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Original Article

Cucumis Melo L. alleviates obesity and insulin resistance in obese human subjects and high fat diet-induced obese mice

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Abstract: Obesity is a major risk factor for insulin resistance, type 2 diabetes mellitus, and dyslipidemia. Recent studies have shown that inflammation is a key mediator between obesity and insulin resistance. Despite intensive efforts to develop pharmaceuticals for treating obesity and insulin resistance, these efforts have not been very successful in managing the symptoms and preventing multiple complications. Here, we discovered a novel therapeutic application of *Cucumis Melo* L. (Cucumis), traditionally used for treating edema and jaundice, as a therapy for obesity and insulin resistance. In a clinical study, oral treatment with Cucumis led to significant weight loss in 22 obese patients without any adverse events. A mouse study demonstrated that Cucumis treatment was effective for rescuing high fat diet-induced changes in body weight, epididymal fat weight, glucose, insulin, cholesterol, and insulin resistance without liver or renal toxicity. Additionally, Cucumis showed an anti-inflammatory effect, as evidenced by reduced adipose tissue macrophages, decreased expression of tumor necrosis factor-α and monocyte chemoattractant protein-1 in fat, and higher gene expression of insulin signaling proteins in the liver. With these findings, we suggest that oral treatment with Cucumis is promising for treating obesity and insulin resistance through an anti-inflammatory mechanism.

Keywords: *Cucumis Melo* L., medicinal herb, obesity, insulin resistance, anti-inflammation

Introduction

Recently, the prevalence of obesity has dramatically increased in many countries including Korea [1, 2]. Many efforts and monetary investments have been devoted to treating obesity; however, obese patients are still suffering from various symptoms and complications. Obesity is an independent risk factor for metabolic disorders including type 2 diabetes (T2DM), hypertension and dyslipidemia [3].

In East Asia, herbal medicine has been widely used to treat various diseases including several endocrine disorders for at least 2,000 years. To treat obesity and/or diabetes-like symptoms, several kinds of herbs and herbal decoctions have been prescribed, and many recent studies have demonstrated their therapeutic effects [4].

Cucumis Melo L. (hereafter referred to simply as Cucumis) is a medicinal herb used broadly in East Asian countries including Korea and China [5]. Cucumis has been traditionally used to remove undigested food in the stomach or obstructed Qi in the upper part of the body [6, 7]. Recently, several novel effects of Cucumis have been discovered, such as therapeutic effects on headache, hepatitis B, jaundice, and tumor formation [8, 9]. A clinical study even reported that Cucumis reduces fasting glucose in patients with diabetes [10], suggesting that Cucumis might have a beneficial effect on treating obesity and insulin resistance. However, the therapeutic effect and the mechanism of Cucumis on obesity and insulin resistance has not been thoroughly investigated yet. In the current study, we showed that the oral administration of Cucumis has a therapeutic effect on obesity and diabetes through an anti-inflammatory mechanism.

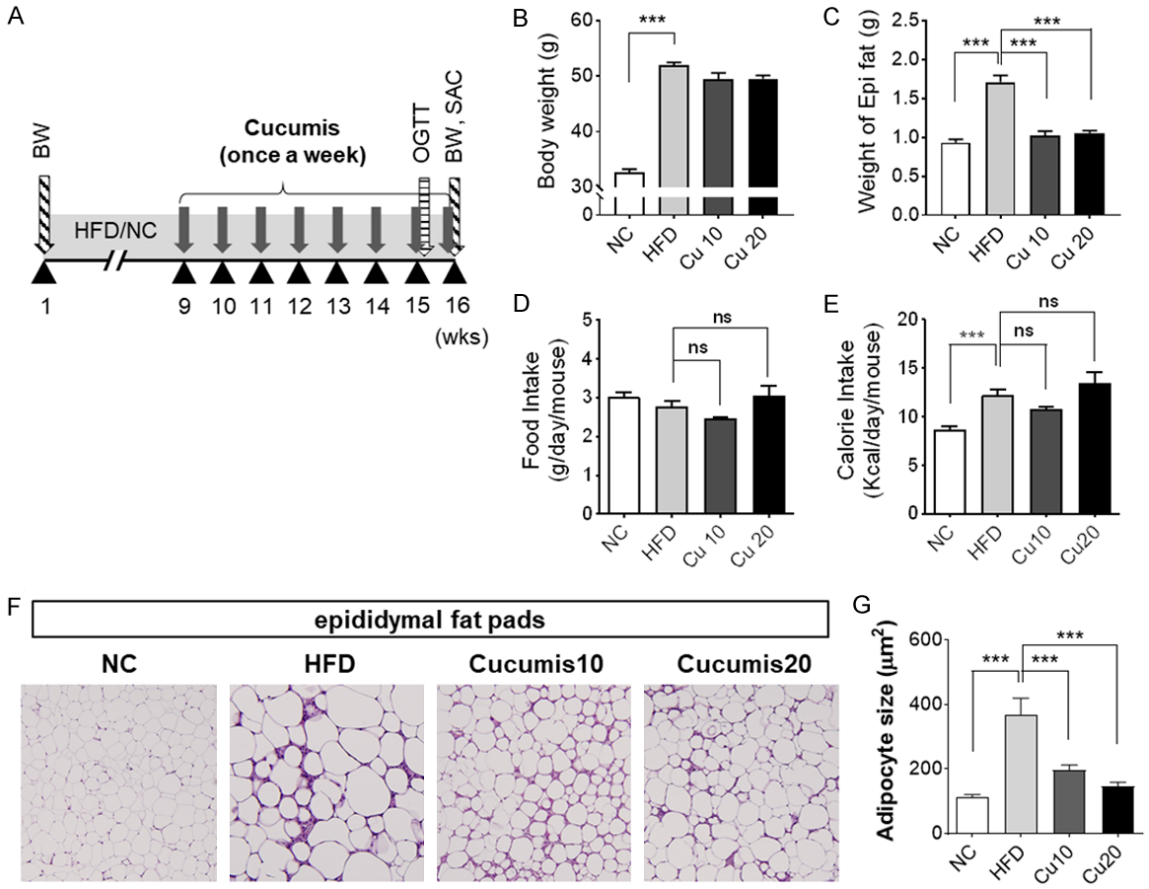

Figure 1. Effect of Cucumis treatment on body weight, fat weight, and adipoycte size. (A) Experimental schedule. (B, C) Cucumis treatment led to epidydimal (Epi) fat pads that were similar in size to the NC group (C), but only slightly attenuated increases in total body weight (B). Cucumis treatment did not alter food intake (D) or calorie intake (E). (F, G) Cucumis treatment led to significantly reduced the size of adipocytes. Values are presented as mean ± SEM (n = 5-6 for each group). ***p < 0.001.

Materials and methods

Preparation of the Cucumis Melo L.

Cucumis was purchased from the Department of Pharmaceutical Preparation of the Hospital of Korean Medicine, Kyung Hee University (Seoul, South Korea). Cucumis (100 g) was extracted with 1,500 mL of 80% ethanol by boiling for 2 hours. The solution was filtered and concentrated with a rotary evaporator (model NE-1, EYELA Co., Tokyo, Japan), and freeze-dried. The final collection rate was 19.28%.

Human study

A clinical study was designed to investigate the therapeutic effects of Cucumis on obesity. Participants were recruited between 2014 and 2015 from the Hospital of Korean Medicine, Kyung Hee University (Seoul, South Korea). Inclusion criteria were people who showed a body mass index (BMI) over 25 kg/m^2, aged 17-75 years, able to give signed informed consent, and considered to be in stable health in the opinion of the investigator. Exclusion criteria were pregnant or lactating women, people who underwent other obesity treatment in the last three months, those who had a clinically significant new illness in the month before screening, those not suitable to participate in the study in the opinion of the investigator due to various reasons including an existing physical or mental condition, those who had significant changes in smoking habits within the last three months, and those who had diseases including epilepsy, uncontrolled hypertension or hypotension, cerebrovascular diseases, and hepatic or renal impairment.

Eligible patients were orally administered a single dose of 1 g Cucumis powder three sequential times at 60 minute intervals for one day. No participants received standardized lifestyle intervention, except Cucumis administration. One month after Cucumis treatment, patients were analyzed for body weight (BW), height, waist-hip ratio (WH ratio), body fat mass and percentage (BFM and BFP), visceral fat area (VFA), and basal metabolic rate (BMR) using a body composition analyzer (Inbody Co., Seoul, Korea). BMI was calculated from the above data. Additionally, in order to assess the safety of Cucumis, aspartic aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltranspeptidase (GGT), alkaline phosphatase (ALP), total and direct bilirubin, blood urea nitrogen (BUN), creatinine, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) were assessed from a blood sample before and one week after Cucumis treatment. This study was approved by the International Review Board of Kyung Hee Hospital (KOMCIRB-2014-06).

Animals, diets, and Cucumis administration

Six-week-old male C57BL/6 mice (Central Lab Animals Inc., Seoul, Korea) were used. The animal room was maintained at 40-70% relative humidity, with 12-hour periods of light and dark. Water and food were supplied *ad libitum*. All experiments were carried out in accordance with guidelines from the Korean National Institute of Health Animal Facility. The Animal Care Committee at Kyung Hee University approved all protocols used in this study (KHMC-IACUC 14-025).

The mice were randomly assigned to one of four groups (5-6 mice per group): normal chow diet (NC), high fat diet (HFD), HFD with 10 mg/kg Cucumis, and HFD with 20 mg/kg Cucumis. The mice were fed HFD (60% fat) or NC (10% fat) for 8 weeks, and we first confirmed a significant difference ($p < 0.001$, data not shown) in body weight between NC and HFD groups. Then we orally administered Cucumis extraction or saline once a week for 8 weeks (**Figure 1A**). In order to determine the adequate dose of Cucumis, we performed a preliminary experiment with 35-40 g, 25-week-old male BALB/c mice. Six different doses of Cucumis were orally administered to five mice in each group: 5, 10, 20, 40, 100, and 150 mg/kg. After one week of a single administration, 10 and 20 mg/kg of Cucumis showed the maximal effect on loss of BW and weight loss of the liver, spleen, and kidney with the fewest side effects (data not shown). Therefore, we used 10 and 20 mg/kg of Cucumis to test its therapeutic effect in this study.

Measurement of weight and food consumption

BW was measured at the first day and the last day of the experiment. The weights of epididymal fat pads were measured at week 16 (**Figure 1A**). Food consumption was measured by weighing the food every morning.

Histology

Adipose tissue was fixed in 10% formalin and processed for paraffin embedding. Paraffin tissue sections were cut at 7 μm and processed for hematoxylin and eosin (H&E) staining. The mean adipocyte area was analyzed from photographs using NIH ImageJ software (https://imagej.nih.gov/ij/).

Oral glucose tolerance test (OGTT)

At week 15, an OGTT was performed after 14 hours of fasting in clean cages with free access to water from newly cleaned bottles. The fasting (baseline) blood glucose measurement was performed by applying a drop of tail blood to a strip-operated blood glucose sensor (ACCU-CHEK Performa, Castle hill, New South Wales, Australia). Glucose (2 g/kg) was dissolved in distilled water and *p.o.* administered. Blood samples were withdrawn from the tail vein at 0, 30, 60, and 120 minutes after glucose administration.

Insulin and serum lipid measurements

At week 15, blood collected from the tail vein of the mouse was withdrawn after 6 hours of fasting. Serum insulin level was determined using an ultrasensitive mouse insulin ELISA kit (Crystal Chem, Downers Grove, IL, USA) according to the manufacturer's instructions. Insulin resistance was calculated by the following equation; HOMA-IR = Fasting Blood Glucose (mg/dl) × Fasting Blood Insulin (mg/ml) × 0.0717225161669606. Serum total cholesterol and high-density lipoprotein (HDL) cholesterol were analyzed at week 16.

Table 1. The sequences of primers for qRT-PCR

	Sense	Anti-sense
TNF-α	5'-TTCTGTCTACTGAACT TCGGGGTGATCGGTCC-3'	5'-GTATGAGATAGCAAATC GGCTGACGGTGTGGG-3'
IFN-γ	5'-ACTGGCAAAAGGATGGTGAC-3'	5'-TGAGCTCATTGAATGCTTGG-3'
IL-6	5'-AACGATGATGCACTTGCAGA-3'	5'-GAGCATTGGAAATTGGGGTA-3'
MCP-1	5'-CCCACTCACCTGCTGCTACT-3'	5'-TCTGGACCCATTCCTTCTTG-3'
Insr	5'-GAGATGGTCCACCTGAAGGA-3'	5'-GGACAGACATCCCCACATTC-3'
Irs1	5'-AAGCACCTGGTGGCTCTCTA-3'	5'-TCAGGATAACCTGCCAGACC-3'
Irs2	5'-ATACCGCCTATGCCTGTCTG-3'	5'-TGGTCTCATGGATGTTCTGC-3'
GAPDH	5'-AGTCCATGCCATCACTGCCACC-3'	5'-CCAGTGAGCTTCCCGTTCAGC-3'

RNA preparation and quantitative real-time polymerase chain reaction (qRT-PCR)

To study the gene expression of tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), insulin receptor (*Insr*), insulin receptor substrate 1 (*Irs1*), and insulin receptor substrate 2 (*Irs2*) in the fat and liver, we extracted RNA from epididymal fat pads and liver using the Mini RNA Isolation II kit (Zymo Research, USA) and amplified the complementary DNA using an Advantage RT for PCR kit (Clontech, Mountain View, CA, USA) according to the manufacturer's instructions. qRT-PCR was performed by following the general protocol for the Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Primer sequences are listed in **Table 1**. Threshold cycle, obtained using SDS Software 2.4 (Applied Biosystems), was converted into relative quantitation based on GAPDH, and a calculated fold-change value was used for gene expression analysis. The fold-changed values of the experimental groups were normalized to the value of the NC group.

Stromal vascular cell (SVC) segregation

To obtain SVC from the epididymal fat pads, the pads were harvested at week 16 and mixed with a solution composed of phosphate buffered saline (PBS, Gibco, Thermo Fisher Scientific, Waltham, MA, USA) and 2% bovine serum albumin (BSA, Gibco). After pulverizing the fat pad into 1-2 mm pieces, collagenase (Sigma-Aldrich, St. Louis, MO, USA) and DNase I (Roche, Sigma-Aldrich) were added. The sample was kept at 37°C for 20-25 minutes while shaking. Subsequently, 2% BSA/PBS and 5 mM EDTA were mixed with the sample, and then uncrushed adipose tissue was filtered using a 100-μM filter (BD Biosciences, San Jose, CA, USA). It was centrifuged at 1,000 rpm for 3 minutes. After elimination of the supernatant containing adipocytes and residual solution, the remaining pellet was mixed with PBS and 2% fetal bovine serum (FBS, Sigma-Aldrich). A 100-μm cell strainer (BD Bioscience) was used to remove unnecessary tissue, and the sample was centrifuged at 200 *g* for 10 minutes. Finally, SVC was obtained from the bottom of the sample.

Fluorescence-activated cell sorting (FACS) analysis of adipose tissue macrophages (ATMs)

The SVC cell count from adipose tissue was performed by a cellometer (Nexcelom Bioscience LLC, Lawrence, MA, USA), and every sample was set at the concentration of 10^6 cells. FcBlock (BD Bioscience) was mixed with the sample at the ratio of 1:100, and the reaction was performed for 10 minutes. Fluorophore-conjugated antibodies were added at the shaded state and reacted for 20 minutes. Antibodies were used as follow: CD45-APC Cy7 CD68-APC, CD11c-phycoerythrin (CD11b-PE), and CD206-FITC (all from Biolegend, San Diego, CA, USA). The samples were washed with 2% FBS/PBS solution and centrifuged at 1,500 rpm. All samples were put into FACS tubes and analyzed with a FACS Calibur (BD Bioscience). FlowJo (Tree Star, Inc., Ashland, OR, USA) was used to analyze the percentages of macrophages with $CD45^+/CD68^+$, $CD45^+/CD68^+/CD206^+$, and $CD45^+/CD68^+/CD11c^+$.

Statistical analysis

All calculations were performed using GraphPad PRISM 5 (Graphpad Software Inc., San Diego, CA, USA). All values are presented as the mean ± SEM (standard error mean). For the human

Table 2. Therapeutic Effects of Cucumis in Obese Patients

	Before	After	*P* value
BW (kg)	79.83 ± 2.96	77.40 ± 2.85	< 0.001
BMI (kg/m^2)	29.92 ± 0.77	28.91 ± 0.71	< 0.001
BFP (%)	37.09 ± 1.45	36.36 ± 1.43	N.S.
BFM (kg)	29.61 ± 1.63	28.10 ± 1.51	< 0.01
WH ratio	0.93 ± 0.01	0.92 ± 0.01	< 0.01
VFA (cm^2)	112.6 ± 5.83	106.7 ± 5.36	N.S.
BMR (kcal/day)	1,458 ± 50.42	1,435 ± 47.84	< 0.01

Table 3. Safety of Cucumis in Obese Patients

	Before	After	*P* value
BUN (mg/dL)	14.82 ± 1.23	14.78 ± 1.37	N.S.
Creatinine (mg/dL)	0.67 ± 0.03	0.65 ± 0.07	N.S.
AST (IU/L)	25.00 ± 2.43	24.23 ± 2.27	N.S.
ALT (IU/L)	18.09 ± 1.97	18.50 ± 1.87	N.S.
GGT (IU/L)	0.93 ± 0.01	0.92 ± 0.01	N.S.
ALP (IU/L)	24.09 ± 3.78	24.24 ± 3.66	N.S.
Total bilirubin (mg/dL)	1.01 ± 0.22	0.76 ± 0.15	< 0.05
Direct bilirubin (mg/dL)	0.32 ± 0.10	0.27 ± 0.08	N.S.
CRP (mg/dL)	0.35 ± 0.05	0.37 ± 0.22	N.S.
ESR (mm/hr)	11.64 ± 2.47	14.85 ± 2.93	N.S.

study, the significance of the differences before and after the intervention was assessed with a paired t-test. For the animal study, the significance of differences between groups was determined using a one-way analysis of variance (ANOVA) followed by a Tukey's post-hoc multiple comparison test (all except BW of animals and IL-6 mRNA) or Dunnett's post-hoc multiple comparison test (for BW of animals and IL-6 mRNA). All *p*-values were two-tailed, and significance was set at $p < 0.05$. Significance compared to NC and HFD groups is displayed with an asterisks (*) and section signs (§), respectively; for instance, * or § for $p < 0.05$; ** or §§ for $p < 0.01$; and *** or §§§ for $p < 0.001$.

Results

Cucumis improves obese condition without side-effects in patients

To study the therapeutic effect of Cucumis on obese patients, we designed a clinical trial with 22 obese patients. The patients were administered 1 g of Cucumis powder three sequential times at a 60-minute interval. We found that BW and BMI, the representative indexes for obesity, were significantly reduced one month after Cucumis treatment. BFM was also significantly decreased, but the decrease in BFP was not significant. Intriguingly, the reduced weight (2.43 ± 0.49 kg) was mainly derived from a decrease in fat mass (1.51 ± 0.48 kg). WH ratio was also slightly yet significantly decreased. Cucumis treatment was effective at reducing VFA as well. In addition, unexpectedly, BMR was slightly decreased with Cucumis treatment (**Table 2**). These findings suggest that oral treatment of Cucumis improves the general condition of obese patients.

Next, to see if Cucumis administration induced any toxic effects, we assessed BUN and creatinine levels for kidney function; AST, ALT, GGT, ALP, and total and direct bilirubin for liver function; and CRP and ESR for inflammation. We found that oral administration of Cucumis reduced all of the above items, and total bilirubin was significantly reduced (**Table 3**). These findings suggest that Cucumis did not induce any toxic effects to the kidney or liver or undesirable inflammation.

Cucumis reduces body weight, fat weight, and size of adipocytes

We first tested the effects of Cucumis on BW and the weight of epididymal fat pads (EFPs), one of the most massive visceral fat tissues in the body. As expected, at the end of the study, the HFD-induced obesity model exhibited significantly greater BW and weight of the EFPs compared to the NC group (**Figure 1B**, **1C**). Surprisingly, although Cucumis treatment led to only a slight lower average BW compared to that in the HFD group (10 mg/kg, $p = 0.07$; 20 mg/kg, $p = 0.08$), Cucumis led to significantly lower EFP weight compared to that in the HFD group, and these weights were similar to those in the NC group (**Figure 1B**, **1C**). In addition, we found that Cucumis treatment did not alter either the amount of food intake or calorie intake (**Figure 1D**, **1E**). These findings suggest that Cucumis treatment minimally affects food consumption and BW in HFD-induced obese mice; however, it markedly attenuates accumulation of body fat mass. Histopathological

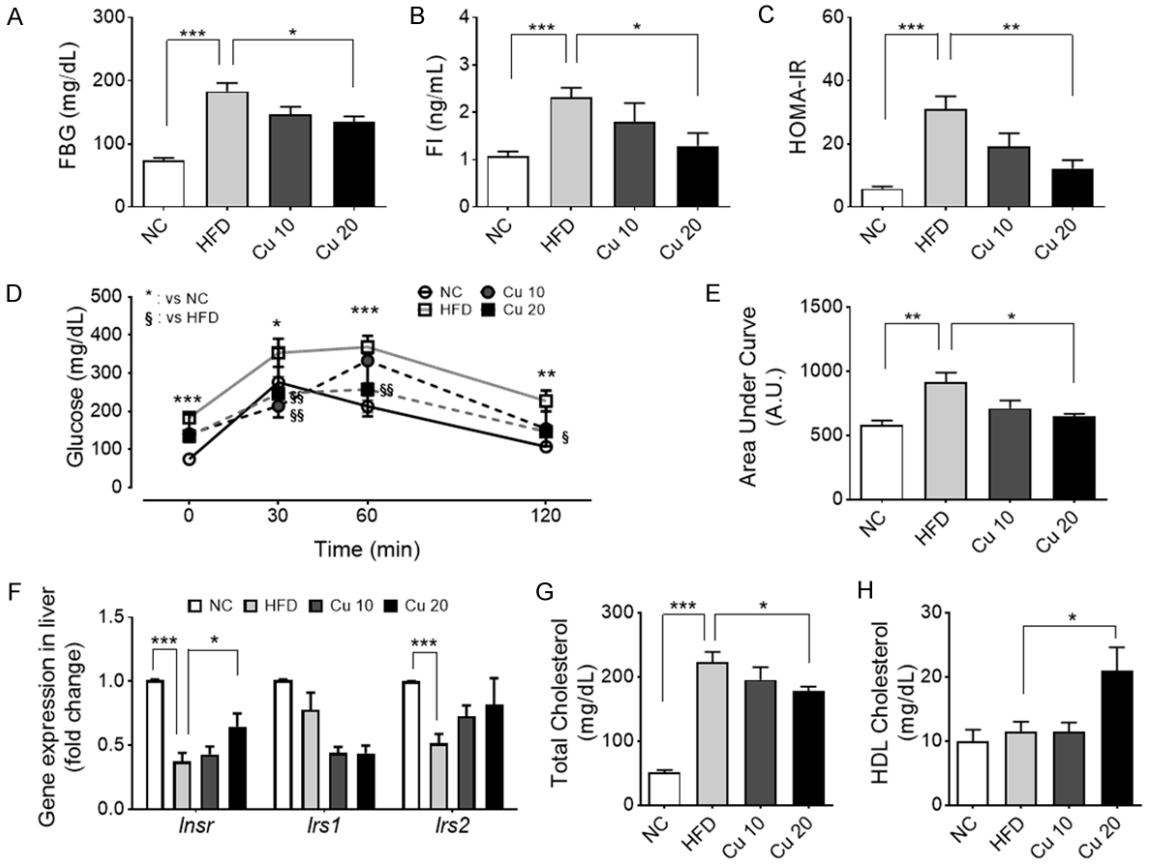

Figure 2. Cucumis treatment attenuated HFD-induced dysregulation of glucose metabolism, insulin resistance, and lipid profiles. Cucumis treatment reduced FBG (A), FI (B), and HOMA-IR (C). In an OGTT, Cucumis treatment led to attenuated glucose levels after 30 minutes of glucose administration (D) and smaller areas under curve (E) compared to those in the HFD group. Cucumis attenuated decreases in *insr* mRNA level (F) and led to more favorable lipid profiles in regard to TC (G) and HDL cholesterol (H). Values are presented as mean ± SEM (n = 5-6 for each group). * or §, $p < 0.05$; ** or §§, $p < 0.01$; ***$p < 0.001$.

observation found that HFD exposure led to larger adipocytes in the EFPs compared to the NC group (**Figure 1F**). Moreover, adipocyte area was significantly smaller in the Cucumis-treated mice compared to those in the HFD group, as measured using ImageJ (**Figure 1G**).

Cucumis improves glucose metabolism

We next investigated the effect of Cucumis on glucose metabolism through the insulin pathway via assessment of fasting blood glucose (FBG), fasting insulin (FI), and HOMA-IR representing insulin resistance. As expected, the HFD-induced obesity model showed significantly higher FBG, FI, and HOMA-IR. Cucumis treatment (20 mg/kg) significantly rescued the HFD-induced increases in FBG, FI, and HOMA-IR, and the effect was dose-dependent (**Figure 2A-C**). To directly see the effect of Cucumis on glucose metabolism, we additionally performed an OGTT. We found that the glucose levels of the HFD group at 0, 30, 60, and 120 min were significantly greater than those of the NC group at the corresponding time points, and that Cucumis treatment (20 mg/kg) led to significantly lower blood glucose at 30, 60, and 120 min (**Figure 2D**). The area under the curve (AUC) of the HFD group was also significantly greater than that of the NC group, and Cucumis treatment (20 mg/kg) significantly recovered an increase in the AUC (**Figure 2E**). Altogether, these data show that Cucumis treatment remarkably recovered the increases in blood glucose, insulin, and insulin resistance and improved glucose metabolism.

Cucumis increases insulin receptor gene expression

Next, to see if the alteration of the insulin receptor genes contributed to the improvement of

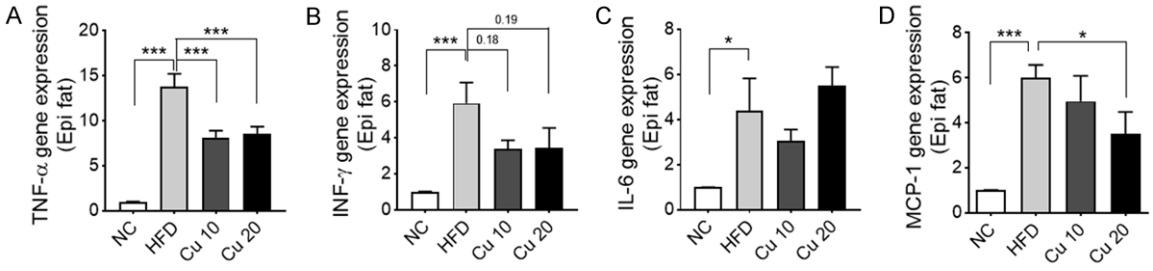

Figure 3. Cucumis treatment enhanced an anti-inflammatory mechanism. Cucumis reduced prevented increases in TNF-α (A) and MCP-1 (D), but not INF-γ (B) or IL-6 (C). Values are presented as mean ± SEM (n = 5-6 for each group). *p < 0.05 and ***p < 0.001.

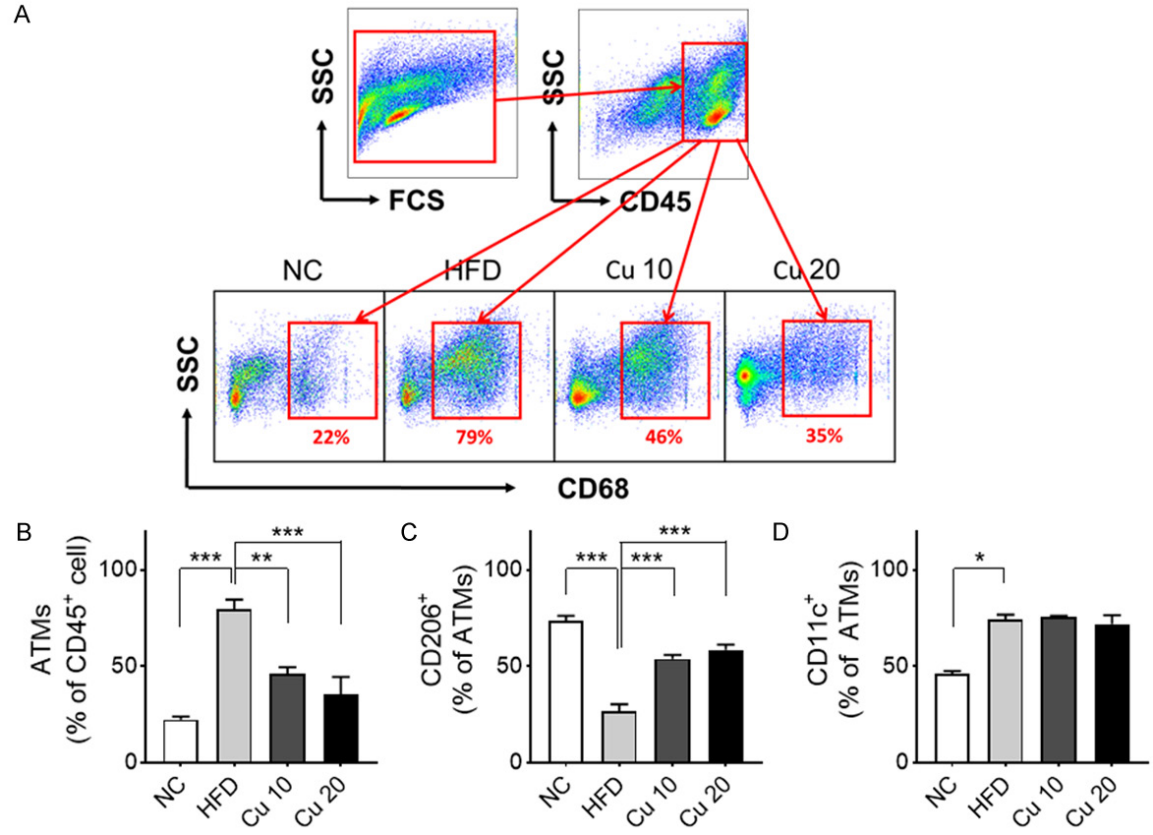

Figure 4. Cucumis-induced anti-inflammatory mechanism in the adipose tissue macrophages. Cucumis attenuated HFD-induced increases in ATMs (A, B). Among ATMs, Cucumis rescued the changes in the proportion of $CD206^+$ ATMs, but not $CD11c^+$ ATMs (C, D). Values are presented as mean ± SEM (n = 5-6 for each group). *p < 0.05, **p < 0.01, and ***p < 0.001.

glucose metabolism by regulating insulin level, we investigated the expression of insulin receptor genes (Insr, Irs1, and Irs2). The HFD group showed significantly lower levels of Insr and Irs2 mRNA, but not Irs1 mRNA (**Figure 2F**), which suggests that decreased expression of insulin receptors might induce an increase of insulin resistance. Cucumis treatment increased mRNA levels of Insr and Irs2 by 1.7- and 1.6-fold, respectively, although there was no statistical significance in *Irs2* (**Figure 2F**).

Cucumis improves lipid profile

Next, we tested whether Cucumis administration also affected serum lipid levels by assess-

ing TC and HDL cholesterol. The HFD-induced obesity model showed a significant increase in TC, but not in HDL. Interestingly, Cucumis treatment (20 mg/kg) significantly reduced TC while significantly increasing HDL compared to the HFD group (**Figure 2G**, **2H**).

Cucumis exert an anti-inflammatory effect

Recent studies have demonstrated that inflammation is the underlying mechanism of obesity and insulin resistance [11-14]. To see whether Cucumis had an anti-inflammatory effect, we assessed mRNA levels of the inflammation-related genes TNF-α, INF-γ, and IL-6. We found that mRNA levels of TNF-α, INF-γ, and IL-6 were significantly higher in the HFD group compared to the NC group. Cucumis treatment, at both 10 and 20 mg/kg, led to significantly lower levels of TNF-α similar to those in the NC group. A similar, marked trend was observed for INF-γ expression, though differences were not statistically significant. Unlike TNF-α and INF-γ, Cucumis treatment did not alter the mRNA level of IL-6 (**Figure 3A-C**).

In addition to the general inflammatory cytokines, we investigated the expression level of MCP-1 mRNA, one of the key chemokines that recruits monocytes and macrophages [15, 16]. The HFD group showed a significantly higher MCP-1 level compared to the NC group, along with higher levels of other inflammatory cytokines. We found that Cucumis treatment (20 mg/kg) led to significantly lower MCP-1 gene level than seen in the HFD group-similar to that of the NC group (**Figure 3D**). Taken together, these results illustrate that Cucumis treatment showed a potent anti-inflammatory effect that might be strongly associated with its anti-obesity and anti-diabetic effect.

Cucumis-induced anti-inflammatory effect in visceral adipose tissue

ATMs are known as prominent sources of pro-inflammatory cytokines, which block insulin action in adipocytes and lead to systemic insulin resistance [15, 16]. To study the profound mechanism of anti-inflammatory action of Cucumis, we investigated the ATM infiltration rate by performing FACS analysis. We first found that the ATM portion of the $CD45^+$ leukocytes was significantly higher in the HFD group compared to the NC group, and Cucumis treatment led to levels more similar to that of the NC group (**Figure 4A**, **4B**). We also found that the HFD was associated with a significantly lower percentage of anti-inflammatory $CD206^+$ ATMs, also known as M2 ATMs, and a significantly higher percentage of pro-inflammatory $CD11c^+$ ATMs, also known as M1 ATMs (**Figure 4C**, **4D**). Intriguingly, Cucumis treatment significantly rescued the proportion of $CD206^+$ M2 ATMs, but did not have this effect on $CD11c^+$ M1 ATMs (**Figure 4**). Together, these findings suggest that the improvement of hyperglycemia and insulin resistance in the Cucumis group could be related to anti-inflammatory effects of Cucumis on visceral adipose tissue.

Discussion

Obesity is a medical condition in which excess body fat has accumulated to the extent that it might have a negative effect on health. The main burden of obesity lies in its inter-connection with a number of diseases including Type 2 diabetes, dyslipidemia, arterial hypertension, and atherosclerosis, leading to substantially increased cardiovascular and cerebrovascular morbidity and mortality [17]. Due to the escalating attention to obesity and insulin resistance, there has been a pressing need for a new therapy. Here, we report for the first time that Cucumis is effective for managing obesity and insulin resistance through an anti-inflammatory action.

In this study, we demonstrated that Cucumis treatment is effective for reducing BW and fat tissue in 22 human obese patients without any adverse events or harmful effects on liver and kidney function. A limitation of this clinical study is the absence of placebo group; therefore, there could have been a placebo effect. However, our reverse-translational study utilizing HFD-induced obesity model mice confirmed the solid therapeutic effect of Cucumis.

Recent studies have investigated the underlying mechanism of obesity and insulin resistance and demonstrated a positive correlation between inflammation and these diseases [11-14]. In the obese condition, ATMs accumulate in adipose tissue along with BW gain and exacerbate inflammation by releasing pro-inflammatory cytokines including TNF-α and IL-6. These cytokines can block insulin action in adipocytes via autocrine/paracrine signaling and cause

systemic insulin resistance via endocrine signaling [18]. The therapeutic effect of Cucumis treatment on obesity and insulin resistance seems to be mediated by an anti-inflammatory mechanism, evidenced by reduced ATM with an increase in anti-inflammatory M2 macrophages and decreases of TNF-α, INF-γ, and MCP-1 gene expression in liver tissue. In addition to the anti-inflammatory effect, Cucumis treatment alleviated the HFD-induced insulin resistance. The Cucumis-induced recovery of Insr gene expression in the HFD model might have been due to the decreased level of TNF-α. TNF-α can inhibit insulin signaling through suppression of the phosphorylation of insulin receptors and increasing activity of its tyrosine kinase, leading to an increase in insulin resistance [19]. Therefore, the anti-inflammatory action of Cucumis can promote the insulin signaling pathway by hindering phosphorylation of its serine residues.

In the study, we found that the anti-inflammatory effects of Cucumis were not limited to fat tissue, but systemically manifested, e.g., the improvement of insulin signaling in the liver. These systemic changes finally resulted in the prevention of aberrant insulin resistance confirmed by OGTT and HOMA-IR; FBG, FI, BW, and epididymal fat mass that more resembled those of mice fed NC; and a healthier lipid profile including lower TC and higher HDL-cholesterol compared to mice consuming a HFD without Cucumis. In conclusion, Cucumis treatment showed a beneficial effect on relieving and preventing the obese condition and insulin resistance via anti-inflammatory actions without toxicity in humans and mice. We propose the oral treatment of Cucumis as an adjunctive therapy for uncontrollable obesity and diabetes.

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Disclosure of conflict of interest

None.

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