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ORGANIC VS. CONVENTIONAL VEGETABLES: INFLUENCE OF CARROTS AND LETTUCE CONSUMPTION ON SERUM AND BONE MINERAL CONTENTS IN RATS

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ABSTRACT

The objective of this study was to assess the minerals content of organic and conventional carrot and lettuce produced in Saudi markets and their effect on some bone minerals and some serum heavy metals content in adult rats. The investigation was performed on 30 adult Wistar rats, fed on organic and conventional vegetables for 2 months. Mineral concentrations were measured using ICP-OES to determine Ca, P, Mg, Zn, Pb, and Cd contents in vegetables, Ca, P, Mg, and Zn contents in the bone and Pb and Cd concentrations contents in serum. Results show statistically insignificant difference in the mineral content between two cultivation systems except for calcium of lettuce. The effect of organic and conventional vegetable intake on bone minerals content shows significant difference in magnesium and phosphorous content between organic and conventional vegetables and as far as heavy metals content in the serum of adult rats is concerned, the data showed insignificant differences between organic and conventional vegetables. Our data didn’t suggested much distinctionsbetween the effect of the intake of conventional and organic vegetables on bone minerals and heavy metals in the serum. Future studies should concentrate on long-term consumption of these vegetables.

KEYWORDS: organic; conventional; carrot; lettuce; minerals; bone.

INTRODUCTION

There are various descriptions of organic agriculture. According to The Codex Alimentarius Commission "Organic farming is a holistic production management system which promotes and enhances agro ecosystem health, including biodiversity, biological cycles, and soil biological activity."[1] International Federation of Organic Agriculture Movements (IFOAM) states that, "Organic agriculture is a production system that sustains the health of soils ecosystems and people."[2] The idea of organic food is cultivation without the use of chemicals and genetically modified organisms (GMOs), where the DNA of the plant seed is modified to make the product more engaging or bigger. Contrariwise, conventional agriculture relies on using pesticides and antibiotics. In the last few decades, the demand for organic products has increased. The fundamental explanation is that organic agriculture can promote human and environmental health.[3] Based on this idea, a study conducted by Gomiero,[4] showed that people believes that organic food has superior health benefits because it is environment friendly. Despite the high cost of organic foods; the main motivation for purchasing organic food was health and environmental. Dimitri and Dettmann,[5] found a strong relationship between education and the likelihood of purchasing organic food products.

The chemical compounds in organic and conventional vegetables may vary due to the carbon and nitrogen balance in the soil. The availability of free nitrogen makes it easy to manufacture compounds to promote vegetable growth. Through the manipulation of the growth differentiation balance hypothesis (GDBH) that states, "plants modulate growth through evaluation of available resources" the addition of synthetically produced nitrogen can promote plant.[3]

Vegetables are one of the most important sources of minerals and trace minerals, which are absorbed from soil and water. Absorption of calcium from various vegetables is either inferior to or comparable with that from milk, with bioavailability estimates ranging from 20% to 40%.[6] In nature, vegetables serve as a significant source of magnesium, with green leafy vegetables being especially abundant in this essential nutrient.[7] Carrot has a high minerals content, which is good for bone growth such as Ca (33 mg/ 100g), Mg (12 mg/ 100g), P (35 mg/ 100g) and Zn (0.24 mg/ 100g), while lettuce contains Ca (35 mg/ 100g), Mg (13 mg/ 100g), P (33 mg/ 100g) and Zn (0.2 mg/ 100g).[8]

Although macro-minerals are required in the body in large amounts ranging from hundreds of milligrams to grams, but micro-minerals, also called trace minerals, are required in amounts so tiny that they are measured in micrograms. They play an important role in various body functions. Calcium and phosphorus are associated with bone formation and deficiency of these minerals causes osteoporosis and rickets and magnesium reduces the state of osteoporosis by osteo-immunomodulation.[9] In recent years it has been recognized that zinc plays an important role in bone homeostasis and development.[10] On the other hand, the accumulation of heavy metals in the blood causes health problems. The presence of cadmium and lead in food is associated with problems in the respiratory system, hematology, and cardiovascular system, and increases the chance of developing various types of cancer.[11,12] Lead and cadmium are abundant in soil.[13] Thus, vegetables absorb the toxic substances present in the soil.[14] Pesticides are known to weaken the immune system and adversely affect nerve receptors. They may also contribute to the development of tumors and negative impact on hormones.[15] Additionally, pesticides have a chemical composition similar to some human hormones, which may affect fertility for men and women.[16] Organic products are expected to witness significant growth in the future in the Kingdom of Saudi Arabia at a compound annual growth rate (CAGR) of 15.9 %, due to the awareness of the harmful effects of using chemicals and pesticides.[17] To the best of our knowledge, hardly any studies are available assessing the effects of organic and conventional vegetable intake (lettuce and carrot) on the bone and serum mineral content of adult rats. Therefore, this study aims to analyze the effect of organic and conventional vegetables (lettuce and carrot) intake on some bone mineral content and some serum heavy metals content in adult rats.

MATERIALS AND METHODS

Vegetable Analysis

Organic and conventional vegetable crops (carrot and lettuce) were purchased from randomly selected hypermarkets in Riyadh's capital city of Saudi Arabia. Carrot and lettuce were washed with distilled deionized water and prepared for freeze-drying. The samples were diced into small pieces and freeze-dried (Christ Freeze Dryer ALPHA 1–2, Milan, Italy) then ground to a fine powder. The temperature of the cold trap and the vacuum pressure in the drying chamber was set according to Chan *et al*.[18] and Kapoor and Aggarwal[19] with some modifications. It has been set at −30 °C and 0.37 mbar for 14h for carrot and −50 °C and 0.12 mbar for 10h for lettuce. The freeze-dried vegetable samples were than digested in microwave acid digestion (CEM MARS 6, CEM Corp., USA). In brief, in a vessel tube, approximately 0.5 gram of the freeze-dried sample was added to 10 mL nitric acid (HNO3) and digested for 30-45 min at 200°C, 800psi. The concentrations of calcium (Ca), phosphorus (P), zinc (Zn), magnesium (Mg), cadmium (Cd), and lead (Pb) was analyzed by the technique of inductively coupled plasma optical emission spectrometry ICP-OES (GBS- Integra; Australia). The mineral concentration was expressed as mg/L.

Experimental Animals

Thirty healthy adult Wistar albino male rats weighed between 180 – 200 g were supplied from the Experimental Animal Care Center, College of Education, King Saud University, Riyadh, Saudi Arabia. Rats were housed in separate cages under standardized controlled conditions (temperature 24 ± 2 ˚C, relative humidity of 40- 70%, 12-h light/dark cycle) with free access to laboratory rat chow diet and tap water.

Experimental Ethics

All experimental procedures were approved by the Research Ethics Committee, King Saud University, Ref. No: KSU-SE-21-12.

Experimental Design

The rats were fed on a basal diet (Crude protein:20.00%; Crude fat:4.00%; Crude fiber:3.50%; Ash:6.00%; Salt:0.50%; Calcium:1.00%; Phosphorus:0.60%; Vitamin A:20.00 IU/g; Vitamin D:2.20 IU/g; Vitamin E:70.00 IU/g) purchased from General Organization for Grain Silos and Flour Mills for one week adaptation period, then continued experimental diets for 2 months. The rats were divided into five groups (each of 6 rats) as follows:

1. Control group (C), fed on a basal diet.
2. Organic carrot (CO) group, fed on basal diet + organic carrot 5g/100g diet.
3. Conventional carrot (CC) group, fed on basal diet +conventional carrot 5g/100g diet.
4. Organic lettuce (LO) group, fed on basal diet + organic lettuce 5g/100g diet.
5. Conventional lettuce (LC) group, fed on basal diet + conventional lettuce 5g/100g diet.

Samples Collection

Rats were anesthetized by carbon dioxide CO2, and thereafter blood samples were collected in clot activator & gel tubes (serum tubes) by cardiac puncture, then centrifuged (Eppendorf centrifuge 5430R) at 3000 rpm for 15min, then stored at – 80 °C until used for analysis.[20] After blood sample collection, the anesthetic dose was increased to sacrifice rats, and then bone samples (femur and tibia) were collected.[21] The soft tissues were removed, then the bones were washed with normal saline and dried with a lint-free paper towel. The bone samples were stored at –80 °C until used for minerals analysis. Bone and serum were selected because bones contain essential bone minerals, and serum was chosen to evaluate heavy metals since the human body absorbs around 70% of the heavy metals from vegetables.[22]

Serum and Bone Analysis

The serum samples were digested by Microwave Acid Digestion (CEM MARS 6 manufactured by CEM Corp., USA). In a digestion vessel, 0.5g of sample was added to 2ml of HNO3 and digested for 30-45 min at 200°C 800psi.The bones were thawed at room temperature, and then both bone epiphyses were removed using a fine saw, thereafter an incision was made along the bone using a sharp non-serrated knife. Bone marrow was removed, and then bone samples were rinsed with normal saline and dried using a lint-free paper towel. An amount of 0.5g from each bone sample was digested in 10 ml of concentrated HNO3 in a microwave digestion system for 30-45 min. Mineral concentrations in serum (Pb and Cd) and bone (Ca, P, Mg, and Zn) were measured using ICP-OES (GBS-Integra; Australia) and expressed as mg/L. Flow chart of the research design has been presented in Figure 1.

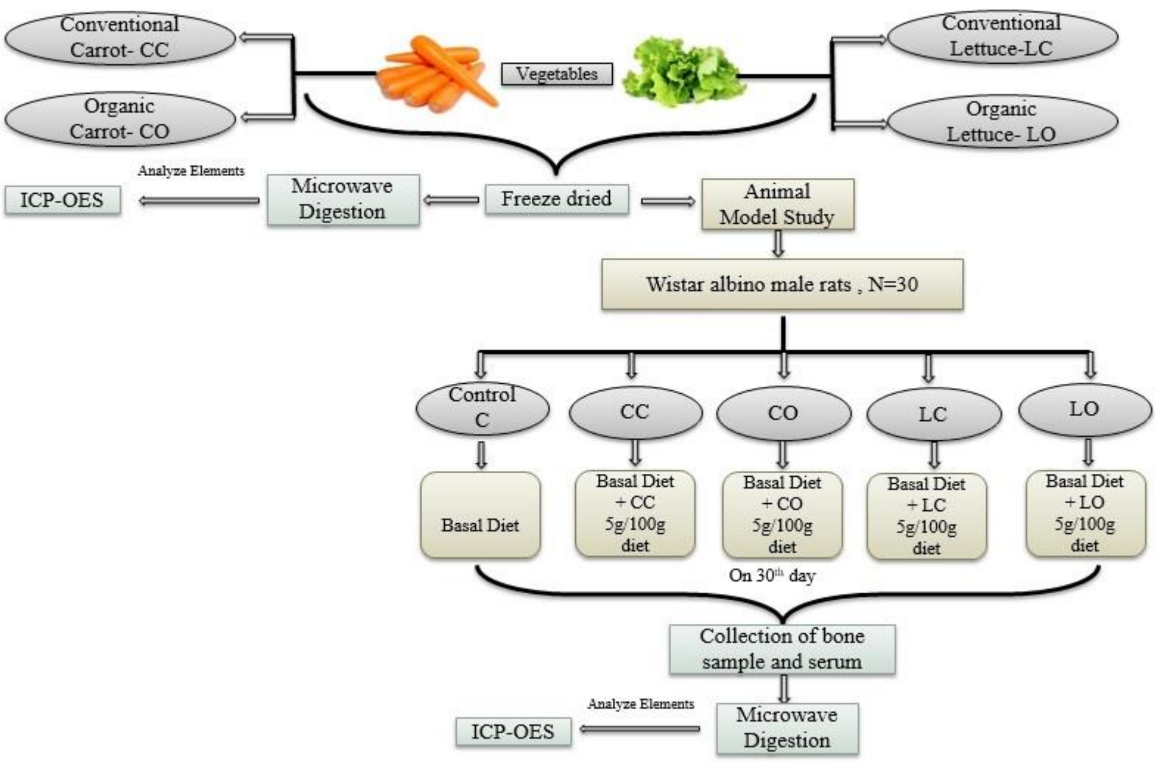

Figure 1: Flow chart of the research design.

Total Food Consumption, Weight Gain, and Feed Efficiency Calculation

Daily feed offered and feed residues were recorded to calculate daily food consumption DFC (g) then it was added to find total food consumption (TFC). To assess weight, gain in the body weight at the end of the experiment was subtracted from the weight at the start of the experiment [weight gain (g) = final body weight (g) - initial body weight (g)]. Feed efficiency was calculated as the ratio (g/g) between weight gain (WG) and total food consumption (TFC).

Statistical Analysis

Data were expressed as means ± standard deviations. The statistical significance of differences among means was estimated by one-way Analysis of variance (ANOVA) at a significance level of $P \leq 0.01$ and $P \leq 0.05$, if significant differences were found, a post-hoc analysis was performed using Tukey's test. All statistical analysis were carried out using SPSS 26.0 for Windows (SPSS, Inc.).

RESULTS

Assessment of Minerals Content in Organic and Conventional Vegetables

In the present study, the mineral content of organic and conventional carrots and lettuce is presented in Table 1. The higher Ca value was obtained in organically grown carrot (187.82mg/L) and lettuce (225.37 mg/L), but statistically significance difference ($p \leq 0.05$) between the two cultivation systems was observed only in lettuce. Statistically insignificance difference has been observed for magnesium and phosphorous between the two cultivation systems. The zinc level in the present research showed the highest value in organic carrot and lettuce (3.28 and 3.83mg/L), but like Mg and P the difference was insignificant between the two cultivation system. The current study found that the ratio of organic and conventional carrot Ca:P was 1.42 mg/L and 0.696 mg/L and the ratio of organic and conventional lettuce Ca:P was 1.90 mg/L and1.32 mg/L respectively. In all the examined vegetable samples, cadmium was not observed in any sample and the highest amount of Pb was found in conventional carrot and lettuce (0.3 and 0.75 mg/L), but the difference was insignificant between the two cultivation systems.

Table 1: Mineral Content in organic and Conventional vegetable samples (mg/L).

Mineral	CO	CC	LO	LC
Ca	187.82± 112.59[a]	128.67±7.19[a]	347.65±98.67[b]	225.37±20.71[ab]
Mg	69.53±90.60	32.68±43.83	135.67±63.01	153.1±88.91
P	135.75±43.173	184.73±8.658	186.75±20.167	176.6±32.23
Zn	3.28±1.13	1.9±1.21	3.83±2.85	3.33±1.08
Pb	0.23±0.40	0.3±0.52	0±0	0.75±0.39
Cd	0±0	0±0	0±0	0±0
Ca:P	1.42±0.83[ab]	0.69±0.07[a]	1.90±0.68[b]	1.32±0.33[ab]

The values represent the Mean ±SD (n=30; rats). *P value < 0.05;* Where: C- control group; CO- organic carrot; CC- conventional carrot; LO- organic lettuce; LC- conventional lettuce; Ca- calcium; P- phosphorous; Mg- magnesium; Zn- zinc; Pb- lead; Cd-cadmium.

Effect of Organic and Conventional Vegetable Intake on Food Consumption, Weight Gain, and Food Efficiency in Adult Rats

Total food consumption, weight gain, and food efficiency is illustrated in Figure 2. There were significant difference between C vs CO ($p \le 0.01$), C vs CC ($p \le 0.01$), C vs LC ($p \le 0.05$), and LO vs LC ($p \le 0.05$) on the rats' weight gain. The highest body weight gain was found in C and LO as compared to other experimental groups. Moreover, a significant difference was found only in C vs CO ($p \le 0.01$) in total food consumption (TFC), while insignificant differences in other groups. Significant difference were found for C vs CC ($p \le 0.05$) in the food efficiency (FE), the highest FE was found in rats fed LO, while the lowest FE was found in groups of rats fed CC as compared to other experimental groups. The highest body weight gain was found in C and LO as compared to other experimental groups. Furthermore, rats' weight was the lowest in both organic and conventional carrots.

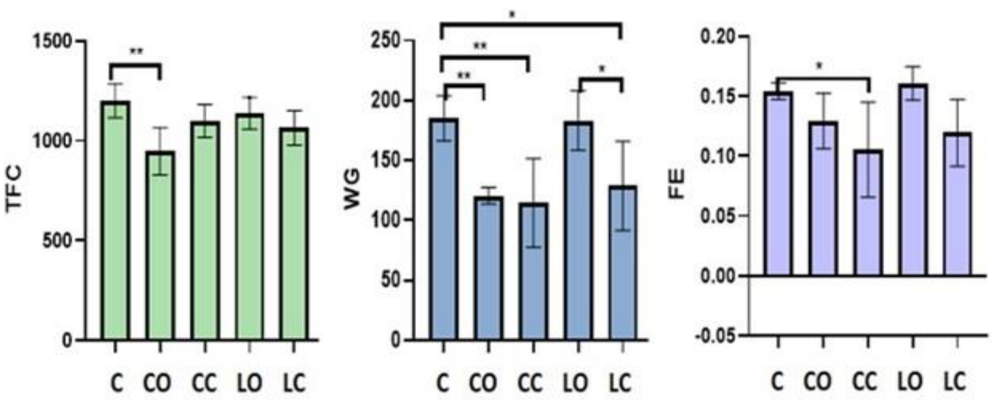

Figure 2: Effect of Organic and Conventional Vegetable Intake on Food Consumption, Weight Gain, and Food Efficiency in Adult Rats.

Where TFC: Total food Consumption; WG: Weight gain; FE: Food efficiency; C- control group; CO- organic carrot; CC- conventional carrot; LO- organic lettuce; LC- conventional lettuce.

Effect of Organic and Conventional Vegetable Intake on Bone Mineral Content and Serum Heavy Metals Content in Adult Rats(mg/L)

Table 2 depicts that Ca, Mg, P, and Zn content was higher in treatment group as compared to control. Although an insignificant difference ($p>0.05$) has been observed in Ca content between control and others, but the difference was significant ($p\leq0.05$) in Mg content between controls and others except organic carrot. A significant different has been reported in P content between LO and LC and also with control group. The analysis of tested heavy metals in serum illustrated in Table 3 revealed statistically insignificant difference between control and treated groups and even between treated groups for Pb and Cd.

Table 2: Effect of Organic and Conventional Vegetable Intake on Bone Mineral Content in Adult Rats.

Mineral	C	CO	CC	LO	LC
Ca	7285±839.30	8423±171.10	8868±271.40	8682±1200	8417±521.70
Mg	173.30±7.4[a]	183.50±1.90[a]	192.70±3.27[ab]	206.80±23.55[b]	190.20±4.82 [ab]
P	2095±198.30[a]	2353±42.32[ab]	2438±67.34[ab]	2501±322.20[b]	2353±143.70[ab]
Zn	9.60±1.94	10.47±0.86	10.89±1.35	11.88±1.56	10.76±0.62
Ca:P	3.47±0.07[a]	3.58±0.008[ab]	3.64±0.03[b]	3.47±0.12[a]	3.58±0.02[ab]

The values represent the Mean ±SD (n=30; rats). *P value < 0.05 ;* Where: C- control group; CO- organic carrot; CC- conventional carrot; LO- organic lettuce; LC- conventional lettuce; Ca- calcium; P- phosphorous; Mg- magnesium; Zn- zinc.

Table 3: Effect of Organic and Conventional Vegetable Intake on Serum Heavy Metals Content in Adult Rats.

Heavy Metals	C	CO	CC	LO	LC
Pb	1.50±0.08	1.24±0.19	1.45±0.16	1.44±0.34	1.46±0.31
Cd	0.12±0.04	0.10±0.05	0.11±0.06	0.10±0.08	0.08±0.08

The values represent the Mean ±SD (n=30; rats). Where: C- control group; CO- organic carrot; CC- conventional carrot; LO- organic lettuce; LC- conventional lettuce.

DISCUSSION

Minerals are substances that are essential for human body development and functioning and people should aim to meet their nutrient requirements through a healthy eating. The higher Ca value in organically grown vegetables is in agreement with the findings of Masamba and Nguyen[23] who reported that carrot and lettuce had significant differences between different cultivation systems. But in this study statistically significance difference ($p \leq 0.05$) between the two cultivation systems was observed only in lettuce. Moreover, Kelly and Bateman[24] found significantly greater concentrations of Ca in organic tomatoes. The results from the study by Masamba and Nguyen[23] showed significant differences in calcium and potassium content in organically and conventionally grown cabbage, carrots, lettuce, and Valencia oranges. Worthington[25] in his analysis had reported higher Mg and P content in organic carrots and lettuce as compared to conventional, which is quite in line with the result obtained in the current study. Even though the content of Mg was little high in conventional lettuce and P content was higher in conventional carrot, but statistically insignificant difference was found between the different cultivation systems. The sources of these minerals in plant derived food are from natural or anthropogenic origin. Minerals from both origins take the way to plants through water and soil. Worthington[25] has reported that phosphorous in conventional plants are due to the reason that the absorption of phosphorous depends on the availability of magnesium (Mg). Zinc is considered as an essential plant micronutrients, and significant component of numerous enzymes and proteins. Glodowska and Krawczyk[26] reported that there was a significant difference in mineral content between the organic and conventional carrot. Similarly, Elmi *et al*.[27] also reported higher Zn content in organic lettuce 5.21mg/L as compared to the conventional one (1.23 mg/L). Likewise, Kapoulas *et al*.[28] also found statistically more amount of Zn in organic lettuce as compared to conventional lettuce. But in contrast, de Souza *et al*.[29] reported that as compared to organic lettuce (0.69 ± 0.08 mg/L), the highest amount of Zn was found in conventional lettuce (1.125 ± 0.064 mg/L).

Previously, it has been reported that Ca to P ratio (Ca:P) is essential for infant's bone development.[30] It is believed that bone mass accumulation during infancy is essential for the prevention of poor childhood growth and adult osteoporosis.[31] Sax[30] in his study on animal studies have previously reported that low Ca:P diets cause low bone densities. Common practice is to have a Ca:P molar ratio between 1:1 and 2:1.[32] The intake of calcium: phosphorus ratio (Ca:P) by consuming more P than Ca has adverse effects on health, the most important effect is loss of bone minerals and a chronically elevated serum parathyroid hormone (PTH).[33] In the current research, vegetables meet the Ca:P ratio to a great extent which has also been reported earlier.[34] Adequate intake (AI) of calcium and phosphorus ratio is 1-2:1 (Ca:P) in infants, while in adults, Ca:P ratio ranges from 0.08:1 to 2.40:1.[30,35] In addition the ratio of Ca:P for rats is 2:1.[36]

The safe limits for lead given by FAO, WHO in 2001 was 0.3 µg/g.[37] In comparison with the results of our study, Hadayat *et al*.[38] reported that the amount of Pb in organic carrots (0.00720 ± 2.79 mg/L) and lettuce (0.0120 ± 3.10 mg/L) was less than the amount in conventional carrot (0.0224 ± 4.79 mg/L) and lettuce (0.0145 ± 0.61 mg/L). Correspondingly, the amount of Cd in organic carrots (0.00520 ± 1.59 mg/L) and lettuce (0.00487 ± 0.49 mg/L) was also less than the amount in conventional carrot (0.0138 ± 1.59) and lettuce (0.0253 ± 5.24 mg/L). A recent study in Poland showed that organic carrots contain the lowest amount of Pb and Cd.[39] Nevertheless, de Souza *et al*.[29] did not reported any significant difference in the Cd and Pb content between the organic and conventional vegetables. A significant risk to the health of potential consumers is food contaminated with heavy metals, such as cadmium (Cd) and lead (Pb), exceeding the maximum permissible limits for food products. Since Pb is not an essential element, so plants do not have channels for Pb uptake. It is believed that this element attaches to carboxylic groups of mucilage uronic acids on root surfaces, however the mechanism is still unknown how it is taken up by root tissues.[40] As stated by Millis *et al*. human consumption of vegetables is the main source of Cd, and soil heterogeneity causes variations in element concentration in plants.[41] The toxicity of Pb arises from inhibition of some metabolic pathways in some species, although some species tolerate Pb through complexation and inactivation.[42] Lead is mostly available for plant uptake in soils with low organic matter, low pH, and low phosphate.[43] Some studies have highlighted an inverse relationship between the intake of fruits and vegetables and chronic inflammatory conditions,[44-45] which are linked to an increased risk of osteoporosis and fractures.[46] One well-known hypothesis suggests that consuming fruits and vegetables may slightly shift the body's acid-base balance toward a more alkaline state. This mild alkalization could enhance calcium reabsorption in the renal tubules, potentially leading to a reduction in bone loss.[47]

As shown in Figure 2, the TFC was more in CC group as compare to CO group and it was more in LO group when compare with LC group, its effect has been observed in the mineral content of bone in adult rats in Ca, Mg, P, and Zn was higher in CC group as compared to CO and even in LO group and compared to LC, although the different was insignificant between them. A significant difference has been reported between C vs CO, C vs CC, C vs LC, CO vs CC and LO vs LC for Ca:P. Some cross-sectional studies revealed a positive association between vegetables or fruit (or both) consumption and bone mass markers.[48-49] Consumption of fruits and vegetables has been linked to enhanced reduction-oxidation (REDOX) capacity, which may boost bone remodeling and bone repair, and decrease bone loss.[50-51] A systematic review on the intake of fruits and vegetables and bone health in women over 45 years did not found a clear association between this group of foods and bone metabolism or fractures.[52] By contrast, a meta-analysis revealed that increased consumption vegetables but not fruits had positive effects on bone density and might decrease fracture risk.[53]

In the human body, heavy metals can be the cause of many chronic diseases whose symptoms are different depending on the level of toxicity of an element, as well as the duration and level of exposure.[54] Kidneys and liver are the main organs especially sensitive to Cd toxicity.[55] Cd most often causes damage to both of these organs, as well as the testicles, lungs and bones. In addition, it causes a carcinogenic effect, initiating cancers of the prostate, kidneys, pancreas and testicles.[56-57] This element negatively affects the function of the skeletal system by disturbing the metabolism of calcium, magnesium, zinc, copper and iron ions.[54,56] Safe production of vegetables is without a doubt one of the most pressing issues in heavy metal contamination of edible vegetables, and as a result, determining cadmium uptake and transit in soil by vegetables is a major challenge.[58-59]

CONCLUSION

Our data suggested that organic vegetable has a higher mineral and lower level of heavy metals content than conventional crop. Statistically insignificant difference in the mineral content between two cultivation systems except for calcium of lettuce has been observed. The effect of organic and conventional vegetable intake on bone minerals content shows significant difference in magnesium and phosphorous content between organic and conventional vegetables and as far as heavy metals content in the serum of adult rats is concerned, the data showed insignificant differences between the organic and conventional vegetables. Our analysis does not present a definitive statement on the long-term health benefits of organic dietary intake. Consumption of organic vegetables is often tied to overall, which is likely to be influential in the results of observational research. Correspondingly, we suggest that additional research on the health effects of long-term intake of organic through diet is needed.

CONFLICTS OF INTEREST: The authors declare no conflicts of interest.

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