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Effect of intermittent fasting during acrylamide exposure on blood copper, zinc and lead levels in rats

Zübeyir Huyut[1*], Okan Arihan[2]

[1]*Van Yuzuncu Yil University, Faculty of Medicine, Department of Biochemistry, Campus, Van*
[2]*Van Yuzuncu Yil University, Faculty of Medicine, Department of Physiology, Campus, Van*

ABSTRACT

Zinc and copper are responsible for certain physiological functions in the body. On the other hand lead is a toxic trace element. In this study, effect of intermittent fasting on blood zinc, copper and lead levels during acrylamide exposure was studied.

Forty female adult Wistar albino rats were divided into 4 groups (n=10 in each group). First group: control group without any administration, second group: acrylamide group in which 20 mg/kg/day acrylamide was administered for 30 days, third group: intermittent fasting group in which 12 hours of fasting were administered overnight for 30 days, fourth group: intermittent fasting and acrylamide group in which 20 mg/kg/day acrylamide and 12 hours of intermittent fasting was administered for 30 days. At the last day of experimental procedure blood was withdrawn under anesthesia. Zinc as well as copper levels were measured from plasma samples in flame unit and lead was measured from erythrocyte packages in combustion unit of Atomic Absorption Spectrophotometer.

In acrylamide group, zinc was lower ($p<0.05$) whereas copper ($p>0.05$) and lead levels ($p<0.05$) were increased. In intermittent fasting group zinc level was higher whereas copper and lead levels were lower compared to acrylamide group ($p<0.05$). In acrylamide+intermittent fasting group lead level was significantly lower ($p<0.05$).

Conclusion: Results indicate an augmentation of toxic copper and lead elements due to acrylamide administration whereas a decrease in zinc which is an important element for growth and immune system. On the contrary, intermittent fasting decreased such untoward effect of acrylamide and increased potential detoxification of toxic chemicals.

Key Words: Intermittent fasting, copper, zinc, lead, erythrocyte, trace element

Introduction

Acrylamide is an important manufacturing material for industry. It is widely use in waste water management, cosmetics, construction as well as laboratory uses. Since it is found as an environmental toxin it can be included into water and food resources. Another inclusion of acrylamide into human food is via reactions occurring during processing. Some of our processed foods are processed above 100 degrees Celcius (frying and baking). It causes production of acrylamide via Maillard reaction which includes degradation reaction of protein and carbohydrates in food (1, 2). Therefore it is an unintended chemical found in many food sources (bread, cookies, coffee, potato crisps, roasted chickpea). Turkey is a leading country in bread consumption in the world and bread is a major acrylamide source for diet in Turkey. Acrylamide intake changes with dietary habits of different populations. Coffee and croissant are among those in some populations whereas it can be beer in some others (3). Acrylamide molecule binds to sugar moiety of DNA molecule. This property raised interest on its potential mutagenic and carcinogenic property. Acrylamide also possess adverse neurological effects (4). Metabolic effects due to different exposure routes to acrylamide (oral, i.p., dermal) are reported. In one of those studies, acrylamide is shown to bind hemoglobin molecule which is crucial for gas transport in blood (5). Acrylamide was reported to attenuate red blood cell deformability which is an important contributor of healthy blood microcirculation (6). Acrylamide was also reported to alter erythrocyte fragility adversely (7).

Caloric restriction is a scientifically proven method for longevity and a healthier life (8, 9). Intermittent fasting was reported to have benefits in certain metabolic disorders and diseases (10). However there are no data for effect of intermittent fasting on blood zinc (Zn), copper (Cu) and lead (Pb) levels during acrylamide exposure.

Zn is among the most common trace elements in human body. 85% of total body Zn is within

***Corresponding Author:** Assist. Prof. Dr. Zubeyir Huyut, Van Yuzuncu Yil University, Faculty of Medicine, Department of Biochemistry
E-mail: zubeyir.huyut@gmail.com, Phone: 0(506) 027 13 00
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muscles and bones, 11% of it is in skin and liver and remaining is found in other tissues (11). Zn is important for biological functioning of enzymes more than 300 as well as DNA synthesis and repair, growth, brain growth, behavioral response, reproduction, fetal growth, membrane stability, bone formation and wound healing (11). Its level in cell is critically related with proliferation, differentiation and apoptosis. In addition it is also essential for neurogenesis, synaptogenesis, neuronal growth and neurotransmission. Zn is stored in specific synaptic vesicles as a counterpart of glutaminergic neurons and also released as an activity dependent neuromodulator (12). Zn at physiological concentrations play role in regulation of immune response, spermatogenesis and steroidogenesis, vitamin A metabolism, insulin release and storage, energy metabolism, protein synthesis, stabilization of macromolecules, regulation of DNA transcription (13). Zn deficiency may cause abnormalities such as insulin resistance, cardiovascular diseases, neurological diseases, retardation of follicular maturation, Menkes syndrome and high homosistein levels (14). Copper may also contribute to protein oxidation, DNA and RNA lysis and reactions causing membrane damage like Fe. Since trace elements such as Zn and Cu have crucial roles in cellular homeostasis, signaling pathways and also involved in pathogenesis of certain diseases it is important to determine Cu and Zn level. In addition, absorption of such elements varies with food ingredients and due to some intestinal pathologic situations. The reason why copper and zinc are evaluated together is that negative correlation in their absorptions after they are taken with diet.

Aim of this study is to investigate effect of intermittent fasting on blood Zn, Cu and Pb levels during acrylamide exposure which is an unintended diet component.

Materials and Methods

Experimental Design: Female, adult, Wistar-Albino rats were divided into four groups including 10 rats in each group. Control group received no administration. Acrylamide group was administered with 20 mg/kg/day acrylamide with gastric gauge. Intermittent fasting group (IF) was let fasting each other day for 12 hours (10). Water was provided ad libitum during fasting. Acrylamide and intermittent fasting group (AIF) was administered with 20 mg/kg/day acrylamide as well as intermittent fasting each other day as explained. Acrylamide doses administered to rats were determined according to the toxic dose ranges applied in previous studies (15-17). Following 30 days of experimental procedure rats were sacrificed under ketamine – xylazine anesthesia. Blood was withdrawn under anesthesia into heparin containing plastic polyethylene tubes. 2 mL of obtained blood sample was centrifuged at 3500 *xg* for 10 minutes and plasma samples were separated. An ethical permission was obtained (date: 01.03.2018, decision no:2018/02).

Trace element measurement: Trace element measurements were performed with partial modified methods of Yahaya et al. (18) and Reyes et al. (19). Prior to study, glassware and plastic containers were cleaned with 20% HNO_3. They were washed with deionized water 3 times and dried. HNO_3, $(NH_4)H_2PO_4$, Triton X 100 used in the study was ultrapure chemical grade (ultrapure reagent grade).

Zn and Cu Measurement: 400 µL from plasma sample was transferred into a tube and 3600 µL 0.2% HNO_3 was added onto it (Total volume 4 mL). Zn and Cu cathode lamps (Holllow Cathode Lamp, Hamamatsu Photonics K.K Made in China) were used. Calibration curves were obtained with standards prepared with 0.2% HNO_3 solution for Zn and Cu. Absorbances were measured with Atomic Absorption Spectrophotometer device (AA-7000, Shimadzu) combustion unit at 213.9 and 324.8 nm wavelengths, respectively for Zn ad Cu. 0.2% HNO_3 solution was used as blind. Results are expressed as µg/dL.

Pb Measurement in Packed Erythrocytes: 500 µL 10% Triton-X 100 was added onto 250 µL packed erythrocytes. 1250 µL, 10% $(NH_4)H_2PO_4$ and 3 mL of deionized water ultrapure water was added on it (total volume 5 mL). Then calibration curve was obtained with different standard concentrations by using Pb cathode lamp (Hollow Cathode Lamp, Hamamatsu Photonics K.K Made in Japan) and measurement was performed at 283.3 nm with atomic absorption combustion unit (Graphite Furnace Atomizer, GFA-7000 Shimadzu).

In addition, hemoglobin was measured according to method used by Huyut et al (20). Hemolyzate (20 µL) was added onto 5 mL drabkin solution and incubated for 10 min at room temperature. Following incubation, % transmittance values were measured with spectrophotometry at 540 nm. Hemoglobin content was calculated as g/dL from previously prepared calibration curve. Results are expressed as ngPb/gHb levels.

Table 1. Plasma Zn and Cu levels and erythrocyte Pb level for control, acrylamide, IF and AIF groups

Groups	Plasma Zn (µg/dL)	Plasma Cu (µg/dL)	Erythrocyte Pb (ng/gHb)
Control	9.21±0.38b	7.54±0.15a	3.15±0.34b
Acrylamide	7.98±0.33c	7.86±0.21a	5.35±0.41a
IF	11.67±0.46a	6.48±0.60b	4.18±0.5b
AIF	7.58±0.51c	7.21±0.8ab	3.43±0.42b
p Value	0.001	0.014	0.016

"[a,b,c]": Values with different letters in the same column are significantly different than others ($p<0.05$). IF: intermittent fasting, AIF: acrylamide + intermittent fasting

Statistical Analysis: Data were analyzed with Kruskal-Wallis, Mann Whitney U test and Bonferonni correction. Statistical analysis was performed using the SPSS 15 statistics package program (SPSS Inc., IL, USA). Data were expressed as mean±S.E.M. P values less than 0.05 was accepted statistical significant.

Results

No deaths were observed during experimental procedure. Results concerning Zn, Cu and Pb are given in Table 1. Highest Zn value was observed in intermittent fasting group (11.67±4.6 µg/dL). Lowest value was observed in Acrylamide (7.98±3.3 µg/dL) and AIF (7.58±5.1 µg/dL) groups ($p<0.001$). Plasma Cu value was found highest in acrylamide group (7.86±2.1 µg/dL) whereas it was found lowest in IF group ($p<0.014$). In addition, Cu level was lower in AIF group compared to acrylamide group although statistically insignificant. Similarly with Cu, Pb which is also a toxic element was highest in acrylamide group (5.35±4.1 ng/gHb) ($p<0.016$). Although statistically insignificant, AIF group Pb level was lower compared to acrylamide group.

Discussion

Acrylamide is a harmful chemical and also an unintended food ingredient. In this study, lowest Zn value in plasma was observed in acrylamide and AIF groups. This finding shows that acrylamide decreases an important trace element in blood tissue. Zn is an important element for maintenance of homeostasis, delaying aging process and preventing cancer (21). Intermittent fasting application possessed an ameliorating effect for this harmful impact of acrylamide. In addition increase in Zn value in lone intermittent fasting process may clarify the mechanisms of protective role of Zn in longevity and protection from diseases. Yerlikaya and Yener (22) investigated effect of acrylamide exposure on serum trace element levels (female 65-70 grams, 3-4 weeks old Wistar-albino rats) and found Zn as 107.7±36.7 µg/L in control group, 41.5±10.9 µg/L in 2 mg/kg acrylamide group and 38.0±9.4 µg/L in 5 mg/kg acrylamide group and showed that both of the acrylamide group Zn content was significantly lower compared to control. Our results correlate with findings in the study of Yerlikaya and Yener (22). Intermittent fasting in our study showed a recovery of Zn values in acrylamide+intermittent fasting administered group.

Cu level in this study was found lowest in intermittent fasting group. Although, Cu is an essential element for processes like cellular respiration it is also an element which is related with toxicity. Thus, observation of low levels of Cu in IF group compared to control can be concluded as another benefit for health. In the study by Yerlikaya and Yener (22) Cu levels were found as 161.2±14.0 µg/L in control group, 178.1±44.4 µg/L in 2 mg/kg acrylamide group and 178.1±21.9 µg/L in 5 mg/kg acrylamide group. No significant difference was reported in the study by Yerlikaya and Yener (22), whereas Cu levels were found lower in IF group compared to other groups. This discrepancy may be due to higher administration dose of acrylamide compared to mentioned study. High Zn levels were reported to be negatively correlated with Cu levels and it may change due to age and exposed dosage (23). Therefore another reason for dissimilarity of our findings with Yerlikaya and Yener (22) may be due to interaction of Cu with increasing Zn concentration.

Pb is a potent neurotoxic element which humans have been exposed since ancient times (24). No beneficial function is known for Pb and is still an important environmental pollutant and an unintended diet ingredient despite the efforts to limit its abundance with applications such as Pb free oil. Although in this experimental procedure no Pb was administered to rats it is highly possible that the provided commercial rat food includes

Pb. Acrylamide administration increased Pb in rats significantly whereas intermittent fasting ameliorated this alteration.

Mechanism of alterations of those three elements due to acrylamide exposure and intermittent fasting are not clear but in similar studies (22), acrylamide was shown to exert prominent effect on some other elements (cobalt, vanadium, magnesium, selenium). Absorption of elements taken with diet is closely related with diet content and substances such as phytates directly affect absorption (25). There are no detailed data concerning effects of acrylamide on those food ingredients. In addition structural disorders and inflammatory diseases also have impact on intestinal absorption (26). Acrylamide may also alter intestinal absorption. There are studies presenting alterations in intestinal absorption due to different mechanisms. 8 weeks of acupuncture treatment was shown to increase iron absorption compared to control group (27). In another study, 3 weeks of bed rest increased Zn and Cu excretion (28). Therefore it can be suggested that acrylamide may interfere with mechanisms other than diet and altered Zn, Cu and Pb absorption and excretion. A certain deficiency of Zn or Cu due to different mechanism ingredients may also change serum levels of other elements. Iron deficiency increases absorption of cadmium, lead, and aluminum. Lead interacts with calcium in the nervous system to impair cognitive development. Lead further replaces zinc on heme enzymes (29). Therefore increase in Pb and decrease in Zn may augment potential health hazards since Zn is an important element for a healthy immune system, sustained growth and neuronal development (30). Due to their important physiological functions without any storage mechanism in body, observed untoward effects of acrylamide on Zn and Cu levels requires further studies on the subject.

Acrylamide exposure (20 mg/kg) causes a significant increase in Pb and Cu level and a significant decrease in Zn level in female Wistar-albino rats. When administered concomitantly with intermittent fasting levels of Pb decreases to control level. A similar but less prominent effect was also observed in Zn and Cu levels. This finding shows important health benefit of intermittent fasting in trace element aspect which also suggests an enhanced detoxification of toxic elements such as Pb.

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