Research Article

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Area of Expertise: Medical Physiology

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

Short title: Quercetin on lipid accumulation in 3T3-L1 cells.

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 µM) 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 ($p \leq 0.05$). The administration of 80 µM quercetin in mature adipocytes caused a significant increase in ADP levels ($p \leq 0.05$). Administration of quercetin to hypertrophic adipocytes caused a significant decrease in ADP levels ($p \leq 0.05$).

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.

Makale başlığı: Quercetin'in 3T3-L1 adipositlerinde lipid birikimi ve adiponektin düzeyi üzerindeki etkisinin araştırılması.

Kısa başlık: 3T3-L1 hücrelerinde quercetinin lipid birikimi ve adiponektin üzerine etkisi.

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, quercetinin olgun ve hipertrofik adipositlerde lipit 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 µM), olgun adipositlere 24 saat, hipertrofik adipositlere ise 48 saat boyunca uygulandı. Oil-Red-O boyama yöntemiyle lipit miktarı görüntülendi. ELISA yöntemi aracılığıyla lipit 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 lipit birikimini anlamlı olarak azalttı ($p \leq 0.05$). Olgun adipositlerde 80 µM quercetin uygulaması, ADP seviyelerinde anlamlı bir artışa yol açtı ($p \leq 0,05$). Hipertrofik adipositlere quercetin uygulaması ise, ADP seviyelerinde anlamlı bir azalmaya yol açtı ($p \leq 0,05$).

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

Anahtar kelimeler: Adiponektin, adiposit, lipit birikimi, obezite, quercetin.

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_2 to create experimental groups. When the cells reached approximately 80% density in the culture dishes, after cell counting, 10^5 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 µM [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.

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 triglyserid 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±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 μM doses of quercetin to mature adipocytes significantly reduced lipid accumulation compared to mature control (C: 0.21±0.01; Q40: 0.14±0.01; Q80: 0.12±0.01; p=0.0001) (Figure 3). Although 40 and 80 μM 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±0.07; Q40: 0.42±0.05; Q80: 0.41±0.07) (Figure 4). Administration of 80 μM quercetin to mature adipocytes significantly increased ADP levels compared to both mature control and 40 μM quercetin groups (C: 1.22±0.15; Q40: 1.37±0.15; Q80: 2.18±0.15; p=0.0001) (Figure 5). Administration of 40 and 80 μM quercetin to hypertrophic adipocytes significantly decreased ADP level compared to hypertrophic control (C:2.08±0.15, Q40:1.19±0.01, Q80:0.91±0.01, p=0.0001). In hypertrophic adipocytes, 80 μM quercetin significantly decreased ADP levels compared to 40 μM quercetin (Q40:1.19±0.01, Q80:0.91±0.01, p=0.001) (Figure 6).

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 µM [25] and 5, 10, and 20 µM quercetin [26] elevated ADP levels in one research. A further investigation revealed that 100 µM quercetin elevated ADP mRNA levels as measured by PCR [27]. Our investigation revealed that the elevation of ADP levels after 24 hours of 80 µM quercetin administration to mature adipocytes consistent with the existing literature. According to Hwang et al. (2009) [28], 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.

References

1. Kopelman PG. Obesity as a medical problem. *Nature*. 2000;404(6778):635-643. doi:10.1038/35007508
2. Oates EH, Antoniewicz MR. Coordinated reprogramming of metabolism and cell function in adipocytes from proliferation to differentiation. *Metab Eng*. 2022;69:221-230. doi:10.1016/j.ymben.2021.12.005

3. Rosen ED, Spiegelman BM. Adipocytes as regulators of energy balance and glucose homeostasis. *Nature*. 2006;444(7121):847-853. doi:10.1038/nature05483
4. Hwang JT, Park IJ, Shin JI, et al. Genistein, EGCG, and capsaicin inhibit adipocyte differentiation process via activating AMP-activated protein kinase. *Biochem Biophys Res Commun*. 2005;338(2):694-699. doi:10.1016/j.bbrc.2005.09.195
5. Rayalam S, Yang JY, Ambati S, DellaFera MA, Baile CA. Resveratrol induces apoptosis and inhibits adipogenesis in 3T3-L1 adipocytes. *Phytother Res*. 2008;22(10):1367-1371. doi:10.1002/ptr.2503
6. Ahn J, Lee H, Kim S, Park J, Ha T. The anti-obesity effect of quercetin is mediated by the AMPK and MAPK signaling pathways [published correction appears in Biochem Biophys Res Commun. 2011 Jan 7;404(1):579]. *Biochem Biophys Res Commun*. 2008;373(4):545-549. doi:10.1016/j.bbrc.2008.06.077
7. Yahfoufi N, Alsadi N, Jambi M, Matar C. The Immunomodulatory and Anti-Inflammatory Role of Polyphenols. *Nutrients*. 2018;10(11):1618. Published 2018 Nov 2. doi:10.3390/nu10111618
8. Herranz López M, Borrás Linares I, Olivares Vicente M, Gálvez J, Segura Carretero A, Micol V. Correlation between the cellular metabolism of quercetin and its glucuronide metabolite and oxidative stress in hypertrophied 3T3-L1 adipocytes. *Phytomedicine*. 2017;25:25-28. doi:10.1016/j.phymed.2016.12.008
9. Herranz López M, OlivaresVicente M, Rodríguez Gallego E, et al. Quercetin metabolites from Hibiscus sabdariffa contribute to alleviate glucolipotoxicity-induced metabolic stress in vitro. *Food Chem Toxicol*. 2020;144:111606. doi:10.1016/j.fct.2020.111606
10. Herranz López M, Fernández Arroyo S, Pérez Sanchez A, et al. Synergism of plant-derived polyphenols in adipogenesis: perspectives and implications. *Phytomedicine*. 2012;19(3-4):253-261. doi:10.1016/j.phymed.2011.12.001
11. Yeop Han C, Kargi AY, Omer M, et al. Differential effect of saturated and unsaturated free fatty acids on the generation of monocyte adhesion and chemotactic factors by adipocytes: dissociation of adipocyte hypertrophy from inflammation. *Diabetes*. 2010;59(2):386-396. doi:10.2337/db09-0925
12. Cave E, Crowther NJ. The Use of 3T3-L1 Murine Preadipocytes as a Model of Adipogenesis. *Methods Mol Biol*. 2019;1916:263-272. doi:10.1007/978-1-4939-8994-2_25
13. Li T, Zhang L, Jin C, Xiong Y, Cheng YY, Chen K. Pomegranate flower extract bidirectionally regulates the proliferation, differentiation and apoptosis of 3T3-L1 cells through regulation of PPARγ expression mediated by PI3K-AKT signaling pathway. *Biomed Pharmacother*. 2020;131:110769. doi:10.1016/j.biopha.2020.110769

14. Blüher M, Aras M, Aronne LJ, et al. New insights into the treatment of obesity. *Diabetes Obes Metab*. 2023;25(8):2058-2072. doi:10.1111/dom.15077
15. Ruiz Ojeda FJ, Rupérez AI, Gomez Llorente C, Gil A, Aguilera CM. Cell Models and Their Application for Studying Adipogenic Differentiation in Relation to Obesity: A Review. *Int J Mol Sci*. 2016;17(7):1040. Published 2016 Jun 30. doi:10.3390/ijms17071040
16. Tung YC, Hsieh PH, Pan MH, Ho CT. Cellular models for the evaluation of the antiobesity effect of selected phytochemicals from food and herbs. *J Food Drug Anal*. 2017;25(1):100-110. doi:10.1016/j.jfda.2016.10.018
17. D'Andrea G. Quercetin: A flavonol with multifaceted therapeutic applications?. *Fitoterapia*. 2015;106:256-271. doi:10.1016/j.fitote.2015.09.018
18. Yang JY, Della Fera MA, Rayalam S, et al. Enhanced inhibition of adipogenesis and induction of apoptosis in 3T3-L1 adipocytes with combinations of resveratrol and quercetin. *Life Sci*. 2008;82(19-20):1032-1039. doi:10.1016/j.lfs.2008.03.003
19. Eseberri I, Miranda J, Lasa A, et al. Effects of Quercetin Metabolites on Triglyceride Metabolism of 3T3-L1 Preadipocytes and Mature Adipocytes. *Int J Mol Sci*. 2019;20(2):264. Published 2019 Jan 11. doi:10.3390/ijms20020264
20. Perdicaro DJ, Rodriguez Lanzi C, Gambarte Tudela J, Miatello RM, Oteiza PI, Vazquez Prieto MA. Quercetin attenuates adipose hypertrophy, in part through activation of adipogenesis in rats fed a high-fat diet. *J Nutr Biochem*. 2020;79:108352. doi:10.1016/j.jnutbio.2020.108352
21. Ting Y, Chang WT, Shiau DK, Chou PH, Wu MF, Hsu CL. Antiobesity Efficacy of Quercetin-Rich Supplement on Diet-Induced Obese Rats: Effects on Body Composition, Serum Lipid Profile, and Gene Expression. *J Agric Food Chem*. 2018;66(1):70-80. doi:10.1021/acs.jafc.7b03551
22. Govindaraj J, Govindaraj K, Jayesh SR, Padmavathy K, Ramalingam M, U V. Role of adipokines in obesity and obesity-related metabolic disorders. *Res J Pharm Technol*. 2021;14(4261-4264). doi:10.52711/0974-360X.2021.00739
23. Prieto Hontoria PL, Fernández Galilea M, Pérez Matute P, Martínez JA, Moreno Aliaga MJ. Lipoic acid inhibits adiponectin production in 3T3-L1 adipocytes. *J Physiol Biochem*. 2013;69(3):595-600. doi:10.1007/s13105-012-0230-7
24. Tzeng YM, Chen K, Rao YK, Lee MJ. Kaempferitrin activates the insulin signaling pathway and stimulates secretion of adiponectin in 3T3-L1 adipocytes. *Eur J Pharmacol*. 2009;607(1-3):27-34. doi:10.1016/j.ejphar.2009.01.023
25. Ahmed QU, Sarian MN, Mat So'ad SZ, et al. Methylation and Acetylation Enhanced the Antidiabetic Activity of Some Selected Flavonoids: In Vitro, Molecular Modelling and

Structure Activity Relationship-Based Study. *Biomolecules*. 2018;8(4):149. Published 2018 Nov 15. doi:10.3390/biom8040149

26. Sannappa Gowda NG, Shiragannavar VD, Karunakara SH, et al. Novel role of Quercetin in ameliorating metabolic syndrome via VDR mediated activation of adiponectin/AdipoR2 signaling. *Biochem Biophys Rep*. 2024;39:101754. Published 2024 Jun 21. doi:10.1016/j.bbrep.2024.101754
27. Noori Z, Sharifi M, Dastghaib S, et al. Quercetin declines LPS induced inflammation and augments adiponectin expression in 3T3-L1 differentiated adipocytes SIRT-1 dependently. *Mol Biol Rep*. 2024;51(1):445. Published 2024 Mar 23. doi:10.1007/s11033-024-09334-7.
28. Hwang JT, Kwon DY, Yoon SH. AMP-activated protein kinase: a potential target for the diseases prevention by natural occurring polyphenols. *N Biotechnol*. 2009;26(1-2):17-22. doi:10.1016/j.nbt.2009.03.005.

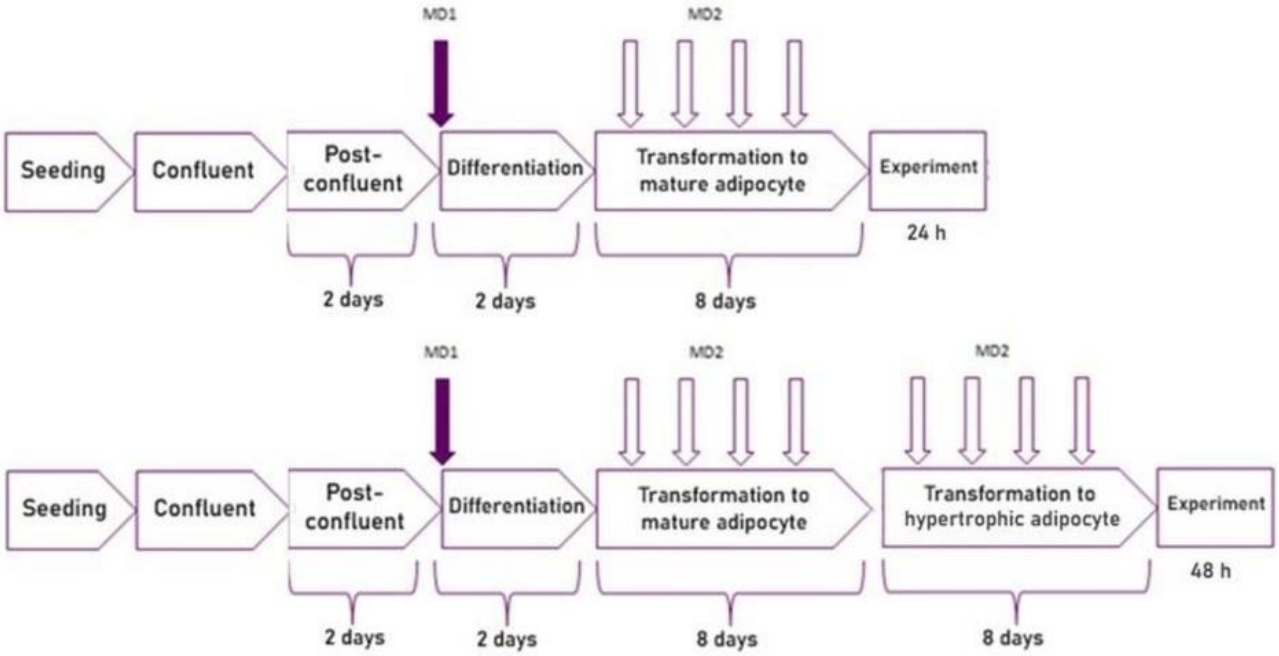

Figure 1. Demonstration of experimental design. Quercetin was applied at 40 and 80 μM dose. MD1: medium 1, MD2: medium 2.

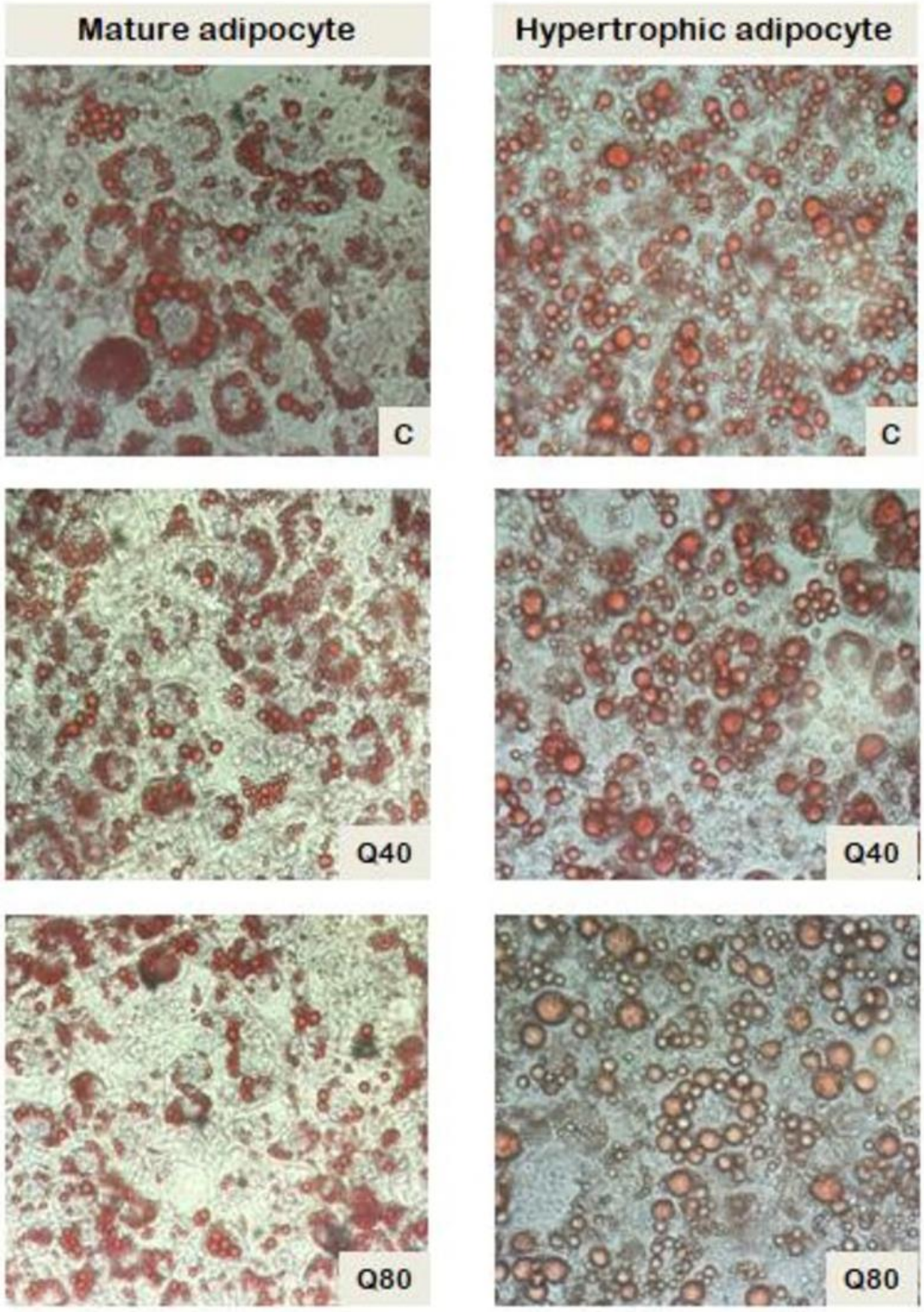

Figure2. Oil-red-O stained inverted microscopy images of quercetin applied to mature and hypertrophic adipocytes (40× magnification) (C: Control, Q40: 40 µM Quercetin, Q80: 80 µM Quercetin)

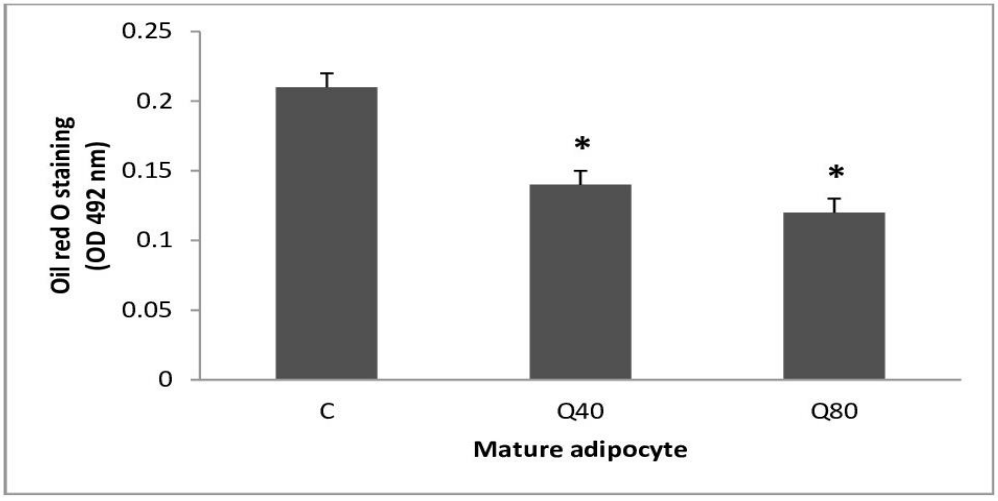

Figure 3. Lipid accumulation results of quercetin in mature adipocytes. Arithmetic means and standard deviation (Mean±SD) were used to express the results.
Statistical significance was determined as $p \leq 0.05$ are represented by the *.
*: Significantly from mature control (p=0.0001, F=125.842).
F: One Way Analysis of Variance, C: Control, Q40: 40 µM Quercetin, Q80: 80 µM Quercetin.

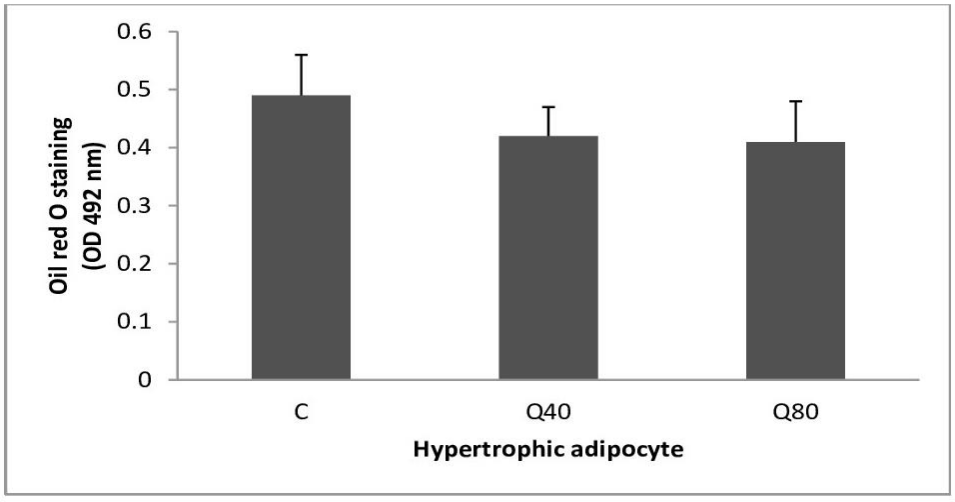

Figure 4. Lipid accumulation results of quercetin in hypertrophic adipocytes. Arithmetic means and standard deviation (Mean±SD) were used to express the results (p=0.369, F=1.181). F: One Way Analysis of Variance, C: Control, Q40: 40 µM Quercetin, Q80: 80 µM Quercetin

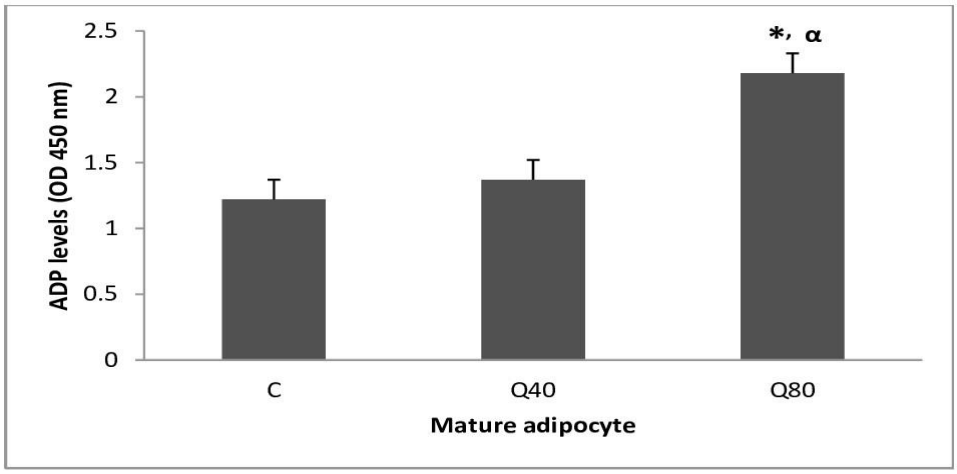

Figure5. Effect of quercetin on ADP level in mature adipocytes. Arithmetic means and standard deviation (Mean±SD) were used to express the results. Statistical significance was determined as $p \leq 0.05$ are represented by the $^{*,\alpha}$.
*: Significantly from mature control.
$^{\alpha}$: Significantly from mature Q40 (p=0.0001, F=45.943).
F: One Way Analysis of Variance, C: Control, Q40: 40 µM Quercetin, Q80: 80 µM Quercetin

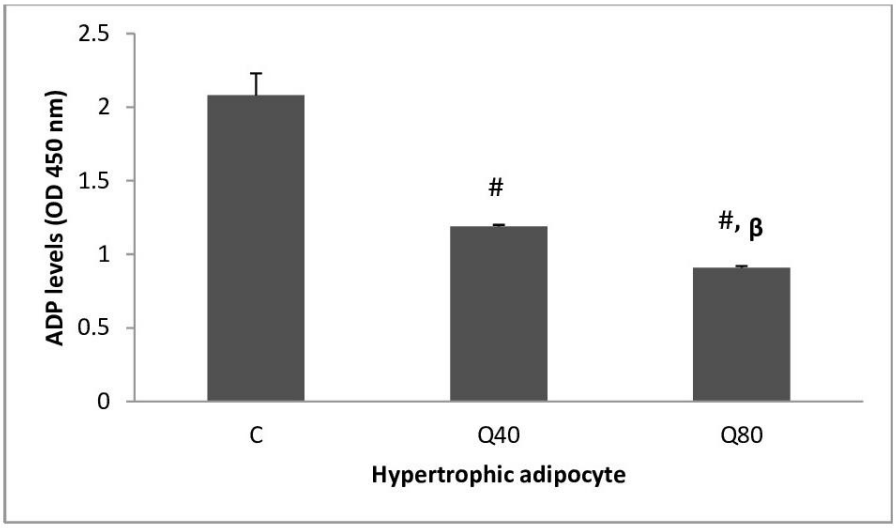

Figure 6. Effect of quercetin on ADP level in hypertrophic adipocytes. Arithmetic means and standard deviation (Mean±SD) were used to express the results. Statistical significance was determined as $p \leq 0.05$ are represented by the $^{\#,\beta}$.
$^{\#}$: Significantly from hypertrophic control.
$^{\beta}$: 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

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Akan G, Tunç Ata M, Kılıç Toprak E. Quercetin'in 3T3-L1 adipositlerinde lipid birikimi ve adiponektin düzeyi üzerindeki etkisinin araştırılması. Pam Tıp Derg 2025;18:…-…

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