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Boron Contents of German Mineral and Medicinal Waters and Their Bioavailability in *Drosophila melanogaster* and Humans

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Scope: Boron is a trace element that naturally occurs in soil, making mineral and medicinal water important contributors to overall intake. Thus, in a systematic screening, the mean boron concentrations of 381 German mineral and medicinal waters are determined.
Methods and Results: Boron concentrations in mineral and medicinal waters are analyzed by inductively coupled mass spectrometry (ICP-MS). Highest boron values find in waters from the southwest of Germany. The boron content of the waters is positively correlated with the concentration of most other analyzed bulk elements, including calcium, potassium, magnesium, and sodium. Mineral waters with either low (7.9 μg L^{-1}), medium (113.9 μg L^{-1}), or high (2193.3 μg L^{-1}) boron content are chosen for boron exposure experiments in fruit flies (*Drosophila melanogaster*) and humans. In flies, boron-rich mineral water significantly increases boron accumulation, with the accumulation predominantly occurring in the exoskeleton. In humans, serum boron and 24-h urinary boron excretion significantly increase only in response to the intake of boron-rich mineral water.
Conclusion: Overall, the current data demonstrate that mineral and medicinal waters vary substantially in the content of boron and that boron-rich mineral water can be used to elevate the boron status, both in flies and humans.

1. Introduction

Given that boron is a mineral component of the Earth's crust, it accumulates in ground and mineral water.[1,2] In 1857, Wittstein and Apoiger identified boron in seeds from flowers of the *Primulaceae* family.[3] Today, it is well documented that plants take up boron from the soil and convert it into uncharged boric acid and small amounts of borate anions. Boron is an important micronutrient for plants, and boron deficiency may lead to developmental impairments.[4,5] In particular, in plant cell walls, boron interacts with polyols such as pectin, thereby improving cross-linking and cell wall integrity.[6,7] However, the optimum range for plant growth is rather narrow, and moderately elevated boron concentrations are toxic. Moreover, the boron requirement of plants was found to be species-specific.[8]

Boron is also present in animal tissues. Although essentiality has not yet been fully proven, similar to plants, adequate boron concentration is suggested to support various biological processes, whereas increased levels are toxic. In the fruit fly *Drosophila melanogaster (D. melanogaster)*, it has been shown that boron body concentrations change during the life cycle, being highest during the egg stage, lowest in the third instar larvae/pupae stages, and increasing steadily thereafter in ageing adult flies.[9]

In humans, boron circulates in the blood predominantly as boric acid,[10] and boron elimination is mainly regulated via renal excretion.[11,12] Notably, compared to blood and soft tissue, boron concentrations in human bone and hair are rather high,[13,14] indicating a tissue-specific distribution. More recent studies suggest a role of boron in bone health.[15]

Data on dietary intake of boron in humans are scarce. The mean daily boron intake of the southeastern US population was estimated to be approximately 1200 μg day^{-1}[16] and 2200–2300 for Australians.[17] Fruits and vegetables were determined to be relatively rich in boron (>2000 μg kg^{-1}), while animal-derived foods, such as meat, dairy products, and eggs, as well as grain products, were relatively low in boron (<1000 μg kg^{-1}). Interestingly, fruits often contain higher amounts of boron than

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vegetables, with the highest amounts in plums, peaches, grapes, and the respective juices as well as wine. High amounts of boron were also found in plant-derived fatty foods such as nuts and avocados with values >11 000 μg kg^{-1}.[16,17] Moreover, it was recently shown that high dietary boron intake was associated with increased renal boron excretion.[11]

Further substantial dietary sources for boron are drinking/tap and mineral water. To date, boron intake via water consumption has been primarily investigated as a risk assessment based on studies that observed reproductive and developmental toxicity of very high boron intake.[18,19] However, no negative effects of boron on reproduction were observed in humans living in geographical areas with boron-rich drinking water, such as in the province of Balıkesir in Turkey (≈29 000 μg L^{-1}),[20] the Argentinean Andes (≈11 000 mg L^{-1})[21] or northern France and Pas-de-Calais in France (≈1000 μg L^{-1}).[2] Based on a recent review, it was concluded that only extreme occupational boron exposure exerts reproductive toxicity in humans.[22] However, blood boron levels of ≥500 μg L^{-1} were not associated with impaired semen quality or sexual hormone parameters in Chinese[23] or Turkish[24] workers with extreme occupational boron exposure. In 2011, the WHO guideline upper acceptable value for boron in drinking water was adjusted from 300 to 2400 μg L^{-1}.[25,26] Little is known about the boron content of mineral water from Germany and its bioavailability in humans. After coffee (166 L per capita), mineral and medicinal waters are the second most consumed beverages (134 L per capita) in Germany.[27] The German Mineral and Drinking Water Ordinance (Min/TafelwV) requires that mineral and medicinal waters originate from mineral springs and be directly bottled without modification of their mineral composition.[28] In contrast to mineral water, medicinal water possesses a minimum of valuable elements and a scientifically proven therapeutic effect that classifies medicinal water as a pharmaceutical.[29] In the present study, a systematic screening of boron concentrations of these waters was conducted to gain more insight into the role of mineral and medicinal waters as a source of boron intake. Previously, our group determined the lithium concentrations of mineral and medicinal waters derived from mineral springs throughout Germany and their bioavailability in humans.[30] In the present study, these waters were reanalyzed for boron concentrations. Accordingly, three different mineral waters with a low, medium, or high boron concentration were chosen for the bioavailability study in healthy male volunteers. To address the question of whether boron bioavailability/retention is evolutionarily conserved across species, the respective mineral waters were initially fed to the fruit fly *D. melanogaster*, which has been established as a versatile model organism in food and nutrition research.[31]

2. Results

2.1. The Boron Content of German Mineral and Medicinal Waters Varies Depending on the Geographical Origin

In total, the boron contents of 360 mineral and 21 medicinal waters from different mineral springs allocated throughout Germany were determined. The mean ± SD boron concentration in the tested waters was 176 ± 309 μg L^{-1}, while the median was 61 μg L^{-1}. The lowest boron concentration found in mineral water was 2 μg L^{-1}, and the highest was 2774 μg L^{-1} (see Supplementary Information). As shown in **Figure 1**a, the majority of the tested waters contained boron values between 10 and 49 μg L^{-1} (34.6%), followed by values between 50 and 100 μg L^{-1} and 100 and 199 μg L^{-1}, with each sharing approximately 14% of the waters. Relatively low (<10 μg L^{-1}) and relatively high (> 900 μg L^{-1}) boron concentrations were represented by 10.8 % and 3.1% of the samples, respectively. To consider the geographical impact of mineral water origin on boron accumulation, the data evaluation was clustered in German federal states and German districts possessing mineral springs. In Figure 1b, individual boron concentrations of mineral and medicinal waters originating from 13 German federal states are scattered. The highest mean boron concentrations were observed in waters from Baden-Wuerttemberg (2774, 1369 μg L^{-1}), Rhineland Palatinate (2193 μg L^{-1}), Nordrhein-Westphalia (1969, 1546 μg L^{-1}) and Hesse (1770 μg L^{-1}). In general, the mean boron concentrations of mineral waters originating from southwestern Germany were higher than those of mineral waters from northeastern and southeastern Germany. The districts with the highest mean boron values in mineral waters were Dortmund (1254 μg L^{-1}), Erlangen Höchstadt (851 μg L^{-1}), Göppingen (775 μg L^{-1}), Mühlheim an der Ruhr (748 μg L^{-1}), and Rhein-Lahn-Kreis (697 μg L^{-1}) (Figure 1c).

2.2. The Boron Content of German Mineral and Medicinal Waters Positively Correlates with the Concentration of Most Bulk Elements and the Trace Elements Lithium and Aluminum

To examine whether boron concentrations in mineral waters were statistically related to concentrations of bulk and other trace elements, linear regression was conducted (**Figure 2**). As shown in Figure 2a,b, boron in mineral water was positively correlated with bulk elements, such as magnesium, potassium, sodium ($p < 0.001$) and calcium ($p = 0.008$), while there was no correlation with phosphorous ($p = 0.169$). Moreover, boron was significantly positively correlated with the trace elements aluminum, iron, lithium ($p < 0.001$) and negatively correlated with manganese ($p = 0.017$) and zinc ($p = 0.005$).

2.3. Boron Retention in Fruit Flies Increases in Response to a Diet Supplemented With Boron-Rich Mineral Water

To examine boron retention across species, the fruit fly *D. melanogaster* was used as a model organism for the first time. Four different sugar yeast (SY) feeds with different boron contents owing to the different waters that were used, namely, deionized water without boron and mineral water with boron contents of 7.9 μg L^{-1} (LB), 113.9 μg L^{-1} (MB), and 2193.3 μg L^{-1} (HB), were prepared. After feeding the flies the experimental diets for 1 week, only the HB treatment resulted in significant body boron accumulation, with body boron contents rising from 665 ± 97.3 μg kg^{-1} at the SY control diet to 2820 ± 228 μg kg^{-1} in male flies, and rising from 313 ± 50.2 μg kg^{-1} for the SY control diet to 1360 ± 116 μg kg^{-1} in the female flies (**Figure 3**). Independent of the experimental diets, male flies contained at least twice as

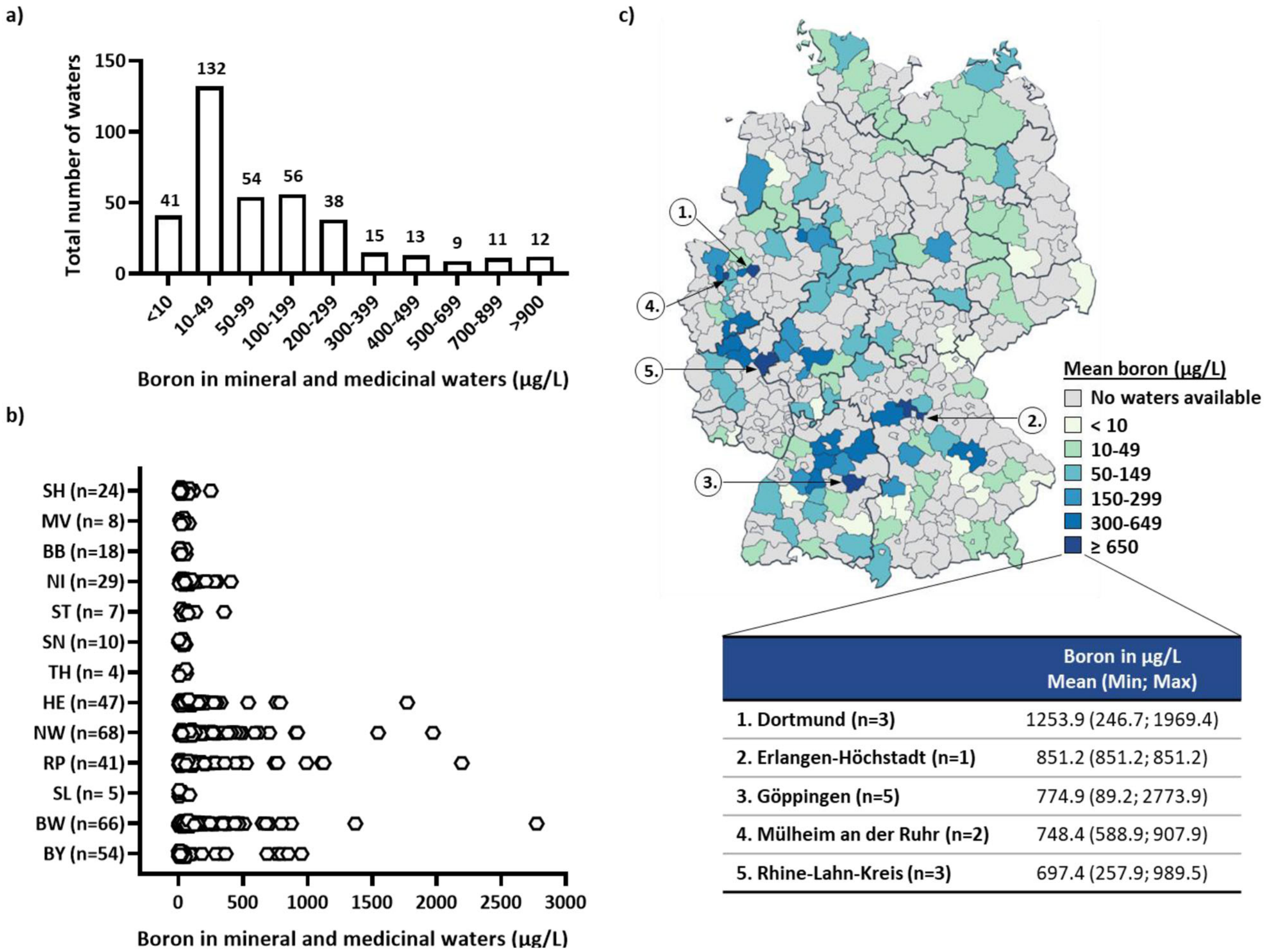


Figure 1. Boron concentrations in mineral and medicinal water from German source locations. a) Frequency distribution of boron concentrations in the total number of tested waters clustered in 10 groups. Boron concentrations between 10 and 49 μg L^{-1} were predominantly represented in 132 of 381 mineral and medicinal water samples. b) Scatter plot of individual boron concentrations from 381 water samples clustered in 13 German federal states. Abbreviations of federal states in Germany: Baden-Württemberg (BW), Bavaria (BY), Brandenburg (BB), Hesse (HE), Lower Saxony (NI), Mecklenburg Western Pomerania (MV), Nordrhein-Westphalia (NW), Rhineland Palatinate (RP), Saarland (SL), Saxony (SN), Saxony-Anhalt (ST), Schleswig Holstein (SH) and Thuringia (TH). c) Mean boron concentrations of mineral and medicinal water bottled from springs in 120 German districts. No water originating from the remaining 174 districts (grey) was available. The highest mean boron values were observed in districts from the southwest (dark blue) compared to the northeast of Germany (bright aquamarine). The chloropleth map was created with Datawrapper.

much boron as female flies. As depicted in Figure 3, the majority of excess boron (≈60% in male flies and 80% in female flies) accumulated in the insoluble fraction of the fly bodies.

2.4. In Humans, Boron-Rich Mineral Water Increases Serum Boron Concentrations and Urinary Boron Excretion

The three mineral waters with low, medium, or high boron contents were selected for a bioavailability study in humans. The characteristics of the 10 healthy male volunteers as well as a linear regression of age and relative fat free mass (FFM) with the respective baseline serum boron values are shown in **Figure 4**.

In total, 10 healthy male volunteers aged between 21 and 32 completed the study. With a body mass index (BMI) between 20 and 30 kg m^{-2} and a relative body fat mass (FM) between 5.5% and 26.5%, the study subjects were rated from very lean (5–8% FM) to obese (21–30% FM). Since the study was conducted in a crossover design comprising three different interventions, baseline circulating boron levels of each participant were measured at three independent time points. As shown in Figure 4b, the mean boron baseline levels in serum were positively correlated with FFM (%), while there was no significant relationship between baseline boron levels and age of the study subjects.

As depicted in **Figure 5**, a 24 h concentration–time profile of boron in serum and 24 h urinary boron excretion was calculated after ingestion of 1.5 L of the respective waters.

For each intervention, the mean baseline serum boron level was approximately 25 μg L^{-1}. After the consumption of LB water, circulating boron levels immediately decreased to 18 ± 5.3 μg L^{-1} at 1 h after ingestion, followed by a further decrease to 4.8 ± 3.0 μg L^{-1} within 24 h after ingestion. Due to the MB water

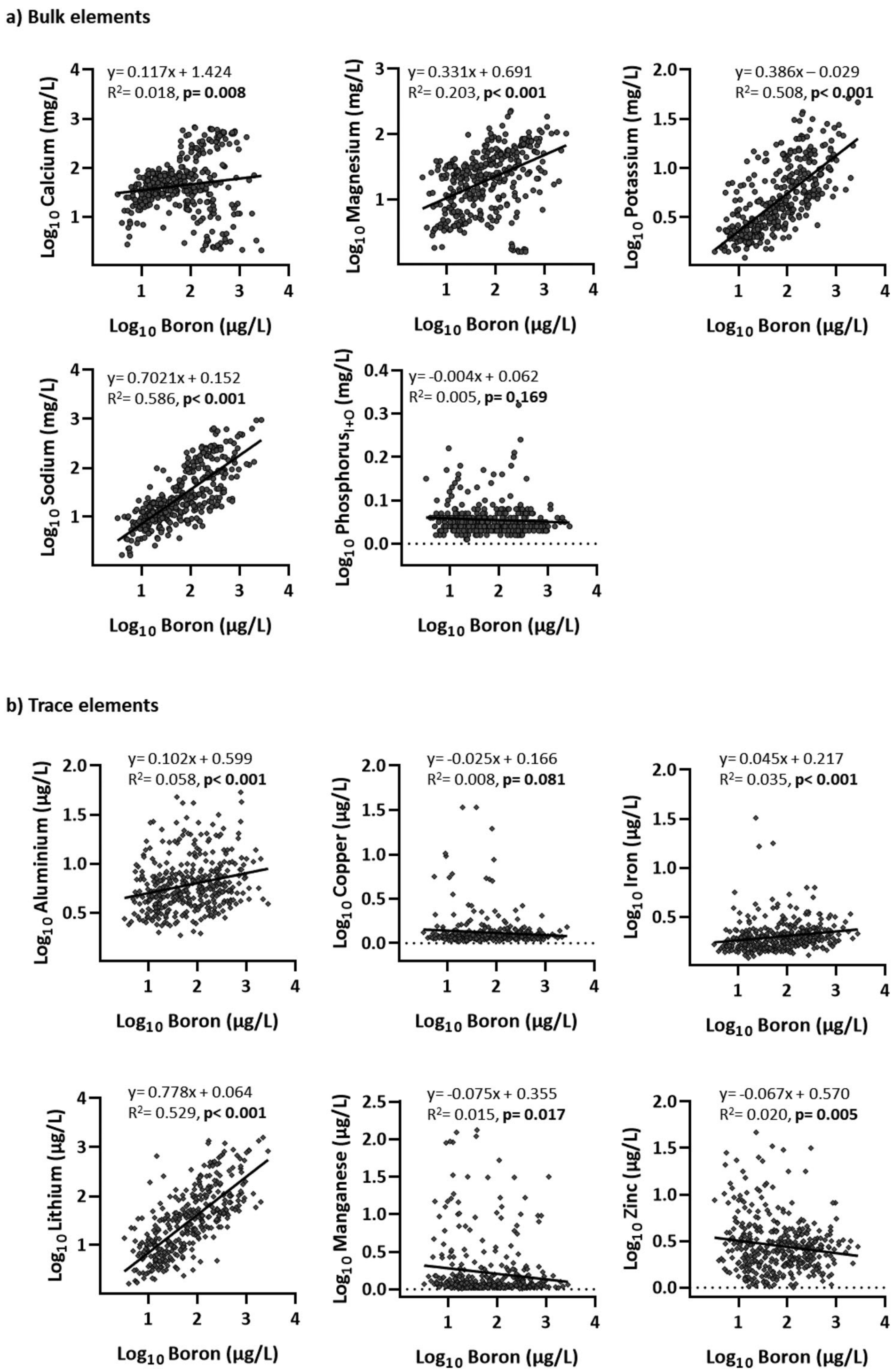


Figure 2. Linear regression of boron (µg L^{-1}) with bulk (mg L^{-1}) and trace elements (µg L^{-1}) determined in mineral and medicinal waters. Values of boron and bulk elements were determined in 381 mineral and medicinal water samples and were log_{10} transformed before linear regression to obtain more normal distributed data. a) Significant positive correlations of boron with calcium, magnesium, potassium, and sodium but not with $phosphorus_{I+O}$ (inorganic+organic). b) Boron in the mineral and medicinal water samples was positively correlated with aluminum, iron, and lithium concentrations of the respective waters. A slightly invers correlation of trace elements in mineral and medicinal water occurred between boron and manganese as well as zinc.

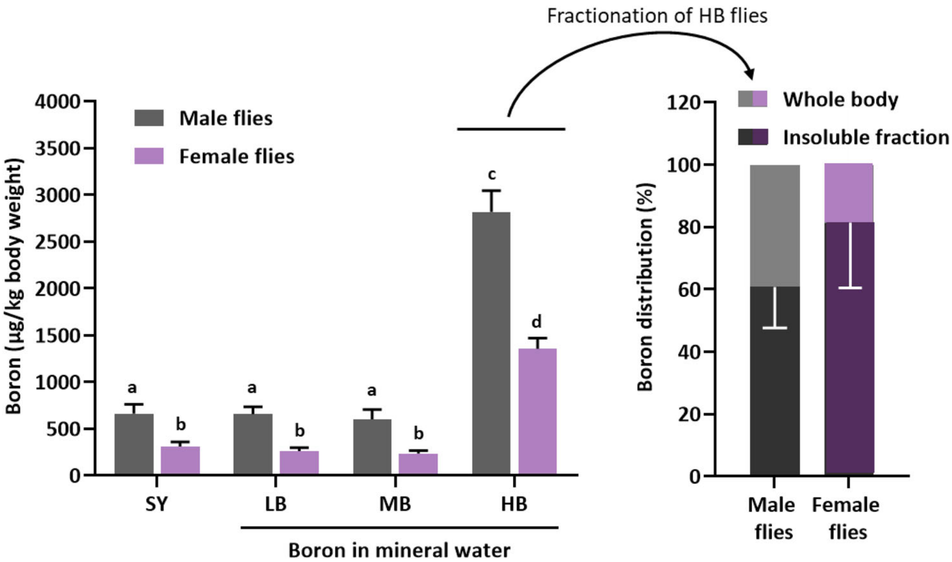


Figure 3. Boron-rich mineral water increases boron accumulation in male and female flies. Diets supplemented with mineral water containing low (LB) boron or median (MB) values did not change boron retention in male and female flies. However, in comparison to the sugar yeast (SY) control diet, the boron accumulation in male and female flies was significantly enhanced after 7 days of feeding a diet supplemented with boron-rich mineral water (HB). In each treatment group, boron concentrations (µg kg^{-1}) in male flies were approximately twice as high as those in female flies. Approximately 60% and 80% of the total boron was found within the insoluble fraction of male and female fly bodies. Data are the means ± SD (n = 2–4 independent experiments per group, comprising 100 flies per group). Different letters indicate significant differences ($p < 0.001$), which were calculated by the Games-Howell *post hoc* test.

intervention serum boron levels started to decrease after 1 h of ingestion and ended up in a final concentration of 14 ± 3.6 µg L^{-1} after 24 h. Only the HB water increased maximum boron serum levels up to 72 ± 5.8 µg L^{-1} (C_{max}) within 0.5 h after consumption. Furthermore, a negative incremental area under curve (iAUC) of −145 ± 31.8 µg L^{-1} x 24 h and -82 ± 22.8 µg L^{-1} x 24 h occurred after the ingestion of LB and MB mineral water, respectively (Figure 5c). While the urinary boron excretion did not differ between the LB and MB intake, it was significantly higher in the HB intervention compared to LB and MB intervention (3262 ± 430 µg d^{-1} vs 968 ± 358 µg d^{-1} and 1081 ± 386 µg d^{-1} resp.; both $p < 0.001$). The dietary boron intake (I) was considerably lower than the urinary excretion (E) following the LB (Δ_{I-E} = -638 ± 318 µg d^{-1}; $p < 0.001$) and MB (Δ_{I-E} = -585 ± 330 µg d^{-1}; $p < 0.001$) interventions, while the total dietary boron intake exceeded the 24 h urinary boron excretion in the HB group (Δ_{I-E} = 347 ± 384 µg d^{-1}; $p = 0.022$) (Figure 5b). For all interventions, individual baseline boron levels were inversely correlated with the 24 h iAUC (Figure 5d). Furthermore, there was a significant positive correlation between baseline boron levels and 24 h urinary boron excretion (Figure 5e).

3. Discussion

Our systematic screening of 360 mineral and 21 medicinal water samples from Germany revealed a strong impact of geographical origin on the boron content, with values ranging between 2.2 and 2774 µg L^{-1} boron. Interestingly, the maximum boron concentration was similar or even appreciably lower than the highest boron values found in ground water from private wells sampled in the Münster region in Western Germany. In several studies, maximum boron values of 2600,[32] 8000,[33] 8600,[34] and 10 600 µg L^{-1} [35] were found in ground water from the so-called "Emscher Mergel" area, which was explained by regional marl layers of the chalk era that are very rich in boron.[35] For the present investigations, operative mineral springs were solely available in the peripheral area of the "Emscher Mergel," namely, the district of Bielefeld, with mean boron values of 345 µg L^{-1} (min: 105 µg L^{-1}; max: 702 µg L^{-1}). Nevertheless, the Münster region is part of the federal state Nordrhein-Westphalia, which includes two districts (Dortmund, Mühlheim an der Ruhr) that exhibited the highest mean boron values of >650 µg L^{-1} in mineral water.

In accordance with our recently published data examining lithium in mineral waters, there was likewise a northeast to southwest gradient in boron concentrations in the tested mineral waters.[30] Out of the six trace elements analyzed (aluminum, copper, iron, lithium, manganese, zinc), three elements, namely aluminum, iron, and lithium were positively associated with the detected boron concentrations in a highly significant manner ($p < 0.001$). The enrichment of mineral water with bulk and trace elements is mainly determined by geological factors such as the mineral composition of the continental crust, which is very diverse throughout the world.[36] Interestingly, some studies observed similar lithium and boron contents of the upper continental crust, which were 22 and 9 µg g^{-1}, respectively, in Canada[37] as well as 20 and 28 µg g^{-1} [38] or 21 and 17 µg g^{-1} in East China,[39] respectively. Moreover, boron concentrations in the tested mineral waters were significantly positively correlated with calcium, magnesium, potassium, and sodium and with the exception of calcium, in accordance with results derived from drinking water analyses in northern France.[2]

a)

	Mean ± SD	Min; Max
Age	26.0 ± 3.0	21; 31
Body mass index (m²/kg)	23.9 ± 2.8	20.3; 30.1
Fat mass (kg)	14.7 ± 7.4	4.3; 26.3
Fat mass (%)	18.4 ± 7.2	5.5; 28.5
Fat free mass (kg)	62.9 ± 7.9	53.2; 74.7
Fat free mass (%)	81.6 ± 7.2	71.5; 94.5

b)

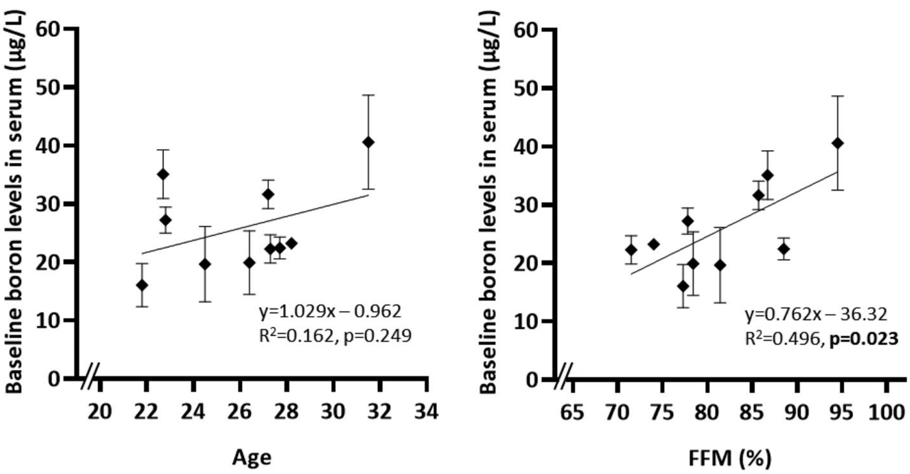
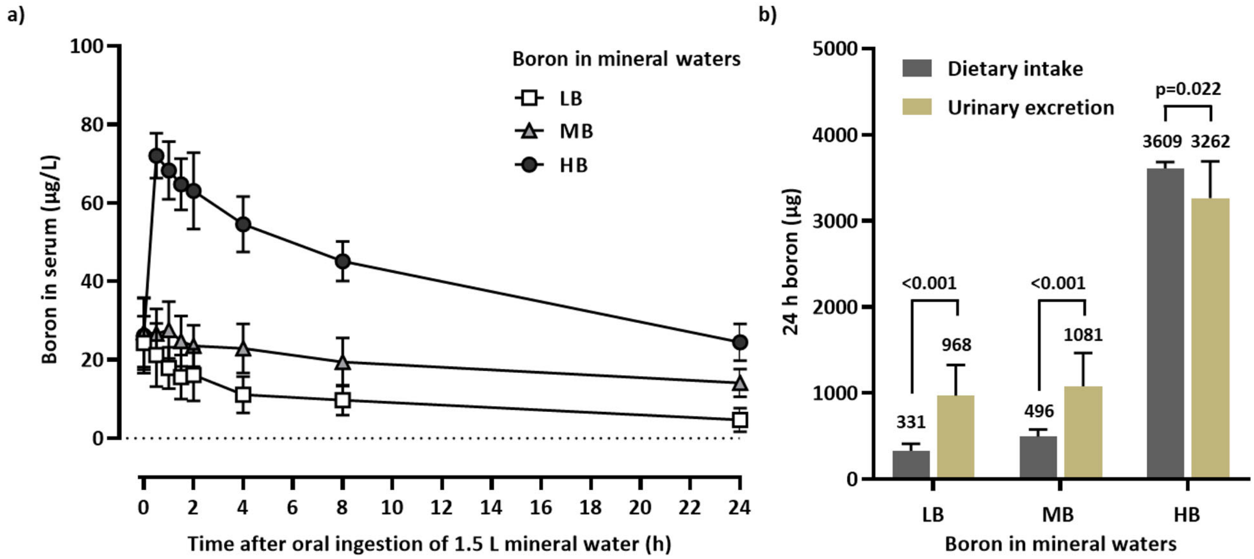


Figure 4. Baseline characteristics of the participants (n = 10). a) Baseline characteristics of 10 healthy male volunteers aged between 21 and 31 years. Body fat mass and fat-free mass were measured via air displacement plethysmography. b) The mean baseline serum boron concentration of each participant was calculated from three different timepoints. The mean baseline boron concentration in serum was positively correlated with the percentage fat-free mass (FFM%) but was not significantly related to the age of the participants.

The current study further demonstrates that even mineral water samples originating from adjacent German districts may accumulate highly diverse boron concentrations. For example, mineral water samples from Dortmund exhibited a mean boron concentration of 1254 µg L^{-1}, while 28 µg L^{-1} boron was found in water samples from the neighboring district of Recklinghausen. Thus, large inter-individual differences in boron intake via mineral or drinking water should be considered for the German population.

To gain further insight into boron retention and distribution across species, different dietary boron concentrations were applied to the model organism *D. melanogaster*. Independent of the diet, male flies accumulated significantly higher boron concentrations than female flies. In general, male flies are smaller and possess a lower body fat content than female flies.[40,41] This is in accordance with the present data in humans showing that a higher lean mass is associated with a higher baseline boron status. A gender effect of boron accumulation was also observed in healthy Croatian adults. Interestingly, the median hair boron concentration in men (2.210 µg g^{-1}) was shown to be considerably higher than that in women (1.040 µg g^{-1}), while the median whole blood boron concentrations in male (40 µg kg^{-1}) and female (41 µg kg^{-1}) subjects did not differ.[42] The authors further suggested that boron plays an essential role in the metabolism of the connective tissue and bone matrix.[42] Insects lack a $CaCO_3$ endoskeleton and instead possess a chitin exoskeleton. In this regard, it is remarkable that in male and female flies that received a boron-rich diet, 60% and 80% of the total body boron was found in the insoluble chitin exoskeleton-enriched fraction of their body lysates, respectively. Thus, we suggest that boron predominantly accumulates in the exoskeleton of *D. melanogaster*, which is similar to the situation in humans, where dietary boron is incorporated to a great extent into endoskeletal bone tissue compared to other human tissues.[13,43] Accordingly, it is tempting to speculate that boron may have a role in cuticle formation and mammalian bone health.[15,44,45] Moreover, Massie et al. observed similar boron levels in *D. melanogaster* and tissues of mice and humans.[9] In conclusion, we suggest that the fruit fly is an appropriate model organism to study boron retention and tissue distribution in response to dietary boron supply.

The bioavailability study of boron after consumption of three different mineral waters in humans revealed mean baseline levels of circulating boron of approximately 25 µg L^{-1}. This was comparable with baseline boron levels found in other studies, e.g., 14 [46] and 22 µg L^{-1}.[47] Moreover, the consumption of HB mineral water led to an increase in serum boron concentrations up to



c)

Mineral water	iAUC	T_{max} (h)	$T_{1/2}$ (h)	C_{max} (µg/L)	C_{min} (µg/L)
LB	- 145.1 ± *31.76* [c]	0.10 ± *0.21* [b]	3.17 ± *1.53* [b]	24.26 ± *6.84* [b]	4.66 ± *3.02* [c]
MB	- 82.3 ± *22.79* [b]	0.65 ± *0.34* [a]	5.71 ± *2.09* [a]	27.02 ± *8.87* [b]	14.10 ± *3.57* [b]
HB	397.6 ± *42.82* [a]	0.55 ± *0.16* [a]	6.98 ± *1.05* [a]	72.02 ± *5.78* [a]	24.43 ± *5.71* [a]

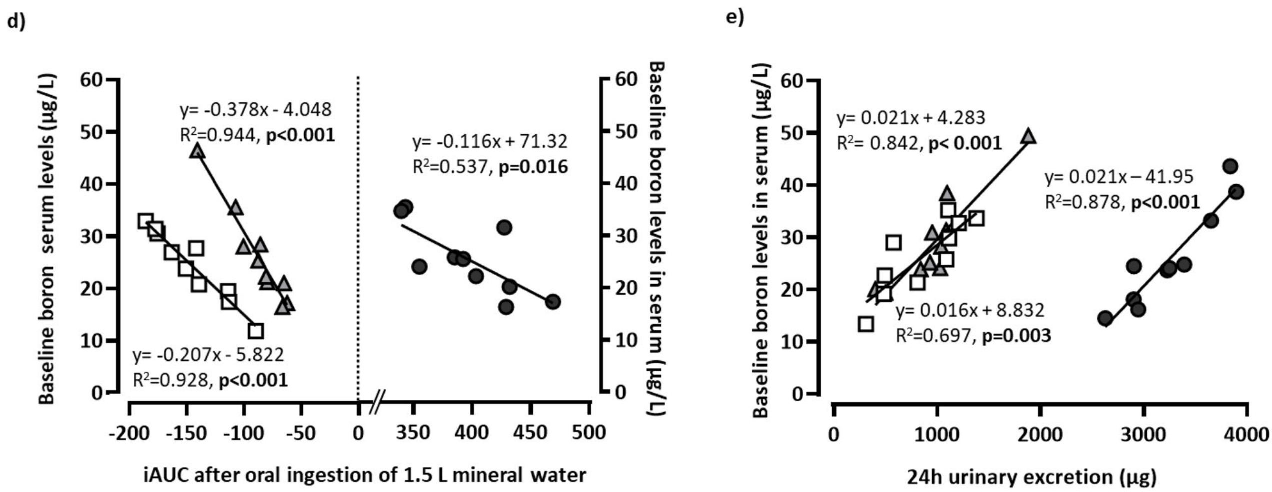


Figure 5. Dose-dependent response in serum boron concentrations and total urinary excretion. a) The mean serum concentration–time profiles of boron 0, 0.5, 1, 1.5, 2, 4, 8, and 24 h after oral ingestion of 1.5 L of mineral water with low (LB), medium (MB), and high boron (HB) contents. Solely HB mineral water led to an increase in serum boron after 0.5 h of ingestion. b) Total dietary intake and urinary excretion of boron within 24 h after consumption of 1.5 L of the three different mineral waters. The 24 h boron uptake was calculated as total boron uptake via mineral water and low-boron food that was provided to the participants. In the groups receiving MB and LB mineral water, urinary excretion exceeded the nutritional uptake of boron. c) Pharmacokinetic parameters of boron calculated as the incremental area under curve (iAUC), time of maximum boron concentration (T_{max}), half-live of boron ($T_{1/2}$), maximum boron concentration (c_{max}), and minimum boron concentration (c_{min}). Consumption of HB mineral water increased mean serum levels of boron up to 72.02 ± 5.78 µg L^{-1} (c_{max}) with a positive iAUC, while consumption of MB and LB waters did not enhance serum boron levels and resulted in a negative iAUC. a–c) Data are expressed as the mean ± SD (n = 10). Different letters indicate significant differences ($p < 0.05$), which were calculated by either Tukey's or Games-Howell *post hoc* tests. d) Linear regression showing a significant inverse correlation of individual boron baseline levels and the iAUC of the participants. e) Linear regression showing a significant positive relationship between individual boron baseline levels and 24 h boron excretion.

72 ± 5.8 μg L^{-1} and a subsequent decline over 24 h to final circulating boron levels that were again comparable to baseline values. Interestingly, the C_{max} of 72 μg L^{-1} was attained approximately 0.5 h after consumption of the HB mineral water, which indicates rapid absorption of boron. Within the normal pH range of the gastrointestinal tract, boron exists in its undissociated/uncharged form as boric acid, $B(OH)_3$, which makes it highly permeable for biological membranes. Therefore, the permeability coefficient is much higher compared to hydrated ions such as calcium or iron.[48] Whether there are additional important channel- or transporter-mediated transport mechanisms through animal epithelial cells is still a matter of debate.[49–51]

The LB and MB mineral waters did not provide sufficient amounts of boron to maintain the baseline boron status, as indicated by decreasing serum boron concentration over time and a negative iAUC. These results may indicate that low dose boron intake is not counter-regulated by mechanism such as increased renal reabsorption of boron. Hence, only HB mineral waters increased boron status in humans, which may reflect adequate boron intake.

In addition to the tested water samples, the volunteers were provided with low-boron meals and 1.5 L of low-boron mineral water. Nevertheless, the daily total mean boron intake was 331 ± 81.2 μg d^{-1} in the LB group, 496 ± 80.1 μg d^{-1} in the MB group, and 3609 ± 75.7 μg d^{-1} in the HB group. In subjects who drank LB and MB waters, 24 h urinary boron excretion exceeded the 24 h dietary boron intake (968 ± 357 μg d^{-1} vs 331 ± 81.2 μg d^{-1}; 1081 ± 386 μg d^{-1} vs 496 ± 80.1 μg d^{-1}). Interestingly, it appeared that the 24 h urinary boron excretion was at least approximately 1000 μg d^{-1}. This concentration was also recommended by Nielson and Meacham as the minimum dietary boron intake for humans.[52] Presumably, in the present study, additional body boron was mobilized when dietary boron intake was less than 1000 μg d^{-1}. Boron intake and excretion seemed to be in balance when the total dietary boron intake was approximately 3500 μg d^{-1}. Based on the current data, it remains to be elucidated whether a boron intake lower than 3500 μg d^{-1} leads to a balanced boron supply. Naghii and Samman determined a urinary boron excretion of ≈1900 μg d^{-1} [53] in Australian subjects, which effectively equals the boron intake of 2200 μg d^{-1} calculated in another study by this group.[17] In contrast, in a study from Sutherland et al., dietary boron intake (1870 μg d^{-1}) was considerably exceeded by urinary excretion (2860 μg d^{-1}). In addition, additional boron losses via faeces need to be taken into consideration.[11] Assuming that there are further unknown boron losses (e.g., via sweat), data indicate a minimum nutritional boron intake of 2000–3000 μg d^{-1} for a balanced boron supply. Merely six German mineral waters analyzed in the current study would provide >2000 μg boron during a water intake of 1.5 L per day. Nevertheless, it should be noted that a healthy eating pattern includes food items that also contain considerable amounts of boron, including fruits, vegetables and nuts. However, further studies are needed to pinpoint the minimal nutritional boron uptake that maintains boron homeostasis. Our data further revealed a negative correlation between individual baseline levels of circulating boron and the respective iAUC, while baseline boron levels were positively correlated with 24 h urinary boron excretion of the participants. Individuals with lower (<20 μg L^{-1}) baseline boron levels excreted less boron via urine compared to individuals with higher (>20 μg L^{-1}) baseline boron levels in serum. Whether this is due to a homeostatic regulation warrants further investigations. The initial boron status of the recruited subjects was also associated with body composition. The percentage FFM (%) was positively correlated with baseline boron levels ($p = 0.023$). In turn, baseline boron levels are inversely associated with the percentage fat mass (FM%). These outcomes may indicate a relation between circulating boron levels, healthy eating habits (a diet rich in nuts, fruits, and vegetables) and body weight control. Apart from that, more detailed information about body composition is needed to explain the relationship between boron status and the distribution of non-adipose tissues such as muscle, bone, or organ masses.

Overall, current data demonstrate that water in Germany varies significantly in the content of boron and that only boron-rich mineral water improves the boron status in both flies and humans.

4. Experimental Section

Sampling of Mineral Waters: The sampling procedure and geographical distribution of the mineral springs were already described in our previous publication.[30] In short, sampling took place between November 2017 and February 2018 throughout Germany. In total, 360 mineral and 21 medicinal water samples were collected. Ten-millilitre aliquots of the corresponding waters were placed into polyethylene Falcon tubes with screw caps (Sarstedt, Nuembrecht, Germany). Tubes were stored in carton boxes at 10 °C until boron analysis.

Analysis of Bulk and Trace Elements: In general, concentrations of bulk and trace elements were determined via an inductively coupled plasma-mass spectrometry (ICPMS) ICAP Q instrument (Thermo Fisher Scientific, Waltham, MA, USA) at SYNLAB (Jena, Germany). Measurements were conducted in accordance with DIN EN ISO 17294-2: 2017-01. Samples were decomposed with a mixture of nitric acid and hydrogen peroxide (4:1) using microwave pressure digestion. For calibration, a multi-element standard was used. Under the selected conditions, for boron the limit of detection (LOD) of the diluted (1:50) samples was 13 μg kg^{-1}, the limit of quantification (LOQ) was 50 μg kg^{-1}, and the recovery and intra-day precision was 101.8% and 2.35%, respectively. To minimize spectroscopic interferences a collision/reaction cell was used for detection. Rhodium (2 μg L^{-1}) was added as the internal standard.

Studies with Fruit Flies: Stocks of the *D. melanogaster* wild-type strain w^{1118} (Bloomington Drosophila Stock Center #5905, Indiana University, Bloomington, IN, USA) were maintained at 25 °C and 60% humidity under a 12/12 h light/dark cycle on standard Caltech fly medium (CT medium: 5.5% dextrose, 3.0% sucrose, 6.0% corn meal, 2.5% inactive dry yeast, 1.0% agar, 0.3% nipagin, and 0.3% propionic acid) according to.[54] For boron supplementation experiments, 250–300 synchronized fly eggs were given into culture bottles filled with 25 mL of CT medium, and animals were raised under standard culture conditions. Three days after hatching from pupae, the adult flies were separated by sex and transferred to SY-based medium (10% sucrose, 2% Drosophila agar, 10% inactive dry yeast, 0.3% nipagin, and 0.3% propionic acid) prepared as recently published.[55] For the control groups, SY was prepared by using boron-free deionized water. For the treatment groups, three different commonly available sparkling mineral waters, namely, "Trendic medium" (Hansa-Heemann AG, Rellingen, Germany), with 7.9 μg boron kg^{-1} (LB); "Gerolsteiner medium" (Gerolsteiner Brunnen GmbH & Co. KG, Gerolstein, Germany), with 113.9 μg boron kg^{-1} (MB); and "Perling medium" (Rhenser Mineralbrunnen GmbH, Rhens, Germany), with 2193.3 μg boron kg^{-1} (HB) were used instead of deionized water. After 7 days of feeding, flies were harvested, transferred to empty bottles and kept under standard conditions for 2 h for gastric emptying. After the first hour of starvation, the bottle was exchanged to reduce coprophagy. Subsequently, the flies were stored at −80 °C. The body weights of 95–100 animals per treatment group were determined before their boron content was analyzed. To elucidate whether dietary boron accumulates in the soluble fraction of fruit fly bodies, animals that had been fed a diet containing 2200 µg kg^{-1} boron were homogenized in boron-free distilled water. After centrifugation at 9000 × *g* for 6 min, the pellet (insoluble fraction including the chitin exoskeleton) was used for boron analysis.

Human Bioavailability Study: The bioavailability study of boron was conducted in a randomized cross-over design with 10 healthy male volunteers aged between 20 and 40 years who were recruited in August 2018. The sequence of the intervention days was block-randomized and each participant went three times through a 24 h intervention phase (3×24). Baseline characteristics of the participants were determined at the beginning of the study period after an overnight fast. Body composition was analyzed by air displacement plethysmography using the BOD POD device (Cosmed, Rome, Italy) to determine fat mass (FM) and fat free mass (FFM), while body weight was assessed by an electronic scale coupled to the BOD POD device.

The study protocol was approved by the ethics committee of the medical faculty, Christian-Albrechts-University Kiel, Germany (D 484/18), and registered with the DRKS-ID DRKS00016063. All subjects provided written informed consent before participation. A detailed study protocol was described in our previous publication.[30] In short, the day before the intervention started, the participants received mineral water (max. 3 L) and a standard dinner both low in boron to ensure equal baseline conditions for the following intervention day. The intervention started after an overnight fast with no beverage consumption. The beverages tested were three mineral waters that were already used for studies in flies: "Trendic medium" (LB); "Gerolsteiner medium" (MB); and "Perling medium" (HB). After fasting blood sampling, subjects consumed 1.5 L of mineral water from the tested beverages within 45 min, and further blood samples were taken after 0.5, 1, 1.5, 2, 4, 8, and 24 h to determine serum boron levels. To assess total boron excretion, the participants also collected 24-h urine samples during the intervention day. The participants received lunch and dinner low in boron, which are both described in Seidel et al.[30] During the whole intervention time the participants only ate and drank food and beverages that were provides by the study mentors. Subjects were also instructed to abstain from vigorous physical activity during the study periods to avoid boron losses due to increased sweating.

Calculations and Statistical Analysis: The geographical distribution of the collected mineral water samples and the chloropleth map were created by datawrapper.de. The kinetics of boron in serum were assessed by the following parameters: iAUC, T_{max}, $T_{1/2}$, and C_{max} as well as C_{min} boron concentrations within 24 h. Total urinary boron excretion was determined as the product of the lithium concentration (µg L^{-1}) in urine and the 24 h urine volume. For bivariate analysis, Pearson's correlation coefficient and linear regression were calculated using GraphPad PRISM software (San Diego, CA, United States). Statistical hypothesis tests were conducted with IBM SPSS Statistics 24 (Ehningen, Germany). Therefore, groups were analyzed for normality of the distribution (Shapiro-Wilk tests). In the case of normally distributed data, Levene's test was conducted to assess the homogeneity of variances. If the null hypothesis was rejected (Levene's test not significant), one-way analysis of variance (ANOVA) with a one-sided Tukey test as *post hoc* analysis was performed. If the null hypothesis was confirmed (Levene's test was significant), the Games–Howell *post hoc* test was used.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

All of the authors contributed significantly to the research. Study design: G.R., M.B. and A.B.W.; methodology, data curation and statistical analysis: U.S., F.H., K.L., K.J., Y.S., D.K., S.B.; collection of water samples: E.B.; bioavailability study with humans: A.B.W., F.H.; bioavailability study with flies: K.L., K.J., Y.S.; writing-original: U.S., G.R., K.L.; writing-review-editing: all authors. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

The data that supports the findings of this study are available in the supplementary material of this article

Keywords

beverages, food database, fruit fly, serum boron, trace elements

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