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Effect of quercetin on oxidative stress in 3T3-L1 mature and hypertrophic cells

Quercetin'in 3T3-L1 olgun ve hipertrofik hücrelerde oksidatif stres üzerine etkisi

Melek Tunç Ata, Emine Kılıç Toprak, Gizem Akan

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Abstract

Purpose: The process of excessive or abnormal accumulation of fat in the body is called obesity, and its prevalence is increasing globally. The imbalance between antioxidants and free radicals, or oxidative stress, can be caused by or result from obesity. Flavonoids with antioxidant potential may help lower the increased oxidative stress associated with obesity. This study aimed to determine how quercetin affected oxidative stress in hypertrophied and mature 3T3-L1 adipocytes.

Materials and methods: After differentiating, 3T3-L1 adipocytes were treated with insulin and a glucose-containing medium to become mature (10 days) and hypertrophic (18 days). The cells were subsequently incubated with 80 µM quercetin for 24 and 48 hours. ELISA was used to determine the levels of total antioxidant total oxidant capacity (TAS/TOS). Using Oil Red O staining, an accumulation of triglycerides in cells was examined.

Results: The results showed that quercetin molecule only increased TAS level on oxidative stress in mature adipocytes (TAS; M-C: 649.37±1.38; M-Q80: 655.87±1.68 *p*=0.0001), whereas it exerted a prooxidative effect in hypertrophic adipocytes (OSI; H-C: 4.90±0.19; H-Q80: 6.20±0.039 *p*=0.0001).

Conclusion: It is believed that the administration of quercetin at the appropriate dose and duration in different fat cell types is crucial for the antioxidant mechanism of action that produces numerous beneficial effects.

Keywords: Obesity, 3T3-L1 cell, quercetin, oxidative stress, oxidant/antioxidant level.

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Öz

Amaç: Bütün dünyada sıklığı giderek artan obezite, vücutta aşırı veya anormal yağ birikmesi sürecidir. Oksidatif stres; serbest radikaller ve antioksidanlar arasındaki dengesizlik olup, bu durum obezitenin bir sonucu olabileceği gibi aynı zamanda obezitenin bir tetikleyicisi de olabilir. Obezite durumunda artan oksidatif stres, antioksidan kapasiteye sahip flavanoidler tarafından azaltılabilir. Bu araştırmanın amacı, olgun ve hipertrofik hale getirilmiş 3T3-L1 adipositlerde quercetinin oksidatif stres üzerine etkisinin belirlenmesidir.

Gereç ve yöntem: Önce farklılaştırılan ve daha sonra glikoz içeren medyum ve insülin ile muamele edilerek mature (10 gün) ve hipertrofik (18 gün) hale getirilen 3T3-L1 adipositleri 80 µM dozda quercetin (24saat, 48saat) ile inkübe edildi. Total antioksidan total oksidan kapasite (TAS/TOS) düzeyleri ELISA aracığılıyla ölçüldü. Oil Red O boyama ile hücrelerde oluşan trigliserit birikimi analiz edildi.

Bulgular: Sonuçlar quercetin molekülünün olgun adipositlerde oksidatif stres üzerine yalnızca TAS düzeyini arttırdığını gösterdi (TAS; M-C: 649,37±1,38; M-Q80: 655,87±1,68 *p*=0,0001), hipertrofik adipositlerde ise prooksidatif etki yaptığını gösterdi (OSI; H-C: 4,90±0,19; H-Q80: 6,20±0,039 *p*=0,0001).

Sonuç: Quercetin'in farklı yağ hücre türlerinde uygun doz ve sürede uygulanmasının çok sayıda yararlı etki üreten antioksidan etki mekanizması için belirleyici olduğuna inanılmaktadır.

Anahtar kelimeler: Obezite, 3T3-L1 cell, quercetin, oksidatif stres, oksidan/antioksidan seviye.

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Melek Tunç Ata, Ph.D. Department of Physiology, Faculty of Medicine, Pamukkale University, Denizli, Türkiye, e-mail: melekt@pau.edu.tr (https://orcid.org/0000-0002-0384-2356) (Corresponding Author)

Emine Kılıç Toprak, Assoc. Prof. Department of Physiology, Faculty of Medicine, Pamukkale University, Denizli, Türkiye, e-mail: ektoprak@pau.edu.tr (https://orcid.org/0000-0002-8795-0185)

Gizem Akan, PT. Department of Physiology, Faculty of Medicine, Pamukkale University, Denizli, Türkiye, e-mail: gakan22@posta.pau.edu.tr (https://orcid.org/0009-0000-3336-102X)

Introductıon

Around the world, there is a rising prevalence of obesity and disorders associated with it. Adipose tissue enlargement, or obesity, is closely linked to long-term metabolic conditions such type 2 diabetes mellitus (T2DM) and cardiovascular illnesses [1]. The combination of hyperplasia and hypertrophy results in an increase in adipose tissue, which is characterized by an excessive accumulation of lipids and ultimately leads to adipose tissue dysfunction [2, 3]. Adipogenesis is the process by which a preadipocyte differentiates into a mature adipocyte through the accumulation of lipid droplets; however, obesity is the result of excessive lipid accumulation [4]. Obese people have high levels of genotoxic stress. Hyperlipidemia is linked to alterations in lipoprotein distribution and systemic oxidative stress in both humans [5] and lab animals [6].

The fundamental mechanism behind the chronic state of systemic inflammation that these individuals display is not entirely understood, however it is commonly related to cellular stress [7]. The methods used today for preventing obesity include increased energy expenditure through physical exercise, medication, bariatric surgery, and calorie restriction by dietary modifications [8]. Quercetin, a flavonoid, is frequently found in onion peels, wine, and tea [9]. Recent studies have demonstrated that Quercetin has the potential to reduce body fat. The suggested mechanism of action for its favorable effects includes its impact on lipolysis, apoptosis, fatty acid absorption, inhibition of adipogenesis, and reduction of lipogenesis [10, 11]. Treatment with quercetin in adipocytes triggers the apoptosis pathway by regulating AMP-activated protein kinase (AMPK) [11]. Alternatively, it inhibits the expression of transcription factors involved in adipocyte differentiation, such as peroxisomal proliferator-activated receptors (PPAR)-γ, CCAAT/enhancer-binding protein (C/EBP) α, fatty acid-binding protein 4, and adipocyte protein 2 (aP2) [12]. The 3T3-L1 preadipocyte cell line is an important tool for investigating the in vitro processes by which obesogens can impact lipid accumulation and adipocyte development [13, 14]. This study aimed to assess the impact of quercetin on oxidative stress in hypertrophic 3T3L-1 cells caused by high glucose.

Therefore, this study may make an important contribution to the literature in terms of revealing the fine details of the antioxidant or prooxidant potential of quercetin.

Materials and methods

Cell culture

In our study, 3T3-L1 cell line (ATCC® CL173™), a fibroblast cell line obtained from mouse (Mus musculus) embryo, which is also known as preadipocyte, was used. Mouse 3T3-L1 fibroblasts were maintained in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, USA, Waltham) containing 25 mM glucose supplemented with 10% calf serum (Cegrogen, Germany) 100 U/ml penicillin (Wisent, Saint-Jean-Baptiste, Canada) 100 lg/ml streptomycin (Wisent, Saint-Jean-Baptiste, Canada) and 2 mM L-glutamine at 37°C in a humidified atmosphere of 5% CO_2. The cells were propagated by transferring them to new culture dishes every 3-4 days until they reached around 80% coverage.

Differentiation of the 3T3-L1 cell line

In brief, cells were first cultured in low-glucose medium to create the experimental groups. Cells were followed with the same medium until confluent. When the cells reached approximately 80% density in the culture dishes, a medium containing a differentiation cocktail 3-Isobutyl-1-methylxanthine (IBMX, Sigma I5879, USA) dexamethasone (DEX, Sigma D4902, USA), insulin (INS) (Sigma I6634, USA) 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.

Mature/Hypertrophic adipocyte model

After incubation, the medium containing (Gibco, USA, Waltham) glucose and INS (MD2) was changed every other day for 10 days mature and 18 days for hypertrophic and the cells were monitored. Thus, the diameter and number of lipid droplets in adipocytes increased.

Oil Red O staining

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) Staining Kit (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 20X and 40X magnifications.

Administration of quercetin

Quercetin 80 µM [15] 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.

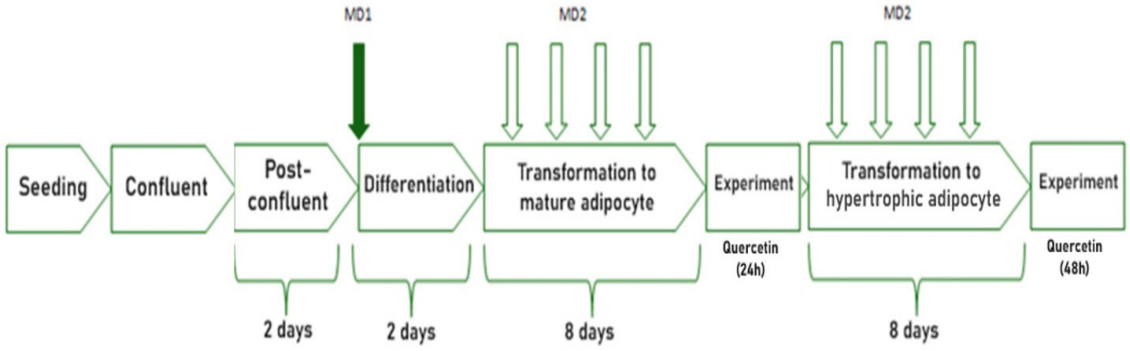

Figure 1. Illustration of experimental design.Quercetin was administered 80 µM dose

For medium 1, DMEM containing high glucose (4.5 g/L) was prepared by adding 0.5 mM IBMX, 1 µM DEX, 10 µg/mL INS, 10% FBS, 1% P/S solution. For medium 2, DMEM containing high glucose (4.5 g/L) was prepared by adding 10µg/ml INS, 10% Fetal bovine serum (FBS), 1% P/S solution. IBMX; 3-Isobutyl-1-methylxanthine, DEX; dexamethasone, INS; insulin, FBS; fetal bovine serum, P/S; penicilin/streptomycin, DMEM; Dulbecco's Modified Eagle Medium

Measurements of total antioxidant status

Following a treatment of 3T3-L1 mature and hypertrophic cells with 80 µM Q for 24 and 48 hours, the cell culture media was removed. The Fenton reaction generates the most powerful biological radical, namely the hydroxyl radical. The hydroxyl radical reacted with the colorless substrate o-dianisidine to produce the diansyl radical, which exhibits a prominent yellowish-brown hue. This approach is utilized to assess the overall antioxidant response to potent free radical reactions. When a sample of cell culture medium is introduced into the reaction mixture, the antioxidant components hinder the oxidative processes initiated by the hydroxyl radicals in the reaction mixture, hence preventing any alteration in color and offering a reliable method to measure the overall level of antioxidant capacity (TAS). The assay results are reported in mmol Trolox Equiv./L (RelAssay, Türkiye, Gaziantep).

Measurements of total oxidant status

The colorimetric examine was used to measure the amounts of total oxidant status (TOS). The ferrous ion-o-dianisidine complex underwent oxidation to form the ferric ion. The presence of glycerol molecules enhanced the oxidation reaction, which was inadequate in the reaction media. At the conclusion of the reaction, an intense complex formed between xylenol orange and the ferric ion in the acidic

environment. The concentration of oxidant molecules in the sample is directly proportional to the intensity of its color, which is quantified using spectrophotometry. The calibrations were conducted utilizing hydrogen peroxide, and the results were expressed in micromoles of H_2O_2 equivalent per liter (RelAssay, Türkiye, Gaziantep).

Determination of oxidative stress index

The oxidative stress index (OSI) was calculated by the following formula: OSI (arbitrary unit) = TOS (μmol)/TAS (mmol)×100.

Statistical analysis

The data were analyzed with the software package SPSS 25.0. Continuous variables are expressed as the mean±standard deviation. Shapiro Wilk test was used for normality testing. Independent samples t test was used for comparing independent groups. In all analyses, $p \leq 0.05$ was considered statistically significant.

Results

In order to analyze the suitability of the experimental model, an Oil Red O was performed on mature and hypertrophic adipocytes, and the results were evaluated qualitatively. It was shown that lipid accumulation increased towards hypertrophic adipocytes. The visual difference between preadipocytes, mature adipocytes, and hypertrophic adipocytes confirmed the suitability of the model for lipid accumulation (Figure 2).

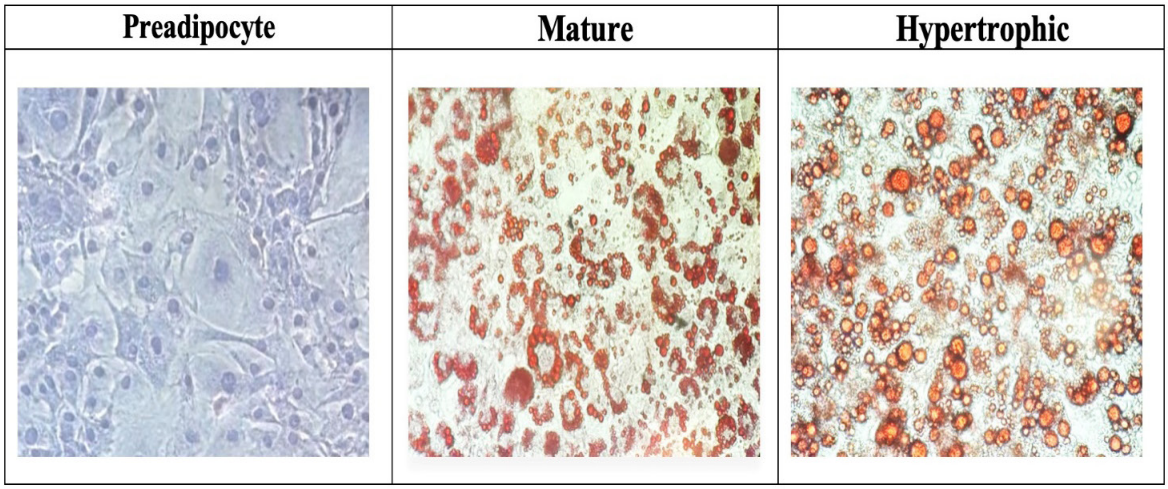

Figure 2. Representative inverted microscopy images (20× and 40× magnification)

Mature and hypertrophic cells before differentiation, after 10 and 18 days of differentiation stained with Oil Red O

The oxidant and antioxidant responses obtained after the application of 80 μM quercetin were examined in mature and hypertrophic 3T3-L1 cells. TAS level was significantly higher in the M-Q80 (655.87±1.68) group compared to the M-C (649.37±1.38) (p=0.0001; t=-6.683) group. No significant difference was seen between groups in terms of total oxidant status (TOS, M-C: 50.39±1.54; M-Q80: 50.62±1.63, p=0.828, t=-0.225), and oxidative stress index (OSI, M-C: 7.76±0.24; M-Q80: 7.72±0.25, p=0.791, t=0.275) levels after a 24-hour treatment with quercetin (Figure 3-5). After a 48-hour application of quercetin to hypertrophic cells, the TAS level was significantly lower in the H-Q80 (804.62±1.85) group compared to the H-C (884.48±1.26) (p=0.0001; t=79.907) group, while the TOS and OSI levels were significantly higher in the H-Q80 (TOS: 49.91±71.15; p=0.0001, t=-7.065; OSI: 6.20±0.39, p=0.0001, t=0.688) group compared to the H-C (TOS: 43.36±1.72, p=0.0001; OSI: 4.90±0.19, p=0.0001).

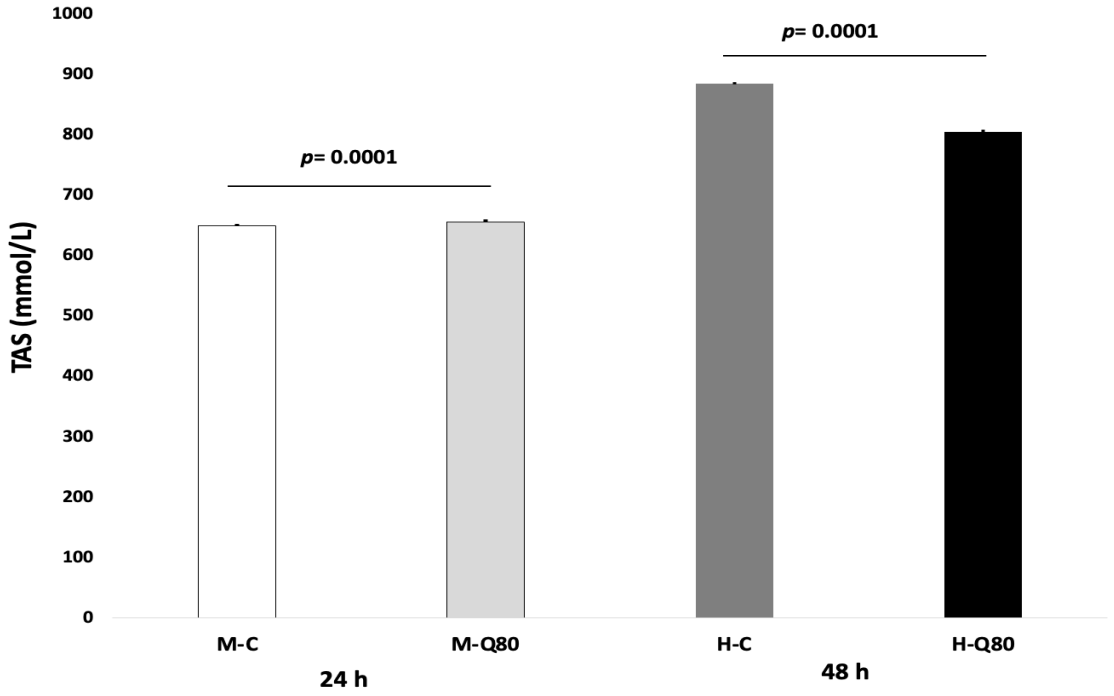

Figure 3. TAS results in mature (24 h) and hypertrophic (48 h) 3T3L-1 cells treated with quercetin

Arithmetic mean and the standard deviation is used to express the results. $p \leq 0.05$ is considered statistically significant. Independent samples t test was used to analyze the data. M-C: Mature control group, M-Q80: Mature 80μM quercetin group, H-C: Hypertrophic control group H-Q80: Hypertrophic 80μM quercetin group

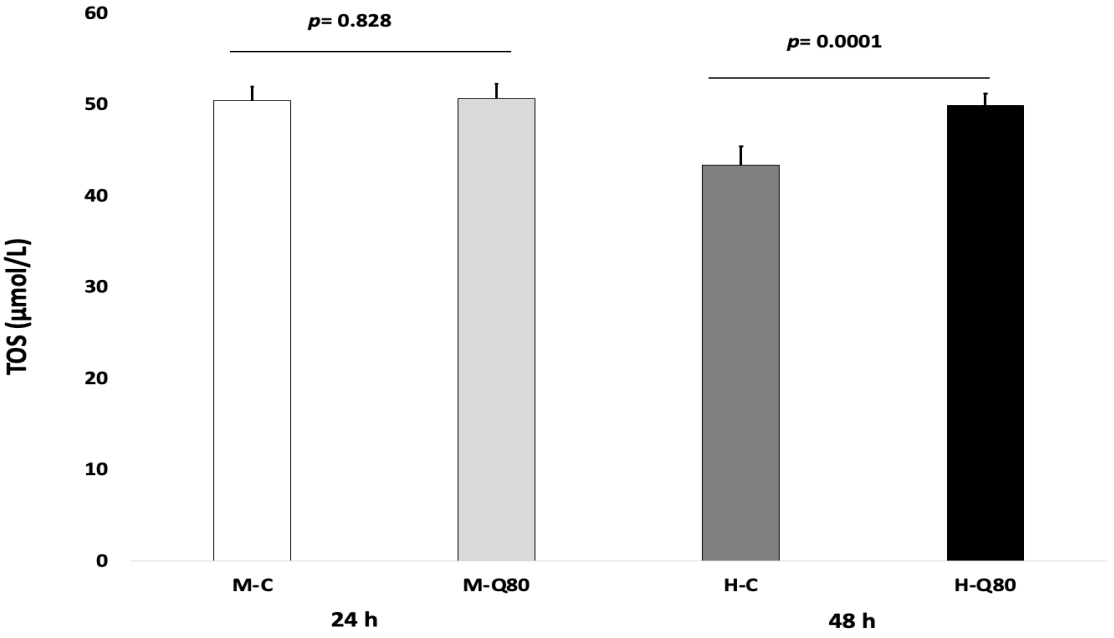

Figure 4. TOS results in mature (24 h) and hypertrophic (48 h) 3T3L-1 cells treated with quercetin

Arithmetic mean and the standard deviation is used to express the results. $p \leq 0.05$ is considered statistically significant. Independent samples t test was used to analyze the data. M-C: Mature control group, M-Q80: Mature 80μM quercetin group, H-C: Hypertrophic control group H-Q80: Hypertrophic 80μM quercetin group

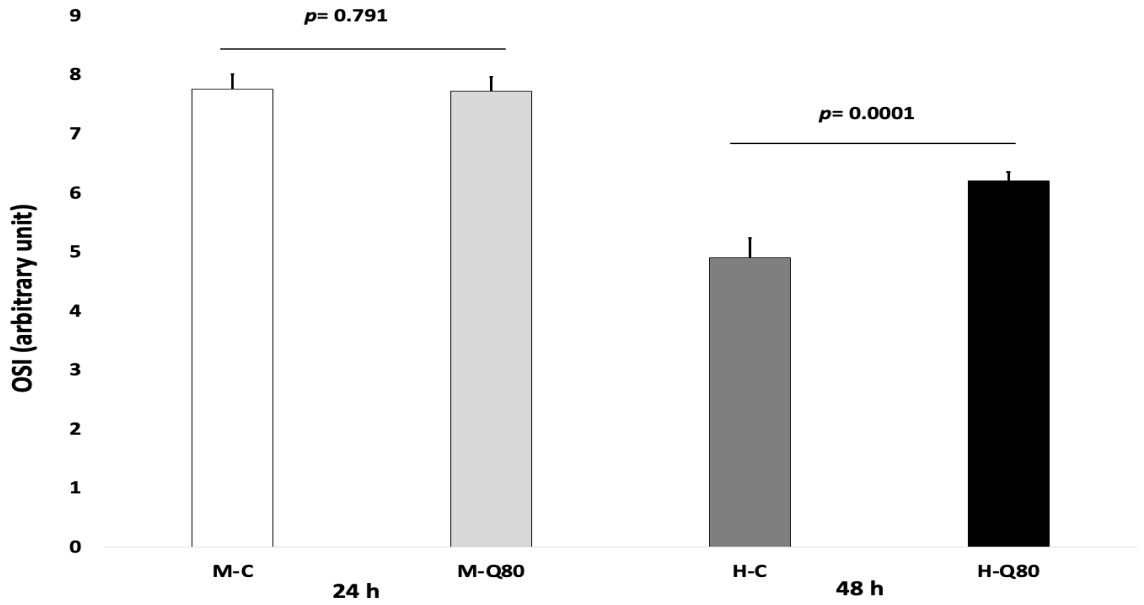

Figure 5. OSI results in mature (24 hours) and hypertrophic (48 hours) 3T3L-1 cells treated with quercetin

Arithmetic mean and the standard deviation is used to express the results. $p \leq 0.05$ is considered statistically significant. Independent samples t test was used to analyze the data. M-C: Mature control group, M-Q80: Mature 80 µM quercetin group, H-C: Hypertrophic control group H-Q80: hypertrophic 80 µM quercetin group

Discussion

Reactive oxygen species (ROS) and the cell's antioxidant defense system are out of balance, which leads to oxidative stress, one of the causes contributing to obesity [16]. It is highly interesting to study the biological effects of naturally occurring micronutrients that may enhance the body's antioxidant capacity because oxidative stress is an important component in obesity and related chronic diseases like T2DM. These substances, known as polyphenols, have demonstrated anti-inflammatory activity both in vitro and in vivo, making them useful as therapeutic tools in a variety of acute and chronic illnesses [17, 18]. In addition, their anti-oxidant features, including scavenging reactive oxygen species, these chemical compounds also have a role in regulating inflammatory signaling by modifying the expression of many pro-inflammatory genes, including lipoxygenase, nitric oxide synthases, and different cytokines [19, 20]. Additionally, they block several of the enzymes that cause ROS to develop [21]. Quercetin is one of these polyphenols. Numerous beneficial effects of quercetin, including anti-poliferative, anti-oxidant, anti-diabetic, and anti-obesity properties, have been documented in the literature through different mechanisms [22, 23]. In light of this, we aimed to examine how quercetin affected oxidative stress in hypertrophic and mature 3T3-L1 adipocyte cells. This study showed that quercetin has a pro-inflammatory effect in hypertrophic adipocytes, but may have a marked effect on oxidative stress by increasing only the total antioxidant level in 3T3-L1 mature adipocyte cells.

In our work, 3T3-L1 preadipocytes were stimulated to transform into mature, hypertrophic adipocytes by use of differentiation media. In matching previous research, the Oil Red O data confirmed an accumulation of lipids in these adipocytes [24, 25]. Quercetin increased antioxidant activity in mature cells compared to control cells. Oxidative stress is determined by the balance between ROS and antioxidants. Optimal redox conditions for most cells are achieved when high levels of antioxidants are present to quench and keep ROS at low levels, and a state of balance is achieved [26]. This

effect is referred to as the oxidant-antioxidant balance. According to this information, it was determined that although quercetin did not have a significant effect on the TOS level in mature cells, it could affect this balance. Quercetin has demonstrated strong antioxidant properties and the ability to scavenge free radicals in vitro studies [27]. Quercetin's antioxidant potential has been revealed through animal experiments. For instance, when quercetin is given to rodents, it results in increased antioxidant activity [28] and reduced lipid peroxidation [29]. A few small-scale human studies on quercetin supplementation have provided inconsistent results about its potential antioxidant properties. Several human investigations have indicated that quercetin does not have any impact on different indicators of antioxidant capacity and oxidative stress [30, 31].

Administration of quercetin to hypertrophic 3T3-L1 adipocytes was found to enhance the oxidative stress index. In cases of obesity and comorbidities, there is an increase in reactive oxygen species (ROS) and a decrease in antioxidants in fat cells. This imbalance leads to oxidative stress and other issues associated with obesity, such as insulin resistance and diabetes [26]. According to reports, quercetin has been found to enhance the activity of free radical scavengers such as superoxide dismutase (SOD), catalase, and glutathione in cases of obesity and related disorders [32, 33].

In vitro experiments on quercetin showed that the dosage and duration of administration of this flavonoid were different. In the study, Boadi et al. [34] demonstrated that administering quercetin (at concentrations ranging from 0 to 25 micromolar) for 24 hours increased the activity of glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rx), and superoxide dismutase (SOD) in oxidatively damaged 3T3L-1 cells. Noh et al. [35] demonstrated that giving 2, 10 micromolar quercetin to 3T3-L1-adipocytes for 6 and 16 hours effectively suppressed the production of macrophage inflammatory protein (MIP)-1α from hypertrophic adipocytes, which is a known trigger for adipose tissue inflammation. According to a report, this result was found to be beneficial in preventing inflammation of adipose tissue caused by obesity. Another study shown that high concentrations of quercetin led to a gradual decrease in the total antioxidant capacity of cell extracts [36]. In this study, we believe that the administration of 80 μM quercetin dose over 48 hours in these cells has a time- and dose-dependent prooxidative effect.

In conclusion, although the mechanisms of action of polyphenols are still unclear, they are seen as an innovative approach for the prevention and treatment of metabolic diseases, including obesity. The use of these polyphenols at the right dose and duration is very important for a clearer explanation of their mechanisms. Future studies should investigate the dose-duration relationship in more detail to better understand the effects of quercetin at the cellular level and determine the optimal conditions for the use of quercetin in cell models. Furthermore, clinical studies evaluating the potential therapeutic effects of quercetin on metabolic health are needed. Such studies may contribute to our understanding of the biological effects of quercetin and clarify the role of this natural compound in health.

This study has limitations. The results of the study are based on ELISA results. These results need to be supported by other experiments (DNA/RNA damage, ROS levels).

Conflict of interest: No conflict of interest was declared by the authors.

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Author contributions

Conceptualization, M.T.A., E.K.T. and G.A. Data curation, M.T.A., E.K.T. and G.A. Formal analysis, E.K.T. and G.A. Investigation, M.T.A., E.K.T. and G.A. Methodology, M.T.A., E.K.T. and G.A. Supervision, E.K.T. Validation, M.T.A., E.K.T and G.A. Visualization, M.T.A. and G.A. Writing – original draft, M.T.A. and G.A. Reviewing, M.T.A. and G.A. All authors have read and agree to the published version of the manuscript.