

Novel CoQ10 Antidiabetic Mechanisms Underlie Its Positive Effect: Modulation of Insulin and Adiponectine Receptors, Tyrosine Kinase, PI3K, Glucose Transporters, sRAGE and Visfatin in Insulin Resistant/Diabetic Rats

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Abstract

As a nutritional supplement, coenzyme Q10 (CoQ10) was tested previously in several models of diabetes and/or insulin resistance (IR); however, its exact mechanisms have not been profoundly explicated. Hence, the objective of this work is to verify some of the possible mechanisms that underlie its therapeutic efficacy. Moreover, the study aimed to assess the potential modulatory effect of CoQ10 on the antidiabetic action of glimepiride. An insulin resistance/type 2 diabetic model was adopted, in which rats were fed high fat/high fructose diet (HFFD) for 6 weeks followed by a single sub-diabetogenic dose of streptozotocin (35 mg/kg, i.p.). At the end of the 7th week animals were treated with CoQ10 (20 mg/kg, p.o) and/or glimepiride (0.5 mg/kg, p.o) for 2 weeks. CoQ10 alone opposed the HFFD effect and increased the hepatic/muscular content/activity of tyrosine kinase (TK), phosphatidylinositol kinase (PI3K), and adiponectin receptors. Conversely, it decreased the content/activity of insulin receptor isoforms, myeloperoxidase and glucose transporters (GLUT4; 2). Besides, it lowered significantly the serum levels of glucose, insulin, fructosamine and HOMA index, improved the serum lipid panel and elevated the levels of glutathione, sRAGE and adiponectin. On the other hand, CoQ10 lowered the serum levels of malondialdehyde, visfatin, ALT and AST. Surprisingly, CoQ10 effect surpassed that of glimepiride in almost all the assessed parameters, except for glucose, fructosamine, TK, PI3K, and GLUT4. Combining CoQ10 with glimepiride enhanced the effect of the latter on the aforementioned parameters. Conclusion: These results provided a new insight into the possible mechanisms by which CoQ10 improves insulin sensitivity and adjusts type 2 diabetic disorder. These mechanisms involve modulation of insulin and adiponectin receptors, as well as TK, PI3K, glucose transporters, besides improving lipid profile, redox system, sRAGE, and adipocytokines. The study also points to the potential positive effect of CoQ10 as an add-on to conventional antidiabetic therapies.

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Introduction

Prevalence of insulin resistant (IR)/type 2 diabetes nowadays depends on the slothful life style on one side and on the high intake of "Westernized" diet, which deemed to be the main environmental trigger [1,2], on the other side.

Pathogenesis of this metabolic disorder emerges from a complicated interplay between several factors, including mitochondrial dysfunction that lies somewhere along a continuum from genetic to environmental abnormalities. Several studies have pointed to the paramount importance of the mitochondrial dysfunction in different metabolic disorders including IR/type 2

diabetes [3,4]. Mitochondrial dysfunction was found to be an underlying mechanism and a complication of diabetes, with a major role played by oxidative stress [5]. Hence, treatment or supplemental strategies that contemplate on improving mitochondrial function and constraining oxidative stress might present important prospect.

Among the mitochondria-linked supplements is the Co-enzyme Q10 (CoQ10), a fat-soluble, vitamin like quinone commonly known as ubiquinone. It exists in the body in 2 forms; the oxidized form that acts as an electron carrier during the mitochondrial respiration and the reduced form that serves as an endogenous

antioxidant [6]. Although CoQ10 is present in most tissues, yet the heart, liver, kidneys, and pancreas possess the highest concentrations [6].

CoQ10 is one of several nutritional components that have been recorded to be inadequate in diabetic states [6–9]. The CoQ10 deficiency is possibly due to the impaired mitochondrial substrate metabolism and/or increased oxidative stress [10] that may be linked with the impairment of β -cells and the development of IR [11].

To this end, CoQ10 appears to be an interesting component that merits supplementation in several diabetes/insulin resistant studies in order to assess its ability to counteract insulin-resistance associated metabolic disorders, and/or diabetes-related complications; studies that were carried either experimentally [6,8,12,13] or clinically [7,14–16].

Albeit several studies show controversial findings regarding the antidiabetic effect of CoQ10, yet those that support its positive effect did not explain the potential mechanisms, which was the goal of this study. Therefore, we have tested the influence of CoQ10 supplementation on metabolic alterations induced by a combination of high fat-high fructose diet (HFFD) and a single subdiabetogenic dose of streptozotocin (STZ) to offer overt diabetic rats. To verify the possible mechanisms that may underlie the antidiabetic/insulin sensitizing effect of CoQ10, the study shed light on the possible involvement of insulin receptors, tyrosine kinase (TK) and phosphatidylinositol 3 kinase (PI3K), as main members of the insulin downstream cascade. Besides, the current work evaluated the potential effect on adiponectin receptors, glucose transporters and myeloperoxidase, in the liver and soleus skeletal muscle. In addition, the effect of CoQ10 on the serum level of endogenous soluble receptor of advanced glycosylated end products (sRAGE) and 2 adipocytokines, *viz.*, adiponectin and visfatin was studied. Glimperide was used as the standard antidiabetic drug to compare the CoQ10 effect and to assess its potential importance when combined with the sulfonylurea derivative.

Materials and Methods

1. Drugs and Chemicals

CoQ10 and STZ were purchased from Sigma-Aldrich Co. (St Louis, MO, USA) and glimepiride (Amaryl) from Sanofi-Aventis Co. (Zeitoun, Cairo, Egypt), while long-acting insulin (Monotard) was obtained from Novo Nordisk Co. (Copenhagen, Denmark). The diet ingredients, such as cholesterol, was procured from Panreac Quimica (Barcelona, Spain), fructose was obtained from El-Nasr Chemical Co. (Abou Zaabal, Cairo, Egypt), while lard was brought from commercial sources and other chemicals used were of analytical grades. Both glimepiride and CoQ10 was administered orally, with the first suspended in distilled water, and CoQ10 was dissolved in 1% Tween 80.

2. Animals

Adult male Wistar albino rats weighing 80–90 g were purchased from the National Research Center Laboratory (Cairo, Egypt) and were housed in standard polypropylene cages and kept under constant environmental conditions and equal light-dark cycles. Rats were acclimatized for 1 week and were fed commercially available rat normal pellet diet and water *ad libitum*, prior to the dietary manipulation.

2.1 Ethics Statement. This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee of the Faculty of Pharmacy, on the Ethics of Animal Experiments of the Cairo University (Permit Number: PT 290). All surgery was performed under deep sodium pentobarbital anesthesia and all efforts were made to minimize suffering.

3. Development of Insulin Resistant/Type 2 Diabetic Rats

The current model was adopted according to a previous study by Schaalan et al. [2]. Briefly, 90 rats were divided into two main groups, where in the first one animals were fed normal fat diet ([NFD], $n = 10$) to serve as the normal control group. In the second group ($n = 80$) animals received an in-house-prepared high-fat diet, combined with fructose in drinking water (20%) for a

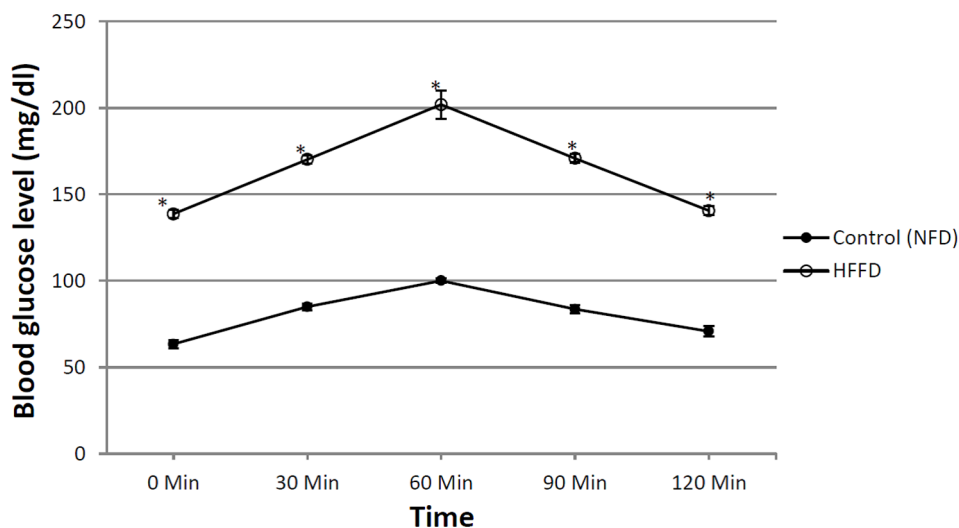


Figure 1. The glucose tolerance curve depicts the changes in glucose (2 g/kg, p.o) response in serum of normal control group (NFD) and non-treated insulin-resistant rats (HFFD), after 6 weeks of food manipulation at 0, 30, 60, 90, and 120 min. Values are means \pm S.E of 6 animals; as compared to the normal control group (*) (one-way ANOVA followed by Tukey-Kramer post hoc test), $P < 0.05$. doi:10.1371/journal.pone.0089169.g001

Table 1. The assessment of food and fructose/water intake, and body weight of the rats before and at the end of the HFFD.

	Body weight (g)	Food intake [HFD] (g)	Food intake [NFD] (g)	Fructose (20%)/ water intake (ml)	Water intake (ml)	Total Kcal/day
1 st Week	81.6±1.12	29.70±1.41	–	43.6±1.51	–	183.57±8.37
5 th Week	290.5±3.58*	34.68±2.14	–	54.1±3.52* [#]	–	216.26±12.7 [#]
6 th Week	297.3±3.6*	39.21±2.66*	–	58.5±2.41* [#]	–	242.9±15.54* [#]
7 th Week	290.2±3.24*	–	37.19±1.6*	–	38.9±2.03	117.14±5.04*

Values are presented as means ± S.E.M. (n=70–80). Animals were fed high fat diet [HFD] and were allowed free access to 20% fructose in water throughout the 6 weeks. Diet manipulation was stopped after the STZ injection on day 1 of the 7th week. During this week animals received normal fat diet [NFD] and water. Caloric intake for NFD=food intake (g) × 3.15 kcal, caloric intake for HFD=food intake (g) × 5.3 kcal and caloric intake for 20% fructose=0.6 kcal/ml. As compared with 1st week (°), and 7th week (#) (one-way ANOVA followed by Tukey-Kramer post hoc test), P<0.05. doi:10.1371/journal.pone.0089169.t001

period of 6 weeks (HFFD). During the 6th week the 2nd group was injected a daily single dose of long-acting human insulin (Monotard) (0.5 IU/kg, i.p) to augment a state of IR. On the 7th week a freshly prepared single sub-diabetogenic dose of STZ (35 mg/kg in citrate buffer, pH 4.5) was injected once after an overnight fasting to produce frank hyperglycemia. During the development of this model a regular estimation of the body weight (BW), food, fructose/water and caloric intake, as well as the levels of fasting serum glucose, triglycerides (TGs), total cholesterol (TC) and insulin were carried out. At the end of the 7th week, animals showing blood glucose level between 200–350 mg/dl, hyperinsulinemia and hyperlipidemia were considered as insulin resistant/type 2 diabetic rats and were included in the study.

4. Oral Glucose Tolerance Test (OGTT)

At the end of the 6th week and before the injection of STZ, the OGTT (2 g glucose/kg) was performed on 6 rats from each group after an overnight fasting. Blood droplets were withdrawn from the tip of the tail vein at 0 time and every 30 min along 2 hours, to assess the blood glucose level using test strips for blood glucose testing device (Lifescan, USA). Insulin resistance was reflected by the OGTT and was further confirmed by the fasting hyperinsulinemia and the HOMA-I value.

5. Experimental Design

Animals that were confirmed to be obese, insulin-resistant, and diabetic were randomly divided into four groups (n = 10–12 rats). The 1st group served as the obese insulin-resistant/type 2 diabetic control, while the 2nd and 3rd groups received either glimepiride (0.5 mg/kg, p.o) [2] or CoQ10 (20 mg/kg, p.o) [17], and their combination was administered to the 4th group. The NFD animals

were set as the last 5th group (n = 10) and received the vehicle to function as the negative control group. All treatments sustained for 2 weeks and the last dose of any treatment was given 24 h before the animals were euthanized. Rats were fasted 18 h before the time of death to minimize the feeding-related variations in the lipid and glucose patterns.

6. Collection of Serum Samples for Analysis

At the time of carnage, animals were weighed and blood was collected from the tail vein under brief ether anesthesia and was centrifuged (700×g, 4°C, 20 min) to separate sera. Sera were used to determine glucose, liver enzymes (AST and ALT) and malondialdehyde (MDA) as a measure for lipid peroxidation, using available colorimetric reagent kits [Randox (Antrim, U.K), Quimica Clinica Aplicada (Amposta, Spain) and Biovision (California, USA), respectively]. ELISA technique was carried out using the corresponding ELISA kit for the assessment of fructosamine (EIA ab, Wuhan, China), reduced glutathione (Cayman Chemical, Michigan, USA), adiponectin (Chemicon, MA, USA), the soluble receptor of advanced glycated end product (sRAGE) and visfatin (Ray Biotech, Georgia USA). Serum lipid profile, viz., triglycerides (TGs), total cholesterol (TC) and free fatty acids (FFAs) were estimated using Synchron CX5 auto-analyzer (Beckman Instruments INC, Brea, USA). Finally, insulin level was measured using rat RIA kit (Sceti Medical Labo K.K, Tokyo, Japan), and Homeostasis Model Assessment-index (HOMA-index) was calculated according to the following equation [Fasting glucose (mg/dl)/18×Fasting insulin (μIU/ml)]/22.5 [18].

Table 2. Effect of 2 weeks daily oral administration of CoQ10 (10 mg/kg) and/or glimepiride (0.5 mg/kg), on serum levels of glucose, fructosamine, insulin, and HOMA-index in HFFD/STZ-induced diabetic rats.

	Normal Control (NFD)	Diabetic Control (HFFD/STZ)	Diabetic+Glimepiride	Diabetic+CoQ10	Diabetic+Glimepiride/ CoQ10
Glucose (mg/dl)	81±1.97	324.60±8.47*	213.22±5.36* [#]	286.10±3.28* [#] , [§]	210.12±5.32* [#] , [@]
Fructosamine (nmol/l)	9.09±0.66	70.17±3.65*	22.97±0.75* [#]	29.38±0.42* [#] , [§]	33.62±0.45* [#] , [§]
Insulin (μIU/ml)	1.61±0.14	44.98±2.50*	27.53±1.10* [#] , [@]	19.46±0.57* [#] , [§]	14.15±0.51* [#] , [§] , [@]
HOMA-index	0.38±0.04	34.47±1.65*	14.45±0.55* [#]	13.77±0.37* [#]	7.35±0.37* [#] , [§] , [@]

Values are means ± S.E of 8–10 animals. As compared with normal control (*), diabetic control ([#]), glimepiride treated ([§]) and CoQ10 treated ([@]) groups (one-way ANOVA followed by Tukey-Kramer post hoc test), P<0.05. doi:10.1371/journal.pone.0089169.t002

Table 3. Effect of 2 weeks daily oral administration of CoQ10 (10 mg/kg) and/or glimepiride (0.5 mg/kg), on serum, hepatic and muscular contents of triglycerides (TGs), total cholesterol (TC) and free fatty acids (FFAs), as well as serum ALT and AST in HFFD/STZ diabetic rats.

	Normal Control (NFD)	Diabetic Control (HFFD/STZ)	Diabetic+Glimepiride	Diabetic+CoQ10	Diabetic+Glimepiride/CoQ10
Serum TGs (mg/dl)	66.8±2.80	164.91±2.8*	142.28±2.18* [#]	123.9±1.6* [#] , [§]	109.57±1.56* [#] , [§] , [@]
Liver TGs (mg/g tissue)	2.9±0.15	15.92±0.6*	11.68±0.25* [#]	9.65±0.21* [#] , [§]	7.27±0.58* [#] , [§] , [@]
Muscle TGs (mg/g tissue)	5.5±0.34	22.75±0.9*	19.35±0.40* [#]	16.32±0.36* [#] , [§]	11.57±0.47* [#] , [§] , [@]
Serum TC (mg/dl)	91.9±3.10	175.6±2.9*	151.84±2.48* [#]	133.41±1.69* [#] , [§]	121.06±0.23* [#] , [§] , [@]
Liver TC (mg/g tissue)	3.5±0.19	16.37±0.6*	11.96±0.19* [#]	9.71±0.20* [#] , [§]	7.31±0.23* [#] , [§] , [@]
Muscle TC (mg/g tissue)	6.4±0.34	23.82±0.8*	20.41±0.40* [#]	17.42±0.36* [#] , [§]	12.55±0.48* [#] , [§] , [@]
Serum FFA (mg/dl)	14.5±1.43	50.05±3.7*	29.11±1.9* [#]	36.81±1.4* [#] , [§]	21.56±1.33* [#] , [§] , [@]
Liver FFA (mg/g tissue)	4.0±0.27	16.88±0.6*	12.77±0.25* [#]	10.76±0.21* [#] , [§]	8.37±0.23* [#] , [§] , [@]
Muscle FFA (mg/g tissue)	7.5±0.35	25.95±0.8*	21.68±0.46* [#]	18.52±0.36* [#] , [§]	13.63±0.48* [#] , [§] , [@]
AST (IU/l)	35.9±0.43	86.23±0.7*	73.32±1.20* [#]	69.77±0.98* [#]	55.88±2.34* [#] , [§] , [@]
ALT (IU/l)	20.9±0.42	53.37±0.4*	49.66±0.47* [#]	38.36±0.49* [#] , [§]	45.01±0.60* [#] , [§] , [@]

Values are means ± S.E of 8–10 animals. As compared with normal control (†), diabetic control ([#]), glimepiride treated ([§]) and CoQ10 treated ([@]) groups (one-way ANOVA followed by Tukey-Kramer post hoc test), P<0.05. doi:10.1371/journal.pone.0089169.t003

7. Tissue Extracts

Following blood collection animals were killed by a deep sodium pentobarbital anesthesia and both the liver and soleus skeletal muscle were dissected out, washed, dried, weighed, homogenized in PBS (10%) and kept in aliquots for the estimation of the following biomarkers.

7.1. Assessment of lipid profile. One part of the aliquot was centrifuged at 1500×g at 4°C for 15 minutes and the supernatant was collected for the assessment of TGs, TC and FFAs using Synchron CX5 auto-analyzer (Beckman Instruments INC, Brea, USA).

7.2. Assessment of adiponectin receptors, glucose transporters, and myeloperoxidase. Two other parts of the aliquots were exposed to 2 repeated freeze-thaw cycle to further break the cell membranes, then centrifuged at 5000×g for 5 minutes and stored at -20°C. These aliquots were used for the estimation of adiponectin receptor 1 and 2 (Adipo R1 & R2) [19], glucose transporter 2 and 4 (GLUT 2 & 4) and myeloperoxidase (MPO) activity, using the corresponding ELISA rat kit (Usen,

Wuhan, China; EIAab, Wuhan, China and Hycult biotech, Uden, Netherlands, respectively).

7.3. Assessment of the activities of tyrosine kinase and PI3K. These two parameters were assayed in one aliquot that was processed as mentioned under 7.2. The activity of both tyrosine kinase (TK) and PI3K was determined by the proper ELISA rat kit (Chemicon, Billerica, USA and Millipore, Massachusetts, USA, respectively).

7.4. Protein Assay. The protein content in the liver and muscle tissue homogenates was assayed using the method described by Bradford [20] and the bovine serum albumin was used as a standard.

7.5. Assessment of High Affinity and Low Affinity Insulin Receptors (HAIR & LAIR). HAIR and LAIR were measured using RIA method, where 2.5 ml aliquot of the sample was incubated in polystyrene tube (12×75 mm) with 3.37×10⁻¹¹ of ¹²⁵I - labeled insulin in 200 µl of HEPES buffer (25 mM, pH 7.8). The latter contains 0.1% Triton X-100, 150 mM sodium chloride, 1% bovine serum albumin (BSA), 100 U/ml bacitracin

Table 4. Effect of 2 weeks daily oral administration of CoQ10 (10 mg/kg) and/or glimepiride (0.5 mg/kg) on serum level of reduced glutathione (GSH), malondialdehyde (MDA), adiponectin, visfatin and soluble receptor of advanced glycated end product (sRAGE) in HFFD/STZ diabetic rats.

	Normal Control (NFD)	Diabetic Control (HFFD/STZ)	Diabetic+Glimepiride	Diabetic+CoQ10	Diabetic+Glimepiride/CoQ10
GSH (µM)	14.64±0.05	2.45±0.23*	8.80±0.13* [#]	12.61±0.24* [#]	12.69±0.55* [#] , [§]
MDA (nmol/ml)	8.88±0.08	15.29±0.32*	11.99±0.14* [#]	11.09±0.09* [#] , [§]	9.98±0.34* [#] , [§] , [@]
Adiponectin (ng/ml)	5.21±0.11	0.64±0.018*	2.47±0.19* [#]	3.84±0.11* [#] , [§]	5.04±0.25* [#] , [§] , [@]
Visfatin (ng/ml)	15.28±2.05	201.55±4.73*	156.60±2.21* [#]	128.57±1.85* [#] , [§]	96.26±.96* [#] , [§] , [@]
sRAGE (ng/ml)	1.77±0.08	1.19±0.03*	2.61±0.13* [#]	3.44±0.03* [#] , [§]	3.88±0.08* [#] , [§] , [@]

Values are means ± S.E of 8–10 animals. As compared with normal control (*), diabetic control ([#]), glimepiride treated ([§]) and CoQ10 treated ([@]) groups (one-way ANOVA followed by Tukey-Kramer post hoc test), P<0.05. doi:10.1371/journal.pone.0089169.t004

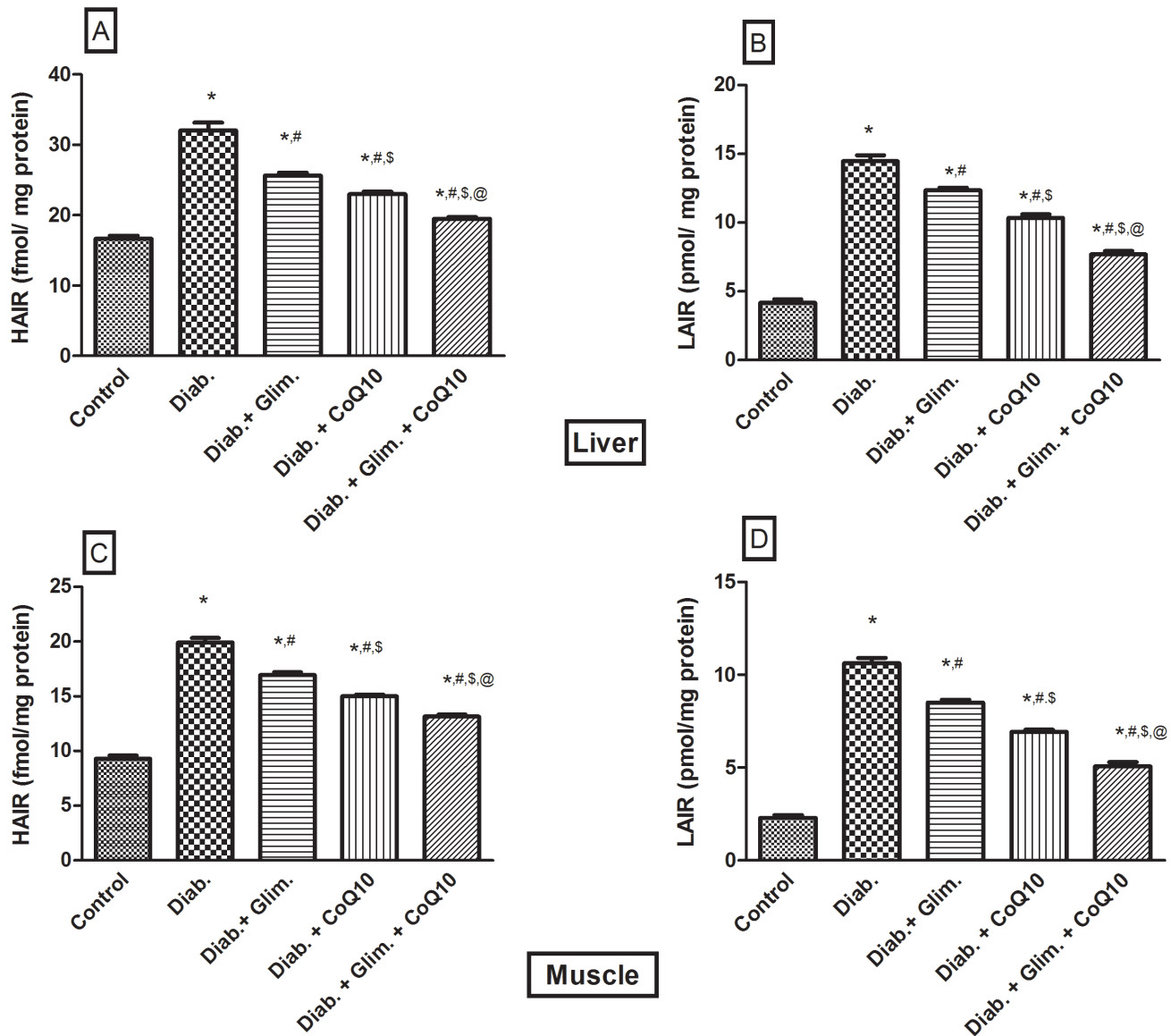


Figure 2. Effect of diabetes and 2 weeks oral administration of CoQ10 (20 mg/kg) and/or glimepiride (0.5 mg/kg) on the hepatic (A, B) and muscular (C, D) insulin receptor isoforms (high affinity, [HAIR, fmol/mg protein] and low affinity [LAIR, pmol/mg protein] insulin receptor). Values are means of 8–10 animals \pm S.E.M. As compared with normal control (*), diabetic control ([#]), glimepiride treated ([®]) and CoQ10 treated (@) groups (one-way ANOVA followed by Tukey-Kramer post hoc test), $P < 0.05$. doi:10.1371/journal.pone.0089169.g002

and varying concentrations of unlabeled insulin for 16 hours at 4°C. At the end of incubation, 100 μ l of 0.3% gamma globulin (w/v) and 300 μ l of 25% polyethylene glycol (w/v, molecular weight 8000) were added to each tube and all tubes were left for another 20 minutes on ice bath. Tubes were then centrifuged at 10,000 \times g for 10 minutes at 4°C to separate the bound hormone-receptor complex from the free hormone. After centrifugation, careful, decantation was carried out and the pellets were washed with 300 μ l of 12.5% of the same polyethylene glycol and centrifuged again at 10,000 \times g for 10 minutes at 4°C. The resulting pellets were counted for two minutes in a gamma counter to get the total binding of each tube. Specific binding was determined by the difference between total binding and non-specific binding and the total count was determined for each assay (it is the count of any tube before centrifugation). A control tube was run with each assay, where it was passed through the same

steps like all other tubes except that the ¹²⁵I- labeled insulin was added just before centrifugation. All counts were corrected for the control tube count before any calculation. The data were analyzed for insulin receptors concentration and dissociation constant (KD) [21].

8. Statistical Analysis

Values are expressed as mean \pm S.E. of 8–10 animals and the differences between groups were tested for significance using analysis of variance (ANOVA), followed by Tukey-Kramer post-hoc test determined by SPSS software program, version 21. Correlation coefficient (r) was carried using linear regression analysis. The level of statistical significance was taken at $P < 0.05$. The graphs were drawn using a prism computer program (GraphPad software Inc. V5, San Diego, CA, USA).

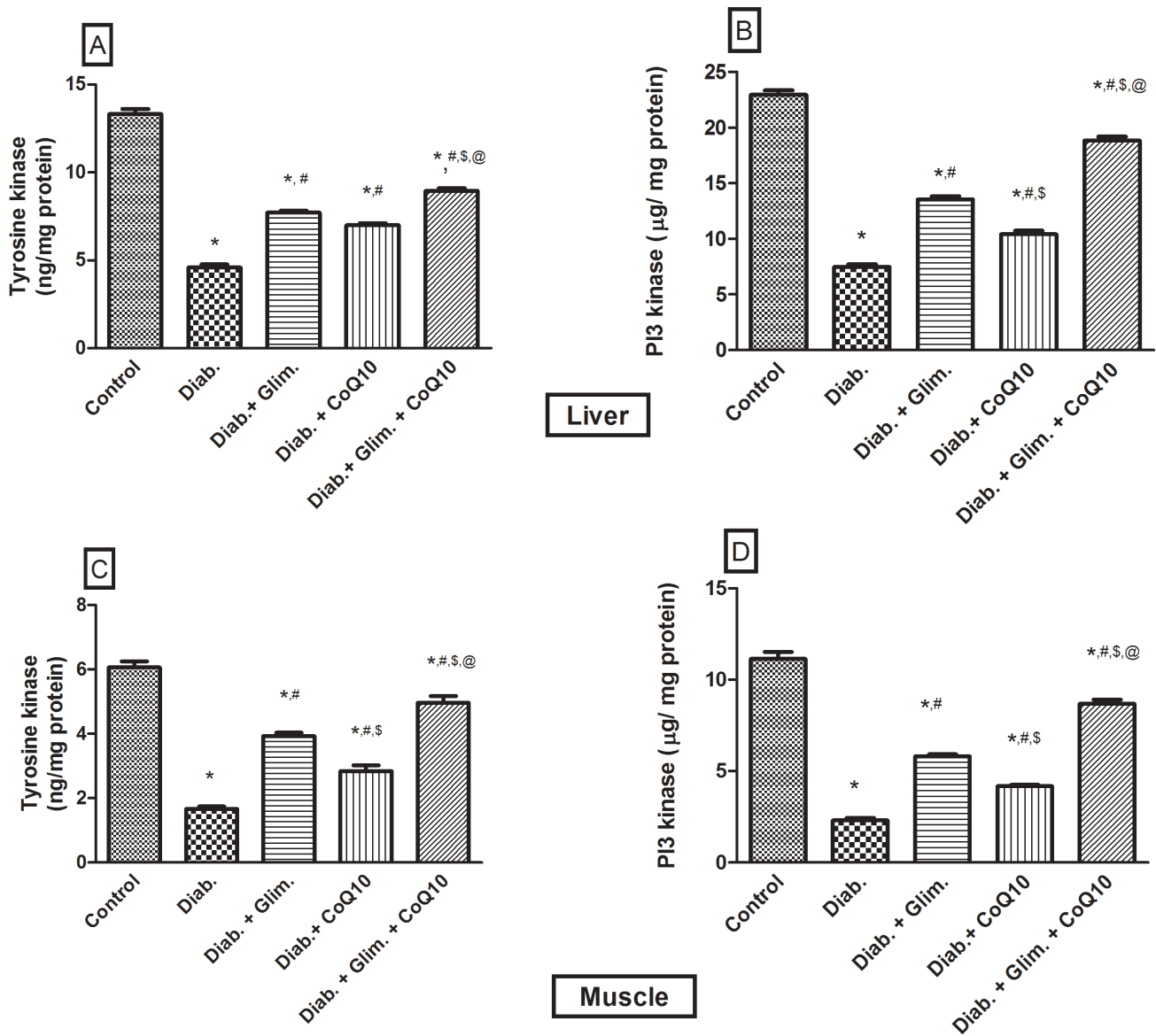


Figure 3. Effect of diabetes and 2 weeks oral administration of CoQ10 (20 mg/kg) and/or glimepiride (0.5 mg/kg) on the hepatic (A, B) and muscular (C, D) activity of tyrosine kinase (ng/mg protein) and PI3K (µg/mg protein). Values are means of 8–10 animals ± S.E.M. As compared with normal control (*), diabetic control (#), glimepiride treated (\$) and CoQ10 treated (@) groups (one-way ANOVA followed by Tukey-Kramer post hoc test), $P < 0.05$. doi:10.1371/journal.pone.0089169.g003

Results

Table 1 showed that the HFFD resulted in a significant increase in the BW compared to the 1st week; however, the difference in the BW from the 5th week to the 7th one was not significant. The food intake was significantly increased in the 6th and 7th weeks, but not the 5th one. Moreover, the intake of the 20% fructose in water was increased in the 5th and 6th weeks as compared with the 4th and 7th weeks. The food and fructose/water intake was reflected on the total Kcal/day, where in the 7th week, during which animals drank water only, was significantly the lowest as compared to the 1st, 5th and 6th weeks. Furthermore, the caloric intake in the 5th and 6th weeks was significantly higher than that in the 1st one. It should be noticed that during the 7th week the animals received NFD and water instead of the HFFD consumed in the previous 6 weeks. In figure 1 the OGTT showed that the HFFD effect presented the

same pattern offered by the NFD along the 2 hours period, but with about 2 folds elevation indicating a state of glucose intolerance/insulin resistance. In relation to the normal control group, the untreated diabetic group showed a discernible increase in serum level of glucose, fructosamine, insulin, and HOMA-I value (Table 2). The model also induced conspicuous increment in the serum, liver and muscle levels of TGs (147, 433 & 313%, respectively), TC (90, 365 & 273%, respectively), and FFAs (3.4, 4.2 & 3.4 folds, respectively) and doubled that of ALT and AST as presented in table 3. HFFD/STZ also increased MDA by 1.7 folds, as well as the adipocytokine visfatin, but it conversely demonstrated a sharp decline in the serum level of adiponectin (88%), glutathione (83%) and sRAGE (32.7%) (Table 4). On the molecular level, the model boosted the insulin receptor isoforms in the liver and muscle (Figure 2 A–D), but inhibited noticeably

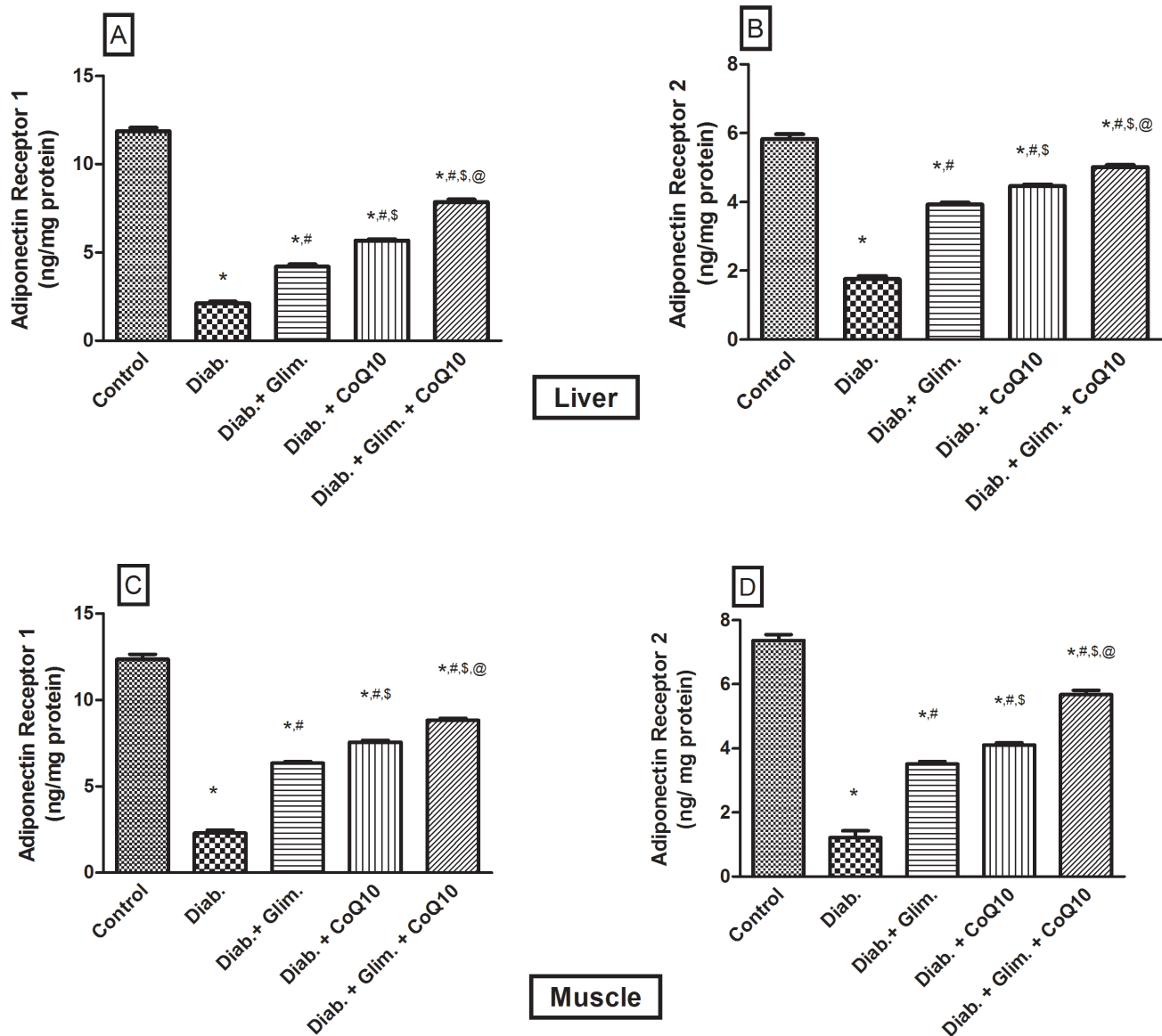


Figure 4. Effect of diabetes and 2 weeks oral administration of CoQ10 (20 mg/kg) and/or glimepiride (0.5 mg/kg) on hepatic (A, B) and muscular (C, D) adiponectin receptors (Adipo-R1, Adipo-R2 [ng/mg protein]). Values are means of 8–10 animals \pm S.E.M. As compared with normal control (*), diabetic control (#), glimepiride treated (\$) and CoQ10 treated (@) groups (one-way ANOVA followed by Tukey-Kramer post hoc test), $P < 0.05$.

doi:10.1371/journal.pone.0089169.g004

those of tyrosine kinase and PI3K in both tissues (Figure 3 A–D), as well as adiponectin receptors (Figure 4 A–D). The model also, as depicted in figure 5, increased the glucose transporters in both the liver (GLUT2, panel A) and muscle (GLUT4, panel C), as well as the MPO activity in both tissues (Panels B and D).

As presented in table 2, treatment with CoQ10 alone for 2 weeks repressed the insulin level (56.7%), HOMA-I (60%) and fructosamine (58.1%), when compared to the diabetic group, with subtle, yet significant effect on the glucose level (11.8%). CoQ10 abated the model-mediated lipid profile elevation in serum, liver and muscle, as well as the two aminotransferases (Table 3); this effect entailed the pro-inflammatory cytokine, visfatin and the MDA level (Table 4). On the other hand, and as presented in the latter table, CoQ10 boosted the levels of adiponectin (6 folds), GSH (5 folds) and sRAGE (2.8 folds). Combining CoQ10 with

glimepiride improved the previous parameters, an effect that was prominent in serum adiponectin (table 4), where it reached that of the NFD control group.

In the liver, the CoQ10 treated groups showed a 28% decrease in the levels of insulin-receptor isoforms (Figure 2 A, B), 25.4% in the GLUT2 by (Figure 5A), and 37.3% in the MPO (Figure 5B); the same pattern was observed in the skeletal muscle (Figures 2 C, D; 5 C, D). In figure 3, the four panels showed an elevation in the activity of both enzymes after the treatment with CoQ10, glimepiride and the combination regimen. The activities were increased in the same order and parallel the improvement in the glucose level. Additionally, CoQ10 caused about 60–70% increase in the adiponectin receptors in the liver (Figure 3 A, B) and muscle (Figure 3 C, D) compared to the non-treated diabetic group. As detected in tables 2–4 and figures 2–5, CoQ10 overrides the effects

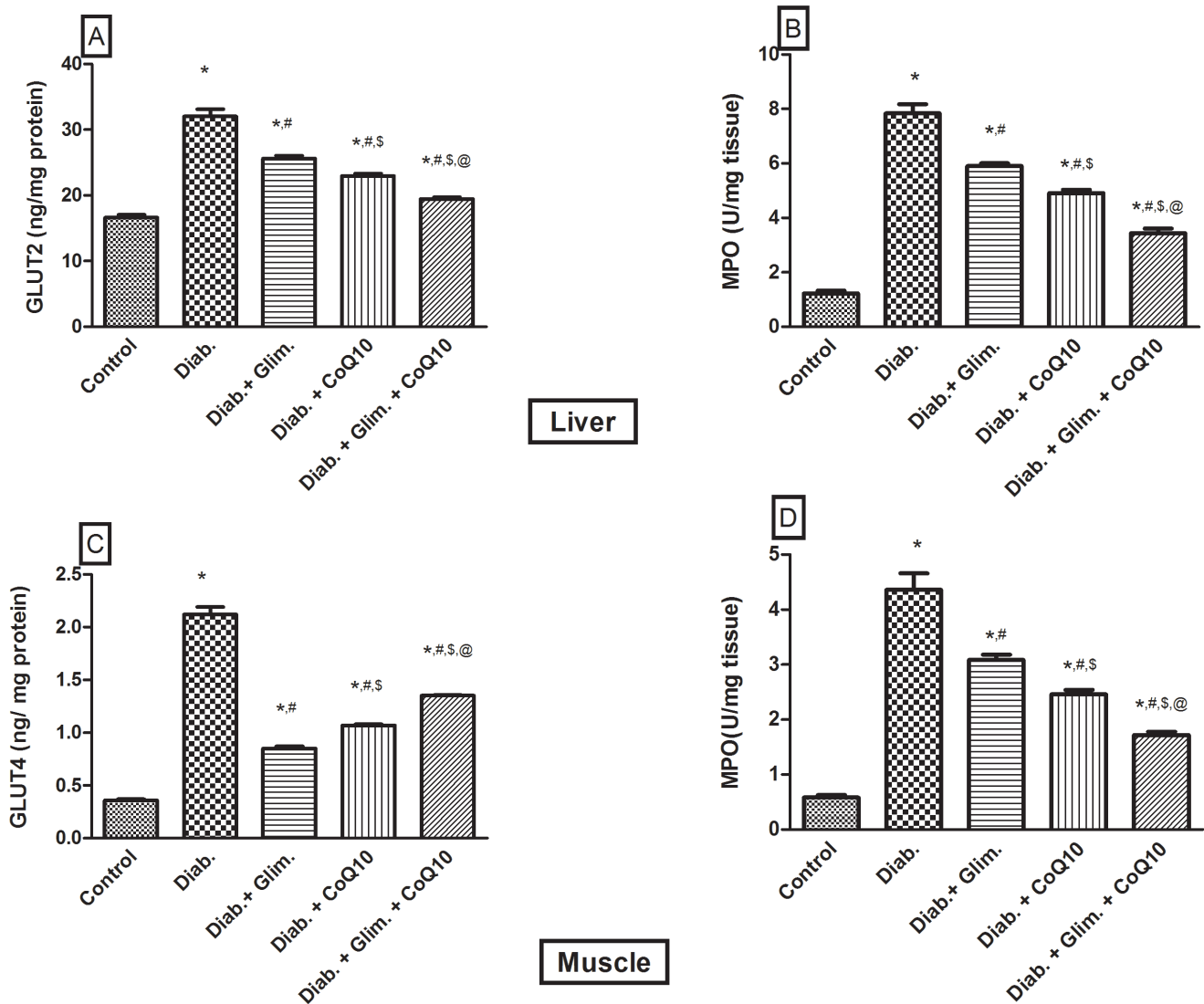


Figure 5. Effect of diabetes and 2 weeks oral administration of CoQ10 (20 mg/kg) and/or glimepiride (0.5 mg/kg) on the hepatic (A) and muscular (C) glucose transporters [ng/mg protein], and (B, D) myeloperoxidase activity [MPO, U/mg]. Values are means of 8–10 animals ± S.E.M. As compared with normal control (*), diabetic control (#), glimepiride treated (\$) and CoQ10 treated (@) groups (one-way ANOVA followed by Tukey-Kramer post hoc test), $P < 0.05$. doi:10.1371/journal.pone.0089169.g005

of glimepiride, in the redox parameters, lipid profile, inflammatory parameters and the molecular markers, except for the glucose, fructosamine, serum FFAs, TK, PI3K, and GLUT4 levels. When CoQ10 was added to the antidiabetic drug, a further improvement was observed in most of the biochemical markers, as well as in the molecular parameters (Tables 2–4; Figures 2–5).

Using the linear regression analysis, HOMA-index correlated negatively with the liver and muscle adiponectin receptors (Adipo-R1, Adipo-R2), as well as tyrosine kinase and PI3K. Conversely, a positive correlation was detected between the HOMA-I value and the glucose transporters [GLUT2/GLUT4] and insulin receptors [LAIR/HAIR] ($P < 0.001$). On the other hand, adiponectin

Table 5. Correlation coefficient (r) between HOMA-index and serum adiponectin level with hepatic GLUT2, adiponectin receptors (Adipo- R1, R2), insulin receptor isoforms (HAIR, LAIR), tyrosine kinase (TK) and PI3K.

	GLUT2	Adipo-R1	Adipo-R2	HAIR	LAIR	TK	PI3K
HOMA-I	0.905 $P < 0.001$	-0.882 $P < 0.001$	-0.957 $P < 0.001$	0.911 $P < 0.001$	0.883 $P < 0.001$	-0.886 $P < 0.001$	-0.876 $P < 0.001$
Adiponectin	-0.890 $P < 0.001$	0.882 $P < 0.001$	0.952 $P < 0.001$	-0.939 $P < 0.001$	-0.884 $P < 0.001$	-0.804 $P < 0.001$	0.847 $P < 0.001$

Correlation was carried out in untreated and treated HFFD-STZ diabetic rats. doi:10.1371/journal.pone.0089169.t005

Table 6. Correlation coefficient (*r*) between HOMA-index and serum adiponectin level with muscular GLUT4, adiponectin receptors (Adipo- R1, R2), insulin receptor isoforms (HAIR, LAIR), tyrosine kinase (TK) and PI3K.

	GLUT4	Adipo-R1	Adipo-R2	HAIR	LAIR	TK	PI3K
HOMA-I	0.858 P<0.001	-0.947 P<0.001	-0.934 P<0.001	0.908 P<0.001	0.911 P<0.001	-0.890 P<0.001	-0.887 P<0.001
Adiponectin	-0.666 P<0.001	0.894 P<0.001	0.912 P<0.001	-0.900 P<0.001	-0.904 P<0.001	0.826 P<0.001	0.832 P<0.001

Correlation was carried out in untreated and treated HFFD-STZ diabetic rats.
doi:10.1371/journal.pone.0089169.t006

correlated negatively with GLUT2/GLUT4, and insulin receptors [HAIR and LAIR], while positively with its receptors (P<0.001), and both tyrosine kinase and PI3K, as depicted in tables 5 and 6.

Discussion

Although CoQ10 has been extensively tested in insulin resistant/diabetic models and patients, yet the current study is the first to address new machineries that may underline its insulin sensitizing effect. CoQ10 herein revoked the HFFD/STZ effects on the content/activity of glucose/lipid panels, insulin receptors, tyrosine kinase, PI3K, adiponectin and its receptors, glucose transporters, visfatin, MPO and sRAGE. Moreover, the study showed that apart from being an insulin secretagogue, glimepiride can act *via* other mechanisms, where it played a significant role on the aforementioned parameters.

CoQ10 is known to be deficient in case of diabetes [6,7] and in some individuals with reduced energy expenditure and skeletal muscle dysfunction [22]. Ample of evidence has shown that CoQ10 is proficient to counteract several metabolic disturbances associated with IR and diabetes [23–24], a fact that applies to the present findings. Mitochondrial respiratory capacity was reported to be reduced by the consumption of high fat diet, besides the aforementioned effects [25]; hence, the obliging effect of CoQ10 relies partly on its pivotal role in maintaining mitochondrial function [26] and on its ability to improve the lipid profile in serum, liver and muscle tissue as detected in the current work. Exposure of the liver to this type of diet not only elevates TGs, TC and favors FFAs esterification, but also accelerates lipogenesis with the accumulation of TGs that contribute, sequentially, to IR/glucose intolerance [27]. This phenomenon enhances the overproduction of hepatic very low density lipoprotein, with the consequent increase in its chain connected lipoproteins [28], the *de-novo* lipogenesis under the influence of high carbohydrate feeding and lipolysis in adipose tissue leading to a high flux of FFAs [28,29]. Moreover, elevated TC can abate the activation of hepatic cholesterol-rich lipoprotein (LDL-C) receptors and/or increases their glycation, resulting in its decreased catabolism [30].

The present model-induced dyslipidemia and precisely the elevated FFAs are responsible partly for the impairment of glucose uptake/utilization with the subsequent increase in hepatic glucose production [2,31]. Furthermore, the increased blood glucose level plays a role in the IR induction through its flux into the hexosamine pathway [32], which expresses IR as an upshot of lipids and carbohydrates utilization imbalance. Therefore, lowering FFAs, TGs and TC is one vital arm in the CoQ10-mediated insulin sensitivity documented by the reduced HOMA-I and insulin level, as compared to the diabetic non-treated group. A similar effect was observed in the glimepiride treated rats and was more pronounced when combined with CoQ10.

Additionally, high fructose over nutrition not only induces the hexosamine pathway [33], but also reduces both insulin-stimulated autophosphorylation and insulin receptor substrate (IRS) phos-

phorylation in both liver and muscle [34], as documented herein by the inhibited tyrosine kinase activity and PI3K. These mechanisms alter the proper insulin function, a fact that was reflected here on the insulin receptors, which were increased in both organs, possibly as a corollary to the impaired insulin task. This finding was previously documented in liver [19] and skeletal muscles [35,36] of prediabetic/diabetic models. Moreover, Sbraccia et al. [37] showed also that in case of IR the expression of LAIR correlates positively with hyperinsulinemia and negatively with insulin sensitivity. CoQ10 by elevating the activity of both tyrosine kinase and PI3K is responsible for improving the insulin cascade. These two enzymes are responsible for the insulin-stimulated autophosphorylation and its downstream flow, which was paralleled by the inhibition of the LAIR and HAIR content. This effect was even better when CoQ10 was combined with glimepiride. Regarding the effect of glimepiride on the insulin receptors we reported a decrease in their levels, possibly due to the improved insulin sensitivity, decreased FFAs, and/or the increased affinity and binding of insulin to its receptors as previously reported [38].

Revert to the hexosamine pathway glucose is diverted from the glycolytic pathway at the level of fructose-6-phosphate *via* the glutamine fructose-6-phosphate amidotransferase enzyme, with the consequent production of glucosamine-6-phosphate and other hexosamine products. As an inducer of IR, glucosamine inhibits the insulin-mediated glucose transport and glucose transporter 4 (GLUT4) translocation [39]. In the present study, however, the content of GLUT4 was increased in skeletal muscle, a finding that is debatable, where some studies showed a negative correlation [40,41], while others [42,43], including the present work, showed a positive correlation. We infer that GLUT4 elevation could be a compensatory reflex for serum hyperglycemia, yet failure of this reparation may be attributed to the impaired GLUT4 translocation to the cell membrane by the IR insult [39]. Previous study by Kruszynska et al. [44] assents with our results, where they stated that the elevated non esterified fatty acids, documented herein, induce IR and impair IRS-1 tyrosine phosphorylation and IRS-1-associated PI3K activation. Hence, the defected insulin signaling pathway can be responsible for the limited muscle glucose uptake and the impaired glucose transport. CoQ10, glimepiride and their combination have inhibited GLUT4 content in the skeletal muscles. This effect is linked to their ability to improve insulin signaling trail by increasing the activity of both tyrosine kinase and PI3K and the subsequent translocation of GLUT4 to cell membrane as evidenced by the decreased glucose level by CoQ10 and/or glimepiride. Previously, CoQ10 [45] and glimepiride [46] have been reported to activate the PI3K enzyme and its pathway in models other than diabetes.

Likewise, hepatic glucose transporter GLUT2 was also increased in the diabetic rats, a finding that coincides with previous studies [47,48]. In contrast, in an earlier work with HFFD-induced IR without overt diabetes [19], hepatic GLUT2

leveled off, a finding that concurs with that of Burcelin et al. [48], who reported that GLUT2 increases with hyperglycemia and decreases with hyperinsulinemia. Postic et al. [49] further clarified that when insulin and glucose are associated the stimulatory effect of glucose on GLUT2 gene expression predominates. CoQ10, as well as glimepiride and their combination brought the GLUT2 level down, *via* improving the hyperglycemia/hyperinsulinemia-associated metabolic disorders including dyslipidemia and the activation of PI3K. IRS-1 tyrosine phosphorylation, modulates on one hand the GLUT2 function to enhance the glucose uptake and on the other hand, it elevates the level of PI3K proven in this work. The downstream of the PI3K is the activation of Akt, which blocks gluconeogenesis and mediates glycogen synthesis [50].

Consequent to the hyperglycemia/IR, and according to the hyperglycemic extent, the formation of polyols and non enzymatic glycoxidation products of proteins and lipids occur, leading to the formation of heterogeneous products termed advanced glycation end-products (AGEs) [51] that are also associated with IR [52]. Although AGEs were not measured directly, yet their soluble receptor type (sRAGE), which reflects the activity of AGE/RAGE axis, decreased greatly in the current model and others [53], possibly as a consequence of eliminating the circulating AGEs burden. This receptor type was described previously as a decoy domain receptor that plays a role in blocking the AGE/RAGE interaction [54,55]. The latter was reported to stimulate certain signaling cascade that leads to the induction of NAD(P)H oxidase with the consequential increase in reactive oxygen species (ROS) [55]. In a mutual scenario, Anderson et al. [56] stated also that AGEs may be generated by oxidative stress and inflammation, where MPO can trigger the production of RAGE ligands by generating different stresses, including NF- κ B, which begets further inflammation and an endless cycle of AGE production. This fact was imitated herein by the marked activation of MPO, decreased GSH level and the 2 fold elevation of the lipid peroxide product and as documented previously [57,58]. These results suggest that hyperglycemia may bridge the ligand-RAGE axis upregulation with increased oxidant stress and inflammation.

CoQ10 is known as a powerful endogenous lipophilic antioxidant [59] that directly protects cellular components from free radicals and indirectly regenerates other antioxidants [60]. This fact explains the restoration of GSH and the decrease of lipid peroxidation proved by the current model and others [57,58], in addition to the inhibition of MPO and the elevation of sRAGE. In a vicious cycle, the CoQ10-mediated sRAGE elevation adds to its antioxidant mechanisms, where inhibition of AGE/RAGE axis entails an elevation in sRAGE and a decrease in the production of ROS [55]. Additionally, the CoQ10 inhibitory action on MPO can be another reason for decreasing AGEs production as mentioned previously [56]. Of note, inhibition of neutrophils recruitment by CoQ10 along with ROS can favor the improved insulin sensitivity. Our results coincide with those of Kunitomo et al. [61] and Tsai et al. [62], who documented that CoQ10 significantly attenuated the increase of oxidative and nitrative stress markers, oxidized LDL, MPO and mitigated the NF- κ B and downstream inflammatory mediators, including the expression of adhesion molecules. Inhibition of the latter molecules can further explain the CoQ10-induced low MPO activity.

Moreover, diabetic animals showed hypo-adiponectinemia that may result from obesity-induced IR in adipose tissue, especially the visceral ones [63], which initiates further metabolic alterations in other peripheral tissues, *viz.*, liver and skeletal muscles [64]. Moreover, Krssak et al. [65] and Shulman [66] stated that the elevated plasma FFAs and TGs in muscles lead to the development of muscle IR, events that correlate, even partially, to hypo-adipo-

nectinemia. Not knowing if altered adiponectin is a cause or a consequent, hypo-adiponectinemia was reported to down regulate adiponectin receptors in the skeletal muscles of ob/ob mice [67], and livers of IR rats [19], findings that accentuate the present decrease in the adiponectin receptors in both organs. On the other hand, other studies [67,68] concluded that the decreased levels of adiponectin receptors participate in the development of hypo-adiponectinemia with the consequent IR. Additionally, hypo-adiponectinemia may be responsible for the pan dyslipidemia observed in this work, where high adiponectin level enhances fatty acids oxidation, reduces muscular TGs, improves muscle fat burn, insulin sensitivity and lowers hepatic glucose output, effects that are abated by hypo-adiponectinemia. Therefore, the ability of CoQ10 and glimepiride to increase the levels of adiponectin and its receptors, in this study, verify another mechanism for their insulin sensitizing effect along with their combination, which showed a better significant effect. The effect of CoQ10 overrides that of glimepiride in correcting the disturbed lipid profile, a finding that may be linked to its action on the adiponectin level.

Another adipocytokine assessed in the current study was the pro-inflammatory visfatin, which was elevated significantly in the present model and previous studies [69], reporting its elevation in many inflammatory diseases including obesity, IR and type 2 diabetes [70]. Visfatin was previously thought to increase in diabetic patients to enhance glucose uptake *in vitro* and *in vivo* *via* its binding to insulin receptor, causing its phosphorylation, as well as the IRS-1 and -2 [71]. However, to share the ambiguity of the other adipocytokines in their metabolic and immune functions, visfatin was found to bind to and activate the insulin receptor, but insulin does not interact with its cytokine-inducing effects [72]. Moreover, it was proven that visfatin plays a pivotal role in the generation of IR, by inducing the expression and production of inflammatory mediators, *viz.*, IL-1 β , TNF- α , and IL-6 *via* NF- κ B activation [70,72]. Additionally, increased visfatin level offers another explanation for the increased neutrophils infiltration, where administration of visfatin inhibitor succeeded to decrease MPO level [73]. Moreover, visfatin was reported to assist leukocyte adhesion to endothelial cells by the induction of the cell adhesion molecules [70]. This effect may be mediated by the ROS-induced NF- κ B activation; hence, linking oxidative stress with inflammatory pathways *via* the released adipocytokines [74]. Therefore, CoQ10 and glimepiride by inhibiting the level of visfatin highlight the improvement of insulin sensitivity, redox balance and inflammatory disorders. The effect of CoQ10 was better than that of glimepiride and the combination regimen offered a far better inhibitory effect on the visfatin content.

Perturbed lipid panel is known to elevate liver aminotransferases, a fact that is exemplified in the present study. Aminotransferases are indicators of hepatic health that is compromised in case of obesity, IR and diabetes [75]. Moreover, fatty changes are associated with the increased level of TNF- α , proven to increase in this model [19], which has a great influence on ALT elevation. Therefore, activated aminotransferases may reflect the role of hepatic gluconeogenesis and/or inflammation in the type 2 diabetes pathophysiology. Therefore, the inhibitory action of CoQ10 on ALT can reflect the inhibition in the hepatic gluconeogenesis and the type 2 associated inflammation indicating a better insulin sensitivity and correction of the diabetic disorders.

To this extent, the improving effect of CoQ10 on the glucose panel and insulin sensitivity can be attributed to its action on the insulin signaling pathway (insulin receptors, tyrosine kinase and PI3K) with the subsequent modulation in the function of the glucose transporters, as well as elevating sRAGE content and adiponectin with its receptors. Additionally, CoQ10 extended its

positive effect by inhibiting ROS, MPO and visfatin. The study, hence, highlighted for the first time the possible mechanisms responsible for the CoQ10-mediated insulin sensitivity and its antidiabetic action. These findings support its useful effect as an add-on supplement with known antidiabetic drugs.

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Author Contributions

Conceived and designed the experiments: HEA M. Amin GA. Performed the experiments: M. Amin GA M. Arbid. Analyzed the data: RAS. Contributed reagents/materials/analysis tools: M. Amin GA RAS M. Arbid. Wrote the paper: HEA.

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