

Esser Philipp (Orcid ID: 0000-0002-7379-9699)
Martin Stefan (Orcid ID: 0000-0002-1617-1280)

Feeding of fat-enriched diet causes the loss of resistance to contact hypersensitivity

Anne-Catherine Rühl-Muth^{1,2*}, Mareike Maler^{1,2}, Philipp R. Esser¹, Stefan F. Martin^{1*}

¹Allergy Research Group, Department of Dermatology, Medical Center-University of Freiburg, Faculty of Medicine, University of Freiburg, Hauptstraße 7, D-79104 Freiburg;

²Faculty of Biology, University of Freiburg

*Correspondence:

Stefan F. Martin, Ph.D
Allergy Research Group
Department of Dermatology
Medical Center - University of Freiburg
Hauptstrasse 7
D-79104 Freiburg
Germany
Phone: +49-761-270-67380
Fax: +49-761-270-66550
E-Mail: stefan.martin@uniklinik-freiburg.de
ORCID: <https://orcid.org/0000-0002-1617-1280>

Abbreviations

Allergic contact dermatitis ACD, Contact hypersensitivity, CHS; control diet, CD; epididymal, epi; high fat diet, HFD; normal chow, NC; pattern recognition receptor, PRR; sub cutaneous, sc; 2,4,6-trinitro-1-chlorobenzene TNCB; wildtype wt;

Acknowledgement

We are grateful to Ursula Voith for excellent technical assistance. This work was supported by a DFG grant, DFG MA 1567/15-1 to S.F.M.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Keywords

allergic contact dermatitis, inflammation, high fat diet, Toll-like receptor

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the [Version of Record](#). Please cite this article as doi: [10.1111/cod.13927](https://doi.org/10.1111/cod.13927)

Abstract

Background:

Low molecular weight chemicals or metal ions can cause allergic contact dermatitis, an inflammatory skin disease. Mice lacking Toll-like receptors 2 and 4 (TLR2/4 mice) are resistant to contact hypersensitivity. In the Western population obesity is increasing, which is known to have a pro-inflammatory impact.

Objectives:

The aim of this study was to investigate the impact of high fat diet on the sensitization and elicitation of CHS. We hypothesized that a pro-inflammatory micro-milieu can be caused by an increase in adipose tissue, which might be sufficient to break the resistance of TLR2/4 mice.

Methods:

Four weeks prior to sensitization wildtype (wt) or TLR2/4 mice were fed normal chow, control diet or high fat diet. The effects on CHS and inflammation were analysed measuring the ear swelling response, using flow cytometry and ELISA.

Results:

The reaction of wt mice to TNCB was increased by high fat diet. While normal-chow fed TLR2/4 mice were still resistant to CHS, high fat diet and, unexpectedly, control diet feeding broke the resistance of TLR2/4 mice to TNCB.

Conclusions:

These experiments suggest that the increased fat content or the different fatty acid composition of the diets increases inflammation and, therefore, the likelihood of developing CHS.

1. Introduction

Allergic contact dermatitis (ACD) is a T cell mediated delayed type hypersensitivity reaction of the skin. Approximately 20% of the European and North American population are affected and some professions like hairdressers, healthcare and metal workers bear a high risk for developing ACD [1,2]. ACD is caused by protein-reactive haptens and metal ions and is divided into two main phases [3]. The first phase is the sensitization phase which is clinically mostly inapparent; the second phase is called elicitation phase which causes clinical symptoms like erythema and eczema [4]. During sensitization the contact allergen penetrates the skin and causes activation of the innate immune system resulting in skin inflammation [5]. Epidermal Langerhans cells and dermal dendritic cells (DCs) are activated and migrate to the local lymph nodes to prime naïve contact allergen-specific T cells, polarize them to a Th1/Tc1 phenotype and induce skin homing receptors. These T cells start to proliferate, enter the blood circulation and differentiate to effector and eventually memory T cells which marks the end of the sensitization phase [6,7]. Upon a second antigen encounter the primed T cells enter the inflamed skin, kill antigen-modified cells including keratinocytes and secrete pro-inflammatory cytokines which causes the clinical symptoms [8].

It has been shown, that exposure to a combination of allergens can lead to a synergistic elicitation response and that the mixing of allergens with an irritant lowers the threshold of the elicitation response [9–11]. The synergistic effect of the combination was shown by an increase in ear thickness compared to the sensitization with the single components and an increased infiltration of CD4 T cells, CD8 T cells and B cells [11].

A crucial role for innate immune receptors in ACD was shown in the mouse contact hypersensitivity (CHS) model. Mice lacking TLR2 and TLR4, TLR4 and IL-12R β 2, NLRP3 or P2X7 are resistant to CHS [12–15]. These studies underline the crucial role of the innate immune response and skin inflammation for sensitization and elicitation of CHS. Interestingly, in these pattern recognition receptor (PRR) deficient mice, the resistance to CHS can be overcome by activation of alternative inflammatory signalling pathways. One such example is the activation of the TLR9 signalling pathway in TLR4/IL-12R β 2 deficient DCs by CpG-ODN, which results in the restoration of the sensitizing capacity of these cells [12].

Interestingly, in a recent publication it was shown by Herbert et al. that mice which were fed a high fat diet (HFD) showed an increased inflammatory phenotype in an imiquimod-induced psoriasis model. They were able to show this effect not only in mice which were fed HFD for 20-25 weeks but as well in mice fed HFD for 5 weeks. The short term fed mice did not show increased bodyweight or fasting blood glucose. Furthermore, Zhang et al. observed the appearance of spontaneous skin lesions in C57BL/6 mice fed a HFD for 9 month [16].

The adipose tissue does not only store energy but is also immunologically active. It is known that obesity, often associated with Western diet rich in fat and sugar, causes a low grade inflammation which contributes to obesity-linked disorders and metabolic dysfunction [17]. Leptin is an adipokine, which is mainly secreted by adipocytes. The level of leptin in the serum directly correlates with the amount of body fat [18]. Leptin is not only involved in energy homeostasis but is also immunologically active [19]. The leptin receptor is expressed on all immune cells and leptin signalling is known to have an anti-apoptotic effect, alter the phagocytic function of macrophages and the proliferative capacity of T cells [20,21]. On this background we hypothesized that feeding mice lacking TLR2 and TLR4 (TLR2/4 mice) a HFD may induce sufficient inflammation to allow for sensitization with contact allergens and subsequent CHS.

To investigate the effect of an increased fat intake on CHS, CHS resistant TLR2/4 or wildtype (wt) mice were fed HFD or control diet (CD) for four weeks starting at an age of four weeks. In normal chow (NC) and CD 10%, in HFD 60% of the calories are derived from fat. NC and CD differ from each other in the composition of the fatty acids. While in NC the ratio of unsaturated fatty acids to saturated fatty acids is 1:0.3, this ratio is in the CD 1:0.6. Interestingly, the CD contains a higher amount of sugar compared to the NC (32% and 10%, respectively) which may contribute to the observed differences. The effects of the diets on the sensitization phase as well as on the elicitation phase of the response to the contact allergen 2,4,6,-trinitrochlorobenzene (TNCB) was analysed.

2. Results

Impact of HFD on wt and TLR2/4 mice

Four week old wt and TLR2/4 mice were fed NC, CD or HFD for four weeks. During four weeks of feeding, the HFD fed wt mice gained more weight compared to CD fed wt mice but there were no significant differences in the weight of TLR2/4 (Figure 1A, 1B). The weight at day 31 of the NC fed mice was similar to the mice fed CD. Furthermore, the amount of subcutaneous (sc) and epididymal (epi) fat was increased in both strains stepwise from NC to CD to HFD (Figure 1C, 1D). While the size of the fat pads was similar in wt and TLR2/4 NC fed mice, the increase upon CD and HFD feeding was reduced in TLR2/4 mice compared to wt mice. The increase in body fat correlated with an increase in serum leptin (Figure 1E, 1F). The lipid content of the liver was determined using Oil Red O staining on cryosections of liver tissue and by measuring the fluorescence of Nile red in lysates. The amount of lipid in the livers of the mice were nearly absent in the NC fed wt and TLR2/4 mice, whereas the CD and HFD fed wt mice showed more lipid in contrast to TLR2/4 mice (Figure 1G, 1H, 1I). As a positive control liver tissue from a mouse with non-alcoholic fatty liver disease (NAFLD) was used. Taken together, these data show that the four weeks of CD feeding result in increased fat mass, leptin levels and enhanced lipid content in the liver compared to NC feeding. These effects were further increased by HFD feeding.

HFD feeding results in an increased CHS in wt mice and the break of resistance of TLR2/4 mice to CHS

After four weeks of feeding with HFD or CD mice were sensitized with TNCB or treated with acetone as vehicle control. 5 days later mice were challenged with TNCB to elicit CHS (see scheme 1). The ear swelling of TNCB-sensitized wt mice was higher compared to the vehicle treated wt mice (Figure 2A). Interestingly, the increase in ear thickness was higher in the vehicle treated HFD fed wt mice compared to CD fed wt mice. The CD and the HFD fed TLR2/4 mice showed a significant increase in ear thickness (Figure 2B) whereas the NC fed TLR2/4 mice showed no significant increase in ear thickness as shown before [12]. This indicates that both, CD and HFD, can lead to the loss of CHS resistance of TLR2/4 mice. Since IFN γ producing CD8 $^{+}$ effector T cells are known to play a major role in CHS [8], we analysed IFN γ production by CD8 $^{+}$ T cells in the ear skin of the mice. We found increased levels of IFN γ in CD8 $^{+}$ T cells from the ears of HFD and CD fed wt mice after TNCB sensitization compared to vehicle controls (Figure 2C). A statistically significant effect was only observed in the HFD fed mice. The level of IFN γ in the T cells from ears of the TLR2/4 mice was increased by trend in the NC and HFD fed mice and statistically significant in the CD fed mice (Figure 2D). No diet-dependent effect was observed for NK and NKT cells (data not shown) which play a role in CHS and obesity [22–24]. Furthermore, the levels of IL-1 β in the ears were determined. In TNCB treated wt mice the local IL-1 β levels were marginally increased compared to the acetone mice and in the HFD mice compared to the CD mice (Figure 2E). In TLR2/4 mice both the IL-1 β levels in the serum were increased upon TNCB sensitization in all three diets (Figure

2F). Interestingly, comparing the acetone CD and the acetone HFD mice, a statistically significant increase in IL-1 β level can be shown in the HFD mice. These results show that HFD increases the CHS reaction of wt mice to TNCB and that both HFD and CD can lead to an increased inflammation that can overcome the resistance of TLR2/4 mice to TNCB.

HFD increases the initial reaction of mice to TNCB

To evaluate whether HFD influences the T cell-independent innate inflammatory process during the sensitization phase, wt mice were fed either HFD or CD for four weeks and treated with TNCB on one ear and acetone as vehicle control on the other ear (see scheme 2). The thickness of the ears was determined 24 h after TNCB treatment. A significant increase upon TNCB treatment in both HFD and CD fed mice was found in wt and TLR2/4 mice (Figure 3A, 3B) indicating an increased inflammation in the TNCB treated ears. This increase was higher in the HFD fed mice compared to CD fed mice (Suppl. Figure 1). No increase in ear thickness in the acetone ears in both strains and both diets was shown, indicating that acetone has no irritative effect. Numbers of ear infiltrating neutrophils as one of the first immune cells entering the skin in CHS [13] were determined. The neutrophil numbers were increased in the TNCB treated ears compared to the acetone treated ears 24 h after TNCB treatment in both strains (Figure 3C, 3D), again indicating an increased inflammation. No significant increase in neutrophils was found in both strains upon HFD feeding. A trend for more pro-inflammatory cytokines IL-1 β , IL-6 and TNF was found in the serum of HFD fed wt and TLR2/4 mice 24 h after TNCB treatment (Figure 3E, 3F, 3G) indicating an increased inflammation. A minor trend for an increase in cytokine levels in the TLR2/4 mice was found compared to wt mice. IL-1 β levels in the ears of the wt and TLR2/4 mice were slightly increased upon TNCB treatment, independent of the diet (Figure 3H). The IL-6 and TNF levels were increased in skin of TNCB treated ears compared to the acetone treated ears (Figure 3I, 3J) independent of the diet.

We could show an increased local inflammation after short term TNCB treatment. A trend for higher levels of pro-inflammatory cytokines in the serum of both mouse strains suggests that HFD increases systemic inflammation 24 h after TNCB treatment.

3. Discussion

So far it is not completely understood why some people develop ACD and others not. It is known that obesity is a risk factor for chronic inflammation and is linked to inflammatory and metabolic diseases like type 2 diabetes [25]. Furthermore, overnutrition and obesity result in so-called metaflammation which is a constitutive low-grade inflammation and associated with the metabolic syndrome [26,27]. Not only is the amount of fat in the diet important for the inflammatory processes but the composition of the fatty acids is important as well. Saturated fatty acids are known to act pro-inflammatory whereas mono-unsaturated fatty acids are considered to be more anti-inflammatory [28].

In this study we were able to show that not only a HFD but a CD with similar lipid levels compared to NC is able to increase CHS. This was not dependent on weight gain. Compared to wt mice, the increase in adipose tissue was much smaller in TLR2/4 mice. This may be due to a protective role of these TLRs in SFA induced obesity [29,30]. The size of the fat pads, the lipid in the livers and the leptin levels were only slightly elevated in CD compared to NC. Therefore we could show an impact of both CD and HFD on CHS even though the feeding time was short and the mice did not show a sign of obesity. However, NC fed TLR2/4 mice were still resistant to TNCB induced CHS, even though the amount of crude fat is nearly the same in the synthetic, experimental CD and NC (Suppl. Table 1). The difference between NC and CD in our experiments could be caused by the higher ratio of saturated to unsaturated fatty acids

in the CD compared to the NC. It remains to be determined if there is also a role for the elevated sugar levels in the CD. It has been published that an increase in intake of sugars can increase inflammation directly [31,32] and via the de novo synthesis of free fatty acids [33,34]. Feeding a Western diet, in which both sugar and fat is increased, leads to the priming of innate immune responses to an inflammatory trigger via epigenetic reprogramming [35].

Some studies have already addressed the question whether HFD increases the reaction of mice to CHS. Savetsky et al. fed mice 10-12 weeks with HFD and were sensitized them with DNFB and showed an increase in ear thickness compared to mice fed NC caused by an decreased vessel density and increased levels of basal skin inflammation [36]. Interestingly, Katagiri et al. showed after four weeks of feeding HFD a reduce in CHS response to TNCB [37]. Furthermore, they sowed an increased irritative effect of TNCB in HFD fed mice. We were able to show that HFD increases the inflammation in wt mice during CHS in contrast to the findings of Katagiri et al [37]. Herbert et al. could show a difference in the imiquimod induced psoriasis-like phenotype when the mice were fed diets with the same amount of fat but with mainly saturated or unsaturated fatty acids, respectively [38]. Furthermore, it has been shown that palmitic acid, a saturated fatty acid, can activate TLR2 and TLR4 whereas polyunsaturated fatty acids act inhibitory [39,40]. This might explain the increased reaction of HFD fed wt mice to TNCB but not the loss of resistance in the TLR2/4 mice lacking these PRRs. There are other pathways for fatty acids action on immune cells. The scavenger receptor CD36 and some of the fatty acid binding proteins (FABPs) are expressed by immune cells and can induce pro-inflammatory signalling due to the binding of fatty acids [16,41]. Fatty acids can also create a pro-inflammatory micromilieu by inducing ROS [42] which are known to be induced after TNCB treatment [43]. Some of these innate immune pathways are well known to play an important role in CHS [44]. Therefore, we hypothesise that the increase in saturated fatty acids of the CD and the HFD creates an inflammatory milieu which increases the inflammation during TNCB sensitization and challenge resulting in the induction of CHS independent of TLR2 and TLR4. This compensatory mechanism might be comparable to the synergistic effects of the combination of certain fragrance allergens which are known to induce higher inflammatory response in combination than alone [11]. The exact mechanisms underlying this alternative activation of innate and adaptive immune responses are currently under investigation.

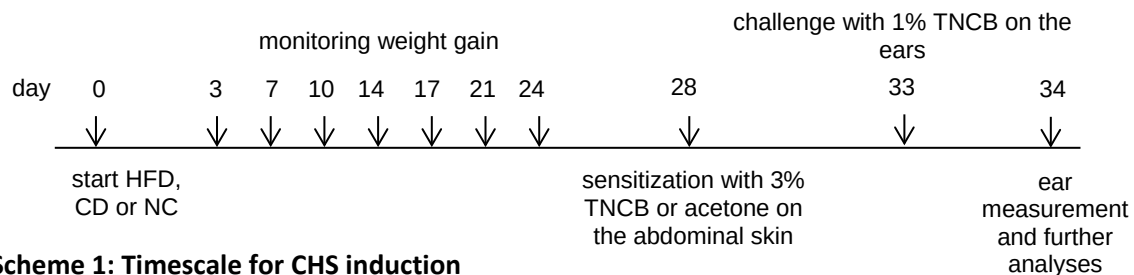
We and others could show that already a short time of HFD feeding, which has only a slight impact on the bodyweight, can increase the inflammatory level in mice [38]. This lowers the threshold for sensitization and compensates for the lack of innate immune system activation via TLR2/4. The effect of HFD feeding compared to CD is observed in both wt and TLR2/4 mice upon a single TNCB application on the ear skin (Suppl. Figure 1). This indicates that the HFD-induced low-grade inflammation indeed lowers the sensitization threshold as visualized by the increased irritant effect of TNCB. Thereby, resistance to CHS is lost. This might be a hint that a short period of malnutrition in humans can already have an impact on the development of ACD or other inflammatory diseases. The malnutrition might have a similar effect on the CHS as the simultaneous application of multiple allergens. Some weak allergens only induce CHS in combination with other weak allergens or an irritant [9–11]. Changing the diet to one that contains less fat might be an option for people who accumulate multiple allergies to prevent them from new sensitizations and reduce the effects of the existing allergies. Our findings point out that diet with an enriched quantity of fat can create a pro-inflammatory milieu in a short period of time. As a consequence, inflammatory diseases can be induced or amplified. Future work will address the question if feeding SFA-rich diet may even be beneficial by overcoming immunodeficiency resulting from defects in the innate immune system and if switching back to low fat diet can reverse the pro-inflammatory effect.

4. Materials & Methods

Mice

C57BL/6 (wt) mice were from Charles River Laboratories, Inc. The C57BL/10 TLR2/TLR4 double knockout mice (TLR2/4) were bred at the Center for Experimental Models and Transgenic Services (CEMT), Freiburg. The mice were housed in a temperature controlled room with a 12 h light/dark cycle. The mice were fed ad libitum a HFD (60% of the kJ from lard, #D12492) or a CD (10% of the kJ from lard, #D12450B). The experimental diets were purchased by ssniff Spezialdiäten GmbH, Germany. The mice were fed starting at an age of 4 weeks and used for experiments at an age of 8 weeks and sacrificed by CO₂ inhalation at the end of the experiments. All animal experiments were approved by the Regierungspräsidium Freiburg (AZ G17/64, G-17/064).

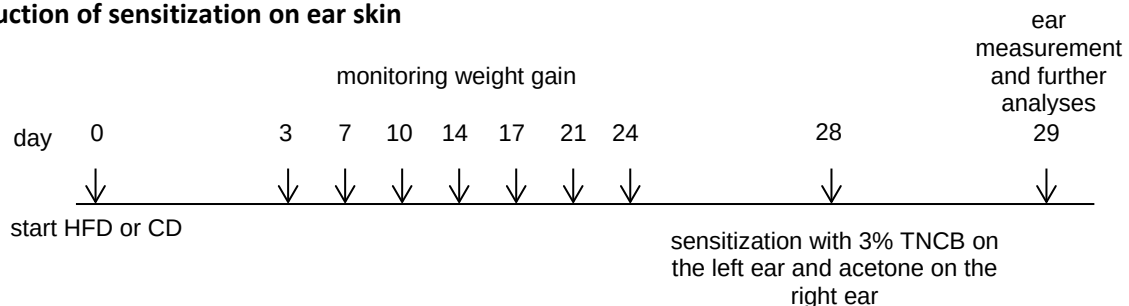
Induction of CHS



Scheme 1: Timescale for CHS induction

Twice a week the mice were weighted. After four weeks of feeding the sensitization was performed by applying 100 µl of 3% TNCB dissolved in acetone or acetone alone (vehicle control) on the abdominal skin. 5 days after sensitization, the ear thickness was measured and the challenge was performed by applying 20 µl of 1% TNCB dissolved in acetone on the back side of the ears. 24 h later, the ear thickness was measured again, the mice were sacrificed and the organs and serum were taken for analysis.

Induction of sensitization on ear skin



Scheme 2: Timescale for short term TNCB treatment

Twice a week the mice were weighted. After 4 weeks of feeding the sensitization was performed by applying 20 µl TNCB dissolved in acetone on the left ear and 20 µl acetone as vehicle control on the right ear. 24 h later the ear thickness was measured again, the mice were sacrificed and the organs were taken for analysis.

Oil Red O staining

The Oil Red O staining was performed to stain lipid droplets in mouse livers. First, 4 µm cryosections were prepared. After a short drying step, the sections were fixed in 10% formalin in PBS for 5 min.

After rinsing for 2 min in tap water the slides were air dried for a few minutes and rinsed with 60% isopropanol for 5 min. The Oil Red O was prepared fresh by mixing two parts of aqua dest. with three parts of Oil Red O, letting it sit for 10 min and filtering with a filter paper. The livers were stained with the freshly prepared Oil Red O working solution for 12 min. The slides were rinsed again shortly in 60% isopropanol, dipped in aqua dest. and then stained in hematoxylin for 10 s. The slides were rinsed again in tap water and mounted in Kaiser's glycerol gelatine.

Nile red lipid analysis

Frozen livers were homogenised using mechanical force using the rough side of histological slides and 25 µl of lysate was mixed with 100 µl 1 µg/ml Nile red solution (Sigma, # 19123 – 10 mg). After 5 min of incubation at RT the fluorescence was measured (extinction 485 nm /emission 565 nm).

ELISA

The amounts of Leptin, IL-1 β , IL-6 and TNF were determined in the serum and ear lysates with the following kits: Leptin (Leptin ELISA, DuoSet development system R&D, DY498), IL-1 β (BD Biosciences San Jose. USA, 559603), IL-6 (BD Biosciences San Jose. USA, 555240) and TNF (BD Biosciences San Jose. USA, 555268). The protocols provided by the manufacturers were followed.

FACS

The ears of the mice were digested with Liberase TM digestion buffer (28 U/ml Roche Liberase TM, 10 mg/ml DNase1, 1 M HEPES in HBSS) for 1 h at 37°C. After blocking the Fc receptors with Fc Block (made in-house clone 2.462) the single cells were stained with anti-mouse CD8 antibody (clone 53-6.7), anti-mouse CD45 antibody (clone 30-F11), anti-mouse IFN- γ antibody (clone XMGL1.2), anti-mouse Ly6C antibody (clone HK1.4 and AL-21) and anti-mouse Ly6G antibody (clone 1A8 and RB6-8C5).

Statistics

The percent fat of bodyweight was calculated by dividing the weight of the fat by the body weight of the individual mouse. Data are shown as means $\pm$ standard error of the mean (SEM). If not stated otherwise, results were pooled from at least three independent experiments. The statistical analysis was performed using Graph Pad Prism V5.03. Data analysis was performed using student's t test. Differences between groups as marked by asterisks were statistically non-significant (ns) at $p > 0.05$ (ns), significant at $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***).

Figure Legends

Figure 1: Impact of HFD on wt and TLR2/4 mice

Mice were fed HFD, CD or NC for four weeks starting at an age of four weeks. (A,B) The weight gain was determined twice a week; wt NC n=9, HFD / CD n= 30; TLR2/4 NC n=9, HFD / CD n=28 (C,D) The sc and epi fat pads were weighted and the % fat of bodyweight was calculated; wt: NC n=2; HFD / CD sc n= 30; epi n=20; TLR2/4 NC n=17; HFD / CD sc n=28 epi n=19 (E,F) the serum leptin level was determined using an ELISA; wt: NC n=2, HFD / CD n= 29; TLR2/4: NC n=12; HFD/CD n=30 (G) Oil red O staining of 4 µm cryosections of liver tissue, one representative experiment of three is shown. (H,I) Lipid content of liver lysates was determined by Nile red fluorescence; NC n=8; CD / HFD n=14 fatty liver n=1; mean values $\pm$ SEM are shown.

Figure 2: HFD feeding results in an increased CHS in wt mice and the break of resistance of TLR2/4 mice to CHS

After four weeks of feeding HFD or CD, mice were sensitized with TNCB or acetone as vehicle control and challenged 5 days later with TNCB. (A,B) The thickness of the ears was measured before challenge and 24 h after challenge. Increase in ear thickness is shown; wt: n=18; TLR2/4 HFD/CD n=30; NC n=10 (C,D) the ear infiltrating cells were analysed by FACS and the MFI of IFN- γ in the CD8 T cells was displayed; wt n=9; TLR2/4 HFD/CD n=10; NC n=5 (E,F) IL-1 β levels in the ear lysates were determined by ELISA; wt: n=4; TLR2/4 HFD/CD n=10, NC N=3

Figure 3: HFD increased the initial reaction of mice to TNCB

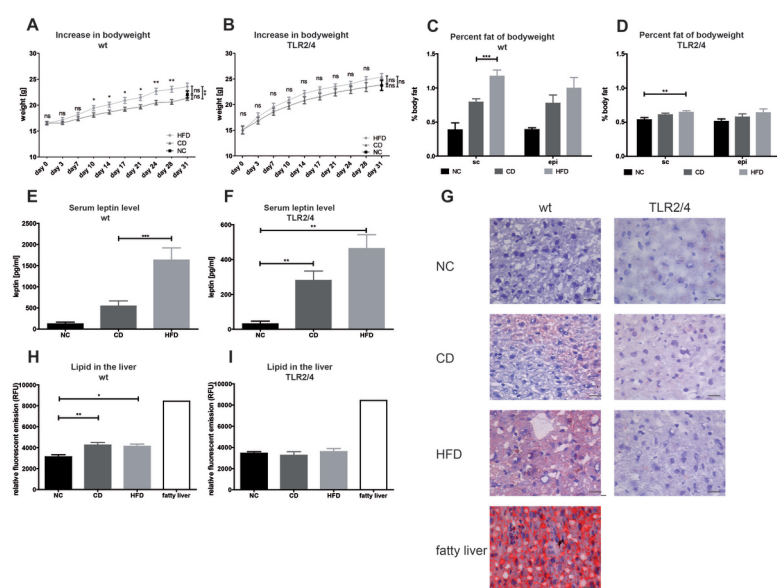
After four weeks of feeding HFD or CD wt mice were sensitized with TNCB or acetone as vehicle control and analysed 24 h later. (A,B) the ear thickness was determined before and 24 h after sensitization; wt n=8; TLR2/4 n=10 (C,D) and the ear infiltrating neutrophils were determined by FACS as percent neutrophils of CD45+ cells 24 h after sensitization; wt n=11; TLR2/4 n=5 and the cytokines IL-1b, IL6 and TNF were determined by ELISA (E, F, G) in the serum; wt n=9; TLR2/4 n=8 and (H,I,J) in the ear lysates; wt n=4; TLR2/4 n=5; mean values $\pm$ SEM are shown.

5. References

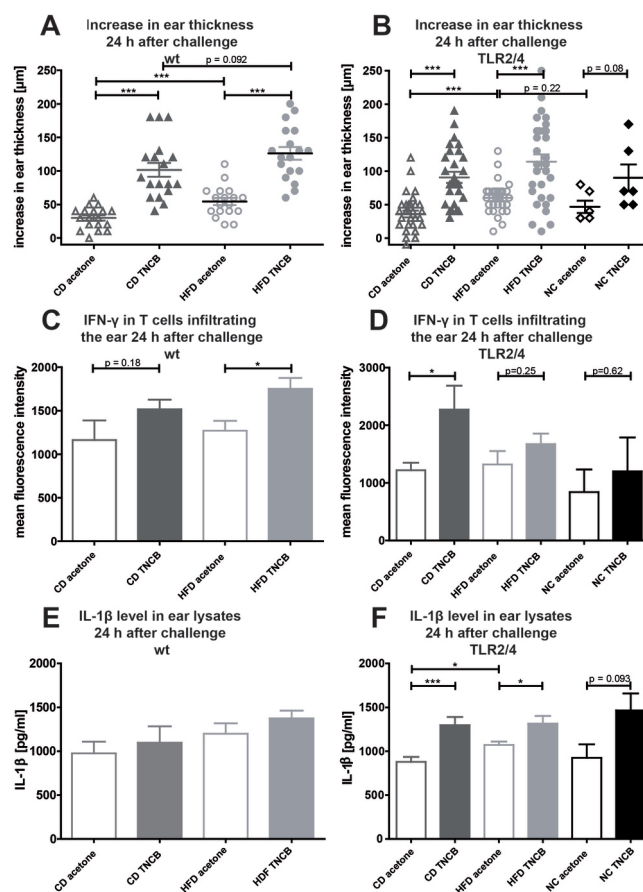
1. Alinaghi, F., Bennike, N.H., Egeberg, A., Thyssen, J.P., and Johansen, J. (2019) Prevalence of contact allergy in the general population: A systematic review and meta-analysis. *Contact Dermatitis*, **80** (2), 77–85.
2. Jakasa, I., Thyssen, J.P., and Kezic, S. (2018) The role of skin barrier in occupational contact dermatitis. *Exp. Dermatol.*, **27** (8), 909–914.
3. Natsch, A., and Emter, R. (2008) Skin sensitizers induce antioxidant response element dependent genes: application to the in vitro testing of the sensitization potential of chemicals. *Toxicol Sci*, **102** (1), 110–9.
4. Fitzpatrick, J.M., Roberts, D.W., and Patlewicz, G. (2017) What determines skin sensitization potency: Myths, maybes and realities. The 500 molecular weight cut-off: An updated analysis. *J Appl Toxicol*, **37** (1), 105–116.
5. Martin, S.F., and Esser, P.R. (2017) Avoiding contact allergens: from basic research to the in vitro identification of contact allergens. *Allergol Select*, **1** (1), 77–84.
6. Gamradt, P., Laoubi, L., Nosbaum, A., Mutez, V., Lenief, V., Grande, S., Redoulès, D., Schmitt, A.-M., Nicolas, J.-F., and Vocanson, M. (2019) Inhibitory checkpoint receptors control CD8+ resident memory T cells to prevent skin allergy. *J Allergy Clin Immunol*, **143** (6), 2147–2157.e9.
7. Schmidt, J.D., Ahlström, M.G., Johansen, J.D., Dyring-Andersen, B., Agerbeck, C., Nielsen, M.M., Poulsen, S.S., Woetmann, A., Ødum, N., Thomsen, A.R., Geisler, C., and Bonefeld, C.M. (2017) Rapid allergen-induced interleukin-17 and interferon- γ secretion by skin-resident memory CD8+ T cells. *Contact Dermatitis*, **76** (4), 218–227.
8. Martin, S.F., Esser, P.R., Schmucker, S., Dietz, L., Naisbitt, D.J., Park, B.K., Vocanson, M., Nicolas, J.-F., Keller, M., Pichler, W.J., Peiser, M., Luch, A., Wanner, R., Maggi, E., Cavani, A., Rustemeyer, T., Richter, A., Thierse, H.-J., and Sallusto, F. (2010) T-cell recognition of chemicals, protein allergens and drugs: towards the development of in vitro assays. *Cell. Mol. Life Sci.*, **67** (24), 4171–4184.
9. Johansen, Skov, Volund, Andersen, and MennÉ (1998) Allergens in combination have a synergistic effect on the elicitation response: a study of fragrance-sensitized individuals. *British Journal of Dermatology*, **139** (2), 264–270.
10. Agner, T., Johansen, J.D., Overgaard, L., Vølund, A., Basketter, D., and Menné, T. (2002) Combined effects of irritants and allergens. *Contact Dermatitis*, **47** (1), 21–26.
11. Bonefeld, C.M., Nielsen, M.M., Rubin, I.M.C., Vennegaard, M.T., Dabelsteen, S., Giménez-Arnau, E., Lepoittevin, J.-P., Geisler, C., and Johansen, J.D. (2011) Enhanced sensitization and elicitation responses caused by mixtures of common fragrance allergens. *Contact Dermatitis*, **65** (6), 336–342.
12. Martin, S.F., Dudda, J.C., Bachtanian, E., Lembo, A., Liller, S., Dürr, C., Heimesaat, M.M., Bereswill, S., Fejer, G., Vassileva, R., Jakob, T., Freudenberg, N., Termeer, C.C., Johnner, C., Galanos, C., and Freudenberg, M.A. (2008) Toll-like receptor and IL-12 signaling control susceptibility to contact hypersensitivity. *J Exp Med*, **205** (9), 2151–2162.

13. Weber, F.C., Esser, P.R., Müller, T., Ganesan, J., Pellegatti, P., Simon, M.M., Zeiser, R., Idzko, M., Jakob, T., and Martin, S.F. (2010) Lack of the purinergic receptor P2X7 results in resistance to contact hypersensitivity. *J Exp Med*, **207** (12), 2609–2619.
14. Gaspari, A.A., Katz, S.I., and Martin, S.F. (2016) Contact Hypersensitivity. *Current Protocols in Immunology*, **113** (1), 4.2.1-4.2.7.
15. Sutterwala, F.S., Ogura, Y., Szczepanik, M., Lara-Tejero, M., Lichtenberger, G.S., Grant, E.P., Bertin, J., Coyle, A.J., Galán, J.E., Askenase, P.W., and Flavell, R.A. (2006) Critical role for NALP3/CIA1/Cryopyrin in innate and adaptive immunity through its regulation of caspase-1. *Immunity*, **24** (3), 317–327.
16. Zhang, Y., Li, Q., Rao, E., Sun, Y., Grossmann, M.E., Morris, R.J., Cleary, M.P., and Li, B. (2015) Epidermal Fatty Acid Binding Protein Promotes Skin Inflammation Induced by High-Fat Diet. *Immunity*, **42** (5), 953–964.
17. Ouchi, N., Parker, J.L., Lugus, J.J., and Walsh, K. (2011) Adipokines in inflammation and metabolic disease. *Nat. Rev. Immunol.*, **11** (2), 85–97.
18. Boguszewski, C.L., Paz-Filho, G., and Velloso, L.A. (2010) Neuroendocrine body weight regulation: integration between fat tissue, gastrointestinal tract, and the brain. *Endokrynologia Polska*, **61** (2), 194–206.
19. Fantuzzi, G., and Faggioni, R. (2000) Leptin in the regulation of immunity, inflammation, and hematopoiesis. *Journal of Leukocyte Biology*, **68** (4), 437–446.
20. Naylor, C., and Petri, W.A. (2016) Leptin Regulation of Immune Responses. *Trends Mol Med*, **22** (2), 88–98.
21. Fantuzzi, G., and Faggioni, R. (2000) Leptin in the regulation of immunity, inflammation, and hematopoiesis. *J. Leukoc. Biol.*, **68** (4), 437–446.
22. Cifaldi, L., Prencipe, G., Caiello, I., Bracaglia, C., Locatelli, F., De Benedetti, F., and Strippoli, R. (2015) Inhibition of natural killer cell cytotoxicity by interleukin-6: implications for the pathogenesis of macrophage activation syndrome. *Arthritis Rheumatol*, **67** (11), 3037–3046.
23. Bähr, I., Jahn, J., Zipprich, A., Pahlow, I., Spielmann, J., and Kielstein, H. (2018) Impaired natural killer cell subset phenotypes in human obesity. *Immunol Res*, **66** (2), 234–244.
24. Martin, S.F. (2015) Immunological mechanisms in allergic contact dermatitis. *Curr Opin Allergy Clin Immunol*, **15** (2), 124–130.
25. Gregor, M.F., and Hotamisligil, G.S. (2011) Inflammatory mechanisms in obesity. *Annu. Rev. Immunol.*, **29**, 415–445.
26. Lumeng, C.N., and Saltiel, A.R. (2011) Inflammatory links between obesity and metabolic disease. *J Clin Invest*, **121** (6), 2111–2117.
27. Christ, A., Lauterbach, M., and Latz, E. (2019) Western Diet and the Immune System: An Inflammatory Connection. *Immunity*, **51** (5), 794–811.
28. Ralston, J.C., Lyons, C.L., Kennedy, E.B., Kirwan, A.M., and Roche, H.M. (2017) Fatty Acids and NLRP3 Inflammasome-Mediated Inflammation in Metabolic Tissues. *Annu. Rev. Nutr.*, **37** (1), 77–102.
29. Davis, J.E., Gabler, N.K., Walker-Daniels, J., and Spurlock, M.E. (2008) Tlr-4 deficiency selectively protects against obesity induced by diets high in saturated fat. *Obesity (Silver Spring)*, **16** (6), 1248–1255.
30. Davis, J.E., Braucher, D.R., Walker-Daniels, J., and Spurlock, M.E. (2011) Absence of Tlr2 protects against high-fat diet-induced inflammation and results in greater insulin-stimulated glucose transport in cultured adipocytes. *J Nutr Biochem*, **22** (2), 136–141.
31. Della Corte, K.W., Perrar, I., Penczynski, K.J., Schwingshackl, L., Herder, C., and Buyken, A.E. (2018) Effect of Dietary Sugar Intake on Biomarkers of Subclinical Inflammation: A Systematic Review and Meta-Analysis of Intervention Studies. *Nutrients*, **10** (5).
32. de Koning, L., Malik, V.S., Kellogg, M.D., Rimm, E.B., Willett, W.C., and Hu, F.B. (2012) Sweetened Beverage Consumption, Incident Coronary Heart Disease and Biomarkers of Risk in Men. *Circulation*, **125** (14), 1735–S1.
33. Softic, S., Cohen, D.E., and Kahn, C.R. (2016) Role of Dietary Fructose and Hepatic de novo Lipogenesis in Fatty Liver Disease. *Dig Dis Sci*, **61** (5), 1282–1293.
34. I. S. Sobczak, A., A. Blindauer, C., and J. Stewart, A. (2019) Changes in Plasma Free Fatty Acids Associated with Type-2 Diabetes. *Nutrients*, **11** (9), 2022.
35. Christ, A., Günther, P., Lauterbach, M.A.R., Duewell, P., Biswas, D., Pelka, K., Scholz, C.J., Oosting, M., Haendler, K., Baßler, K., Klee, K., Schulte-Schrepping, J., Ulas, T., Moorlag, S.J.C.F.M., Kumar, V., Park, M.H., Joosten, L.A.B., Groh, L.A., Riksen, N.P., Espevik, T., Schlitzner, A., Li, Y., Fitzgerald, M.L., Netea, M.G., Schultze, J.L., and Latz, E. (2018) Western Diet Triggers NLRP3-Dependent Innate Immune Reprogramming. *Cell*, **172** (1–2), 162–175.e14.
36. Savetsky, I.L., Albano, N.J., Cuzzone, D.A., Gardenier, J.C., Torrisi, J.S., García Nores, G.D., Nitti, M.D., Hespe, G.E., Nelson, T.S., Kataru, R.P., Dixon, J.B., and Mehrara, B.J. (2015) Lymphatic function regulates contact hypersensitivity dermatitis in obesity. *J Invest Dermatol*, **135** (11), 2742–2752.

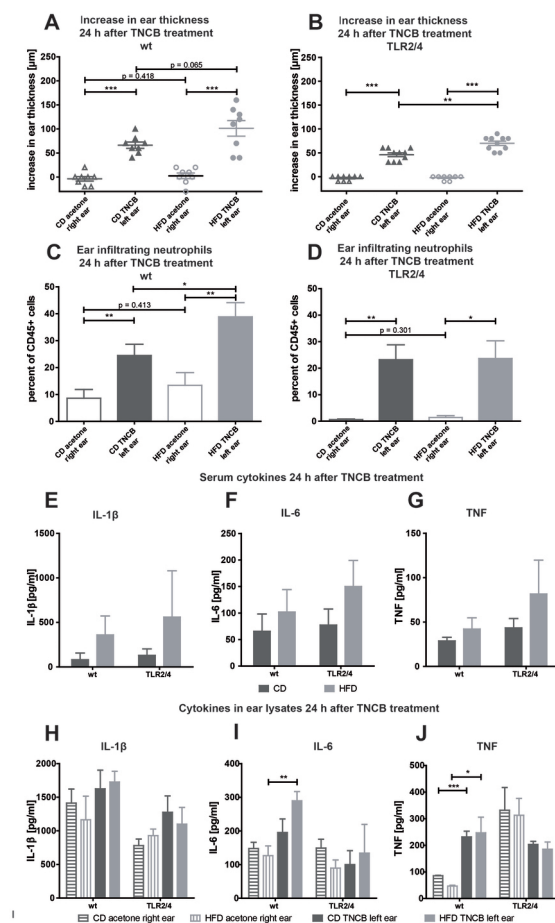
37. Katagiri, K., Arakawa, S., Kurahashi, R., and Hatano, Y. (2007) Impaired contact hypersensitivity in diet-induced obese mice. *Journal of Dermatological Science*, **46** (2), 117–126.
38. Herbert, D., Franz, S., Popkova, Y., Anderegg, U., Schiller, J., Schwede, K., Lorz, A., Simon, J.C., and Saalbach, A. (2018) High-Fat Diet Exacerbates Early Psoriatic Skin Inflammation Independent of Obesity: Saturated Fatty Acids as Key Players. *J. Invest. Dermatol.*, **138** (9), 1999–2009.
39. Hwang, D.H., Kim, J.-A., and Lee, J.Y. (2016) Mechanisms for the activation of Toll-like receptor 2/4 by saturated fatty acids and inhibition by docosahexaenoic acid. *Eur. J. Pharmacol.*, **785**, 24–35.
40. Snodgrass, R.G., Huang, S., Choi, I.-W., Rutledge, J.C., and Hwang, D.H. (2013) Inflammasome-mediated secretion of IL-1 β in human monocytes through TLR2 activation; modulation by dietary fatty acids. *J. Immunol.*, **191** (8), 4337–4347.
41. Park, Y.M. (2014) CD36, a scavenger receptor implicated in atherosclerosis. *Exp. Mol. Med.*, **46**, e99.
42. Wen, H., Gris, D., Lei, Y., Jha, S., Zhang, L., Huang, M.T.-H., Brickey, W.J., and Ting, J.P.-Y. (2011) Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling. *Nat. Immunol.*, **12** (5), 408–415.
43. Esser, P.R., Wölfle, U., Dürr, C., von Loewenich, F.D., Schempp, C.M., Freudenberg, M.A., Jakob, T., and Martin, S.F. (2012) Contact sensitizers induce skin inflammation via ROS production and hyaluronic acid degradation. *PLoS ONE*, **7** (7), e41340.
44. Martin, S.F., Esser, P.R., Weber, F.C., Jakob, T., Freudenberg, M.A., Schmidt, M., and Goebeler, M. (2011) Mechanisms of chemical-induced innate immunity in allergic contact dermatitis. *Allergy*, **66** (9), 1152–1163.



COD_13927_COD-OM-04-21-2880 Figure 1.jpg

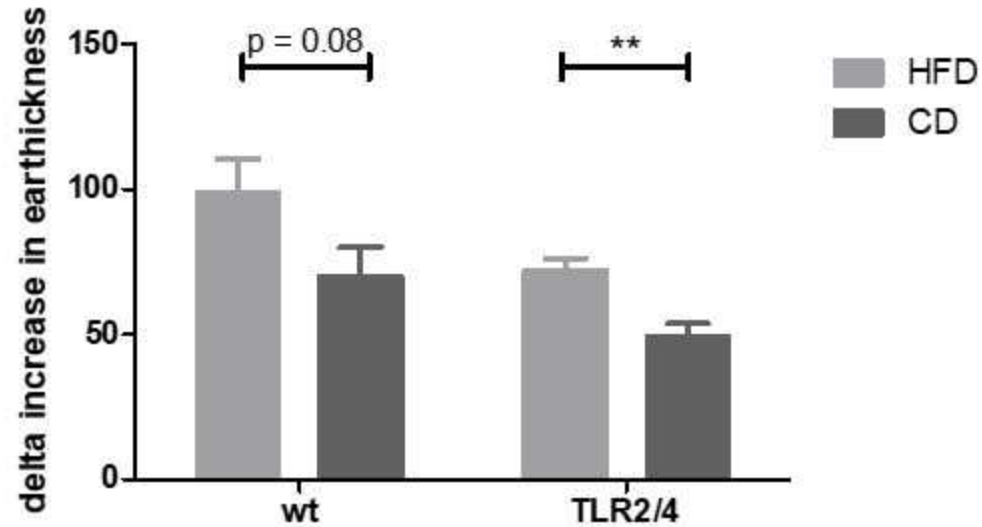


COD_13927_COD-OM-04-21-2880 Figure 2 .jpg



COD_13927_COD-OM-04-21-2880 Figure 3.jpg

Supplementary figure 1



The delta increase in ear thickness was calculated by subtracting the increase in ear thickness of the acetone treated ear from the increase in ear thickness of the TNCB treated ear of the same mouse 24 h after treatment; wt n=8; TLR2/4 n=10 mean values $\pm$ SEM are shown

Suppl. Table 1: Dietary composition

		NC	CD		HFD	
Ref. Nr.: 3437			D12450B		D12492	
Crude Protein		18,50%	18,20%		24,40%	
Crude fibre		4,50%	4,80%		6,00%	
Crude ash		6,30%	5,40%		5,50%	
Starch		38,00%	25,00%		0,10%	
Sugar		4,45%	32,80%		9,40%	
N free extracts		54,20%	63,90%		26,30%	
Crude Fat		4,50%	4,10%		34,60%	
Ratio unsaturated FAs / saturated FAs		1:0.3	1:0.6		1:2.1	
			times increased compared to NC		times increased compared to NC	
C12	Lauric acid	0,00%	0,01%	5,00	0,07%	35,00
C14	Myristic acid	0,01%	0,04%	5,00	0,44%	55,00
C16	Palmitic acid	0,72%	0,66%	0,92	7,70%	10,71
C16:1	Palmitoleic acid	0,05%	0,06%	1,15	0,94%	18,08
C17	Heptadecanoic acid	0,00%	0%	0,00	0%	0,00
C18	Stearic acid	0,16%	0,33%	2,10	4,34%	27,64
C18:1	Oleic acid	1,06%	1,31%	1,24	13,57%	12,81
C18:2	Linoleic acid	2,11%	1,44%	0,68	4,75%	2,25
C18:3	α -Linoleic acid	0,21%	0,17%	0,80	0,51%	2,41
C20	Arachidic acid	0,01%	0,01%	1,00	0,02%	2,00
C20:1	Paullinic acid	0,01%	0%	0,00	0,01%	0,77
C20:4	Arachidonic acid	0,01%	0,03%	3,33	0,53%	58,89
C20:5	Eicosapentaenoic acid	0%	n/a		n/a	
C22	Behenic acid	0,00%	n/a		n/a	
C22:1	Erucic acid	0,00%	n/a		n/a	
C22:6	Docosahexaenoic acid	0,00%	n/a		n/a	
C24	Lignoceric acid	0,01%	n/a		n/a	

Table 1: Detailed composition of the diets normal chow (NC), control diet (CD) and high fat diet (HFD)