The Influence of Catechin on Adiponectin Gene Expression and Insulin Resistance in Obese Rats

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Abstract

Obesity represented as an abnormal accumulation of visceral fat, which is closely related with insulin resistance, adipokine expression. The generation of adiponectin (AdipoQ) has been linked to visceral fat accumulation. Obesity, insulin resistance, and cardiovascular disease have all been linked to decreased levels of AdipoQ. Fifteen white female rats were used in the experiment; they were divided into three primary groups equally as negative control, positive control and Catechin-fed group. After 28 days of restricted diet for negative control group the weight became 150g, while the weight of the positive control group with high fat diet change to 217g significantly. First group and positive control group killed after the 28 days from starting day. While, the five remaining rats treated with (+) Catechin for another 28 days as Catechin treated group then killed for getting samples. The blood and visceral adipose tissue samples from all groups used to measure glucose, insulin and lipid profile, and AdipoQ. Treatment with (+) Catechin increased the levels of AdipoQ in visceral WAT as well as improved fasting glucose and insulin levels resulting in amelioration of Insulin Resistance in Cat-fed group compared to positive control group. A significant difference of circulating glucose, insulin, and AdipoQ levels and gene expression inside visceral WAT obtained between means of positive control with Catechin treatment group.

Keywords: Catechin, Glucose, Insulin, Adiponectin Gene Expression, Obesity.

Introduction

Obesity represented as an abnormal accumulation of subcutaneous and visceral fat, which is closely related with changes in lipids metabolism, endothelial cells dysfunction, insulin resistance, adipokine expression, and inflammation [1]. These alterations are thought to enlarge the likelihood of initial metabolic syndrome, diabetes, atherosclerosis and cardiovascular disease [2].

Additionally, being overweight heightens the severity of preexisting renal injury and is a risk factor for kidney failure [3]. Obesity’s negative consequences are mostly caused by a pair of variables; excessive adipose tissue enlargement and a higher production of pathogenic products from larger cells of fat [4]. It is a result of multiple factors interacting, including genetic as well as environmental factors [5].

Adiponectin as single-chain protein comprised of 244 amino acids with a molecular weight of around 26 kilo Daltons (kDa) released by white adipose tissue (WAT). The human adiponectin gene (AdipoQ), which is located on chromosome 3q27, encodes the AdipoQ protein [6]. The generation of AdipoQ has been linked to visceral fat accumulation. Insulin resistance, obesity and cardiovascular disease have all been linked to decreased levels of AdipoQ [7]. Hypoadiponectinemia was detected in obese humans and animal models, while higher AdipoQ levels were observed after weight loss. Obesity prevention becomes essential for public health and has been treated with medication and surgical techniques, but these approaches have clear negative consequences [8].

It might be safer to address the issue of obesity by using dietary supplements to prevent or treat the condition [9]. A number of recent investigations in Iraq have investigated the effects of certain plant extracts and compounds on models of animals. Among the studies mentioned are Moringa oleifera [10].

Hylocereus polyrhizus peel, zinc oxide and chromium oxide nanoparticles on diabetes, and
Costus-loaded silver nanoparticles and its impact on atherosclerosis. Treatment with Green Tea Catechin (GTC) increased the levels of Peroxisome proliferation activated receptor gamma (PPAR-γ) inside brown adipose tissue, visceral WAT, subcutaneous WAT, as well as expression of genes implicated in fatty acid metabolism these results show that Catechin has an opposing obesity effect by shifting the Peroxisome proliferation activated receptor-signaling route [11]. Mentioned that heart disease is the primary source of disability, morbidity and death globally. Coronary heart disease with atherosclerosis can be reduced by Catechin due to low density lipoprotein (LDL)-cholesterol-lowering action [12].

Discovered that gallate-catechin interferes with the biliary micelle system in the colon, forming insoluble cholesterol precipitates and increasing cholesterol fecal output. This notable reduction in cholesterol absorption and concentration in the liver promotes LDL-receptor activation and its expression [13]. This receptor exists upon most cells, particularly hepatic cells, and is capable of removing circulating LDLs lipoproteins [14]. However, some study discovered that GTC ensues a discriminating action [15]. GTC dramatically decreases the amount of serum LDL [16].

Furthermore, catechin has varied implications on cancer prevention triggered apoptosis because of reactive oxygen species (ROS) generation and activating caspase-9 and 3, usually resulting in obstruction of cell-cycle during gape one phase by modulating Cyclin dependent kinase 1 (cyclin D1) expression, potent-cyclin dependent kinase inhibitor-1 (p21), and Cyclin-dependent kinase 4 cdk4. [17]. Tea and its components can exhibit antibacterial effects by blocking intracellular enzymes and nucleic acid synthesis, damaging cell walls and membranes [18].

Anti- Diabetes mellitus effect has been shown to sustain β-cell by decreasing radicals and inflammation factors in vitro or decreasing Nitric oxide synthesis. Green tea was also able to reduce blood sugar levels in diabetic rats by stimulating β-cells to secrete more insulin Catechin based anti-inflammatory therapy decreased responses to inflammation in adipocytes, normalized AdipoQ levels, protected the liver cells from obesity induced damage and recovered normal functioning of the liver [19].

Anti-hypertensive effects reduce the chances of budding hypertension by 46%, thought to be mediated due to antioxidant effect [20]. The main objective of this study determines the impact of a catechin supplement on the expression pattern of AdipoQ, a gene implicated in mitigating IR in visceral WAT.

Material and Methods

Experimental design

Fifteen white female rats were used in the experiment; they were divided into three primary groups based on the initial body weights listed in the table (1) below. For a duration of 28 days, two groups of animals—one administered (+) Catechin and the other obese as a positive control—were fed a regular diet and allowed unrestricted access to water. Five normal rats were kept under food restriction for the duration of the experiment in order to maintain a weight consistency near to their starting body weight (designated as negative control rats).

The (+) catechin-fed group rats were given daily doses of 1.7 mg of crude (+) catechin (dissolved in cold water) for a period of 28 days. Five obese rats in the positive control group are not given any medication [20].

Blood samples

Following overnight fasting, physicochemical and endocrine markers were evaluated using a plasma sample obtained from the animal heart via sterile syringe. Animal anesthetized by Ketamine 10 milligram/Kg of body wight and Xylazine 90 milligram/Kg of body weight (0.2ml/animal) and collecting 5 ml of blood. The serum was obtained from blood used for laboratory analysis.

For total-cholesterol, triglyceride, high density lipoprotein (HDL) and glucose the kits used from Bio research for diagnosis Germany to determine them spectrophotometric. In addition, insulin and AdipoQ determined by the enzyme-linked immunosorbent assay (ELISA) technique using Rat Insulin Elisa kit SUNLONG-Chin and Rat adiponectin Elisa kit SUNLONG-Chin, respectively.

Visceral adipose tissue and mRNA of adiponectin extraction

The obtained visceral adipose tissues used for adiponectin gene expression detection using Transgenbiotech (ET101). The total mRNA from adipose tissue was extracted matching to company instruction. Purity of total mRNA estimated as the absorbance ratio A260/A280 was 1.97. The estimated mRNA concentration, assumed from reading the absorbance of mRNA samples at 260nm was 35µg/ml. Reverse transcription polymerase chain reaction (RT-PCR) was performed using (English-Promega) and the procedure applied agreeing with the constructor’s protocols. Adiponectin primers sequences were designed (Table 2). [21]

Preparation of Primers

The primers manufactured in Korea by Macrogen Company is dryer and prepared by add 250 microns of deionized water to obtained concentration 100 picomole and is considered as stock solution. After dilution 10 times of the primers stock solution RT-PCR performed with the following mixture in PCR tubes of each sample; MgCl2 (1.6 μL), Master Max microns, reverse transcriptase (0.4 μL), Carboxy-X-Rhodamine (CXR) Reference Dye (0.3 μL), primer forward 2 μL, primer reverse 2 μL and sample RNA (6-7) μL of each sample (Table 3).

Statistical analysis

Statistical data analysis was conducted according to SPSS version 25, were the T-test and a one-way Analysis of variance (ANOVA) were employed to determine significant differences among and within groups. Data were provided as mean ± SD, with P values ≤ 0.05 indicating statistical significance for biochemical parameters. Polymerase chain reaction results were statistically significant (P value ≤ 0.001). [22]

Ethics approval

Every experiment was authorized by the Animal Ethics Committee. Of University of Kufa named Institutional Animal Care and Use Committee (IACUC) according to the book, University of Kufa, No1686 for the Data 10-3-2024.

Statistical analysis

The gathered information was statistically analyzed using the Analysis of Variance (ANOVA) to identify mean differences and the Chi-square test for categorical variables. The SPSS software was used to conduct the analysis [13].

Results

The mean ± SD of all biochemical and hormonal levels as well as adiponectin gene expression are explained in (Table 1). This investigation discovered that the levels of glucose were significantly different (p ≤ 0.001) between means of negative and positive control groups. As well, a significant difference (p ≤ 0.001) of glucose level obtained between means of positive control group with Catechin treatment group. Insulin levels were also differed significantly (p ≤ 0.001) between negative and positive control groups. Also, a significant difference (p ≤ 0.001) insulin levels obtained between positive controls groups with Catechin treatment group.

The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) value was significantly (p ≤ 0.001) increase to 2.8 (A value greater than 2 indicates IR) in obese positive control group compared to negative control. Moreover, the HOMA-IR value of catechin-fed group decreased significantly (p ≤ 0.001) to 0.85 compared to positive control group. Adiponectin levels had significant differences (p ≤ 0.001) obtained between means of negative and positive control groups.

Moreover, a significant difference (p ≤ 0.001) of AdipoQ level obtained between means of negative control group with Catechin treatment group as shown in Fig. 1 and 2. Finally, there was a significant decrease (p ≤ 0.001) of gene expression as fold changes obtained in mean of positive control (0.07) compared to negative control (1.38). But, fold change of catechin-fed group was (1.23) that return to near the value of negative control.

Discussion

Glucose homeostasis

The significant differences of glucose results may be due to the prolonged exposure to high fat diets eventually impairs glucose tolerance and β-cells function, resulting in obvious diabetes [23]. Whereas, improving glucose levels after catechin treatment for 28 days represents the positive effect of catechin on glucose homeostasis. The mechanism of action underlying Epigallocatechin Gallates (EGCG’s) anti-hyperglycemic actions in rats fed a high sugar and high fat diet is unclear. Epigallocatechin gallate (EGCG) has been shown to considerably improve blood glucose homeostasis in obese diabetic rats and reduced Type 2 Diabetes Mellitus (T2DM) via enhancing β-cell function [7].

As well as raised weights in diabetes-induced mice and prolonged EGCG therapy lowers blood glucose levels and promotes insulin sensitivity in a diabetic mice model [24]. It is known that pancreas and duodenum home box 1 (PDX-1) and transcription factor [MAF bZIP transcription factor A (MafA)] perform significant roles into sustaining cell maturation in rat pancreatic duct. Also, MafA promotes maturation of pancreatic cells, differentiations, as well as survival/proliferation, while PDX-1 which mostly yielded by β-cells and causes their differentiation besides insulin production [25]. Another study recorded that it activates Transport receptor potential channel -3 (TRPC-3) as well as TRPC-6 of Ca-channels, stimulates cyclin D plus Extracellular signal regulated protein kinase 1/2 (ERK1/2), along with increasing β-cell multiplying. The PDX-1 supports pancreatic development and modulates islet cell function during maturation [26,27].

Expression of MafA onsets at E13.5 where appears just inside β-cells until maturity. In diabetic model animals, the levels of PDX-1 along with MafA at pancreas were significantly lowered, whereas later on EGCG consumption, PDX-1 and MafA levels increased significantly, showing that EGCG has effects in both the early and late phases of pancreatic development, as well as the main phase of insulin production [28]. Furthermore, EGCG’s anti-diabetes...
actions may increase Glucose transporter-2 (GLUT2), peroxisome proliferation receptor gamma coactivator -1 Beta (PGC-1β), Sterol regulatory element binding protein -1c (SREBP-1c), and fatty acid synthase (FAS) communication to enhance insulin sensitivity by reducing Insulin Resistance-induce oxidative damage, inflammation, and fatty acids synthesis. [29].

**Adiponectin and Insulin**

In this study of rat models, high fat diet intake resulted in circulating lipid disorder, IR, decreased circulating AdipoQ, obesity, besides adipose tissue inflammation. Dietetic Catechin supplement improved all of these measures. Catechin in 3T3-L1 adipocytes reduced tumor necrosis alpha (TNF-α) provoked protein carboxylation, pro-inflammatory cytokine production Mobile colistin resistance (MCP-1), plus AdipoQ concentrations. Catechin’s ability to reduce Jun N-terminal kinase (JNK) and potent cyclin dependent kinase inhibitor (p38) activation and prevent PPAR-γ downregulation contributes to its preventive effects on adipose inflammation. [30]

Reduced plasma AdipoQ contributes to the development of lipid-induced IR in the organs of the liver and skeletal muscles. Blood AdipoQ levels in humans show a strong correlation with overall sensitivity to insulin [31, 32]. Overexpression or injection of AdipoQ in rodents reduces hyperglycemia and enhances systemic insulin sensitivity Adiponectin-deficient rat have reduced sensitivity to insulin and are more prone to develop diabetes [33]. Putting all of this together, circulating AdipoQ may indicate the existence of functional adipose tissue in the body, which is part of the WAT’s mechanism for fat storage.

In normal physiological processes, healthful adipose tissue produces enough AdipoQ so as to encourage the storing of Triacylglycerol's (TAG) accessible WAT and signal a modification toward increased fatty acid degradation inside skeletal muscle; though, with being overweight, because adipose tissue possesses a limited size for storage, decline WAT production of AdipoQ [34].

This disruption in fat storage space and muscle fat metabolism may result in amplified ectopic lipid TAG and membranous Diacylglycerol (DAGs) formation inside the skeletal muscle along with liver, as well as the development of IR in these tissues, which conducts to the metabolic syndrome, liver disease. Steatosis of Nonalcoholic fatty liver disease (NASH), with heart disease. [34]

**The gene expression of Adiponectin for all group**

This study found that there was a significant deference (p ≤ 0.001) gene expression level obtained between means of negative, positive control, Catechin groups. These findings are consistent with previous study finding that Catechin improves AdipoQ expression and secretion in adipocytes. The expression of AdipoQ increased adipocyte growth and insulin-responsive glucose transport. As a result, Catechin-induced AdipoQ expression is associated with increased insulin-stimulated glucose transfer. Its treatment increased insulin-stimulated absorption of glucose in Fibroblast 3T3-L1 adipocytes, suggesting that it serves as a diabetic sensitizer. Catechin treatment reduced Kruppel like factor -7 (KLF7) expression and did not change PPARγ, The transcription factor CCAAT/Enhancer binding protein alpha (C/EBPα), or The transcription factor CCAAT/Enhancer Binding Protein α (C/EBPα) levels of entirely differentiated adipocytes. This data shows the route is account for the rise expression of AdipoQ arbitrated by means of catechin care [35].

These result in accordance with another study several extracellular signaling factors regulate AdipoQ gene expression, it involves the hormone insulin, β-adrenergic agonists plus TNF-α, PPARγ stimulates the AdipoQ promoter through an unidentified region, while C/EBPα controls AdipoQ gene transcription through an intronic enhancer [36]. The protein SREBP interacts with the AdipoQ promoter, affecting dependent on insulin AdipoQ expression. KLF7 was demonstrated to inhibit adiponectin gene expression in adipocytes [37].

**Catechin and Adiponectin expression**

In this study the effect of (+) catechin on adiponectin gene expression was significantly increased in catechin fed obese rats compared to high fat diet obese group. Catechin may augments the expression of adiponectin and rises glucose entrance into 3T3-L1 adipocytes. The special effects are come with the KLF7 downregulation, which is recognized as transcription factor counted in the pathogenesis of type 2 diabetes [35].

However, catechin promotes adipocyte differentiation and improved sensitivity to insulin partially by straight activation of PPARγ, which may possibly be at the root of the detected pharmacological values of green tea ingestion in reduction the threat of type 2 diabetes [38]. Green tea catechins show to diminish hepatic steatosis in a PPARα-related mode, and particularly epigallocatechin and epicatechin be able to in some way activate PPARα, while it appears they are not absolute ligands [39].

Therefore, catechin present a good promotion for adiponectin gene expression of obese animals which give potential uses as obesity metabolic abnormalities especially when the exact mechanism of action is elucidated by researches.

**Conclusion**

(+ ) Catechin has positive effect on adiponectin gene expression in obese female rats where increased
expression of this gene after four weeks administration. Additionally, after (+) Catechin administration the IR improved significantly in these rats, where it is evidently due to minimizing serum glucose concentrations in obese rats. The other most important effects of Catechin were observed by decreasing fasting insulin in obese rats, as well as AdipoQ that both participate to attenuate IR and obesity adverse effects.

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Conflict of Interest

There are no conflicts of interest.

Author’s contributions

All Authors designed the entire work and worked simultaneously to collect data and statistically analyses it.

| TABLE 1. Weight, biochemical, hormonal and adiponectin gene expression results for all experiments |
|---------------------------------|----------------|------------------|---------------|----------------|---------------|
| Biochemistry                    | Groups represented as (Mean ± SD) | Negative Control | Positive Control | P-value | Cat-fed Group | P-value |

| Initial BW (g) | 140.6 ± 3.78 | 147.6 ± 2.51 | 0.19 | 143.8 ± 2.86 | 0.69 |
| BW 30 d (g)    | 150.2 ± 2.86 | 217.6 ± 4.28 | < 0.001 | 212.8 ± 4.44 | 0.52 |
| BWG30(g)       | 9.6 ± 3.21   | 66 ± 10.2 | < 0.001 | 69 ± 5 | 0.82 |
| BW 60 d(g)     | 158.4 ± 9.4  | 205.4 ± 5.55 | < 0.001 | 185.4 ± 4.22 | < 0.001 |
| Glucose mg/dL  | 122.72 ± 14.4 | 225.51 ± 24.47 | < 0.001 | 135.68 ± 4.72 | < 0.001 |
| Insulin mU/L   | 1.84 ± 0.16  | 4.99 ± 1.28 | < 0.001 | 2.54 ± 0.37 | 0.0002 |
| AdipoQ ng/ml   | 25.59 ± 2.81 | 14.06 ± 0.73 | < 0.001 | 15.68 ± 0.55 | 0.44 |
| HOMA-IR*       | 0.56 ± 0.08  | 2.8 ± 0.85 | < 0.001 | 0.85 ± 0.11 | < 0.001 |
| Gene expression† | 1.38 ± 0.41 | 0.07 ± 0.02 | < 0.001 | 1.23 ± 0.35 | < 0.001 |

*A value greater than 2 indicates insulin resistance
† Gene expression as fold change of AdipoQ according to house-keeping gen GAP-DH.

| TABLE 2. Primers of gene Expression experiment |
|-----------------------------------------------|---------------------------------|----------------|----------------|----------------|---------------|
| Organism                                      | Gene                            | Primer name      | Sequence        | Accession No   |
| Rattus rarus                                   | AdipoQ                          | Adiponectin-RF   | CTACTGTTGCAAACGCTCTCC |            |
|                                               |                                 | Adiponectin-RR   | CTTACATCTTTTCATGTCAACC |            |
| Rattus rarus                                   | GAP-DH                          | GAPDH –F         | AGTGGCCAGCTCGTCTCTATA | N017008.4   |
|                                               |                                 | GAPDH-R          | GATGTTGATGGTTTTCCCGT |            |

| TABLE 3. Preparation of Real time PCR solution |
|-----------------------------------------------|----------------|----------------|---------------|
| Component                                    | Volume | Concentration |
| MgCl₂                                        | 1.6 µL | 1x             |
| Master mix                                   | 10 µL  | 1x             |
| Reverse transcriptase                        | 0.4 µL | 1x             |
| CXR                                          | 0.3 µL | 1x             |
| Forward primer                               | 2 µL   | 10 Pmol        |
| Reverse primer                               | 2 µL   | 10 Pmol        |
| Sample                                       | (6-7) µL |                |
| ddH₂O                                        | up of 25 µL |            |
Fig. 1. Role of orally intubated 1.7mg (Kg/day) of Catechin on Glucose Adiponectin and insulin level for all group. The different letters indicate significant difference while similar letter indicate no significant difference.

Fig. 2. Role of orally administrated 1.7mg (Kg/day) of Catechin on the gene expression of Adiponectin for all group.

References


Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. *Diabetes*, **50**(5), 1126-1133 (2001).


Effect of Catechin on the Adipogenic Environment and Adiponectin in the Rodent Perfume

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The objective of the present study was to investigate the effect of Catechin, a green tea extract, on adipose tissue and adiponectin expression in rat perfumers, as well as the correlation of adiponectin with adipose tissue weight and insulin sensitivity. The study was conducted on adult Wistar rats weighing 150-175 g. The rats were divided into five groups: control, Catechin (+), high-fat diet, and control + Catechin (+) groups. Blood samples were collected from the abdominal aorta at the end of the experiment. The results showed that the Catechin (+) group had significantly lower adipose tissue weight and higher adiponectin levels compared to the control group. The study concluded that Catechin has a positive effect on adipose tissue and insulin sensitivity. Further studies are needed to investigate the underlying mechanisms of these effects.

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