Research Article

Comparative Study on Absorption of Heavy Metal Residues in Broiler Chicken Available in the University Campus Peshawar, Pakistan

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Abstract | The study was conducted to analyze three different tissues (liver, gizzard and muscle tissue) of the broiler chicken for absorption of four heavy metal contents (Cu, Zn, Pb and Ni). In addition, analysis of five electrolytes (Ca, Na, K, P and Mg), moisture content, protein, lipids and ash were carried out. The samples were obtained from five different sites of the University Campus, Peshawar- Pakistan. The Atomic Absorption Spectrometry was employed for determination of Cu, Zn, Pb, Ni, Ca and Mg. Furthermore, a spectrophotometer was also used for the purpose of phosphorus (P). However, the analysis of sodium (Na) and potassium (K) content was done through a flame photometer. It was observed that gizzard had the highest concentration of Zn (24.84mg/kg), Pb (2.25mg/kg) and Ni (2.34mg/kg) while, had lowest concentration of Cu (3.30mg/kg) Ca (8.05mg/kg) Na (74.29mg/kg), P (130.67mg/kg) and Mg (15.85mg/kg). Liver had highest concentration of Cu (7.74mg/kg) and Mg (24.23mg/kg) while lowest concentration of Pb (1.24mg/kg) Ni (0.64mg/kg), K (205.20mg/kg) and Na (74.32mg/kg). The muscle tissue come up with highest concentration of Ca, K, Na, P and Mg were 18.65mg/kg, 258.13mg/kg, 110.59mg/kg, 455.53mg/kg and 27.06mg/kg respectively, whereas lowest concentration of Zn (13.13mg/kg). It is concluded that muscle tissue of the broiler chicken is comparatively a good source of macro-nutrients (Protein, Lipid) and micro-nutrients (Ca, K, Na, P and Mg) and less contaminated with heavy metals.

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Introduction

Poultry meat is one of the most important animal sources of protein in food mesh, which is considered to be as healthier meat choice (Akan, *et al*., 2010). Meat or poultry is extremely vulnerable to exposure of heavy metal intoxication, particularly the cadmium as it occurs by feeding on the plant origin food. Its accumulation in body leads to renal failure, osteoporosis and mutagenic effects beyond the threshold limits established by the WHO. Copper is an essential metal element for the looking after of body homeostasis as well act as cofactor in many enzymatic activities inside the body (Strausak *et al*., 2011).

Poultry meat is a substantial source of macronutrients and micronutrients. The nutritional and chemical composition of meat depends on multiple factors such as age, feeding of animals, the living condition as well as physiological condition of the animals (Babiker *et al*., 1996).

Those foods which are free from all types of health hazards are considered Healthy foods and are also good in taste. It is estimated that chicken meat is the main source for the heavy metals towards human body (Linda *et al*., 2011). Poultry industry of Pakistan is deficient in producing protein substances from animal sources. In order to increase the protein content of meat, various metals are added to poultry feed which makes the poultry meat hazardous for intake.

All living organisms have heavy metal in their body where they play various functions. They may be structural components of control mechanism, (e.g., in nerves and muscles) and, or redox systems, enzyme activator systems, enzyme activator. There are two primary categories of heavy metals, essential and non-essential metals. The essential metals are (Cr, cu, Fe, Ni, Zn) and nonessential are (As, Cd, Hg, Pb.) Pollution of meat by heavy metals is a trouble and great alarm for food safety and human health. Even in a very small amount present in foods may cause hostile effects. Different investigators had stated the instances of heavy metal pollution while treating the meat products. The feeding of cattle's and poultry by polluted feed was found to be accountable for heavy metal contamination (Bilal, 2010). Poultry is very susceptible to the intoxication with heavy metals and its accumulation in the body leads to mutagenic implications if they are beyond the permitted limits established by World Health Organization. Many metals are intentionally added in poultry feed to increase the level of protein (Demirezen and Uruc, 2006). The diet of broiler chicken is primarily composed of nutrients like Zinc, Arsenic, Manganese, Copper and phosphorous which are needed to promote growth of birds and also facilitate the production of healthy birds. Lead, cadmium and arsenic are very toxic metal which have toxic consequence while compile in the form of food chain. Heavy metals are stored in the tissues of living organisms and have direct physiological toxic results. Lead is a poison that gets bonds with essential enzymes and many other active situations of cellular ingredients and deactivates them and creates serious troubles in metabolic pathway and neurotoxin. Lead has also toxic consequences on hemolytic, nervous, renal and gastrointestinal system of the body.

Materials and Methods

There are numerous selling spots for meat in the university campus. For the current research, samples of chicken were collected from four most popular and main selling locations at university campus Peshawar and later on were evaluated for heavy metals (Cu, Zn, Pb and Ni), electrolytes (Na, K, Ca, Mg and P) and moisture, lipids, protein and ash contents. Three samples of each type were collected from each location. All collected samples were stored in clean polythene bags and labeled according to their type and transported to the laboratory for analysis. The samples were stored at refrigerator temperature till the final analysis was performed.

Mineral analysis

For mineral analysis an acid digest of each sample was prepared according to (AOAC, 2000). The technique involved was wet digestion for which 1g of dry sample was taken in a digestion flask. The digest was used for analysis of heavy metals (i.e., Cu, Zn, Pb, and Ni) and electrolytes i.e. Na, K, Ca, Mg and P.

Heavy metals (Cu, Zn, Pb and Ni) were determined with Atomic Absorption Spectrometry. Atomic Absorption Spectrometry (AAS) is one of the most commonly used instrumental techniques of analysis for the quantitation of metals and metalloid in water and food samples. The Atomic Absorption Spectrophotometer Model (Perkins Elmer, 200) was used for the analysis of water samples.

The digest was introduced into the Atomic Absorption Spectrophotometer by mean of capillary tube. The device was adjusted for the analysis specific elements by setting light source of desired wave length. The instrument was calibrated with the standards of respective elements before the sample digest was introduced. The absorbance appeared on the screen was noted. The concentration was calculated as follow:

$$\text{Amount of Heavy metal } \left(\frac{\text{mg}}{\text{kg}}\right) = \frac{\text{Absorbance reading} \times \text{ dilution factor}}{\text{Weight of sample}} \times 100$$

Proximate analysis

The powdered samples were analyzed for moisture, protein, lipid and ash content according to standard

methods of the Association of Official Analytical Chemist (AOAC, 2000).

Determination of moisture content

Moisture content was determined by taking 5.0g of sample in a clean, dried and pre weighted petri dish. The petri dish was placed in oven at 100-105c till constant weight was obtained. After cooling in desiccator and reweighting the moisture percentage was calculated as follow:

$$\% \text{ Moisture} = \frac{W1 - W2}{W1} \times 100$$

Where; W1= Initial weight of sample; W2= Final weight of sample.

Determination of protein

Protein was determined by Kjeldahl method using Kjeldtherm digestion unit and Gerhardt vadopest 3 automatic steam heated distillation apparatus. The samples were digested in Kjheldtherm digestion flask by heating with concentrated sulphuric acid in the presence of digestion mixture (K_2SO_4+$CuSO_4$). The percent protein was calculated by multiplying the nitrogen content with the factor 6.25 (Demirezen and Uruc, 2006).

$$\%Protein = \%\,Nitrogen \times 6.25$$

Determination of lipids

Soxhlet intermittent method was used. Accurately weighed 1g of dried sample was taken in thimble. It was taken extracted repeatedly with anhydrous petroleum ether. The ether was distilled by rotary evaporator and the lipids was dried in an oven at 70°C. Percentage of lipids in the samples was calculated as:

$$\%\text{lipids} = \frac{\text{Weight of lipid}}{\text{Weight of sample}} \times 100$$

Determination of ash

Dried samples 1g were taken in the pre –weighed crucible for the determination of ash. They were charred over a slow burning flame with the help of blowpipe and then kept muffle furnace at 550-600°C for two hours or till the appearance of a grey-white ash. The crucibles were transferred to a desiccator to cool. They were then weighted. The percentage of ash was calculated as follow.

$$\% \text{ Ash} = \frac{\text{Weight of Ash}}{\text{Weight of sample}} \times 100$$

Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) was applied through Completely Randomized Design (CRD). Differences between the means were tested by LSD tests. The level of significance was chosen at $p \leq 0.05$ and the results were calculated with the help of mean and standard deviation in duplicate. Bars and Charts were prepared through latest Excel software.

Results and Discussion

Heavy metals determination

The data regarding heavy metals revealed higher concentrations of heavy metal deposition in chicken meat sold at various shops of university campus as the most of meat is sold in open areas and vehicular emissions are deposited in the chicken meat during slaughtering process.

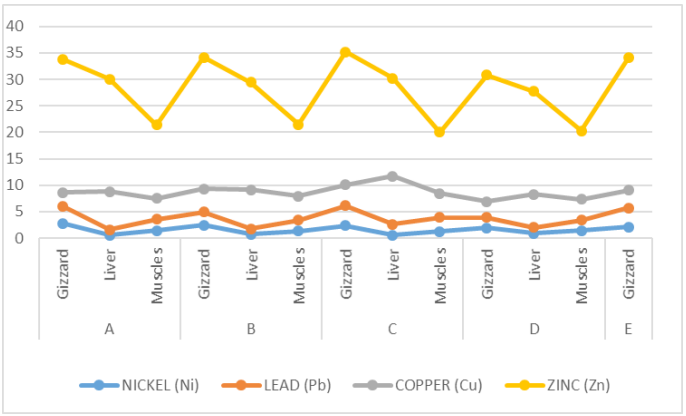

Figure 1: *Mean concentrations of heavy metals in different organs of broiler chicken sold in various locations of university campus (Mg/Kg).*

The mean value of lead (Pb) concentration in different tissues liver, gizzard and muscle tissues of broiler chicken samples collected from five areas A (forest bazar), B (Lalazar), C (Coffee shop), D (Madina Market), E (Masjid-e-wasta) of University Campus, Peshawar are represented in Table 1 and graph no 1, showed the highest mean value of Pb were found in gizzard (3.00mg/kg) while the mean value were found in liver (1.24mg/kg). however, the highest concentration of lead (Pb 3.76mg/kg) were found in gizzard samples collected from area C (coffee shop) and lowest found in liver (1.03) at area A (forest bazar). The table also showed that the mean values of lead (Pb) of different areas i-e A, B, C, D and E are 2.11, 1.87, 2.83, 1.65 and 2.37, respectively. Lead (Pb) is stored mainly in kidney and liver and its toxicity directly affect the performance of different enzymes

working in human body. The sources of lead pollution may be due to the use of lead containing vessels or lead-based pottery glazes.

Table 1: *Mean concentrations of heavy metals in different organs of broiler chicken at various locations of university campus. (Mg/Kg).*

Locations	Organs	Nickel (NI)	Lead (Pb)	Copper (Cu)	Zinc (Zn)
A=(Forest bazar)	Gizzard	2.83	3.22	2.54	25.20
	Liver	0.56	1.03	7.25	21.25
	Muscles	1.44	2.10	3.96	13.87
B=(Lalazar)	Gizzard	2.46	2.48	4.36	24.90
	Liver	0.70	1.06	7.34	20.37
	Muscles	1.34	2.06	4.55	13.54
C=(Coffee shop)	Gizzard	2.34	3.76	3.97	25.12
	Liver	0.57	2.04	9.14	18.51
	Muscles	1.25	2.68	4.53	11.58
D=(Madina market)	Gizzard	1.93	1.99	2.96	23.95
	Liver	0.93	1.06	6.26	19.48
	Muscles	1.42	1.96	4.00	12.88
E=(Masjid-e-wasta)	Gizzard	2.14	3.60	3.30	25.02
	Liver	0.46	1.04	8.73	18.35
	Muscles	1.07	2.46	5.32	13.67

Mean followed by similar letters are not significantly different at p ≤ 0.05. LSD at 5% level of probability for tissues =0.0292; LSD at 5% level of probability for locations =0.0377; LSD at 5% level of probability for tissues × locations = 0.0652

Maximum and minimum mean values of Nickel (Ni) concentration in the various tissues of broiler chicken collected from five different locations A (Forest bazar), B (Lalazar), C (Coffee shop), D (Madina Market) and E (Masjid-e-wasta) of university campus Peshawar are represented in Table 2. Permitted dose limit for Nickel in meat according to World Health Organization is 0.5mgkg-1. The concentrations of nickel in research conducted ranged from 0.64mg/kg to 2.83mg/kg for meat of liver, muscle and gizzard, respectively. The data showed highest mean value (2.34mg/kg) of Nickel (Ni) in gizzard and lowest mean value (0.64mg/kg) in liver. However, the data also showed that the mean values of Nickel (Ni) (2.83mg/kg) was found at area A (Forest bazar) in gizzard and lower (0.46mg/kg) at area E (Masjid-e-wasta) in liver. (Chowdhury *et al*, 2012). The concentration of copper (Cu) in these tissues are significantly different p≤0.05 in different tissues as well as in various locations. The data revealed that overall copper concentration was higher in (7.74mg/kg) in liver and lowered (3.30mg/kg) in gizzard. However, the highest concentration of copper in liver was found in the sample collected from area C (coffee shop) of university campus Peshawar. That high concentration of copper in liver at area C (coffee shop) might be due to the contaminated slaughtering tools or may be due to the environmental condition of that area.

Proximate analysis

Data regarding proximate contents of broiler chickens collected from the five different locations A (Forest bazar), B (Lalazar), C (Coffee shop), D (Madina market) and E (Masjid-e-wasta) of university campus Peshawar were presented in Table 2. The data indicated that highest moisture content (81.46%) was found in chicken at area C (coffee shop) whereas the lowest (72.40%) at area B (Lalazar). However, the maximum protein content (25.46%) was found at area C (coffee shop) while the minimum (20.35%) was at area B (Lalazar). The increased lipid level (3.2233%) was found at area C (coffee shop) and D (Madina market), respectively where as the decreased level was found at area A (Forest bazar) (2.5%) B (2.3%) and E (2.7%). The data showed higher degree of ash (3.4% and 3.2%) at area C and D, respectively while lower degree (1.15%) at area A. These values were significantly p<0.05 different from each other while compared the samples of all areas (A, B, C, D and E). These changes in the proximate content due to the breed, age, feed supplementation. Especially broiler meat gives large amount of protein trace element findings are in accordance with study of (Demirezen and Uruc, 2006). Meat and meat products are important for human diet because they provide a great part of nutrients (Abdu-el-Salam, 2013). The study conducted may be due to the various farm out of city from where they brought the similar work was done by (Mariam *et al*., 2004). Calcium is essential constitution of body and is important intracellular action. It plays as a second messenger in various signal transduction cascades (Mariam *et al*., 2004). Inadequate intake of calcium lowered the utilization of mineral during fast growth and genetic factors the findings of this research are in line with (Hamasalim, 2013).

Mineral determination

The mean values of calcium, sodium, magnesium, potassium and phosphorus concentration in liver, gizzard and muscle tissue of broiler chicken collected from five different locations of University Campus Peshawar are given in Table 3 and Graph 2. The concentrations of calcium varied significantly in the liver, gizzard and muscle tissue of the broiler chickens collected from the five different areas A (Forest

bazar), B (Lalazar), C (Coffee shop), D (Madina market) and E (Masjid-e-wasta). The data presented the highest mean value of calcium (Ca) (18.65mg/kg) in muscle tissue while the lowest mean value of liver, gizzard and muscle tissue at area A (12.07mg/kg), B (11.89mg/kg), C (14.12mg/kg), D (11.62mg/kg) and E (11.49mg/kg). However, the maximum concentration of calcium (Ca) (20.23mg/kg) was found in muscle tissue at area C while the minimum was found in gizzard (7.52mg/kg) at area D. The overall mean values of calcium (Ca) in different tissues of broiler were significantly ($p \leq 0.05$) different.

Table 2: *Moisture, protein, lipids and ash contents (%) of muscles tissues of broiler's chicken collected from various locations at university campus, Peshawar.*

Locations	Moisture %	Protein (gms)	Lipids (gms)	Ash %
A= (Forest bazar)	73.430	21.933	2.5967	1.1533
B= (Lalazar)	72.407	20.353	2.3733	1.7633
C= (Coffee shop)	81.463	25.463	3.2233	3.4267
D= (Madina market)	76.277	23.507	3.3033	3.2867
E= (Masjid-e-wasta)	74.990	22.657	2.7000	1.5567

Means in the same columns followed by same letters are not significantly different at $p \leq 0.05$.

Table 3: *Mean concentrations of minerals in different organs of broiler chicken at various locations of university campus (Mg/Kg).*

Locations	Organs	Potassium (K)	Calcium (Ca)	Sodium (Na)	Phosphorus (P)	Magnesium (Mg)
A= (Forest bazar)	Gizzard	235.00	8.09	76.37	133.33	16.57
	Liver	236.33	11.37	76.57	174.33	24.44
	Muscles	262.33	18.54	118.17	465.00	42.00
B=(Lalazar)	Gizzard	219.33	7.57	74.57	126.33	15.27
	Liver	219.33	8.55	67.50	165.22	22.43
	Muscles	256.34	19.57	83.27	433.36	25.57
C=(Coffee shop)	Gizzard	253.00	9.41	73.11	137.00	16.73
	Liver	226.67	12.73	78.13	179.67	26.47
	Muscles	275.67	20.56	118.97	442.36	19.98
D=(Madina market)	Gizzard	195.67	8.64	78.61	132.67	14.53
	Liver	154.00	7.06	78.90	185.35	25.27
	Muscles	246.00	20.06	117.35	466.00	21.43
E=(Masjid-e-wasta)	Gizzard	225.67	Z.60	68.80	124.00	16.13
	Liver	212.67	10.04	70.50	162.33	22.10
	Muscles	256.36	18.46	115.17	451.00	27.67

Mean followed by similar letters are not significantly different at $p \leq 0.05$. LSD at 5% level of probability for tissues =11.103; LSD at 5% level of probability for locations =14.333; LSD at 5% level of probability for tissues × locations = 24.826

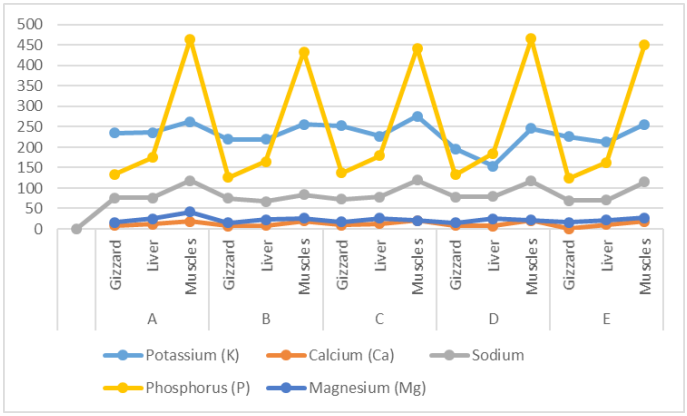

Figure 2: *Mean concentrations of major minerals in different organs of broiler chicken at various locations of university campus (mg/Kg).*

Data regarding to potassium (K) of the different tissues of liver, gizzard and muscle tissue of the broiler chicken collected from the five different areas A (Forest bazar), B (Lalazar), C (Coffee shop), D (Madina market) and E (Masjid-e-wasta) at university campus Peshawar are represented in Table 3. The data showed the mean values of potassium (K) concentration in liver, gizzard and muscle tissue were 205.20 mg/kg, 225.73mg/kg and 258.13mg/kg respectively. The maximum concentration (275.67) was found in muscle tissue at area C while the minimum (154mg/kg) in liver at area D. Table 3 also showed the mean values of liver gizzard and muscle tissue at location A (244.56) B(223mg/kg) C(251.78mg/kg) D(198.58mg/kg) and E(230.56mg/kg). The mean values of potassium (K) concentration in different tissues of broiler chickens at university campus are significantly $p \leq 0.05$ different.

The data concerning the concentration of sodium in the muscle tissue revealed that it comprised of highest mean value of sodium (Na) concentration. While the mean values of liver, gizzard and muscle tissue at area A, B, C, D and E were (90.37mg/kg), (75.11mg/kg), (90.07mg/kg), (91.62mg/kg) and (84.82mg/kg), respectively. All the mean values of different tissues were significantly ($p \leq 0.05$) different. However, the maximum concentration of sodium (Na) (118.97mg/kg) was shown in muscle tissue of the broiler chicken collected from area C while the minimum (67.50mg/kg) was shown in liver of broiler chicken collected from area B.

The concentration of phosphorous (P) in the liver, gizzard and muscle tissue of broiler chickens collected from the five different areas A (Forest bazar), B (Lalazar), C (Coffee shop), D (Madina market) and

E (Masjid-e-wasta) of university campus Peshawar were shown in Table 3. The data in Table 3 had shown the mean values of P (phosphorous) concentration in liver (173.27mg/kg), gizzard (130.67mg/kg) and muscle tissue (455.53mg/kg). Whereas, the mean values of phosphorus concentration in liver gizzard and muscle tissue at each area were A (257.56mg/kg), B (248.33mg/kg), C (252.89mg/kg), D (261.22mg/kg) and E (245.78mg/kg). The data also shown the highest concentration of phosphorous (P) (466mg/kg) in muscle tissue collected from area D whereas the lowest (124mg/kg) in gizzard collected from area E. The overall mean values of phosphorous concentration were significantly ($p \leq 0.05$) different.

The data regarding magnesium content in the different tissues of broiler chickens collected from the five different areas A (Forest bazar) B (Lalazar) C (coffee shop) D (Madina market) and E (Masjid-e-wasta). The data showed the mean concentration in the liver (24.23mg/kg), gizzard (15.85mg/kg) and muscle tissues (25.08mg/kg). While the mean values of liver, gizzard and muscle tissues at area A (27.82mg/kg), B (20.64mg/kg), C (21.06mg/kg), D (20.41mg/kg) and E (21.97mg/kg). However, the maximum Mg concentration (42mg/kg) was found in muscle tissue collected from area A and the minimum concentration (14.53mg/kg) was found in gizzard at area D.

Data regarding proximate contents of broiler chickens collected from the five different locations A (Forest bazar), B (Lalazar), C (Coffee shop), D (Madina market) and E (Masjid-e-wasta) of university campus. The data indicated that highest moisture content (81.46%) was found in chicken at area C (coffee shop) whereas the lowest (72.40%) at area B (lalazar). However, the maximum protein content (25.46%) was found at area C (coffee shop) while the minimum (20.35%) was at area B (Lalazar). The increased lipid level (3.2233%) was found at area C (coffee shop) and D (Madina market), respectively whereas the decreased level was found at area A (Forest bazar) (2.5%) B (2.3%) and E (2.7%). The data showed higher degree of ash (3.4% and 3.2%) at area C and D respectively while lower degree (1.15%) at area A. These values were significantly $p<0.05$ different from each other while compared the samples of all areas (A, B, C, D and E). These changes in the proximate content due to the breed, age, feed supplementation. Especially broiler meat gives large amount of protein trace element.

Conclusions and Recommendations

It was concluded from the results of research that the chicken meat had significantly higher concentration of heavy metals permissible by the CODEX alimantarous. The study was focused to compare three different tissues (liver, gizzard and muscle tissue) of the broiler chicken for the levels of four heavy metals (Cu, Zn, Pb and Ni), five electrolytes (Ca, Na, K, P and Mg), moisture content, protein, lipids and ash. Analysis of the liver, gizzard and muscle tissue of the broiler chicken was carried out as it is an integral part of human diet, it can be concluded that muscle tissue of the broiler chicken is a good source of macro nutrients (Protein, Lipid) and micronutrients (Ca, K, Na, P and Mg).

After the analysis it was concluded that gizzard had the highest concentration of Zn, Pb and Ni while had lowest concentration of Cu and Mg However liver had highest concentration of Cu and Mg while, lowest concentration of Pb Ni, K and Na The muscle tissue showed highest concentration of Ca, K, Na, P and Mg Analyzing the liver, gizzard and muscle tissue of the broiler chicken which is frequently used in human diet. Conclusions of this research reveals that muscle tissue of the broiler chicken is comparatively a good source of macro nutrients (Protein, Lipid) and micronutrients (Ca, K, Na, P and Mg) and less contaminated with heavy metals.

Novelty Statement

Significance of this study is to screen out absorption of heavy metals in different organs of broiler chicken and associated health risk.

Author's Contribution

Zahin Anjum: Supervisor and wrote draft of the manuscript.
Mubashra Tarana: Data collection, Lab work and analysis
Shaista Ali: Helped in manuscript writing
Faryal Yousaf: Helped in statistical analysis
Amina Rahat: Assisted in sampling techniques
Sumbla Yousaf: Helped in proofreading and format setting

Conflict of interest
The authors have declared no conflict of interest.

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