

# Investigation of the effect of quercetin on the accumulation of lipids and the level of adiponectin in 3T3-L1 adipocytes

## *Quercetin'in 3T3-L1 adipositlerinde lipid birikimi ve adiponektin düzeyi üzerindeki etkisinin araştırılması*

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### Abstract

**Purpose:** Obesity disrupts the homeostasis of adipose tissue, leading to an increase in the number and size of adipose cells. Many positive effects of quercetin polyphenol on health through various mechanisms are known in the literature. The aim of our study was to investigate the effects of quercetin on lipid accumulation and adiponectin (ADP) levels in mature and hypertrophic adipocytes.

**Materials and methods:** In this study, 3T3-L1 differentiated preadipocyte cells were exposed to high glucose media for 8 days to generate mature adipocytes, then for 18 days to produce hypertrophic adipocytes. Quercetin (40 and 80  $\mu$ M) was administered to mature adipocytes for 24 hours and to hypertrophic adipocytes for 48 hours. Lipid content was visualized by Oil-Red-O staining method. Lipid accumulation and ADP level were measured by ELISA method. For statistical analysis, one-way analysis of variance (post hoc: Tukey test) was used.  $p \leq 0.05$  was considered statistically significant.

**Results:** The administration of both quercetin doses to mature adipocytes significantly reduced lipid accumulation ( $p=0.0001$ ). The administration of 80  $\mu$ M quercetin in mature adipocytes caused a significant increase in ADP levels ( $p=0.0001$ ). Administration of quercetin to hypertrophic adipocytes caused a significant decrease in ADP levels ( $p=0.0001$ ).

**Conclusion:** Our study revealed that quercetin decreased lipid accumulation and increased ADP levels in mature adipocytes. However, in hypertrophic adipocytes, quercetin had no significant effect on lipid accumulation and decreased ADP levels. These findings suggest that quercetin has protective effects on health in the early stages of obesity, but its efficacy is limited in the later stages of obesity.

**Keywords:** Adiponectin, adipocyte, lipid accumulation, obesity, quercetin.

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### Öz

**Amaç:** Obezite, adipoz dokunun homeostazını bozarak adipoz hücrelerinin sayısında ve büyüklüğünde artışa neden olur. Literatürde quercetin polifenolünün çeşitli mekanizmalar aracılığıyla sağlık üzerinde pek çok olumlu etkileri bilinmektedir. Araştırmamızın amacı, quercetin'in olgun ve hipertrofik adipositlerde lipid birikimi ve adiponektin (ADP) seviyeleri üzerindeki etkilerini araştırmaktır.

**Gereç ve yöntem:** Çalışmada, 3T3-L1 preadiposit hücreler farklılaştırıldıktan sonra yüksek glikoz içeren ortamda 8 gün muamele edilerek olgun adipositler, 18 gün muamele edilerek ise hipertrofik adipositler elde edildi. Quercetin (40 ve 80  $\mu$ M), olgun adipositlere 24 saat, hipertrofik adipositlere ise 48 saat boyunca uygulandı. Oil-Red-O boyama yöntemiyle lipid miktarı görüntülendi. ELISA yöntemi aracılığıyla lipid birikimi ve ADP seviyesi ölçüldü. İstatistiksel analiz için; tek yönlü varyans analizi (post hoc: Tukey testi) kullanıldı.  $p \leq 0,05$  değeri istatistiksel olarak anlamlı kabul edildi.

**Bulgular:** Olgun adipositlere her iki quercetin dozunun uygulaması lipid birikimini anlamlı olarak azalttı ( $p=0,0001$ ). Olgun adipositlerde 80  $\mu$ M quercetin uygulaması, ADP seviyelerinde anlamlı bir artışa yol açtı ( $p=0,0001$ ). Hipertrofik adipositlere quercetin uygulaması ise, ADP seviyelerinde anlamlı bir azalmaya yol açtı ( $p=0,0001$ ).

**Sonuç:** Çalışmamız, quercetin'in olgun adipositlerde lipid birikimini azaltıp ADP seviyesini artırdığını ortaya koydu. Ancak, hipertrofik adipositlerde ise quercetin'in lipid birikiminde anlamlı bir etkisi olmayıp ADP seviyesini azalttığı gözlemlendi. Bu bulgular, quercetin'in obezitenin erken döneminde sağlık üzerindeki koruyucu etkilerini ortaya koymakla birlikte, obezitenin daha ileri döneminde etkinliğinin sınırlı kaldığını göstermektedir.

**Anahtar kelimeler:** Adiponektin, adiposit, lipid birikimi, obezite, quercetin.

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## Introduction

Obesity is defined by a confluence of genetic susceptibility, the intake of high-calorie meals, and diminished physical activity. Currently, it is regarded as the main cause for numerous chronic conditions, including cardiovascular diseases, type 2 diabetes, and specific forms of cancer. It is regarded as both a personal issue and an epidemic that threatens global well-being [1]. Obesity, characterized by an excessive accumulation of adipose tissue, disrupts energy balance at a pathological level. Adipocytes in adipose tissue accumulate excess nutrients through hyperplasia, hypertrophy or a combination of both. During increased energy demand, they release energy substrates via lipolysis. Thus, adipose tissue plays a crucial part in the regulation of energy balance [2]. Adipose tissue not only serves as a store of energy and regulates energy homeostasis but also performs significant endocrine roles by secreting adipokines, including resistin, leptin, and adiponectin (ADP). Furthermore, these hormones are crucial in various physiological processes within the body, including insulin sensitivity, inflammation, and lipid metabolism. In particular, ADP produced in adipocytes is a hormone involved in various metabolic processes and ADP levels are closely related to the size and composition of adipose tissue [3].

Current obesity interventions focus on either diminishing caloric consumption (diets, pharmacotherapy, bariatric surgery) or enhancing energy expenditure via physical exercise. Moreover, the processes by which polyphenols operate in the treatment of obesity have been extensively studied in recent years. Polyphenols are significant bioactive chemicals that modulate lipid metabolism and energy balance in adipocytes. Polyphenols, particularly catechins, resveratrol, and quercetin, inhibit adipogenesis and restrict the development of adipocytes. These polyphenols enhance lipolysis in adipocytes, diminish triglyceride accumulation, and stimulate energy expenditure [4-6]. Moreover, the anti-inflammatory properties of polyphenols protect metabolic health by reducing inflammation linked to obesity [7]. Consequently, polyphenols

restrict the proliferation of adipose tissue and contribute to preventing obesity-related problems. The literature has a limited number of studies investigating the effects of quercetin polyphenol on mature adipocytes [8, 9]. No research were identified in the literature analysis that investigated the impact of quercetin on ADP levels in hypertrophic adipocytes. Mature adipocytes represent obesity and hypertrophic adipocytes represent insulin resistant/advanced obesity model [10, 11]. Consequently, our work aimed to explore the effects of quercetin administration on lipid accumulation and ADP levels in both mature and hypertrophic adipocytes.

## Materials and methods

### Cell culture

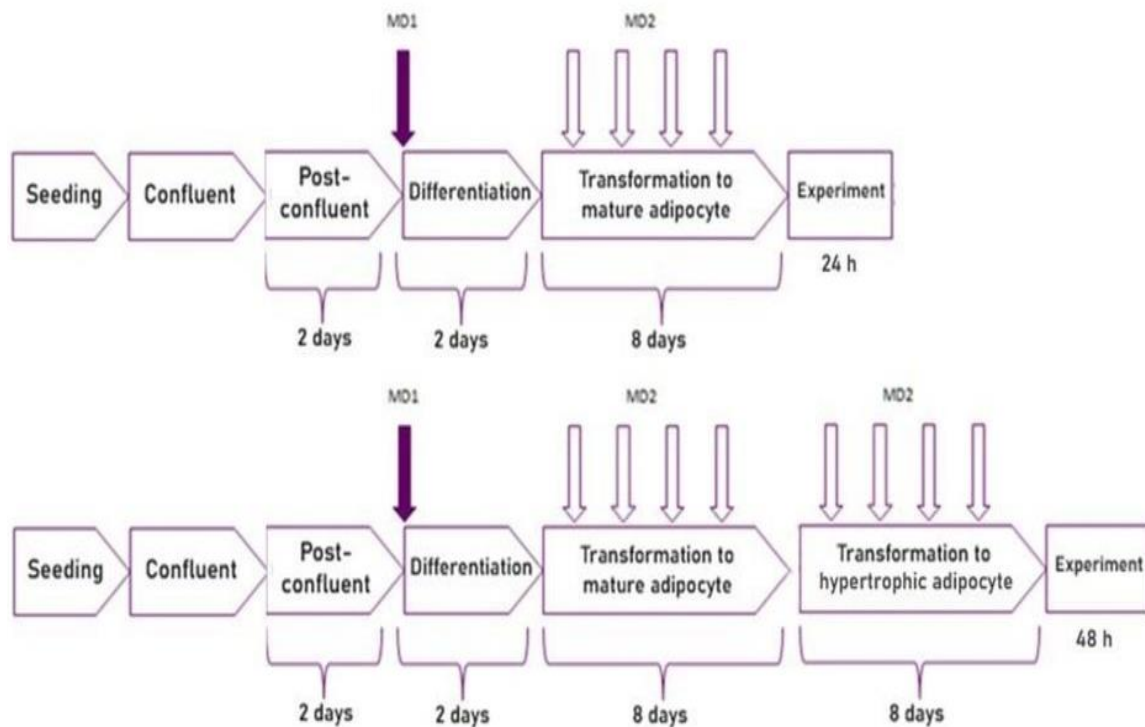
#### Differentiation and establishment of the 3T3-L1 hypertrophic adipocyte model

In this study, we used the 3T3-L1 cell line (ATCC® CL173™), a fibroblast cell line derived from a mouse (*Mus musculus*) embryo known as a preadipocyte. The cells were cultured in a Dulbecco's Modified Eagle Medium (DMEM) (Gibco, USA, Waltham) containing 25 mM glucose supplemented with 10% heat inactivated calf serum (Cegrogen, Germany), and antibiotics (penicillin and streptomycin; Wisent, Saint-Jean-Baptiste, Canada) at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> to create experimental groups. When the cells reached approximately 80% density in the culture dishes, after cell counting, 10<sup>5</sup> cells were seeded in 6-well plate. The 2 ml of medium was added to each well and the growth of the cells. The cells were cultured in the same medium until they reached confluence. After that a medium containing a differentiation cocktail 0.5 Mm 3-Isobutyl-1-methylxanthine (IBMX, Sigma I5879, USA), 1 µM dexamethasone (DEX, Sigma D4902, USA), 10 µg/mL insulin (INS) (Sigma I6634, USA), 10% fetal bovine serum (FBS, Biowest, South America) was added to the medium to induce the differentiation of preadipocytes into adipocytes and the cells were incubated in this medium (MD1) for 48 hours [12]. After incubation, the medium containing

high glucose, FBS and INS (MD2) (Gibco, Waltham, MA, USA) was changed every 2 days. After this stage, the cells will become mature adipocytes after 8 to 10 days and hypertrophic adipocytes after 18 to 21 days [11]. Adipocyte differentiation was evaluated by Oil Red O staining. Our experiments were performed on adipocytes that completed differentiation on day 10 and 18 became mature and hypertrophic.

### Administration of quercetin

Quercetin 40 and 80  $\mu\text{M}$  [13] dose was applied to mature 3T3-L1 cells at day 10 post-differentiation for 24 hours and hypertrophic 3T3-L1 cells at day 18 post-differentiation for 48 hours. Details of the experimental timeline are shown in Figure 1.



**Figure 1.** Demonstration of experimental design

Quercetin was applied at 40 and 80  $\mu\text{M}$  dose. MD1: medium 1, MD2: medium 2

### Oil red o staining and measurements of adiponectin level

To confirm the transformation of preadipocytes into mature (day 10) and hypertrophic (day 18), they were analysed microscopically using the Oil Red O staining kit [Biovision Lipid (Oil Red O) (Catalog #K580-24)]. Differentiated 3T3-L1 adipocytes were fixed with 10% formalin in Phosphate Buffered Saline (PBS, Wisent, Saint-Jean-Baptiste, Canada) for 1 h and washed twice with 60% isopropanol. The fixed cells were then stained using Oil Red O solution for 30 min and washed with distilled water. After drying, the cells were imaged by scanner. The Oil Red O solution taken up by the cells was then extracted using 100% isopropanol and its optical intensity was measured at 490 nm.

A microscope was used to see the triglycerid accumulation at 40X magnifications. ADP levels were measured in 3T3-L1 mature and hypertrophic cell lysates using an ELISA kit according to the manufacturer's instructions (BTLAB E0246Mo, China).

### Statistical analysis

The data were analyzed with the software package SPSS 25.0. Continuous variables are expressed as the mean  $\pm$  standard deviation. One-way analysis of variance (post hoc: Tukey test) was used to compare group differences.  $p \leq 0.05$  was considered statistically significant.

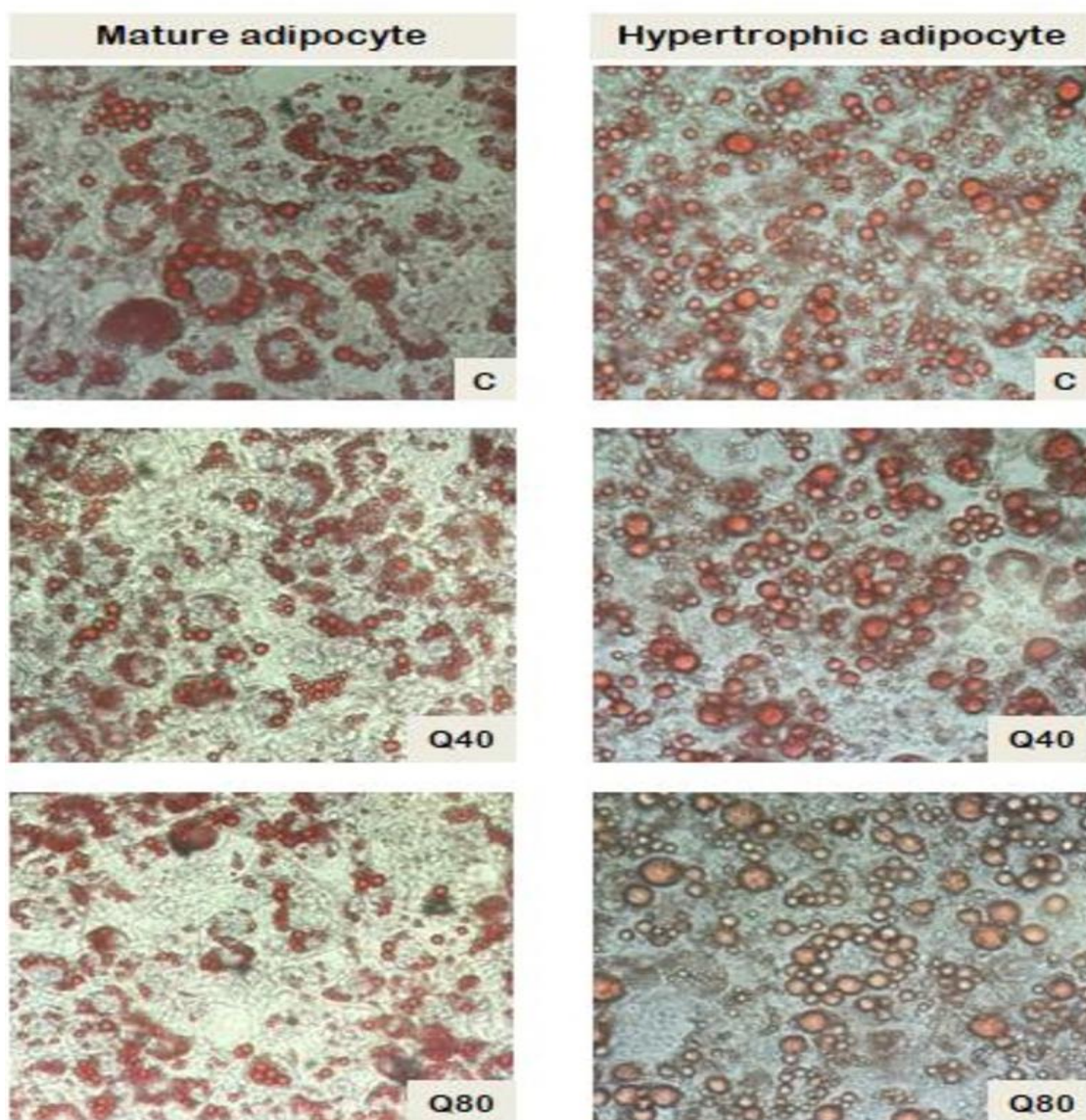
The study is a cell culture study that does not require ethics committee approval.



## Results

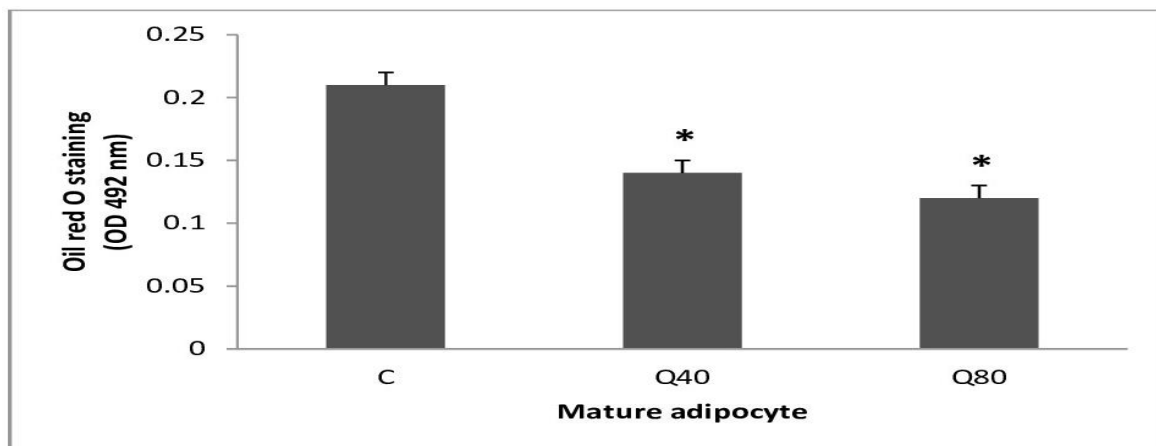
Images taken from mature and hypertrophic cells at different stages after cultivation of 3T3-L1 preadipocyte cells showed that these cells had different morphologies in terms of lipid accumulation (Figure 2). Administration of 40 and 80  $\mu\text{M}$  doses of quercetin to mature adipocytes significantly reduced lipid accumulation compared to mature control (C:0.21 $\pm$ 0.01; Q40:0.14 $\pm$ 0.01; Q80:0.12 $\pm$ 0.01;  $p=0.0001$ ) (Figure 3). Although 40 and 80  $\mu\text{M}$  doses of quercetin administered to hypertrophic adipocytes demonstrated a tendency to decrease lipid accumulation compared to hypertrophic control, this difference was

not statistically significant (C:0.49 $\pm$ 0.07; Q40:0.42 $\pm$ 0.05; Q80:0.41 $\pm$ 0.07) (Figure 4). Administration of 80  $\mu\text{M}$  quercetin to mature adipocytes significantly increased ADP levels compared to both mature control and 40  $\mu\text{M}$  quercetin groups (C:1.22 $\pm$ 0.15; Q40:1.37 $\pm$ 0.15; Q80:2.18 $\pm$ 0.15;  $p=0.0001$ ) (Figure 5). Administration of 40 and 80  $\mu\text{M}$  quercetin to hypertrophic adipocytes significantly decreased ADP level compared to hypertrophic control (C:2.08 $\pm$ 0.15, Q40:1.19 $\pm$ 0.01, Q80:0.91 $\pm$ 0.01,  $p=0.0001$ ). In hypertrophic adipocytes, 80  $\mu\text{M}$  quercetin significantly decreased ADP levels compared to 40  $\mu\text{M}$  quercetin (Q40:1.19 $\pm$ 0.01, Q80:0.91 $\pm$ 0.01,  $p=0.001$ ) (Figure 6).



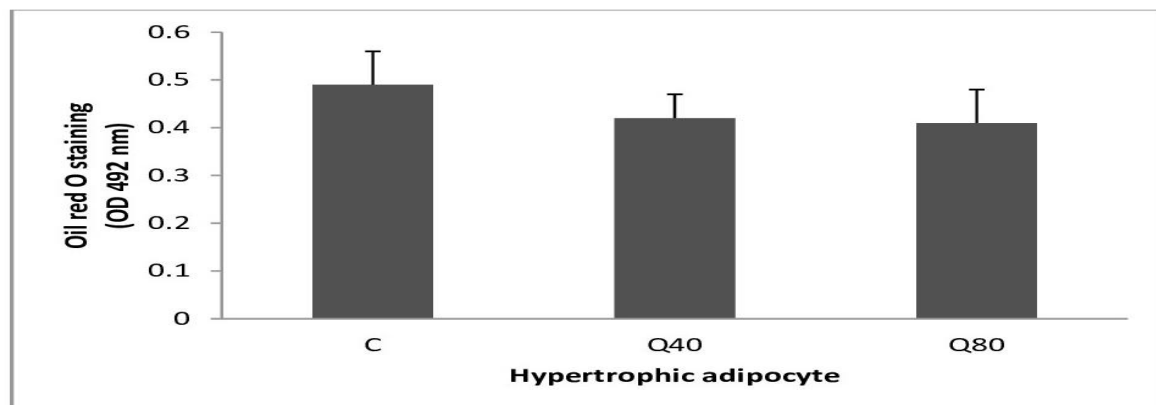
**Figure 2.** Oil-red-O stained inverted microscopy images of quercetin applied to mature and hypertrophic adipocytes (40 $\times$  magnification)

C: Control, Q40: 40  $\mu\text{M}$  Quercetin, Q80: 80  $\mu\text{M}$  Quercetin



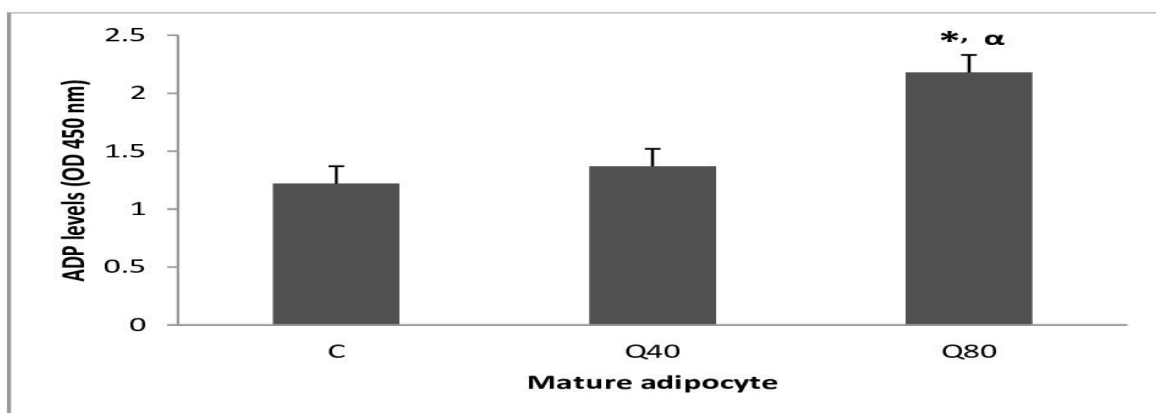
**Figure 3.** Lipid accumulation results of quercetin in mature adipocytes

Arithmetic means and standard deviation (Mean±SD) were used to express the results  
 Statistical significance was determined as  $p \leq 0.05$  are represented by the \*. \*: Significantly from mature control ( $p=0.0001$ ,  $F=125.842$ )  
 F: One Way Analysis of Variance, C: Control, Q40: 40  $\mu\text{M}$  Quercetin, Q80: 80  $\mu\text{M}$  Quercetin



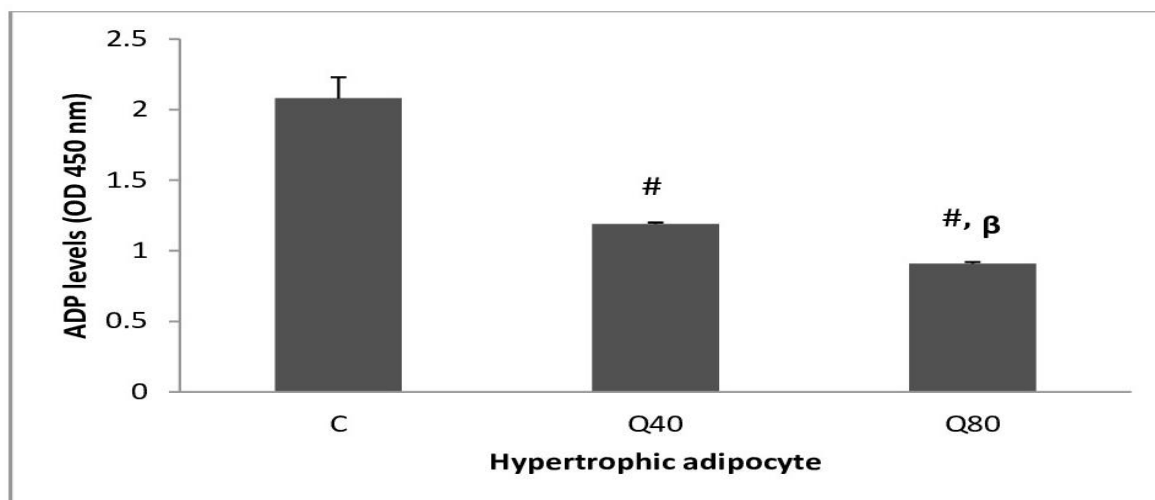
**Figure 4.** Lipid accumulation results of quercetin in hypertrophic adipocytes

Arithmetic means and standard deviation (Mean±SD) were used to express the results ( $p=0.369$ ,  $F=1.181$ ). F: One Way Analysis of Variance  
 C: Control, Q40: 40  $\mu\text{M}$  Quercetin, Q80: 80  $\mu\text{M}$  Quercetin



**Figure 5.** Effect of quercetin on ADP level in mature adipocytes

Arithmetic means and standard deviation (Mean±SD) were used to express the results  
 Statistical significance was determined as  $p \leq 0.05$  are represented by the \*, $\alpha$ . \*: Significantly from mature control  
 $\alpha$ : Significantly from mature Q40 ( $p=0.0001$ ,  $F=45.943$ ). F: One Way Analysis of Variance, C: Control, Q40: 40  $\mu\text{M}$  Quercetin  
 Q80: 80  $\mu\text{M}$  Quercetin



**Figure 6.** Effect of quercetin on ADP level in hypertrophic adipocytes

Arithmetic means and standard deviation (Mean±SD) were used to express the results

Statistical significance was determined as  $p \leq 0.05$  are represented by the #, β. #: Significantly from hypertrophic control

β: Significantly from hypertrophic Q40 ( $p=0.0001$ ,  $F=110.717$ ), F: One Way Analysis of Variance, C: Control, Q40: 40 μM Quercetin

Q80: 80 μM Quercetin

## Discussion

An energy imbalance between caloric intake and expenditure results in excessive fat formation, resulting in obesity. Obesity is an important and widespread public health problem globally due to its increasing incidence and adverse effects [14]. Obesity results in an increase in both the amount and size of adipocytes in the body. Adipocytes originate from preadipocytes and subsequently enlarge as they accumulate lipids. This process is often examined using models like the 3T3-L1 cell line [15].

Due to the challenges and long nature of managing obesity through diet, exercise, pharmacological interventions, and surgical procedures, alternative treatment methods have been explored, particularly the anti-obesity effects of polyphenols, which have long been studied for their health benefits in adipocytes. Tung et al. [16] demonstrated in their research that phytochemicals, such as polyphenols, reduce obesity in 3T3-L1 adipocytes by decreasing lipid accumulation via the activation of energy sensors (Adenosine monophosphate-activated protein kinase (AMPK), Mitogen-activated protein kinase (MAPK). Quercetin, a flavonoid belonging to the polyphenol group, is present in numerous vegetables, fruits, and plants, and has several health benefits, including anti-obesity actions [17]. Research on the 3T3-L1 cell line indicates that quercetin

administration decreases lipid accumulation during adipocyte differentiation [18] and in completely distinct adipocytes [19]. Research on obese rats indicates that quercetin reduces adipocyte size, enhances adipose tissue hypertrophy [20], and lowers lipid accumulation [21]. Our study's observation of reduced lipid levels following quercetin administration to mature adipocytes aligns with previous studies.

Furthermore, only one study examined the impact of quercetin on lipid accumulation in hypertrophic adipocytes, revealing that the application of 100 μM quercetin decreased triglyceride levels in these cells [9]. In contrast to these data, quercetin administration to hypertrophic adipocytes did not affect lipid accumulation according to this study. The data indicate that this may result from the increase in cell density and size when cells undergo hypertrophy (progressive obesity), and that the administered doses may be inadequate to diminish lipid accumulation. Consequently, we suggest that future studies ought to focus on the administration of quercetin treatment during the initial or initial stages of obesity.

Adipose tissue is thought to have both endocrine and metabolic capabilities, significantly contributing to the development of obesity and related metabolic problems through the secretion of adipokines [22]. ADP is a polypeptide containing 244 amino acids, produced by adipose tissue and generated

and released in elevated quantities by mature adipocytes. In recent years, there has been an increase in studies investigating the effect of ADP on adipose cells, which is closely related to the size and structure of adipose tissue and is an important indicator of metabolic health [23-27].

Research investigating the impact of polyphenols on ADP levels in mature adipocytes revealed that the application of lipoic acid [23] inhibited ADP secretion, but the application of kaempferitrin [24] enhanced ADP secretion. Furthermore, the investigation of quercetin's impact on ADP levels in mature adipocytes revealed using ELISA that concentrations of 12.5  $\mu$ M [25] and 5, 10, and 20  $\mu$ M quercetin [26] elevated ADP levels in one research. A further investigation revealed that 100  $\mu$ M quercetin elevated ADP mRNA levels as measured by PCR [27]. Our investigation revealed that the elevation of ADP levels after 24 hours of 80  $\mu$ M quercetin administration to mature adipocytes consistent with the existing literature. According to Hwang et al. [28] (2009), AMPK is a crucial sensor for energy metabolism. In the literature, through dose-dependent phosphorylation, quercetin activated AMPK [9]. We speculate that by triggering AMPK signaling, quercetin may have raised ADP.

In the literature, adipocytes becoming mature is defined as the early stage of obesity, and becoming hypertrophic is defined as advanced obesity or insulin-resistant obesity model [10, 11]. In line with these findings, our study showed that the use of quercetin in the early stage of obesity may be more appropriate in terms of targeted effects. In advanced obesity, it can be said that quercetin is insufficient to produce the expected beneficial effects in terms of dose, duration and changing metabolic processes. This situation can be considered as one of the limitations of our study, in order to make clearer comments, it would be appropriate to test hypertrophic cells with quercetin at higher doses and different treatment durations and also to evaluate other pathways (such as AMPK, hormone sensitive lipase (HSL), Perilipin-1 (PLIN1), etc.). Our study's results from the mouse cell line could lead to variations in human adaption, which is another limitation.

As a result, it shows that similar metabolic functions may occur through different mechanisms in adipocytes undergoing hypertrophy and that further studies are needed to elucidate these mechanisms.

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**Authors contributions:** Conceptualization, E.K.T., M.T.A. and G.A. Formal analysis, M.T.A. and G.A. Investigation, G.A., M.T.A. and E.K.T. Methodology, E.K.T. Project administration, E.K.T. and G.A. Supervision, E.K.T. and M.T.A. Writing – original draft, E.K.T., M.T.A. and G.A. Writing – review & editing, E.K.T., M.T.A. and G.A.

**Conflict of interest:** The authors declare that there is no conflict of interest.

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