Journal of Trace Elements in Medicine and Biology 51 (2019) 91–97

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

Nutrition

Effects of green tea polyphenols on trace metals level of rats on food restriction and high-fat diet

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ARTICLE INFO

Keywords:
Green tea polyphenols
Trace elements
Food restriction
High-fat diet
Zn
Se

ABSTRACT

Little evidence showed the interplay between tea and diet in the regulation of trace metal. Here, we examined the effects of green tea polyphenols (GTPs) on the level of trace elements (TEs) in rats on food restriction or high-fat diet. Thirty-six rats (Wistar, male) were randomly divided into 6 groups and fed on standard diet, food restriction and high-fat diet with or without GTPs (200 mg/kg bw/day) supplementation, respectively. Levels of vanadium (V), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), selenium (Se), molybdenum (Mo) and cobalt (Co) in feed, whole blood, femur and urine were measured by inductively coupled plasma mass spectrometry (ICP-MS). Blood glucose, total cholesterol (TC), triglycerides (TG), high and low density lipoprotein-cholesterol (LDL-C, HDL-C) in serum were determined. Decreased daily intakes of TEs were observed in rats on food restriction and high-fat diet. Decreased whole blood level of Zn, femur level of Co and increase urinary excretion of Se were observed in rats fed on high-fat diet. GTPs altered the whole blood level of several TEs in rats on food restriction (V, Zn, Co) or high-fat diet (V, Se), respectively, but not in rats fed on standard diet. The level of several TEs in femur and the daily urinary excretion of V and Mo were altered by GTPs in rats on all of the three diets. In addition, rats fed on high-fat diet developed dyslipidemia, which was ameliorated by GTPs. The data indicated that diet status played a role in the effects of GTPs on TEs and lipid metabolism, and trace elements may play a role in the modulation of lipid metabolic disturbances by high-fat diet and GTPs.

1. Introduction

Consumption of tea to benefit from its medicinal properties has been practiced by mankind since ancient times. Nowadays, tea has become the most widely consumed beverage. Green tea Polyphenols (GTPs), the major functional components in green tea, were demonstrated anti-oxidative, anti-inflammatory and anticancer activities [1–3]. Chemically, GTPs are a class of natural compounds with phenolic structural features, which makes them potential chelators for metal ions. With the development of electrochemical and optical technology [4], GTPs had been shown to interact with Iron ions [5], Copper ions [6] and Zinc ions and form polyphenol-metal-complexes in vitro [4,7]. Evidences in cell lines also revealed that (-)-epigallocatechin-3-gallate (EGCG), the predominant catechin component of GTPs, redistributed

Abbreviations: GTPs, green tea polyphenols; TEs, trace elements; V, vanadium; Mn, manganese; Fe, iron; Cu, copper; Zn, zinc; Se, selenium; Mo, molybdenum; Co, cobalt; EGCE, (-)-epigallocatechin-3-gallate; TC, total cholesterol; TGs, triglycerides; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein Cholesterol; ALT, alanine Aminotrans; GSH-PX, glutathione peroxidase; ICP-MS, inductively coupled plasma mass spectrometry; C, standard diet group; L, food restriction group; H, high-fat diet group; C + G, standard diet supplemented with GTPs group; L + G, food restriction supplemented with GTPs group; H + G, high-fat diet supplemented with GTPs group

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https://doi.org/10.1016/j.jtemb.2018.10.002
Received 13 December 2017; Received in revised form 24 September 2018; Accepted 2 October 2018

the intracellular Copper ions and Zinc ions [8–10].

Study also suggested that the effects of tea on metals depends both on the meal matrix and its components [11], indicating that GTPs might affect trace elements status differently under different dietary patterns. In a number of eastern countries, such as China, where tea and plant foods are popular, iron and zinc deficiency is a major nutritional problem in the 20th century [12]. While in Japan where tea is also popular, people not only have a lower rate of micronutrient deficiencies than China, but also have the world's longest life expectancy. Whether tea consumption is related to iron and zinc deficiency and whether dietary patterns play a role in those different effects of GTPs on trace elements remain unsettled.

Moreover, little is known about the in vivo effects of GTPs on the status of TEs other than iron and dietary patterns were not considered in most of the previous studies [13–16]. Western diet, characterized by too much refined sugar and saturated fatty acids, and food restriction (FR), a 30%–50% reduction in food intake relative to ad libitum, have been reported to increase risk of micronutrient deficiencies [17,18]. Since a high number of people worldwide face restricted or high-fat diet, an exploration of the effects of tea on metals under those dietary patterns is needed.

Thus, this study aims to investigate the effects of GTPs on the levels of eight TEs in rats fed on standard, restricted and high-fat diet, which were to mimic the balanced, dietary restriction and western diet in humans.

2. Materials and methods

2.1. Ethics statement

This study was approved by the Huazhong University of Science and Technology Institutional Animal Care and Use Committee, in compliance with NIH guidelines (permit number: S412).

2.2. Materials and chemicals

Green tea polyphenols (GTPs, purity > 98%) were obtained from Fuzhou Rimian Inc. (Fuzhou, Fujian, China). Glucose, total cholesterol (TC), triglycerides (TGs), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein Cholesterol (LDL-C), Alanine Aminotrans (ALT), and Glutathione peroxidase (GSH-PX) assay kits were provided by Nanjing Jiancheng Bioengineering Co. Ltd. (Nanjing, Jiangsu, China). Multi-element Plasma Inductively Coupled Mass Spectrometry (ICP-MS) standard solution Std.3 (1000 mg/L Fe, K, Ca, Mg; 10 mg/L Ag, Al, As, Ba, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, T, V, Zn, Th, U) and Internal Standard (100 mg/L Sc, Ge, Rh, In, Tb, Bi, 6Li, Lu) were from O2si Smart Solutions (Charleston, South Carolina, USA). Other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA)

2.3. Animals and experimental design

Thirty-six male Wistar rats (SPF level, 160 g–180 g) were obtained from Hubei Provincial Center for Disease Control and Prevention, China and then housed on a 12-h light/12-h dark cycle at our animal facility.

Rats were randomly divided into 6 groups (C: standard diet group, L: restricted diet group, H: high-fat diet group, C + G: standard diet supplemented with GTPs group, L + G: restricted diet supplemented with GTPs group, H + G: high-fat diet supplemented with GTPs group.) and fed on standard diet (C and C + G group; containing 52% Fiber, 23% Dry Matter, 20% Crude Protein and 5% Fat), restricted diet (L and L + G group; containing 52% Fiber, 23% Dry Matter, 20% Crude Protein and 5% Fat), high-fat diet (H and H + G group; 60% standard chow, 12% lard, 10% sugar, 8% yolk powder, 6% peanuts powder, 3% Casein powder and 1% milk powder) with or without GTPs supplementation (200 mg/kg bw/day; C, L, H, C + G, L + G, and H + G group, n = 6 in each group) for 18 weeks, respectively. The standard and high-fat diet group (C and C + G, H, H + G group) had free access to food; while the rats of the restricted diet group (L and L + G group) were served with 70% of the average food intake of the standard group. The percentages of calories from carbohydrates, fat and protein were 62%, 14%, 24% in standard chow and 38%, 45%, 17% in high-fat diet. GTPs were added to the feed in C + G, L + G, H + G group based on the food intake and body weight. In C + G, L + G and H + G groups, GTPs were administrated at a dose of 200 mg per kilogram of body weight per day, a dose that reduced fat deposits in high fat-fed rats in our previous study [19]. Daily food intake and body weight were recorded; and 24 h urine of rats were collected using metabolic cages. At the 18th week, rats were sacrificed by decapitation; whole blood was collected and serum was separated.

2.4. Determination of biochemical indexes

Serum levels of fast blood glucose, TC, TG, HDL-C, LDL-C, ALT and GSH-PX were measured by spectra Max M2 versatile microplate reader (Molecular Devices Corporation, USA) using the commercial kit package according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Co. Ltd).

2.5. Histological study

Liver tissues were removed and parts of the liver tissue were fixed with 4% paraformaldehyde. Liver tissues fixed in 4% paraformaldehyde were then dehydrated, embedded in paraffin and stained with hematoxylin and eosin (HE). The samples were scanned under an IX-71 inverted fluorescence microscope (Olympus Corporation, Japan) at 200 × magnifications.

2.6. Sample preparation and trace element determination

Femur were removed from rats. Femur and feed were dried in electrothermal constant-temperature dry box (Shanghai Pudong Rongfeng Scientific Instrument Co., Ltd, China) for 48 h. Overnight digestion of samples (0.050 g of solid specimens, 0.1 ml of liquid specimens) were conducted in plastic centrifuge tube with nitric acid (purity = 69%, Sigma-Aldrich), followed by heating in ET358B Hot Plate (LabTech Corporation, USA) at 180 °C until no smoke and near dry, then diluted with ultrapure water to a final volume of 10 mL.

Calibration of the system was performed using solutions of trace elements with a final concentration of 0.5, 5, 10, and 50 μg/l by dilution of a stock solution with 1% HNO3. The r value of the calibration curve of all analyzed trace elements were greater than 0.9995.

The instrumental parameters of the 7900 ICP-MS (Agilent Technologies, Inc., USA) used were as follows: radiofrequency power (1850 W), peristaltic pump (0.1 rpm), sampling depth (9.0 mm), sampling cone (0.2 mm), plasma gas flow rate (15.0 L/min), auxiliary gas flow rate (0.8 L/min), carrier gas flow rate (0.8 L/min), nebulizer pump (40 rpm), S/C temperature (2.0 °C), oxide ions (156/140) ≤2.0%, doubly charged (70/140) ≤3.0%.

The internal standards (Charleston, South Carolina, USA) were used for quality control. Reference materials of whole blood (Seronorm™ Trace Elements Whole Blood L-3, Sweden) and urine (Seronorm™ Trace Elements Urine L1, Sweden) were tested together with the samples. The recoveries of all those analyzed metals were in the range of 90.4–99.7% of certified values of reference materials.

2.7. Statistics

Data were presented as median (25 and 75 percentile boundaries). The Shapiro -Wilk method was used to evaluate data normality. Significance of the difference was assessed by the Mann-Whitney U test using SPSS (version 12.0, SPSS, Inc, Chicago, USA). Level of significance was $P < 0.05$.

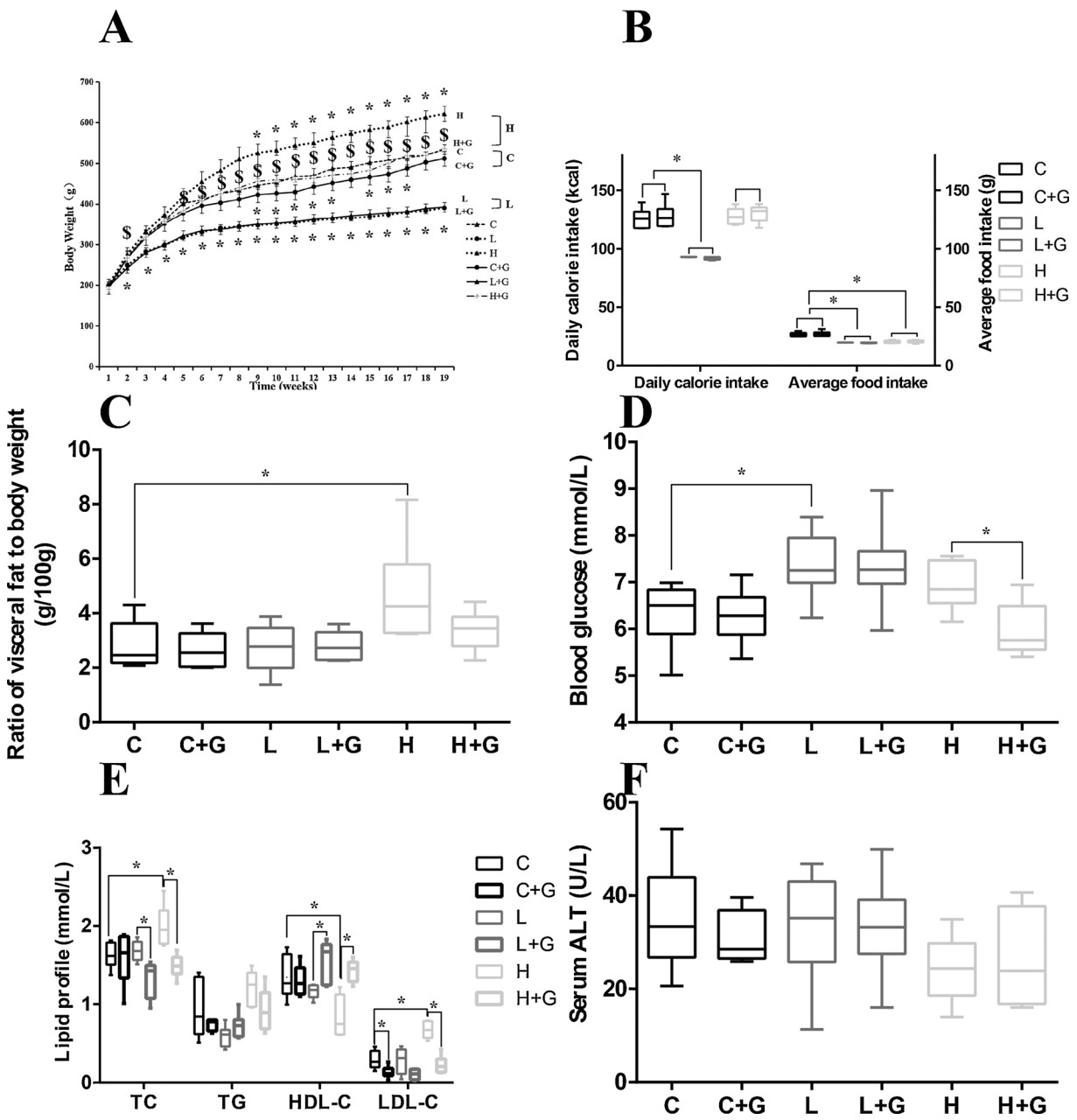

Fig. 1. Physiological and biochemical status of rats fed on different diets.
(A) The changes of body weight during 18 weeks; (B) Average food intake and daily calorie intake; (C) The ratio of visceral fat to body weight; (D) Blood glucose levels; (E) Lipid profiles; (F) Serum ALT levels; The daily calorie intakes of rats on standard chow, restricted and high-fat diet were calculated by the average food intake, percentages of calories from carbohydrates, fat and protein of different diets and energy coefficients of carbohydrates, fat and protein, respectively. C: standard diet group, L: restricted diet group, H: high-fat diet group, C + G: standard diet supplemented with GTPs group, L + G: restricted diet supplemented with GTPs group, H + G: high-fat diet supplemented with GTPs group. Data expressed as median (25–75), N = 6, * $P < 0.05$. The results of L + G and H + G group in compare with C group were not included.

3. Results

3.1. Physiological and biochemical status of rats treated with different diets

Compare with the C group, the body weight of rats was significantly lower in L group and significantly higher in the H group ($P < 0.05$, Fig. 1A). GTPs supplement reduced the body weight of rats in both C and H group ($P < 0.05$, Fig. 1A). Food intake in L and H group were 26% and 24% lower than the C group. The daily calorie intakes of rats on standard chow, restricted and high-fat diet were 125.87 kcal (117.69–131.60), 92.92 kcal (92.83–93.06), 127.00 kcal (121.44–134.06), respectively. GTPs supplement had no significant effects on the food intake and daily calorie intake ($P < 0.05$, Fig. 1B). Ratio of visceral fat to body weight (g/100 g) of rats in H group were significantly higher than C group ($P < 0.05$, Fig. 1C).

Rats in L group exhibited higher blood glucose ($P < 0.05$, Fig. 1D); while rats in H group showed higher TC, LDL-C and lower HDL-C ($P < 0.05$, Fig. 1E). GTPs supplement reduced the LDL-C in C group, TC in L group and blood glucose, LDL-C and TC in H group ($P < 0.05$). In addition, GTPs supplement increased the HDL-C in rats from both L and H group ($P < 0.05$) (Fig. 1E). There was no significant difference in ALT levels of all groups (Fig. 1F).

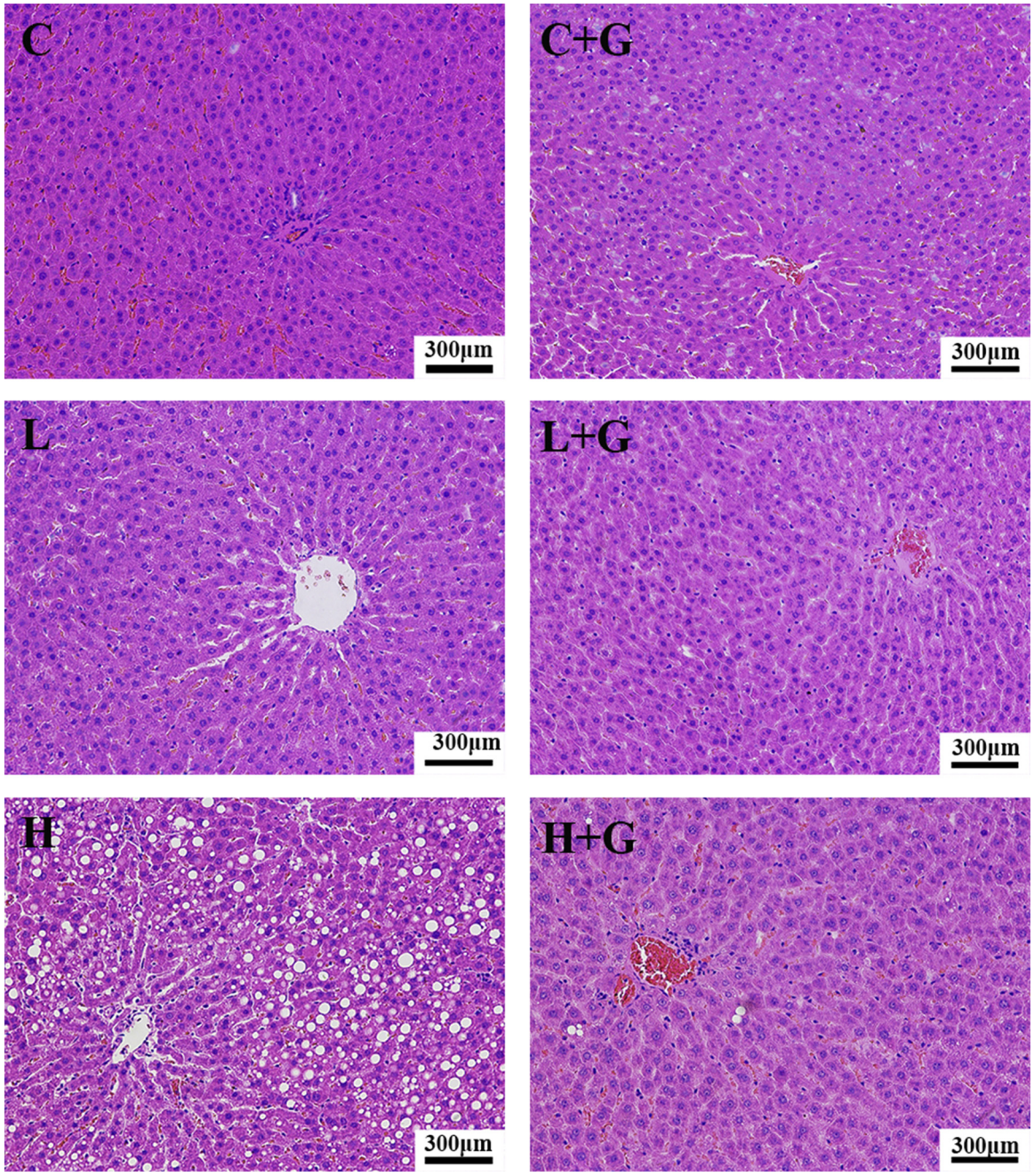

Fig. 2. Liver histology of rats fed on different diets.
H&E staining of the liver (10 × 20); C: standard diet group, L: restricted diet group, H: high-fat diet group, C + G: standard diet supplemented with GTPs group, L + G: restricted diet supplemented with GTPs group, H + G: high-fat diet supplemented with GTPs group.

3.2. Liver histology of rats treated with different diets

Fat vacuoles were observed in the liver specimen from H and H + G group but not in other groups. However, the amount of fat vacuoles was significantly less in H + G group than in H group (Fig. 2).

3.3. Dietary restriction and high-fat diet per se caused a lower elements intake

The average daily intake of trace elements (V, Mn, Fe, Co, Cu, Zn, Se, Mo) in L group were74% of C group (Table 1; $P < 0.05$); the V, Mn, Fe, Co, Cu, Zn, Se and Mo intake of rats in H group were 97% ($P > 0.05$), 45%, 45%, 59%, 46%, 58%, 84%, 65% ($P < 0.05$) of C group, respectively. GTPs supplement had no significant effects on the daily intake of TEs (Table 1; $P > 0.05$).

3.4. GTPs supplement altered the whole blood level of trace elements in rats fed on restricted and high-fat diet, but not in rats fed on standard diet

Compared with rats on standard diet, whole blood level of Zn decreased by 47% in rats on high-fat diet ($P < 0.05$, Table 2). GTPs supplement decreased the whole blood level of V by 58%, increased the level of Zn and Co by 46% and 54% in rats on restricted diet, decreased the whole blood level of V by 42% and increased Se by 21% in rats on high-fat diet (Table 2; $P < 0.05$). However, GTPs supplement exhibited no effects on the whole blood level of any TEs in rats on standard diet (Table 2; $P > 0.05$).

As selenium is an element with low vaporization temperature, high

Table 1
Daily trace elements intake of rats fed on different diets through feed.

elements	C	C+G	L	L+G	H	H+G
V (μg/d)	19.01(17.78–19.88)	19.07(18.03–20.23)	14.04(14.02–14.06)*	13.89(13.70–14.05)	18.49(17.68–19.52)*	19.24(18.07–19.70)
Mn (mg/d)	2.81(2.63–2.94)	2.82(2.67–2.99)	2.08(2.07–2.08)*	2.06(2.03–2.08)	1.27(1.21–1.34)*	1.32(1.24–1.35)
Fe (mg/d)	11.05(10.33–11.55)	11.08(10.48–11.75)	8.16(8.15–8.17)*	8.07(7.96–8.16)	5.06(4.84–5.34)*	5.26(4.49–5.39)
Co (μg/d)	6.16(5.76–6.44)	6.18(5.84–6.55)	4.55(4.54–4.55)*	4.50(4.44–4.45)	3.66(3.50–3.86)*	3.81(3.57–3.90)
Cu (mg/d)	0.46(0.43–0.48)	0.46(0.44–0.49)	0.34(0.34–0.34)*	0.34(0.33–0.34)	0.21(0.19–0.21)*	0.21(0.19–0.21)
Zn (mg/d)	1.67(1.56–1.74)	1.67(1.58–1.77)	1.23(1.23–1.23)*	1.22(1.20–1.23)	0.96(0.92–1.01)*	1.00(0.94–1.02)
Se (μg/d)	7.23(6.76–7.56)	7.25(6.86–7.69)	5.34(5.33–5.35)*	5.28(5.21–5.34)	6.10(5.83–6.44)*	6.34(5.96–6.50)
Mo (μg/d)	44.45(41.57.76–46.48)	44.59(42.16–47.29)	32.82(32.79–32.87)*	32.49(32.02–32.85)	29.06(27.78–30.67)*	30.23(28.40–30.96)

Daily food intake of rats and the concentrations of eight trace elements in feed were used to calculate the daily trace elements intake. C: standard diet group, L: restricted diet group, H: high-fat diet group, C+G: standard diet supplemented with GTPs group, L+G: restricted diet supplemented with GTPs group, H+G: high-fat diet supplemented with GTPs group. Data expressed as median (25–75), N = 6, * $P < 0.05$ vs. C; # $P < 0.05$ vs. L; $ $P < 0.05$ vs. H. The results of L+G and H+G group in compare with C group were not included.

temperature in the process of sample preparation may induce inaccurate results, we further evaluated the GSH-PX activity in whole blood to assess selenium nutritional status to confirm the results. GTPs supplementation increased the GSH-PX activity by 12% in rats on high-fat diet, which was consistent with the results from ICP-MS ($P < 0.05$) (Fig. 3).

3.5. GTPs supplement affect level of several trace elements in femur and reduced the daily urinary excretion of V and Mo in rats on all three kinds of diets

To further explore the effects of GTPs on the deep pool of trace elements, level of TEs in the femur and urine were also determined. Compared with rats on standard diet, femur level of Co decreased by 9% in rats on high-fat diet ($P < 0.05$, Table 3). In the results from the femur, GTPs supplement decreased V in rats on all diets (by 45%, 46% and 55% in rats on standard, restricted and high-fat diet, respectively. $P < 0.05$, Table 3), decreased Cu and Co by 55% and 27% in rats on standard diet ($P < 0.05$, Table 3), and decreased Mo by 73% and 77% in rats on restricted and high-fat diet, respectively ($P < 0.05$, Table 3).

The daily urinary excretion of Se in H group was lower than in C group by 52% ($P < 0.05$, Table 3); no significant difference was observed in the daily urinary excretion of V, Mn, Fe, Co, Cu, Zn or Mo among C, L and H group ($P > 0.05$, Table 3). GTPs supplement reduced the daily urinary excretion of V by 52%, 39%, 73% and Mo by 61%, 75%, 81% in rats on standard, restricted and high-fat diet, respectively ($P < 0.05$, Table 3).

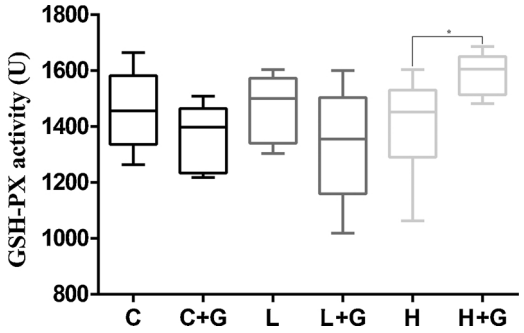

Fig. 3. GSH-PX activity in whole blood of rats fed on different diets.
GSH-PX activity in whole blood is measured to further assess the nutritional status of selenium. C: standard diet group, L: restricted diet group, H: high-fat diet group, C+G: standard diet supplemented with GTPs group, L+G: restricted diet supplemented with GTPs group, H+G: high-fat diet supplemented with GTPs group. Data expressed as median (25–75), N = 6, * $P < 0.05$.

4. Discussion

In the current study, we found that 200 mg/kg bw/day GTPs decreased the whole blood level of V in rats fed on both restricted and high-fat diet, increased Zn, Co in rats fed on restricted diet, and increased Se in rats fed on high-fat diet. However, no effects on the whole blood level of V, Mn, Fe, Cu, Zn, Se, Mo or Co were observed in rats fed on standard chow. Meantime, GTPs supplement decreased several TEs in femur and decreased daily urinary excretion of V and Mo in rats on all of the three diets. In addition, decreased whole blood level of Zn and abnormal lipid metabolism were observed in high-fat diet rats and GTPs ameliorated high-fat diet induced dyslipidemia and body weight gain. These finding indicated that the effects of GTPs on trace elements status are modified by dietary patterns and Zn, Se may play a role in the modulation of lipid metabolic disturbances by high-fat diet andGTPs, respectively.

The effects of dietary restriction on the level of trace metals had been rarely investigated. In our dietary restriction animal models, despite the almost 30% lower overall food intake, the rats still maintained the homeostasis of eight TEs in whole blood without affecting the daily urinary excretion and femur content of those elements except the

Table 2
Effects of GTPs on concentrations of eight trace elements in whole blood of rats fed on different diets.

elements	C	C+G	L	L+G	H	H+G
V (μg/L)	19.35(10.5–45.17)	13.80(9.88–37.62)	24.02(15.00–35.18)	10.05(8.61–16.78)#	7.82(6.71–16.53)	4.54(3.93–6.30)$
Mn (mg/L)	0.83(0.57–1.54)	1.02(0.78–1.98)	0.38(0.24–0.65)	0.74(0.56–1.14)	0.23(0.15–0.69)	0.34(0.29–0.56)
Fe (g/L)	0.55(0.42–0.64)	0.64(0.61–0.66)	0.53(0.50–0.61)	0.67(0.57–0.70)	0.53(0.50–0.61)	0.59(0.56–0.61)
Co (μg/L)	10.65(6.64–24.26)	13.60(9.1–18.36)	3.29(2,12–4.90)	7.10(4.95–14.91)#	2.03(1.18–12.83)	7.27(4.08–27.53)
Cu (mg/L)	1.35(0.70–2.50)	0.72(0.46–1.35)	0.78(0.61–0.97)	0.37(0.22–0.64)	1.00(0.80–5.47)	1.52(0.96–47.73)
Zn (mg/L)	12.19(8.99–18.14)	13.22(10.46–16.69)	6.74(4.53–10.24)	12.58(10.43–14.64)#	6.43(4.67–8.85)*	6.94(6.56–9.20)
Se (mg/L)	0.39(0.21–0.44)	0.27(0.22–0.47)	0.30(0.18–0.33)	0.20(0.16–0.26)	0.41(0.37–0.45)	0.52(0.47–0.53)$
Mo (μg/L)	18.21(12.21–48.83)	20.54(13.50–36.83)	31.82(25.33–40.23)	18.82(13.84–33.55)	17.75(15.86–26.46)	21.15(18.22–25.39)

C: standard diet group, L: restricted diet group, H: high-fat diet group, C+G: standard diet supplemented with GTPs group, L+G: restricted diet supplemented with GTPs group, H+G: high-fat diet supplemented with GTPs group. Data expressed as median (25–75), N = 6, * $P < 0.05$ vs. C; # $P < 0.05$ vs. L; $ $P < 0.05$ vs. H. The results of L+G and H+G group in compare with C group were not included.

Table 3
Effects of GTPs on trace elements concentrations in femur and urinary excretion of trace elements of rats fed on different diets.

Sample	Element	C	C+G	L	L+G	H	H+G
Femur (μg/g)	V	0.11(0.09–0.17)	0.06(0.0–0.08)*	0.13(0.12–0.16)	0.07(0.06–0.09)#	0.11(0.09–0.13)	0.05(0.03–0.07)$
	Mn	0.98(0.71–1.37)	0.84(0.78–1.05)	0.91(0.78–1.22)	1.33(1.08–2.63)	0.87(0.75–0.98)	0.89(0.64–1.18)
	Fe	64.94(58.42–99.61)	75.37(59.37–90.48)	111.98(63.36–128.30)	108.54(95.63–130.88)	69.97(43.84–86.15)	58.94(37.68–72.63)
	Co	0.11(0.11–0.12)	0.08(0.08–0.09)*	0.11(0.10–0.11)	0.11(0.08–0.14)	0.10(0.09–0.11)*	0.08(0.055–0.14)
	Cu	1.32(0.80–1.59)	0.60(0.47–0.79)*	1.05(0.87–1.28)	1.43(.63–2.51)	0.78(0.68–2.18)	0.58(0.33–1.35)
	Zn(mg/g)	0.13(0.11–0.15)	0.13(0.13–0.14)	0.15(0.14–0.16)	0.11(0.08–0.13)	0.12(0.11–0.13)	0.14(0.13–0.15)
	Se	0.23(0.19–0.30)	0.23(0.21–0.26)	0.26(0.23–0.28)	0.24(0.22–0.33)	0.20(0.17–0.21)	0.22(0.14–0.23)
	Mo	0.37(0.32–0.61)	0.43(0.19–0.48)	0.48(0.35–0.65)	0.13(0.22–0.33)#	0.39(0.24–0.64)	0.09(0.05–0.15)$
Urine (μg/d)	V	0.23(0.20–0.33)	0.11(0.08–0.13)*	0.23(0.19–0.27)	0.14(0.09–0.19)#	0.22(0.17–0.27)	0.06(0.05–0.09)$
	Mn	4.04(2.29–5.33)	2.10(1.52–3.63)	2.92(2.32–3.95)	3.20(2.02–4.18)	2.18(1.66–2.68)	1.55(1.04–3.59)
	Fe	30.78(24.75–248.73)	59.10(42.26–86.82)	59.16(40.52–116.63)	87.88(57.22–183.29)	47.04(18.06–68.90)	40.62(34.37–67.71)
	Co	0.13(0.12–0.18)	0.15(0.11–0.88)	0.17(0.10–0.20)	0.15(0.13–0.20)	0.15(0.11–0.21)	0.13(0.11–0.80)
	Cu	3.44(1.75–14.35)	4.41(1.65–7.47)	2.66(0.96–3.48)	3.05(2.20–3.37)	2.30(1.10–3.13)	2.64(1.51–3.00)
	Zn	13.79(9.48–19.58)	14.58(12.06–18.45)	23.34(17.65–27.66)	30.19(18.31–42.43)	26.96(15.36–31.28)	17.20(12.61–44.44)
	Se	4.34(3.25–5.15)	3.65(3.34–3.82)	4.59(3.72–4.83)	3.50(2.90–4.35)	2.10(1.57–2.89)*	1.98(1.61–2.11)
	Mo	18.87(16.67–31.45)	7.46(6.49–9.10)*	25.81(21.41–27.42)	6.45(4.18–6.93)#	15.8(14.56–18.62)	2.93(2.30–3.34)$

The concentrations of eight trace elements in urine and 24 h urine output of rats were used to calculate the daily urinary excretion of trace elements. C: standard diet group, L: restricted diet group, H: high-fat diet group, C+G: standard diet supplemented with GTPs group, L+G: restricted diet supplemented with GTPs group, H+G: high-fat diet supplemented with GTPs group. Data expressed as median (25–75), N = 6, * $P < 0.05$ vs. C; # $P < 0.05$ vs. L; $ $P < 0.05$ vs. H. The results of L+G and H+G group in compare with C group were not included.

decreased body weight. While the daily intake of Zn was obviously lower (58.0% of rats on standard diet) in the high-fat diet fed rats, and the Zn level in whole blood was decreased after long-term high-fat diet intervention, which explained why rats on high-fat diet are prone to develop Zn deficiency [20–22]. Meanwhile, the decrease of Zn was accompanied with increased body weight and dyslipidemia. In consistent with our results, erythrocyte Zn level was also found to be negatively associated with BMI in population [23]. Since no significant difference in the calorie intake was observed between the control and the high-fat diet group, Zn insufficiency may be one of the causes of weight gain and dyslipidemia.

The results that GTPs had no effects on whole blood levels of trace elements (V, Mn, Fe, Cu, Zn, Se, Mo and Co) in rats fed on standard diet are in agreement with the results from Ganji V et al. [24], and Basu A et al. [25]. Although the whole blood levels of eight TEs were not altered by GTPs, the levels of V, Co and Cu in femur were reduced by GTPs as well as the daily urinary excretion of V and Mo. Since the daily trace elements intakes were the same as the control and GTPs were reported to interact with Cu ion in vitro [26,27], GTPs may affect the absorption of V, Co and Cu in intestinal and the reduction of those trace elements in the femur and urine may be the regulatory mechanism of the body to maintain the homeostasis of trace elements in the blood. The dose of 200 mg/kg bw/day used in our animal model is equivalent to the consumption of 8–13 cups of green tea for a 60 kg man a day, which was higher than the recommended daily consumption of human (4–6 cups of green tea, 540 mg of total green tea catechins per day) [28]. Thus, tea consumption is unlikely to affect minerals levels in whole blood of population following normal diet. The zinc and iron deficiencies observed in eastern countries (China, in 20th century) may not be related to the consumption of tea. As GTPs decreased the LDH-C level as well, it may benefit individuals following normal diet without causing those eight trace element deficiency.

With GTPs supplementation, decreased V and increased Zn, Co levels in whole blood, decreased V and Mo levels in femur were observed in rats fed on restricted diet as well as decreased urinary excretion of V and Mo. Those data indicated that despite reducing the urinary excretion and the femur levels of certain trace elements; the body loses the maintenance of the V content in whole blood under the dual effects of food reduction and GTPs. Vanadium, an essential trace element, plays a role in promoting glucose transport and metabolism, lipid, DNA, and protein synthesis [29]. That may partly explain why higher blood glucose was observed in both L and L+G group. For zinc in the whole blood, we found a phenomenon. GTPs do not affect the whole blood level of zinc when the zinc intake is sufficient (standard diet), but increased whole blood zinc when zinc intake drops to 70% of normal (dietary restriction). However, when the zinc intake drops to 58% (high-fat diet), GTPs no longer show any zinc increasing effect. An explanation for this phenomenon may be that polyphenols are known to chelate zinc which might alter their bioaccessibility during intestinal absorption and improve zinc absorption [30,31]. Those zinc atoms that enter the cells may not suffice to produce significant increments of total zinc content when there is excess or too little zinc (available zinc has been fully utilized) present in feed.

In our high-fat diet animal models, we found that GTPs increased GSH-PX activity and the whole blood level of Se simultaneously in rats fed on high-fat diet, neither increase in GSH-PX activity or Se level by GTPs supplementation was observed in rats on normal or restricted diet. Increased of the GSH-PX activity and serum selenium concentration by green tea consumption was also observed by Hamdaoui MH in rats on basic diet [32]. Generally, GTPs is believed to exert antioxidative effects by inhibiting the body's oxidant enzymes, improving the body's antioxidant enzyme activity, chelating transition metal ions, anti-lipid oxidation in vitro and scavenging ROS directly [33]. As the GSH-PX activity is an important component of the body's antioxidant system [34] and GTPs supplementation ameliorated high-fat induced lipid metabolism variation and body weight gain, the data reported here may add a new dimension to the understanding of the possible mechanisms underlying the anti-oxidative effects of GTPs. The modulation of GTPs on Se may be a reason for their health promotion benefits in high-fat induced lipid metabolism and obesity [35].

Although GTPs supplementation increased the whole blood level of Zn, Co in rats fed on restricted diet and Se, GSH-PX activity in rats fed on high-fat diet, and ameliorated high-fat induced dyslipidemia. GTPs also reduced the content of V in whole blood of rats fed on both restricted and high-fat diet. Since GTPs supplements can be taken without professional supervision, and often by people intent to lose weight or to prevent other health problems, the effects of GTPs on trace element status observed in rats on food restriction and high-fat diet deserves considerable public attention. Thus, further researches about the effects of GTPs consumption on trace elements in humans on different diets are in need. From a "first do no harm" point of view, individuals on food restriction or high-fat diet should monitor their TEs status if they consume GTPs regularly.

5. Conclusion

Green tea polyphenols did not alter the trace elements levels in whole blood of rats on standard diet, but altered trace elements homeostasis in rats fed on food restriction and high-fat diet; Meantime, GTPs supplement decreased several TEs in femur and increased daily urinary excretion of V and Mo in rats on all of the three diets. In addition, rats fed on high-fat diet developed dyslipidemia and body weight gain, which was ameliorated by GTPs. The results indicate that the interaction between nutrients and non-nutrients in organisms were extremely complex. More researches are needed to clarify the complex relationship and the underlying mechanisms.

Conflict of interest

None.

Sources of funding

This work was supported by the National Natural Science Foundation of China (No. 81373007, 81302423), and the Fundamental Research Funds for the Central Universities (HUST: 2016YXMS222).

Acknowledgments

Nannan Wu performed the experiments and wrote the manuscript; Weijie Yi designed the study; Xiao Xie, Siyun Xiang, Miying Du and Yongjun Bu performed the animal experiments; Guangyu Yang, Chong Tian, Getachew Eskedar, Fangyi Xu and Shuiqing He contributed to the revision of the manuscript; Chenjiang Ying and Guangyu Yang designed and supervised the study. We thank all the anonymous reviewers for their helpful suggestions on the quality improvement of our paper.

References

[1] S. Molino, M. Dossena, D. Buonocore, F. Ferrari, L. Venturini, G. Ricevuti, M. Verri, Polyphenols in dementia: from molecular basis to clinical trials, Life Sci. 161 (2016) 69–77.
[2] S. Visnja, G. Ana Cipak, T. Koraljka Gall, A. Dragan, Z. Neven, Selected attributes of polyphenols in targeting oxidative stress in cancer, Curr. Top. Med. Chem. 15 (5) (2015) 496–509.
[3] R. Colomer, A. Sarrats, R. Lupu, T. Puig, Natural polyphenols and their synthetic analogs as emerging anticancer agents, Curr. Drug Targets 18 (2) (2017) 147–159.
[4] R.F. de Souza, W.F. De Giovani, Synthesis, spectral and electrochemical properties of Al(III) and Zn(II) complexes with flavonoids, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 61 (9) (2005) 1985–1990.
[5] P. Tamilmani, M.C. Pandey, Iron binding efficiency of polyphenols: comparison of effect of ascorbic acid and ethylenediaminetetraacetic acid on catechol and galloyl groups, Food Chem. 197 (Pt B) (2016) 1275–1279.
[6] B. Zhang, X.R. Cheng, I.S. da Silva, V.W. Hung, A.J. Veloso, L. Angnes, K. Kerman, Electroanalysis of the interaction between (-)-epigallocatechin-3-gallate (EGCG) and amyloid-beta in the presence of copper, Metallomics 5 (3) (2013) 259–264.
[7] Y. Liu, M. Guo, Studies on transition metal-quercetin complexes using electrospray ionization tandem mass spectrometry, Molecules (Basel, Switzerland) 20 (5) (2015) 8583–8594.
[8] H.Y. Khan, H. Zubair, M. Faisal, M.F. Ullah, M. Farhan, F.H. Sarkar, A. Ahmad, S.M. Hadi, Plant polyphenol induced cell death in human cancer cells involves mobilization of intracellular copper ions and reactive oxygen species generation: a mechanism for cancer chemopreventive action, Mol. Nutr. Food Res. 58 (3) (2014) 437–446.
[9] E.Y. Kim, T.K. Pai, O. Han, Effect of bioactive dietary polyphenols on zinc transport across the intestinal Caco-2 cell monolayers, J. Agric. Food Chem. 59 (8) (2011) 3606–3612.
[10] I.M. Quesada, M. Bustos, M. Blay, G. Pujadas, A. Ardèvol, M.J. Salvadó, C. Bladé, L. Arola, J. Fernández-Larrea, Dietary catechins and procyanidins modulate zinc homeostasis in human HepG2 cells, J. Nutr. Biochem. 22 (2) (2011) 153–163.
[11] P.B. Disler, S.R. Lynch, R.W. Charlton, J.D. Torrance, T.H. Bothwell, R.B. Walker, F. Mayet, The effect of tea on iron absorption, Gut 16 (3) (1975) 193–200.
[12] L.S. Stephenson, M.C. Latham, E.A. Ottesen, Global malnutrition, Parasitology 121 (Suppl) (2000) S5–22.
[13] M. Nelson, J. Poulter, Impact of tea drinking on iron status in the UK: a review, J. Hum. Nutr. Diet. 17 (1) (2004) 43–54.
[14] A.B. Beverly, L. Zhu, T.L. Fish, T. Thannhauser, M.A. Rutzke, D.D. Miller, Green tea ingestion by rats does not affect iron absorption but does alter the composition of the saliva proteome, J. Food Sci. 77 (5) (2012) H96–H104.
[15] A. Singh, K. Bains, H. Kaur, Effect of inclusion of key foods on in vitro iron bioaccessibility in composite meals, J. Food Sci. Technol. 53 (4) (2016) 2033–2039.
[16] S.F. Ahmad Fuzi, D. Koller, S. Bruggraber, D.I. Pereira, J.R. Dainty, S. Mushtaq, A 1-h time interval between a meal containing iron and consumption of tea attenuates the inhibitory effects on iron absorption: a controlled trial in a cohort of healthy UK women using a stable iron isotope, Am. J. Clin. Nutr. (2017).
[17] F.M. Cerqueira, A.J. Kowaltowski, Commonly adopted caloric restriction protocols often involve malnutrition, Ageing Res. Rev. 9 (4) (2010) 424–430.
[18] C. Hutchinson, A review of iron studies in overweight and obese children and adolescents: a double burden in the young? Eur. J. Nutr. 55 (7) (2016) 2179–2197.
[19] C. Tian, X. Ye, R. Zhang, J. Long, W. Ren, S. Ding, D. Liao, X. Jin, H. Wu, S. Xu, C. Ying, Green tea polyphenols reduced fat deposits in high fat-fed rats via erk1/2-PPARgamma-adiponectin pathway, PLoS One 8 (1) (2013) e53796.
[20] A.A. Tinkov, E.R. Gatiatulina, E.V. Popova, V.S. Polyakova, A.A. Skalnaya, E.F. Agletdinov, A.A. Nikonorov, A.V. Skalny, Early high-fat feeding induces alteration of trace element content in tissues of juvenile male Wistar rats, Biol. Trace Elem. Res. 175 (2) (2017) 367–374.
[21] E. Krol, Z. Krejpcio, A. Chmurzynska, Folic acid and protein content in maternal diet and postnatal high-fat feeding affect the tissue levels of iron, zinc, and copper in the rat, Biol. Trace Elem. Res. 144 (1-3) (2011) 885–893.
[22] L. Frommelt, M. Bielohuby, B.J. Stoehr, D. Menhofer, M. Bidlingmaier, E. Kienzle, Effects of low-carbohydrate, high-fat diets on apparent digestibility of minerals and trace elements in rats, Nutrition (Burbank, Los Angeles County, Calif.) 30 (7-8) (2014) 869–875.
[23] J. Olechnowicz, A. Tinkov, A. Skalny, J. Suliburska, Zinc status is associated with inflammation, oxidative stress, lipid, and glucose metabolism, J. Physiol. Sci. 68 (1) (2018) 19–31.
[24] V. Ganji, C.V. Kies, Zinc bioavailability and tea consumption. Studies in healthy humans consuming self-selected and laboratory-controlled diets, Plant foods for human nutrition (Dordrecht, Netherlands) 46 (3) (1994) 267–276.
[25] A. Basu, N.M. Betts, A. Mulugeta, C. Tong, E. Newman, T.J. Lyons, Green tea supplementation increases glutathione and plasma antioxidant capacity in adults with the metabolic syndrome, Nutr. Res. (New York, N.Y.) 33 (3) (2013) 180–187.
[26] K.F. Pirker, M.C. Baratto, R. Basosi, B.A. Goodman, Influence of pH on the speciation of copper(II) in reactions with the green tea polyphenols, epigallocatechin gallate and gallic acid, J. Inorg. Biochem. 112 (2012) 10–16.
[27] L. Zhang, I.D. Sahu, M. Xu, Y. Wang, X. Hu, Effect of metal ions on the binding reaction of (-)-epigallocatechin gallate to beta-lactoglobulin, Food Chem. 221 (2017) 1923–1929.
[28] W. Dekant, K. Fujii, E. Shibata, O. Morita, A. Shimotoyodome, Safety assessment of green tea based beverages and dried green tea extracts as nutritional supplements, Toxicol. Lett. 277 (2017) 104–108.
[29] D. Rehder, The role of vanadium in biology, Metallomics 7 (5) (2015) 730–742.
[30] K. Sreenivasulu, P. Raghu, K.M. Nair, Polyphenol-rich beverages enhance zinc uptake and metallothionein expression in Caco-2 cells, J. Food Sci. 75 (4) (2010) H123-8.
[31] Y.S. Tarahovsky, Y.A. Kim, E.A. Yagolnik, E.N. Muzafarov, Flavonoid-membrane interactions: involvement of flavonoid-metal complexes in raft signaling, Biochim. Biophys. Acta 1838 (5) (2014) 1235–1246.
[32] M.H. Hamdaoui, A. Chahed, S. Ellouze-Chabchoub, N. Marouani, Z. Ben Abid, A. Hedhili, Effect of green tea decoction on long-term iron, zinc and selenium status of rats, Ann. Nutr. Metab. 49 (2) (2005) 118–124.
[33] Z. Zou, W. Xi, Y. Hu, C. Nie, Z. Zhou, Antioxidant activity of Citrus fruits, Food Chem. 196 (2016) 885–896.
[34] J.Q. Wu, T.R. Kosten, X.Y. Zhang, Free radicals, antioxidant defense systems, and schizophrenia, Prog. Neuro-psychopharmacol. Biol. Psychiatry 46 (2013) 200–206.
[35] S.A. Nido, S.A. Shituleni, B.M. Mengistu, Y. Liu, A.Z. Khan, F. Gan, S. Kumbhar, K. Huang, Effects of selenium-enriched probiotics on lipid metabolism, antioxidative status, histopathological lesions, and related gene expression in mice fed a high-fat diet, Biol. Trace Elem. Res. 171 (2) (2016) 399–409.