploded' [1–3]. Allergic asthma accounts for over half of cases and results from aberrant immunity to environmental antigens in susceptible individuals whereby allergen exposure results in airway hyperresponsiveness, goblet-cell hyperplasia, and lung inflammation. The immune response involved induces

Type-2 CD4⁺ T-helper (Th2) lymphocytes, IgE antibodies and lung eosinophilia [4].

Many factors have been implicated in increased allergic asthma but possibly one of the most compelling is the simultaneous increase in dietary fat content in the developed world. However, human data on dietary fat content and fatty acid constituents and links to asthma are controversial [5–7] and confounded by association of high-fat intake with obesity [8, 9].

Obesity has been linked to decreased lung function [10–12] and asthma in human [13–16] and experimental studies [17]. However, a clear-cut causal link between

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obesity and Th2 immunity is controversial [16]. Indeed obesity is reported to induce low-grade inflammation associated with increased TNF- α and MCP-1 [18] and to increase Type-1 CD4⁺ T-helper (Th1) activity and IFN- γ in a murine diet-induced model [19].

Few studies have attempted to dissect how high-fat diet in the absence of obesity might affect allergic inflammation. One Dutch study showed that children given full fat dairy products early in life had decreased incidence of later allergies compared with those given skimmed milk products [20]. High-fat diet was reported to increase life-time incidence of asthma in a Swedish study [21]. Varying dietary fat content by increasing omega 3:6 ratios has been reported to reduce asthma incidence [22] whilst other studies report no protective effect [23].

Given these controversies, we wished to distinguish effects high-fat diet *per se* may have on immune function relevant to allergic asthma. To avoid distinction between types of dietary fatty acid and to look simply at effects of increasing total fat calories, we compared effects of isocaloric high-saturated-fat (58% fat as calories) and control (11% fat as calories) diets containing similar amounts of mono- (MUFAs) and poly-unsaturated fatty acids (PUFAs) on immune responses in a non-obese mouse model of allergic airway inflammation (AAI).

To test a null hypothesis, we chose to use C57Bl/6 mice as a model of 'mild to moderate' AAI rather than the 'severe' Th2-skewed-BALB/c strain model [24, 25] to have the best opportunity of seeing either improvement or worsening of disease markers. C57Bl/6 mice were fed high-fat or control-diet from weaning and throughout sensitization and challenge in ovalbumin (OVA)-induced AAI. Like allergic asthma, this generates Th2 immunity resulting in lung eosinophilia, goblet-cell hyperplasia and IgE antibodies, similar to that we reported for house-dust mite allergen [26]. Unexpectedly, high-fat fed mice had less lung eosinophilia than control fed animals. The data support the idea that high-fat diet affects immune responses and indicate that high-fat content in diet *per se* does not predispose towards allergic inflammation.

Materials and methods

Animals and diets

Animals were bred and maintained in specific and opportunistic pathogen free facilities at the University of Edinburgh. Experiments were performed with UK Home Office approval. C57BL/6J female mice were fed isocaloric control (11% fat as calories; D12328; Research Diets Inc., New Brunswick, NJ, USA) or high-fat (58% fat as calories; D12331; Research Diets Inc.) diet from weaning at 3–4 w (see Table 1 for details). Both diets contained similar amounts of MUFAs and PUFAs. Mice ate approximately 3 g/day, giving a daily intake of 45 mg PUFAs, well below

Table 1. Diet constituents

Ingredient	Control (kcal%) D12328	HF (kcal%) D12330
Protein	16.4	16.4
Carbohydrate	73.1	25.5
Fat	10.5	58
Total	100	100
	Control (kcal)	HF (kcal)
Casein, 80 mesh	912	912
Maltodextrin 10	680	680
Corn starch	3340	700
Soybean oil	225	225
Coconut oil, hydrogenated	360	3001.5
Vitamin mix V10001	40	40
Total	5557	5558.5
	LF (mg/g)	HF (mg/g)
Saturated fatty acids	43.2	334
Mono-unsaturated fatty acids	6.4	8.7
Poly-unsaturated fatty acids	15.3	15.4

doses (250 mg–10 g/day) reported to have anti-inflammatory effects [27, 28]. Animals were randomly assigned to dietary group.

Sensitization, airway challenge and serum collection

At 8 and 10 w (experimental days 1 and 14), mice were sensitized by intra-peritoneal injection of 100 μ L phosphate-buffered saline (PBS) containing 300 μ g OVA (Sigma-Aldrich, Poole, UK) absorbed on 2 mg aluminium hydroxide (Imject-ALUM; Pierce, Rockford, IL, USA). Airway inflammation was induced under anaesthetic (0.1 mg/g Avertin, 2,2,2-tribromo-ethanol) by intra-tracheal instillation of 50 μ L PBS containing 10 μ g OVA on experimental days 28 and 31 ($n = 4$ –8/group). Four days later (experimental day 35), animals were killed in random order. Blood samples were collected, clotted, serum separated by centrifugation and stored at -20°C until use. At the end of the protocol, mice had been fed high-fat or control-diet for 8–9 w.

Bronchoalveolar lavage

As described [26], the trachea was exposed and cannulated. Lungs were lavaged twice with 800 μ L PBS and samples centrifuged at 300 g/5 min. BAL fluid was removed and stored at -20°C . Cell pellets were resuspended in PBS, counted, cyto-centrifuged at 300 r.p.m. (10g)/3 min onto glass slides, air-dried, methanol fixed, and stained with DiffQuik (CellPath Store, Newtown, Powys, UK). Differential cell counts were performed (at least 300 cells/slide) by the same individual (SEMh) blinded to experimental details. ELISA DuoSet kits (R&D

Systems, Abingdon, UK) were used to measure eotaxin, IL-5 and IL-13 in BAL fluid.

Analysis of lung tissue

As described [26], after BAL, lungs were transcardially perfused with PBS, inflated with and fixed in 4% neutral-buffered formalin before paraffin embedding. Sections (3 µm) were stained with haematoxylin and eosin (H&E) or Periodic Acid Schiff (PAS). Inflammation was scored blindly [26] on H&E sections at 200× magnification on an increasing severity score of 1–4 in the peri-vascular compartment (1 = no, 2 = < 20, 3 = < 100, 4 = > 100 cells), bronchiolar epithelium (1 = no, 2 = < 5, 3 = 5–10, 4 = > 10 cells), peri-bronchiolar alveolar tissue (1 = no, 2 = < 20, 3 = 20–100, 4 = > 100 cells) and alveolar walls (1 = no expansion, 2 = focal cellular expansion of alveolar walls by 2–3 cells, 3 = by 4–5 cells, 4 = by > 5 cells). Eosinophilia was determined as percentage of infiltrating cells in lung tissue (not airspaces) of the same fields and was confirmed by immunohistochemistry [26] using monoclonal eosinophil-specific antibody [29] (kindly supplied by Dr J. Lee, Mayo Clinic, Rochester, MN, USA). The average score in each compartment for 10 consecutive fields/mouse, each containing a complete transection of a small bronchiole > 300 µm diameter, and from this the average score/experimental group was calculated. Goblet-cell hyperplasia was scored on PAS sections in 10 small completely transected bronchioles > 300 µm diameter at 400× magnification and expressed as average percentage goblet-cells/bronchiole.

Fat-pad examination

Inguinal fat-pads were weighed, fixed in 4% neutral-buffered formalin and paraffin embedded. Sections (3 µm) were dewaxed, antigen retrieved and stained for the macrophage marker F4/80 (AbD Serotec, Kidlington, UK) using secondary biotinylated-anti-rat IgG antibody (DAKO, Ely, UK) and visualized with ABC-HRP and DAB (Vector Laboratories, Peterborough, UK).

Lymph node and splenocyte culture

As described [26], before lung fixation, lung draining mediastinal lymph nodes (dLN) and spleen were removed. Single-cell suspensions were washed, resuspended in RPMI1640 supplemented with 10% FCS, 100 U/mL penicillin, 100 µg/mL streptomycin, 2 mM L-glutamine and 50 µM 2-ME (all Sigma-Aldrich) and counted using trypan blue exclusion. Cells (always > 96% viable) were adjusted to $5 \times 10^6 \text{ mL}^{-1}$, plated in 96-well round bottom plates (100 µL/well) and stimulated with 100 µL medium alone, or containing different concentrations of OVA or concanavalin-A (Con-A). Supernatants were harvested after

48 h and stored at -80°C before cytokine analysis using flow cytometric bead assay for TNF- α , MCP-1, IFN- γ and IL-10 (BD Biosciences, Oxford, UK), and ELISA DuoSet kits for IL-13 and IL-5 (R&D Systems). At 48 h, cells were pulsed with 0.5 µCi ^3H -thymidine/well (GE Healthcare Ltd, Little Chalfont, UK), harvested at 72 h and incorporation read on a betaplate scintillation counter (Wallac UK, Milton Keynes, UK).

Flow cytometric analysis of lymphocytes

Spleen and dLN cells stained with appropriate isotype controls, CD3-FITC, or CD19-PE or triple-stained with CD4-FITC, CD25-PECY5.5 (BD Biosciences) and intracellular anti-Foxp3-APC (eBioscience, Wembley, UK), as recommended by the manufacturers, were acquired on a BD FACS Calibur using a viable lymphocyte gate and analysed using CELLQuestTM software.

Enzyme-linked immunosorbent assays for ovalbumin-specific antibody

Microtitre wells were coated with 0.5 µg OVA in 50 µL 0.05 M carbonate/bicarbonate buffer, pH 9.6, overnight at 4°C , blocked with BSA, serum dilutions added and bound antibody detected using biotinylated-rat-anti-mouse IgG1 (Clone LO-MG1-2; Serotec) or mouse-anti-mouse IgG2a^b (Clone 5.7; BD Biosciences) or rat-anti-mouse IgE (clone R35-72, BD Biosciences). For OVA-specific IgE, serum aliquots were depleted of IgG using Protein-G beads (Amersham Pharmacia, UK). Plates were developed using streptavidin-HRP and tetramethylbenzidine substrate (R&D Systems) and read on a BIOTEKTM plate reader.

Statistics

Data were analysed using unpaired Student's *t*-test with Welch's correction on Graphpad PrismTM software and are presented as mean ± SEM; $P < 0.05$ was regarded as significant.

Results

Ovalbumin-induced allergic airway inflammation and serum antibody responses

OVA-induced AAI was first established in control-diet fed mice. From preliminary experiments, lung inflammation peaked 4 days after OVA challenge (not shown), which was used as the end point for all following experiments. Figure 1 shows that compared with PBS challenge, total BAL cells, BAL eosinophils, BAL lymphocytes, lung inflammation, tissue eosinophilia and goblet-cell hyperplasia were all significantly higher after OVA challenge. OVA-specific

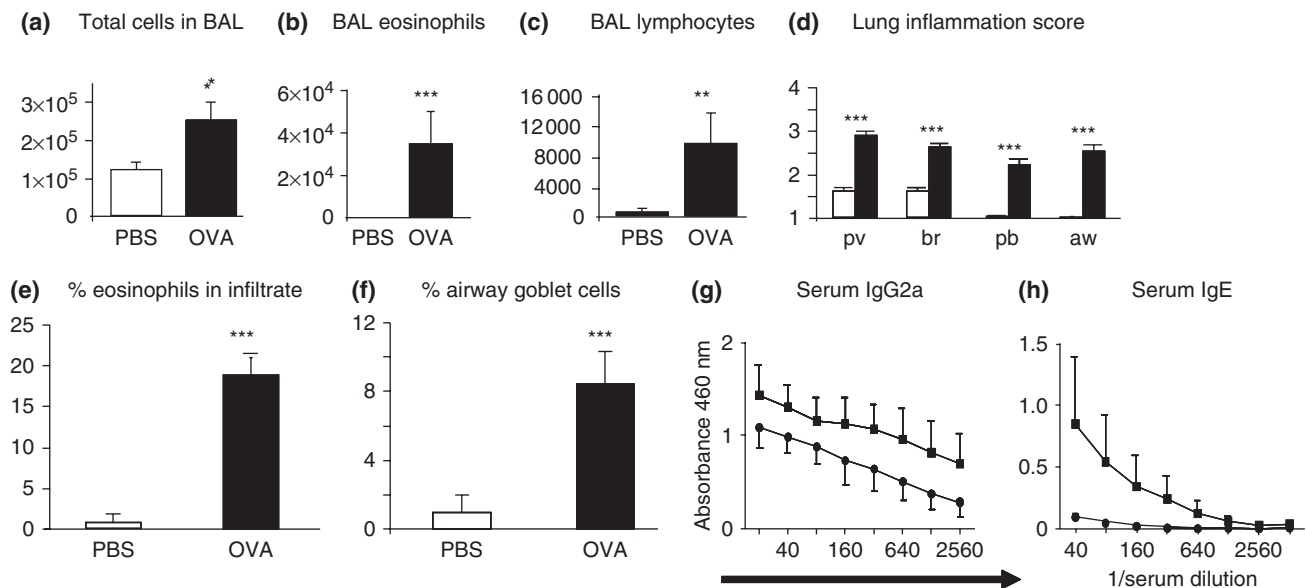


Fig. 1. Ovalbumin (OVA)-induced allergic airway inflammation (AAI). All mice fed control-diet, sensitized with OVA and phosphate-buffered saline (PBS) (striped boxes) or OVA (filled boxes) challenged. Representative of three experiments. Total bronchoalveolar lavage (BAL) cells (a), eosinophils (b), and lymphocytes (c); (d) inflammation scores in peri-vascular (pv), peri-bronchiolar (pb), bronchiolar (br) and alveolar wall (aw) lung compartments; (e) percentage eosinophils in lung tissue infiltrate; (f) percentage goblet-cells in bronchioles. OVA-specific serum IgG2a (g) and IgE (h) after challenge with OVA (square symbols) or PBS (round symbols). * $P < 0.05$; ** $P < 0.03$, *** $P < 0.005$.

IgG2a was present in all sera and was boosted by OVA challenge, IgE was boosted only in OVA challenged mice.

Effect of high-fat diet on weight and fat-pad inflammation

Mice with AAI on high-fat diet were heavier than those on control-diet (22.2 ± 1.2 g, vs. 19.4 ± 0.3 g). Although this was a significant increase ($P < 0.05$), animals were not obese and both groups were within 1 SD of the expected weight for 13 w old female C57Bl/6 mice (22.21 ± 1.80 g, <http://jaxmice.jax.org/support/phenotyping/B6data000664.pdf>). Inguinal fat-pads were all macroscopically normal, there was no difference in their weight and no inflammation in stained sections. On average, < 1 cell was positive for the macrophage marker F4/80 per high power ($\times 630$) field (not shown).

Effect of high-fat diet on lung inflammation and serum-antibody responses

All mice sensitized and challenged with OVA had lung inflammation. BAL showed no difference in total cells (Fig. 2a) but high-fat fed mice had more macrophages (Fig. 2b) and fewer lymphocytes (Fig. 2c) and eosinophils (Figs 2d and e). The Th2 cytokine IL-13 was undetectable in BAL fluid (not shown), whereas IL-5 was significantly decreased in high-fat fed mice (Fig. 2f). There was significantly less eosinophilia in the high-fat group (Fig. 2g). Despite decreased eosinophilia, the percentage of goblet-cells was unchanged by diet (Fig. 2i) and there was no

difference in serum OVA-specific IgG2a (Fig. 2j) or IgE (Fig. 2k). Figures 3a and b show representative histology, Fig. 3c eosinophil immunohistochemistry and Fig. 3d BAL eosinophilia in control fed mice. Histology of the predominantly mononuclear inflammation in high-fat fed mice is illustrated in Figs 3e and f.

Interleukin-13 release from draining mediastinal lymph node but not spleen cells is down-regulated by high-fat diet

There was no significant difference in IFN- γ release by dLN or spleen cells from OVA sensitized and challenged mice cultured with Con-A or OVA regardless of diet in any of three experiments (not shown).

The local lymphocyte response was measured by *in vitro* stimulation of dLN cells. Allergen-specific responses were measured after OVA stimulation and general responses after stimulation with the T cell mitogen, Con-A. As seen in Fig. 4, IL-13 release after Con-A treatment was significantly less after high-fat feeding, whereas IL-4 was unchanged. The Type-2 cytokine IL-5 showed a trend for decreased release on OVA re-stimulation (control-diet dLN 149.4 ± 56.2 , high-fat diet dLN 105.480 ± 63.7 pg/mL, $n = 5$) as did the regulatory cytokine IL-10 (control-diet dLN 35.1 ± 19.3 pg/mL, high-fat diet dLN 6.0 ± 3.1 pg/mL, $n = 5$), but these did not reach significance.

The systemic lymphocyte response was determined by culturing spleen cells which released the same Type-2 and regulatory cytokines when incubated with OVA (IL-5 control-diet 55.7 ± 6.8 pg/mL, high-fat diet 65 ± 5.9 pg/

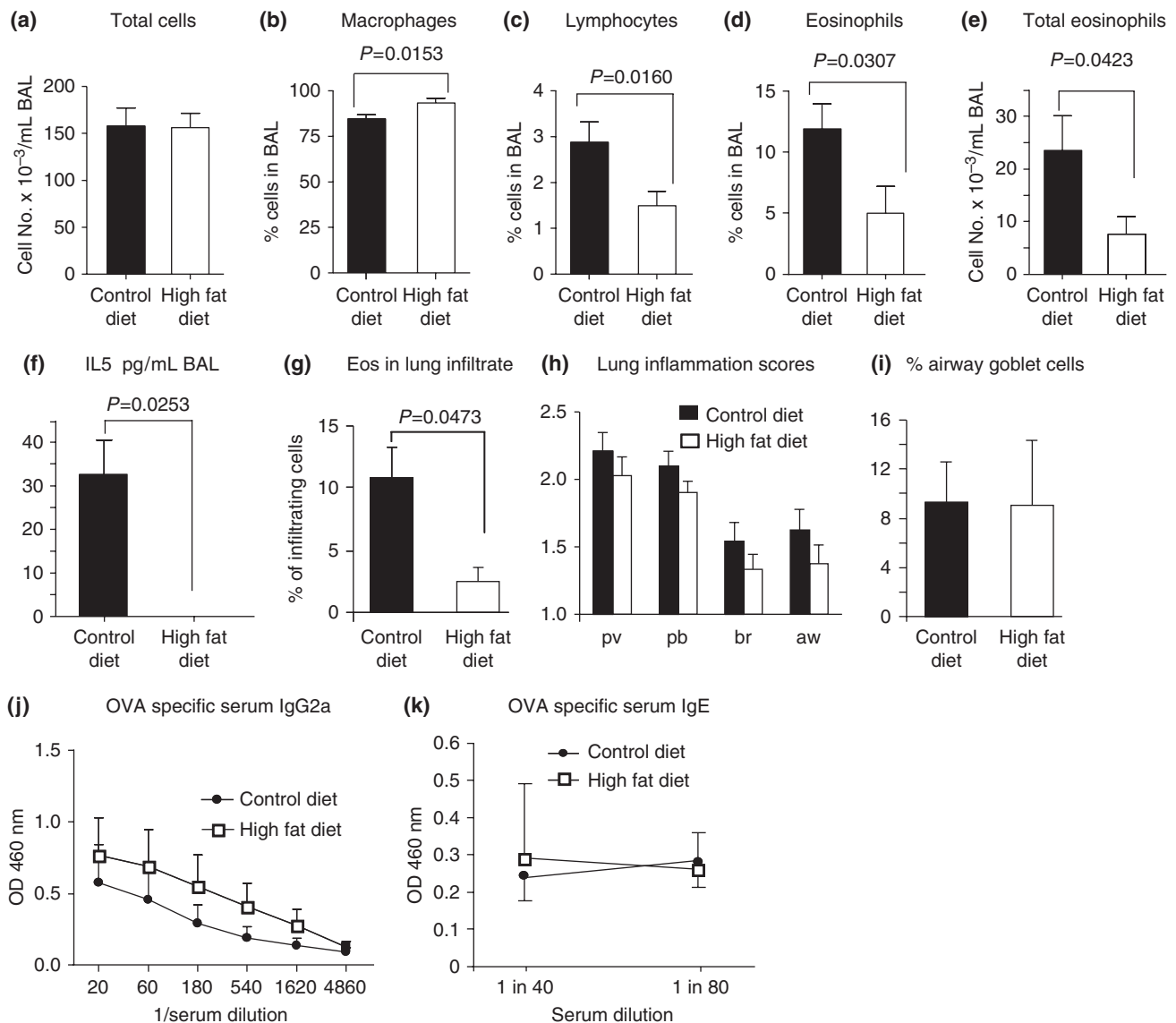


Fig. 2. Eosinophilia in airways and lung tissue and IL-5 in bronchoalveolar lavage (BAL) is reduced by high-fat diet. All mice sensitized and challenged with ovalbumin (OVA). a–f) Pooled data from four experiments: (a) total cell number; (b) percentage macrophages; (c) percentage lymphocytes; (d) percentage eosinophils; (e) total eosinophil number; (f) IL-5; in BAL. (g–k) Representative of four experiments: (g) percentage of lung tissue infiltrating cells that were eosinophils; (h) inflammation in peri-vascular (pv), peri-bronchiolar (pb), bronchiolar (br) and alveolar wall (aw) compartments; (i) percentage of bronchiolar cells that were mucin+ve goblet-cells; (j, k) OVA-specific IgG2a (j) and IgE (k) serum antibodies.

mL; IL-10 control-diet 265 ± 41.7 pg/mL, high-fat diet 331.7 ± 76.5 pg/mL) or Con-A (not shown), but these showed no differences induced by diet.

Differential effects of high-fat diet on pro-inflammatory cytokine release by ovalbumin-stimulated splenocytes and draining mediastinal lymph nodes lymphocytes

As both MCP-1 and TNF- α are associated with inflammation in high-fat diet-induced obesity [30, 31], we determined whether they were affected in our model. After re-stimulation, splenocytes from high-fat fed

mice released significantly more MCP-1 and there was a non-significant trend for more TNF- α release (Figs 5a and b).

Fewer cells were obtained from lung dLNs so only one dose of OVA was tested. In contrast to splenocytes, both MCP-1 and TNF- α were significantly decreased after re-stimulation of dLN cells from high-fat diet mice (Figs 5c and d).

Differences in cytokine secretion were not due to proliferative changes as spleen and dLN cells from high-fat and control fed mice incorporated similar amounts of ^3H -thymidine whether incubated in medium alone, different amounts of OVA, or Con-A (Figs 5e and f).

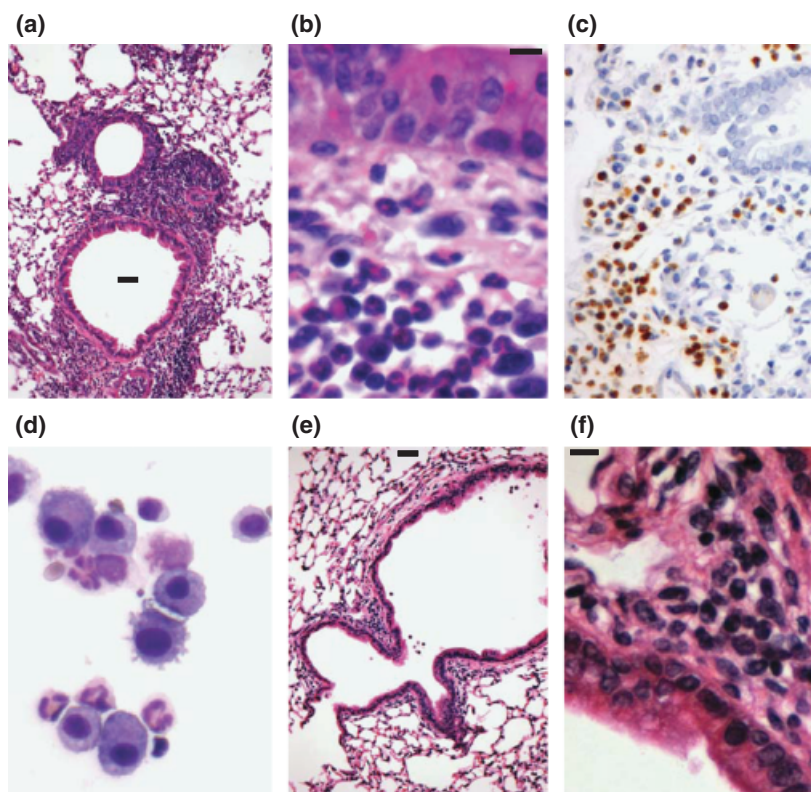


Fig. 3. Histology of allergic airway inflammation (AAI). Representative pictures. All mice sensitized and challenged with ovalbumin (OVA). (a, b) H&E staining of control-diet lungs at (a) $\times 100$ and (b) $\times 630$ magnification showing eosinophilia in infiltrate; (c) immunohistochemistry with eosinophil-specific antibody; (d) cytopsin of bronchoalveolar lavage (BAL) cells from control fed mice showing eosinophilia; (e, f) H&E staining of high-fat diet lungs at (e) $\times 100$ and (f) $\times 630$ magnification showing lack of eosinophils in infiltrate. (a, e) bar = 100 μm ; (b, f) bar = 20 μm .

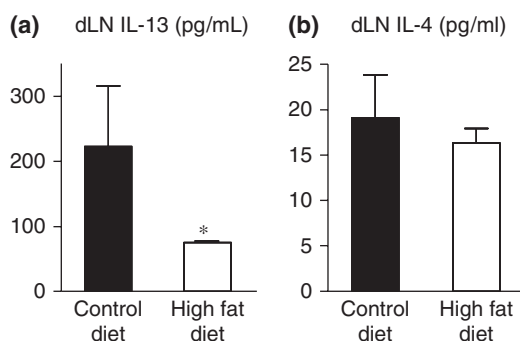


Fig. 4. Type-2 cytokine release. Representative of three experiments. All mice sensitized and challenged with ovalbumin (OVA). IL-13 (a) and IL-4 (b) in supernatants from lung draining mediastinal lymph node (dLN) cells stimulated *in vitro* with Con-A for 48 h. * $P < 0.05$.

Decreased eosinophilia is not associated with increased Treg cells

There was no difference in numbers or percentages of T cells ($\text{CD}3^+$) or B cells ($\text{CD}19^+$) in lung dLN or spleens between control or high-fat fed mice (not shown). Neither was there any difference in $\text{CD}4^+\text{CD}25^+\text{foxp}3^+$ Tregs in lung dLN (Fig. 6) or spleens (not shown). This is consistent

with the lack of difference in IL-10 secretion referred to above.

Decreased eosinophilia is associated with decreased serum and bronchoalveolar lavage eotaxin

As eotaxin is a major eosinophil chemo-attractant, we determined whether or not there was a local or systemic decrease in eotaxin in mice on high-fat diet. There was significantly less serum eotaxin (Fig. 7a) in high-fat fed mice. Eotaxin was undetectable in all BALs from high-fat fed mice but was present in BAL from control fed mice (Fig. 7b).

Discussion

Here we demonstrate that high-fat diet attenuated lung eosinophilia in a non-obese murine model of mild to moderate AAI. Without altering serum antibody responses, or goblet-cell hyperplasia, high-fat diet also significantly reduced BAL IL-5, serum and BAL eotaxin, and IL-13 secretion by lung dLN cells.

Reduced eotaxin is consistent with data showing that its release is stimulated by IL-13 [32]. The reduction in dLN cell IL-13 secretion was not compensated by increased

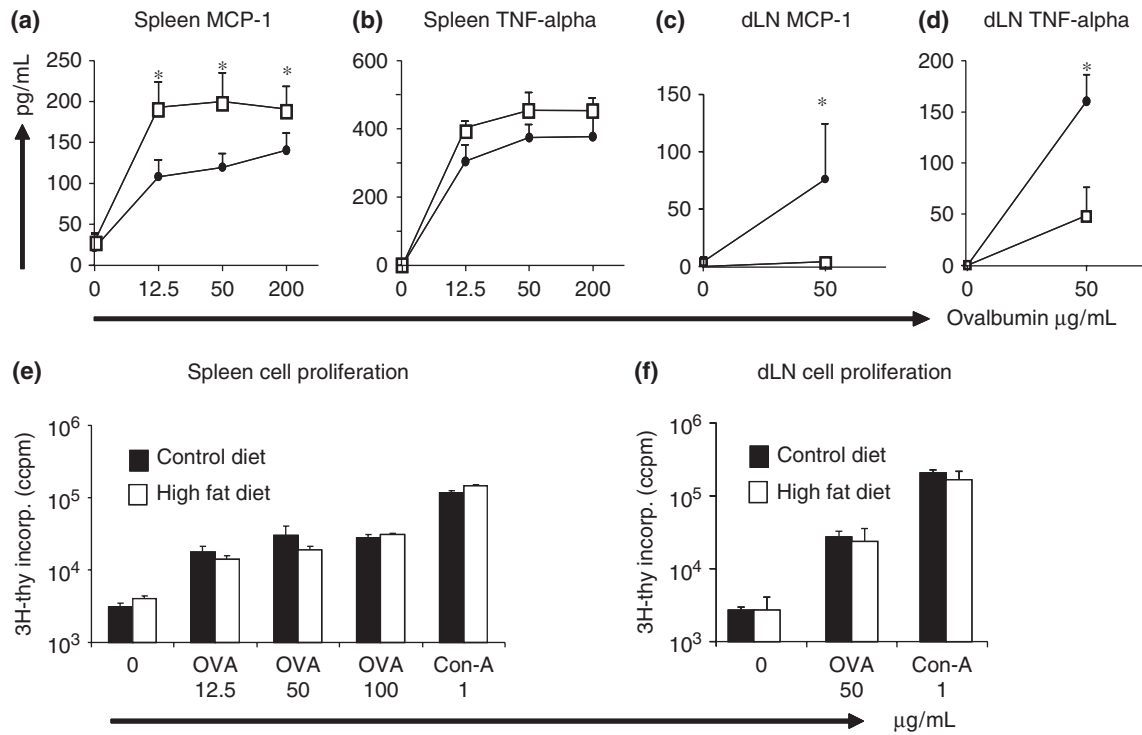


Fig. 5. Spleen and draining mediastinal lymph node (dLN) pro-inflammatory cytokine release is differentially modified by high-fat diet whilst proliferation is unchanged. Representative of three experiments. All mice sensitized and challenged with ovalbumin (OVA). (a–d) MCP-1 and TNF- α in supernatants from spleen (a, b) and lung dLN cells (c, d) stimulated *in vitro* for 48 h with medium only or different concentrations of OVA. Open square symbols, high-fat fed mice; closed round symbols, control fed mice. * $P < 0.05$. (e, f) Proliferation measured by ³H-thymidine incorporation by spleen (e) and dLN (f) cells after 72 h culture.

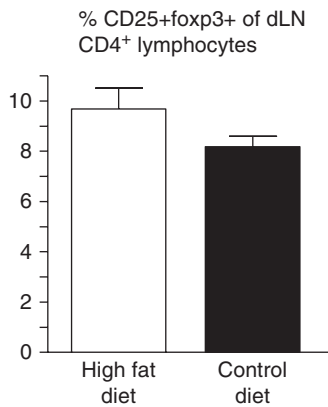


Fig. 6. CD4⁺CD25⁺foxP3⁺ Treg cells are unchanged by diet. Representative of three experiments. All mice sensitized and challenged with ovalbumin (OVA). Percentage of CD4⁺CD25⁺foxP3⁺ lymphocytes in lung draining mediastinal lymph node (dLN) cells determined by triple-colour flow cytometry.

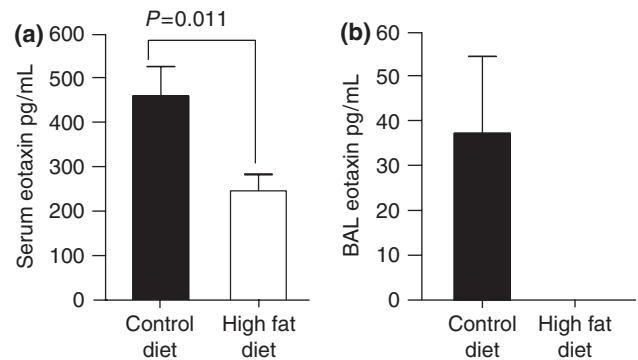


Fig. 7. Decreased eotaxin in serum and bronchoalveolar lavage (BAL) of high-fat fed mice. Pooled data from three experiments. All mice sensitized and challenged with ovalbumin (OVA). (a) Eotaxin is significantly decreased in sera from the high-fat diet group. (b) Eotaxin is only detectable in BAL from control fed mice.

Type-1 or pro-inflammatory cytokine release as IFN- γ was not altered and TNF- α and MCP-1 levels were lower from dLN cells of high-fat fed mice. In contrast, high-fat diet had a differential effect on spleen responses and OVA re-stimulation induced significantly higher MCP-1 release from splenocytes of high-fat fed mice. Thus, high-fat diet

appeared to alter the 'setting' of the spleen response towards increased production of pro-inflammatory cytokines.

Obesity has been well studied in rodents [33] and obese adipose tissue is described as a pro-inflammatory environment [34, 35]. Johnston and colleagues reported

decreased airway eosinophilia in murine monogenic obesity [36] and diet-induced obesity has been linked with increased Th1 activity [19]. In humans, obesity has been associated with increased asthma severity [37, 38] and been postulated to predispose towards decreased immune tolerance and increased Th2 responses [39]. Obese children were reported to have increased serum IL-6 and decreased post-vaccination anti-tetanus antibodies, which was suggested to be due to generalized low-grade inflammation [40].

However, there are few studies on effects of high-fat diet on immune responses and inflammation in the absence of obesity. Puertollano et al. [41] showed increased serum TNF- α in *Listeria monocytogenes* infected mice fed high-fat diet for 4 weeks, although no data regarding weight change is given.

The high-fat fed mice in our study were not obese. At the end of the experimental protocol, mice had been on the diets for just over 8 w and those on high-fat diet were on average 14% heavier than control fed animals. Both groups were within 1 SD of the expected weight for 13 w female C57Bl/6 mice. Mice on the C57Bl/6 background in the same animal house do become significantly obese after 13–14 w on the same high-fat diet [42]. Thus, the high-fat fed mice sensitized and challenged here could be regarded as being in a 'pre-obese state' at the end of the experiments. There was no sign of inflammation in inguinal fat-pads in high-fat fed animals. Our data show that increased dietary fat promotes pro-inflammatory responses in spleen and this does not require induction of overt obesity.

We speculated that attenuation of airway eosinophilia in high-fat fed mice could be caused by increased Tregs, which can decrease lung eosinophilia [26, 43–45]. However, we found no evidence for increased Tregs in lung dLN or spleens of high-fat fed mice or for any difference in IL-10 secretion.

Perinodal fat around lymph nodes is highly specialized [46] and can attenuate lymphocyte activation in proportion to rate of lipolysis [47]. IL-4 abolishes adipocyte lipolysis [48], whereas guinea-pigs fed saturated fats show increased lipolysis of perinodal fat adipocytes [47, 48], indicating that high-fat diet and Type-2 cytokines can have opposing effects on the potential of perinodal fat to affect immune responses. If the high-saturated-fat diet used here did increase lipolysis in perinodal fat, this may in part account for the decreased ability of lung dLN cells to release IL-13, MCP-1 and TNF- α .

The finding that eotaxin was decreased in serum and BAL from high-fat fed compared with control fed mice is intriguing as serum eotaxin/CCL11 was reported to be increased in diet-induced obese C57Bl/6 mice after 26 weeks [49, 50]. Thus, our data suggest that high-fat diet *per se* might influence local inflammation induced by allergens by different mechanisms to those induced by overt obesity. It remains to be established whether there is

a critical point in obesogenesis where a 'switch' from one type of inflammatory immune response to another occurs.

In conclusion, we show that obesogenic high-saturated-fat diet in mice that were not yet obese unexpectedly attenuated lung eosinophilia in a mouse model of mild to moderate AAI. This was accompanied by significantly decreased release of the Type-2 cytokine IL-13 and the pro-inflammatory cytokines MCP-1 and TNF- α by local, lung dLN cells whilst pro-inflammatory cytokine release was up-regulated from spleen cells. The reduced eosinophilia may be explained by reduced serum and BAL eotaxin and reduced BAL IL-5 as both eotaxin and IL-5 are eosinophil chemo-attractants. Thus, high-fat diet appears to compromise the ability to mobilize eosinophils in response to local allergen challenge in the lungs.

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