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Capsaicin Modulates Adipocyte Cell Differentiation and Inflammatory Gene Expression

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ABSTRACT

Objective: Adipose tissue stores lipids necessary for the maintenance of nutritional homeostasis. It is also an endocrine organ that reacts to changes in inflammation and energy status. Capsaicin, the principal bioactive compound in red pepper, has garnered significant attention for its reported anti-obesity, anti-diabetic, anti-oxidant, and anti-inflammatory properties. In this study, we aimed to elucidate the influence and most efficacious dose of capsaicin on the expression of lipid metabolism-related inflammatory proteins and the inhibition of adipocyte cell differentiation.

Materials and Methods: Cell viability analysis was performed using CCK-8, cell differentiation was assessed using Oil Red O, and gene expression levels of peroxisome proliferator-activated receptor gamma (PPARγ), CCAAT/enhancer binding protein alpha (C/EBPα), adiponectin, leptin, cyclooxygenase-2 (COX-2), interleukin-6 (IL-6), nuclear factor kappa B1 (NF-κB1), tumor necrosis factor-alpha (TNF-α), sirtuin-1 (SIRT-1), transient receptor potential vanilloid receptor 1 (TRPV1), and uncoupling protein 2 (UCP2) were evaluated using quantitative real time polymerase chain reaction (qRT-PCR). Statistical analyses were conducted using GraphPad Prism 5. One-way ANOVA was performed to compare quantitative data between the groups.

Results: Capsaicin suppressed preadipocyte-to-adipocyte differentiation and mitigated the release of pro-inflammatory cytokines, particularly at low concentrations. Capsaicin effectively suppressed adiponectin levels at all concentrations but decreased leptin levels at lower concentrations (0.5 μM and 1 μM). Capsaicin stimulated the expressions of SIRT1 and TRPV-1 in adipocytes. According to our findings, the most effective capsaicin dose for the regulation of SIRT1 and TRPV-1 expressions appears to be 20 μM.

Conclusion: Capsaicin's effect on proteins regulating adipogenesis is not dose-related, but its inhibitory effect on adiposity-dependent inflammation was more pronounced at low concentrations.

Keywords: Adipogenesis, capsaicin, cytokines, differentiation and inflammation

INTRODUCTION

One of the common health problems worldwide is the increasing prevalence of obesity and obesity-related chronic conditions. The excessive storage of fat in adipose tissue and increase in body weight are the main causes of obesity. Adipose tissue plays a crucial role in controlling body weight, as it stores the lipids necessary for maintenance of nutritional homeostasis. Additionally, it is an endocrine organ that reacts to changes in inflammation and energy status (1).

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Red pepper (Chili pepper, Capsicum annuum L.) is a flavoring spice used in food. It has been demonstrated that some of the active components of red pepper have many physiological roles (2). Capsaicin, the primary and extensively studied bioactive component of red pepper, has been documented to possess anti-obesity, anti-diabetic, anti-oxidant, and anti-inflammatory properties (2, 3). Many epidemiological and animal studies have shown that the anti-obesity effect of capsaicin is related to its potential to stimulate the transient receptor potential vanilloid receptor 1 (TRPV1) receptor. The mechanism by which capsaicin affects TRPV1 is not fully understood, but recent studies suggest that TRPV1 plays a significant role in the regulation of body weight, glucose, and lipid metabolism (4, 5). Zheng et al. have suggested that one of the potential mechanisms exposing the anti-obesity effects of capsaicin is to increase lipid oxidation and to inhibit adipogenesis (6). Studies have reported that capsaicin induces the transformation of white adipose tissue (WAT) into brown adipose tissue (BAT) through sirtuin-1 (SIRT-1) dependent deacylation (7). A recent study showed that capsaicin also has a receptor-independent effect, which can trigger nuclear factor κB (NF-κB) inactivation and prevent inflammation associated with adipose tissue (8).

Adipogenesis is an essential metabolic pathway for the storage of lipids in adipose tissue. Previous studies have suggested that the reduction of preadipocyte differentiation, proliferation, and lipogenesis could prevent obesity (9). Hsu et al. reported that capsaicin suppresses the expression of adipogenesis-related transcription factors such as peroxisome proliferator-activated receptor gamma (PPAR-γ) and CCAAT/enhancer binding protein alpha (C/EBP-α). It was also shown that capsaicin controls the protein expression of leptin and adiponectin, which are two hormones primarily produced by adipose tissue (6, 9). Uncoupling protein 2 (UCP2), a protein situated in the inner mitochondrial membrane responsible for regulating lipid metabolism, was found to be diminished both in the omental and subcutaneous adipose tissues among obese individuals (10-12).

Adipose tissue and lipid storage represent an insidious source of chronic low-grade inflammation (13, 14). Adipose tissue-related inflammation facilitates adipogenesis (15). Capsaicin is

Table 1: List of primers used for qRT–PCR

Gene Name	Gene bank #	Primer Sequences	bp
PPARG	NM_138712.5	F: 5′-AGGATGCAAGGGTTTCTTCCG-3′ R: 5′-CCGCCAACAGCTTCTCCTTC-3′	200
CEBPA	NM_001285829.1	F: 5′-CACCGCTCCAATGCCTACTG-3′ R: 5′-CTAAGGACAGGCGTGGAGGA-3′	200
NFKB1	NM_003998.4	F: 5′-ACTGCTGGACCCAAGGACAT-3′ R: 5′-CGCCTCTGTCATTCGTGCTT-3′	105
TNFA	NM_000594.4	F: 5′- AGAACTCACTGGGGCCTACA-3′ R: 5′- GCTCCGTGTCTCAAGGAAGT-3′	177
UCP2	NM_001381944.1	F: 5′-CTTCTGCACCACTGTCATCG-3′ R: 5′-GTGACGAACATCACCACGTT-3′	195
TRPV1	NM_080704.4	F: 5′-ACCCTGTTTGTGGACAGCTA-3′ R: 5′-CAAGGCCAGGGAGAATACCA-3′	129
SIRT1	NM_012238.5	F: 5′-TATGCTCGCCTTGCTGTAGA-3′ R: 5′-TGGCTGGAATTGTCCAGGAT-3′	132
COX2	NM_000963.4	F: 5′-GCTTCCATTGACCAGAGCAG-3′ R: 5′-CTCCACAGCATCGATGTCAC-3′	159
LEP	NM_000230.3	F: 5′-TGGAGAAGCTGATGCTTTGC-3′ R: 5′-GGACCATTCAGAGGGTCACA-3′	196
ADIPOQ	NM_001177800.2	F: 5′-GGATGTGAAGGTCAGCCTCT-3′ R: 5′-TACACCTGGAGCCAGACTTG-3′	141
GAPDH	NM_002046.7	F: 5′-ACCCAGAAGACTGTGGATGG-3′ R: 5′-TCAGCTCAGGGATGACCTTG-3′	124

PPARG: Peroxisome proliferator-activated receptor gamma, CEBPA: CCAAT/enhancer-binding protein, NFKB1: Nuclear factor-kappa B1, TNFA: Tumor necrosis factor alpha, UCP2: Uncoupling protein 2, TRPV1: Transient receptor potential vanilloid receptor-1, SIRT1: Sirtuin 1, COX2: Cyclooxygenase-2, LEP: Leptin, ADIPOQ: Adiponectin, GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

an effective anti-inflammatory agent that helps to suppress the inflammatory process by reducing the expression of some pro-inflammatory proteins, such as tumor necrosis factor-alpha (TNF-α) and cytokines (16). However, the capacity of capsaicin to inhibit adipocyte differentiation and cellular inflammatory status is not yet well understood.

The primary aim of this study was to explore the influence of capsaicin on the expression of inflammatory markers associated with lipid metabolism, and to identify the most effective dosage.

MATERIALS AND METHODS

Cell Culture

Human preadipocyte cells (HPAd) from heart tissue were acquired from Cell Applications (San Diego, USA) and cultured in preadipocyte medium (Cell Applications, San Diego, USA) in 25 cm^2 tissue culture flasks from Corning (Charlotte, USA). The flask was then incubated for 24 h and placed in an incubator containing 5% CO_2 at 37°C (Sanyo, Osaka, Japan). In the following day, microscopy examination revealed that the cells had adhered to the flask and continued to proliferate in the subsequent days. When the cells reached 70-80% confluence, they were passaged according to the manufacturer's protocol (Cell Applications, San Diego, USA). The remaining procedures followed the methodology outlined in the experiment by Cetinalp et al. (17). On the 15th day, microscopy examination (Leica, Wetzlar, Germany) revealed that the cells contained granulated oil droplets and had successfully differentiated into adipocyte cells. 0.5μM, 1μM, 10μM, 20μM, and 50μM doses of capsaicin as ≥95% purity (Sigma, Taufkirchen, Germany) was applied.

Oil Red O Staining of Differentiated Adipocyte Cells to Quantify Lipid Accumulation

The cells were differentiated using Oil Red O (Sigma, Taufkirchen Germany) staining method. The remaining procedures followed the methodology outlined in the experiment by Cetinalp et al. (17).

Cell Viability Analysis Using CCK-8

Cell viability analysis was performed using a CCK-8 Kit (Abbkine, Georgia, USA). All groups were seeded with 15x10^3 cells per well in 6-well plates, transferred to a 96-well plate, and incubated at 37°C with 5% CO_2 for 1 day to allow the cells to attach to the plate. The following day, 10 μL of CCK-8 reagent was pipetted into all wells, and the plate was incubated at 37°C in an incubator containing 5% CO_2 for 3 h. After the incubation period, the absorbance of the wells was measured at 450 nm using a microplate reader (BioTek Instruments, Vermont, USA). Experiments were repeated three times for each group, and measurements were performed in triplicates.

Quantifying mRNA Expression in Cells Using qRT–PCR

For mRNA expression quantification, total RNA was extracted from the cells using RNAzol RT solution (MRC, Cincinnati, USA) according to the manufacturer's protocol. The concentrations and purities of the RNA samples were evaluated using a NanoDrop 2000 (Thermo Scientific, Massachusetts, USA). Prior to reverse transcription, RNA concentrations were standardized. Reverse transcription was performed using a Script cDNA Synthesis Kit (Jena Bioscience, Jena, Germany) following the kit protocol. The resulting cDNA was then amplified by quantitative

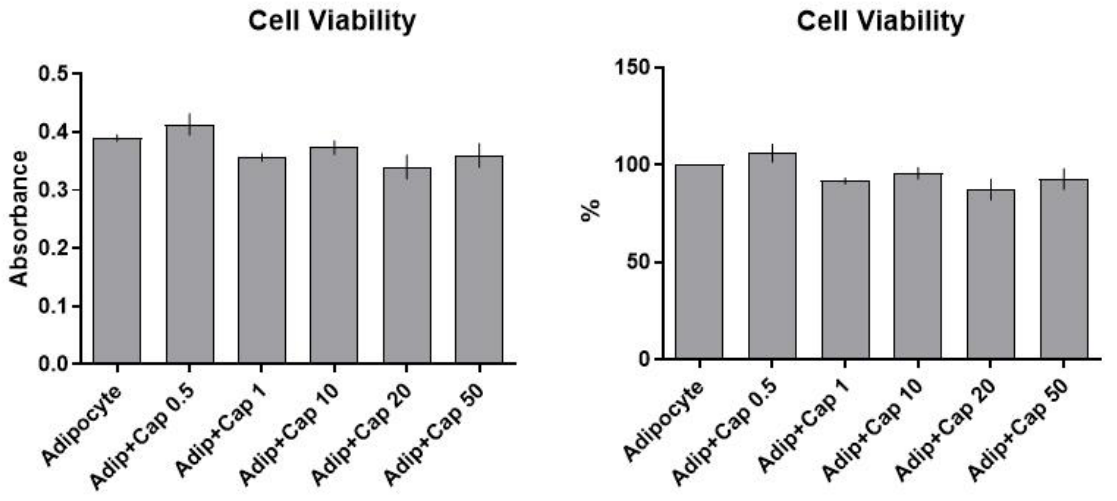

Figure 1. Cell viability assay following stimulation with different doses of capsaicin. Cap: Capsaicin ; Adip: Adipocyte.

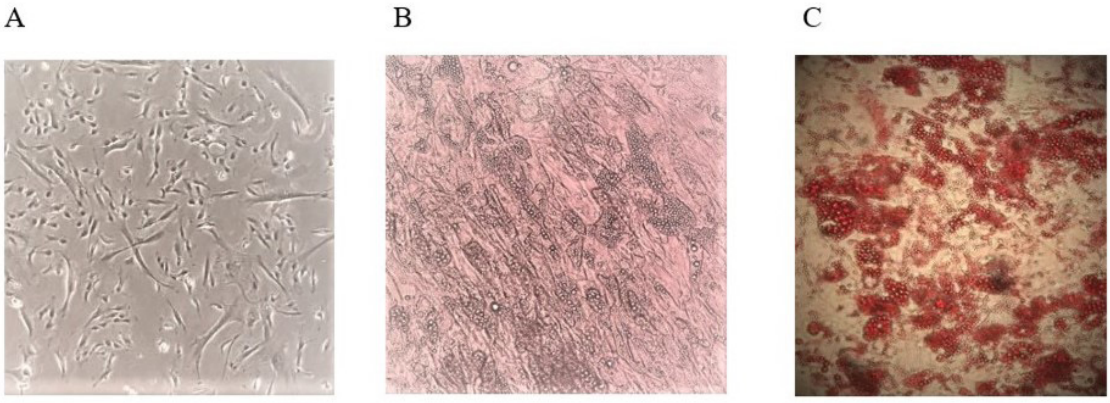

Figure 2. Differentiation of preadipocyte cells into mature adipocytes starting from day 0 and on day 15 and appearance of lipid droplets stained with Oil Red O dye under light microscopy. A. Preadipocyte, B. Mature adipocyte cells and C. Oil Red O staining of mature adipocyte cells.

reverse transcription polymerase chain reaction (qRT–PCR) using the qPCR GreenMaster with the UNG Kit (Jena Bioscience, Jena, Germany). The remaining procedures followed the methodology outlined in the experiment by Cetinalp et al. (17). The relative mRNA transcript levels were calculated using the $2^{-\Delta\Delta Ct}$ method and the relative expression of each gene was normalized to that of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping gene. Primers were sourced from LGC Standards (Middlesex, UK), and all qRT–PCR analyses were performed in triplicates. The primers used are detailed in Table 1.

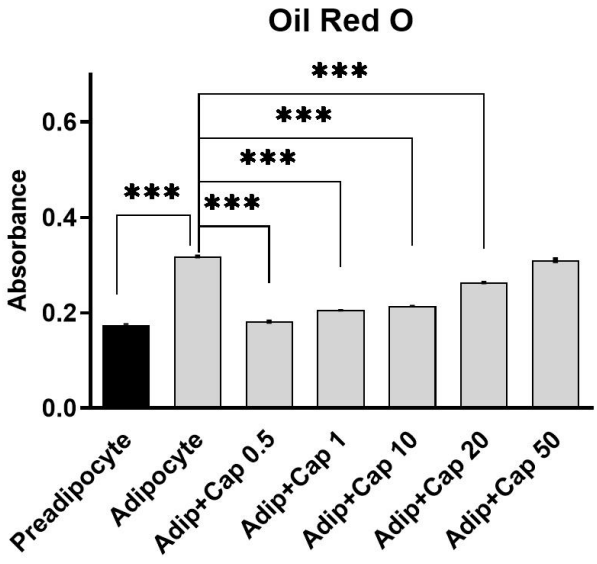

Figure 3. Comparison of the differentiation absorbance values of preadipocyte, adipocytes, and different doses of capsaicin-added adipocytes stained with Oil Red O dye. ***p<0.001; Cap: Capsaicin; Adip: Adipocyte.

Statistical Analyses

The results were presented as means ± standard deviation (SD). Statistical analyses were conducted using GraphPad Prism 5 software (San Diego, USA). One-way ANOVA was performed to compare quantitative data between the groups. If the ANOVA results were significant, the Kruskal-Wallis Test was used to compare means between groups. The statistical significance level for all analyses was set at a significance level of $p<0.05$.

RESULTS

In this study, we investigated the molecular mechanisms responsible for the inhibitory effects of various capsaicin doses on differentiation. We used the CCK-8 assay to determine the cytotoxic effect of capsaicin at different doses and found no significant difference in cell viability ($p=0.0620$, Figure 1).

During this period, the morphology of the cells was examined under a microscope, and differentiation was determined on day 15. At the end of 15th day, Oil Red O staining method was applied for lipid accumulation in preadipocytes, adipocytes, and capsaicin-treated adipocytes (Figure 2). During differentiation, there was a notable increase in lipid accumulation in untreated adipocytes ($p<0.001$). Nevertheless, all capsaicin doses except for 50 µM inhibited lipid accumulation, presenting statistically significant differences compared with untreated adipocytes ($p<0.001$, Figure 3).

The effect of capsaicin on the expression of C/EBP-α and PPAR-γ genes

C/EBP-α levels experienced a notable increase in adipocytes during the differentiation process ($p<0.001$). However, treatment of cells with different doses of capsaicin (ranging from 0.5 to 50 µM) during differentiation resulted in reduced C/EBP-α levels in comparison with adipocytes ($p<0.001$, Figure 4).

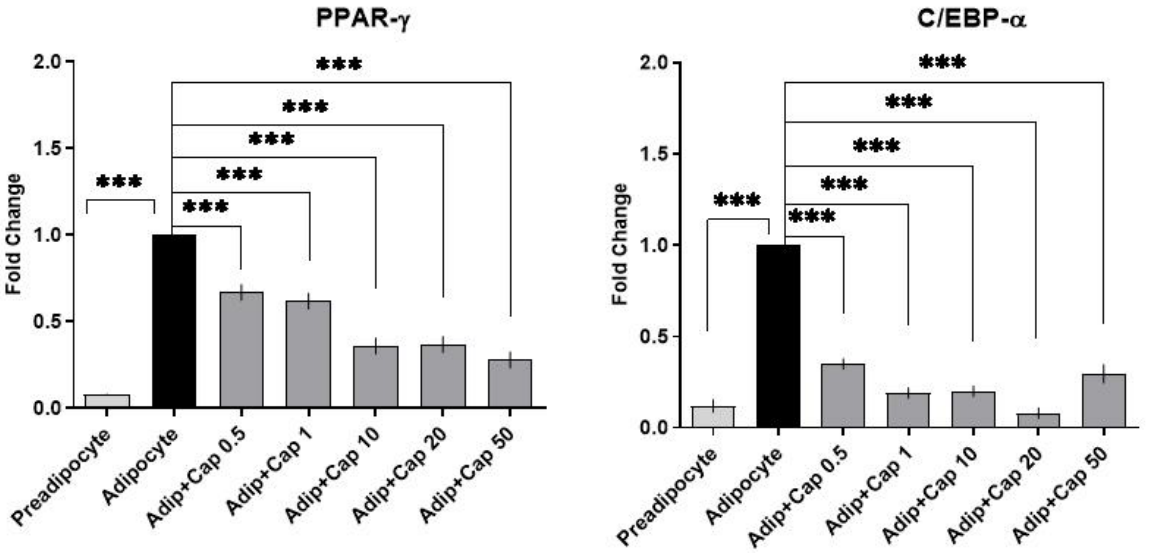

Figure 4. Effects of capsaicin on the expression of PPAR-γ (A) and C/EBP-α (B) during differentiation of preadipocyte to adipocytes ***p<0.001; Cap: Capsaicin; Adip: Adipocyte.

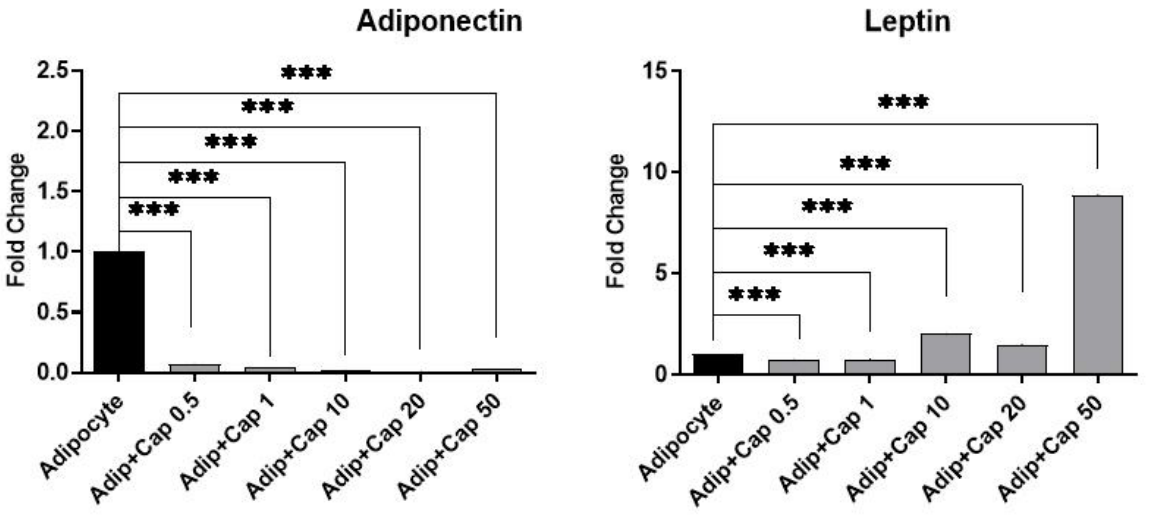

Figure 5. Effects of different doses of Capsaicin on adipokine gene expression. ***p<0.001, Cap: Capsaicin; Adip: Adipocyte.

Similarly, there was a significant increase in PPAR-γ expression in adipocytes during differentiation compared to preadipocyte. However, treatment with different concentrations of capsaicin (0.5-50 μM) during differentiation resulted in decreased PPAR-γ levels compared with adipocytes (p<0.001, Figure 4).

Impact of Capsaicin on Adipokine Gene Expression

Adiponectin levels exhibited a decrease in all capsaicin groups compared with adipocytes (p<0.001). However, no dose-dependent pattern was observed for the reduction of adiponectin levels (Figure 5).

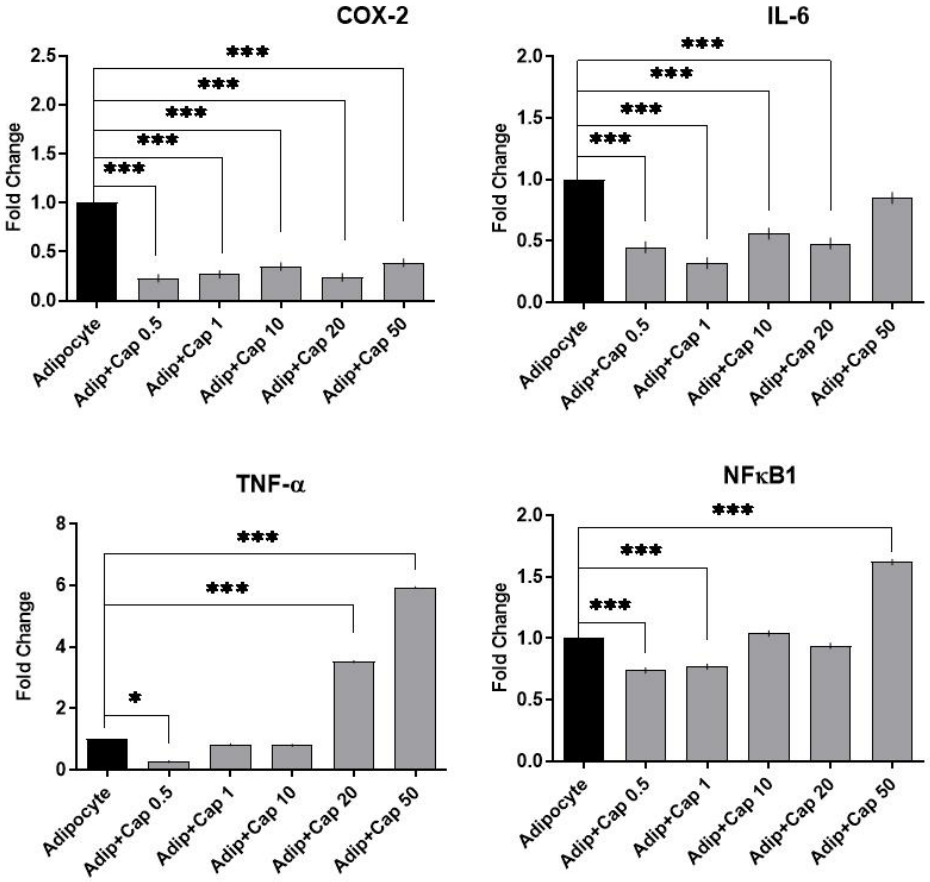

Figure 6. Effects of different doses of Capsaicin treatment to proinflammatory cytokines levels.
*p<0.05; ***p<0.001; Cap: Capsaisin; Adip: Adipocyte.

Leptin levels were significantly decreased in lower doses of capsaicin (0.5μM and 1μM) capsaicin-treated adipocytes and significantly increased in higher doses of capsaicin (10μM, 20μM, and 50μM) capsaicin-treated adipocytes in comparison with adipocytes (p<0.001, Figure 5).

Impact of Capsaicin on Proinflammatory Cytokines

In comparison with control adipocytes, COX-2 levels were significantly reduced in all adipocytes treated with capsaicin (p<0.001). Additionally, IL-6 gene expression levels were significantly decreased in all capsaicin-treated adipocytes except for the 50μM group when compared to untreated adipocytes (p<0.001). TNF-α levels were significantly increased in the capsaicin 20 and capsaicin 50 groups compared with untreated adipocytes (p<0.001) and significantly decreased in the capsaicin 0.5 group (p<0.05, Figure 6).

NF-kB1 levels were significantly decreased in lower doses of capsaicin (0.5μM and 1μM) capsaicin-treated adipocytes and significantly increased in the highest dose of capsaicin (50μM) capsaicin-treated adipocytes, in comparison with untreated adipocytes (p<0.001, Figure 6).

Impact of Capsaicin on TRPV-1, UCP-2, and SIRT-1 Expression

Except for capsaicin 20, significant decreases in TRPV-1 levels were observed in all capsaicin groups compared with untreated adipocytes (p<0.001). Conversely, in the capsaicin 20 group, TRPV-1 levels significantly increased (p<0.001, Figure 7).

Additionally, UCP-2 levels exhibited significant decreases in all capsaicin groups (p<0.001). Similarly, SIRT-1 levels were decreased significantly in all Capsaicin groups, except for capsaicin 20, when compared with untreated adipocytes (p<0.001). Notably, SIRT-1 levels were significantly increased in the capsaicin 20 group (p<0.001, Figure 7).

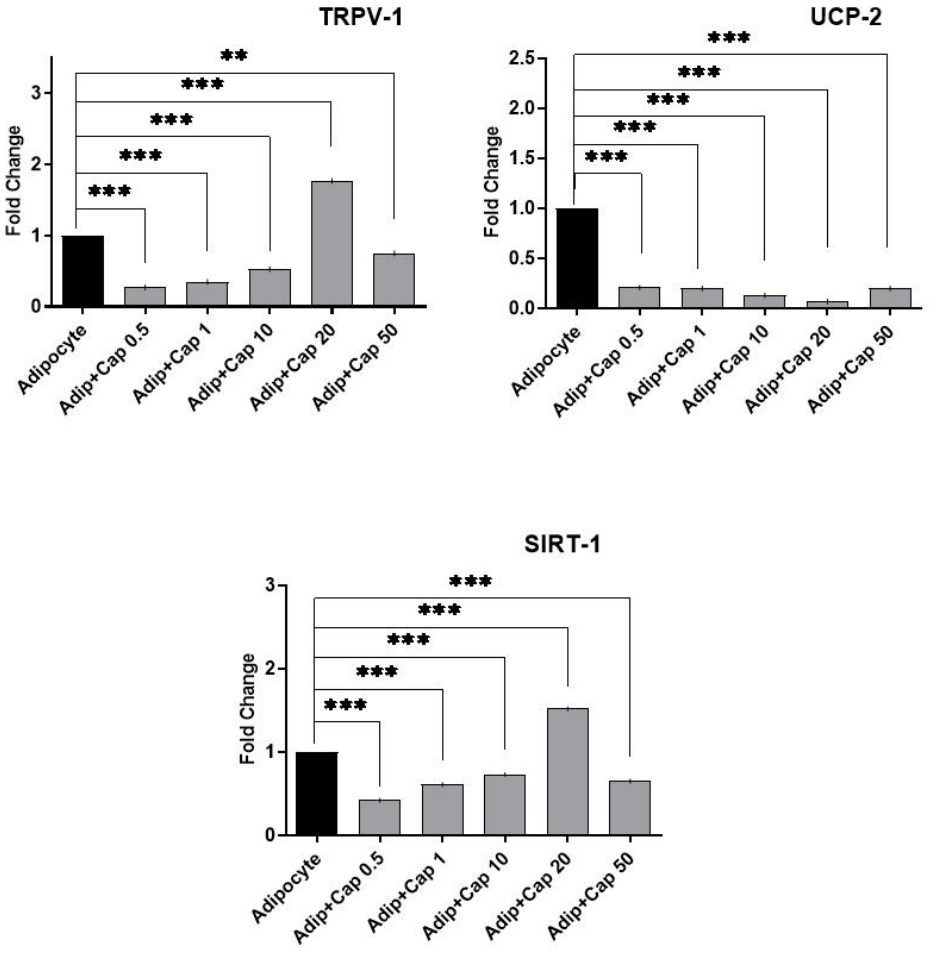

Figure 7. Effects of different doses of capsaicin treatment on TRPV-1, UCP-2, and SIRT-1 gene expression levels. **p<0.01; ***p<0.001; Cap: Capsaisin; Adip: Adipocyte.

DISCUSSION

It is well known that weight loss is very effective in reducing the complications of obesity-related disorders. For this purpose, many studies have focused on the investigation of food supplements that suppress body fat storage. Recent studies have shown that many natural food components have beneficial effects on weight loss and can prevent obesity (18, 19). One of them, capsaicin, was reported to be effective in reducing body weight and fat storage in vivo and suppressing adipogenesis in vitro (9, 20-22).

As adipose tissue is an active source of inflammation, illumination of the effect of capsaicin on adipocyte differentiation is of great importance. In this study, we demonstrated that adipocyte differentiation and adipose tissue-related inflammatory protein expression were suppressed by dose-dependent capsaicin supplementation.

The main role of adipose tissue is to store fat for energy uptake (23). There are two types of adipose tissue: the first is WAT which stores lipids and oxidizes them when energy is required. The second type is BAT, which is rich in mitochondria and burns lipids to generate heat by activating TRPV1 (23). Capsaicin induces TRPV1-mediated Ca^{2+} influx and reduces adipogenesis and obesity. Zhang et al. reported that long-term feeding with capsaicin prevented fat storage in WAT, whereas TRPV1 null-mice feeding with a high-fat diet + capsaicin was not effective on adipogenesis and obesity (21). Baskaran et al. demonstrated that capsaicin stimulates SIRT1-dependent deacetylation of PPARγ and induces BAT activation (23). It has been reported that capsaicin has generated WAT browning by inducing SIRT-dependent deacetylation by TRPV1 activation (7). Our results are consistent with those of previous studies. Capsaicin has stimulated the protein expressions of SIRT1 and TRPV-1 in adipocytes. According to our findings, the most effective capsaicin dosage for the regulation of SIRT1 and TRPV-1 expressions seems to be 20 µM.

The transcription factors PPARγ and C/EBP-α are known key proteins in the regulation of adipogenic differentiation (24). Ibrahim et al. have shown that capsaicin significantly reduced the mRNA expression of PPARγ and C/EBP-α (25). A recent study has reported that capsaicin decreased the PPARγ, C/EBP-α, and leptin expression and inhibited preadipocyte differentiation (9). In this study, capsaicin supplementation suppressed PPARγ, C/EBP-α, and leptin expression. According to our findings, capsaicin appears to effectively reduce PPARγ and C/EBP-α expressions at lower and higher concentrations.

Leptin expressions were found to be decreased at 0.5 μM and 1 μM capsaicin concentrations in this study. The decreased expression of leptin in a dose-dependent manner by capsaicin is a remarkable finding for its anti-lipogenic ability, especially at lower doses of capsaicin. The upper doses (10, 20, and 50 μM) have seemed to stimulate leptin expression and induce an inflammatory response. According to our findings, capsaicin reduced adiponectin expression. Babbota et al. reported that adiponectin secretion remained unchanged in adipocytes supplemented with capsaicin (16). Recent studies have reported contradictory results regarding adiponectin secretion. Several studies have reported its pro-inflammatory effects (26, 27), while others have supported its anti-inflammatory effects (28, 29). In this study, adiponectin seems to act as a pro-inflammatory mediator, and its expression was decreased with capsaicin supplementation.

Another important point is to focus on the response of NF-κB, a major transcription factor in inflammation, to capsaicin during adipocyte differentiation. Kang et al. have shown that capsaicin inhibits NF-κB activation in adipocytes (8). In the present study, NF-κB expression and inflammatory adipokines, such as IL-6 and TNF-α reduced by capsaicin supplementation. Consistent with these results, cyclooxygenase-2 (COX-2), which is an inflammatory marker, was significantly decreased in a dose-independent manner. NF-κB and IL-6 seem to be sensitive to lower doses of capsaicin, but TNF-α expression was reduced by 0.5 μM capsaicin, whereas the opposite effect was observed at 20 and 50 μM doses. Our findings are consistent with those by Baboota et al. (16). The inhibitory effect of capsaicin on the inflammatory state appears to be effective at lower capsaicin doses (0.5-1 μM).

UCP2, which controls energy expenditure, is highly expressed in human white adipose tissue (30). UCP2 is a mitochondrial proton transporter that influences thermogenesis and is related to lipid accumulation (31). Oliveira et al. have shown that UCP2 gene expression is decreased in patients with obesity (32). Heinitz et al. reported significant downregulation of UCP2 in the skeletal muscle of patients with obesity (33). In contrast, Vidal-Puig et al. detected that UCP2 expression was not altered in the adipose tissues of obese and lean individuals (34). According to our current knowledge, there is a single recent study that has evaluated the association between UCP2 and capsaicin in patients with obesity. Lee et al. reported that UCP2 expression was increased with 0.1, 1, and 10 μM capsaicin doses in cultured adipocyte cells (35). They have informed that UCP2 is related to mitochondrial antioxidant mechanisms and controls reactive oxygen species (ROS) production (35, 36). In another study, Ding et al. demonstrated that UCP2 ameliorated mitochondrial dysfunction by reducing the inflammatory state and oxidative stress in an experimental acute kidney injury model. UCP2 decreased cytokine release and inhibited NF-κB activation (37). In previous studies, UCP2 was shown to reduce adipogenesis and regulate genes related to lipid metabolism (9, 35). It has been reported to exert anti-obesity effects by activating the TRPV1 receptor. However, a previous study has shown that its anti-inflammatory effect was independent of TRPV1 (8). In the present study, in contrast to the results reported by Lee et al., UCP2 expression was decreased in the presence of capsaicin. The regulation of UCP2 expression with capsaicin is still not completely understood. According to the findings of our study, the regulatory anti-inflammatory mechanism of capsaicin may be completely independent of its adipogenesis-regulating mechanism.

These findings indicate that capsaicin exerts an inhibitory influence on adipocyte differentiation. This finding is consistent with that of a previous study that reported the anti-inflammatory effect of capsaicin through NF-κB inactivation (8). However, the inhibitory effects of capsaicin on cytokine secretion and UCP2 expression appear to be more complex and involve different pathways. According to our study, it has been concluded that although the effect of capsaicin on proteins regulating adipogenesis is not dose-related, however, its inhibitory effect on adiposity-dependent inflammation was more pronounced at low doses. We would like to point out that this study also has some limitations. One important point is that protein expression levels cannot be determined. Furthermore, we could not measure triglyceride levels, which is another indicator of lipid levels, along with oil red o staining. However, this study is important because it is the first to investigate the effects of capsaicin on human adipocyte cells.

Ethics Committee Approval: For this type of study formal ethical consent is not required.

Peer-review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- S.D., P.C., M.S., S.T.K., H.K., Y.O.I.; Data Acquisition: S.D., P.C., S.T.K.; Data Analysis/Interpretation: M.S., Y.O.I., H.K.; Drafting Manuscript- S.D., Y.O.I.; Critical Revision of Manuscript- S.D., P.C., M.S., S.T.K., H.K., Y.O.I.; Final Approval and Accountability- S.D., P.C., M.S., S.T.K., H.K., Y.O.I.

Conflicts of Interests: The authors declare that they have no competing interests.

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