

Article

# Quercetin and Green Tea Extract Supplementation Downregulates Genes Related to Tissue Inflammatory Responses to a 12-Week High Fat-Diet in Mice

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**Abstract:** Quercetin (Q) and green tea extract (E) are reported to counter insulin resistance and inflammation and favorably alter fat metabolism. We investigated whether a mixture of E + Q (EQ) could synergistically influence metabolic and inflammation endpoints in a high-fat diet (HFD) fed to mice. Male C57BL/6 mice ( $n = 40$ ) were put on HFD (fat = 60%kcal) for 12 weeks and randomly assigned to Q (25 mg/kg of body weight (BW)/day), E (3 mg of epigallocatechin gallate/kg BW/day), EQ, or control groups for four weeks. At 16 weeks, insulin sensitivity was measured via the glucose tolerance test (GTT), followed by area-under-the-curve (AUC) estimations. Plasma cytokines and quercetin were also measured, along with whole genome transcriptome analysis and real-time polymerase chain reaction (qPCR) on adipose, liver, and skeletal muscle tissues. Univariate analyses were conducted via analysis of variance (ANOVA), and whole-genome expression profiles were examined via gene set enrichment. At 16 weeks, plasma quercetin levels were higher in Q and EQ groups vs. the control and E groups ( $p < 0.05$ ). Plasma cytokines were similar among groups ( $p > 0.05$ ). AUC estimations for GTT was 14% lower for Q vs. E ( $p = 0.0311$ ), but non-significant from control ( $p = 0.0809$ ). Genes for cholesterol metabolism and immune and inflammatory response were downregulated in Q and EQ groups vs. control in adipose tissue and soleus muscle tissue. These data support an anti-inflammatory role for Q and EQ, a result best captured when measured with tissue gene downregulation in comparison to changes in plasma cytokine levels.

**Keywords:** cytokines; fat metabolism; flavonoids; inflammation; insulin resistance; immune function; obesity; metabolic syndrome; phytochemicals

## 1. Introduction

High-fat Western diets are associated with insulin resistance, inflammation, and de novo lipogenesis [1,2], which are factors that contribute to the development of metabolic syndrome. Flavonoid ingestion has the potential to partially offset these effects. In particular, quercetin and

epigallocatechin gallate (EGCG) from green tea have been reported to attenuate insulin resistance, counter inflammation, and favorably alter fat metabolism [2–5]. However, the effect of a mixture of quercetin and EGCG has been examined in only a few studies.

Quercetin is a flavonoid that is found in many plant and foods such as onions, green tea, apples, peppers, and berries [6]. Both in vitro and rodent models provide evidence that quercetin supplementation reduces various measures related to metabolic syndrome [2,3,7]. Specifically, quercetin has been reported to blunt pro-inflammatory signaling via regulation of NF- $\kappa$ B-associated mechanisms in adipocytes, macrophages, and other cell lines [8–13], decrease insulin intolerance in primary human adipocytes and 3T3-L1 cells [8,14], and inhibit adipogenesis in 3T3-L1 cells [14–16] and lipid body formation in macrophages [17]. In rodents, quercetin has been reported to lower levels of circulating inflammatory-related plasma cytokines [18], inhibit pro-inflammatory signals [11,19–21], and improve insulin sensitivity [20–27] and dyslipidemia [20,21,24,26–28]. Very few human studies have examined the relationship between quercetin supplementation and metabolic syndrome risk factors in overweight adults. In a double-blinded, placebo-controlled study, Egert et al. [29] reported that six weeks of supplementation of quercetin at 150 mg/day reduced systolic blood pressure and plasma oxidized low-density lipoprotein (LDL) concentrations in overweight adults ( $n = 93$ ; mean age = 45.1 years), but had no effect on inflammation. However, the effect of quercetin supplementation on lipid markers appears to vary based on apolipoprotein (APOE) genotype. Similarly, six weeks of onion-extract supplementation (quercetin of 162 mg/day) was associated with a reduction in 24-h ambulatory blood pressure in overweight/obese adults ( $n = 68$ , mean age = 47.4 years) with central obesity and pre-hypertension [30]. However, quercetin supplementation had no impact on endothelial function, inflammation, oxidative stress, and lipid and glucose metabolism in these individuals [30]. In large community studies including both normal weight and overweight female adults, quercetin supplementation at 500 mg/day or 1000 mg/day for 12 weeks was reported to have no influence on innate immune function or inflammation [31], body composition [32], or disease risk factors [33]. Quercetin supplementation was, however, associated with a reduction in the severity and number of sick days associated with upper respiratory tract infections (URTI) in adults [34]. To our knowledge, only two studies have examined the influence of quercetin supplementation on insulin sensitivity. In one study, a 17.5% improvement in the homeostatic model assessment of insulin resistance (HOMA-IR) was reported in women with polycystic ovary syndrome (PCOS;  $n = 82$ , age = ~30 years) after 12 weeks of quercetin supplementation (1000 mg/day) [35]. In contrast, four weeks of quercetin supplementation (500 mg/day) had no impact on fasting blood glucose levels in healthy males ( $n = 22$ , age = 29.9 years) [36].

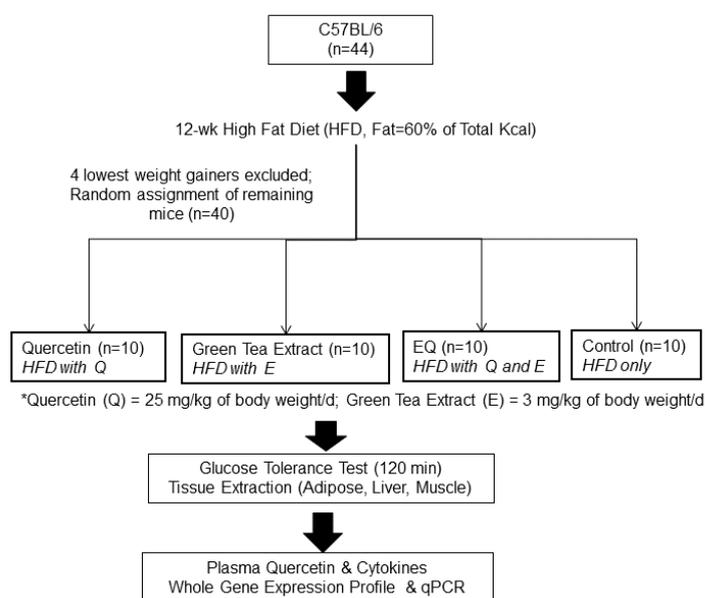
EGCG, a catechin, is the most abundant flavonoid found in green tea [6] and has been reported to have anti-obesity, anti-diabetic, and anti-inflammatory properties [2,3,37]. Notably, in vitro studies indicate that EGCG suppressed insulin resistance [38,39] and promoted glucose uptake via enhanced GLUT4 translocation [39,40] in skeletal muscle cells, attenuated  $\beta$ -cell release of insulin from mouse and human islet cells [39], and improved insulin sensitivity in human hepatocytes (HepG2 cells) [41]. Furthermore, EGCG was associated with decreased glucose uptake [42], lipid accumulation [43–45], adipogenesis [46], and adipocyte differentiation [44] in 3T3-L1 adipocytes, and reduced inflammation by reactive oxygen species generation in macrophages [47]. In rodents, EGCG and green tea extract have been shown in most studies to reduce total body and adipose tissue weights [37,48,49], decrease blood/plasma glucose and insulin levels [37,48,50], improve insulin sensitivity [37,48], blood pressure, and lipid profile [37,48,51], and reduce unfavorable obesity-associated changes in gut microbiota [52]. Epidemiological research and meta-analyses in general support the anti-obesity and health effects of EGCG [53]. In randomized controlled studies in humans, three studies found a small but significant decrease in body weight, waist circumference, and body fat with green tea supplementation [54–56], while two studies found no change [57,58]. Several meta-analyses of randomized controlled trials with green tea indicate a possible reduction in blood pressure [59–61], total and low-density lipoprotein cholesterol [60,62,63], and fasting blood glucose and insulin insensitivity [64].

Given the independent effects of quercetin and EGCG on metabolic syndrome, we aimed to elucidate whether the combined effort of quercetin and green tea extract supplementation would improve blood glucose tolerance, decrease inflammation, and favorably alter metabolism in mice fed a high-fat diet. Previous studies by our research group suggest that ingestion of both quercetin and EGCG-enriched green tea extract have a greater anti-inflammatory effect than quercetin alone [65–68]. We utilized whole genome transcriptome and real-time polymerase chain reaction (qPCR) analysis of adipose, liver, and skeletal muscle tissues in mice fed high-fat diets to improve our ability to measure potential metabolic and anti-inflammatory effects related to flavonoid ingestion.

## 2. Materials and Methods

### 2.1. Animals and Experimental Design

Forty C57BL/6 mice (male, 5 weeks old,  $n = 44$ ), purchased from a commercial vendor (Jackson Laboratory, Bar Harbor, ME, USA), were provided ad libitum access to a high-fat diet (HFD, fat = 60% kcal; BioServ, Frenchtown, NJ, USA) and water and maintained in 12 h light/dark cycle for the first 12 weeks at the animal facility of the North Carolina Research Campus. The experimental design is depicted in Figure 1. After 12 weeks on HFD, the four mice with the least weight gain were excluded from the second phase of the study, and the remaining mice ( $n = 40$ ) were randomly assigned to one of four treatment groups ( $n = 10$  per group): quercetin only (Q, 25 mg/kg of body weight (BW)/day of quercetin), green tea extract only (E; 3 mg/kg BW/day of EGCG), quercetin and green tea extract (EQ; 25 mg/kg BW of quercetin plus 3 mg/kg of EGCG), or control. All mice were maintained on HFD and with the exception of the control group were also supplemented with Q, E, or both for four weeks. Body weight was monitored weekly. At 16 weeks, mice underwent a glucose tolerance test and then were sacrificed. Tissue and plasma samples were collected for further analysis (Figure 1). All protocols utilized were approved by The Institutional Animal Care and Use Committee (IACUC) of the North Carolina Research Campus.



**Figure 1.** Study Design: C57BL/6 mice ( $n = 40$ ) were placed on a high-fat diet (fat = 60% of total kcal) for 12 weeks and then randomly assigned to a diet supplemented with quercetin only (Q), green tea extract only (E), quercetin + green tea extract (EQ), or control (i.e., high fat diet only) for four weeks. The quercetin dosage was 25 mg of quercetin/kg of body weight (BW) per day, and green tea extract dosage was 3 mg of epigallocatechin gallate/kg BW per day.

## 2.2. Glucose Tolerance Test and Blood and Tissue Collection

Following the four-week treatment period, mice fasted for 14 h and then were anesthetized and placed on a warming blanket. Next, mice were injected intraperitoneally with 2 g of glucose/kg BW. Blood (~3  $\mu$ L) was collected from the tail vein, and blood glucose levels were measured at 0, 15, 30, 60 and 120 min using OneTouch Ultra<sup>®</sup> blood glucometer (LifeScan, Johnson & Johnson, Chesterbrook, PA, USA).

Upon completion of the glucose tolerance test, mice were sacrificed, and whole blood was collected by cardiac puncture and centrifuged at  $1000\times g$  for 10 min at 4 °C. Plasma samples were aliquoted, snap frozen in liquid nitrogen, and stored at  $-80$  °C for later analysis. The following tissue was harvested from the mice: left lobes of kidney and liver, pancreas, visceral adipose, subcutaneous adipose, and skeletal muscle tissue (soleus, gastrocnemius, plantaris, EDL, and quadriceps). All tissue was weighed. Tissue was either stored in RNeasy Lysis Buffer (Qiagen, Crawley, UK) per manufacturer's instructions for genomics or frozen in liquid nitrogen and stored at  $-80$  °C for later analysis.

## 2.3. Biochemical Assays

Plasma samples were pooled to assess quercetin, which was measured following solid-phase extraction via reversed-phase high-performance liquid chromatography with UV detection as previously described [65–68]. Plasma cytokines (IFN- $\gamma$ , IL-1 $\beta$ , IL-6, IL-10, KC/GRO/CINC, and TNF- $\alpha$ ) were measured using Mouse ProInflammatory 7-Plex Base Kit (Meso Scale Discovery, Rockville, MD, USA) per manufacturer's instructions.

## 2.4. Genomic Analysis

Whole genome expression profiling was conducted with total RNA isolated from adipose, liver and skeletal muscle from mice in the Q, EQ and control groups. RNA was isolated and quantified, and quality control (QC) was performed on all samples. Expression profiling was performed on Mouse ST 1.1 PEG array (Affymetrix, ThermoFischer Scientific, Waltham, MA, USA) as per the manufacturer's instructions. Signal extraction and background was subtracted for normalization utilizing Robust Multichip Average [69]. Samples that were considered outliers were excluded based on the QC report and scatter plots. Both the mean signal per treatment group and fold-change (log ratio) were calculated. CyberT was used to identify differentially expressed genes [70]. Pathways affected by each treatment relative to the control was determined using overrepresentation analysis via Ingenuity Pathway Analysis (IPA) software (Qiagen, Redwood City, CA, USA).

To quantify the expression of individual genes ( $n = 27$ ), qPCR was performed in tissue samples from fat, liver, and soleus for the four experimental groups using Applied Biosystems<sup>™</sup> TaqMan<sup>®</sup> Gene Expression Assays (ThermoFischer Scientific, Waltham, MA, USA) as per the manufacturer's instructions. Genes examined include those involved in cholesterol regulation (Abca1, Apoa1, Cyp3a41a, Srebf1, and Srebf2), fatty acid metabolism (Lpl, Ppara, Pparag. and Scd1), inflammatory and immune response (Cc12, Cd68, Ikbkb, Il1r1, Nfkb1, and Nr1h3), adipokines (Adipoq and Lep), oxidative stress (Ppargc1a), stress response (Hspa1a, Hspa2, Mapk8, and Sirt1), transcription (Atf2 and Nfact3), and xenobiotics (Cyp2e1).

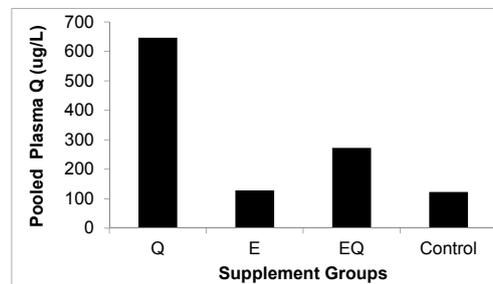
## 2.5. Statistical Analysis

Data was summarized using means and standard error. To detect significant differences between groups, one-way ANOVA (time  $\times$  treatment) was used for blood analysis and gene expression. Whole-genome expression profiles were examined via gene-set enrichment analysis (GSEA) [71]. A  $p$ -value was set at  $<0.05$  for significance. Analysis was conducted using SAS 9.3 (SAS Institute, Cary, NC, USA).

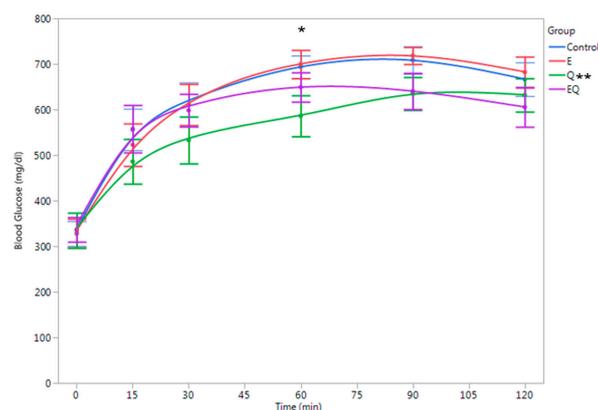
### 3. Results

#### 3.1. Body Mass and Biochemical Analysis

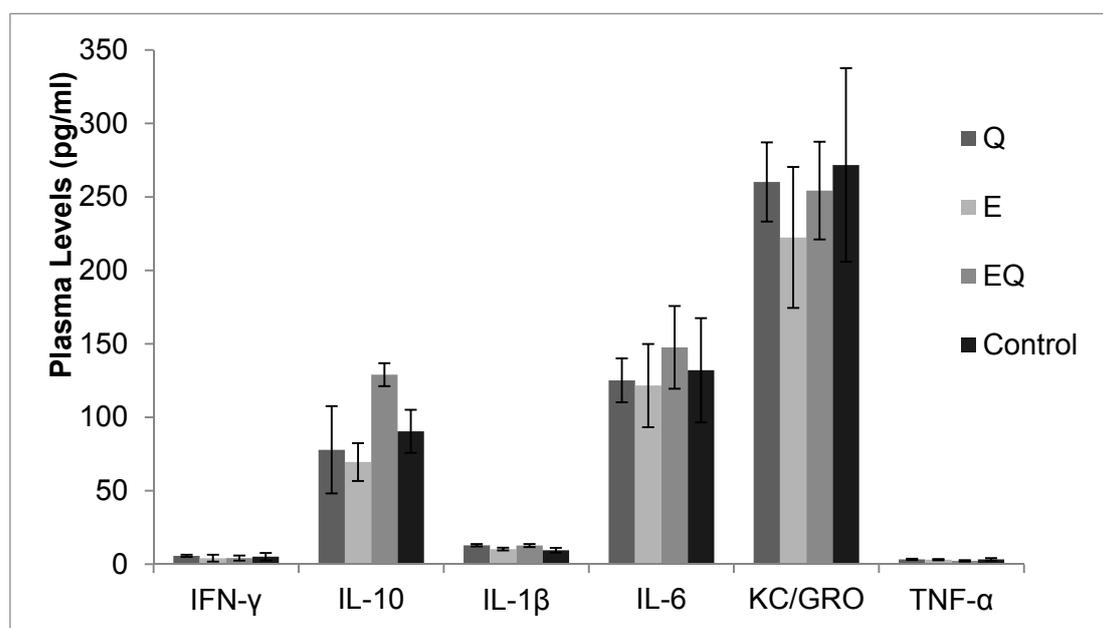
At the beginning of the study, the body mass for all mice was  $20.0 \pm 0.0$  g with no differences among groups ( $p > 0.05$ ). Body mass was also similar among groups at 12 weeks ( $Q = 47.3 \pm 0.7$  g,  $E = 47.1 \pm 0.8$  g,  $EQ = 47.1 \pm 0.8$  g, and control =  $47.1 \pm 1.0$  g;  $p > 0.05$ ) and at 16 weeks (i.e., after four weeks of supplementation ( $Q = 51.1 \pm 0.6$  g,  $E = 50.6 \pm 0.8$  g,  $EQ = 50.5 \pm 0.5$  g, and control =  $50.2 \pm 0.7$  g;  $p > 0.05$ ). At 16 weeks, pooled plasma quercetin levels were ~fivefold higher in Q and twofold higher in the EQ group compared to the control group (Figure 2). Glucose tolerance test (GTT) results are presented in Figure 2. Area-under-the-curve (AUC) estimations for plasma glucose were 14% lower for Q vs. EQ ( $p = 0.031$ ) and trended 11% lower than control, but did not reach significance ( $p = 0.081$ ). Plasma glucose was lower for Q vs. control at 60 min ( $p = 0.032$ ; Figure 3). No other differences among groups were detected ( $p > 0.05$ ; Figure 3). Plasma cytokines levels were also similar among groups ( $p > 0.05$ , Figure 4).



**Figure 2.** Pooled plasma quercetin at 16 weeks by experimental groups. C57BL/6 mice ( $n = 40$ ) were placed on a high-fat diet (fat = 60% of total kcal) for 12 weeks and then randomly assigned to a diet supplemented with quercetin only (Q), green tea extract only (E), quercetin + green tea extract (EQ), or control (i.e., high-fat diet only) for four weeks. The dosage for quercetin was 25 mg of quercetin/kg of body weight (BW) per day and green tea extract dosage was 3 mg of epigallocatechin gallate/kg BW per day. Plasma samples were pooled for each group and analyzed for quercetin. At 16 weeks, plasma quercetin levels were 525% higher in Q, and 225% higher in EQ compared to control.



**Figure 3.** Glucose tolerance curve at 16 weeks by supplement groups. C57BL/6 mice ( $n = 40$ ) were placed on a high-fat diet (fat = 60% of total kcal) for 12 weeks and then randomly assigned to a diet supplemented with quercetin only (Q), green tea extract only (E), quercetin + green tea extract (EQ), or control (i.e., high-fat diet only) for four weeks. The dosage for quercetin was 25 mg of quercetin/kg of body weight (BW) per day and green tea extract dosage was 3 mg of epigallocatechin gallate/kg BW per day. \* Q lower than control at 60-min ( $p < 0.05$ ). \*\* Area-under-the-curve (AUC) estimations lower for Q vs. EQ ( $p < 0.05$ ).

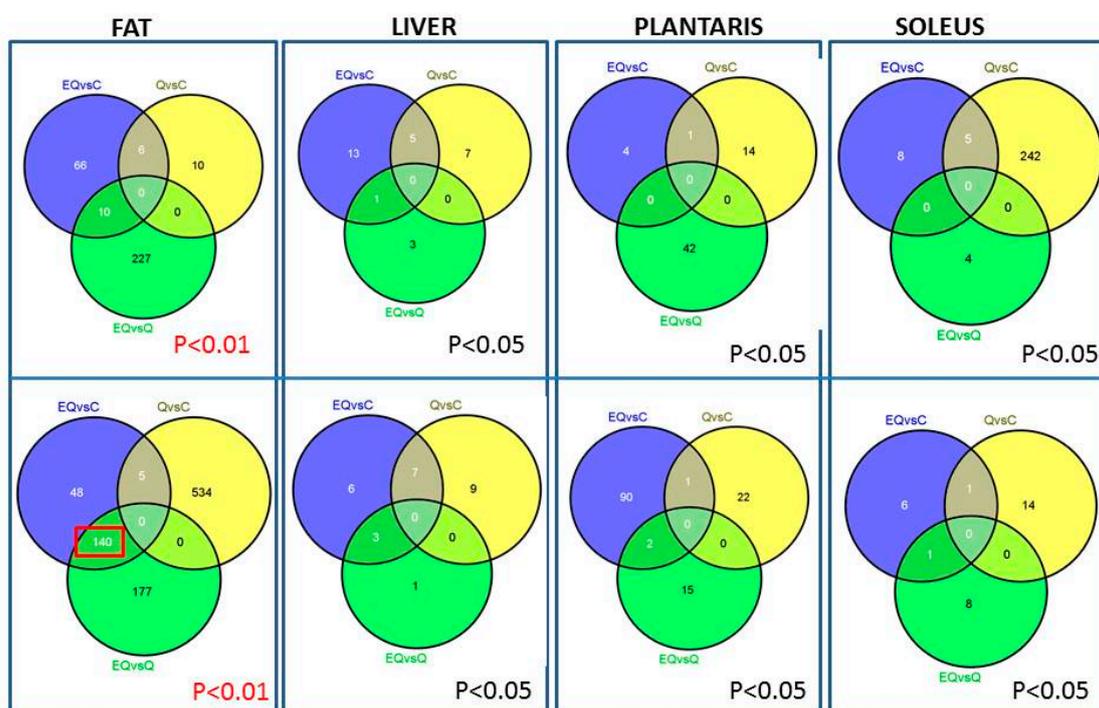


**Figure 4.** Plasma cytokine levels at 16 weeks by supplement groups. C57BL/6 mice ( $n = 40$ ) were placed on a high-fat diet (fat = 60% of total kcal) for 12 weeks and then randomly assigned to a diet supplemented with quercetin only (Q), green tea extract only (E), quercetin + green tea extract (EQ), or control (i.e., high fat diet only) for four weeks. The dosage for quercetin was 25 mg of quercetin/kg of body weight (BW) per day and green tea extract dosage was 3 mg of epigallocatechin gallate/kg BW per day. Plasma cytokine levels did not differ between supplement groups and control ( $p > 0.05$ ).

### 3.2. Genomic Analysis

Both microarray and IPA analysis revealed downregulation of genes associated with cholesterol metabolism and immune/inflammation in adipose tissue and soleus muscle tissue, fatty acid metabolism in soleus muscle tissue, and CYP450 metabolism in the liver. EQ resulted in downregulation of over 100 genes in adipose tissue compared to both control and quercetin alone ( $p < 0.01$ ; Figure 5). The specific pathways downregulated by EQ and Q are depicted in Table 1. In skeletal muscle, protein ubiquitination, the pathway responsible for marking proteins for degradation, was upregulated by Q treatment relative to the control.

In Table 2, gene expression changes are presented related to the plasma cytokines assessed. Of these, KC/GRO (i.e., Cxcl1) gene was expressed in adipose tissue and liver with an upregulation of KC/GRO detected in the liver of the EQ group compared to control (Table 2). The IL-1 $\beta$  gene was also expressed in the liver, but no difference was found among treatments (Table 2). Q was associated with the downregulation of the IL-1 $\beta$  receptor gene in adipose (Table 2) vs. control, and a downregulation trend was observed for other cytokine receptors genes in adipose tissue and soleus muscle tissue ( $p > 0.05$ , Table 1). For the EQ treatment, the IL-10 receptor gene was downregulated while the TNF- $\alpha$  receptor gene was upregulated in comparison to the control (Table 2). No differences were detected between Q and EQ groups for the genes presented in Table 2.



**Figure 5.** Overlap among differentially expressed genes by tissue. Top panel shows the number of downregulated genes and the bottom panel shows upregulated genes. C57BL/6 mice ( $n = 40$ ) were placed on a high-fat diet (fat = 60% of total kcal) for 12 weeks and then randomly assigned to a diet supplemented with quercetin only (Q), green tea extract only (E), quercetin + green tea extract (EQ), or control (i.e., high fat diet only) for four weeks. The dosage for quercetin was 25 mg of quercetin/kg of body weight (BW) per day, and green tea extract dosage was 3 mg of epigallocatechin gallate/kg BW per day. EQ treatment result in the upregulation of 140 genes compared to the control and Q groups.

**Table 1.** Top canonical pathways altered by four-week supplementation vs. control as identified by Ingenuity Pathway Analysis (IPA) <sup>1</sup>.

Downregulated Pathways	Fat	Liver	Muscle	Comments
Steroid Biosynthesis		Q; EQ		Target of Statins
Phagocytosis/ leukocyte extravasation	EQ			Innate Immune Response
EIF2 signaling			Q; EQ	Stress Response
Mitochondrial dysfunction			Q	Associated with disease
eIF4/p70S6K signaling			Q; EQ	Insulin Signaling
Oxidative phosphorylation			Q; EQ	Energy Production
PPAR $\alpha$ /RXR $\alpha$ activation			Q	Gene Expression

<sup>1</sup> C57BL/6 mice ( $n = 40$ ) were placed on a high-fat diet (fat = 60% of total kcal) for 12 weeks and then randomly assigned to a diet supplemented with quercetin only (Q), green tea extract only (E), quercetin + green tea extract (EQ), or control (i.e., high fat diet only) for four weeks. The dosage for quercetin was 25 mg of quercetin/kg of body weight (BW) per day and green tea extract dosage was 3 mg of epigallocatechin gallate/kg BW per day. IPA analysis was only conducted on tissue collected from the EQ, Q and control groups.

**Table 2.** Fold change in genes associated with cytokines assessed in plasma vs. control, based on microarray analysis <sup>1</sup>.

Description	Q Change	p	EQ Change	p	Description
Adipose					
Ifngr1	-0.28	0.340	-0.18	0.659	interferon gamma receptor 1
Ifngr2	-0.14	0.820	-0.54	0.090	interferon gamma receptor 2
Il10ra	-0.36	0.550	-0.63	0.194	interleukin 10 receptor, alpha
Il10rb	-0.29	0.550	-0.73	0.047	interleukin 10 receptor, beta
Il1r1	-0.60	0.037	-0.53	0.087	interleukin 1 receptor, type I
Il1rap	-0.10	0.908	0.10	0.916	interleukin 1 receptor accessory protein
Il1rn	-0.31	0.783	-1.22	0.052	interleukin 1 receptor antagonist
Il6ra	-0.29	0.466	0.32	0.428	interleukin 6 receptor, alpha
Il6st	-0.18	0.645	-0.04	0.977	interleukin 6 signal transducer
Cxcl1	-0.17	0.679	-0.11	0.873	chemokine (C-X-C motif) ligand 1
Tnfrsf1a	-0.47	0.196	0.99	0.188	tumor necrosis factor receptor superfamily, member 1a
Tnfrsf1b	-0.33	0.565	0.20	0.046	tumor necrosis factor receptor superfamily, member 1b
Soleus					
Ifngr1	-0.27	0.423	-0.15	0.775	interferon gamma receptor 1
Il10rb	-0.10	0.876	0.10	0.879	interleukin 10 receptor, beta
Il6ra	-0.18	0.692	-0.12	0.845	interleukin 6 receptor, alpha
Il6st	-0.31	0.151	-0.16	0.602	interleukin 6 signal transducer
Tnfrsf1a	-0.42	0.162	0.21	0.968	tumor necrosis factor receptor superfamily, member 1a
Liver					
Ifngr1	-0.07	0.935	-0.13	0.805	interferon gamma receptor 1
Ifngr2	0.15	0.678	-0.05	0.961	interferon gamma receptor 2
Il10rb	0.00	0.999	-0.06	0.964	interleukin 10 receptor, beta
Il1b	0.07	0.985	0.07	0.986	interleukin 1 beta
Il1r1	0.31	0.799	0.79	0.243	interleukin 1 receptor, type I
Il1rap	-0.01	0.998	0.00	0.999	interleukin 1 receptor accessory protein
Il1rn	0.06	0.971	0.32	0.413	interleukin 1 receptor antagonist
Il6ra	-0.24	0.550	0.55	0.055	interleukin 6 receptor, alpha
Il6st	-0.07	0.929	0.12	0.801	interleukin 6 signal transducer
Cxcl1	0.72	0.222	1.15	0.030	chemokine (C-X-C motif) ligand 1
Tnfrsf1a	-0.42	0.162	0.21	0.968	tumor necrosis factor receptor superfamily, member 1a

<sup>1</sup> C57BL/6 mice ( $n = 40$ ) were placed on a high-fat diet (fat = 60% of total kcal) for 12 weeks and then randomly assigned to a diet supplemented with quercetin only (Q), green tea extract only (E), quercetin + green tea extract (EQ), or control (i.e., high fat diet only) for four weeks. The dosage for quercetin was 25 mg of quercetin/kg of body weight (BW) per day and green tea extract dosage was 3 mg of epigallocatechin gallate/kg BW per day. Individual genes ( $n = 27$ ) were assessed in soleus, liver, and fat. \* Significantly different than the control group ( $p < 0.05$ ).

Of the 27 individual genes evaluated in adipose, soleus, and liver via qPCR, Q was associated with downregulation of three genes in adipose tissue, and no gene changes in the soleus or liver tissue compared to the control group (Table 3). In the soleus tissue, EQ and Q were associated with the downregulation of genes (4 and 2 genes, respectively) in the soleus vs. control with no other changes observed in adipose or liver tissue (Table 3).

**Table 3.** Fold change in genes downregulated in adipose and soleus tissue compared to control by supplement groups as assessed via real-time quantitative polymerase chain reaction (qPCR) analysis.

Description	Q	E	EQ	Pathways
Adipose				
Srebf2	0.44 *	0.57	0.82	Sterol biosynthesis
Atf2	0.51 *	0.83	1.10	Transcriptional activator
Sirt1	0.40 *	0.92	0.74	Stress response
Soleus				
Srebf2	0.60	0.54 *	0.62 *	Sterol biosynthesis
Pparag	1.04	0.71	0.69 *	Fatty acid storage and Glucose metabolism
Scd1	0.44	0.97	0.40 *	Fatty Acid metabolism
Cd68	0.88	0.71	0.57 *	Promote phagocytosis and activation of macrophages
Atf2	1.23	0.62 *	0.85	Transcriptional activator

<sup>1</sup> C57BL/6 mice ( $n = 40$ ) were placed on a high-fat diet (fat = 60% of total kcal) for 12-weeks and then randomly assigned to a diet supplemented with quercetin only (Q), green tea extract only (E), quercetin + green tea extract (EQ), or control (i.e., high fat diet only) for four weeks. The dosage for quercetin was 25 mg of quercetin/kg of body weight (BW) per day and green tea extract dosage was 3 mg of epigallocatechin gallate/kg BW per day. Individual genes ( $n = 27$ ) were assessed in soleus, liver, and fat tissue. \* Significantly different than the control group ( $p < 0.05$ ).

#### 4. Discussion

In mice on a 12-week HFD, four weeks of EQ supplementation were associated with the downregulation of over 100 genes in adipose tissue, including those involved in phagocytosis and leukocyte extravasation or trafficking pathways. Recruitment of leukocytes, specifically neutrophils, to adipose has been implicated in chronic inflammation in adipose tissue [72,73] and has been linked to insulin resistance in mice on HFD [73]. Traditional biomarkers for inflammation and glucose tolerance, however, were not different between EQ and control groups, but a mild improvement in blood glucose tolerance was detected with the Q treatment. In adipose and muscle tissue, EQ was associated with a downregulation of cholesterol metabolism compared to control. Cholesterol accumulation in adipose and muscle tissue have been associated with obesity and sarcopenia [74,75]. Genes associated with drug metabolism were also downregulated in EQ vs. control in the liver. The implications, however, are unclear, as changes in drug metabolism vary by metabolic and excretion pathways in obese individuals [76]. Thus, four weeks of EQ supplementation in mice on a 12-week HFD resulted in changes in tissue gene expression suggestive of reduced inflammation and cholesterol metabolism, while blood markers of glucose tolerance and inflammation were largely unaltered.

In the EQ group, the changes in tissue gene expression are indicative of reduced inflammation and leukocyte trafficking, which has been examined as a treatment target for inflammatory diseases [77]. Cytokine levels in the present study were not different among the experimental groups. Our findings in mice (~age in human = 50 years) [78] parallel previous studies in middle-aged humans. In overweight and obese women ( $n = 48$ , age = 56 years), 10 weeks of supplementation with mixed flavonoid-nutrient-fish oil supplement (Q-mix; 1000 mg quercetin, 400 mg isoquercetin, 120 mg EGCG, 220 mg EPA, and 180 mg DHA, 1000 mg vitamin C, 40 mg niacinamide, and 800 µg folic acid) did not alter biomarkers of inflammation, oxidative stress, and blood lipid levels, but was associated with gene alterations suggestive of enhanced antiviral defense and decreased leukocyte trafficking [79]. Similarly, in a randomized, double-blinded, crossover study in overweight men ( $n = 26$ , age = 46 years), Bakker et al. [78] reported no change in traditional biomarkers, but did report a shift in nutrigenomic profiles, which was associated with a reduction in inflammation after a five-week, anti-inflammatory dietary mix supplementation (AIDM, 6.3 mg resveratrol, 3.75 mg lycopene, ~38 mg EGCG, 300 mg EPA, 260 mg DHA, 125 mg vitamin C, and 90.7 mg  $\alpha$ -tocopherol) vs. placebo [80]. The relative dose of both quercetin and EGCG was higher in the present study compared to the human studies (Q = 25 mg/kg BW vs. AIDM = 0 mg/kg BW and Qmix = ~15 mg/kg BW; and E = 3 mg/kg BW

vs. AIDM = ~0.4 mg/kg BW and Q<sub>mix</sub> = ~1.3 mg/kg BW). In addition, the respective duration of the supplementation was longer in the present study (i.e., four weeks of supplementation in mice is equivalent to ~10 years in human). Finally, the supplements in the human studies also contained fish oil, which has been associated with improvement in inflammatory biomarkers [81,82]. Taken together, these studies provide evidence that a mixture of flavonoids may be a promising treatment for reducing inflammation in overweight/obese individuals. Further research is needed to elucidate the optimal combination of flavonoids and/or whether the inclusion of fish oil in the supplementation provides additive benefits. Part of the challenge is the inclusion of novel outcome measures that capture metabolic and anti-inflammatory effects that are missed by basic plasma inflammation biomarkers [79]. The tissue-specific transcriptomic change observed in the present study may possibly reflect an earlier stage of tissue response to supplementation. Systemic changes may follow, and further research is thus warranted that examines a prolonged period of supplementation and/or supplementation at higher doses.

In the present study, plasma quercetin levels were lower in the EQ vs. Q group, despite the same dosage of quercetin being provided to both groups. Our findings are consistent with a mouse study conducted by Wang et al. [83], in which the authors reported that total quercetin levels in tissue were lower with the co-administration of EGCG and quercetin in mice. In the intestine, quercetin in humans is absorbed via passive diffusion as a primary route, and organic anion transporting polypeptide (OATPs) as a secondary route [84,85]. EGCG has been speculated to interfere with quercetin absorption via OATPs by acting as a non-competitive inhibitor or decreasing the activity of the transporter [83,86]. Given the high dose of Q administered in the present study, decreased absorption via OATPs could explain the lower plasma Q levels observed in the EQ group compared to Q and thus supporting the hypothesis of EGCG's interaction with this transporter.

A mild improvement in blood glucose tolerance was associated with quercetin alone in this study. As previously discussed, very few human studies [35,36] have examined the impact of quercetin on blood glucose control and insulin resistance. Mehta et al. [82] reported that male Swiss albino mice (age not reported) had less stress-induced hyperglycemia and insulin-resistance following three weeks of quercetin supplementation (30 mg/kg) vs. control. Henagan et al. [87] reported that eight weeks of a low dose of quercetin (~1.6 g/kg BW) compared to a placebo resulted in improved insulin tolerance in male C57BL/6J mice (~14 weeks of age at sacrifice) on HFD, while the high dose (20 g/kg BW) did not alter insulin tolerance. The mice in the present study were older and had a higher dosage of quercetin compared to Henagan et al. [87]. In the EQ group, blood glucose levels were similar to both the control and E groups, but were higher than the Q group. As discussed previously, plasma quercetin levels were lower in the EQ vs. Q, which was possibly related to the interference of EGCG on quercetin's absorption. Thus, the observed differences support quercetin's role in improving glucose tolerance. A potential limitation in the current study is that the glucose tolerance test was conducted while the mice were under general anesthesia, which may have caused stress-induced hyperglycemia in all groups [88]. In addition, blood glucose was measured utilizing a glucometer, which has been reported to overestimate blood glucose levels in hyperglycemic states [89]. Thus, the measured blood glucose may have been higher than actual levels. Furthermore, it is difficult to separate hyperglycemia caused by the stress of anesthesia vs. HFD, and this may have confounded the potential impact of flavonoid supplementation on HFD-induced hyperglycemia.

Finally, the metabolism of flavonoids in human and mice differ, and more research is needed to determine the applicability of our results to human populations. The agreement between our prior human trial and the current mouse-based study indicating a downregulation in expression of genes related to leukocyte trafficking following mixed flavonoid supplementation is one indicator of similar responses between species [79]. In humans, polyphenols are transformed into metabolites with diminished biological impact [90–92]. Unabsorbed polyphenols can undergo bacterial bioconversion by gut microbiota into more bioactive forms [90–92]. Mice have different species of gut bacteria compared to humans, which limits the applicability of this model [92]. Humanized mice models have

been suggested that utilize human fecal microbiota transplants (FMT) in mice to create a similar gut microbiome [92]. In addition, mice expressing the human drug metabolizing enzymes, cytochromes P450, may also prove to be a useful animal model in examining flavonoids [93]. Despite these differences, the plasma and urine content of quercetin metabolites are similar between humans and rats in type and number [94], and the bioavailability of EGCG have been reported as similar between human and mice [95]. Taken together, future studies on polyphenol mixtures could compare humanized and standard mouse models (e.g., those with FMT) to determine both similarities and differences on metabolic and inflammation outcome measures.

## 5. Conclusions

Supplementation with EQ for four weeks in mice fed a high fat diet for 12 weeks was associated with tissue gene expression changes suggestive of reduced inflammation and diminished leukocyte cell trafficking, a result we have previously demonstrated in human participants [79]. Traditional inflammatory biomarkers and glucose tolerance were not altered by EQ, but a mild improvement in glucose tolerance was observed with Q only. Future research should consider comparing flavonoid biotransformation in humanized mouse models to standard mouse models. Furthermore, lower doses and different flavonoid mixtures should be examined in both sedentary and physically active rodent models.

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## References

1. Lee, C.Y. The effect of high-fat diet-induced pathophysiological changes in the gut on obesity: What should be the ideal treatment? *Clin. Transl. Gastroenterol.* **2013**, *4*, e39. [CrossRef] [PubMed]
2. Cherniack, E.P. Polyphenols: Planting the seeds of treatment for the metabolic syndrome. *Nutrition* **2011**, *27*, 617–623. [CrossRef] [PubMed]
3. Siriwardhana, N.; Kalupahana, N.S.; Cekanova, M.; LeMieux, M.; Greer, B.; Moustaid-Moussa, N. Modulation of adipose tissue inflammation by bioactive food compounds. *J. Nutr. Biochem.* **2013**, *24*, 613–623. [CrossRef] [PubMed]
4. Lee, S.G.; Parks, J.S.; Kang, H.W. Quercetin, a functional compound of onion peel, remodels white adipocytes to brown-like adipocytes. *J. Nutr. Biochem.* **2017**, *42*, 62–71. [CrossRef] [PubMed]
5. Kim, H.M.; Kim, J. The effects of green tea on obesity and type 2 diabetes. *Diabetes Metab. J.* **2013**, *37*, 173–175. [CrossRef] [PubMed]
6. Bhagwat, S.; Haytowitz, D.B.; Holden, J.M. USDA Database for the Flavonoid Content of Selected Foods. Available online: <http://www.ARS.USDA.Gov/nutrientdata/ flav> (accessed on 15 May 2017).
7. Li, Y.; Yao, J.; Han, C.; Yang, J.; Chaudhry, M.T.; Wang, S.; Liu, H.; Yin, Y. Quercetin, inflammation and immunity. *Nutrients* **2016**, *8*, 167. [CrossRef] [PubMed]
8. Chuang, C.C.; Martinez, K.; Xie, G.; Kennedy, A.; Bumrungpert, A.; Overman, A.; Jia, W.; McIntosh, M.K. Quercetin is equally or more effective than resveratrol in attenuating tumor necrosis factor- $\alpha$ -mediated inflammation and insulin resistance in primary human adipocytes. *Am. J. Clin. Nutr.* **2010**, *92*, 1511–1521. [CrossRef] [PubMed]
9. Overman, A.; Chuang, C.C.; McIntosh, M. Quercetin attenuates inflammation in human macrophages and adipocytes exposed to macrophage-conditioned media. *Int. J. Obes.* **2011**, *35*, 1165–1172. [CrossRef] [PubMed]

10. Comalada, M.; Ballester, I.; Bailon, E.; Sierra, S.; Xaus, J.; Galvez, J.; De Medina, F.S.; Zarzuelo, A. Inhibition of pro-inflammatory markers in primary bone marrow-derived mouse macrophages by naturally occurring flavonoids: Analysis of the structure-activity relationship. *Biochem. Pharmacol.* **2006**, *72*, 1010–1021. [[CrossRef](#)] [[PubMed](#)]
11. Dias, A.S.; Porawski, M.; Alonso, M.; Marroni, N.; Collado, P.S.; Gonzalez-Gallego, J. Quercetin decreases oxidative stress, NF- $\kappa$ B activation, and iNOS overexpression in liver of streptozotocin-induced diabetic rats. *J. Nutr.* **2005**, *135*, 2299–2304. [[PubMed](#)]
12. Comalada, M.; Camuesco, D.; Sierra, S.; Ballester, I.; Xaus, J.; Galvez, J.; Zarzuelo, A. In vivo quercitrin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF- $\kappa$ B pathway. *Eur. J. Immunol.* **2005**, *35*, 584–592. [[CrossRef](#)] [[PubMed](#)]
13. Nair, M.P.; Mahajan, S.; Reynolds, J.L.; Aalinkeel, R.; Nair, H.; Schwartz, S.A.; Kandaswami, C. The flavonoid quercetin inhibits proinflammatory cytokine (tumor necrosis factor alpha) gene expression in normal peripheral blood mononuclear cells via modulation of the NF- $\kappa$ B system. *Clin. Vaccine Immunol.* **2006**, *13*, 319–328. [[CrossRef](#)] [[PubMed](#)]
14. Fang, X.K.; Gao, J.; Zhu, D.N. Kaempferol and quercetin isolated from euonymus alatus improve glucose uptake of 3T3-L1 cells without adipogenesis activity. *Life Sci.* **2008**, *82*, 615–622. [[CrossRef](#)] [[PubMed](#)]
15. Hsu, C.L.; Yen, G.C. Induction of cell apoptosis in 3t3-L1 pre-adipocytes by flavonoids is associated with their antioxidant activity. *Mol. Nutr. Food Res.* **2006**, *50*, 1072–1079. [[CrossRef](#)] [[PubMed](#)]
16. 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. *Biochem. Biophys. Res. Commun.* **2008**, *373*, 545–549. [[CrossRef](#)] [[PubMed](#)]
17. Lara-Guzman, O.J.; Tabares-Guevara, J.H.; Leon-Varela, Y.M.; Alvarez, R.M.; Roldan, M.; Sierra, J.A.; Londono-Londono, J.A.; Ramirez-Pineda, J.R. Proatherogenic macrophage activities are targeted by the flavonoid quercetin. *J. Pharmacol. Exp. Ther.* **2012**, *343*, 296–306. [[CrossRef](#)] [[PubMed](#)]
18. Stewart, L.K.; Soileau, J.L.; Ribnicky, D.; Wang, Z.Q.; Raskin, I.; Poulev, A.; Majewski, M.; Cefalu, W.T.; Gettys, T.W. Quercetin transiently increases energy expenditure but persistently decreases circulating markers of inflammation in C57BL/6 mice fed a high-fat diet. *Metabolism* **2008**, *57*, S39–S46. [[CrossRef](#)] [[PubMed](#)]
19. Milenkovic, M.; Arsenovic-Ranin, N.; Stojic-Vukanic, Z.; Bufan, B.; Vucicevic, D.; Jancic, I. Quercetin ameliorates experimental autoimmune myocarditis in rats. *J. Pharm. Pharm. Sci.* **2010**, *13*, 311–319. [[CrossRef](#)] [[PubMed](#)]
20. Hu, Q.H.; Zhang, X.; Pan, Y.; Li, Y.C.; Kong, L.D. Allopurinol, quercetin and rutin ameliorate renal NLRP3 inflammasome activation and lipid accumulation in fructose-fed rats. *Biochem. Pharmacol.* **2012**, *84*, 113–125. [[CrossRef](#)] [[PubMed](#)]
21. Rivera, L.; Moron, R.; Sanchez, M.; Zarzuelo, A.; Galisteo, M. Quercetin ameliorates metabolic syndrome and improves the inflammatory status in obese Zucker rats. *Obesity* **2008**, *16*, 2081–2087. [[CrossRef](#)] [[PubMed](#)]
22. Hu, Q.H.; Wang, C.; Li, J.M.; Zhang, D.M.; Kong, L.D. Allopurinol, rutin, and quercetin attenuate hyperuricemia and renal dysfunction in rats induced by fructose intake: Renal organic ion transporter involvement. *Am. J. Physiol. Renal. Physiol.* **2009**, *297*, 1080–1091. [[CrossRef](#)] [[PubMed](#)]
23. Kannappan, S.; Anuradha, C.V. Insulin sensitizing actions of fenugreek seed polyphenols, quercetin & metformin in a rat model. *Indian J. Med. Res.* **2009**, *129*, 401–408. [[PubMed](#)]
24. Kobori, M.; Masumoto, S.; Akimoto, Y.; Oike, H. Chronic dietary intake of quercetin alleviates hepatic fat accumulation associated with consumption of a western-style diet in C57/BL6J mice. *Mol. Nutr. Food Res.* **2011**, *55*, 530–540. [[CrossRef](#)] [[PubMed](#)]
25. Shao, L.; Liu, K.; Huang, F.; Guo, X.; Wang, M.; Liu, B. Opposite effects of quercetin, luteolin, and epigallocatechin gallate on insulin sensitivity under normal and inflammatory conditions in mice. *Inflammation* **2013**, *36*, 1–14. [[CrossRef](#)] [[PubMed](#)]
26. Zhou, M.; Wang, S.; Zhao, A.; Wang, K.; Fan, Z.; Yang, H.; Liao, W.; Bao, S.; Zhao, L.; Zhang, Y.; et al. Transcriptomic and metabolomic profiling reveal synergistic effects of quercetin and resveratrol supplementation in high fat diet fed mice. *J. Proteome Res.* **2012**, *11*, 4961–4971. [[CrossRef](#)] [[PubMed](#)]
27. Snyder, S.M.; Zhao, B.; Luo, T.; Kaiser, C.; Cavender, G.; Hamilton-Reeves, J.; Sullivan, D.K.; Shay, N.F. Consumption of quercetin and quercetin-containing apple and cherry extracts affects blood glucose concentration, hepatic metabolism, and gene expression patterns in obese C57/BL6J high fat-fed mice. *J. Nutr.* **2016**, *146*, 1001–1007. [[CrossRef](#)] [[PubMed](#)]

28. Jung, C.H.; Cho, I.; Ahn, J.; Jeon, T.I.; Ha, T.Y. Quercetin reduces high-fat diet-induced fat accumulation in the liver by regulating lipid metabolism genes. *Phytother. Res.* **2013**, *27*, 139–143. [[CrossRef](#)] [[PubMed](#)]
29. Egert, S.; Bosy-Westphal, A.; Seiberl, J.; Kurbitz, C.; Settler, U.; Plachta-Danielzik, S.; Wagner, A.E.; Frank, J.; Schrezenmeir, J.; Rimbach, G.; et al. Quercetin reduces systolic blood pressure and plasma oxidised low-density lipoprotein concentrations in overweight subjects with a high-cardiovascular disease risk phenotype: A double-blinded, placebo-controlled cross-over study. *Br. J. Nutr.* **2009**, *102*, 1065–1074. [[CrossRef](#)] [[PubMed](#)]
30. Brull, V.; Burak, C.; Stoffel-Wagner, B.; Wolfram, S.; Nickenig, G.; Muller, C.; Langguth, P.; Altheld, B.; Fimmers, R.; Naaf, S.; et al. Effects of a quercetin-rich onion skin extract on 24 h ambulatory blood pressure and endothelial function in overweight-to-obese patients with (pre-)hypertension: A randomised double-blinded placebo-controlled cross-over trial. *Br. J. Nutr.* **2015**, *114*, 1263–1277. [[CrossRef](#)] [[PubMed](#)]
31. Heinz, S.A.; Henson, D.A.; Nieman, D.C.; Austin, M.D.; Jin, F. A 12-week supplementation with quercetin does not affect natural killer cell activity, granulocyte oxidative burst activity or granulocyte phagocytosis in female human subjects. *Br. J. Nutr.* **2010**, *104*, 849–857. [[CrossRef](#)] [[PubMed](#)]
32. Knab, A.M.; Shanely, R.A.; Jin, F.; Austin, M.D.; Sha, W.; Nieman, D.C. Quercetin with vitamin C and niacin does not affect body mass or composition. *Appl. Physiol. Nutr. Metab.* **2011**, *36*, 331–338. [[CrossRef](#)] [[PubMed](#)]
33. Knab, A.M.; Shanely, R.A.; Henson, D.A.; Jin, F.; Heinz, S.A.; Austin, M.D.; Nieman, D.C. Influence of quercetin supplementation on disease risk factors in community-dwelling adults. *J. Am. Diet. Assoc.* **2011**, *111*, 542–549. [[CrossRef](#)] [[PubMed](#)]
34. Heinz, S.A.; Henson, D.A.; Austin, M.D.; Jin, F.; Nieman, D.C. Quercetin supplementation and upper respiratory tract infection: A randomized community clinical trial. *Pharmacol. Res.* **2010**, *62*, 237–242. [[CrossRef](#)] [[PubMed](#)]
35. Rezvan, N.; Moini, A.; Janani, L.; Mohammad, K.; Saedisomeolia, A.; Nourbakhsh, M.; Gorgani-Firuzjaee, S.; Mazaherioun, M.; Hosseinzadeh-Attar, M.J. Effects of quercetin on adiponectin-mediated insulin sensitivity in polycystic ovary syndrome: A randomized placebo-controlled double-blind clinical trial. *Horm. Metab. Res.* **2017**, *49*, 115–121. [[CrossRef](#)] [[PubMed](#)]
36. Shi, Y.; Williamson, G. Quercetin lowers plasma uric acid in pre-hyperuricaemic males: A randomised, double-blinded, placebo-controlled, cross-over trial. *Br. J. Nutr.* **2016**, *115*, 800–806. [[CrossRef](#)] [[PubMed](#)]
37. Sae-tan, S.; Grove, K.A.; Lambert, J.D. Weight control and prevention of metabolic syndrome by green tea. *Pharmacol. Res.* **2011**, *64*, 146–154. [[CrossRef](#)] [[PubMed](#)]
38. Deng, Y.T.; Chang, T.W.; Lee, M.S.; Lin, J.K. Suppression of free fatty acid-induced insulin resistance by phytopolyphenols in C2C12 mouse skeletal muscle cells. *J. Agric. Food Chem.* **2012**, *60*, 1059–1066. [[CrossRef](#)] [[PubMed](#)]
39. Pournourmohammadi, S.; Grimaldi, M.; Stridh, M.H.; Lavallard, V.; Waagepetersen, H.S.; Wollheim, C.B.; Maechler, P. Epigallocatechin-3-gallate (EGCG) activates AMPK through the inhibition of glutamate dehydrogenase in muscle and pancreatic  $\beta$ -cells: A potential beneficial effect in the pre-diabetic state? *Int. J. Biochem. Cell Biol.* **2017**, *88*, 220–225. [[CrossRef](#)] [[PubMed](#)]
40. Ueda, M.; Nishiumi, S.; Nagayasu, H.; Fukuda, I.; Yoshida, K.; Ashida, H. Epigallocatechin gallate promotes glut4 translocation in skeletal muscle. *Biochem. Biophys. Res. Commun.* **2008**, *377*, 286–290. [[CrossRef](#)] [[PubMed](#)]
41. Ma, S.B.; Zhang, R.; Miao, S.; Gao, B.; Lu, Y.; Hui, S.; Li, L.; Shi, X.P.; Wen, A.D. Epigallocatechin-3-gallate ameliorates insulin resistance in hepatocytes. *Mol. Med. Rep.* **2017**, *15*, 3803–3809. [[CrossRef](#)] [[PubMed](#)]
42. Sung, H.Y.; Hong, C.G.; Suh, Y.S.; Cho, H.C.; Park, J.H.; Bae, J.H.; Park, W.K.; Han, J.; Song, D.K. Role of (–)-epigallocatechin-3-gallate in cell viability, lipogenesis, and retinol-binding protein 4 expression in adipocytes. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2010**, *382*, 303–310. [[CrossRef](#)] [[PubMed](#)]
43. Lee, M.S.; Kim, C.T.; Kim, I.H.; Kim, Y. Inhibitory effects of green tea catechin on the lipid accumulation in 3T3-L1 adipocytes. *Phytother. Res.* **2009**, *23*, 1088–1091. [[CrossRef](#)] [[PubMed](#)]
44. Moon, H.S.; Chung, C.S.; Lee, H.G.; Kim, T.G.; Choi, Y.J.; Cho, C.S. Inhibitory effect of (–)-epigallocatechin-3-gallate on lipid accumulation of 3T3-L1 cells. *Obesity* **2007**, *15*, 2571–2582. [[CrossRef](#)] [[PubMed](#)]
45. Sakurai, N.; Mochizuki, K.; Kameji, H.; Shimada, M.; Goda, T. (–)-epigallocatechin gallate enhances the expression of genes related to insulin sensitivity and adipocyte differentiation in 3T3-L1 adipocytes at an early stage of differentiation. *Nutrition* **2009**, *25*, 1047–1056. [[CrossRef](#)] [[PubMed](#)]

46. Wu, M.; Liu, D.; Zeng, R.; Xian, T.; Lu, Y.; Zeng, G.; Sun, Z.; Huang, B.; Huang, Q. Epigallocatechin-3-gallate inhibits adipogenesis through down-regulation of PPAR $\gamma$  and FAS expression mediated by PI3K-AKT signaling in 3T3-L1. *Eur. J. Pharmacol.* **2017**, *795*, 134–142. [[CrossRef](#)] [[PubMed](#)]
47. Li, M.; Liu, J.T.; Pang, X.M.; Han, C.J.; Mao, J.J. Epigallocatechin-3-gallate inhibits angiotensin II and interleukin-6-induced C-reactive protein production in macrophages. *Pharmacol. Rep.* **2012**, *64*, 912–918. [[CrossRef](#)]
48. Bose, M.; Lambert, J.D.; Ju, J.; Reuhl, K.R.; Shapses, S.A.; Yang, C.S. The major green tea polyphenol, (–)-epigallocatechin-3-gallate, inhibits obesity, metabolic syndrome, and fatty liver disease in high-fat-fed mice. *J. Nutr.* **2008**, *138*, 1677–1683. [[PubMed](#)]
49. Cunha, C.A.; Lira, F.S.; Rosa Neto, J.C.; Pimentel, G.D.; Souza, G.I.; Da Silva, C.M.; De Souza, C.T.; Ribeiro, E.B.; Sawaya, A.C.; Oller do Nascimento, C.M.; et al. Green tea extract supplementation induces the lipolytic pathway, attenuates obesity, and reduces low-grade inflammation in mice fed a high-fat diet. *Mediat. Inflamm.* **2013**, *2013*. [[CrossRef](#)] [[PubMed](#)]
50. Sampath, C.; Rashid, M.R.; Sang, S.; Ahmedna, M. Green tea epigallocatechin 3-gallate alleviates hyperglycemia and reduces advanced glycation end products via NRF2 pathway in mice with high fat diet-induced obesity. *Biomed. Pharmacother.* **2017**, *87*, 73–81. [[CrossRef](#)] [[PubMed](#)]
51. Szulinska, M.; Stepien, M.; Kregielska-Narozna, M.; Suliburska, J.; Skrypnik, D.; Bak-Sosnowska, M.; Kujawska-Luczak, M.; Grzymislawska, M.; Bogdanski, P. Effects of green tea supplementation on inflammation markers, antioxidant status and blood pressure in NaCl-induced hypertensive rat model. *Food Nutr. Res.* **2017**, *61*. [[CrossRef](#)] [[PubMed](#)]
52. Remely, M.; Ferk, F.; Sterneder, S.; Setayesh, T.; Roth, S.; Kepcija, T.; Noorizadeh, R.; Rebhan, I.; Greunz, M.; Beckmann, J.; et al. Egcg prevents high fat diet-induced changes in gut microbiota, decreases of DNA strand breaks, and changes in expression and DNA methylation of DNMT1 and MLH1 in C57BL/6J male mice. *Oxidative Med. Cell. Longev.* **2017**, *2017*. [[CrossRef](#)] [[PubMed](#)]
53. Grove, K.A.; Lambert, J.D. Laboratory, epidemiological, and human intervention studies show that tea (*camellia sinensis*) may be useful in the prevention of obesity. *J. Nutr.* **2010**, *140*, 446–453. [[CrossRef](#)] [[PubMed](#)]
54. Nagao, T.; Hase, T.; Tokimitsu, I. A green tea extract high in catechins reduces body fat and cardiovascular risks in humans. *Obesity* **2007**, *15*, 1473–1483. [[CrossRef](#)] [[PubMed](#)]
55. Hase, T.; Komine, Y.; Meguro, S.; Takeda, Y.; Takahashi, H.; Matsui, Y.; Inaoka, S.; Katsuragi, Y.; Tomitsu, J.; Shimasaki, H.; et al. Anti-obesity effects of tea catechins in humans. *J. Oleo Sci.* **2001**, *50*, 599–605. [[CrossRef](#)]
56. Basu, A.; Sanchez, K.; Leyva, M.J.; Wu, M.; Betts, N.M.; Aston, C.E.; Lyons, T.J. Green tea supplementation affects body weight, lipids, and lipid peroxidation in obese subjects with metabolic syndrome. *J. Am. Coll. Nutr.* **2010**, *29*, 31–40. [[CrossRef](#)] [[PubMed](#)]
57. Basu, A.; Du, M.; Sanchez, K.; Leyva, M.J.; Betts, N.M.; Blevins, S.; Wu, M.; Aston, C.E.; Lyons, T.J. Green tea minimally affects biomarkers of inflammation in obese subjects with metabolic syndrome. *Nutrition* **2011**, *27*, 206–213. [[CrossRef](#)] [[PubMed](#)]
58. Brown, A.L.; Lane, J.; Coverly, J.; Stocks, J.; Jackson, S.; Stephen, A.; Bluck, L.; Coward, A.; Hendrickx, H. Effects of dietary supplementation with the green tea polyphenol epigallocatechin-3-gallate on insulin resistance and associated metabolic risk factors: Randomized controlled trial. *Br. J. Nutr.* **2009**, *101*, 886–894. [[CrossRef](#)] [[PubMed](#)]
59. Khalesi, S.; Sun, J.; Buys, N.; Jamshidi, A.; Nikbakht-Nasrabadi, E.; Khosravi-Boroujeni, H. Green tea catechins and blood pressure: A systematic review and meta-analysis of randomised controlled trials. *Eur. J. Nutr.* **2014**, *53*, 1299–1311. [[CrossRef](#)] [[PubMed](#)]
60. Onakpoya, I.; Spencer, E.; Heneghan, C.; Thompson, M. The effect of green tea on blood pressure and lipid profile: A systematic review and meta-analysis of randomized clinical trials. *Nutr. Metab. Cardiovasc. Dis.* **2014**, *24*, 823–836. [[CrossRef](#)] [[PubMed](#)]
61. Peng, X.; Zhou, R.; Wang, B.; Yu, X.; Yang, X.; Liu, K.; Mi, M. Effect of green tea consumption on blood pressure: A meta-analysis of 13 randomized controlled trials. *Sci. Rep.* **2014**, *4*. [[CrossRef](#)] [[PubMed](#)]
62. Kim, A.; Chiu, A.; Barone, M.K.; Avino, D.; Wang, F.; Coleman, C.I.; Phung, O.J. Green tea catechins decrease total and low-density lipoprotein cholesterol: A systematic review and meta-analysis. *J. Am. Diet. Assoc.* **2011**, *111*, 1720–1729. [[CrossRef](#)] [[PubMed](#)]

63. Zheng, X.X.; Xu, Y.L.; Li, S.H.; Liu, X.X.; Hui, R.; Huang, X.H. Green tea intake lowers fasting serum total and LDL cholesterol in adults: A meta-analysis of 14 randomized controlled trials. *Am. J. Clin. Nutr.* **2011**, *94*, 601–610. [[CrossRef](#)] [[PubMed](#)]
64. Liu, K.; Zhou, R.; Wang, B.; Chen, K.; Shi, L.Y.; Zhu, J.D.; Mi, M.T. Effect of green tea on glucose control and insulin sensitivity: A meta-analysis of 17 randomized controlled trials. *Am. J. Clin. Nutr.* **2013**, *98*, 340–348. [[CrossRef](#)] [[PubMed](#)]
65. Nieman, D.C.; Henson, D.A.; Davis, J.M.; Dumke, C.L.; Gross, S.J.; Jenkins, D.P.; Murphy, E.A.; Carmichael, M.D.; Quindry, J.C.; McAnulty, S.R.; et al. Quercetin ingestion does not alter cytokine changes in athletes competing in the western states endurance run. *J. Interferon Cytokine Res.* **2007**, *27*, 1003–1011. [[CrossRef](#)] [[PubMed](#)]
66. Nieman, D.C.; Henson, D.A.; Davis, J.M.; Angela Murphy, E.; Jenkins, D.P.; Gross, S.J.; Carmichael, M.D.; Quindry, J.C.; Dumke, C.L.; Utter, A.C.; et al. Quercetin's influence on exercise-induced changes in plasma cytokines and muscle and leukocyte cytokine mRNA. *J. Appl. Physiol.* **2007**, *103*, 1728–1735. [[CrossRef](#)] [[PubMed](#)]
67. Nieman, D.C.; Henson, D.A.; Maxwell, K.R.; Williams, A.S.; McAnulty, S.R.; Jin, F.; Shanely, R.A.; Lines, T.C. Effects of quercetin and EGCG on mitochondrial biogenesis and immunity. *Med. Sci. Sports Exerc.* **2009**, *41*, 1467–1475. [[CrossRef](#)] [[PubMed](#)]
68. Nieman, D.C.; Henson, D.A.; Gross, S.J.; Jenkins, D.P.; Davis, J.M.; Murphy, E.A.; Carmichael, M.D.; Dumke, C.L.; Utter, A.C.; McAnulty, S.R.; et al. Quercetin reduces illness but not immune perturbations after intensive exercise. *Med. Sci. Sports Exerc.* **2007**, *39*, 1561–1569. [[CrossRef](#)] [[PubMed](#)]
69. Irizarry, R.A.; Hobbs, B.; Collin, F.; Beazer-Barclay, Y.D.; Antonellis, K.J.; Scherf, U.; Speed, T.P. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* **2003**, *4*, 249–264. [[CrossRef](#)] [[PubMed](#)]
70. Baldi, P.; Long, A.D. A bayesian framework for the analysis of microarray expression data: Regularized *t*-test and statistical inferences of gene changes. *Bioinformatics* **2001**, *17*, 509–519. [[CrossRef](#)] [[PubMed](#)]
71. Subramanian, A.; Tamayo, P.; Mootha, V.K.; Mukherjee, S.; Ebert, B.L.; Gillette, M.A.; Paulovich, A.; Pomeroy, S.L.; Golub, T.R.; Lander, E.S.; et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 15545–15550. [[CrossRef](#)] [[PubMed](#)]
72. Dam, V.; Sikder, T.; Santosa, S. From neutrophils to macrophages: Differences in regional adipose tissue depots. *Obes. Rev.* **2016**, *17*, 1–17. [[CrossRef](#)] [[PubMed](#)]
73. Talukdar, S.; Oh, D.Y.; Bandyopadhyay, G.; Li, D.; Xu, J.; McNelis, J.; Lu, M.; Li, P.; Yan, Q.; Zhu, Y.; et al. Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. *Nat. Med.* **2012**, *18*, 1407–1412. [[CrossRef](#)] [[PubMed](#)]
74. Krause, B.R.; Hartman, A.D. Adipose tissue and cholesterol metabolism. *J. Lipid Res.* **1984**, *25*, 97–110. [[PubMed](#)]
75. Parr, E.B.; Coffey, V.G.; Hawley, J.A. 'Sarcobesity': A metabolic conundrum. *Maturitas* **2013**, *74*, 109–113. [[CrossRef](#)] [[PubMed](#)]
76. Brill, M.J.; Diepstraten, J.; Van Rongen, A.; Van Kralingen, S.; Van den Anker, J.N.; Knibbe, C.A. Impact of obesity on drug metabolism and elimination in adults and children. *Clin. Pharmacokinet.* **2012**, *51*, 277–304. [[CrossRef](#)] [[PubMed](#)]
77. Luster, A.D.; Alon, R.; Von Andrian, U.H. Immune cell migration in inflammation: Present and future therapeutic targets. *Nat. Immunol.* **2005**, *6*, 1182–1190. [[CrossRef](#)] [[PubMed](#)]
78. Dutta, S.; Sengupta, P. Men and mice: Relating their ages. *Life Sci.* **2016**, *152*, 244–248. [[CrossRef](#)] [[PubMed](#)]
79. Cialdella-Kam, L.; Nieman, D.C.; Knab, A.M.; Shanely, R.A.; Meaney, M.P.; Jin, F.; Sha, W.; Ghosh, S. A mixed flavonoid-fish oil supplement induces immune-enhancing and anti-inflammatory transcriptomic changes in adult obese and overweight women—a randomized controlled trial. *Nutrients* **2016**, *8*, 277. [[CrossRef](#)] [[PubMed](#)]
80. Bakker, G.C.; Van Erk, M.J.; Pellis, L.; Wopereis, S.; Rubingh, C.M.; Cnubben, N.H.; Kooistra, T.; Van Ommen, B.; Hendriks, H.F. An antiinflammatory dietary mix modulates inflammation and oxidative and metabolic stress in overweight men: A nutrigenomics approach. *Am. J. Clin. Nutr.* **2010**, *91*, 1044–1059. [[CrossRef](#)] [[PubMed](#)]

81. Schmidt, S.; Stahl, F.; Mutz, K.O.; Scheper, T.; Hahn, A.; Schuchardt, J.P. Different gene expression profiles in normo- and dyslipidemic men after fish oil supplementation: Results from a randomized controlled trial. *Lipids Health Dis.* **2012**, *11*, 105. [[CrossRef](#)] [[PubMed](#)]
82. Skulas-Ray, A.C. Omega-3 fatty acids and inflammation: A perspective on the challenges of evaluating efficacy in clinical research. *Prostaglandins Other Lipid Mediat.* **2015**, *116–117*, 104–111. [[CrossRef](#)] [[PubMed](#)]
83. Wang, P.; Heber, D.; Henning, S.M. Quercetin increased bioavailability and decreased methylation of green tea polyphenols in vitro and in vivo. *Food Funct.* **2012**, *3*, 635–642. [[CrossRef](#)] [[PubMed](#)]
84. D'Andrea, G. Quercetin: A flavonol with multifaceted therapeutic applications? *Fitoterapia* **2015**, *106*, 256–271. [[CrossRef](#)] [[PubMed](#)]
85. Glaeser, H.; Bujok, K.; Schmidt, I.; Fromm, M.F.; Mandery, K. Organic anion transporting polypeptides and organic cation transporter 1 contribute to the cellular uptake of the flavonoid quercetin. *Naunyn Schmiedebergs Arch. Pharmacol.* **2014**, *387*, 883–891. [[CrossRef](#)] [[PubMed](#)]
86. Roth, M.; Timmermann, B.N.; Hagenbuch, B. Interactions of green tea catechins with organic anion-transporting polypeptides. *Drug Metab. Dispos.* **2011**, *39*, 920–926. [[CrossRef](#)] [[PubMed](#)]
87. Henagan, T.M.; Lenard, N.R.; Gettys, T.W.; Stewart, L.K. Dietary quercetin supplementation in mice increases skeletal muscle PGC1alpha expression, improves mitochondrial function and attenuates insulin resistance in a time-specific manner. *PLoS ONE* **2014**, *9*, e89365. [[CrossRef](#)] [[PubMed](#)]
88. Palermo, N.E.; Gianchandani, R.Y.; McDonnell, M.E.; Alexanian, S.M. Stress hyperglycemia during surgery and anesthesia: Pathogenesis and clinical implications. *Curr. Diabetes Rep.* **2016**, *16*, 33. [[CrossRef](#)] [[PubMed](#)]
89. Togashi, Y.; Shirakawa, J.; Okuyama, T.; Yamazaki, S.; Kyohara, M.; Miyazawa, A.; Suzuki, T.; Hamada, M.; Terauchi, Y. Evaluation of the appropriateness of using glucometers for measuring the blood glucose levels in mice. *Sci. Rep.* **2016**, *6*. [[CrossRef](#)] [[PubMed](#)]
90. Gonzalez-Gallego, J.; Garcia-Mediavilla, M.V.; Sanchez-Campos, S.; Tunon, M.J. Fruit polyphenols, immunity and inflammation. *Br. J. Nutr.* **2010**, *104*, 15–27. [[CrossRef](#)] [[PubMed](#)]
91. Mereles, D.; Hunstein, W. Epigallocatechin-3-gallate (EGCG) for clinical trials: More pitfalls than promises? *Int. J. Mol. Sci.* **2011**, *12*, 5592–5603. [[CrossRef](#)] [[PubMed](#)]
92. Van Duynhoven, J.; Vaughan, E.E.; Jacobs, D.M.; Kemperman, R.A.; Van Velzen, E.J.J.; Gross, G.; Roger, L.C.; Possemiers, S.; Smilde, A.K.; Doré, J.; et al. Metabolic fate of polyphenols in the human superorganism. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 4531–4538. [[CrossRef](#)] [[PubMed](#)]
93. Gonzalez, F.J. Cytochrome P450 humanised mice. *Hum. Genom.* **2004**, *1*, 300–306. [[CrossRef](#)] [[PubMed](#)]
94. Graf, B.A.; Ameho, C.; Dolnikowski, G.G.; Milbury, P.E.; Chen, C.Y.; Blumberg, J.B. Rat gastrointestinal tissues metabolize quercetin. *J. Nutr.* **2006**, *136*, 39–44. [[PubMed](#)]
95. Lambert, J.D.; Lee, M.J.; Lu, H.; Meng, X.; Hong, J.J.; Seril, D.N.; Sturgill, M.G.; Yang, C.S. Epigallocatechin-3-gallate is absorbed but extensively glucuronidated following oral administration to mice. *J. Nutr.* **2003**, *133*, 4172–4177. [[PubMed](#)]

