

GRIM-19 exerts anti-obesity effects in obese patients by regulating inflammation and inducing brown adipocytes

Subtitle: Anti-inflammatory and anti-obesity effects of GRIM-19

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Abstract

Background

Obesity is a chronic metabolic disease caused by inflammation and imbalance between WAT and BAT. Brown adipocytes internalize glucose and fatty acids for heat production. Therefore, the activation of BAT may represent a valid treatment strategy for obesity. In this study, we explored the correlation between the status of GRIM-19, BMI and inflammation in obese patients.

Method

To investigate GRIM-19 can modulate WAT and BAT, we knocked down GRIM-19 in human MSC-derived adipocytes. Additionally, we developed a patient-derived fat-transplantation mouse model to determine whether GRIM-19 can attenuate obesity and inflammation.

Results

The results showed that GRIM-19 expression decreased in the adipose tissues of obese patients, but the number of M1 macrophages, STAT3 and STAT6 were increased compared with controls. Furthermore, GRIM-19 expression in adipose tissue was negatively correlated with patient BMI. When GRIM-19 was knocked down in human MSC-derived adipocytes, WAT-related genes were increased while BAT-related genes were decreased. Also, when metformin was administered to patient-derived fat-transplanted mice, we found GRIM-19 expression levels increased and adipocytes size and inflammation decreased in patient-derived adipose tissue.

Conclusion

These results suggest that GRIM-19 attenuates obesity by promoting brown adipogenic differentiation and regulating inflammation.

Background

Obesity, a chronic metabolic disease caused by various factors, is characterized by an imbalance between white adipose tissue (WAT) and brown adipose tissue (BAT) [1, 2]. In adults, subcutaneous fat is populated by classic white adipocytes, whereas classic brown adipose fat is located in the deep neck, armpits, and areas adjacent to skeletal muscle [3]. In murine and human BAT, mitochondria are powerful generators of heat that safely metabolize fat; this process plays a key role in systemic regulation of lipid metabolism and glucose [4]. The activation of BAT may represent a valid treatment strategy for obesity and type 2 diabetes mellitus [1, 5]. Therefore, explorations of regulatory mechanisms involving brown and beige adipose tissues may provide new treatment targets for obesity and diabetes.

Obesity is also linked to inflammation, mitochondrial dysfunction and insulin resistance [6–8]. In obesity, pro-inflammatory macrophages, also known as M1 macrophage, are attracted to adipose tissue depots where they release pro-inflammatory cytokines; these cytokines trigger inflammation and cause increases in oxidative stress and mitochondrial dysfunction [9, 10]. Macrophages (M0) can be polarized into pro-inflammatory (M1) and anti-inflammatory (M2) types in response to various microenvironmental stimuli [11]. M1 type macrophage polarization is induced by interleukin (IL)-6 induced by activation of signal transducer and activator of transcription 3 (STAT3) signaling [12, 13]. Excess levels of dietary nutrients provide mitochondria with large amounts of free fatty acids and glucose, thus impairing multiple mitochondrial functions [9]. The reduced level of beta-oxidation leads to increased reactive oxygen species (ROS) production, enhancing the existing mitochondrial damage and contributing to insulin resistance and adipose tissue dysfunction.

STAT3 signaling is known to be chronically increased in obese patients, promoting inflammation and adipogenesis [14, 15]. The Gene associated with the Retinoid-Interferon-induced Mortality-19 (GRIM-19) protein, located in the mitochondrial inner membrane, is a dual function gene that is involved in mitochondrial metabolism and IFN- β /RA-induced cell death [16–18]. Recent studies provide evidence that GRIM-19 can regulate STAT3 activity [19, 20]. Also, GRIM-19 overexpression reportedly reduced WAT differentiation and induced BAT differentiation in high fat diet-fed mice [16]. In this study, we explored the correlation between obesity and GRIM-19 expression level in adipose tissues of obese patients. Additionally, we developed a patient-derived fat-transplanted mouse model to investigate whether GRIM-19 attenuates the progression of obesity and inflammation.

Materials and Methods

Study population

Morbidly obese patients who underwent bariatric surgery in Seoul St. Mary's hospital and Incheon St. Mary's Hospital between September 2020 and January 2023 were enrolled in this study. All obese patients either had a body mass index (BMI) $> 30 \text{ kg/m}^2$ and ≥ 1 comorbidity related to obesity, or a BMI $> 35 \text{ kg/m}^2$. Twenty-two obese patients yielded omental fat and subcutaneous fat tissues; these tissues were compared with tissues from 8 control patients who underwent laparoscopic surgery for benign diseases. All control patients had a BMI $< 30 \text{ kg/m}^2$. Five grams of each omental and subcutaneous fat tissue sample were used in experiments.

Ethics approval and consent to participate

The Institutional Review Board of the College of Medicine, Catholic University of Korea (KC KC20TISI0728), approved this study, in line with the Declaration of Helsinki and Good Clinical Practice guidelines. All procedures conformed to the ethical standards of the responsible committee on human experimentation (institutional and national) and the Helsinki Declaration of 1964 and later versions. Informed consent or an appropriate substitute was obtained from all patients participating in the study.

Histological assessment of adipose tissue

Fat tissues were fixed with 10% formalin for 24 h and embedded in paraffin. The tissues were sectioned at 5- μ m thickness and placed on standard microscopy slides. Deparaffinized and dehydrated sections were stained with hematoxylin and eosin (H&E).

Immunohistochemistry

Paraffin-embedded sections were incubated at 4°C with the following primary monoclonal antibodies: anti-GRIM-19 (catalog no. ab110240; Abcam, Cambridge, UK), anti-CD80 (catalog no. PA5-85913; Invitrogen, Middlesex County, MA, USA), anti-CD206 (catalog no. ab64693; Abcam), anti-STAT3 (catalog no. ab68153; Abcam), and anti-interleukin (IL)-6 (catalog no. NB600-1131; Novus Biologicals, Littleton, CO, USA). All primary antibodies were detected using a biotinylated secondary antibody, followed by incubation with a streptavidin-peroxidase complex for 30 min. Color development was conducted using the chromogen 3,3'-diaminobenzidine (Dako, Santa Clara, CA, USA).

Isolation of adipose tissue-derived mesenchymal stem cells and differentiation of adipocytes

Mesenchymal stem cells (MSCs) were isolated from donor adipose tissues. Samples of adipose tissue (3–5 mg) were washed with sterile phosphate-buffered saline to remove contaminating debris and red blood cells. After removal of the phosphate-buffered saline, the remaining tissue was added to a Petri dish. Each adipose tissue sample was cut into small pieces using a sterile scalpel and digested with 20 mL of 0.1% type I collagenase (catalog no. LS004197; Worthington Biochemical Products, Lakewood, NJ, USA) at 37°C for 40 min in a water bath with gentle agitation. The resulting mixture was centrifuged at 1,500 rpm for 5 min; adipose tissue debris and supernatant were discarded. Each cellular pellet was resuspended in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (Pan-Biotech, Aidenbach, Germany) and filtered through a 40- μ m strainer to remove debris. Isolated MSCs were cultured for 4 days at 37°C with 5% CO₂ until they reached 90% confluence (passage 0), then expanded for 2–3 passages and used in experiments. For adipogenesis studies, 4×10^4 MSCs were seeded in the wells of a 24-well plate and allowed to reach 100% confluence. Then, the medium was replaced with α -MEM (catalog no. LM008-01; Welgene, Geongsan-si, Korea) containing 10% fetal bovine serum and an adipogenic supplement (catalog no. CCM001; R&D Systems, Minneapolis, MN, USA). The medium was changed every 3 days for 2 weeks. After 2 weeks, differentiated cells were used for experiments.

Single-guide RNA

To generate lentiviruses for transduction, HEK293T cells were transfected with plasmids encoding sgRNA and packaging vectors (pMD2.G, #12259 and psPAX2, #12260; Addgene) using Lipofectamine 2000 (catalog no. 11668030; Thermo Fisher Scientific, Waltham, MA, USA). Culture supernatants were collected at 48 and 72 h post-transfection, then used to infect MSC cells via the spin-infection method. The sequences of the sgRNAs are described in Table 1.

Western blotting

Cells were lysed with RIPA Lysis and Extraction Buffer (catalog no. 89901; Thermo Fisher Scientific) including the HaltTM Protease Inhibitor Cocktail (catalog no. 78410; Thermo Fisher Scientific); protein concentrations

were determined using a Bradford reagent kit (Molecular Devices, Downingtown, PA, USA). Protein samples were separated via 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to Hybond membranes (Amersham Pharmacia Biotech, Piscataway, NJ, USA). Proteins were incubated with antibodies to GRIM-19 (catalog no. ab110240; Abcam) and GAPDH (catalog no. ab181602; Abcam), then detected using an enhanced chemiluminescence detection kit (Pierce, Rockford, IL, USA) and HyperFilm (Agfa, Mortsel, Belgium); GAPDH served as a loading control.

Gene expression analysis via real-time quantitative PCR

Gene expression analysis was performed as previously described. Briefly, total cellular mRNA was extracted using TRI reagent (catalog no. TR118; Molecular Research Center, Cincinnati, OH, USA). cDNA was synthesized using the Superscript Reverse Transcription system (Takara, Shiga, Japan). Real-time quantitative PCR (qPCR) was performed using the LightCycler FastStart DNA Master SYBR Green I (Takara) system **with the specific primers listed in Table 1**, in accordance with the manufacturer's instructions. Expression levels were normalized to the level of β -actin.

Cell culture and metformin treatment

3T3-L1 cells were obtained from the Korean Cell Line Bank and cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum at 37°C with 5% CO₂. Cells were cultured with 10 mM metformin for 48 h.

Animals

Seven-week-old NOD/scid/IL-2R $\gamma^{-/-}$ female mice (NOD.Cg-Prkdc^{scid}IL2rg^{tm1Wjl}/SzJ; NSG) were obtained from the Jackson Laboratory (Bar Harbor, ME, USA). They were fed standard mouse chow (Ralston Purina, St. Louis, MO, USA) and given water ad libitum. All experimental procedures were reviewed and approved by the Animal Research Ethics Committee of the Catholic University of Korea (Seoul, South Korea).

Transplantation of fat tissue and metformin treatment

After 6-week-old NSG mice had been anesthetized, 0.8410 g of adipose tissue from a patient was transplanted into the scapular fat pad of each mouse. After suturing and disinfection, a heat lamp was placed above the pad. Mice were observed daily; weight changes were recorded twice weekly.

Statistical analysis

Data are presented as means $\pm$ standard deviations. Statistical analyses were performed using Prism version 8 for Windows (GraphPad Software, Inc., San Diego, CA, USA). Normally distributed continuous data were compared between two groups by Student's t-test and among ≥ 3 groups by one-way analysis of variance. In all analyses, P-values < 0.05 were considered statistically significant.

Results

GRIM-19 expression is reduced in adipose tissues of obese patients

It is known that in obese patients, WAT is mainly increased while mitochondrial function is decreased [7, 21]. GRIM-19 plays an important role in mitochondrial function [17]. Therefore, we used an immunohistochemical technique to investigate the expression level of GRIM-19 in adipose tissues of obese patients. We found that the expression level of GRIM-19 was lower in subcutaneous fat and omental fat of obese patients compared with controls (Ctrls) (Figure 1A). H&E staining of subcutaneous fat and omental fat from obese patients revealed an increased adipocyte diameter and decreased adipocyte count per high-power field (HPF) compared with Ctrls (Figure 1B). Then, we assessed the relationships between GRIM-19 expression and adipocyte diameter. We found negative correlations between GRIM-19 expression and adipocyte diameter in subcutaneous fat or omental fat (Figure 1C). These results indicated that GRIM-19 has a key role in obesity.

The M1/M2 macrophage ratio increases as GRIM-19 expression decreases in adipose tissues of obese patients

There is evidence that the pro-inflammatory M1 macrophage number increases and mitochondrial function decreases in obese patients compared with other individuals. Therefore, we used an immunohistochemical technique to assess the relationship between GRIM-19 expression and macrophage number in adipose tissues of obese patients. The numbers of cells expressing the M1 macrophage marker CD80 and the percentages of CD80⁺ cells/adipocytes were increased in the subcutaneous and omental fats of obese patients compared with Ctrls (Figure 2A). Conversely, in the subcutaneous and omental fats of obese patients, the numbers of cells expressing CD206, a marker of anti-inflammatory M2 macrophages, and the percentages of CD206⁺ cells/adipocytes were lower compared with Ctrls (Figure 2B). These results showed that the M1/M2 ratio was increased in the adipose tissues of obese patients (Figure 2C). Next, we evaluated the relationship between GRIM-19 expression and macrophage number. We observed a negative correlation between GRIM-19 expression and the M1 macrophage marker CD80 and a positive correlation between GRIM-19 expression and the M2 macrophage marker CD206 in adipose tissues (Figure 2D, E).

As GRIM-19 expression decreases in the adipose tissues of obese patients, the expression levels of STAT3 and IL-6 increase

There is evidence that GRIM-19 reduces the levels of the inflammatory markers STAT3 and IL-6 [20, 22-25]. Therefore, we used immunohistochemical techniques to assess the relationships of GRIM-19 expression with STAT3 and IL-6 levels in adipose tissues of obese patients. The results showed that the numbers of STAT3-expressing cells and the percentages of STAT3⁺ cells/adipocytes were increased in the subcutaneous and omental fats of obese patients compared with Ctrls (Figure 3A). Additionally, the numbers of IL-6-expressing cells and the percentages of IL-6⁺ cells/adipocytes were increased in the subcutaneous and omental fats of obese patients compared with Ctrls (Figure 3B). These results confirmed that the expression level of GRIM-19 was negatively correlated with the expression levels of STAT3 and IL-6 in adipose tissues (Figure 3C, D).

Correlations with BMI

Next, we investigated correlations between each adipose tissue marker and BMI. We found that BMI was positively correlated with adipocyte diameter (Figure 4A), as well as the levels of CD80 (Figure 4C), STAT3 (Figure 4E), and IL-6 (Figure 4F) in adipose tissues. In contrast, BMI was negatively correlated with the levels

of GRIM-19 (Figure 4B) and CD206 (Figure 4D) in adipose tissues. These results showed that GRIM-19 is associated with obesity and inflammation.

GRIM-19 KO represses expression of brown adipocyte markers in human MSC-derived adipocytes

To determine whether GRIM-19 regulates the differentiation of adipocytes into brown adipocytes, we knocked down GRIM-19 in human MSC-derived adipocytes. In this experiment, human MSC-derived adipocytes were transfected with scrambled (Scr) control or GRIM-19 KO sgRNA. The data showed that the expression level of GRIM-19 was lower in GRIM-19 KO cells than in Scr cells (Figure 5A). Furthermore, the transcription levels of the WAT-related genes adiponectin and angiotensinogen (AGT) were higher in GRIM-19 KO cells than in Scr cells (Figure 5B). In contrast, the transcription levels of the BAT-related genes fibroblast growth factor 21 (FGF21), cytochrome C oxidase polypeptide 7A1 (COX7A1), cell death-inducing DNA fragmentation factor alpha-like effector A (CIDEA), and ELOVL fatty acid elongase 3 (ELOVL3) were lower in GRIM-19 KO cells than in Scr cells (Figure 5C). These results suggested that GRIM-19 regulates brown adipogenic differentiation.

Metformin increases GRIM-19 expression and decreases adipocyte size in patient-derived transplanted fat

Next, we developed a patient-derived fat-transplanted mouse model to investigate whether GRIM-19 could attenuate obesity. In this experiment, we used metformin, which reportedly increases GRIM-19 expression [26]. Indeed, we confirmed that metformin-treated 3T3-L1 cells exhibited increased GRIM-19 expression (Figure 6A). The patient-derived fat-transplanted mouse model was developed by transplanting patient fat into the subcutaneous region of the right leg of NOD.Cg-PrkdcscidIL2rgtm1Wjl/SzJ (NSG) mice (0.25 g fat/mouse). Beginning 1 day after transplantation, metformin was administered orally daily for 25 days (Figure 6B). The results showed that metformin increased GRIM-19 expression in patient-derived transplanted fat tissue, compared with vehicle treatment (Figure 6C). Immunohistochemical analysis confirmed that GRIM-19 expression was greater in patient-derived transplanted fat tissue of metformin-treated mice, compared with vehicle-treated mice (Figure 6D). Additionally, H&E staining of patient-derived transplanted fat tissue showed that metformin reduced the diameter of adipocytes compared with vehicle (Figure 6E). Macrophage staining showed that expression of the M1 macrophage marker CD80 was decreased and expression of the M2 macrophage marker CD206 was increased upon treatment with metformin (Figure 6F). Finally, the expression levels of STAT3 and IL-6 were decreased, indicating that metformin treatment improved the overall inflammatory status (Figure 6F). These data suggest that increasing Grim-19 via metformin suppresses obesity and inflammation.

Discussion

We found that adipocyte size increased in the adipose tissues of obese patients. Substantial decreases in GRIM-19 expression were observed in subcutaneous and omental fat tissues of obese patients. Moreover, a negative correlation was evident between adipocyte diameter and GRIM-19 expression. These findings highlight the potential regulatory role of GRIM-19 in adipose tissue homeostasis. GRIM-19, known for its roles in mitochondrial metabolism and apoptosis, has emerged as a key component of adipocyte function. The inverse correlation suggests that decreased GRIM-19 expression contributes to the adipocyte hypertrophy and adipose tissue dysfunction observed in obesity. The M1/M2 ratio increased as GRIM-19 expression decreased in obese patients. Next, we investigated the levels of key inflammatory markers STAT3 and IL-6 in

obese and control adipose tissues. Although the expression levels per HPF did not significantly differ, the percentages of STAT3 and IL-6 expression areas per adipocyte were significantly greater in obese patients. Correlation analysis also indicated an inverse relationship between GRIM-19 expression and levels of inflammatory markers. These results suggest that increased M1 macrophage by IL-6 (secreted by STAT3 activity) may be regulated by GRIM-19. Also, these results are consistent with previous reports that adipose tissue inflammation is associated with obesity-related complications, including insulin resistance and metabolic dysfunction, suggesting a role for GRIM-19 in modulation of the inflammatory milieu within adipose tissue [16, 27, 28]. The findings imply that decreased GRIM-19 expression participates in the enhanced inflammation linked to obesity-associated complications. Our in vitro analysis confirming the involvement of GRIM-19 in adipocyte browning strengthens the overall understanding of the regulatory role of GRIM-19 during adipose tissue differentiation. Furthermore, our in vivo analysis of the impact of metformin on GRIM-19 expression in transplanted adipose tissue suggests a promising therapeutic approach. Metformin has been widely used for many years and is considered an effective and generally well-tolerated medication for the treatment of type 2 diabetes [26]. Metformin, known for its metabolic effects, has emerged as a potential modulator of GRIM-19 expression, supporting a novel approach to mitigate dysfunction in obesity-associated adipose tissue [19].

The present study highlights the importance of brown fat in managing obesity and diabetes via thermogenesis; GRIM-19 and metformin both activate brown fat, possibly suppressing obesity and improving metabolic function. This paradigm supports important new research directions that emphasize the importance of brown fat-assisted treatments in obesity management. Considering the interactions between inflammatory status and mitochondrial function in adipose tissue, the present results provide important guidance for effective treatment strategies focused on inhibiting the onset and progression of obesity and diabetes. They may also offer new insights in terms of preventing and improving obesity-related metabolic diseases. In particular, considering the effectiveness of metformin in terms of GRIM-19 expression and inflammation, preventative strategies using similar substances or other existing treatments could be explored. Further research is needed to support a comprehensive and effective approach regarding health issues related to obesity management.

The greatest strength of this study is that, to our knowledge, it is the first report of a mouse model involving transplanted patient-derived adipose tissue. This approach mimics the in vivo conditions of obese patients and reflects the complex clinical environment involved in evaluating novel treatment strategies or drug efficacies. Existing experimental animal models often do not sufficiently match the complexity of metabolic abnormalities associated with obesity [29-31]. However, the mouse model developed in the present study uses adipose tissues from obese patients, enhancing the reliability and implications of the findings by mimicking a situation that is similar to the metabolic abnormalities associated with obesity. The model confirmations of the effects of GRIM-19, and metformin might contribute to the development of customized treatments that reflect the biological conditions of individual patients.

Another strength is that the experiments were conducted using subcutaneous and omental fat samples from obese patients. Visceral fat tissue exhibits higher cellularity, vascularity, innervation, and a greater presence of inflammatory and immune cells compared to subcutaneous fat tissue. Additionally, visceral fat has a reduced capacity for preadipocyte differentiation and a higher percentage of large adipocytes. The adipocytes in

visceral fat are more metabolically active, display increased sensitivity to lipolysis, and show greater insulin resistance in comparison to those in subcutaneous fat tissue [3, 32]. Although it is widely accepted that the risks associated with abdominal obesity (i.e., visceral obesity) are greater than the risks associated with simple weight gain, many studies have only evaluated subcutaneous fat, possibly because the acquisition of visceral fat tissue is particularly invasive [33-36]. In the present study, we used omental fat tissues obtained from patients undergoing bariatric surgery, which enabled analysis of subcutaneous and visceral fat tissues; to our knowledge, this is the first study to demonstrate that existing and future obesity treatments can also be utilized to manage abdominal obesity.

This study has several limitations. First, the control group was relatively small ($n = 8$) compared with the obese group ($n = 22$), which may limit statistical power; additionally, potential confounding variables such as age and sex were not formally adjusted for in the correlation analyses, and these factors should be accounted for in future studies with larger, matched cohorts. Second, although the patient-derived fat-transplantation model in NSG mice offers a more clinically relevant system than conventional diet-induced obesity models, NSG mice lack mature T, B, and NK cells; because the observed effects were mediated primarily by macrophages, which remain intact in this strain, we consider the model appropriate for the present study, but results should be interpreted with this immunological context in mind. Third, the metformin intervention was evaluated over a relatively short period (25 days), and longer-term studies are needed to confirm the durability of the observed effects on GRIM-19 expression and adipose tissue inflammation. Finally, while our data demonstrate that metformin increases GRIM-19 expression concurrently with improved adipose tissue phenotypes, direct evidence that metformin acts specifically through GRIM-19 (e.g., loss-of-function studies combining GRIM-19 knockdown with metformin treatment) was not obtained in this study and represents an important direction for future research.

Future research will yield a greater understanding of the interactions between GRIM-19 and drugs such as metformin, facilitating the development of effective treatment strategies that exploit such interactions. Additionally, it is important to comprehensively investigate the interactions of and roles played by GRIM-19 in brown adipocytes and white adipocytes; this investigation will yield an in-depth understanding of the regulatory mechanisms involved. Such findings will provide new insights into adipose tissue and its regulation, which will serve as starting points in the development of new treatment strategies for obesity and diabetes.

Conclusion

In conclusion, the results suggest that the metformin-induced increase in GRIM-19 can control the progression of obesity by eliciting conversion of WAT to BAT and improving inflammatory status.

Abbreviations

WAT

white adipose tissue

BAT

brown adipose tissue

GRIM-19

Gene associated with Retinoid-Interferon-induced Mortality-19

BMI

body mass index

MSCs

mesenchymal stem cells

STAT3

signal transducer and activator of transcription 3

STAT6

signal transducer and activator of transcription 6

IL-6

interleukin-6

ROS

reactive oxygen species

IFN- β

interferon-beta

RA

retinoic acid

H&E

hematoxylin and eosin

sgRNA

single-guide RNA

GAPDH

glyceraldehyde 3-phosphate dehydrogenase

qPCR

quantitative polymerase chain reaction

AGT

angiotensinogen

FGF21

fibroblast growth factor 21

COX7A1

cytochrome C oxidase polypeptide 7A1

CIDEA

cell death-inducing DNA fragmentation factor alpha-like effector A

ELOVL3

ELOVL fatty acid elongase 3

NSG

NOD.Cg-PrkdcscidIL2rgtm1Wjl/SzJ (NOD/scid/IL-2R γ -/-)

HPF

high-power field

Scr, scrambled control

KO

knockout
Ctrl
control
OB
obese
Met
metformin

Declarations

Acknowledgments

Not applicable.

Author contributions

Y.J.J., K.H.L., JY.J. and M.L.C. designed the experiments. Y.J.J., K.H.L., JY.J., J.S.W., Y.S.L., S.Y.L., S.G.H., S.H.C., Y.J.L. and H.J.H. collected the data. K.H.L., JY.J., J.S.W., Y.S.L., S.Y.L., S.G.H., S.H.C., Y.J.L. and H.J.H. performed the experiments. Y.J.J., K.H.L. and JY.J. analyzed and interpreted the data. Y.J.J., K.H.L., JY.J. and M.L.C wrote the manuscript. K.Y.S and M.L.C

supervised the study.

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Availability of data materials

The data used to support the findings of this study are included within the article. The data and materials in the current study are available from the corresponding author on reasonable request.

Ethics approval and informed consent

All experimental procedures were performed in accordance with the Laboratory Animals Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Guidelines and Policies for Rodent Experiment provided by the Institutional Animal Care and Use Committee of the School of Medicine of the Catholic University of Korea (approval no. CUMC 2023-0074) and the study was approved by the institutional review board of Seoul St. Mary's Hospital and was performed in accordance with the Helsinki II Declaration (approval no. KC20TISI0728).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1
Primers used in this study.

Gene name	Forward primer	Reverse primer	Application
Adiponectin	AACATGCCCATTCGCTTTACC	TAGGCAAAGTAGTACAGCCCA	qPCR
AGT	CCCCAGTCTGAGATGGCTC	GACGAGGTGGAAGGGGTGTA	qPCR
FGF21	CTGTGGGTTTCTGTGCTGG	CCGGCTTCAAGGCTTTCAG	qPCR
COX7a1	CCGCTTTCAGAACCGAGTG	CCCTTCAGGTACAACGGGA	qPCR
CIDEA	GATGCCCTCGTCATCGCTAC	GCGTGTTGTCTCCCAAGGTC	qPCR
ELOVL3	TGGGGCATTATGGGGACTGT	AGGACCAGAATTTGACTGTGGA	qPCR
GRIM-19	CGGGCACTGGAGCATAATGA	CTGTAACAGTGGCAACAGCG	qPCR
β-actin	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT	qPCR
GRIM-19	CACCGCCCTTGATCGGGGAGCTGTA	AAACTACAGCTCCCCGATCAAGGGC	sgRNA
AGT: Angiotensinogen; FGF21: fibroblast growth factor 21; COX7a1: cytochrome C oxidase polypeptide 7A1; CIDEA: cell death-inducing DNA fragmentation factor alpha-like effector A; ELOVL3: ELOVL fatty acid elongase 3; GRIM-19: Gene associated with Retinoid-Interferon-induced Mortality-19.			

Figures

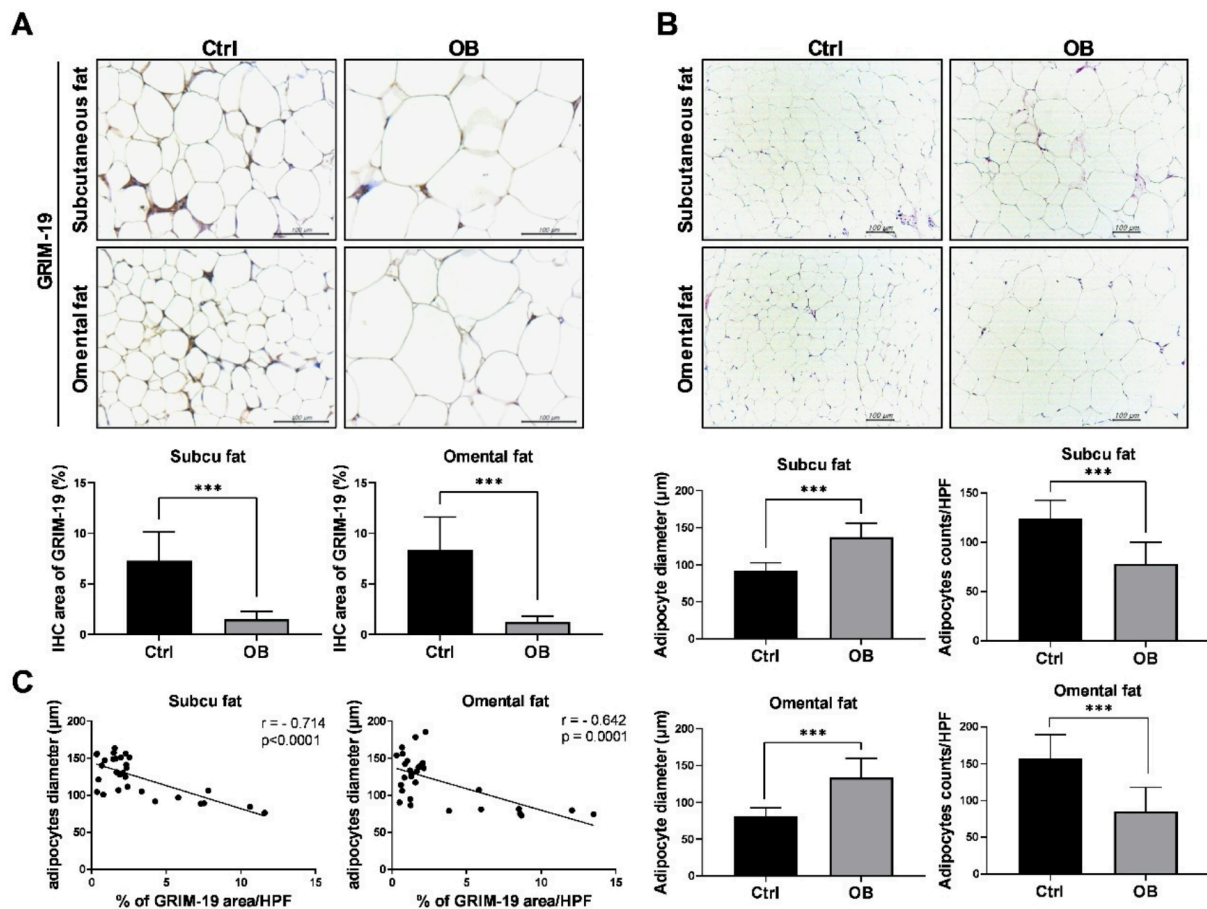


Figure 1

GRIM-19 levels are reduced in adipose tissues of obese patients.

(A) Immunohistochemical assays of GRIM-19 levels in subcutaneous and omental fat tissues of control (Ctrl) and obese (OB) patients. Scale bars = 100 μ m. **(B)** H&E staining revealing adipocyte diameters in subcutaneous and omental fat tissues of Ctrl and OB patients. Scale bars = 100 μ m. **(C)** Correlations between adipocyte diameter and the percentage of GRIM-19-positive area/HPF in subcutaneous and omental fat tissues. Values are means $\pm$ SEMs from three independent experiments. *** $P < 0.001$.

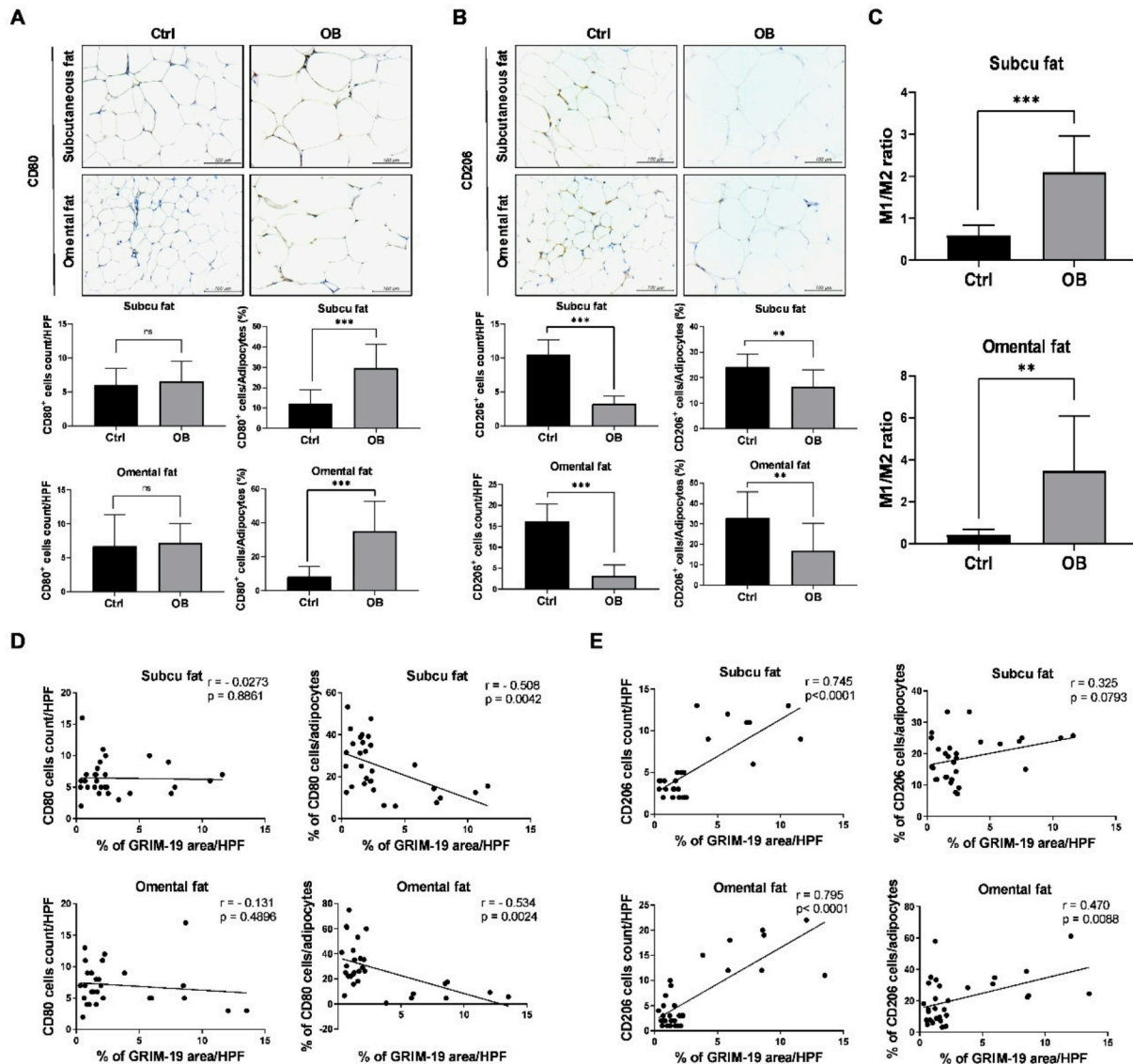


Figure 2

The M1/M2 macrophage ratio increases as GRIM-19 expression decreases in adipose tissues of obese patients.

(A) Immunohistochemical assays of CD80 levels in subcutaneous and omental fat tissues of Ctrl and OB patients. Scale bars = 100 μ m. **(B)** Immunohistochemical assays of CD206 levels in subcutaneous and omental fat tissues of Ctrl and OB patients. Scale bars = 100 μ m. **(C)** M1/M2 ratios in subcutaneous and omental fat tissues of Ctrl and OB patients. **(D)** Correlations of the percentage of GRIM-19-positive area/HPF with the CD80 cell count/HPF and the percentage of CD80 cells/all adipocytes in subcutaneous and omental fat tissues. **(E)** Correlations of the percentage of GRIM-19-positive area/HPF with the CD206 cell count/HPF and the percentage of CD206 cells/all adipocytes in subcutaneous and omental fat tissues.

and the percentage of CD206 cells/all adipocytes in subcutaneous and omental fat tissues. Values are means $\pm$ SEMs from three independent experiments. **P < 0.01 and ***P < 0.001.

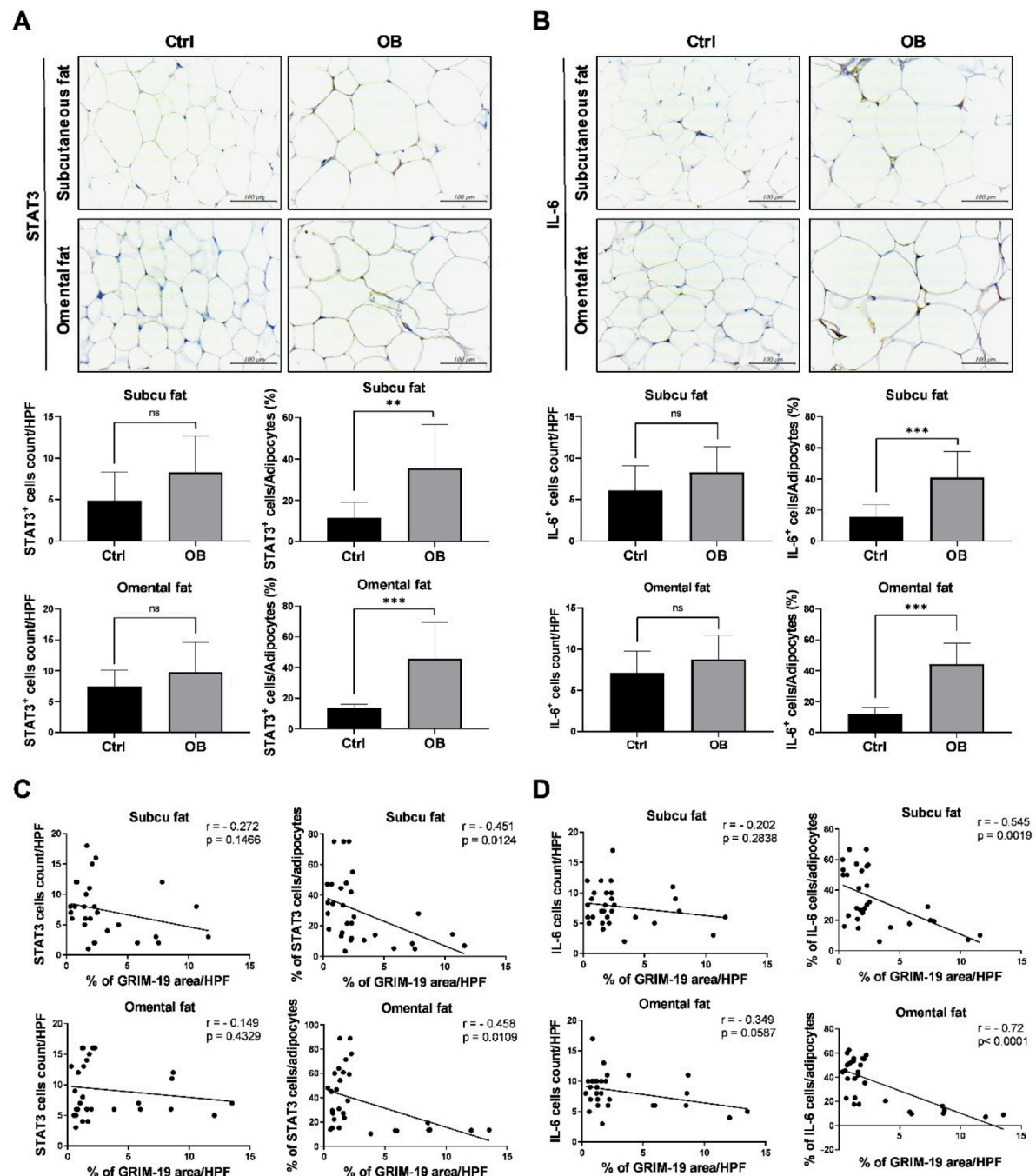


Figure 3

STAT3 and IL-6 levels are higher, and the GRIM-19 level is lower, in fat tissues of obese patients compared with control patients.

(A) Immunohistochemical assays of STAT3 levels in fat and omental fat tissues of Ctrl and OB patients. Scale bars = 100 μ m. **(B)** Immunohistochemical assays of IL-6 levels in subcutaneous and omental fat tissues of Ctrl and OB patients. Scale bars = 100 μ m. **(C)** Correlations of the percentage of GRIM-19-positive area/HPF with the STAT3-positive cell count/HPF and the percentage of STAT3-positive cells/all adipocytes in subcutaneous and omental fat tissues. **(D)** Correlations of the percentage of GRIM-19-positive area/HPF with the IL-6-positive cell count/HPF and the percentage of IL-6-positive cells/all adipocytes in subcutaneous and omental fat tissues. Values are means $\pm$ SEMs from three independent experiments. **P < 0.01 and ***P < 0.001.

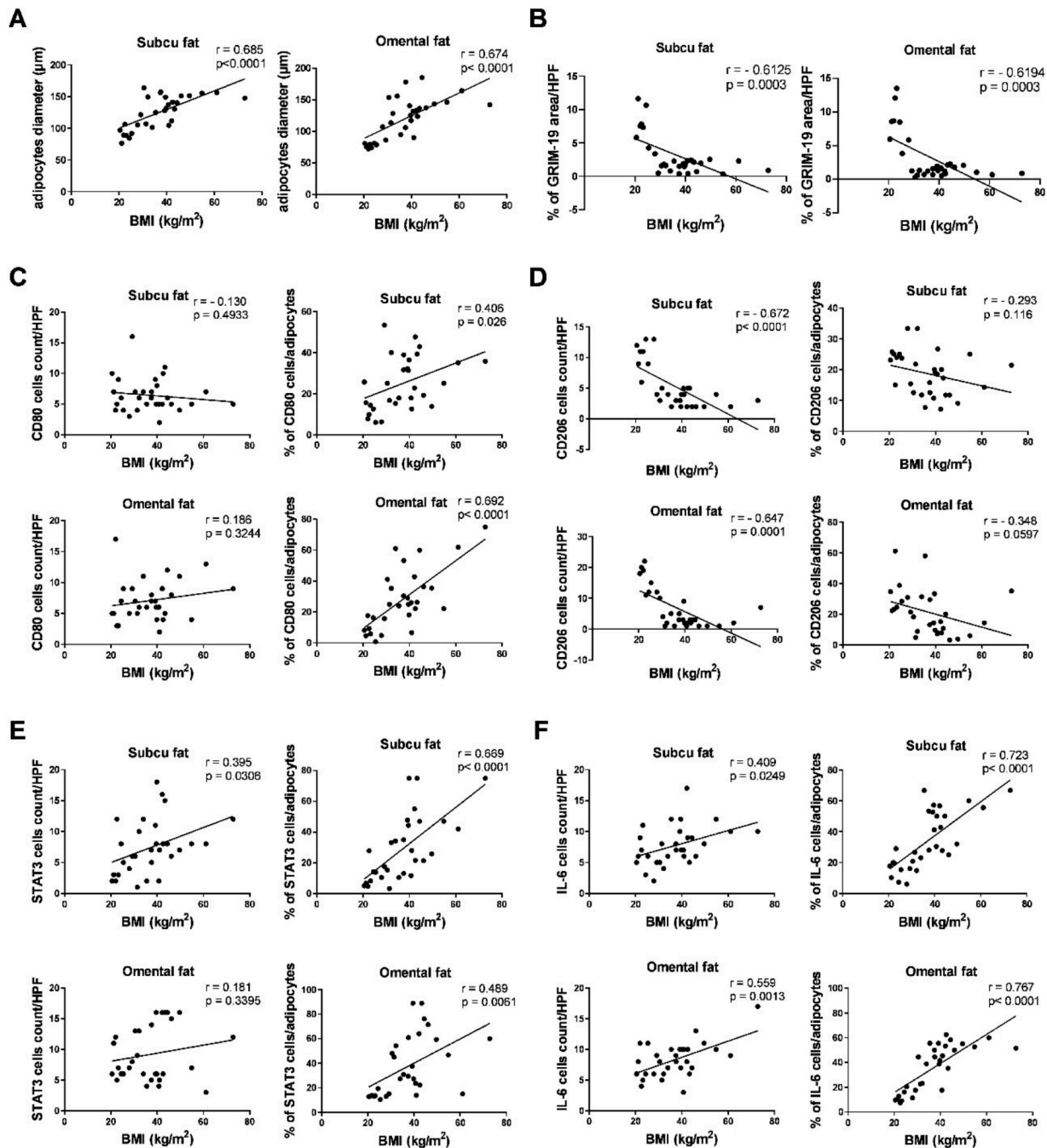


Figure 4

Correlations with BMI.

(A) Correlations between BMI and adipocyte diameter in subcutaneous and omental fat tissues. **(B)** Correlations between BMI and the percentage of GRIM-19-positive area/HPF in subcutaneous and omental fat tissues. **(C)** Correlations of BMI with the CD80-positive cell count/HPF and the percentage of CD80 cells/all adipocytes in subcutaneous and omental fat tissues. **(D)** Correlations of BMI with the CD206-positive cell

count/HPF and the percentage of CD206 cells/all adipocytes in subcutaneous and omental fat tissues. **(E)** Correlations of BMI with the STAT3-positive cell count/HPF and the percentage of STAT3 cells/all adipocytes in subcutaneous and omental fat tissues. **(F)** Correlations of BMI with the IL-6-positive cell count/HPF and the percentage of IL-6 positive cells/all adipocytes in subcutaneous and omental fat tissues.

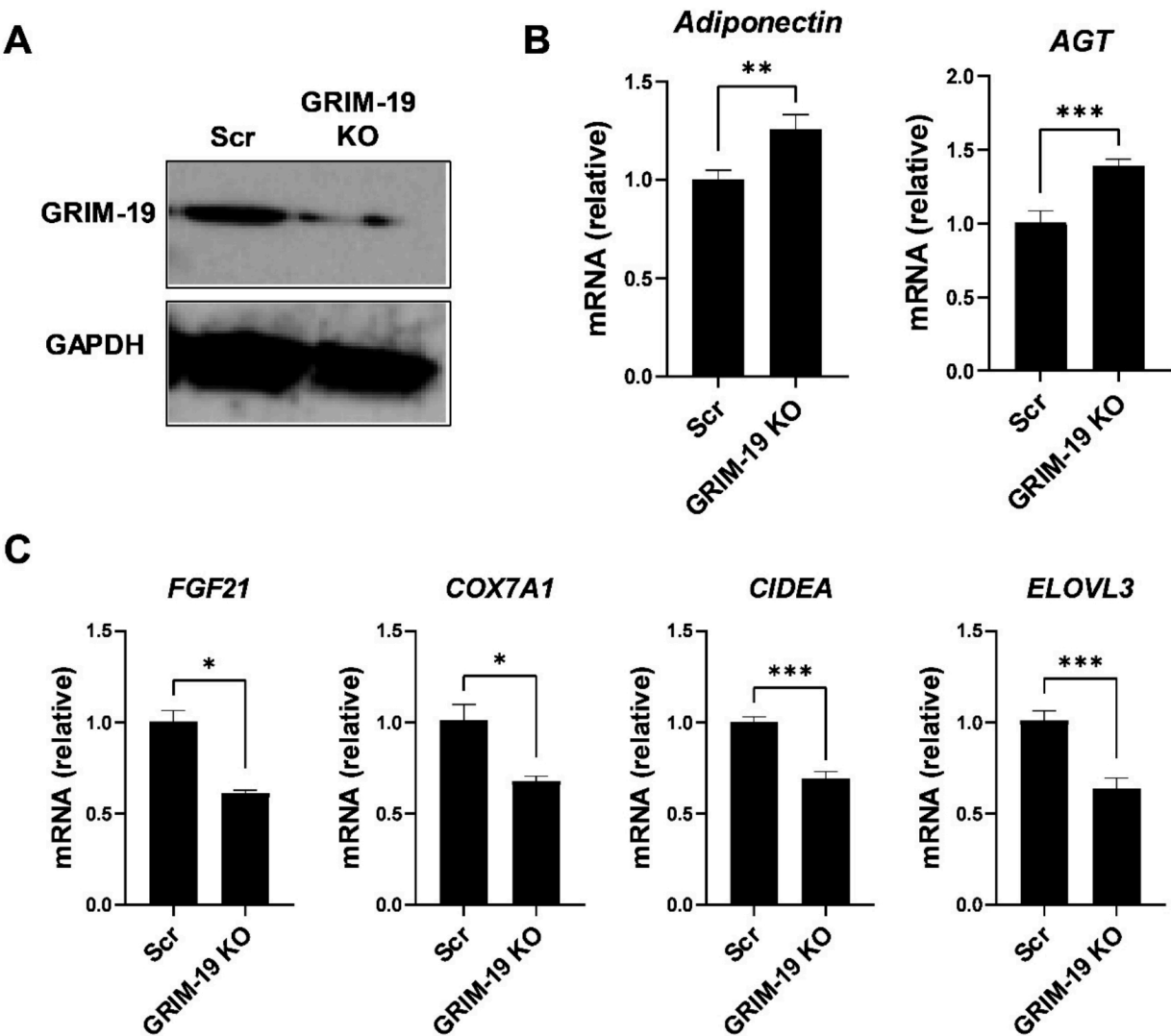


Figure 5

GRIM-19 KO represses the expression of brown adipocyte markers in human MSC-derived adipocytes.

(A) Expression levels of GRIM-19 in scrambled control (Scr) cell and GRIM-19 sgRNA (GRIM-19 KO) cells. **(B)** Transcriptional levels of adiponectin and AGT in Scr and GRIM-19 KO cells. **(C)**Transcriptional levels of fibroblast growth factor 21 (FGF21), cytochrome C oxidase polypeptide 7A1 (COX7A1), cell death-inducing

DNA fragmentation factor alpha-like effector A (CIDEA), and ELOVL fatty acid elongase 3 (ELOVL3) in Scr and GRIM-19 KO cells. Values are means $\pm$ SEMs from three independent experiments.

* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

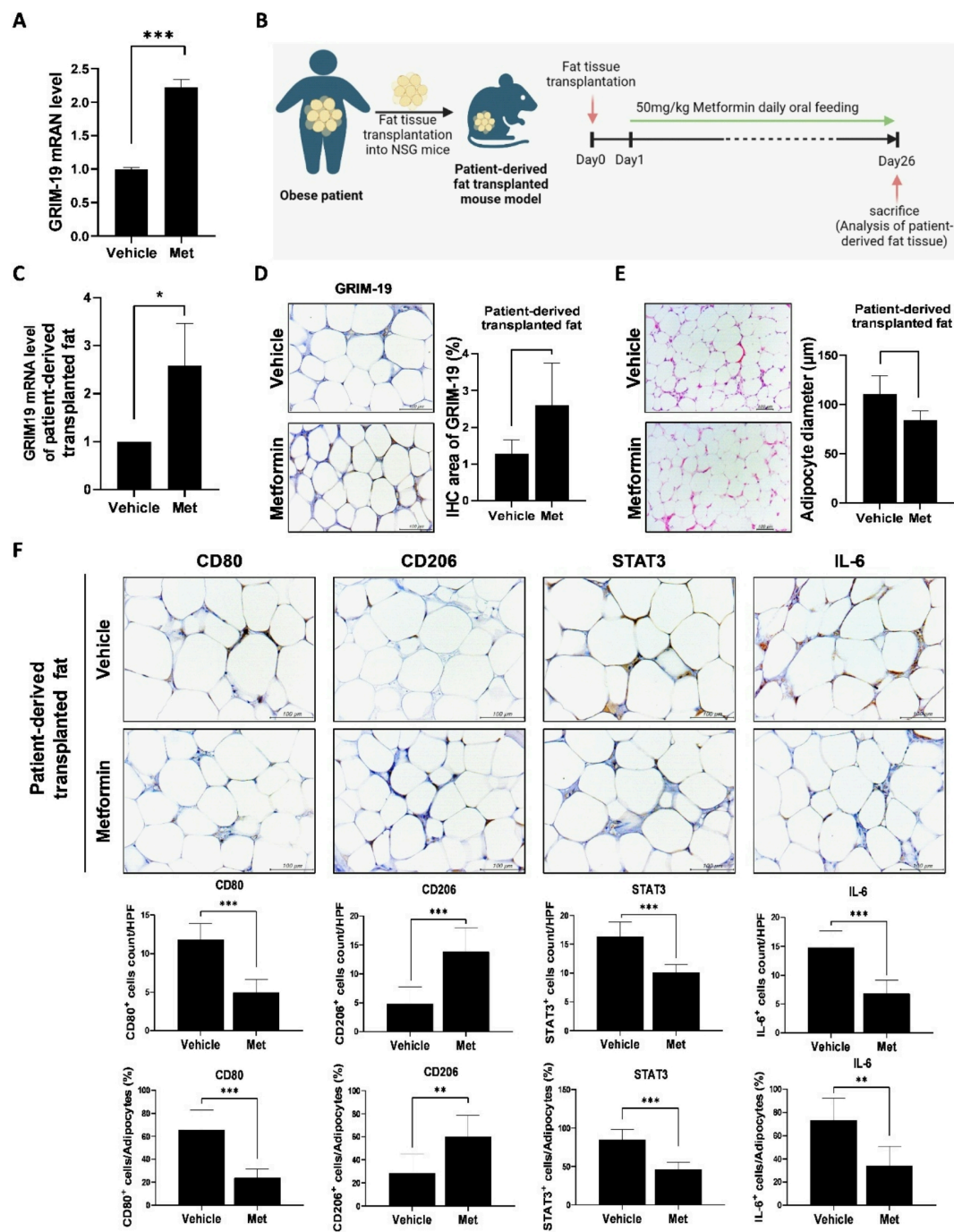


Figure 6

Metformin increases GRIM-19 expression and reduces adipocyte size in patient-derived transplanted fat tissues.

(A) Transcriptional levels of GRIM-19 in control (vehicle) and metformin (Met)-treated 3T3-L1 cells. **(B)** Schematic of the patient-derived fat transplantation mouse model. **(C)** Transcriptional levels of GRIM-19 in control (vehicle) and metformin (Met)-treated patient-derived transplanted fat. **(D)** Immunohistochemical assays of GRIM-19 levels in vehicle- and Met-treated mice with patient-derived transplanted fat. Scale bars = 100 μ m. **(E)** H&E staining revealing adipocyte diameter in vehicle- and Met-treated mice with patient-derived transplanted fat. Scale bars = 100 μ m. **(F)** Immunohistochemical assays of CD80, CD206, STAT3, and IL-6 levels in vehicle- and Met-treated mice with patient-derived transplanted fat. Scale bars = 100 μ m. Values are means $\pm$ SEMs from three independent experiments.

*P < 0.05, **P < 0.01, and ***P < 0.001.

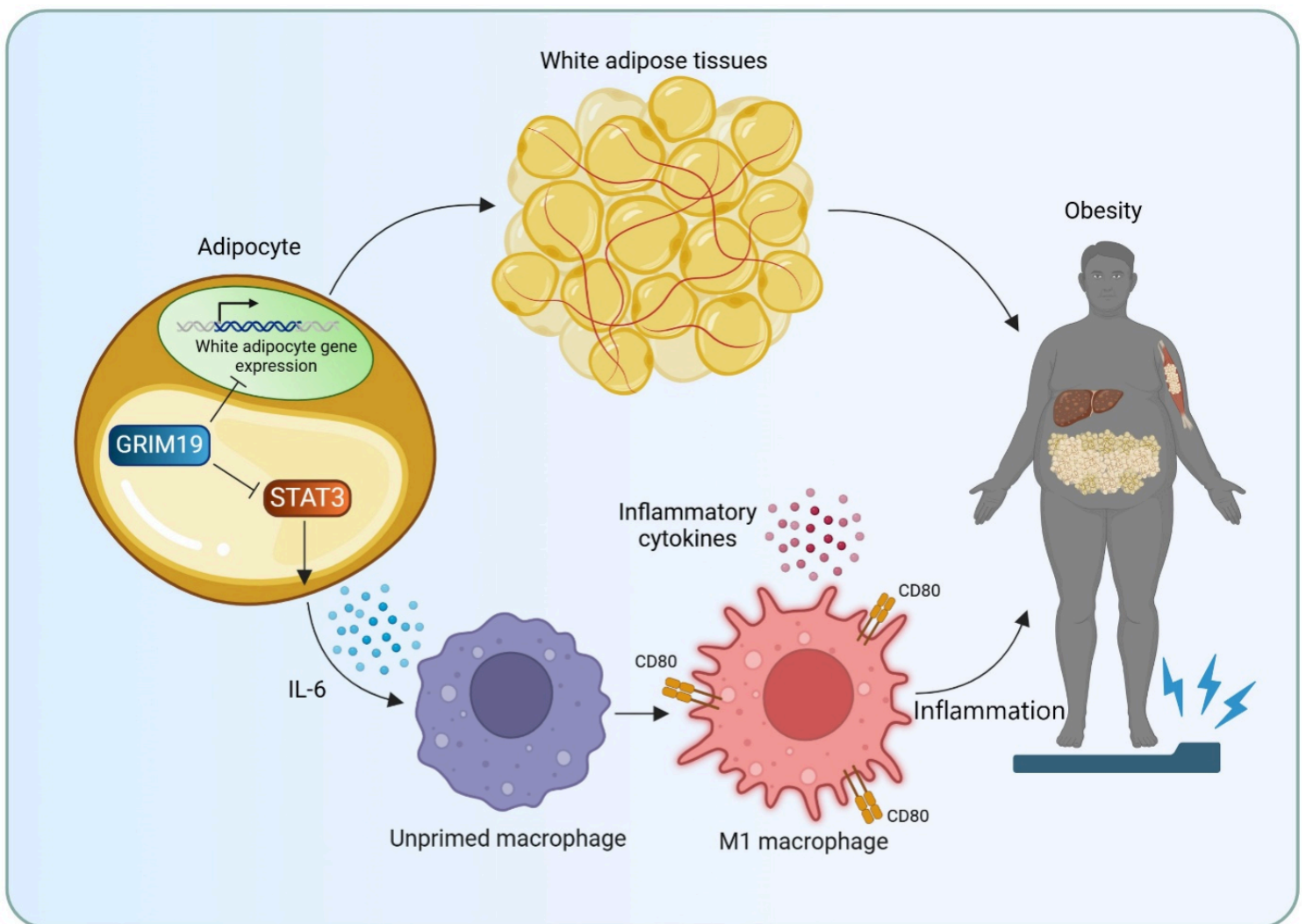


Figure 7

Graphical summary of the findings.

White adipocyte gene expression is suppressed by GRIM-19. Additionally, GRIM-19 suppresses inflammation in obese patients through STAT3 inhibition.

Supplementary Files

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- [OriginalWesternBlotsFigure5A.docx](#)