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Research

Association of Different Doses of Curcumin with Preadipocyte to Adipocyte Differentiation

Farklı Kurkumin Dozları ile Preadiposit-Adiposit Farklılaşması Arasındaki İlişki

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ABSTRACT

Objective: Human adipose tissue participates in fat storage and immune response. Curcumin (CUR) decreases adipocyte differentiation by inhibiting inflammatory cytokines and by activating anti-inflammatory cytokines. In this study, we aimed to determine the suppressor effects of different doses of CUR (0.5 µM, 5 µM, 10 µM, 20 µM, 50 µM) on preadipocyte-adipocyte differentiation and its anti-inflammatory role in adipocytes.

Methods: Differentiation of cells was performed using Oil Red O. The mRNA expression levels of adiponectin, C/EBPα, COX-2, IL-6, leptin, NFκB1, PPARγ, SIRT-1, TNF-α, TRPV1, UCP2, VEGF-A, VEGF-RI, and VEGF-RII were evaluated in preadipocyte and adipocytes.

Results: CUR decreased the differentiation of preadipocytes-adipocytes and the release of proinflammatory cytokines by regulating the expression of *C/EBPα* and *PPARγ* gene expressions.

Conclusion: CUR inhibited adipogenic transcription factors and adipocyte differentiation at all concentrations. The anti-inflammatory effect was greatest at 50 µM.

Keywords: Curcumin, differentiation, adipocytes, anti-inflammatory

ÖZ

Amaç: İnsan yağ dokusu yağ depolanmasının yanısırada immun cevapda da rol oynar. Kurkuminin (CUR) enflamatuvar sitokinleri inhibe ederek ve anti-enflamatuvar sitokinlerin aktive ederek adiposit farklılaşmasını azalttığı gösterilmiştir. Bu çalışmada farklı dozlardaki CUR'un (0,5 µM, 5 µM, 10 µM, 20 µM, 50 µM) preadiposit-adiposit farklılaşmasındaki baskılayıcı etkisini ve adipositlerdeki anti-enflamatuvar rolünü ortaya çıkarmayı hedefledik.

Gereç ve Yöntem: Hücrelerin farklılaşması Oil Red O kullanılarak, adiponektin, C/EBPα, COX-2, IL-6, leptin, Nükleer NFκB1, PPARγ, SIRT-1, TNF-α, TRPV1, UCP2, VEGF-A, VEGF-RI ve VEGF-RII mRNA ekspresyon düzeyleri preadipositlerde ve adipositlerde saptandı.

Bulgular: CUR'un, preadipositlerin adipositlere farklılaşmasını ve proinflamatuar sitokinlerin salınımını azalttı. Bunu, *C/EBPα* ve *PPARγ* gen ekspresyonlarını düzenleyerek yaptı

Sonuç: Sonuç olarak, CUR, 0,5-50 µM arasındaki tüm dozlarda adipojenik transkripsiyon faktörlerini ve adiposit farklılaşmasını inhibe etti. Anti-enflamatuvar etkisini en fazla 50 µM'de gösterdi.

Anahtar Kelimeler: Kurkumin, farklılaşma, adipositler, anti-enflamatuvar

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INTRODUCTION

Human adipose tissue is involved in fat storage and also plays a role in immune response. Adipose tissue includes various cell types such as preadipocyte cells, adipocyte cells, endothelial cells, mast cells, fibroblasts, diverse immune cells, stem cells (1,2). It may also inhibit weight earning and metabolic diseases through the activation of specialized heat-productive adipocytes (3,4). The expression of transcriptional regulators such as peroxisome proliferator-activated receptor gamma (*PPAR-γ*) and CCAAT/enhancer binding protein α (C/EBPα), sirtuin (SIRT), and transient receptor potential vanilloid 1 (TRPV1) receptor and 2, receptors induce the differentiation of adipocytes and adipogenesis. Previous studies have revealed that TRPV1 channels participate in weight loss by enhancing intracellular Ca^{2+} levels (5-10).

Brown adipose tissue (BAT) comprises preadipocyte that express high levels of thermogenic genes. These preadipocyte are located in special stores and play a role in providing energy equality in all body parts by participating in thermogenesis, converting excess amounts of chemical/nutritional energy into heat energy. In contrast, brown-like adipocyte cells, also termed beige cells, grow in white adipocyte cells in response to various activators (5). The activities of beige and brown fat cells are important for reducing metabolic diseases, including obesity, in humans and mice (11). White adipose tissue (WAT) consists of adipocytes that store energy as triglycerides. However, BAT is functionally and physically different from WAT, and body mass index is adversely proportional to the amount of BAT suppression of adipocyte differentiation (from brown cells to white cells) and could be an effective strategy for preventing and treating obesity (12).

Activation and excessive expression of the SIRTs family are contained in the trans-differentiation or "browning" course of WAT to BAT (13). Beige adipocytes specialize in the dissipation of heat as energy. It does this by increasing their high mitochondrial content and the expression of mitochondria-related genes such as uncoupling proteins (UCPs). UCPs are a family of the mitochondrial anion carrier protein family that are targeted for weight loss therapy, with a role in controlling body temperature and energy balance (11).

White adipocytes produce and secrete a large number of adipokine, such as growth factors, cytokines, vasodilators, hormones, and others, including signal molecules (1,2). Adipokines have several functions. Regulation of appetite and energy, glucose and fat and metabolism, endothelial cell function, insulin function, inflammation, blood pressure, atherosclerosis, hemostasis, metabolic syndrome, and so on are some of these functions (14). Leptin, adiponectin (visfatin), inflammatory cytokines such as tumor necrosis factor-α (TNF-α), cyclooxygenase-2 (COX-2), iterleukin-6 (IL-6), interleukin-1 (IL-1), platelet activator inhibitor-1 (PAI-1), angiogenic proteins such as vascular endothelial growth factor (VEGF) and its receptor as VEGF-receptor (VEGF-R) are produced and secreted from white adipocytes. The molecular mechanisms of the effects of these adipokines are not fully understood, and research on this is still being conducted (9,15-17).

Various thermogenic dietary factors have been shown to prevent obesity and metabolic syndrome through antioxidant and anti-inflammatory mechanisms. Curcumin (CUR) is a polyphenolic compound derived from turmeric (Curcuma longa) plants belonging to the gingerol (Zingiberaceae) family. It has a wide variety of biological and pharmacological effects (18).

The thermogenic function of CUR was previously described as inhibiting the differentiation of adipocytes from preadipocyte (19). It has been reported that CUR inhibits adipocyte differentiation by affecting various regulators. CUR can lower the expression of PPARγ and C/EBPα leading to a decrease in lipid accumulation in adipocytes and ameliorating obesity and hyperlipidemia in patients with metabolic syndrome (20,21). CUR plays a potential role in reducing triglycerides. It does this by interacting with various targets, including cholesteryl ester transfer protein, peroxisome proliferator-activated receptor alpha, PPAR-γ and lipoprotein lipase (22). CUR, a natural polyphenol, is suggested to suppress adipocyte differentiation in the early stage by inhibiting the secretion of some regulators and inflammatory cytokines and by activating the secretion of anti-inflammatory cytokines (7,22,23). CUR is also reported to induce both the production and breakdown of triglyceride-rich lipoproteins to reduce plasma cholesterol and triglyceride concentrations by attenuating the expression of lipogenic genes (22). It can affect TRPV receptor 1 and TRPV2 receptor located in the intestinal jejunum and thus may have effects on both WAT and BAT (24,25).

The current study aimed to explore the molecular mechanisms of the inhibitory effects of different CUR doses on human preadipocyte-adipocyte (HPAd) cell differentiation. We also examined an adequate dose of CUR supplements to prevent adipocyte-related oxidative and inflammatory status. This study is the first to examine the effect of CUR on the HPAd cell differentiation.

In our study, we used the following methods with all CUR doses we prepared, and CUR doses were studied in triplicate in all methods.

METHODS

Procedures

Thawing, Detection, and Culturing Human Preadipocyte Cells

A vial containing $5x10^5$ human preadipocyte cells (HPAd from heart tissue, Catalog no: 802h-05a, cell Applications, San Diego CA, mycoplasma testing has been carried out for the cell line) seeded in preadipocyte growth medium (Cell Applications, San Diego CA) in 25 cm^2 (Corning, NY) tissue culture flasks under standard conditions and 25 cm^2 flask (Corning, NY) with preadipocyte growth medium in a 37 °C incubator containing 5% CO_2 (Sanyo MCO-20AIC, Moriguchi, Osaka, Japan) was incubated for 24 h. Microscopic examination showed that the cells were attached to the flask the next day and multiplied in the following days. When the flask surface contained 70-80% cells (confluen), the other passages were made with a Subculture Reagent Kit (cell applications, San Diego CA) according to the manufacturing protocol until the cells reached a sufficient number, and then the preadipocyte differentiation step was initiated.

Differentiation of Preadipocytes into Adipocytes

In order to differentiate $16x10^6$ cells into adipocyte cells, $2x10^5$ cells/2 mL preadipocyte cells were seeded into 5 of 6-well plates with growth medium. The next day, it was observed that the cells were attached to the wells, and the growth medium was changed. Adipocyte differentiation medium (cell applications, San Diego CA) was added to the cells into each well. During the addition of the adipocyte differentiation medium, different concentrations of CUR were also pipetted into the respective wells. In the first plates 3 wells formed the control cells, and in the other plates, different doses of CUR were added to 3 wells and plates were incubated in a 37 °C incubator containing 5% CO_2. Preadipocyte cells were followed for 15 days until they differentiated into adipocyte cells, and their medium was changed every 3 days. At the end of the 15^{th} day, when the cells were examined under a microscope (Leica Wetzlar, Germany), granulated oil drops were observed in the cells, which differentiated into adipocyte cells. The different doses of CUR were diluted with dimethyl sulfoxide and prepared. The applied CUR (Sigma, Germany) doses were 0.5 µM, 5 µM, 10 µM, 20 µM and 50 µM.

Staining of Differentiated Adipocyte Cells Using Oil Red O

Differentiation of cells was performed using the Oil Red O dye method. $15x10^3$ control cells/3 well stained with Oil Red O dye (Lipid Oil Red O Staining kit, Sigma-Aldrich, USA) according to kit procedure. Finally, ultrapure water was added to the cells and examined under a microscope. It was determined that they were painted with Oil Red O, and photographs were taken. Additionally, Oil Red O staining was quantified for all groups of adipocytes and preadipocyte in a 96-well plate reader (BioTek Instruments, USA) at 492 nm according to the kit procedure. For each group, experiments were repeated three times and measurements were performed in triplicate.

Quantifying mRNA Expression in Cells Using qRT-PCR

For mRNA expression quantification, total RNA was extracted from the cells using RNAzol RT solution (MRC, USA) according to the manufacturer's protocol. The concentration and purity of the RNA samples were then evaluated using a NanoDrop 2000 (Thermo Scientific, USA), where 1 µl of RNA was pipetted into the device. Prior to reverse transcription, RNA concentrations were standardized. Reverse transcription was performed using the Script cDNA Synthesis Kit (Jena Bioscience, Germany) following the kit protocol. The resulting cDNA was then amplified by qRT-PCR using the qPCR GreenMaster with the UNG Kit (Jena Bioscience, Germany). The remaining procedures followed the methodology outlined in the experiment by Değirmencioğlu et al. (17). Primers were sourced from LGC (Denmark), and all calculations were performed in triplicate. The primers used are detailed in Table 1.

Statistical Analysis

Results are presented as means±standard deviation of three experiments. Statistical analyses were performed using GraphPad Prism 5 software (San Diego, CA). One-way analysis of variance (ANOVA) was used to compare quantitative data among the groups. If the results of the ANOVA were significant, Tukey's multiple comparison test was used to compare groups' means ($p<0.05$).

RESULTS

The effects of different CUR doses on lipid accumulation during preadipocyte-adipocyte differentiation were determined using the Oil Red O dye method. Lipid accumulation was significantly increased during differentiation in control adipocytes ($p<0.001$). All different doses of CUR inhibited lipid accumulation, and the difference was statistically significant compared with the control adipocytes ($p<0.001$) (Figure 1).

Effects of CUR on *C/EBP-α* and *PPAR-γ* Gen Expresion

C/EBP-α expression levels were significantly increased in adipocytes during differentiation ($p<0.001$). Different doses

of CUR treatments (CUR 0.5-50 μM) during differentiation decreased C/EBP-α mRNA levels compared with adipocytes ($p<0.001$) (Figure 2).

Also *PPAR-γ* gen expression in adipocytes is enhanced significantly during differentiation compared with that in preadipocyte. CUR treatment during differentiation showed different effects on *PPAR-γ* gen expressions. In comparison to adipoyctes; Expression of *PPAR-γ* significantly decreased in CUR 0.5, CUR 10 ($p<0.01$) and CUR 50 ($p<0.05$) groups and significantly increased in the CUR 20 group ($p<0.001$) (Figure 2).

Effect of CUR on Adipokine Gene Expression

Adipokine mRNA expression was decreased in all CUR groups compared with adipocytes ($p<0.001$). The reduction in adipokine levels was not dose dependent (Figure 3). Leptin mRNA levels were significantly increased in all CUR-treated adipocytes except the CUR 50 group compared with the control adipocytes ($p<0.01$). In the CUR 50 group, leptin mRNA levels were significantly decreased ($p<0.01$) (Figure 3).

Effects of CUR on Proinflammatory Cytokines

COX-2 mRNA expression was significantly increased in all CUR-treated adipocytes compared with control adipocytes ($p<0.05$ for CUR 0.5, $p<0.001$ for CUR 5, CUR 10, CUR 20 and CUR 50). *IL-6* gene expression levels significantly changed during differentiation of CUR-added adipocytes. While a statistically significant increase was found in the CUR 0.5 and CUR 5 groups ($p<0.001$), IL-6 mRNA levels were significantly decreased in the CUR 10, CUR 20 and CUR 50 groups ($p<0.001$) in comparison with the control adipocytes. TNF-α mRNA levels were significantly increased in all CUR groups except the CUR 50 group in comparison with the control adipocytes ($p<0.05$ for CUR 0.5, $p<0.001$ for CUR 5, CUR 10 and CUR 20). In the CUR 50 group, mRNA levels of TNF-α significantly decreased ($p<0.001$) (Figure 4). The reduction of NFkB1 mRNA levels in the CUR 5 group and increase in the CUR 10 group were statistically significant compared with the control adipocytes ($p<0.001$ for both) (Figure 4).

Table 1. Primer list for RT-PCR

Gene name	Gene bank	Primer sequences	Product size (bp)
PPARG	NM_138712.5	Forward 5′-AGGATGCAAGGGTTTCTTCCG-3′ Reverse 5′-CCGCCAACAGCTTCTCCTTC-3′	200
CEBPA	NM_001285829.1	Forward 5′-CACCGCTCCAATGCCTACTG-3′ Reverse 5′-CTAAGGACAGGCGTGGAGGA-3′	200
NFKB1	NM_003998.4	Forward 5′-ACTGCTGGACCCAAGGACAT-3′ Reverse 5′-CGCCTCTGTCATTCGTGCTT-3′	105
TNF-α	NM_000594.4	Forward 5′-ACTGCTGGACCCAAGGACAT-3′ Reverse 5′-CGCCTCTGTCATTCGTGCTT-3′	81
UCP2	NM_001381944.1	Forward 5′-CTTCTGCACCACTGTCATCG-3′ Reverse 5′-GTGACGAACATCACCACGTT-3′	195
VEGFA	NM_001025366.3	Forward 5′-CCCACTGAGGAGTCCAACA -3′ Reverse 5′-CTCTCCTATGTGCTGGCCTT-3′	72
VEGFR1	NM_002019.4	Forward 5′-TTACCGAATGCCACCTCCAT-3′ Reverse 5′-CTTGGGTTTGCTGTCAGTCC-3′	105
VEGFR2	NM_002253.4	Forward 5′-CTCAGCAGGATGGCAAAGAC-3′ Reverse 5′-AGGTGAGGTAGGCAGAGAGA-3′	92
TRPV1	NM_080704.4	Forward 5′-ACCCTGTTTGTGGACAGCTA-3′ Reverse 5′-CAAGGCCAGGGAGAATACCA-3′	129
SIRT-1	NM_012238.5	Forward 5′-TATGCTCGCCTTGCTGTAGA-3′ Reverse 5′-TGGCTGGAATTGTCCAGGAT-3′	132
COX2	NM_000963.4	Forward 5′-GCTTCCATTGACCAGAGCAG-3′ Reverse 5′-CTCCACAGCATCGATGTCAC-3′	159
LEP	NM_000230.3	Forward 5′-TGGAGAAGCTGATGCTTTGC-3′ Reverse 5′-GGACCATTCAGAGGGTCACA-3′	196
ADIPOQ	NM_001177800.2	Forward 5′-GGATGTGAAGGTCAGCCTCT-3′ Reverse 5′-TACACCTGGAGCCAGACTTG-3′	141
GAPDH	NM_002046.7	Forward 5′-ACCCAGAAGACTGTGGATGG-3′ Reverse 5′-TCAGCTCAGGGATGACCTTG-3′	124
ACTB	NM_001101.5	Forward 5′-CCCTGGAGAAGAGCTACGAG-3′ Reverse 5′-GGAAGGAAGGCTGGAAGAGT-3′	96

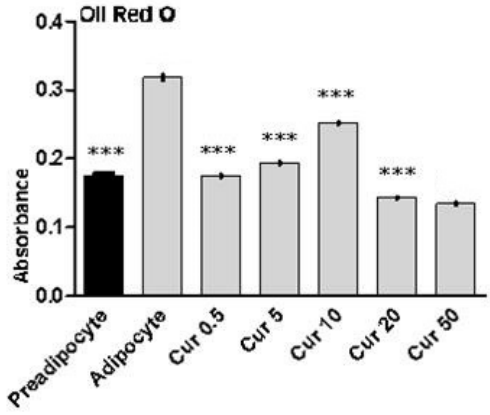

Figure 1. Comparison of the differentiation of absorbance values of different doses of CUR-added adipocytes, adipocytes, and preadipocyte stained with Oil Red O dye method

***p<0.001 in comparison to adipocytes. Data are expressed as mean±SD

Cur 0.5: Differentiated adipocytes in the presence of 0.5 µM Curcumin (CUR)

Cur 5: Differentiated adipocytes in the presence of 5 µM Curcumin (CUR)

Cur 10: Differentiated adipocytes in the presence of 10 µM Curcumin (CUR)

Cur 20: Differentiated adipocytes in the presence of 20 µM Curcumin (CUR)

Cur 50: Differentiated adipocytes in the presence of 50 µM Curcumin (CUR)

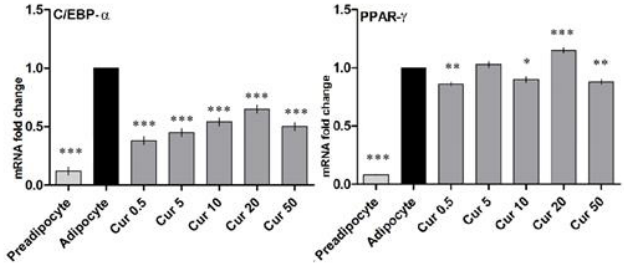

Figure 2. Effects of Curcumin on the expression of *C/EBP-α* and *PPAR-γ* genes during the differentiation of preadipocyte to adipocytes

*p<0.05, **p<0.01, ***p<0.001 in comparison to adipocytes. Data are expressed as mean±SD

Cur 0.5: Differentiated adipocytes in the presence of 0.5 µM Curcumin (CUR)

Cur 5: Differentiated adipocytes in the presence of 5 µM Curcumin (CUR)

Cur 10: Differentiated adipocytes in the presence of 10 µM Curcumin (CUR)

Cur 20: Differentiated adipocytes in the presence of 20 µM Curcumin (CUR)

Cur 50: Differentiated adipocytes in the presence of 50 µM Curcumin (CUR)

PPAR-γ; Peroxisome proliferator-activated receptor gamma

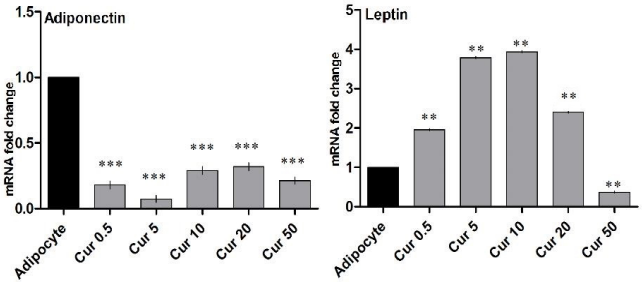

Figure 3. Adiponectin and Leptin mRNA levels in control adipocytes and different dose of Curcumin added adipocytes

**p<0.01, ***p<0.001 in comparison to adipocytes. Data are expressed as mean±SD.

Cur 0.5: Differentiated adipocytes in the presence of 0.5 µM Curcumin (CUR)

Cur 5: Differentiated adipocytes in the presence of 5 µM Curcumin (CUR)

Cur 10: Differentiated adipocytes in the presence of 10 µM Curcumin (CUR)

Cur 20: Differentiated adipocytes in the presence of 20 µM Curcumin (CUR)

Cur 50: Differentiated adipocytes in the presence of 50 µM Curcumin (CUR)

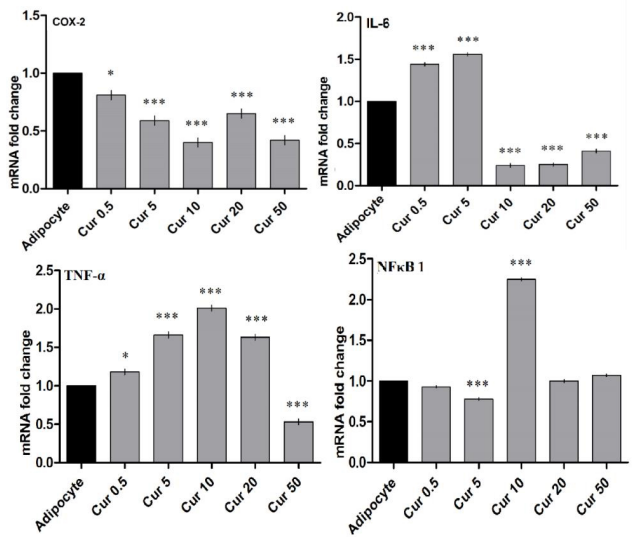

Figure 4. COX2, IL6, TNF-α and NFκB1 mRNA levels in control adipocytes and different dose of Curcumin added adipocytes

*p<0.05, ***p<0.001 in comparison to adipocytes. Data are expressed as mean±SD.

Cur 0.5: Differentiated adipocytes in the presence of 0.5 µM Curcumin (CUR)

Cur 5: Differentiated adipocytes in the presence of 5 µM Curcumin (CUR)

Cur 10: Differentiated adipocytes in the presence of 10 µM Curcumin (CUR)

Cur 20: Differentiated adipocytes in the presence of 20 µM Curcumin (CUR)

Cur 50: Differentiated adipocytes in the presence of 50 µM Curcumin (CUR)

Effects of CUR on Angiogenic Proteins

VEGF-A mRNA levels of CUR-added adipocytes significantly decreased during differentiation in CUR 0.5, CUR 20, and CUR 50 groups ($p<0.01$ for CUR 0.5, CUR 20, $p<0.001$ for CUR 50) and significantly increased in the CUR 10 group ($p<0.001$) in comparison to control adipocytes. VEGF-R1 mRNA levels were significantly increased in all CUR-treated adipocytes except CUR 50 compared with control adipocytes ($p<0.001$). In the CUR 50 group, VEGF-RI mRNA levels were significantly decreased ($p<0.001$) (Figure 5). Elevated VEGF-R2 mRNA levels were found in all CUR groups except for CUR 5. Statistically significant increase in gene expression in CUR 0.5 ($p<0.05$), CUR 10 ($p<0.001$), CUR 20 ($p<0.01$) and CUR 50 ($p<0.001$) adipocytes compared with control adipocytes (Figure 5).

Effects of CUR on TRPV-1, UCP-2, and SIRT-1 expression

TRPV-1 mRNA levels changed significantly in all CUR groups except for CUR 50 compared with control adipocytes ($p<0.001$). UCP-2 mRNA levels were significantly decreased in all CUR groups ($p<0.001$). SIRT-1 mRNA levels reduction was only statistically significant in the CUR 0.5 ($p<0.01$) and CUR 5 ($p<0.05$) groups CUR 50 compared with control adipocytes (Figure 6).

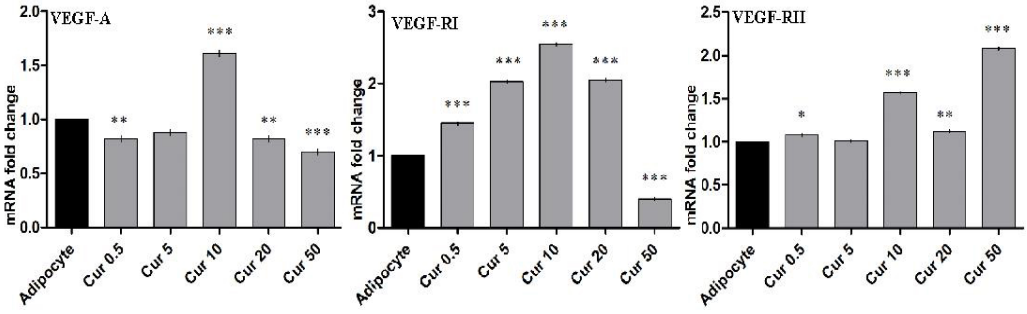

Figure 5 VEGF-A, VEGF-RI, and VEGF-RII mRNA levels of control adipocytes and different dose of Curcumin added adipocytes
*p<0.05, **p<0.01, ***p<0.001 in comparison to adipocytes. Data are expressed as mean±SD
Cur 0.5: Differentiated adipocytes in the presence of 0.5 μM Curcumin (CUR)
Cur 5: Differentiated adipocytes in the presence of 5 μM Curcumin (CUR)
Cur 10: Differentiated adipocytes in the presence of 10 μM Curcumin (CUR)
Cur 20: Differentiated adipocytes in the presence of 20 μM Curcumin (CUR)
Cur 50: Differentiated adipocytes in the presence of 50 μM Curcumin (CUR)

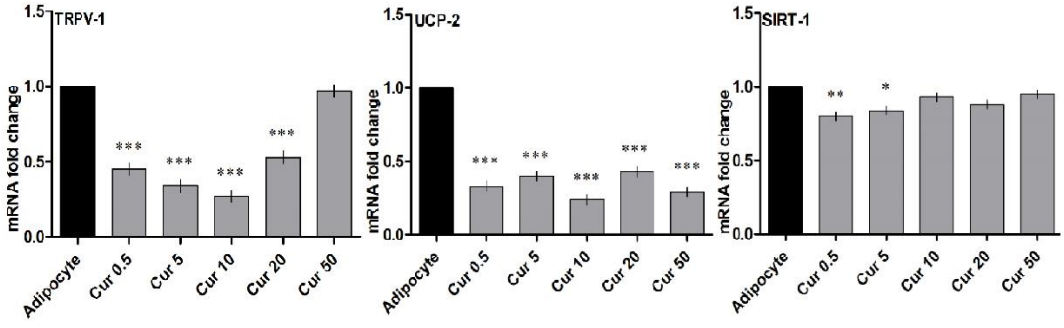

Figure 6. TRPV-1, UCP-2, and SIRT-1 mRNA levels of control adipocytes and different dose of Curcumin added adipocytes
p<0.05, ***p<0.01, ***p<0.001 in comparison to adipocytes. Data are expressed as mean±SD.
Cur 0.5: Differentiated adipocytes in the presence of 0.5 μM Curcumin (CUR)
Cur 5: Differentiated adipocytes in the presence of 5 μM Curcumin (CUR)
Cur 10: Differentiated adipocytes in the presence of 10 μM Curcumin (CUR)
Cur 20: Differentiated adipocytes in the presence of 20 μM Curcumin (CUR)
Cur 50: Differentiated adipocytes in the presence of 50 μM Curcumin (CUR)

DISCUSSION

Adipocyte differentiation is an important process in the identification of adipocytes during the development of adipogenesis and obesity. Augmented adiposity is a risk factor for chronic inflammation and related metabolic disorders. CUR, a natural polyphenol obtained from the rhizomes of the CUR longa plant, exhibits antioxidant and anti-inflammatory properties (26,27). CUR also plays a role as a thermogenic and anti-adipogenesis factor and suppresses adipocyte differentiation by inhibiting some regulators in the early stages of adipocyte differentiation pathways (18,19,24). Therefore, it is important to clarify the mechanism by which CUR regulates adipose differentiation. Clarifying the molecular and cellular mechanisms that regulate adipogenesis and inflammatory factor expression will help prevent and treat inflammatory diseases, obesity, insulin resistance, and metabolic syndrome.

In the present study, human adipocytes incubated with CUR significantly decreased C/EBPα mRNA expression in a dose-independent manner. Zhao et al. (20) also found lipid accumulation in doses independent of CUR in 3T3-L1 cells. This result is consistent with the results of our study. However especially 50 μM CUR treatment lowered PPARγ mRNA expression levels. CUR has been shown to decrease the expression of C/EBPα and PPARγ leading to reduced lipid accumulation in adipocytes and improved obesity and hyperlipidemia in patients with metabolic syndrome (20,21). Additionally, one of the pathways that suppresses adipocyte differentiation is the inhibition of mRNA levels of adipogenic transcription factors such as C/EBPa and PPARγ (22,28). CUR inhibits the adipogenic differentiation of human bone marrow mesenchymal stem cells. This effect ocCURs by inhibiting C/EBPa and PPARγ (29). Our results are similar to those of recent studies. On the other hand, PPARγ is also associated with SIRT-1 protein expression. It has been reported that PPARγ transactivation activity is inhibited by the direct or indirect effects of SIRT-1 protein (30). However, SIRT mRNA levels were not affected by CUR treatment. This suggests that the effect of PPARγ on adipogenesis differentiation with CUR treatment may be via a non-transcriptional mechanism, and this result also indicates that the anti-adipogenic effect of CUR is due to transition cellular events that CUR in the early stage of adipocyte differentiation.

CUR was informed to be affected by TRPV1 located in the intestinal jejunum and in this wise has affected both WAT and BAT differentiations (25). UCP-2 has now been identified as an important molecule in metabolic thermogenesis, such as diet-induced and cold heat production, which is an important component of energy expenditure, and it was also found that its dysfunction contributes to the development of obesity (11). *UCP-2* gene expression is upregulated by through a prostaglandin/PPARg-mediated pathway (11). In our study, UCP-2 and TRPV1 mRNA levels were significantly reduced in adipocytes following incubation with different concentrations of CUR. This reduction may be related to cellular lipid accumulation. According to our CUR knowledge, there are not enough studies showing the effects of CUR on UCP2 and TRPV1 levels. We demonstrated for the first time that CUR has no effect on UCP2 expression during adipocyte differentiation. Mahadik et al. (11) found that obese subjects showed decreased *UCP2* gene expression in adipose tissue. CUR applied during adipocyte differentiation does not seem to be effective on TRPV1 levels. This suggests that the pathways of metabolic thermogenesis and adipogenesis are not related. The lack of correlation between C/EBPα and thermogenesis parameters strengthens our hypothesis. The specific role of adipokine and related pathways in the inflammatory state is not understood. The inflammation may be the cause or the inductive effect of adipokine secretion. It has been reported that CUR has beneficial effects against obesity-related inflammation (12). Therefore, it is important to underly the relationship between CUR and inflamed adipocyte differentiation. CUR prevents inflammation by modulating proinflammatory cytokines, such as COX-2, IL-6, and -α and also related pathways, such as NF-κB and PPARγ (9,15,16). A recent study showed that CUR downregulates the expression of COX-2 and NF-κB and suppresses inflammation in colistin-induced toxic neuroblastoma-2a cells (15,16). In our study, NF-κB did not change during adipocyte differentiation, but CUR decreased the expression of the proinflammatory markers COX-2, TNF-α and IL-6 mRNA in adipocytes. 50 μM treatment of CUR seem to be more effective in suppressing inflammation. This finding suggests that the anti-inflammatory effect of CUR may be associated with a decrease in PPARγ mRNA levels and that CUR plays no role in suppressing NF-κB. Leptin and adiponectin are important adipokines for energy balance and metabolism. There are many confusing results regarding the levels of leptin and adiponectin in adipocytes (17). In our study, leptin mRNA levels in adipocytes decreased with 50 μM CUR administration, whereas adiponectin mRNA levels decreased significantly with dose-independent CUR administration. Because both adipokine are secreted from adipose tissue, their low levels may be associated with decreased fat accumulation resulting from CUR in adipocytes. Our results are consistent with those by Kim et al. (24).

In this study, it was first concluded that CUR suppresses the differentiation of human preadipocyte to adipocytes and decreases the release of proinflammatory cytokines such as COX-2, TNF-α and IL-6, but it does so by regulating *C/EBPα* and *PPARγ* gene expressions outside the NF-κB pathway. One of our important findings was that CUR effectively suppressed adipogenic transcription factors and adipocyte differentiation at all doses between 0.5-50 μM, but showed its anti-inflammatory effect especially in the application of CUR of 50 μM, the highest dose in our study.

Study Limitations

We would like to define that this study also has some limitations. We couldn't determine the protein expression levels. Also, we could not measure another indicator of lipid levels as triglyceride levels which is, along with Oil Red O staining. However, this study is important because it is the first to investigate that CUR suppresses the differentiation of human preadipocyte to adipocytes and decreases the release of proinflammatory cytokines.

CONCLUSION

In conclusion, the inhibitory effect of CUR on adipocyte differentiation is associated with its anti-inflammatory effect, and this beneficial effect is more pronounced, especially at high concentrations.

ETHICS

Ethics Committee Approval: Since this study was on genes, no ethics committee was required.

Informed Consent: Since this study was about genes, patient consent was not required.

Authorship Contributions

Surgical and Medical Practices: P.Ç., S.D., S.T.K., Concept: P.Ç., S.D., S.T.K., M.S., Y.Ö.İ., H.K., Design: P.Ç., S.D., S.T.K., M.S., Y.Ö.İ., H.K., Data Collection or Processing: P.Ç., S.D., S.T.K., M.S., Y.Ö.İ., H.K., Analysis or Interpretation: P.Ç., S.D., S.T.K., M.S., Y.Ö.İ., H.K., Literature Search: P.Ç., S.D., S.T.K., Writing: P.Ç., Y.Ö.İ.

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