Obesity is a major public health issue, with the principal cause of morbidity a result of metabolic dysfunction (e.g., type 2 diabetes, atherosclerosis). Progression of clinical pathology has been strongly linked to chronic inflammation in white adipose tissue (WAT) (1,2), where hypertrophic adipocytes fail to efficiently store excess energy, leading to adipose tissue dysfunction, dyslipidemia, and insulin resistance. Increased tissue inflammation through adipocyte release of cytokines [e.g., tumor necrosis factor-α (TNF-α)] (3,4), chemokines [e.g., monocyte chemoattractant protein 1 (MCP1/CCL2)] (5), and proinflammatory saturated fatty acids (FAs) (6) drives monocyte recruitment and differentiation to M1-polarized macrophages, thereby expanding the inflammatory environment within adipose tissue beds. Proinflammatory factors produced by activated macrophages act reciprocally on adipocytes, thereby perpetuating adipose tissue inflammation and dysfunction (7).

WAT secretes several adipokines that not only are important hormonal regulators of systemic metabolism but also possess either pro- or anti-inflammatory properties. Adipokine production is disrupted in hypertrophic adipose tissue, resulting in obesity-associated inflammation (8). For example, circulating levels of adiponectin are consistently decreased in obese human subjects and experimental animals. A protective role for adiponectin in models of diabetes, dyslipidemia, and atherosclerosis has been reported (9), and in obese human subjects, plasma adiponectin concentrations are inversely correlated with circulating inflammatory markers, including C-reactive protein (CRP) and TNF-α (8,10). In line with these findings, direct anti-inflammatory activity of adiponectin has been demonstrated. Adiponectin inhibits transformation of

Adiponectin Induces A20 Expression in Adipose Tissue to Confer Metabolic Benefit

Laura E. Hand, Paola Usan, Garth J.S. Cooper, Lance Y. Xu, Basil Ammori, Peter S. Cunningham, Reza Aghamohammadzadeh, Handrean Soran, Adam Greenstein, Andrew S.I. Loudon, David A. Bechtold, and David W. Ray

1Faculty of Life Sciences, University of Manchester, Manchester, U.K.
2Faculty of Medical and Health Sciences, University of Manchester, Manchester, U.K.
3School of Biological Sciences, University of Auckland, Auckland, New Zealand
4Centre for Advanced Discovery and Experimental Therapeutics, University of Manchester, Manchester, U.K.

Corresponding author: David W. Ray, david.w.ray@manchester.ac.uk, or David A. Bechtold, david.bechtold@manchester.ac.uk.

Received 5 December 2013 and accepted 10 August 2014.
This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db13-1835/-/DC1.
© 2015 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered.
macrophages into foam cells (11) and reduces macrophage migration and chemokine production (12,13). Adiponectin also promotes macrophage polarization toward an anti-inflammatory M2 phenotype (14) and desensitizes macrophages to Toll-like receptor (TLR) signaling (15,16).

The nuclear hormone receptor Reverbα is a ligand-sensitive transcription factor that negatively regulates the expression of core clock proteins (17,18). Reverbα has previously been implicated in adipocyte differentiation (19), lipid metabolism (20), and regulation of the inflammatory response (21). In the current study, we identify an obese phenotype in Reverbα<sup>−/−</sup> mice characterized by a lack of the predicted M1 macrophage infiltration of WAT depots, a paradoxical increase in adiponectin production, and preservation of insulin sensitivity. We further demonstrate that adiponectin suppression of inflammatory signaling in macrophages and adipose tissue depots is mediated through the cytoplasmic ubiquitin-modifying enzyme and a negative regulator of TLR signaling, A20, which is enhanced in WAT from Reverbα<sup>−/−</sup> mice. We also demonstrate that A20 expression in WAT from obese human subjects is correlated significantly with measures of insulin sensitivity, and in subjects after bariatric surgery, we observed an increase in WAT A20 expression, which was positively correlated with elevated serum adiponectin levels and associated with improved levels of metabolic and inflammatory markers, such as CRP. Taken together, these findings identify a novel target pathway for modulating adipose tissue inflammation in obesity.

**RESEARCH DESIGN AND METHODS**

**Animal Maintenance**

Experimental procedures were licensed under the Animals Act, 1986, and local animal welfare committee. Reverbα<sup>−/−</sup> mice were provided by Ueli Schibler (University of Geneva), as described (22). These mice were subsequently bred onto the Per2:Luciferase reporter line generated by Joe Takahashi (University of Texas Southwestern Medical Center, Dallas, TX) (23) and maintained as an inbred line at the University of Manchester. The control and knockout (KO) mice for all experiments were littermates because the line was bred using heterozygotic parent mice. Adult male mice were maintained in a 12-h light, 12-h dark lighting schedule; housed at an ambient temperature of 20–22°C; and fed standard rodent chow and water supplied ad libitum (except for the study shown in Fig. 1C–E and H, where the animals received diet-induced obesity [DIO] rodent purified diet with 60% energy from fat [International Product Supplies Ltd]). Glucose tolerance tests were carried out as in Bechtold et al. (24).

**Cell Culture**

Peritoneal exudates cells were isolated and the CD11b<sup>+</sup> macrophage population positively selected using anti-CD11b MicroBeads and MS autoMACS Columns (both Miltenyi Biotec) according to the manufacturer’s specifications. Cells were resuspended in RPMI medium and plated.

**Reagents**

Recombinant human adiponectin was produced at the University of Auckland as described previously (25,26). Multimeric adiponectin forms in the purified protein were confirmed by native SDS-PAGE. Mouse TNF-α, lipopolysaccharide (LPS), SB216763, and FAs (sodium stearate, sodium palmitate, sodium myristate, and sodium dodecanoate) were purchased from Sigma. Antibodies to A20 (D13H3) and glucose synthase kinase 3β (GSK3β) (27C10) were from Cell Signaling, to β-actin (AC-15) from Abcam, to TNF-α (MP6-XT22) from R&D Systems, to laminin B (C20) from Santa Cruz, to tubulin from Sigma, and to inducible nitric oxide synthase (iNOS) from Abcam. Secondary antibodies were horseradish peroxidase–conjugated sheep anti-mouse or donkey anti-rabbit IgGs (GE Healthcare) and fluorescein isothiocyanate–conjugated goat anti-rabbit (Jackson ImmunoResearch).

**Preparation of FAs**

FAs were solubilized in ethanol stock solutions of 100 mmol/L and stored at −20°C. FA-albumin complex solutions were freshly prepared before each experiment. Five percent FA-free and low-endotoxin BSA (27) (Sigma) was dissolved in RPMI medium and filtered with 0.22 μmol/L low-protein–binding filter (Millipore). Stock solutions of FAs were added to the BSA medium to achieve an FA:BSA molar ratio of 3:1 and incubated at 40°C for 1 h. Cells were treated with individual FAs, whereas control cells received BSA only.

**Quantitative Real-Time PCR**

Tissue was homogenized in TRIzol reagent (Invitrogen) using lysing matrix D tubes (MP Biomedicals), and total RNA was isolated according to the manufacturer’s specifications. RNA from macrophages was extracted with the RNaseq Mini Kit (QIAGEN). After reverse-transcription using the High-Capacity RNA-to-cDNA Kit (Applied Biosystems), quantitative RT-PCR was performed using Power SYBR Green PCR Master Mix and StepOnePlus Real-Time PCR System (both Applied Biosystems). Mouse housekeeping gene 18S rRNA was used as an internal control.

**RNA Interference**

A20-specific small interfering RNA (siRNA) and non-targeting scrambled control siRNA were designed and purchased from Eurofins MWG Operon (siMAX siRNA). siRNAs were transfected into freshly isolated primary CD11b<sup>+</sup> murine macrophages with the Amaya Nucleofector device set to program Y001 using the Mouse Macrophage Nucleofector Kit. Cells were washed once 1 h after transfection and cultured for an additional 2 days before adiponectin and LPS stimulation.

**Protein Analysis**

Analysis of adiponectin isoform expression was as previously described (25,26). ELISAs were performed with paired antibody sets as recommended by the manufacturer (R&D Systems). For immunoblot analysis, tissue
was homogenized in Tissue Protein Extraction Reagent (Thermo Scientific) using lysing matrix D tubes (MP Biomedicals). Cytoplasmic and nuclear cell extracts were prepared by lysing cells in buffer 1 (30 mmol/L Tris-HCl [pH 7.4], 0.5 mmol/L EDTA, 150 mmol/L NaCl, 0.2% vol/vol NP40), centrifuging nuclear pellets and resuspending these in buffer 2 (30 mmol/L Tris-HCl [pH 7.4], 0.5 mmol/L EDTA, 400 mmol/L NaCl, 1% vol/vol Triton X-100, 0.1% wt/vol SDS). Protein concentration was measured with a BCA protein quantification kit (Pierce Biotechnology). Proteins were subjected to SDS-PAGE using a Mini-PROTEAN 3 apparatus (Bio-Rad) according to the manufacturer’s instructions before transfer to nitrocellulose membrane using an iBlot gel transfer system (Invitrogen). Immunoblots were incubated with the appropriate antibody and imaged using enhanced chemiluminescence (ECL Prime detection system; GE Healthcare) and quantified using ImageJ software. Immunohistochemistry

**Figure 1**—Phenotype of Reverba−/− mice. A and B: Body weight (A) and visceral WAT (B) mass of 12-week-old male Reverba−/− mice and WT littermates maintained on an NC diet (n = 16–20/group). C: Histological sections of WAT from WT and Reverba−/− mice fed NC and WT mice fed HFD to induce DIO. iNOS immunoreactivity demonstrates characteristic macrophage cuffs in WAT of WT DIO mice, which are virtually absent in Reverba−/− mice despite their obese phenotype. D and E: Body weight (D) and intraperitoneal glucose tolerance test results (E) of WT and Reverba−/− mice fed NC or HFD for 10 weeks (n = 5–6/group). F and G: Serum leptin (F) and adiponectin (G) concentrations of 12-week-old Reverba−/− mice were significantly higher than in WT controls (n = 18–21/group) and positively correlated with adipose tissue mass (P < 0.05, Pearson correlation). H: ELISA analysis of serum adiponectin in WT and Reverba−/− mice fed NC vs. HFD. I: Immunoblot analysis of monomeric (top) and multimeric (bottom) adiponectin in sera of WT and Reverba−/− mice (black arrowhead, high molecular weight; white arrowhead, hexamer; gray arrowhead, trimer). J: ELISA analysis of high–molecular weight adiponectin in sera of WT and Reverba−/− mice expressed as a ratio of total adiponectin. Data are mean ± SEM; statistical significance was determined using Student t test or one-way ANOVA with Bonferroni post hoc test (C). *P < 0.05, **P < 0.01, ***P < 0.001. Adn, adiponectin; BW, body weight; HMW, high molecular weight.
was carried out as previously described (24) using paraformaldehyde-fixed and paraffin-embedded tissue epididymal fat pads.

**Human Adipose Tissue Biopsies and Biochemical Analyses**

Patients with severe obesity (BMI >35 kg/m², n = 12) who were awaiting gastric bypass surgery were recruited after full informed written consent in accordance with local research ethics committee approval. Participants were invited to return for a follow-up assessment 6 months after bariatric surgery. On both occasions, patients provided gluteal subcutaneous adipose samples (0.125 cm³) by undergoing a surgical biopsy under local anesthesia (28). The biopsy samples were immediately frozen in liquid nitrogen. Fasting venous blood samples were also collected for the measurement of CRP, adiponectin, and leptin levels as previously described (28).

**Statistical Analysis**

Data are presented as mean ± SEM. The statistical test used is specified in each data section. Student t test was used when only two groups were studied; one-way ANOVA was used when more than two groups were studied and only one factor investigated, followed by post hoc analysis; and two-way ANOVA was used when more than two factors were analyzed, followed by Bonferroni post hoc analysis.

**RESULTS**

**Obesity in *Reverba*−/− Mice Is Not Associated With WAT Inflammation and Insulin Resistance**

On normal chow (NC), 12-week-old male *Reverba*−/− mice have comparable body weight to wild-type (WT) littermates (Fig. 1A) but have significantly increased WAT mass (WT: 0.8 ± 0.13 g; KO: 1.6 ± 0.14 g; n = 16–20/group; P < 0.01, Student t test) (Fig. 1B) and adipocyte hypertrophy (Fig. 1C). WT mice were fed a high-fat diet (HFD) to promote a comparable degree of WAT tissue mass (2.3 ± 0.15 g) and adipocyte morphology to *Reverba*−/− mice maintained on NC. Despite showing similar WAT hypertrophy, obese WT mice exhibited characteristic inflammatory cuffs surrounding adipocytes, which were rarely observed in the NC-fed *Reverba*−/− animals (Fig. 1C). Maintenance of the *Reverba*−/− mice on HFD resulted in a profound increase in body weight and adiposity compared with HFD-fed WT mice (Fig. 1D). Despite this, *Reverba*−/− mice did not exhibit obesity-related deterioration in glucose tolerance compared with WT littermates, suggesting preservation of insulin sensitivity (Fig. 1E). As expected, WT mice exhibited a significant increase in adipose inflammatory markers in response to 16 weeks of HFD feeding (*TNFα*: NC 1.1 ± 0.1 vs. HFD 3.0 ± 0.6 relative mRNA expression, P < 0.01; *IL6*: NC 1.2 ± 0.3 vs. HFD 2.0 ± 0.3, P = 0.06; *MCP1*: NC 1.2 ± 0.3 vs. 5.8 ± 0.7, P < 0.001; n = 6/group). In contrast, expression of inflammatory markers (*TNFα*, *IL6*, *MCP1*) were not increased by HFD feeding in the *Reverba*−/− mice (TNFα: NC 1.0 ± 0.2 vs. HFD 1.8 ± 0.3 relative mRNA expression; *IL6*: NC 1.3 ± 0.3 vs. HFD 1.0 ± 0.3; *MCP1*: NC 1.1 ± 0.5 vs. 1.4 ± 0.5; n = 6/group). The absence of adipose inflammation in the *Reverba*−/− mice was not due to a general lack of inflammatory response, as we have previously demonstrated that these animals exhibit robust responses to systemic endotoxin administration (21). Furthermore, *Reverba*−/− mice exhibited a pronounced elevation in serum free FA concentrations (WT NC: 165.5 ± 20.7 µmol/L; KO NC: 274.0 ± 17.6; P < 0.05, Student t test) indicative of increased adipose tissue lipolysis, a known proinflammatory signal.

In line with increased adiposity, NC-fed *Reverba*−/− mice exhibited increased circulating leptin concentrations (Fig. 1F). Unexpectedly, these animals also showed an elevation in circulating adiponectin (Fig. 1G), which remained significantly elevated in HFD-fed *Reverba*−/− mice compared with WT littermates (Fig. 1H). This finding contrasts many animal models of obesity and obese human subjects in whom circulating levels of adiponectin are reduced. The oligomerization state is critical to adiponectin function, with the high–molecular weight forms being the most bioactive (29). Circulating levels of the high–molecular weight form of adiponectin were significantly increased in *Reverba*−/− mice compared with WT littermates (Fig. 1I and J).

**Adiponectin Drives GSK3β-Mediated Induction of A20 to Attenuate Macrophage Inflammation**

A number of studies have highlighted the ability of adiponectin to improve aspects of metabolic disturbance, including insulin resistance and vascular dysfunction (8). Furthermore, mice lacking adiponectin expression showed elevated WAT inflammation in response to DIO (30). We therefore investigated the anti-inflammatory actions of adiponectin using multimeric, appropriately posttranslationally modified human adiponectin (25).

In agreement with previous work (31), adiponectin pretreatment (6–18 h, 3 µg/mL) rendered CD11b+ primary murine macrophages refractory to subsequent stimulation by the TLR4 agonist LPS as assessed by diminished inflammatory gene induction of *IL6*, *TNFα* (Fig. 2A), *iNOS*, *MCP1*, *CCL5*, and *IL10* (Supplementary Fig. 1). In the context of obesity-related inflammation, TLR4 activation can result from elevated production of saturated medium-chain FAs (e.g., C18:0, C16:0) released in response to DIO (30). We therefore investigated the anti-inflammatory actions of adiponectin using multimeric, appropriately posttranslationally modified human adiponectin (25).

In agreement with previous work (31), adiponectin pretreatment (6–18 h, 3 µg/mL) rendered CD11b+ primary murine macrophages refractory to subsequent stimulation by the TLR4 agonist LPS as assessed by diminished inflammatory gene induction of *IL6*, *TNFα* (Fig. 2A), *iNOS*, *MCP1*, *CCL5*, and *IL10* (Supplementary Fig. 1). In the context of obesity-related inflammation, TLR4 activation can result from elevated production of saturated medium-chain FAs (e.g., C18:0, C16:0) released from hypertrophic adipocytes (32). Importantly, adiponectin pretreatment (18 h, 3 µg/mL) significantly reduced macrophage inflammatory responses to C18:0 and C16:0 (Supplementary Fig. 2).

Attenuated nuclear factor (NF-κB) signaling has been previously implicated in models of induced immunotolerance (e.g., tolerance to subsequent LPS stimulation following an initial proinflammatory stimulus of TNF-α or LPS) (33). Therefore, we measured the expression of negative regulators of NF-κB signaling, including A20, SOCS1,
SOCS3, IRAKM, and SHIP1, following adiponectin administration. Treatment of macrophages with adiponectin (3 μg/mL) caused a rapid and pronounced induction of the cytoplasmic ubiquitin-modifying enzyme A20 (Supplementary Fig. 3). SOCS1, SOCS3, and IRAK transcripts were also induced to a lesser extent, whereas SHIP1 expression was not affected (Supplementary Fig. 3). Because of its particularly strong induction in response to adiponectin, we characterized further the role of A20 in adiponectin anti-inflammatory responses. Constitutive expression of A20 protein in murine primary macrophages was minimal; however, adiponectin treatment caused a strong induction of

Figure 2—Adiponectin-induced cross-tolerance to endotoxin is mediated by A20. A: Quantitative RT-PCR analysis of IL6 and TNFα induction in macrophages pretreated with adiponectin for the indicated times. B: Immunoblot analysis of A20 expression in murine primary macrophages cultured for the indicated times with 3 μg/mL adiponectin. C: Quantitative RT-PCR analysis of A20 expression in primary murine macrophages pretreated with adiponectin (3 μg/mL, 6 h) and challenged with LPS (100 ng/mL, 4 h). D: Quantitative RT-PCR and immunoblot analyses of A20 expression of primary murine macrophages transfected with control or A20-specific siRNA. E: Quantitative RT-PCR analysis of macrophages transfected with control or A20-specific siRNA and subject to adiponectin (3 μg/mL, 18 h) pretreatment, followed by LPS challenge (100 ng/mL, 4 h). F: Immunoblot analysis of cytosolic (left) and nuclear (right) extracts of primary macrophages treated with 3 μg/mL adiponectin for the indicated times. G–I: Quantitative RT-PCR analysis of primary murine macrophages treated with vehicle control [dimethyl sulfoxide] or 50 μmol/L SB216763 and stimulated with 3 μg/mL adiponectin (G and H) or pretreated with adiponectin (3 μg/mL, 18 h) before LPS challenge (100 ng/mL, 4 h) (I). Data are mean ± SEM from three independent experiments normalized to mouse 18S rRNA control and fold change relative to control untreated cells. Statistical significance was determined using the Student t test (D: ***P < 0.001), two-way ANOVA (E: *P < 0.05, **P < 0.01, ***P < 0.001 A20 siRNA vs. control siRNA; ###P < 0.001 adiponectin pretreatment vs. no adiponectin treatment), or one-way ANOVA (A, F, and I: *P < 0.05, **P < 0.01, ***P < 0.001 adiponectin pretreatment vs. no adiponectin treatment) with Bonferroni post hoc test. Adn, adiponectin; Nucl., nuclear; SB, SB216763; siA20, A20 siRNA; siCtrl, control siRNA.
A20 within 3 h of stimulation, which remained elevated beyond 24 h posttreatment (Fig. 2B). A20 expression is also induced following stimulation of macrophages with LPS (Fig. 2C). Of note, the induction of A20 in macrophages to a secondary LPS challenge was not attenuated by pretreatment with adiponectin (Fig. 2C), a time at which induction of IL6 and TNFα expression have become unresponsive to LPS stimulation, suggesting that A20 is not subject to prestimulation-induced tolerance.

A direct role for A20 in adiponectin-induced macrophage quiescence was next demonstrated by targeting A20 by siRNA (Fig. 2D, Supplementary Fig. 4). Knockdown of A20 enhanced the induction of IL6 and TNFα in naïve macrophages treated with LPS, demonstrating a constitutive role of A20 in limiting the magnitude of TLR-driven inflammatory responses in macrophages. Importantly, knockdown of A20 expression also blocked the ability of adiponectin pretreatment to attenuate IL6 and TNFα expression in macrophages following secondary LPS challenge (Fig. 2E).

GSK3β is a critical enzyme upstream of A20 expression (34), and in line with this, immunoblot analysis revealed that adiponectin treatment increased nuclear localization of GSK3β in macrophages (Fig. 2F). Preincubation of murine macrophages with the specific GSK3β inhibitor SB216763 blunted the induction of A20 mRNA in macrophages in response to adiponectin treatment (Fig. 2G) and attenuated the adiponectin impairment of TLR4 activation (Fig. 2I), demonstrating a role for GSK3β in adiponectin-induced inflammatory tolerance. Of note, SB216763 treatment did not diminish acute induction of TNFα by adiponectin (Fig. 2H), demonstrating that adiponectin-induced tolerance was not secondary to TNF-α induction. Therefore, adiponectin signaling stimulates GSK3β activity to drive an A20-mediated anti-inflammatory program.

Enhanced Expression of A20 Is Associated With an Attenuation of WAT Inflammation in Reverba−/− Mice and Improved Metabolic Profile in Bariatric Surgery Patients

We next examined the potential role of A20 in regulating WAT inflammation in Reverba−/− mice. Reverba−/− mice exhibited significantly higher expression of A20 protein in WAT beds compared with WT controls, which was maintained in profoundly obese KO mice maintained on HFD (Fig. 3A). WAT explants derived from Reverba−/− mice also produced more adiponectin than WT explants (Fig. 3B) and in either basal or LPS-stimulated states, exhibited an attenuated expression of IL6 and TNFα (Fig. 3C). Furthermore, adiponectin treatment of WT WAT explant tissue significantly increased A20 expression (Fig. 3D) and reduced induction of both TNFα and IL6 in response to LPS challenge (Fig. 3E). Adiponectin was also effective at inducing the expression of A20 in differentiated 3T3-L1 adipocytes (vehicle treated: 1.0 ± 0.02 relative A20 expression; adiponectin treated: 5.1 ± 1.03; P < 0.01), indicating that its anti-inflammatory actions are not limited to macrophages. These results identify adiponectin and A20 as important modulators of WAT inflammation in vitro and in vivo.

Our studies predict that hypertrophic human adipose tissue depots will show loss of adiponectin action, resulting in reduced A20 protein in both macrophages and adipocytes, permitting unrestrained inflammatory signaling to progress. To investigate this, we studied a cohort of obese subjects before and after bariatric surgery. In support of the animal data revealing a beneficial effect of A20 in obesity, presurgery circulating adiponectin levels and WAT expression of A20 in the obese subjects exhibited a significant negative correlation with insulin resistance (HOMA-IR: n = 12, P < 0.05, Pearson correlation) (Fig. 4K and L). Furthermore, A20 expression in patient WAT samples was positively correlated with serum adiponectin both pre- and postsurgery (P < 0.05, Pearson correlation) (Fig. 4M) and exhibited a significant rise postsurgery (Fig. 4J), concomitant with significant weight loss (Fig. 4A), and improved levels of serum markers of metabolism (i.e., adiponectin, leptin) (Fig. 4G and I) and inflammation (e.g., CRP) (Fig. 4H).

DISCUSSION

A major pathological characteristic of obesity is adipose tissue inflammation, which drives local dysfunction as well as systemic consequences, such as insulin resistance. We identify a novel role for A20 in controlling adipose tissue inflammation and obesity-related pathology. The results indicate that rapid and prolonged induction of A20 by adiponectin can drive macrophage quiescence and reduced WAT inflammation. Furthermore, using a murine model of obesity and, importantly, obese human subjects pre- and postbariatric surgery, we confirm beneficial effects of increased circulating adiponectin levels and enhanced WAT A20 expression associated with attenuated adipose tissue inflammation and improvement of metabolic disease markers and insulin sensitivity.

Reverbaα is a key link between the circadian clockwork and metabolic pathways (35–37) and has been strongly implicated in adipocyte differentiation (19) and lipid handling (20). We show in the current study that mice lacking Reverbaα demonstrated enhanced fat storage yet without the expected loss of insulin sensitivity or WAT inflammation. Delezie et al. (20) reported a similar phenotype, although the inflammatory status of the mice was not explored. The findings imply that obesity in Reverba−/− mice lacks typical proinflammatory stimuli and is accompanied by enhanced anti-inflammatory signaling. The absence of adipose inflammation in the Reverba−/− mice is not a result of a general lack of inflammatory response because these animals exhibit robust responses to systemic endotoxin administration, as we have previously demonstrated (21). Furthermore, Reverba−/− mice exhibit profoundly hypertrophic adipocytes and elevated production of free FAs similar to obese WT mice. Therefore, we focused on the enhanced production of adiponectin as a mediating factor
of the noninflamed fat phenotype. The underlying cause of increased adiponectin production from adipose tissue in Reverbα−/− mice remains unclear. Adiponectin production exhibits a pronounced diurnal variation (38), suggesting that it is regulated by the circadian clockwork. However, no differences were observed in the mRNA expressions of key enzymes that regulate adiponectin assembly or secretion between WT and Reverbα−/− mice (e.g., disulfide-bond-A oxidoreductase-like protein [DsBA-L], endoplasmic reticulum, oxidoreduct 1-like protein [Ero1-L], endoplasmic reticulum resident protein 44 [ERP44], receptor interacting protein 140 [RIP140]). Future studies to explore the molecular coupling between Reverbα and adiponectin will be valuable.

Beneficial effects of adiponectin in models of obesity-related metabolic and cardiovascular diseases have been widely reported (8,9), supporting the potential of adiponectin as an anti-inflammatory agent. Indeed, adiponectin rapidly suppressed proinflammatory signaling in primary murine macrophages through increased A20 expression, which is in line with previous work demonstrating increased A20 expression in human macrophages following adiponectin exposure (31). Adipose tissue beds from Reverbα−/− mice displayed increased adiponectin production and basal expression of A20 protein and an attenuated inflammatory response to ex vivo challenge with LPS. Enhanced A20 expression and reduced inflammatory response was also achieved in WT WAT explant cultures following treatment with adiponectin. A20 activity restricts TLR-directed NF-κB and interferon regulatory factor-3–dependent gene expression by modulating the K48- and K63-polyubiquitination of upstream signaling proteins, respectively, triggering their proteasomal degradation or interaction with other signaling molecules (39). Polymorphisms and mutations in the A20 gene locus are associated with multiple inflammatory, autoimmune, and malignant diseases (40). Our work now demonstrates a distinct role for an adiponectin-A20 axis regulating adipose tissue inflammation.

A “safe” increase in adiposity caused by forced adiponectin overexpression in ob/ob mice has previously been reported (41), resulting in animals with significantly more adipose tissue than their ob/ob littermates but with their inflammatory and diabetic phenotype completely reversed. The authors suggested that increased adiponectin level directs lipid accumulation to adipose rather than to liver or muscle tissue, ameliorating metabolic dysfunction. Therefore, the increased adiposity demonstrated by Reverbα−/− mice might be a result of elevated adiponectin;
however, it can also be argued that increased adipose mass that is noninflamed and thus remains viable drives the increased production of adiponectin.

Results obtained from the present preclinical studies highlight roles for adiponectin and A20 expression in mitigating the negative consequences of obesity on WAT inflammation and insulin sensitivity. Importantly, these findings are supported in studies of obese human subjects before and in response to bariatric surgery. The current human volunteer studies demonstrate that A20 expression in WAT from the obese subjects exhibited a significant negative correlation with HOMA-IR. Furthermore, we observed that weight loss in response to bariatric surgery was associated with a fall in circulating CRP level and a rise in serum adiponectin level, as expected, but in paired WAT samples, we also observed a rise in A20 expression. Both pre- and postsurgery, A20 gene expression was correlated with circulating levels of adiponectin. Taken together, the findings demonstrate that local adipose inflammation can be controlled by adiponectin through a mechanism involving A20. In an animal model of safe fat accumulation, paradoxical induction of adiponectin production by adipose tissue beds is associated with increased A20 expression, and the beneficial effects of adiponectin are shown to depend on A20. Targeting this pathway may offer a novel approach to mitigating obesity-related pathology.

Acknowledgments. The authors thank Marie Corcoran (University of Auckland) for assistance in producing the adiponectin used for these studies and Anthony Heagerty (University of Manchester) for help and support in patient recruitment and sample acquisition.

Funding. This work was supported by grants from the Endocore Research Trust; Maurice and Phyllis Paykel Trust; Health Research Council (New Zealand); Ministry of Business, Innovation and Employment (New Zealand); and Biotechnology and Biological Sciences Research Council (U.K.) (grant BB/I018654/1 to D.A.B.). D.W.R. was supported by the Wellcome Trust. This work was facilitated in part by the National Institute of Health Research Manchester Biomedical Research Unit in musculoskeletal disease and the Greater Manchester Comprehensive Local Research Network.

Duality of Interest. This work was facilitated in part by GlaxoSmithKline. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. L.E.H. carried out the experiments and wrote the manuscript. P.U. and P.S.C. carried out experiments. G.J.S.C. provided reagents. L.Y.X. carried out the experiments and provided reagents. B.A., R.A., H.S., and A.G. provided the human tissue. A.S.L. supervised the project. D.A.B. and D.W.R. supervised the project and wrote the manuscript. D.W.R. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
References