INFLAMED FAT: immune modulation of adipose tissue and lipid metabolism

Dam, A.D. van; Dam A.D. van

Chapter 3

IgG is elevated in obese white adipose tissue but does not induce glucose intolerance via Fcγ-receptor or complement

Andrea D. van Dam, Lianne van Beek, Amanda C.M. Pronk, Susan M. van den Berg, Frits Koning, Cees van Kooten, Patrick C.N. Rensen, Mariëtte R. Boon, J. Sjef Verbeek, Ko Willems van Dijk, Vanessa van Harmelen

Submitted

ABSTRACT

During the development of obesity, B cells accumulate in white adipose tissue (WAT) and produce IgG, which may contribute to the development of glucose intolerance. IgG signals by binding to Fcγ receptors (FcγR) and by activating the complement system. The aim of our study was to investigate whether activation of FcγR and/or complement C3 mediates the development of high fat diet-induced glucose intolerance. We studied mice lacking all four FcγRs (FcγRI/II/III/IV$^{-/-}$), only the inhibitory FcγRIIb (FcγRIIb$^{-/-}$), only the central component of the complement system C3 (C3$^{-/-}$), and mice lacking all FcγRs as well as C3 (FcγRI/II/III/IV/C3$^{-/-}$). These mouse models and wild-type control mice were fed a high-fat diet (HFD) for 15 weeks to induce obesity. Body weight and composition as well as glucose metabolism were assessed. The adipose tissue was characterized for adipocyte functionality and inflammation. In obese WAT of wild-type mice, B cells (+142%, $p<0.01$) and IgG (+128% $p<0.01$) were increased compared to lean WAT. Of all mouse models lacking IgG effector pathways, only C3$^{-/-}$ mice showed reduced HFD-induced weight gain as compared to controls (-18%, $p<0.01$). Surprisingly, FcγRI/II/III/IV$^{-/-}$ mice had deteriorated glucose tolerance (AUC +125%, $p<0.001$) despite reduced leukocyte number (-30%, $p<0.05$) in gonadal WAT (gWAT), whereas glucose tolerance and leukocytes within gWAT in the other models were unaffected compared to controls. Although IgG in gWAT was increased (+44 to +174%, $p<0.05$) in all mice lacking FcγRIIb (FcγRI/II/III/IV$^{-/-}$, FcγRIIb$^{-/-}$ and FcγRI/II/III/IV/C3$^{-/-}$), only FcγRI/II/III/IV/C3$^{-/-}$ mice exhibited appreciable alterations in immune cell infiltrate in gWAT, *e.g.* increased macrophages (+36%, $p<0.001$). In conclusion, FcγRIIb deficiency increases adipose tissue IgG, but FcγR and/or C3 deficiency does not protect against HFD-induced glucose intolerance or reduce adipose tissue inflammation. This indicates that if obesity-induced IgG contributes to the development of glucose intolerance, this is not mediated by FcγR or complement activation.

INTRODUCTION

White adipose tissue (WAT) inflammation is a hallmark of obesity-associated insulin resistance (1). Expanding adipose tissue releases increased levels of fatty acids and inflammatory (adipo)cytokines, which impair insulin signaling proteins and thereby decrease insulin sensitivity (2). These inflammatory mediators originate from hypertrophic adipocytes as well as from several types of immune cells residing in the adipose tissue (3). During obesity, additional immune cells infiltrate and accumulate in the adipose tissue (4, 5). Macrophages are the most abundant immune cells in mouse adipose tissue. They acquire a more pro-inflammatory phenotype during obesity and aggregate around necrotic adipocytes in so-called crown-like structures (6). Neutrophils, eosinophils and mast cells also contribute to inflammation and insulin resistance in adipose tissue (7-9). Besides, T cell infiltration occurs during obesity and various T cell populations differently affect obesity-associated insulin resistance (10), although they mostly contribute to metabolic dysfunction by recruitment of macrophages (11, 12). Moreover, B cells infiltrate obese adipose tissue (5, 13) and promote obesity-associated glucose intolerance by several mechanisms (13). Firstly, they induce secretion of the pro-inflammatory cytokine IFNγ by T cells, which induces pro-inflammatory macrophage polarization. In addition, B cells themselves produce cytokines that modulate insulin resistance. However, a unique function of B cells is the production of antibodies that have recently also been demonstrated to promote insulin resistance (13).

Of the 5 classes of antibodies produced by B cells in mammals, IgG is the major class of antibodies in the blood. IgG activates complement, and binds Fc receptors (FcRs) on macrophages and neutrophils, upon which pathogens coated with IgG are phagocytosed by these cells (14). Intriguingly, obesity is associated with higher IgG production and IgG-positive B cells in adipose tissue. Furthermore, transfer of IgG from obese high-fat diet (HFD)-fed mice to obese HFD-fed mice lacking B cells not only altered local inflammatory cytokine production and promoted pro-inflammatory macrophage polarization, but also deteriorated glucose tolerance. The latter seems to be mediated by an Fc-mediated process (*i.e.* FcRs or complement), since transfer of IgG lacking the Fc-binding portion did not affect glucose tolerance (13).

Innate immune effector cells express different FcRs specific for the diverse antibody subclasses. Fcγ receptors (FcγRs) bind IgG and the murine FcγR family includes FcγRI, FcγRIIb, FcγRIII and FcγRIV. FcγRI is the receptor with the highest affinity for the Fc fragment of IgG. FcγRI, FcγRIII and FcγRIV are activating receptors, which mediate phagocytosis, antigen presentation and killing of infected cells, whereas FcγRIIb is an inhibitory receptor. IgG also has different subclasses with varying affinity and specificity for the FcγRs (*i.e.* IgG1, IgG2a, IgG2b and IgG3) (15). In obese adipose tissue, IgG is enriched in crown-like structures, implicating that they are involved in the clearance of necrotic adipocytes. It is possible that in these crown-like structures, interactions between antibodies and FcRs on innate immune effector cells induce pro-inflammatory cytokine release. However, whether and which of the FcR are responsible for the effects of IgG on glucose metabolism remains

unknown (13). In addition to FcR binding and activation, antigen-bound IgG activates complement, which has also been shown to mediate insulin resistance by affecting adipose tissue macrophages (16).

In the current study, we aimed to elucidate whether activation of FcγR and/or complement mediates the development of adipose tissue inflammation and high fat diet-induced glucose intolerance. To this end, we investigated glucose metabolism in several mouse models lacking either all four FcγRs, only the inhibitory FcγR, the central component of the complement system C3, or all four FcγRs combined with C3. We show that absence of IgG effector pathways rather deteriorates than improves glucose intolerance. This implicates that if obesity-associated IgG contributes to the development of glucose intolerance, IgG does not seem to act via FcγR or complement activation.

MATERIALS AND METHODS

Mice and diet

Wild-type male mice (C57Bl/6J background) were purchased from Charles River (Maastricht, The Netherlands) and used as controls for the experiments with FcγRI/II/III/IV$^{-/-}$, C3$^{-/-}$ and FcγRI/II/III/IV/C3$^{-/-}$ mice. FcγRI/II/III/IV$^{-/-}$ (Fransen & Van Maaren 2016, submitted) and FcγRIIb$^{-/-}$ (17) mice were obtained as previously described. FcγRIIb$^{fl/fl}$ mice were used as controls for the FcγRIIb$^{-/-}$ mice. The complement C3$^{-/-}$ strain (18) was kindly provided by Mike Carroll (Harvard Medical School, Boston, MA). This strain was crossed with the FcγRI/II/III/IV$^{-/-}$ mice to obtain FcγRI/II/III/IV/C3$^{-/-}$ mice. FcγRIIb$^{fl/fl}$ mice and all knockout models had a C57Bl/6J background and were bred at the Leiden University Medical Center (Leiden, The Netherlands). At the start of each experiment, male mice were 8-12 weeks of age and housed under standard conditions with a 12:12 h light-dark cycle and free access to food and water. Mice were fed a high-fat diet (HFD; lard, 45% kcal% fat (D12451) or 60% kcal% fat (D12492) in the study with FcγRI/II/III/IV$^{-/-}$ mice; Research Diet Services, Wijk bij Duurstede, The Netherlands). After 15 weeks, mice were terminated and perfused with ice-cold PBS through the heart. Mouse experiments were performed in accordance with the Institute for Laboratory Animal Research Guide for the Care and Use of Laboratory Animals and had received approval from the University Ethical Review Board (Leiden University Medical Center, The Netherlands).

Body weight and composition measurements

At indicated time points, body weight was measured weekly with a scale and body composition was measured using an EchoMRI-100 analyzer (EchoMRI, TX, USA).

Adipocyte, stromal vascular fraction and leukocyte isolation

Gonadal white adipose tissue was dissected, rinsed in PBS and minced. Tissues were digested in a collagenase mixture (0.5 g/L collagenase (Type 1) in DMEM/F12 (pH 7.4) with 20 g/L of dialyzed bovine serum albumin (BSA, fraction V, Sigma, St Louis, USA)) for 1 h at

Table 1. *Antibodies used for flow cytometry.*

Antibody	Fluorochrome	Dilution	Clone, supplier
CD45.2	FITC	1:100	104, BioLegend
CD3	APC	1:100	145-2C11, eBioscience
CD4	Qdot605	1:1 000	RM-4-5, BioLegend
CD8a	PerCPCy5.5	1:100	53-6.7, BioLegend
CD19	PE	1:200	1D3, eBioscience
CD22.2	PE	1:200	Cy34.1, BD Biosciences
Cd11b	Pacific Blue	1:150	M1/70, BioLegend
F4/80	PE	1:200	BM8, eBioscience

37°C, and filtered through a 236-µm nylon mesh. Upon centrifugation of the suspension (10 min, 200 g), adipocytes were isolated from the surface for assessment of lipolysis. The pelleted stromal vascular fraction and blood samples were treated with red blood cell lysis buffer (BD Biosciences, CA, USA) and cells were counted using an automated cell counter (TC10, Bio-Rad Laboratories Inc., Veenendaal, The Netherlands). Cells were fixed in 0.5% paraformaldehyde and stored in FACS buffer (PBS, 0.02% sodium azide, 0.5% FCS) in the dark at 4°C for analysis by flow cytometry within one week.

Flow cytometry

Stromal vascular cells and circulating white blood cells were stained for 30 min at 4°C in the dark with the fluorescently labelled antibodies listed in **Table 1**. Cells were measured on an LSR II flow cytometer (BD Biosciences, CA, USA). Data were analysed using FlowJo software (Treestar, OR, USA).

Gonadal white adipose tissue histology

Gonadal white adipose tissue was fixed in 4% paraformaldehyde, dehydrated in 70% EtOH, and embedded in paraffin. Immunohistochemical detection of IgG and F4/80 was done on paraffin-embedded sections (5 µm). For detection of IgG, a biotinylated horse-anti-mouse IgG (BA-2000, 1/1250, Vector Laboratories Inc., CA, USA) and Vectastain ABC (PK-6100, Vector Laboratories Inc., CA, USA) were used. Detection of F4/80 was done by using a primary rat anti-mouse F4/80 monoclonal Ab (MCA497; 1/600, Serotec, UK) and a secondary goat anti-rat immunoglobulin peroxidase (MP-7444, Vector Laboratories Inc., CA, USA). Peroxidase activity in both stainings was revealed with NovaRed (SK-4800, Vector Laboratories Inc.). Slides were counterstained with hematoxylin. The areas positive for IgG or F4/80 were quantified using ImageJ Software.

Determination of plasma parameters

At the indicated time points, 6 h-fasted (from 8:00 am to 14:00 pm) blood samples were collected by tail vein bleeding into chilled capillaries that were coated with paraoxon (Sigma-Aldrich) to prevent ongoing lipolysis (19) and isolated plasma was assayed for

glucose, insulin, triglycerides and free fatty acids. Glucose was measured using an enzymatic kit from Instruchemie (Delfzijl, The Netherlands), and insulin by ELISA (Crystal Chem Inc., Downers Grove, IL). TG was measured by a commercially available enzymatic kit (Roche Diagnostics, Germany). Free fatty acids were measured using the NEFA C kit (Wako Diagnostics; Instruchemie, Delfzijl, The Netherlands).

Intravenous glucose tolerance test

After 6 or 8 weeks of HFD, mice were fasted for 6 h (from 8:00 am to 14:00 pm), a baseline blood sample was obtained by tail vein bleeding and mice were intravenously injected with D-glucose (2 g/kg body weight). Additional blood samples were drawn at indicated time points after glucose injection and plasma glucose was measured as described above.

Adipocyte lipolysis assay

Basal and stimulated lipolysis and the anti-lipolytic effect of insulin on gonadal adipocytes was measured by incubating isolated adipocytes (approx. 10 000 cells/mL) for 2 h at 37°C, with DMEM/F12 with 2% (v/v) BSA and 8-bromo-cAMP (10^{-3} M; Sigma, St. Louis, MO) with or without insulin (10^{-9} M). Glycerol concentrations were determined as a measure for lipolysis, using a free glycerol kit (Sigma, St. Louis, MO) and the hydrogen peroxide sensitive fluorescence dye Amplex Ultra Red, as previously described (20).

RNA purification and quantitative RT-PCR

RNA was extracted from snap-frozen gonadal white adipose tissue using Tripure RNA Isolation reagent (Roche, The Netherlands) according to manufacturer's instructions. RNA concentrations were measured using NanoDrop and RNA was reverse transcribed using Moloney Murine Leukemia Virus Reverse Transcriptase (Promega, The Netherlands) for quantitative RT-PCR (qRT-PCR) to produce cDNA. Expression levels of genes were determined by qRT-PCR, using gene-specific primers (**Table 2**) and SYBR green supermix (Biorad, The Netherlands). mRNA expression was normalized to *36b4* and *mCyclo* mRNA content and expressed as fold change compared with control mice using the $\Delta\Delta$CT method.

Statistical analysis

All data are expressed as means ± SD. Groups were compared with a two-tailed unpaired Student's t-test or a two-way ANOVA for repeated measurements, as indicated. Differences were considered statistically significant if $p<0.05$.

Table 2. *Primer sequences of forward and reverse primers (5' → 3').*

Gene	Forward primer	Reverse primer
36b4	GGACCCGAGAAGACCTCCTT	GCACATCACTCAGAATTTCAATGG
Il-6	ACCACGGCCTTCCCTACTTC	CTCATTTCCACGATTTCCCAG
Il-10	GACAACATACTGCTAACCGACTC	ATCACTCTTCACCTGCTCCACT
Mcp1	CACTCACCTGCTGCTACTCA	GCTTGGTGACAAAAACTACAGC
mCyclo	ACTGAATGGCTGGATGGCAA	TGTCCACAGTCGGAAATGGT
Tnfα	GATCGGTCCCCAAAGGGATG	CACTTGGTGGTTTGCTACGAC

RESULTS

B cells and IgG are enriched in obese adipose tissue

To examine the presence of B cells and IgG in gWAT, we used flow cytometry to quantify the percentage of B cells ($CD19^+$) within the leukocyte population ($CD45^+$ cells) of the stromal vascular fraction (SVF) of gWAT of obese (>40 g) compared to lean (<30 g) mice that had been fed an HFD. In addition, we quantified IgG by immunohistochemistry in gWAT of these mice. The percentage of B cells was increased in gWAT of obese compared to lean mice (+142%, p<0.01, **Fig. 1A**). IgG staining in gWAT as percentage of total WAT area was also higher in obese versus lean mice (+128%, p<0.01, **Fig. 1B**). IgG was located around adipocytes and co-localized with cells positive for the macrophage marker F4/80 (**Fig. 1C**), suggesting that IgG primarily interacts with macrophages in the adipose tissue.

FcγRI/II/III/IV deficiency deteriorates glucose metabolism during HFD-induced obesity

We next studied the necessity of FcγRs in the development of HFD-induced obesity and glucose intolerance. Hereto, mice lacking all four FcγRs ($FcγRI/II/III/IV^{-/-}$) were fed an HFD for 15 weeks. Body weight (**Fig. 2A**), fat mass (**Fig. 2B**) and weight of several WAT depots (**Fig. 2C**) of $FcγRI/II/III/IV^{-/-}$ mice were not different from control mice. $FcγRI/II/III/IV^{-/-}$ mice had slightly more lean mass upon 15 weeks of HFD (+6%, p<0.05, **Fig. 1D**). Fasting plasma levels of glucose (**Fig. 2E**), triglycerides (**Fig. S1A**) and free fatty acids (**Fig. S1B**) during HFD did not differ between the two groups. However, after 6 weeks of HFD-feeding, plasma insulin levels were increased (+84%, p<0.05, **Fig. 2F**) and glucose tolerance as measured by an intravenous glucose tolerance test was deteriorated (AUC +125%, p<0.001, **Fig. 1G**) in $FcγRI/II/III/IV^{-/-}$ mice compared to controls. To assess whether the reduced whole-body glucose tolerance was due to insulin resistance in white adipocytes, intracellular lipolysis was assessed *ex vivo*. Adipocytes isolated from gWAT of $FcγRI/II/III/IV^{-/-}$ and control mice showed similar basal lipolysis and 8b-cAMP-stimulated lipolysis. Inhibition of lipolysis by

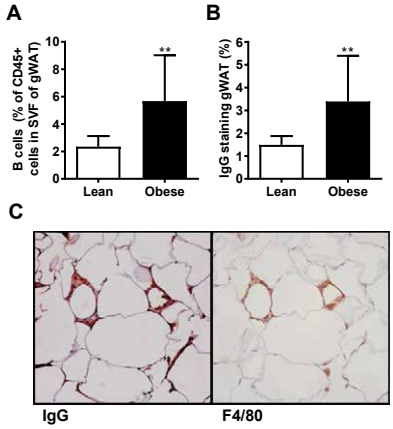


Figure 1. B cells and IgG in gWAT increase during HFD-induced obesity. *Male wild-type C57BL/6J mice were fed an HFD for different amounts of time varying between 4 and 34 weeks. Mice were categorized by body weight, with <30 g defined as lean (n=6-7) and >40 g defined as obese (n=12-13). The percentage of B cells ($CD19^+$) of $CD45^+$ cells in SVF of gWAT was determined by flow cytometry (A). IgG staining of gWAT sections was performed and % of IgG staining was quantified (B). Histological IgG staining (left) and F4/80 staining (right) on gWAT. Values represent means ± SD. **p<0.01 vs. lean.*

insulin was also comparable between groups (**Fig. S1C**), suggesting that WAT may not be responsible for the deteriorated glucose tolerance in FcγRI/II/III/IV$^{-/-}$ mice.

FcγRI/II/III/IV deficiency increases adipose tissue IgG without affecting immune cell composition

To assess whether the absence of FcγRs affects adipose tissue IgG, IgG was quantified by immunohistochemistry on gWAT from FcγRI/II/III/IV$^{-/-}$ and control mice. Interestingly, adipose tissue IgG was increased in the FcγRI/II/III/IV$^{-/-}$ group (+44%, p<0.05, **Fig. 2H**). Immune cell composition in gWAT was not affected as FcγRI/II/III/IV$^{-/-}$ and control mice exhibited comparable percentages of leukocytes (CD45^{+} cells) within the SVF (data not shown), and B cells (CD19^{+}), T cells (CD3^{+}) and macrophages (F4/80^{+}) within the leukocyte population (**Fig. 2I**). However, total cell count of the isolated SVF from gWAT was lower in the FcγRI/II/III/IV$^{-/-}$ group (-30%, p<0.01, **Fig. 2J**), indicating that the absolute numbers of immune cells in gWAT were lower than in control mice. This did not result in a less inflammatory milieu in gWAT, as expression of several inflammatory genes (*Mcp1, Tnfα, Il-6, Il-10*) was similar in FcγRI/II/III/IV$^{-/-}$ and control mice (**Fig. S1D**).

In blood, total cell count (data not shown) and percentages of B cells (CD22.2^{+}) and T cells within the leukocyte population were unaffected by FcγRI/II/III/IV deficiency (**Fig. S1E**), but within the T cell population, percentage of T helper cells (CD4^{+}) was lower (-8%, p<0.05) and the percentage of cytotoxic T cells (CD8^{+}) was higher (+8%, p<0.001) in FcγRI/II/III/IV$^{-/-}$ compared to control mice (**Fig. S1F**). This resulted in a lower CD4:CD8 ratio in FcγRI/II/III/IV$^{-/-}$ than in control mice (-15%, p<0.001, **Fig. 2K**), indicating increased systemic inflammation. In summary, FcγRI/II/III/IV$^{-/-}$ mice exhibited similar body weight but deteriorated glucose metabolism compared to controls, together with higher IgG levels but similar immune cell composition in WAT.

FcγRIIb deficiency does not affect glucose metabolism during HFD-induced obesity

We then assessed whether deficiency of only the inhibitory FcγR (FcγRIIb) would affect HFD-induced obesity and glucose intolerance. To this end, FcγRIIb$^{fl/fl}$ (control) and FcγRIIb$^{-/-}$ mice were fed an HFD for 15 weeks. Body weight (**Fig. 3A**) and total fat mass (**Fig. 3B**) were not different between FcγRIIb$^{-/-}$ and control mice, but weight of the gWAT fat pad was lower in FcγRIIb-deficient animals (-18%, p<0.05, **Fig. 3C**). FcγRIIb$^{-/-}$ mice showed higher lean mass on chow diet before the diet intervention (+9%, p<0.05, **Fig. 3D**), but this difference was nullified after HFD feeding. Fasting plasma glucose (**Fig. 3E**), insulin (**Fig. 3F**), triglyceride (**Fig. S2A**) and free fatty acid (**Fig. S2B**) levels did not differ between the two groups. Glucose tolerance after 8 weeks of HFD-feeding (**Fig. 3G**) and *ex vivo* lipolysis of gWAT adipocytes (**Fig. S2C**) were not different between the FcγRIIb$^{-/-}$ and control groups, suggesting that the inhibitory FcγR does not play a role in HFD-induced glucose intolerance.

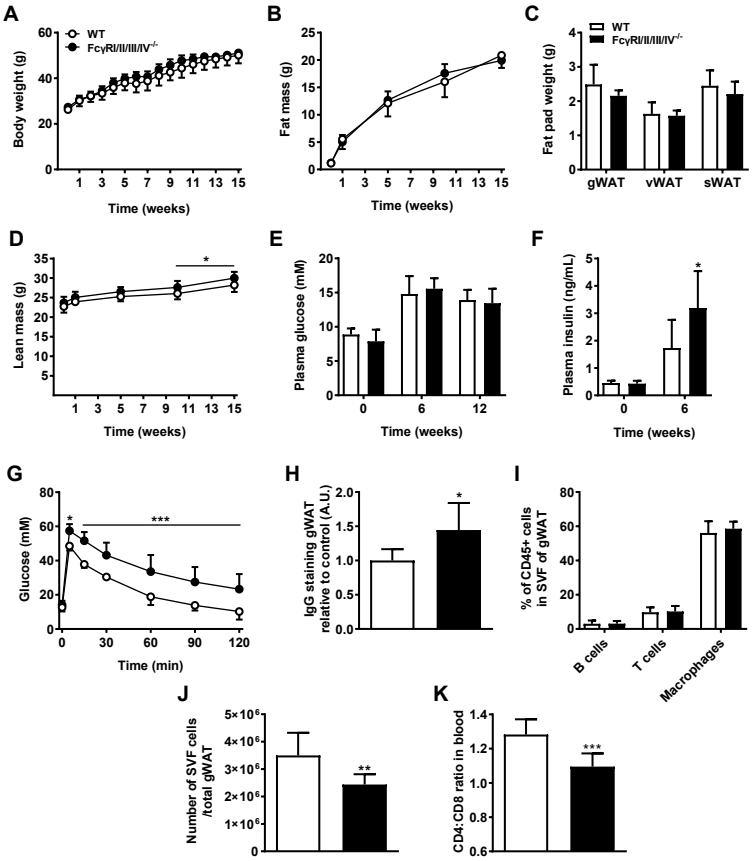


Figure 2. FcγRI/II/III/IV deficiency deteriorates glucose tolerance, increases IgG and decreases immune cells in gWAT during HFD-induced obesity. *FcγRI/II/III/IV$^{-/-}$ and wild-type control mice were fed an HFD for 15 weeks. Body weight (A), fat mass (B), WAT depot weight (C), lean mass (D), plasma glucose (E) and insulin (F) were analysed at the indicated time points. After 6 weeks, mice were injected intravenously with glucose and clearance from plasma was determined by measuring plasma glucose at 5, 15, 30, 60, 90 and 120 min after injection (G). IgG staining of gWAT sections was performed and quantified (H). The percentage of B cells (CD19^{+}), T cells (CD3^{+}) and macrophages (F4/80^{+}) of CD45^{+} cells in SVF of gWAT was determined by flow cytometry (I). Total numbers of SVF cells per gWAT were counted (J). Within the CD3^{+} T cell fraction in blood, the ratio of CD4^{+} and CD8^{+} cells was determined (K). Values represent means ± SD. *p<0.05, **p<0.01, ***p<0.001 vs. control (n=7-8) as measured by a two-way ANOVA in D, G or by a two-tailed unpaired Student's t-test in F, H, J, K. WT, wild-type; gWAT, gonadal white adipose tissue; vWAT, visceral white adipose tissue; sWAT, subcutaneous white adipose tissue; SVF, stromal vascular fraction.*

FcγRIIb deficiency increases adipose tissue IgG without affecting immune cell composition

Like in FcγRI/II/III/IV$^{-/-}$ mice, presence of IgG in gWAT of the FcγRIIb$^{-/-}$ mice was higher compared to controls (+94%, p<0.05, **Fig. 3H**), suggesting that lack of the inhibitory FcγR mediated this effect. FcγRIIb$^{-/-}$ mice had similar percentages of leukocytes (CD45^{+} cells) within the SVF (data not shown), but exhibited a higher percentage of B cells (CD19^{+}, +95%, p<0.05, **Fig. 3I**) within the leukocyte population in gWAT. The percentage of T cells (CD3^{+}) within the leukocyte population of gWAT did not differ between FcγRIIb$^{-/-}$ mice and controls, but the percentage of macrophages (F4/80^{+}) was decreased in the FcγRIIb$^{-/-}$ group (-13%, p<0.05, **Fig. 3I**). Total cell count of the isolated SVF from gWAT was similar in FcγRI/II/III/IV$^{-/-}$ and control mice (**Fig. 3J**), as was the expression of inflammatory genes (*Mcp1, Tnfα, Il-6, Il-10*) in gWAT (**Fig. S2D**). Thus, FcγRIIb$^{-/-}$ mice had similar body weight and glucose tolerance compared to controls, together with higher IgG levels and slight alterations within the leukocyte population in WAT.

Complement C3 deficiency protects against HFD-induced obesity but does not improve glucose intolerance

In addition to signaling through FcγRs, IgG activates complement. To study the involvement of complement C3 in the development of HFD-induced obesity and glucose intolerance, mice lacking the central component of the complement system (C3$^{-/-}$) were fed an HFD for 15 weeks. Lack of C3 decreased HFD-induced body weight gain compared to control mice (-18%, p<0.01, **Fig. 4A**). This effect was primarily due to decreased accumulation of fat mass (**Fig. 4B**), reflected by less weight of the various WAT depots (-23 to -11%, p<0.06, **Fig. 4C**) of C3$^{-/-}$ compared to control mice. Lean mass of C3$^{-/-}$ mice was already lower at the beginning of the diet intervention (-5%, p<0.05), and this difference remained throughout the study (-6%, P<0.01 after 15 weeks of HFD, **Fig. 4D**). Both triglyceride (-29%, p<0.01, **Fig. S3A**) and free fatty acid (-22%, p<0.05, **Fig. S3B**) levels were lower in C3$^{-/-}$ mice before the diet intervention. Despite lower body weight, fasting plasma glucose (**Fig. 4E**) and insulin (**Fig. 3F**) levels were not different in C3$^{-/-}$ mice compared to controls. C3$^{-/-}$ mice did not exhibit different glucose tolerance (**Fig. 4G**) or *ex vivo* lipolysis of gWAT adipocytes, indicating that C3 deficiency does not affect glucose tolerance or adipocyte lipid metabolism.

Complement C3 deficiency does not alter immune cell composition

Even though complement C3$^{-/-}$ mice showed pronounced differences in body composition compared to controls, C3$^{-/-}$ mice exhibited similar percentages of leukocytes (CD45^{+} cells) within the SVF (data not shown), and similar fractions of B cells (CD22.2^{+}), T cells (CD3^{+}) and macrophages (F4/80^{+}) within the leukocyte population of gWAT (**Fig. 4H**). Total cell count of the stromal vascular fraction isolated from gWAT was also the same between the two groups (**Fig. 4I**), as was inflammatory gene expression (*Mcp1, Tnfα, Il-6, Il-10*) in gWAT (**Fig. S3D**).

In blood, total cell count (data not shown) and percentages of B cells and T cells within the leukocyte population were unaffected in C3$^{-/-}$ mice (**Fig. S3E**), but within the T

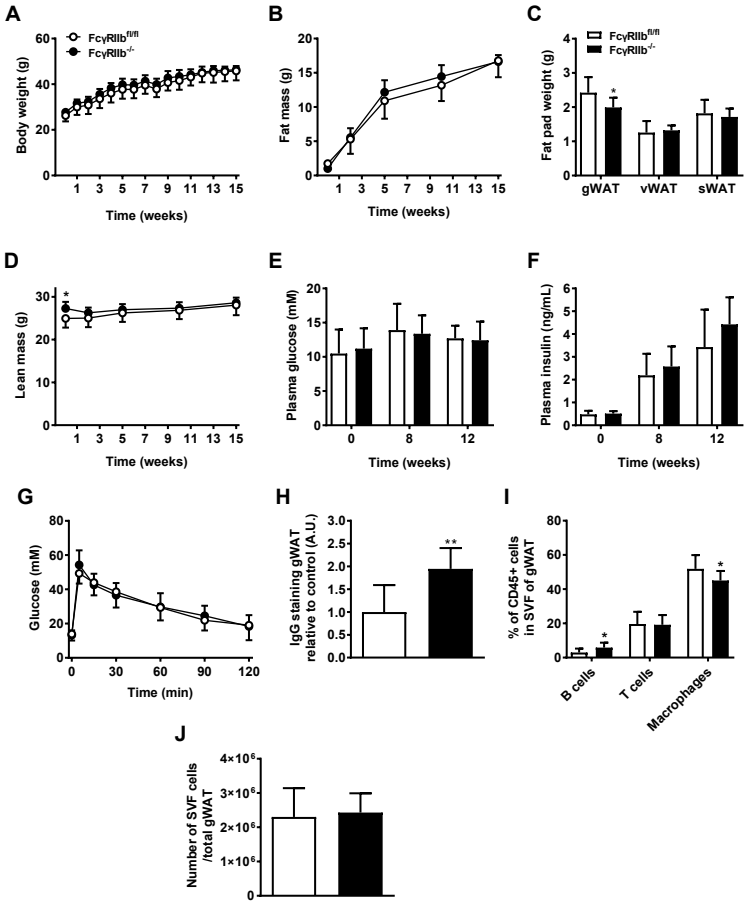


Figure 3. FcγRIIb deficiency does not affect glucose tolerance but increases IgG and slightly alters immune cell composition in gWAT during HFD-induced obesity. *FcγRIIb$^{-/-}$ and FcγRIIb$^{fl/fl}$ control mice were fed an HFD for 15 weeks. Body weight (A), fat mass (B), WAT depot weight (C), lean mass (D), plasma glucose (E) and insulin (F) were analysed at the indicated time points. After 8 weeks, mice were injected intravenously with glucose and clearance from plasma was determined by measuring plasma glucose at 5, 15, 30, 60, 90 and 120 min after injection (G). IgG staining of gWAT sections was performed and quantified (H). The percentage of B cells (CD19$^+$), T cells (CD3$^+$) and macrophages (F4/80$^+$) of CD45$^+$ cells in SVF of gWAT was determined by flow cytometry (I). Total numbers of SVF cells per gWAT were counted (J). Values represent means ± SD. *p<0.05, **p<0.01, ***p<0.001 vs. control (n=11-12) as measured by a two-way ANOVA in D or by a two-tailed unpaired Student's t-test in C, H, I. gWAT, gonadal white adipose tissue; vWAT, visceral white adipose tissue; sWAT, subcutaneous white adipose tissue; SVF, stromal vascular fraction.*

cell population, the percentage of cytotoxic T cells ($CD8^+$) was higher in $C3^{-/-}$ compared to control mice (+10%, p<0.01, **Fig. S3F**). As a result, $C3^{-/-}$ mice exhibited a lower CD4:CD8 ratio (-14%, p<0.05, **Fig. 4J**), representative of increased systemic inflammation. All in all, $C3^{-/-}$ mice had lower body weight but similar glucose tolerance and immune cell composition in WAT as compared to controls.

Deficiency of FcγRI/II/III/IV as well as complement C3 decreases insulin sensitivity during HFD-induced obesity

To exclude the possibility that either FcγRs or complement C3 are sufficient to mediate the effects of IgG signaling on glucose tolerance or that one of the pathways could compensate for lack of the other, mice lacking both C3 and all FcγRs (FcγRI/II/III/IV/$C3^{-/-}$) were fed an HFD for 15 weeks. Body weight, fat mass and lean mass of FcγRI/II/III/IV/$C3^{-/-}$ and control mice was similar after 15 weeks of HFD (**Fig. 5A-D**), although FcγRI/II/III/IV/$C3^{-/-}$ mice showed a transient increase in fat mass compared to controls (**Fig. 5B**). Plasma triglyceride levels (**Fig. S4A**) were not different between the two groups and free fatty acid levels transiently decreased (-18%, p<0.01, **Fig. S4B**) in FcγRI/II/III/IV/$C3^{-/-}$ mice compared to controls. Interestingly, FcγRI/II/III/IV/$C3^{-/-}$ mice also had transiently higher glucose levels after 6 weeks of HFD (+23%, p<0.001, **Fig. 5E**). Plasma insulin levels were higher at baseline (+18%, p<0.05) and after 6 weeks of HFD (+40%, p<0.01, **Fig. 5F**), indicating decreased insulin sensitivity in FcγRI/II/III/IV/$C3^{-/-}$ mice. However, glucose tolerance was not different after 6 weeks of HFD (**Fig. 5G**), suggesting that the increased insulin levels sufficiently compensated for the reduced insulin sensitivity to maintain normoglycemia. *Ex vivo* lipolysis of gWAT adipocytes did not differ between FcγRI/II/III/IV/$C3^{-/-}$ and control mice (**Fig. S4C**), suggesting that the decreased insulin sensitivity was not specific to WAT.

FcγRI/II/III/IV and complement C3 deficiency increases adipose tissue IgG and macrophages

Similar to FcγRI/II/III/$IV^{-/-}$ and FcγRIIb$^{-/-}$ mice, the quantity of IgG in gWAT of the FcγRI/II/III/IV/$C3^{-/-}$ mice was higher compared to controls (+174%, p<0.001, **Fig. 5H**). The leukocyte fraction ($CD45^+$ cells) within the SVF (data not shown) and percentage of B cells ($CD22.2^+$) within the leukocyte population in gWAT were comparable between the two groups. The percentage of T cells ($CD3^+$) was decreased (-20%, p<0.05) and the percentage of macrophages ($F4/80^+$) was increased (+36%, p<0.001) in FcγRI/II/III/IV/$C3^{-/-}$ mice compared to controls (**Fig. 5I**), while the total number of stromal vascular cells isolated from gWAT (**Fig. 5J**) and inflammatory gene expression (*Mcp1, Tnfα, Il-6, Il-10*) in gWAT (**Fig. S4D**) did not differ between the groups.

In blood, no differences were found in total cell count (data not shown), percentages of B and T cells within the leukocyte population (**Fig. S4E**), nor in the ratio of T helper ($CD4^+$) and cytotoxic T cells ($CD8^+$) within the T cell population (**Fig. S4F** and **5K**), indicating that systemic inflammation was not different between the groups. In summary, FcγRI/II/III/IV/$C3^{-/-}$ mice had similar body weight and glucose tolerance compared to controls, but higher IgG levels and altered in immune cell composition in WAT.

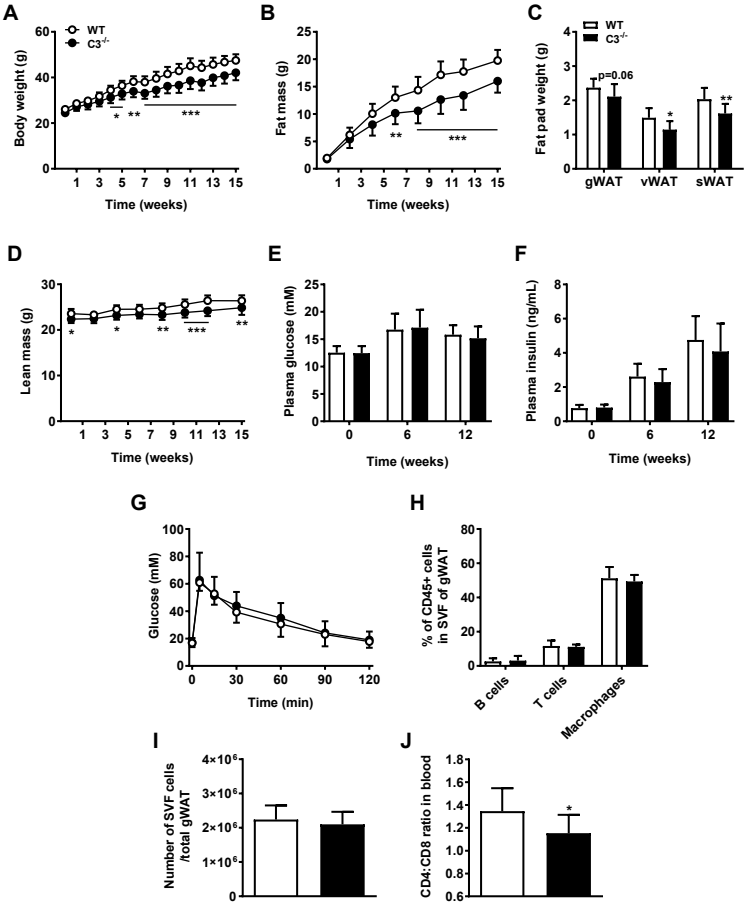


Figure 4. Complement C3 deficiency protects against weight gain but does not affect glucose tolerance or immune cell composition in gWAT during HFD-induced obesity. *Complement C3$^{-/-}$ and wild-type control mice were fed an HFD for 15 weeks. Body weight (A), fat mass (B), WAT depot weight (C), lean mass (D), plasma glucose (E) and insulin (F) were analysed at the indicated time points. After 6 weeks, mice were injected intravenously with glucose and clearance from plasma was determined by measuring plasma glucose at 5, 15, 30, 60, 90 and 120 min after injection (G). The percentage of B cells (CD22.2^{+}), T cells (CD3^{+}) and macrophages (F4/80^{+}) of CD45^{+} cells in SVF of gWAT was determined by flow cytometry (H). Total numbers of SVF cells per gWAT were counted (I). Within the CD3^{+} T cell fraction in blood, the ratio of CD4^{+} and CD8^{+} cells was determined (J). Values represent means ± SD. *p<0.05, **p<0.01, ***p<0.001 vs. control (n=10-12) as measured by a two-way ANOVA in A, B, D or by a two-tailed unpaired Student's t-test in C, J. WT, wild-type; gWAT, gonadal white adipose tissue; vWAT, visceral white adipose tissue; sWAT, subcutaneous white adipose tissue; SVF, stromal vascular fraction.*

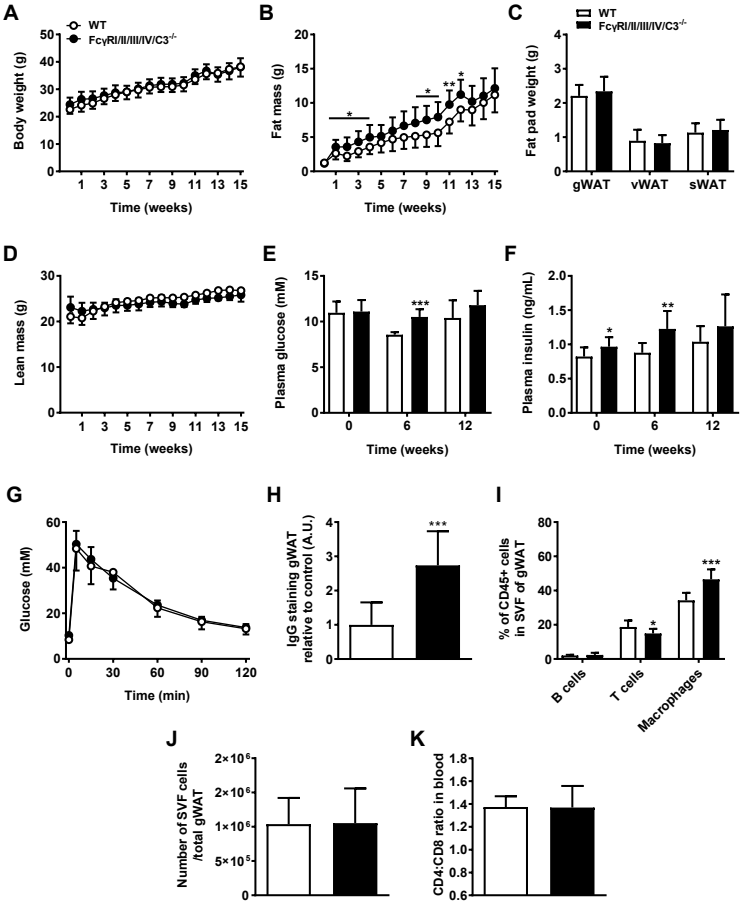


Figure 5. Deficiency of both FcγRI/II/III/IV and complement C3 elevates plasma insulin and increases IgG and macrophages in gWAT during HFD-induced obesity. *FcγRI/II/III/IV/C3$^{-/-}$ and wild-type control mice were fed an HFD for 15 weeks. Body weight (A), fat mass (B), WAT depot weight (C), lean mass (D), plasma glucose (E) and insulin (F) were analysed at the indicated time points. After 6 weeks, mice were injected intravenously with glucose and clearance from plasma was determined by measuring plasma glucose at 5, 15, 30, 60, 90 and 120 min after injection (G). IgG staining of gWAT sections was performed and quantified (H). The percentage of B cells (CD22.2^{+}), T cells (CD3^{+}) and macrophages (F4/80^{+}) of CD45^{+} cells in SVF of gWAT was determined by flow cytometry (I). Total numbers of SVF cells per gWAT were counted (J). Within the CD3^{+} T cell fraction in blood, the ratio of CD4^{+} and CD8^{+} cells was determined (K). Values represent means ± SD. *p<0.05, **p<0.01, ***p<0.001 vs. control (n=9-10) as measured by a two-way ANOVA in A, B, D or by a two-tailed unpaired Student's t-test in C, J. WT, wild-type; gWAT, gonadal white adipose tissue; vWAT, visceral white adipose tissue; sWAT, subcutaneous white adipose tissue; SVF, stromal vascular fraction.*

DISCUSSION

IgG in obese adipose tissue has been shown to decrease glucose tolerance, but the signaling pathway mediating these effects remains unknown (13). By using mouse models deficient for all four FcγRs (FcγRI/II/III/IV$^{-/-}$), only the inhibitory FcγRIIb (FcγRIIb$^{-/-}$), only the central component of the complement system C3 (C3$^{-/-}$), and all FcγRs combined with C3 (FcγRI/II/III/IV/C3$^{-/-}$), we show that deficiency for FcγR and/or complement C3, both of which are important for the effector functions of IgG, does not ameliorate the development of glucose intolerance in diet-induced obesity.

We observed that obese adipose tissue is enriched for B cells and IgG, as compared to lean adipose tissue, and we found that IgG co-localizes with macrophages in crown-like structures within the adipose tissue, which confirms previous reports (13, 21). It would be interesting to uncover to which antigens IgG responds in the adipose tissue. Adipocytes are known to present lipid antigens to immune cells (22) and it has also been reported that HFD-fed mice have higher levels of circulating IgG against modified LDL compared to mice fed a low fat diet (23). Winer *et al.* (13) searched for IgG antigens in serum of obese insulin-resistant *vs.* insulin-sensitive men and found mostly differences in intracellular self-antigens that are ubiquitously expressed in many tissues. Thus, the antigens to which obesity-induced IgG responds remain unknown and uncovering them is of high therapeutic value.

In our study, FcγRI/II/III/IV$^{-/-}$ mice lacking all IgG receptors exhibited deteriorated rather than improved glucose tolerance. This is in contrast to what we expected based on the data of Winer *et al.* (13). They showed that transfer of IgG from diet-induced obese wild-type mice to diet-induced obese B cell deficient mice deteriorated glucose tolerance whereas transfer of IgG lacking the Fc region did not, indicating that the pathogenic effects of IgG are mediated by FcγR or complement. However, the Fc region is important for extension of the half-life of IgG (24), raising the possibility that the lack of effect in the latter experiment is due to increased clearance of IgG. Since insulin sensitivity in the white adipocytes was not affected in our FcγRI/II/III/IV$^{-/-}$ mice, we speculate that other organs such as muscle could be responsible for the deterioration of glucose tolerance we found. FcRs are important for immune complex clearance (25) and lack of FcγRs may lead to accumulation of immune complexes in tissues and subsequent induction of pro-inflammatory responses and pathology (26). Indeed, we show that FcγRI/II/III/IV$^{-/-}$ mice had increased systemic inflammation as evidenced by more cytotoxic T cells in blood. To our knowledge, deficiency of all four FcγR in relation to obesity has not been studied to date. We have previously shown that in mice lacking the FcRγ-chain, the γ-subunit necessary for signaling and cell surface expression of activating FcγR and FcεRI, the development of obesity and glucose intolerance was reduced. However, these effects could be due to IgE (the ligand for FcεRI) or other non-IgG-effects, as the γ-subunit is not exclusively associated with Fc-receptors (27). The FcγRI/II/III/IV$^{-/-}$ model is Fc-receptor-specific and therefore suitable to study specific effects of IgG signaling. FcγRIIb deficient mice did not show abnormal glucose

tolerance, indicating that the stimulating FcγRs were responsible for the deteriorated glucose metabolism that we observed in FcγRI/II/III/IV$^{-/-}$ mice.

Since absence of FcγRs did not improve glucose tolerance in obese mice, we studied whether complement C3 is the pathway mediating the pathogenic effects of IgG. We found decreased body weight and fat mass due to less fat in several WAT depots in C3$^{-/-}$ mice, which is in accordance with previous reports establishing that C3$^{-/-}$ mice on an HFD have reduced weight of the WAT depots (28). We found that the decreased total body weight was partly due to lower lean mass of C3$^{-/-}$ mice compared to controls. C3$^{-/-}$ mice are known to have decreased birth weight (29), which is associated with reduced lean mass later in life (30) and thus coincides with our finding. Despite lower body weight, C3$^{-/-}$ mice did not exhibit different glucose tolerance compared to controls after HFD. Disparate data on the effects of C3 deficiency on glucose tolerance have been reported. C3$^{-/-}$ compared to wild-type mice on different backgrounds (C57Bl/6 and 129Sv) displayed similar insulin but lower plasma glucose levels (31). Murray *et al.* (28) also found lower fasting glucose levels in female C3$^{-/-}$ mice on a chow diet and after 4 weeks of HFD compared to controls. However, after 16 weeks of HFD, fasting glucose and glucose tolerance did not differ anymore (28). The same group has also reported comparable fasting glucose and insulin levels in C3$^{-/-}$ and wild-type mice on a chow diet (32), which is in line with what we found and suggests that complement C3 does not act on glucose levels or tolerance.

Mice lacking both FcγRs and C3 showed increased plasma insulin, possibly due to absence of FcγRs as FcγRI/II/III/IV$^{-/-}$ mice also exhibited increased plasma insulin. However, no effects on glucose tolerance were found. This excludes the possibility that presence of either FcγRs or C3 is sufficient to mediate the development of glucose intolerance during obesity. Redundant roles for FcγR and C3 have been demonstrated before in autoimmune hypopigmentation. In the model for this pathology, FcR or C3 deficiency alone did not prevent development of antibody-mediated hypopigmentation, whereas a combined deficiency did (33).

Of note, all our models lacking the inhibitory FcγRIIb displayed higher IgG levels in adipose tissue compared to control mice. This is probably explained by the fact that this receptor mediates a negative feedback loop to dampen B cell activity and antibody production (15) and in agreement with the higher antibody titres in FcγRIIb$^{-/-}$ mice upon immunization with antigens (34). Strikingly, FcγRI/II/III/IV$^{-/-}$, FcγRIIb$^{-/-}$ and C3$^{-/-}$ mice did not exhibit any or only showed negligible alterations in percentage of adipose tissue B cells, T cells and macrophages. FcγRI/II/III/IV$^{-/-}$ mice even had reduced numbers of SVCs in adipose tissue, which is unexpected given the deteriorated glucose tolerance we found and therefore reinforces our hypothesis that other organs besides the adipose tissue may be the cause of the phenotype observed. Only FcγRI/II/III/IV/C3$^{-/-}$ mice exhibited an appreciable increase in adipose tissue macrophages, but this was not accompanied by metabolic derangements. The lack of differences in inflammation between our immune deficient models and controls suggests that if obesity-associated IgG signals via FcγRs or complement, these pathways may be inferior to or compensated by other inflammatory processes occurring during obesity. Mice lacking B and T cells show a marked increase in macrophages and natural

killer cells in adipose tissue while the onset of obesity and the state of insulin resistance is unaffected compared to wild-type mice fed an HFD (21). In this light, one could argue that the role of B cells as a whole and therefore also IgG effector pathways are expendable in the development of glucose intolerance. Counter-regulatory mechanisms within the immune system may stretch beyond up- or downregulation of entire immune cell populations, as elevated inflammatory cytokine responses can already rescue deficiency of others (35). The higher cytotoxic T cell percentages we observed in blood of FcγRI/II/III/IV$^{-/-}$and C3$^{-/-}$ mice may be the result of such compensatory inflammatory mechanisms.

In conclusion, our data demonstrate that B cells and IgG in adipose tissue increase in diet-induced obesity, and lack of FcγRIIb further increases IgG in adipose tissue. However, FcγR and/or complement C3 deficiency does not ameliorate the development of HFD-induced glucose intolerance. These data suggest that if HFD-induced IgG contributes to the development of glucose intolerance, this is not mediated by FcγRs or complement. Future research is necessary to identify the antigens that IgG in adipose tissue binds and will also reveal whether IgG-induced pathology in obesity is mediated by other mechanisms, for example the deposition of immune complexes.

ACKNOWLEDGEMENTS

We thank Fatiha el Bouazzaoui, Mattijs Heemskerk, Jill Claassens (Dept. of Human Genetics, LUMC, Leiden, The Netherlands), Lianne van der Wee-Pals and Trea Streefland (Dept. of Medicine, Div. of Endocrinology, LUMC, Leiden, The Netherlands) for their excellent technical assistance.

FUNDING

This work was supported by grants from the Center of Medical Systems Biology (CMSB), The Netherlands Consortium for Systems Biology (NCSB) established by the Netherlands Genomics Initiative/Netherlands Organization for Scientific Research (NGI/NWO), the Leiden University Medical Center and Rembrandt Institute of Cardiovascular Science (RICS). Mariëtte R. Boon is supported by the Dutch Diabetes Foundation (grant 2015.81.1808). Patrick C.N. Rensen is an Established Investigator of the Netherlands Heart Foundation (grant 2009T038).

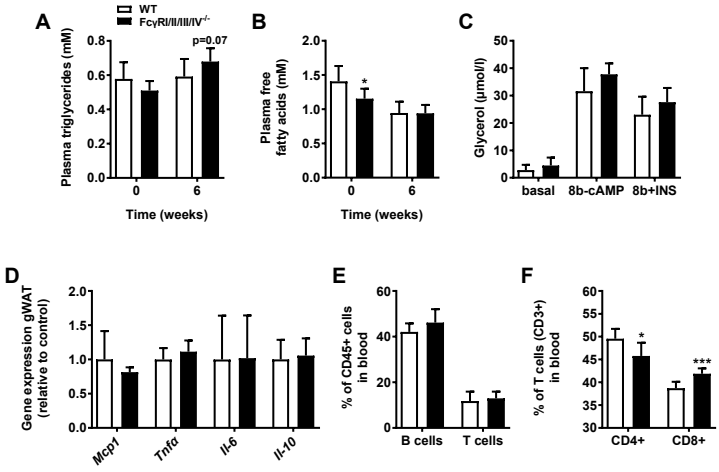
REFERENCES

1. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H: Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. The Journal of clinical investigation 2003;112:1821-1830
2. Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM, Wynshaw-Boris A, Poli G, Olefsky J, Karin M: IKK-beta links inflammation to obesity-induced insulin resistance. Nature medicine 2005;11:191-198
3. Ouchi N, Parker JL, Lugus JJ, Walsh K: Adipokines in inflammation and metabolic disease. Nature reviews Immunology 2011;11:85-97
4. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, Jr.: Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003;112:1796-1808
5. van Beek L, van Klinken JB, Pronk AC, van Dam AD, Dirven E, Rensen PC, Koning F, Willems van Dijk K, van Harmelen V: The limited storage capacity of gonadal adipose tissue directs the development of metabolic disorders in male C57Bl/6J mice. Diabetologia 2015;58:1601-1609
6. Lumeng CN, Bodzin JL, Saltiel AR: Obesity induces a phenotypic switch in adipose tissue macrophage polarization. The Journal of clinical investigation 2007;117:175-184
7. Talukdar S, Oh DY, Bandyopadhyay G, Li D, Xu J, McNelis J, Lu M, Li P, Yan Q, Zhu Y, Ofrecio J, Lin M, Brenner MB, Olefsky JM: Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. Nature medicine 2012;18:1407-1412
8. Liu J, Divoux A, Sun J, Zhang J, Clement K, Glickman JN, Sukhova GK, Wolters PJ, Du J, Gorgun CZ, Doria A, Libby P, Blumberg RS, Kahn BB, Hotamisligil GS, Shi GP: Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. Nature medicine 2009;15:940-945
9. Wu D, Molofsky AB, Liang HE, Ricardo-Gonzalez RR, Jouihan HA, Bando JK, Chawla A, Locksley RM: Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. Science (New York, NY) 2011;332:243-247
10. Seijkens T, Kusters P, Chatzigeorgiou A, Chavakis T, Lutgens E: Immune cell crosstalk in obesity: a key role for costimulation? Diabetes 2014;63:3982-3991
11. Winer S, Chan Y, Paltser G, Truong D, Tsui H, Bahrami J, Dorfman R, Wang Y, Zielenski J, Mastronardi F, Maezawa Y, Drucker DJ, Engleman E, Winer D, Dosch HM: Normalization of obesity-associated insulin resistance through immunotherapy. Nature medicine 2009;15:921-929
12. Nishimura S, Manabe I, Nagasaki M, Eto K, Yamashita H, Ohsugi M, Otsu M, Hara K, Ueki K, Sugiura S, Yoshimura K, Kadowaki T, Nagai R: CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nature medicine 2009;15:914-920
13. Winer DA, Winer S, Shen L, Wadia PP, Yantha J, Paltser G, Tsui H, Wu P, Davidson MG, Alonso MN, Leong HX, Glassford A, Caimol M, Kenkel JA, Tedder TF, McLaughlin T, Miklos DB, Dosch HM, Engleman EG: B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. Nature medicine 2011;17:610-617
14. Alberts B. JA, Lewis J., Raff M., Roberts K., Walter P.: *Molecular Biology of the Cell*. New York, Garland Science, 2002
15. Nimmerjahn F, Ravetch JV: Fcgamma receptors as regulators of immune responses. Nature reviews Immunology 2008;8:34-47

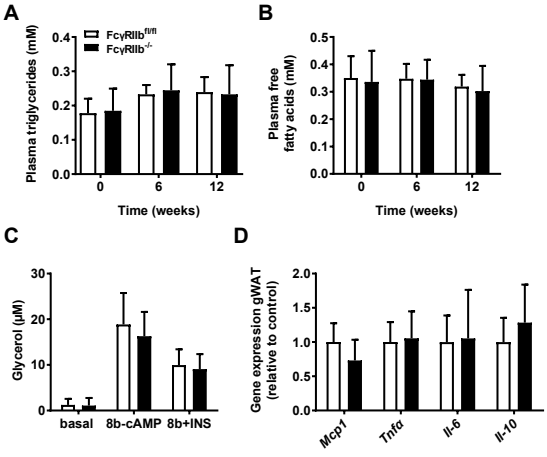
16. Mamane Y, Chung Chan C, Lavallee G, Morin N, Xu LJ, Huang J, Gordon R, Thomas W, Lamb J, Schadt EE, Kennedy BP, Mancini JA: The C3a anaphylatoxin receptor is a key mediator of insulin resistance and functions by modulating adipose tissue macrophage infiltration and activation. Diabetes 2009;58:2006-2017
17. Boross P, Arandhara VL, Martin-Ramirez J, Santiago-Raber ML, Carlucci F, Flierman R, van der Kaa J, Breukel C, Claassens JW, Camps M, Lubberts E, Salvatori D, Rastaldi MP, Ossendorp F, Daha MR, Cook HT, Izui S, Botto M, Verbeek JS: The inhibiting Fc receptor for IgG, FcgammaRIIB, is a modifier of autoimmune susceptibility. Journal of immunology 2011;187:1304-1313
18. Wessels MR, Butko P, Ma M, Warren HB, Lage AL, Carroll MC: Studies of group B streptococcal infection in mice deficient in complement component C3 or C4 demonstrate an essential role for complement in both innate and acquired immunity. Proceedings of the National Academy of Sciences of the United States of America 1995;92:11490-11494
19. Zambon A, Hashimoto SI, Brunzell JD: Analysis of techniques to obtain plasma for measurement of levels of free fatty acids. Journal of lipid research 1993;34:1021-1028
20. Clark AM, Sousa KM, Jennings C, MacDougald OA, Kennedy RT: Continuous-flow enzyme assay on a microfluidic chip for monitoring glycerol secretion from cultured adipocytes. Anal Chem 2009;81:2350-2356
21. Duffaut C, Galitzky J, Lafontan M, Bouloumie A: Unexpected trafficking of immune cells within the adipose tissue during the onset of obesity. Biochemical and biophysical research communications 2009;384:482-485
22. Huh JY, Kim JI, Park YJ, Hwang IJ, Lee YS, Sohn JH, Lee SK, Alfadda AA, Kim SS, Choi SH, Lee DS, Park SH, Seong RH, Choi CS, Kim JB: A novel function of adipocytes in lipid antigen presentation to iNKT cells. Molecular and cellular biology 2013;33:328-339
23. Wolf D, Jehle F, Ortiz Rodriguez A, Dufner B, Hoppe N, Colberg C, Lozhkin A, Bassler N, Rupprecht B, Wiedemann A, Hilgendorf I, Stachon P, Willecke F, Febbraio M, von zur Muhlen C, Binder CJ, Bode C, Zirlik A, Peter K: CD40L deficiency attenuates diet-induced adipose tissue inflammation by impairing immune cell accumulation and production of pathogenic IgG-antibodies. PloS one 2012;7:e33026
24. Ghetie V, Ward ES: Multiple roles for the major histocompatibility complex class I- related receptor FcRn. Annual review of immunology 2000;18:739-766
25. van Lent P, Nabbe KC, Boross P, Blom AB, Roth J, Holthuysen A, Sloetjes A, Verbeek S, van den Berg W: The inhibitory receptor FcgammaRII reduces joint inflammation and destruction in experimental immune complex-mediated arthritides not only by inhibition of FcgammaRI/III but also by efficient clearance and endocytosis of immune complexes. The American journal of pathology 2003;163:1839-1848
26. Rodriguez-Iturbe B, Haas M: Post-Streptococcal Glomerulonephritis. In *Streptococcus pyogenes: Basic Biology to Clinical Manifestations* Ferretti JJ, Stevens DL, Fischetti VA, Eds. Oklahoma City, University of Oklahoma Health Sciences Center, 2016
27. van Beek L, Vroegrijk IO, Katiraei S, Heemskerk MM, van Dam AD, Kooijman S, Rensen PC, Koning F, Verbeek JS, Willems van Dijk K, van Harmelen V: FcRgamma-chain deficiency reduces the development of diet-induced obesity. Obesity (Silver Spring, Md) 2015;23:2435-2444
28. Murray I, Havel PJ, Sniderman AD, Cianflone K: Reduced body weight, adipose tissue, and leptin levels despite increased energy intake in female mice lacking acylation-stimulating protein. Endocrinology 2000;141:1041-1049
29. Chow WN, Lee YL, Wong PC, Chung MK, Lee KF,

Yeung WS: Complement 3 deficiency impairs early pregnancy in mice. Molecular reproduction and development 2009;76:647-655

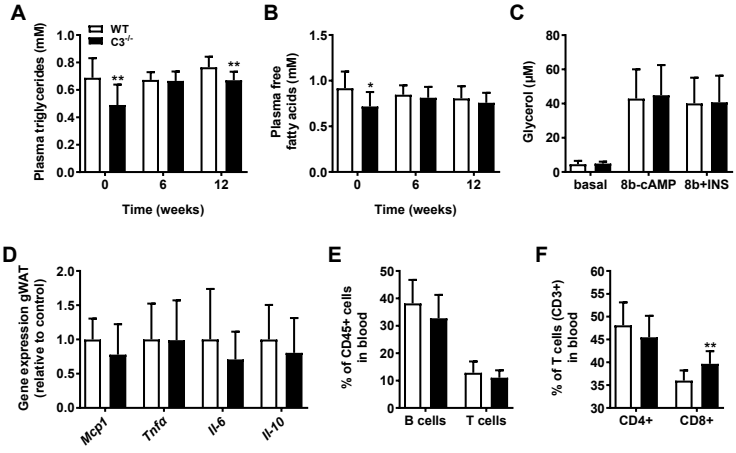
30. Yliharsila H, Kajantie E, Osmond C, Forsen T, Barker DJ, Eriksson JG: Birth size, adult body composition and muscle strength in later life. International journal of obesity (2005) 2007;31:1392-1399
31. Cianflone K, Xia Z, Chen LY: Critical review of acylation-stimulating protein physiology in humans and rodents. Biochimica et biophysica acta 2003;1609:127-143
32. Murray I, Sniderman AD, Cianflone K: Mice lacking acylation stimulating protein (ASP) have delayed postprandial triglyceride clearance. Journal of lipid research 1999;40:1671-1676
33. Trcka J, Moroi Y, Clynes RA, Goldberg SM, Bergtold A, Perales MA, Ma M, Ferrone CR, Carroll MC, Ravetch JV, Houghton AN: Redundant and alternative roles for activating Fc receptors and complement in an antibody-dependent model of autoimmune vitiligo. Immunity 2002;16:861-868
34. Takai T, Ono M, Hikida M, Ohmori H, Ravetch JV: Augmented humoral and anaphylactic responses in Fc gamma RII-deficient mice. Nature 1996;379:346-349
35. Mayer-Barber KD, Yan B: Clash of the Cytokine Titans: counter-regulation of interleukin-1 and type I interferon-mediated inflammatory responses. Cellular & molecular immunology 2017;14:22-35



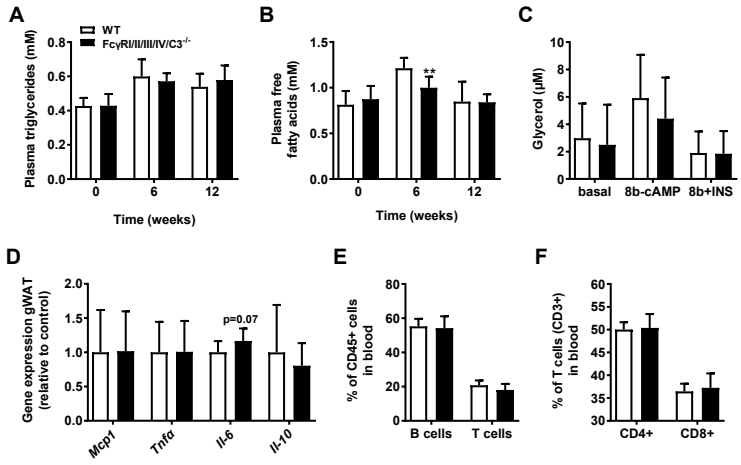
Supplementary figure 1. *FcγRI/II/III/IV$^{-/-}$ and wild-type control mice were fed an HFD for 15 weeks. Plasma triglycerides (A) and free fatty acids (B) were analysed at the indicated time points. Basal, 8b-cAMP-stimulated and insulin inhibition of 8b-cAMP-stimulated ex vivo glycerol release (index of lipolysis) from isolated gonadal white adipocytes were determined (C). GWAT mRNA expression of the indicated inflammatory genes was determined (D). Within the CD45^{+} cell population in blood, the percentages of B cells (CD19^{+}) and T cells (CD3^{+}) were determined (E). Within the CD3^{+} T cell fraction in blood, the percentages of CD4^{+} and CD8^{+} cells were determined (K). Values represent means ± SD. *p<0.05, ***p<0.001 vs. control (n=7-8) as measured by a two-tailed unpaired Student's t-test. WT, wild-type; gWAT, gonadal white adipose tissue.*



Supplementary figure 2. *FcγRIIb$^{-/-}$ and FcγRIIb$^{fl/fl}$ control mice were fed an HFD for 15 weeks. Plasma triglycerides (A) and free fatty acids (B) were analysed at the indicated time points. Basal, 8b-cAMP-stimulated and insulin inhibition of 8b-cAMP-stimulated ex vivo glycerol release (index of lipolysis) from isolated gonadal white adipocytes were determined (C). GWAT mRNA expression of the indicated inflammatory genes was determined (D). Values represent means ± SD. gWAT, gonadal white adipose tissue.*



Supplementary figure 3. *Complement C3$^{-/-}$ and wild-type control mice were fed an HFD for 15 weeks. Plasma triglycerides (A) and free fatty acids (B) were analysed at the indicated time points. Basal, 8b-cAMP-stimulated and insulin inhibition of 8b-cAMP-stimulated ex vivo glycerol release (index of lipolysis) from isolated gonadal white adipocytes were determined (C). GWAT mRNA expression of the indicated inflammatory genes was determined (D). Within the CD45$^+$ cell population in blood, the percentages of B cells (CD22.2$^+$) and T cells (CD3$^+$) were determined (E). Within the CD3$^+$ T cell fraction in blood, the percentages of CD4$^+$ and CD8$^+$ cells were determined (K). Values represent means ± SD. *p<0.05, **p<0.01 vs. control (n=10-12) as measured by a two-tailed unpaired Student's t-test. WT, wild-type; gWAT, gonadal white adipose tissue.*



Supplementary figure 4. *FcγRI/II/III/IV/C3$^{-/-}$ and wild-type control mice were fed an HFD for 15 weeks. Plasma triglycerides (A) and free fatty acids (B) were analysed at the indicated time points. Basal, 8b-cAMP-stimulated and insulin inhibition of 8b-cAMP-stimulated ex vivo glycerol release (index of lipolysis) from isolated gonadal white adipocytes were determined (C). GWAT mRNA expression of the indicated inflammatory genes was determined (D). Within the CD45^{+} cell population in blood, the percentages of B cells (CD22.2^{+}) and T cells (CD3^{+}) were determined (E). Within the CD3^{+} T cell fraction in blood, the percentages of CD4^{+} and CD8^{+} cells were determined (K). Values represent means ± SD. **p<0.01 vs. control (n=10) as measured by a two-tailed unpaired Student's t-test. WT, wild-type; gWAT, gonadal white adipose tissue.*