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# The Association Between Trace Elements and Osmolality in Plasma and Aqueous Humor Fluid in Diabetic Rabbits

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## Abstract

Trace element metabolism plays an important role in the formation of diabetes and complications of diabetes. Although trace elements changes in lenses in diabetic cataract and glaucoma formation have been investigated, there were few studies evaluating trace elements levels in plasma and aqueous humor fluid in diabetic and non-diabetic conditions. Therefore, we aimed to investigate zinc (Zn), copper (Cu), and chromium (Cr) levels in plasma and aqueous humor fluids of rabbits in the diabetic rabbit model. New Zealand male rabbits were divided into two groups as control ($n = 8$), and diabetes ($n = 8$) induced by alloxane. At the end of the experimental period, the osmolality in blood, plasma, and aqueous humor fluids from rabbits were measured by osmometer and Zn, Cu, and Cr levels in plasma and aqueous humor fluid were measured by inductively coupled plasma–optical emission spectrophotometer (ICP-OES). The osmolality in blood, plasma, and aqueous humor fluid of the diabetic group was significantly increased compared to the control group (respectively $p < 0.01$, $p < 0.001$, $p < 0.001$). It was analyzed that plasma Zn and Cu levels of diabetic rabbits increased significantly (respectively, $p < 0.01$; $p < 0.001$), whereas Cr level significantly decreased according to the control group ($p < 0.01$). It was observed that Cr and Zn levels in aqueous humor fluid in diabetes group decreased (respectively $p < 0.001$ and $p < 0.01$), and a significantly increased in Cu level ($p < 0.001$) compared to the control group. Related with these changes that may occur in the eye due to the measured parameters, we consider that comparative studies of these types of diabetic animal models would be useful in the evaluation of diabetes and its complications.

**Keywords** Diabetes · Aqueous humor fluid · Osmotic pressure · Trace elements · Alloxan

## Introduction

Diabetes is a metabolic disease caused by insufficient insulin secretion or increased insulin resistance in tissues [1, 2]. It has been reported that the production and content of the aqueous humor fluid are deteriorated in diabetes and may cause various complications. In relation with the diabetes microaneurysm, hemorrhage, lipid and lipoprotein accumulation, retinal edema, macular edema, and new vessel formation may be observed in the eye; as a result, an increase is reported in the risk of developing diseases that cause blindness such as diabetic retinopathy, cataracts, and glaucoma [3–6]. It was determined

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that protein and amino acid metabolism deteriorate, the level of various amino acids in plasma increases, and plasma osmolality increases in consequence of diabetes and the complications related to diabetes [7–9].

It is known that trace element metabolism deteriorates as a consequence of diabetes and the complications related to diabetes [10–13]. Zinc (Zn) is an essential element that plays an important role in the storage and secretion of insulin in pancreas beta cells. Therefore, Zn, which is necessary for insulin secretion and storage, is released together with insulin. Copper (Cu) is an essential element found in the structure of many enzymes and proteins. It was reported that as a result of hyperglycemia, Cu binds to glycalized proteins and these proteins play a significant role in vascular dysfunction for diabetic patients. Chromium (Cr), which is a related element with carbohydrate, protein, and lipid metabolism, was shown to affect insulin and insulin-sensitive cell membranes; it was determined that glucose tolerance deteriorates and the risk of developing diabetes increases in case of its deficiency [11, 12]. It was showed that Cr plays a role in stimulating the insulin receptor and in glucose regulation in animal models [14–16].

In various clinical and experimental studies, it has been presented that the trace element levels change in different tissue, blood, and urine samples in relation to diabetes. Particularly, the significance of the deterioration of trace element homeostasis in aqueous humor fluid in the formation of diabetes-related cataracts has been emphasized. Although changes in element levels in lenses have been investigated in diabetes-induced cataract formation in several studies, there are very few studies that comparatively evaluate the number of elements in aqueous humor fluid, lens, and plasma in patients with or without diabetes. The osmotic pressure and therefore the fluidity of the aqueous humor fluid play a significant role in the nutrition of intraocular tissues [17–20]. For this reason, in our study, it was aimed to investigate the changes of Zn, Cu, and Cr metabolism in plasma and aqueous humor fluid in the diabetes model created by using alloxan in rabbits and to reveal out the relationship between the issues that may develop in the eye in relation to diabetes and trace elements and osmolality.

## Materials and Methods

### Chemicals

Alloxan monohydrate, sodium pentobarbital, nitric acid, ketamine hydrochloride, and xylazine hydrochloride were purchased from Sigma (Germany). Zn, Cu, and Cr standard solutions were obtained from Chem-Lab NV (Belgium).

### Animals

In the study, 16 male rabbits of New Zealand origin that were provided from Saki Yenilli Laboratory Animals Production Center (Ankara-Turkey) with an average weight of 2.05 ± 0.12 kg were used. All experimental procedures complied with international guidelines for care and use of laboratory animal and were approved by the Ethical Committee of the Experimental Animals of Istanbul University-Cerrahpasa, Istanbul (Decision no: 146). The care and shelter of the rabbits was carried out in Istanbul University-Cerrahpasa, Cerrahpasa Medical Faculty Experimental Animal Production and Research Laboratory. The procedures for the care and feeding of the animals were carried out according to the ethics committee approval. Rabbits were individually placed in galvanized stainless steel cages. Rabbits were fed with normal chow (Eris Chow Industry, Istanbul, Turkey) and tap water ad libitum at 20-]22°C under standard conditions (12-h day/12-h night cycle) to provide their adaptation to the environment. At the end of the 1 week, experimental animals were divided into two groups as control ($n = 8$) and diabetes group ($n = 8$).

### Experimental Procedure

**Control Group ($n = 8$)** The rabbits in this group were fed with normal feed and tap water for the duration of the experiment (6 weeks).

**Diabetes Group ($n = 8$)** To experimentally generate a diabetic rabbit model, rabbits were calmed by intramuscular 35 mg/kg xylazil, and 50 mg/kg ketamine. A single-dose (90–100 mg/kg) intravenous injection of alloxan monohydrate was administered to the rabbits. Three days after the injection of alloxan monohydrate, fasting blood glucose level was measured using a glucometer (Contour TS-BAYER, Germany) with blood samples taken from the ear vein of rabbits. While normal blood glucose value was accepted as 90–110 mg/dL, those with blood glucose levels above 200 mg/dL were included in the diabetes group. After the formation of diabetes, the 6-week experiment period was initiated and the animals were fed with normal feed and tap water during this process.

### Measurement of Blood Glucose Level and Body Weights

After the formation of the experimental groups, the glucose levels of the blood samples taken from the ear veins of the animals in both groups were monitored at the beginning of the experiment and weekly throughout of the experiment period by using a glucometer. On the same days, body weights of the rabbits were measured using the counting scales (Jadever JWEL-6K, Taiwan).

### Collection of Samples and Preparation of Blood Samples

At the end of the experimental period, rabbits were sacrificed under general anesthesia by injecting 200 mg/kg sodium pentobarbital intramuscularly. After opening the thorax of the rabbits, blood samples were collected from their hearts with a 10-mL syringe and transferred to the tube contain anticoagulant. The aqueous humor fluids of the rabbits were obtained by perforating the anterior chamber of the eyes with an insulin syringe. One hundred and fifty microliters of the blood sample and aqueous humor fluid was separated for osmotic pressure measurement, and the remaining portion of the blood sample was centrifuged at 3000 rpm for 5 min to obtain plasma. Aqueous humor fluids and plasma samples were immediately transferred to polyethylene vials, and stored at − 80 °C until further analysis of trace elements (Zn, Cu, and Cr). Hemolyzed and lipemic samples were excluded.

### Measurement of Osmotic Pressure (Osmolality)

The osmotic pressure (osmolality) measurement in plasma and aqueous humor fluid samples was realized with a Semi-Micro Osmometer device (KNAUER K-7400) from the Osmotic Pressure Measurement in Body Fluids Laboratory situated in Department of Biophysics, Cerrahpasa Medical Faculty, Istanbul University-Cerrahpasa. Measurements were realized within average 2–3 h on the day the samples were taken. All measurements were repeated three times and mean values were taken and osmolality results were expressed in mOsm/kg.

### Analysis of Trace Elements Levels

Trace element measurements (Zn, Cu, and Cr) in plasma and aqueous humor fluid were measured after dilution by inductively coupled plasma–optical emission spectrophotometer (ICP-OES, Thermo iCAP 6000 series) at the Trace Element Analysis Laboratory at Department of Biophysics, Cerrahpasa Medical Faculty, Istanbul University-Cerrahpasa.

The aqueous humor fluid samples were diluted 1/5 (v/v) for trace elements analysis with deionized water. The favorable wavelengths were selected as $\lambda_{Zn} = 206.200$ nm, $\lambda_{Cu} = 327.396$ nm, $\lambda_{Cr} = 267.716$ nm in order to analyze the trace elements. ICP-OES was operated with a plasma gas flow rate of 5 L/min, argon carrier flow rate of 0.5 L/min, sample flow rate of 1.51/min, flow rate of elusion, and peristaltic pump speed of 100 rpm. Transport lines were made using a 1.25-mm-i.d. polytetrafluoroethylene tubing. Each measurement was performed three times and averages were used for analysis and results of measurements were expressed in micrograms per deciliter (μg/dL) of the sample. The element solutions (Redoks Laboratory Analytical Systems, Istanbul/Turkey) were used for calibration and standard solutions (Table 1). The test standards used in the ICP-OES analysis were prepared from suitable stock solutions containing 1000 ppm for each tested element obtained from Chem-Lab NV (Belgium). Deionized water was used as a blank solution. The chemicals used were analyzed blank to check whether there was an elemental contribution from the agents. All glass materials were sterilized before using for trace elements measurements. Stock solutions of Cu, Zn, and Cr were prepared by taking appropriate amounts of standards in deionized water. Standard solutions were prepared just before use. The calibration graph was obtained from a software program using blank and standard solutions and the Zn, Cu, and Cr levels were analyzed of the prepared plasma and aqueous humor fluid (Fig. 1).

The data obtained as a result of the analysis were evaluated by using an SPSS-21.0 (Statistical Package for Social Science) package program. The statistical comparison of the measured parameters was realized by using a One-Way ANOVA Student *t* test. All results were expressed as mean ± standard deviation (M ± SD). $p < 0.05$ iconsidered as significant.

## Results

At the beginning and the end of the experiment, the same values of the diabetes group were statistically higher ($p < 0.001$) compared with the blood glucose levels of the control group. While the average body weights of the control group did not show a statistically significant change during the 6-week experiment period, the same data decreased significantly ($p < 0.05$) during the experiment for the diabetes group (Fig. 2).

### Osmotic Pressure Levels

It was found that the osmotic pressure values measured in the diabetic rabbits' blood increased significantly ($p < 0.01$) in comparison with the control group. When the plasma osmotic pressure values of the control group were compared with the plasma osmotic pressure levels of the diabetes group, a significant ($p < 0.001$) increase was observed in the diabetes group. It was detected that the osmotic pressure levels of the aqueous

**Table 1** Standard values of trace elements

| Trace elements | Standard-1 | Standard-2 |
|---|---|---|
| Zn | 0.5 μg/mL | 1 μg/mL |
| Cu | 1 μg/mL | 2 μg/mL |
| Cr | 0.2 μg/mL | 0.4 μg/mL |

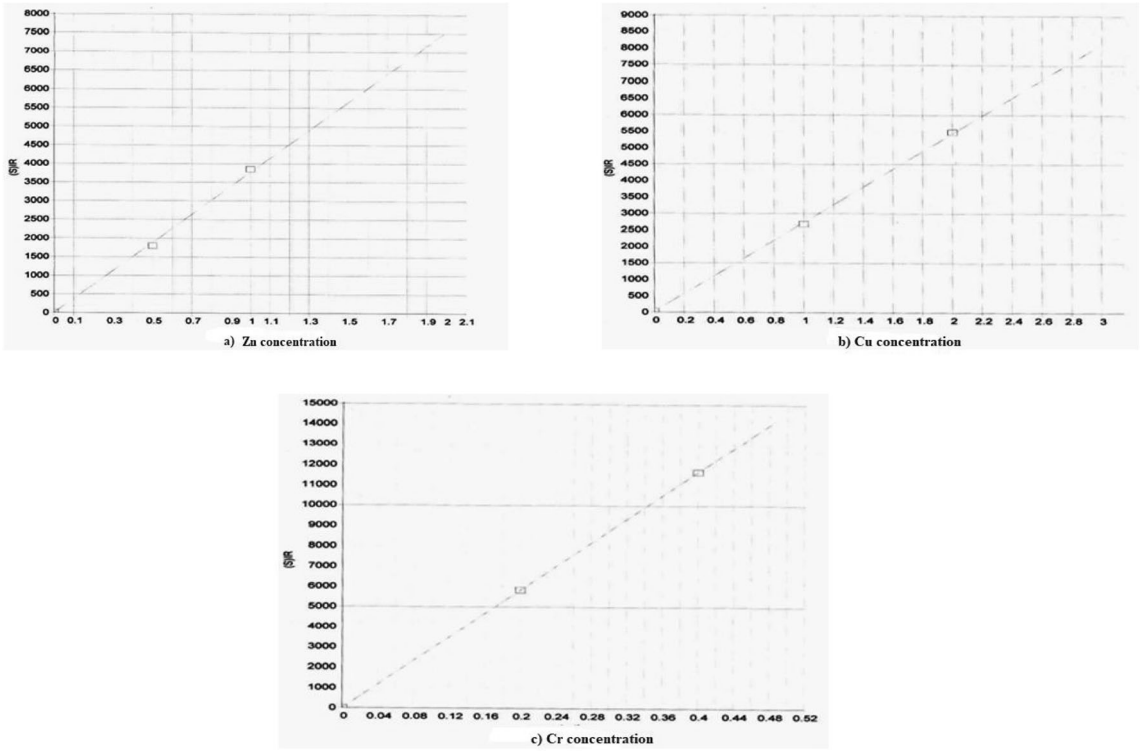


**Fig. 1** Zn, Cu, and Cr calibration graph

humor fluids of diabetic rabbits increased significantly ($p < 0.001$) in comparison with the control group (Fig. 3).

### Trace Element Levels

When the plasma trace element values of the diabetes group were compared with the control group's values, the plasma Zn and Cu levels of the diabetic rabbits were determined to be significantly increased in comparison with the same parameters of the control group ($p < 0.01$ and $p < 0.001$, respectively). It was indicated that the plasma Cr level of the diabetic group showed a statistically significant ($p < 0.01$) decrease when compared with the control group (Fig. 4). In the diabetes group, it was observed that the Zn and Cr levels measured in aqueous humor fluid decreased significantly ($p < 0.01$ and $p < 0.001$) in comparison with the control group and that Cu level increased significantly ($p < 0.001$) in comparison with the control group (Fig. 5).

## Discussion

Diabetes is a metabolic disease characterized by chronic hyperglycemia, which leads to a lack of insulin secretion or a decrease in the tissues' sensitivity to insulin and results with an impaired carbohydrate, fat, and protein metabolism. Chronic hyperglycemia damages the vessels and nerve cells and causes functional disorders in many tissues. In addition to

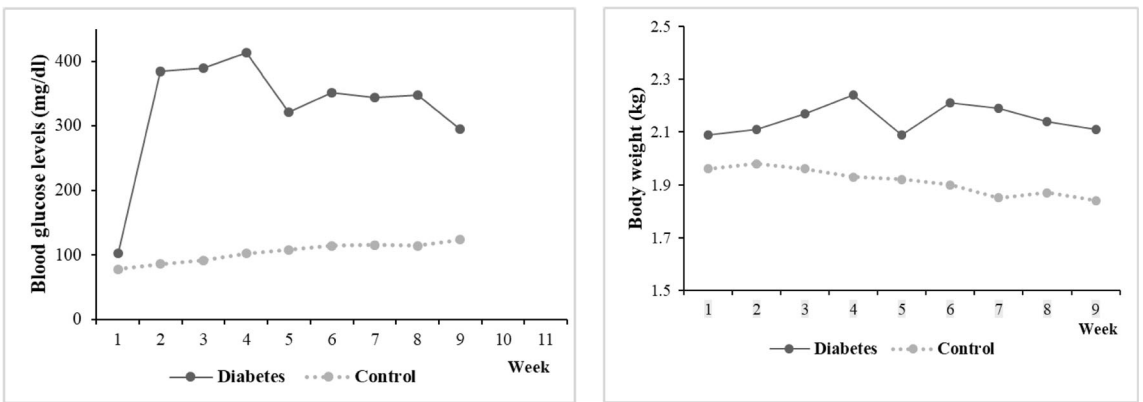


**Fig. 2** Weekly changing of blood glucose levels and body weights in control and diabetes groups during the experiment

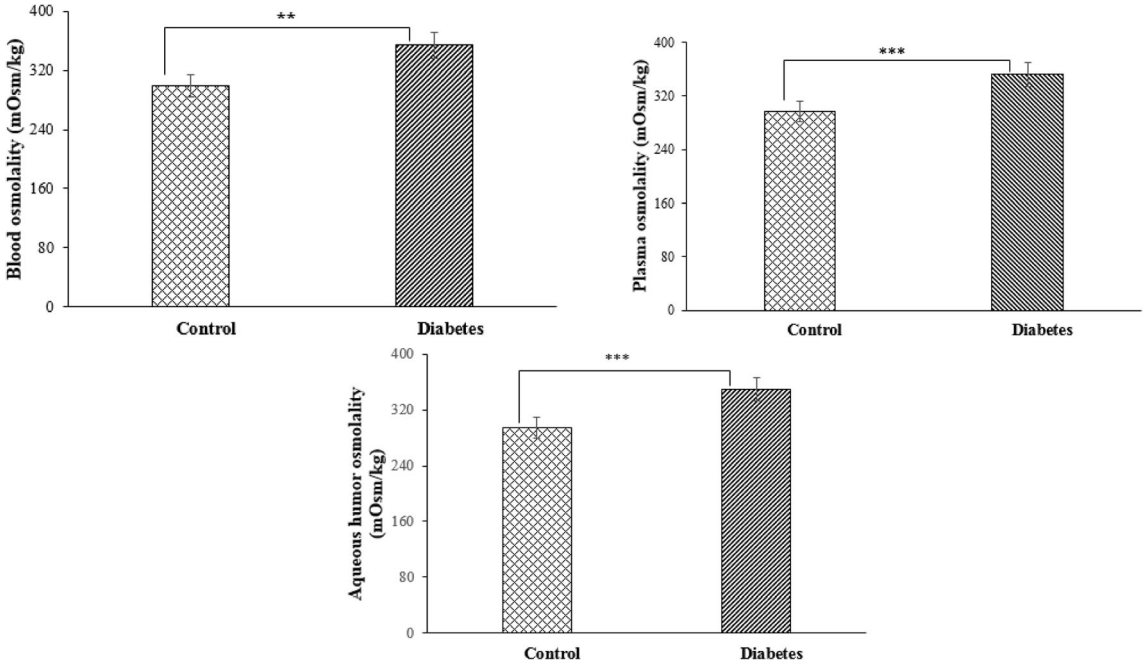


**Fig. 3** Blood, plasma, and aqueous humor osmolality values of the experiment groups. The results are expressed as the mean ± S.D. ($n = 8$) (($^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$)

acute complications such as hypoglycemic and hyperglycemic coma, various microvascular complications such as diabetic retinopathy, neuropathy, and nephropathy and chronic macrovascular complications such as cardiovascular, cerebrovascular, and atherosclerotic diseases have been occured [10–12].

It was reported that the homeostasis of trace elements that plays a significant role in diabetes and the pathogenesis of diabetes has been impaired. It was stated that elements specifically such as Zn, Cu, Cr, selenium (Se), and magnesium (Mg) have important effects on glucose metabolism. It has been specified that these elements function as component or cofactor of metalloenzymes related to glucose metabolism and increase insulin sensitivity and activation. Deficiency of essential elements trigger dysfunctions in the antioxidant defense mechanism and glucose tolerance, which are effective in diabetes. In studies investigating the etiogenesis of diabetes, it is controversial whether the change of trace element levels are

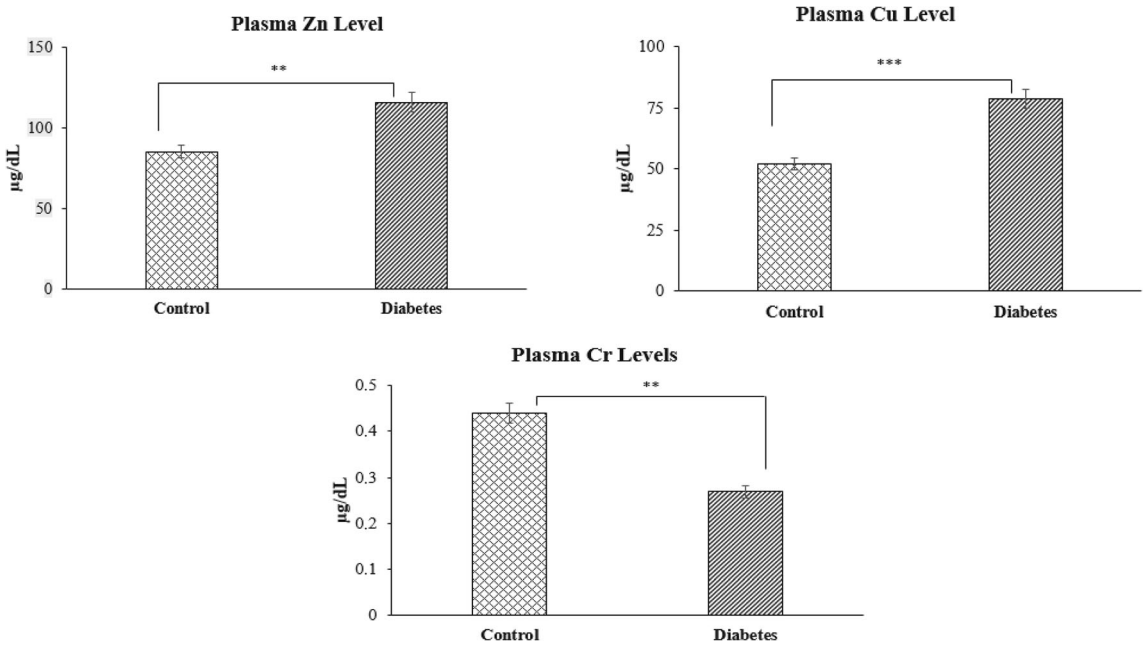


**Fig. 4** Plasma Zn, Cu, and Cr levels of the experiment groups. The results are expressed as the mean ± S.D. ($n = 8$) ($^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$)

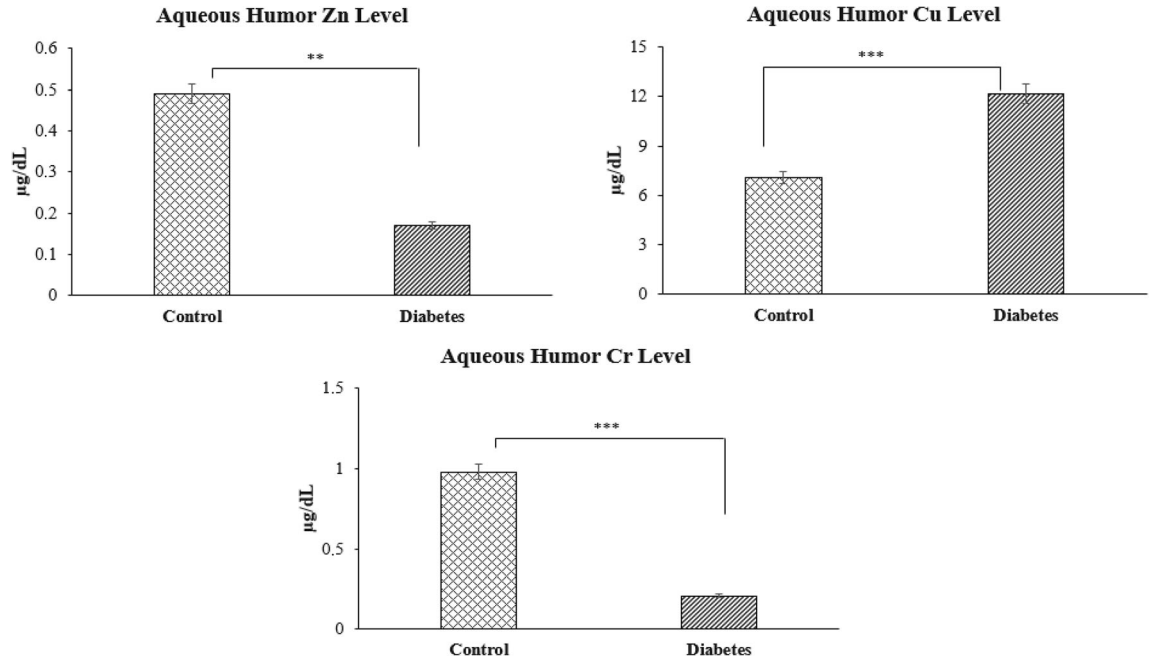


**Fig. 5** Aqueous humor Zn, Cu, and Cr levels of the experiment groups. The results are expressed as the mean ± S.D. ($n = 8$) (($^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$)

the result of hyperglycemia or whether deficiency of the elements contributes to the formation of diabetes [15–18].

Zn is an essential element required for insulin synthesis, storage, and secretion. In most mammals, insulin and Zn are stored together in pancreatic beta cells. Therefore, it is possible for insulin to be secreted together with Zn. Zn, which is known to play a significant role in immune system regulation, is closely related to immune system dysfunction observed with diabetes [21, 22].

Cu is an essential element necessary for the regulation of various enzymes such as the cytochrome c oxidase, superoxide dismutase, and tyrosine. It was declared that the plasma and tissue Cu levels increase in diabetes [11, 15]. In some researches, it was reported that as a result of hyperglycemia, Cu binds to glycalized proteins and these proteins play a significant role in vascular dysfunction of diabetic patients. Also, Cu plasma level was reported to be high in diabetic patients having diabetic retinopathy, hypertension, and macrovascular disease [10, 23, 24].

Cr has been suggested as a therapeutic agent to increase insulin sensitivity and to regulate the lipid and carbohydrate metabolism in experimental animal models. The molecular mechanisms of Cr in insulin resistance in humans are under investigation [25]. Although there are studies recommending Cr supplementation for glucose control in diabetes, there is insufficient evidence to reach a definitive conclusion. While studies with Cr on rodents have shown that Cr has a pharmacological role on insulin resistance, there are conflicting results regarding the effect of Cr on diabetes in chemical, genetic models of diabetes, also present in human clinical studies. Although there are unclear results regarding the effect of Cr supplementation on glucose intolerance, clinical studies suggest that higher doses may be effective [25–27]. It was determined that the insulin resistance of tissues increases in Cr deficiency [28–31]. It was reported that in diabetes, the Cr metabolism changes due to excessive secretion or dysfunctional absorption of Cr that affects glucose metabolism. It was showed that decreasing level of Cr in blood, hair, and nail tissues in patients with diabetes [24, 32].

The carbonic anhydrase enzyme which is a polypeptide that contains Zn was reported to be associated with aqueous humor secretion. Even though there are very few studies realized on this subject, it was reported that the production of aqueous humor decreases with the inhibition of the carbonic anhydrase enzyme [33–36]. Zn, which affects cell metabolism through various mechanisms, plays a significant role in maintaining normal ocular functions. High Zn level is found primarily in the retina and choroid and also in the ciliary body, iris, optic nerve, sclera, cornea, and lens. It was determined that visual deficiency and some degenerative retinal diseases may develop due to Zn deficiency [37–40].

Changes in the metabolism of elements that are significant in diabetes and its complications are controversial. Zargar et al. [41] reported that the Cu levels increased in diabetic patients' plasma, but there was no significant change in Zn and Mg levels. Flores et al. [42] indicated that the serum Cu level increased, the Cr level decreased in diabetic patients but found no significant difference in Zn level. Viktorinova et al. [14] reported that Zn and Mg levels decreased and Cu levels increased in diabetic patients in comparison with healthy

individuals. On the other hand, contrary to the research results mentioned above, some researchers stated that Zn and Cu levels decrease in plasma and blood. Marjani et al. [43] showed that plasma Zn levels decreased in diabetic patients. It was stated that the Zn level measured in the aqueous humor fluid of the patients with cataract due to old age was lower than their serum Zn level [44, 45]. In the elemental studies realized in the aqueous humor fluid of patients with glaucoma and cataracts, it was specified that the Cu level increased, and the Zn level decreased [35, 39, 40]. Gündüz et al. [19] determined that the lens Zn level was higher in cataracts caused by diabetes in comparison with cataracts caused by the old age. Whereas, Cumhurcu et al. [33] did not find a significant difference in the cataract caused by diabetes and non-diabetic cataract. They also stated that diabetic patients have decreased Cr level in both serum and aqueous humor fluids. Aydın et al. [34] reported that the Cu, Zn levels measured in the aqueous humor fluid of the diabetic patients did not change significantly when compared with the control group.

In our study, it was analyzed that plasma Zn and Cu levels showed a statistically significant increase and the Cr level decreased in diabetic rabbits in comparison with the control group. In the aqueous humor fluid, it was determined that the levels of Zn and Cr decreased and the level of Cu increased in the diabetic group in comparison with the control group. It is possible to express that the increasing of Cu in the aqueous humor fluid in diabetic patients may be a result of the deterioration of the diabetic blood-aqueous barrier. Increased vascular permeability caused by diabetes can also affect the transition of Cu to the aqueous fluid. Also, it is possible to express that the release of Cu from the enzymes containing Cu in tissues related to hyperglycemia causes this element to increase in plasma and aqueous humor. It is also possible that the Cu level increases in diabetes by passing into the aqueous humor due to the microvascular dysfunction of the systemic circulation.

In our study, it was observed that as a result of osmotic pressure measurements in blood, plasma, and aqueous humor fluids of both groups together with trace element measurements, the osmotic pressure of the diabetic group increased significantly in blood, plasma, and aqueous humor fluids in comparison with the control group.

Plasma osmotic pressure varies depending on many factors such as liquids and electrolytes taken with food and also sweating, diuresis, gastrointestinal losses, reabsorption, and excretion [46, 47]. It is known that in osmotic balance, nutrient intake and changing ion concentrations play a significant role. Although changes in element levels in lenses have been investigated in several studies, there have been very few studies that comparatively evaluating the number of elements in aqueous humor fluid, lens, and plasma in patients with or without diabetes.

Trace elements play an important role in the pathogenesis and development of several serious ophthalmological and degenerative retinal disorders, such as age-related macular degeneration that causes a progressive loss of central vision, diabetic retinopathy, cataract, glaucoma, retinoblastoma, etc. The deficiency or excess of various trace elements can help understanding the metabolism of many diseases at the molecular level. Our study was aimed to measure the alterations of Zn, Cu, and Cr, also to search relationship between this elements and osmotic pressure in aqueous humor and plasma in diabetes. According to the results, we suggested that the change of trace elements levels and osmolality in the aqueous humor could be associated with cataracts formation. Lens opacity may deteriorate due to many factors such as smoking, ultraviolet, radiation, age, and diabetes. The deterioration of lens opacity is one of the leading causes of vision loss around the world. It is known that the lens takes all of its essential nutrients from aqueous humor fluid. Therefore, the role and significance of trace elements in the cataract formation have been investigated by many researchers.

It has been suggested that changing of the levels of these elements especially Zn and Cu contribute to the formation of cataract. We can say that the deterioration of trace element homeostasis due to diabetes affects osmotic pressure. In our study, we evaluated that the Cu accumulation in plasma and aqueous humor fluid was effective in the increase of osmotic pressure in the diabetic animal model that we created through the application of Alloxan. Changing the element metabolism in aqueous humor fluid may disrupt oxidative balance and may cause various diseases such as cataract formation. Also, changing the osmotic pressure in aqueous humor fluid due to diabetes may have an effect on the development of complications such as glaucoma, as this will disturb the flow balance. Considering the changes that occured in the eye depending on the parameters that we measured, we emphasize that it would be useful to realize similar studies in different diabetic models in a comparative way, in order to evaluate diabetes and the complications related to diabetes.

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## Compliance with Ethical Standards

All experimental procedures complied with international guidelines for care and use of laboratory animal and were approved by the Ethical Committee of the Experimental Animals of Istanbul University-Cerrahpasa, Istanbul (Decision no: 146). The procedures for the care and feeding of the animals were carried out according to the ethics committee approval.

**Conflict of Interest** The authors declare that they have no conflict of interest.

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