Deficiency of FcεR1 increases body weight gain but improves glucose tolerance in diet-induced obese mice

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Abstract

Prior studies demonstrated increased plasma immunoglobulin E (IgE) in diabetic patients, but the direct participation of IgE in diabetes or obesity remains unknown. This study found that plasma IgE levels correlated inversely with body weight, body mass index, and body fat mass among a population of randomly selected obese women. IgE receptor FcεR1-deficient (*Fcer1a*$^{-/-}$) mice and diet-induced obesity (DIO) mice demonstrated that FcεR1 deficiency in DIO mice increased food intake, reduced energy expenditure, and increased body weight gain, but improved glucose tolerance and glucose-induced insulin secretion. White adipose tissue (WAT) from *Fcer1a*$^{-/-}$ mice showed increased expression of phospho-AKT, C/EBPα, PPARγ, Glut4, and Bcl-2, but reduced UCP1 and phospho-JNK expression, tissue macrophage accumulation, and apoptosis, suggesting that IgE reduces adipogenesis and glucose uptake, but induces energy expenditure, adipocyte apoptosis, and WAT inflammation. In 3T3-L1 cells, IgE inhibited the expression of C/EBPα and PPARγ, and preadipocyte adipogenesis, and induced adipocyte apoptosis. IgE reduced 3T3-L1 cell expression of Glut4, phospho-AKT, and glucose uptake, which concurred with improved glucose tolerance in *Fcer1a*$^{-/-}$ mice. This study established two novel pathways of IgE in reducing body weight gain in DIO mice by suppressing adipogenesis and inducing adipocyte apoptosis, while worsening glucose tolerance by reducing Glut4 expression, glucose uptake, and insulin secretion.

Introduction

Immunoglobulin E (IgE) activates mast cells by binding to its high affinity receptor Fcε receptor-1 (FcεR1). This activity of IgE is essential to allergic responses (1), such as asthma. Recent studies demonstrated that IgE also activates macrophages and T cells (2, 3). All these IgE-targeting cells play detrimental roles in obesity and diabetes (4-6), suggesting the participation of IgE in these metabolic diseases. Although the direct role of IgE in obesity and diabetes remains untested, asthma associates with increased plasma IgE (7) and acts as an important risk factor of obesity and diabetes. Of 4,773 subjects aged 20 and older randomly selected from 10,348 individuals from 2005 to 2006 in the National Health and Nutrition Examination Survey (NHANES) in the United States, IgE concentrations correlated positively with obesity risk but not insulin resistance in asthmatic patients (8). Of 4,321 children aged 2 to 19 from the same population, obese and overweight children had higher plasma total IgE levels, driven largely by allergic sensitivity to foods (9). A respective study of 246 adults with asthma and other atopic disorders revealed that asthmatic patients had higher body mass indices (BMI) than non-asthmatics. Obesity associated with increased serum IgE among those patients (10). Yet in a population study of 666 patients with severe asthma, plasma IgE levels correlated negatively with BMI (11). These studies therefore do not prove the direct participation of IgE in body weight gain. Previous studies investigated IgE in patients and animals with diabetes. A linear regression analysis of a population study of 340 patients aged 55 to 75 revealed a positive correlation between plasma IgE and type-2 diabetes mellitus and pre-diabetes status. Ordinal logistic regression demonstrated that plasma IgE correlates with the incidence of type-2 diabetes before and after adjusting for common diabetes risk

factors (12, 13). In non-obese diabetic (NOD) mice, anti-FcεR1 antibody therapy activated basophils and MCs, but delayed type 1 diabetes (14). These observations from diabetic patients and mice highlight the role of IgE in diabetes.

This study design was twofold: to test the direct role of IgE in obesity and diabetes using FcεR1-deficient *Fcer1a*$^{-/-}$ mice in diet-induced obese and diabetic mice; and to understand the molecular and cellular mechanism by which this immunoglobulin molecule contributes to these metabolic diseases.

Materials and Methods

Patients

A random selection from a bariatric surgery program from the Institute of Cardiometabolism and Nutrition (ICAN), Pitié-Salpêtrière Hospital (Paris, France) yielded a cohort of 50 Caucasian women with morbid obesity. These patients met the criteria for bariatric surgery (BMI $\geq$ 40 kg/m^2, or $\geq$ 35 kg/m^2 with at least one comorbidity: hypertension, type-2 diabetes, dyslipidemia, or obstructive sleep apnea syndrome), but without allergic or autoimmune diseases or anti-allergy and anti-autoimmunity medications that may affect plasma IgE levels. Subjects had stable weights (±3 kg) for at least 3 months before the surgery. Of the 50 patients, 18 (36 %) had type 2 diabetes as defined by a fasting glycemia >7 mmol/L and/or the use of an anti-diabetic drug. The ethics committees of the CPP Ile de France 1 (number 0611351) approved the clinical investigations. All subjects gave written informed consent. Spearman's correlation test helped test the correlation between plasma IgE concentration and clinical and biological parameters at baseline. Patient body composition was determined by dual-

energy X-ray absorptiometry (DEXA, Hologic, Bedford, MA). Blood samples were obtained before the bariatric surgery after 12 hours of fasting to measure total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, insulin, glucose, hemoglobin (HbA1c), leptin, adiponectin, inflammatory markers (highly sensitive C-reactive protein (hs-CRP) and interleukin-6 (IL6), and IgE as previously described (12, 13, 15, 16).

Mice

We used C57BL/6 (Jackson Laboratory, Bar Harbor, ME) and *Fcer1a*$^{-/-}$ mice (C57BL/6, N9) (2, 3). All mice used in this study were littermates. Males (or female) mice at 6 weeks of age from each group were fed a high-fat diet (HFD, D12492: 60 kcal% fat, Research Diets Inc. New Brunswick, NJ) for 17 weeks. Mouse body weight was monitored weekly. After 17 weeks on a HFD, mouse total body fat and lean masses were assessed by dual energy X-ray absorptiometry (DEXA; PIXImus, Fitchburg, WI). For calorimetric analysis, these mice were placed individually in an indirect open circuit calorimeter (Oxymax System; Columbus Instruments, Columbus, OH). Oxygen and carbon dioxide concentrations by volume were monitored at the inlet and outlet parts of a partially sealed chamber, through which a known flow of ambient air was forcibly ventilated. The concentration difference measured between the parts was used to compute oxygen consumption (VO_2) and carbon dioxide production (VCO_2). The consumption and production information were presented in units of ml/kg/h and normalized to 25 °C and 760 mmHg. Food intake was investigated by using the Oxymax Feed Scale Device (Columbus Instruments) for three continuous days and data were presented as the average food intake per day of the last two days without considering the first day acclimation

period. The physical activity of the mice was monitored with OPTO-M3 Activity Application Device (Columbus Instruments). The movements (other than scratching, grooming, digging, etc.) of each animal were determined by infrared beams in x, y, and z axes. After 17 weeks on a HFD, an intraperitoneal glucose tolerance test (1.5 g glucose/kg body weight) and an insulin tolerance test (ITT) (1.5 U/kg body weight) were also performed after an overnight (16 hours) and daytime 5-hour fast, respectively. Mice were sacrificed and fat tissue was collected. Mice were bred and maintained according to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. Harvard Medical School Standing Committee on Animals approved all animal protocols.

Cell culture

3T3-L1 (ATCC, CL-173) cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Life Technologies, Woburn, MA) including 10% calf serum and L-glutamine. To induce adipogenesis, complete confluent 3T3-L1 cells were cultured in induction media containing DMEM (Life Technologies), 10% fetal bovine serum, L-glutamine, MEM sodium pyruvate, 0.0115 g/ml 3-isobutyl-1-methylxanthine (IBMX; Sigma, St. Louis, MO), 1 mM dexamethasone (Sigma), and 167 μM insulin (Sigma) for 2 days and for additional 6 days without IBMX and dexamethasone.

Islet isolation and glucose-stimulated insulin secretion (GSIS) by islets

Islets were isolated from 2-month-old male C57BL/6J mice (Jackson Laboratory) by the intraductal collagenase digestion method, as described previously (17). For GSIS assay,

after culturing for 12 hours in RPMI 1640 medium containing 5.6 mM glucose and supplemented with 10% fetal calf serum, ten size-matched islets were incubated at 37°C for 1.5 hours in Krebs-Ringer bicarbonate buffer containing 2.8, 8.3 or 22.2 mM glucose with or without IgE (0.01, 0.1, 1, 10, 100 μg/ml). The insulin levels in the culture media were measured using an insulin ELISA kit (Crystal Chem Inc., Downers Grove, IL). FcεR1α mRNA levels were measured in total RNA extracted from 50 islets that were incubated at 37°C for 24 hours in RPMI 1640 medium containing 5.6 mM glucose with 10% fetal calf serum in the presence or absence of IgE (0.01, 0.1, 1, 10, 100 μg/ml). Each quantitative reaction was performed in duplicate.

Quantitative real-time PCR

Total RNA was extracted from WAT, 3T3-L1 cells, bone marrow-derived macrophages, or islets using a Qiagen RNA extraction kit (Qiagen, Valencia, CA). Quantified total RNA using Nanodrop 2000 (Thermo Fisher Scientific Inc., Waltham, MA) was transcribed into first strand cDNA using Superscript First Strand kit (Life Technologies). Real-time PCR (RT-PCR) was performed using SYBR green super mix (Bio-Rad) in Bio-Rad iCycler iQ to determine the mRNA levels of C/EBPα, PPARγ, and three FcεR1 chains (α, β, and γ) using 36B4 (acidic ribosomal phosphoprotein PO) and β-actin as internal controls to normalize gene expression. RT-PCR data was analyzed based on delta delta CT calculation and presented as the fold of change obtained from the value of 2^(-ΔΔCT). All RT-PCR primer sequences are listed in Supplementary Table 1.

Immunoblotting and immunohistochemistry

WAT, brown adipose tissue (BAT), and cells were lysed in a RIPA buffer containing 50 mM Tris, pH 7.4, 150 mM NaCl, 2 mM EDTA, 1% NP-40, 0.1% SDS, proteinase inhibitor (Roche Diagnostics Corporation, Indianapolis, IN), and phosphatase inhibitor cocktail (Roche). Tissue lysates were centrifuged at 20,000 xg for 15 min. Supernatant was removed without interrupting the upper layer fat for protein concentration determination using the DC protein assay kit (Bio-Rad, Hercules, CA). Tissue or cell lysate was separated by SDS-PAGE, blotted, and detected with different antibodies, including FcεR1a, glucose transporter-4 (Glut4), Bcl-2, p-JNK, total JNK, p-AKT, total AKT, CCAAT/enhancer binding protein-α (CEBPα), peroxisome proliferator-activated receptor-γ (PPARγ), uncoupling protein 1 (UCP1), and β-actin or glyceraldehyde 3-phosphate dehydrogenase (GAPDH). WAT paraffin sections (6 μm) were prepared for immunohistochemistry with antibodies to detect macrophages (Mac-2), T cells (CD3), and FcεR1, and TUNEL staining (In Situ Cell Death Detection Kit, Roche Diagnostics Corp) to detect apoptotic cells. We used AlexaFluor conjugated with different fluorochromes (Invitrogen) to show localization of FcεR1 to inflammatory cells. All antibodies are listed in Supplementary Table 2.

ELISA

ELISA determined plasma IL6 (eBioscience), monocyte chemotactic protein-1 (MCP-1) (eBioscience), IgE (BD Biosciences, Bedford, MA), insulin (Crystal Chem Inc.) and serum amyloid A (Life Technologies), according to the manufacturers' instructions.

2-Deoxyglucose (2DG) uptake assay

Preadipocyte 3T3L1 cells were differentiated to adipocytes in a 48-well plate with and without IgE (0, 1, 10, 50 μg/ml). After 2 days, glucose uptake was performed using a 2-deoxyglucose (2DG) uptake measurement kit (Cosmo Bio Co. Ltd., Tokyo, Japan), according to manufacturer's instructions.

siRNA transfection

Both FcεR1α and scramble control siRNAs (100 nM, Santa Cruz) were transfected to preadipocyte 3T3-L1 cells in a 12 well-plate after electroporation with an Amaxa® Cell Line Nucleofector® Kit (Lonza, Allendale, NJ). After 24 hours, cells were differentiated in an induction medium and cultured for 4 days followed by starvation and stimulation with 25 μg/ml IgE for 10 min. Cells were lysed for protein analysis.

Cell Cytotoxicity assay

Preadipocyte 3T3-L1 cells were differentiated to adipocytes on an 8-well chamber slide or a 96-well plate with and without IgE (50 μg/ml) for 2–8 days before TUNEL staining (In Situ Cell Death Detection Kit, Roche Diagnostics Corp.), cell counting kit-8 (CCK-8), cell viability assay (Dojindo Molecular Technologies, Inc, Rockville, MD), or lactate dehydrogenase cytotoxicity assay (LDH, Promega, Madison, WI), according to the manufacturers' instructions.

Oil-red O staining

Differentiated 3T3-L1 cells with and without IgE (50ug/ml) in a 96-well plate were fixed with 10% formalin for one hour, washed with 100% propylene glycol, and stained with 0.5% oil-red O for 4 hours. This procedure was followed by washing with 85% propylene

glycol. For quantitative analysis, stained cell layers were extracted with isopropanol and measured at $OD_{510\ nm}$.

Statistical analysis

All human data are expressed as means ± SD. Correlation analyses between IgE concentration and clinical parameters were performed using Spearman's correlation. Regression plots were built after log transformation of IgE values for normalization purpose. All *P*-values are two-sided, and *P*-values of <0.05 were considered to be statistically significant. All analyses were performed using R software, version 3.0.1. All mouse data were expressed as mean ± SEM. Due to our small sample sizes and often skewed data distributions, we performed a pairwise non-parametric Mann-Whitney test followed by Bonferroni corrections to examine the statistical significance.

Results

Inverse correlation between human plasma IgE and obesity

Data obtained from the 50 obese women (age: 42±11 years, BMI: 50.67±8.26 kg/m^2) showed that serum IgE correlated negatively with BMI (*P*=0.018, Rho= -0.33) (Figure 1A), body weight (*P*= 0.016, Rho= -0.34) (Figure 1B), and fat mass (*P*=0.023, Rho= -0.34) (Figure 1C). Fasting glycemia, insulin, HbA1C, triglyceride, high-density lipoprotein HDL, ApoA1, ApoB, aspartate amino-transferase AST, alanine amino-transferase ALT, γ-glutamyl transpeptidase γGT, leptin, adiponectin, IL6, and hs-CRP did not associate with IgE levels. Only total cholesterol correlated positively with IgE (*P*= 0.028, Rho=0.31) (Supplementary Table 3). Of the 50 severely obese patients, 18

had type 2-diabetes. Diabetic obese patients were significantly older and exhibited a higher BMI, fasting glycemia, fasting insulin, and HbA1C as expected. These patients also had lower HDL and higher triglyceride, ALT, γGT, IL6 and hs-CRP levels than non-diabetic obese patients. Diabetic and non-diabetic obese patients did not exhibit significantly different plasma IgE levels, however (data not shown).

FcεR1 deficiency increases body weight gain, but improves glucose tolerance in mice

This study monitored the body weight and included glucose and insulin tolerance assays in both male and female WT and FcεR1-deficient *Fcer1a*$^{-/-}$ mice. Male (Figure 2A) or female (data not shown) FcεR1-deficient *Fcer1a*$^{-/-}$ mice gained significantly more body weight than WT control mice on a HFD. *Fcer1a*$^{-/-}$ mice consumed significantly more food and gained more lean and fat mass, as determined by DEXA analysis (Figure 2B). *Fcer1a*$^{-/-}$ mice demonstrated significantly improved glucose tolerance but exhibited no difference in insulin tolerance when compared to WT control mice (Figure 2C), suggesting that *Fcer1a*$^{-/-}$ mice had improved glucose metabolism but a similar degree of insulin resistance to that of WT mice. Consistently, overnight-fasted *Fcer1a*$^{-/-}$ mice exhibited elevated glucose-induced insulin release, which showed no significant difference from WT mice at 90 minutes after the first glucose stimulation (Figure 2D). Islets from WT mice released insulin responding to glucose in a dose-dependent manner, but IgE did not affect islet insulin production at any tested doses of up to 100 μg/mL (Figure 2E). The low level expression of FcεR1 on islets possibly triggered insignificant insulin induction responding to IgE. RT-PCR revealed about 6-fold lower FcεR1α expression on islets than that on bone marrow-derived macrophages (Figure 2F). Non-

fasted WT and *Fcer1a*$^{-/-}$ mice on a HFD showed no difference between the basal levels of plasma IgE or insulin (Figure 2G). WAT from *Fcer1a*$^{-/-}$ mice, however, had significantly lower IgE levels than WT mice (Figure 2H). Although a direct comparison remains impossible, WAT milieu may have much higher IgE concentrations (about 1,600 ng per mg WAT protein from WT mice) than the plasma (about 150 ng/mL from WT mice). Consistent with increased body weight gain, *Fcer1a*$^{-/-}$ mice had higher plasma serum amyloid A (SAA), IL6, and MCP-1 than WT control mice after consuming a HFD, although the difference in MCP-1 levels did not reach statistical significance (Figure 2I). Yet data showed significantly fewer Mac2-positive macrophages in both subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) from *Fcer1a*$^{-/-}$ mice than those from WT control mice (Figure 3A/3B). VAT from *Fcer1a*$^{-/-}$ mice also contained fewer $CD3^+$ T cells than VAT from WT mice (Figure 3C). TUNEL staining also revealed significantly fewer apoptotic cells in WAT from *Fcer1a*$^{-/-}$ mice than in WT mice (Figure 3D), which possibly contributed to increased body weight gain and body fat mass in *Fcer1a*$^{-/-}$ mice (Figure 2A/2B). Metabolic characterization demonstrated that *Fcer1a*$^{-/-}$ mice on a HFD had lower O_2 consumption and CO_2 production than WT control mice (Figure 3E/3F), although the two groups did not reach statistical significance in regards to respiratory exchange ratio (RER) and body heat (Figure 3G/3H). Although *Fcer1a*$^{-/-}$ mice showed more food intake than WT control mice (Figure 2B), their plasma leptin levels and WAT adipocyte sizes did not significantly differ (Figure 3I/3J). Reduced energy expenditure in HFD-fed *Fcer1a*$^{-/-}$ mice (Figure 3E/3F) may contribute to increased body weight gain in these mice (Figure 2A). Immunoblot analysis

demonstrated much lower UCP1 expression in BAT from *Fcer1a*$^{-/-}$ mice than that from WT control mice (Figure 3K).

FcεR1 deficiency in mice affects the expression of molecules involved in WAT adipogenesis, apoptosis, and glucose uptake

Immunoblot analysis (Figure 4A) and immunostaining (Figure 4B) confirmed comparable FcεR1 expression in WAT from mice following a chow diet and a HFD. In WAT, both Mac2-positive macrophages and CD3-positive T cells all express FcεR1 (Figure 4C). WAT from HFD-fed *Fcer1a*$^{-/-}$ mice revealed a significantly enhanced expression of phospho(p)-AKT (Figure 4D), supporting the suppressive role of FcεR1 on AKT activation (18), which requires the expression and activation of C/EBPα and PPARγ (19, 20). Increased AKT activation in WAT from *Fcer1a*$^{-/-}$ mice led to a concurrent increase of C/EBPα and PPARγ expressions (Figure 4E/4F).

Both C/EBPα and PPARγ participate in the control of adipocyte terminal differentiation and maintenance (21, 22) and macrophage apoptosis (23). Reduced macrophage and T-cell contents (Figure 3A-3C) and reduced cell apoptosis (Figure 3D) in WAT from *Fcer1a*$^{-/-}$ mice may therefore associate with increased expression of C/EBPα and PPARγ (Figure 4E/4F). Immunoblot analysis of the same WAT from WT and *Fcer1a*$^{-/-}$ mice revealed reduced expression of the apoptosis-signaling molecule p-JNK (24) (Figure 4G) and increased expression of cell apoptosis and necrosis inhibitory molecule Bcl2 (25) (Figure 4H), which might also contribute to increased fat mass and body weight gain in *Fcer1a*$^{-/-}$ mice (Figure 2A/2B). Improved glucose tolerance and increased release of plasma insulin after a glucose challenge in *Fcer1a*$^{-/-}$ mice (Figure

2C/2D) might also associate with increased PPARγ expression (26, 27). Immunoblot analysis revealed significantly increased Glut4 expression in WAT from *Fcer1a*$^{-/-}$ mice (Figure 4I).

IgE suppresses adipogenesis in mice

Increased fat mass in *Fcer1a*$^{-/-}$ mice (Figure 2A/2B) and increased expression of C/EBPα and PPARγ in WAT from *Fcer1a*$^{-/-}$ mice (Figure 4E/4F) suggest that IgE participates in adipogenesis. WAT from mice on a chow or HFD expressed similar levels of FcεR1 (Figure 4A/4B). Data consistently revealed elevated levels of FcεR1 expression (all three chains: α, β, and γ) during adipogenesis of preadipocyte 3T3-L1 (Figure 5A). Partially differentiated 3T3-L1 cells (2 days) that had increased FcεR1 expression (compared with pre-adipocytes) helped treat cells with 50 μg/mL IgE, which revealed no differences in FcεR1 (α, β, and γ) expression (Figure 5B) with and without IgE stimulation. The addition of different concentrations of IgE (10, 50, and 100 μg/mL) to 3T3-L1 cells during the first 2 days and the whole duration of 8 days of adipogenesis tested IgE participation in adipogenesis. At either treatment, 50~100 μg/mL IgE significantly blocked 3T3-L1 adipogenesis, as determined by oil-red O staining (Figure 5C). IgE inhibited increased expression of C/EBPα and PPARγ after 2 days of 3T3-L1 differentiation, although IgE-induced PPARγ reduction did not reach statistical significance (Figure 5D/5E), likely because cells still existed at an early stage of differentiation and C/EBPα acts upstream of PPARγ (28).

IgE activity on 3T3-L1 adipocyte apoptosis and glucose uptake

Increased fat mass in *Fcer1a*$^{-/-}$ mice (Figure 2A/2B) but reduced apoptosis in WAT from the same mice (Figure 3D) suggest that IgE participates in inducing adipocyte apoptosis. The treatment of 3T3-L1 preadipocytes with IgE during differentiation helped test this hypothesis. IgE treatment induced adipocyte apoptosis in a time-dependent manner (Figure 6A). At both 6- and 8-day time points, IgE completely suppressed adipogenesis, but cell apoptosis reached 7% at the 6-day time point and 45% at the 8-day time point, respectively (Figure 6A/6B), suggesting that IgE inhibited adipogenesis before inducing apoptosis. Indeed, IgE only induced apoptosis of adipocytes but not preadipocytes (Figure 6C). IgE toxicity to the cells did not engender IgE-induced adipocyte apoptosis. In 3T3-L1 cells after 2 days differentiation, when cells exhibited increased FcεR1 expression (Figure 5A/5B) but no evident apoptosis (Figure 6A), a range of IgE concentrations did not affect 3T3-L1 cell viability or cytotoxicity as determined by assay of CCK-8 and LDH (Figure 6D/6E).

Improved glucose tolerance in *Fcer1a*$^{-/-}$ mice (Figure 2C) increased insulin secretion to the plasma from *Fcer1a*$^{-/-}$ mice after a glucose challenge (Figure 2D), and increased Glut4 expression in WAT from *Fcer1a*$^{-/-}$ mice (Figure 4I), which suggests the participation of IgE in inhibiting glucose uptake. This study tested this hypothesis in 3T3-L1 cells after 2 days differentiation, when 3T3-L1 increased FcεR1 expression (Figure 5A/5B) but IgE treatment did not induce 3T3-L1 apoptosis (Figure 6A) to interfere with glucose uptake. While preadipocytes took up 2-deoxy-D-glucose (2DG) at baseline, as determined by measuring intracellular hexokinase-phosphorylated 2DG-6-phosphate (2DG6P) (29), differentiated 3T3-L1 cells showed a significant increase in glucose uptake. IgE treatment inhibited glucose uptake in a concentration-dependent manner

(Figure 6F). A reduced expression of glucose transporters likely mediated IgE-suppressed glucose uptake. In differentiated 3T3-L1 cells, IgE reduced the expression of Glut4 and its upstream signaling molecule p-AKT (Figure 6G). In differentiated 3T3-L1 cells, the silencing of FcεR1 expression with its siRNA, as confirmed by FcεR1 immunoblot analysis, increased the expression of both Glut4 and p-AKT, compared with those transfected with scrambled control siRNA (Figure 6H). This result establishes that IgE suppresses glucose uptake by reducing the expression of glucose transporters.

Discussion

This study revealed the dual participation of IgE action in obesity and diabetes, which remained consistent to observations from several human studies. Plasma IgE levels correlated negatively with BMI among patients with severe asthma (11). Data showed that plasma IgE levels also correlated negatively with body weight, BMI, and fat mass among severely obese women, although the current study observed a relatively small cohort with a power of 0.691 compared with other similar studies (11). However, this is the first correlation study linking IgE to obesity without the confounding from asthma or other allergic or autoimmune diseases. These human studies point to the role of IgE in modulating obesity. We reported previously that human plasma IgE correlated positively with type-2 diabetes (12, 13). Interruption of IgE action with an anti-FcεR1 antibody delayed the onset of type-1 diabetes in mice (14), suggesting IgE increases plasma glucose levels.

This study also demonstrated that the interruption of IgE action in *Fcer1a*$^{-/-}$ mice increased food intake and reduced energy expenditure (O_2 consumption and CO_2

production), as reflected by reduced UCP1 expression in BAT from the *Fcer1a*$^{-/-}$ mice, without exhibiting significant changes in RER and body heat. These physiological changes may contribute to increased body weight gain in these mice. Although previous data suggest that high RER and body heat reduce body weight, the presence of diabetes may affect such values. In obese humans, non-diabetic patients have lower RMR than diabetic patients (30). Increased body weight but improved glucose tolerance in *Fcer1a*$^{-/-}$ mice may trigger insignificant differences in RMR and body heat between WT and *Fcer1a*$^{-/-}$ mice, although this hypothesis merits further investigation. At the molecular and cellular levels, however, this study proposed two possible mechanisms by which IgE reduced fat mass; one is the C/EBPα and PPARγ pathway. Activation of C/EBPα is a prerequisite for PPARγ activation (28). Several signaling pathways can activate C/EBPα, including the MAP kinase (e.g. p38 and ERK1/2) (31), the cAMP-associated protein kinase-A pathway (32), and the AKT pathway (19, 20). Activation of IgE receptor FcεR1 activates the MAP kinase pathway, but suppresses AKT activation (18). This study found that WAT from *Fcer1a*$^{-/-}$ mice showed increased p-AKT, C/EBPα, and PPARγ, supporting a negative action of IgE on AKT activation, downstream C/EBPα and PPARγ (19, 20), and consequent adipocyte differentiation (21, 22). The second mechanism by which IgE can influence obesity involves its activity in promoting the apoptosis of adipocytes while preadipocytes were fully protected. IgE can furnish survival signals to MCs and basophils (1), death signals to macrophages and vascular cells (2), but does not affect $CD4^+$ and $CD8^+$ T cell survival or death (3). This study revealed that IgE induces adipocyte apoptosis but not preadipocytes. Cell differentiation occurred before apoptosis in cultured 3T3-L1 cells. Why IgE promotes survival or apoptosis differently from one

cell type to another (1-3) remains unclear. Prior studies show that PPARγ activation inhibits monocyte/macrophage migration, accumulation, and apoptosis in atherosclerotic lesions (23) and in WAT from HFD-fed mice (33). Reduced PPARγ activation in these WAT may contribute to reduced macrophages in WAT from *Fcer1a*$^{-/-}$ mice. Reduced JNK activation in WAT from *Fcer1a*$^{-/-}$ mice, however, may correlate with reduced macrophage accumulation and inflammation in WAT (34), although interrupted FcεR1 signaling and Syk activation may also directly suppress JNK activation. JNK activation contributes to body weight gain and glucose tolerance. The protection of JNK-deficient mice from obesity and diabetes occurred in DIO mice (35). Reduced apoptosis and p-JNK expression in WAT from *Fcer1a*$^{-/-}$ mice support the role of JNK in regulating adipocyte apoptosis (24). Although the signaling pathways that control adipocyte apoptosis have considerable complexity, IgE-mediated JNK activation, AKT/Bcl-2 suppression, and AKT-C/EBPα-PPARγ reduction may all contribute to adipocyte apoptosis, a hypothesis that merits further investigation.

In contrast to the inhibitory effect of IgE on obesity, IgE promotes experimental type 2 diabetes. IgE-suppressed p-AKT may directly impair the expression and distribution of glucose transporters, such as Glut 4 (36, 37), and indirectly *via* reduced PPARγ expression and activation (38). Therefore, in 3T3-L1 cells, IgE reduced p-AKT and Glut4 expression and FcεR1 knockdown by its siRNA increased p-AKT and Glut4 expression, all of which may explain concentration-dependent suppression of glucose uptake in 3T3-L1 cells by IgE. A similar pattern occurred *in vivo*. FcεR1 deficiency increased WAT Glut4 expression. These observations may explain why *Fcer1a*$^{-/-}$ mice

had improved glucose tolerance and glucose-induced insulin release. Figure 7 summarizes our hypothesis.

Several questions remain unresolved. *Fcer1a*$^{-/-}$ mice consumed more food and had lower levels of energy expenditure and BAT expression of UCP1 than WT control mice. Although these observations may explain why *Fcer1a*$^{-/-}$ mice gained more body weight than WT mice, the mechanism by which IgE activity controls food uptake and energy expenditure remains unknown. Prior studies showed that leptin infusion augmented plasma IgE levels in allergen-challenged mice (39), but the absence of IgE receptor FcεR1 did not affect plasma leptin levels. Therefore, IgE may control food uptake and energy expenditure with mechanisms other than leptin. For example, gut microbiota-associated metabolic endotoxemia and inflammation impair food uptake (40, 41). IgE may reduce food uptake by inducing gut inflammation (42, 43), although this study did not test this hypothesis. We further found that *Fcer1a*$^{-/-}$ mice exhibited improved glucose tolerance and glucose-induced insulin secretion, but these mice showed similar degrees of insulin resistance and similar basal levels of plasma insulin to those from WT control mice. This study did not reveal a direct participation of IgE in suppressing glucose-induced insulin secretion in isolated islets, suggesting that elevated glucose-induced insulin secretion in *Fcer1a*$^{-/-}$ mice indirectly participated in IgE (44, 45).

All *in vitro* experiments in this study used IgE at concentrations (10~100 μg/mL) much higher than its physiological concentrations in human or mouse plasma (100~400 ng/mL). At 10 μg/mL, IgE showed a negligible effect in suppressing adipogenesis and weak suppression of glucose uptake. Therefore, this study used 50 μg/mL IgE to

demonstrate inhibition of 3T3-L1 cell adipogenesis, promoting adipocyte apoptosis, and suppressing Glut4 expression and glucose uptake. The physiological relevance of these *in vitro* experiments remains a key question. As described in our prior studies of atherosclerosis and AAAs (3, 4), low plasma IgE levels may not necessarily reflect the actions of tissue IgE *in situ*. The tissue milieu may harbor higher levels of IgE than previously surmised. This possibility remains consistent to our observation that each milligram of WAT extract from DIO mice contained about 1,600 ng of IgE.

This study provides the first direct evidence of IgE participation in obesity and diabetes, although many observations still remain incompletely understood. As atopic diseases can affect certain disadvantaged populations who remain particularly vulnerable to obesity and diabetes (8-11, 46, 47), these observations have public health implications beyond providing novel mechanistic insight. The results of this study, showing divergent actions of IgE on obesity and glucose tolerance, require consideration in the context of therapeutic targeting of IgE as well.

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Figure Legends

Figure 1. Spearman's correlations between logarithmized human plasma IgE and BMI (**A**), body weight (**B**), and body fat mass (**C**).

Figure 2. FcεR1 deficiency increased obesity but improved glucose tolerance in mice. **A.** Body weight gain of male WT and *Fcer1a*$^{-/-}$ mice on a HFD. **B.** DEXA determined food intake, whole body lean and fat masses after mice consumed a HFD for 17 weeks. Glucose and insulin tolerance test (**C**) and glucose-induced insulin release (**D**) from male WT and *Fcer1a*$^{-/-}$ mice after 17 weeks of a HFD. **E.** Insulin production from islets treated with and without different doses of glucose and IgE. Insulin secretion is expressed as a percent of the islet insulin content (n=4). **F.** The mRNA expression levels of FceR1a in the islets treated with different doses of IgE (n = 4). Bone marrow-derived macrophages were used as positive control. Plasma IgE and insulin levels (**G**), WAT extract IgE (**H**), and plasma SAA, IL6, and MCP-1 levels (**I**) from male WT and *Fcer1a*$^{-/-}$ mice after 17 weeks of a HFD. The number of mice per group is indicated in the parenthesis.

Figure 3. FcεR1 deficiency in WAT macrophage and T-cell accumulation, WAT cell apoptosis, and in mouse energy expenditure. Mac2 immunostaning detected macrophages in VAT (**A**) and SAT (**B**) and CD3 immunostaining detected total T cells (**C**) from male WT and *Fcer1a*$^{-/-}$ mice after 17 weeks of a HFD. **D**. TUNEL staining detected apoptotic cells in WAT from the same groups of mice. Area under curve (AUC) of O_2 consumption volume (**E**), CO_2 production volume (**F**), respiratory exchange ratio

(**G**), and body heat (**H**) from both male WT and *Fcer1a*$^{-/-}$ mice consumed a HFD for 17 weeks (n=6~8 per group). Representative data are shown to the left (**A**-**C**) or right (**D**-**G**) panels. Plasma leptin levels as determined by ELISA (**I**), WAT adipocyte size (**J**), and BAT immunoblot analysis detected UCP1 expression (**K**) in both WT and *Fcer1a*$^{-/-}$ mice consumed a HFD for 17 weeks. The number of mice per group is indicated in the parenthesis.

Figure 4. Immunoblot analysis of adipogenesis-associated proteins in WAT from WT and *Fcer1a*$^{-/-}$ mice after 17 weeks on a HFD. FcεR1a immunoblot analysis (**A**), FcεR1a immunostaining (**B**), and FcεR1a immunofluorescent double staining together with Mac-2 or CD3 (**C**) of WAT from mice fed a chow diet and a HFD. Immunoblots or RT-PCR determined the expression of p-AKT and total AKT (**D**), C/EBPα (**E**), PPARγ (**F**), p-JNK and total JNK (**G**), Bcl-2 (**H**), and Glut4 (**I**) in WAT from WT and *Fcer1a*$^{-/-}$ mice that consumed a HFD. β-Actin immunoblots were used to ensure equal protein loading. Data are mean ± SEM of five independent experiments. Representative immunoblots for panels **B**-**I** are shown to the left.

Figure 5. IgE activity in reducing 3T3-L1 cell adipogenesis. **A**. RT-PCR determined mRNA levels of three FcεR1 chains (α, β, and γ) in 3T3-L1 cells at three time points (0, 2, and 8 days) during the differentiation. **B**. RT-PCR determined the expression of FcεR1 three chains (α, β, and γ) in 3T3-L1 cells differentiated for 2 days with and without 50 μg/mL IgE. **C**. Oil-red O staining and quantification of 3T3-L1 cells treated with different doses of IgE for the first 2 days or throughout the whole course of

differentiation (8 days). Preadipocytes and fully differentiated 3T3-L1 cells without IgE treatment were used as negative and positive controls. Representative data are shown to the right. RT-PCR determined the mRNA levels of C/EBPα (**D**), and PPARγ (**E**) in 3T3-L1 cells before differentiation and after 2 days of differentiation, meanwhile treated with and without 50 μg/mL IgE. Data are mean ± SEM of 3 to 5 independent experiments.

Figure 6. IgE activities in promoting adipocyte apoptosis and suppressing adipocyte glucose uptake. **A**. TUNEL staining and quantification of apoptotic cells in 3T3-L1 cells differentiated in the presence and absence of 50 μg/mL IgE for indicated days. Representative data are shown to the right. **B**. Oil-red O staining of the same experiment from Day-6 and day-8 experiments of panel **A**. **C**. TUNEL staining of preadipocytes and fully differentiated adipocyte treated with and without IgE (50 μg/mL) to induce cell apoptosis. CCK-8 assay determined cell viability (**D**) and LDH assay determined cytotoxicity (**E**) of 3T3-L1 cells after two days of differentiation with and without different amount of IgE. **F**. Glucose uptake assay of 3T3-L1 cells after two days of differentiation with and without different amount of IgE. Preadipocytes were used as experimental negative control. **G**. Glut4, p-AKT, and total AKT immunoblots of differentiated 3T3-L1 cells and treated with and without 50 μg/mL IgE for 30 min. **H**. FcεR1α siRNA- and scramble siRNA-transfected 3T3-L1 cells and differentiated for 4 days, followed by treatment with 25 μg/mL of insulin or IgE for 10 min. β-Actin immunoblots were used for protein loading controls. Data are mean ± SEM of three independent experiments.

Figure 7. Possible mechanisms of FcεR1-mediated IgE actions in regulating the expressions of Glut4, C/EBPα, PPARγ, and Bcl2, and associated pathophysiological activities in obesity and diabetes.

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