Kinetics of sulforaphane in mice after consumption of sulforaphane-enriched broccoli sprout preparation

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Scope: Sulforaphane (SF) is a natural isothiocyanate in broccoli sprouts with cancer chemopreventive activity. This study is aimed to use different methods to develop broccoli sprout preparations to compare their ability to deliver SF to the mice and to evaluate the kinetics and biodistribution of SF.

Methods and results: The SF-enriched sprout preparation generated by two-step procedure (quick-steaming followed by myrosinase treatment) contained the highest level of SF, which was 11 and 5 times higher than the freeze-dried fresh broccoli sprouts and the quick-steamed, freeze-dried broccoli sprouts, respectively. After oral administration of 2.5 mg/g body weight of the broccoli sprout preparations, SF was quickly absorbed and distributed throughout the tissues. The SF-rich preparation resulted in the highest exposure, with peak plasma SF concentration of 337 ng/mL, which is 6.0 times and 2.6 times higher compared to the other two preparations. A whole body physiologically based pharmacokinetic model (developed with ADAPT 5 software) suggests that distribution of SF is perfusion-limited in all organs.

Conclusion: This study provides a broccoli sprout preparation that can serve as a good source of SF, and the model can be utilized to guide the dose designed for the use of broccoli sprout preparation in chemoprevention.

Keywords: Broccoli sprout / Cancer chemoprevention / Kinetics / Mouse / Sulforaphane

1 Introduction

Numerous studies continue to support that dietary intake of cruciferous vegetables, especially broccoli and broccoli sprouts, may reduce the risk of different types of malignancies [1]. The cancer chemopreventive properties of these vegetables have been primarily attributed to isothiocyanates that occur naturally as the glucosinolate precursors in the plant [1, 2]. In particular, sulforaphane (SF), a member of the isothiocyanate family, has received extensive attention for its potent chemopreventive activity [3, 4]. SF has been shown to be not only effective in preventing chemically induced cancers in animal models [4–7], but also inhibit the growth of established tumors [8, 9]. Early research focused on induction of Phase 2 enzymes and inhibition of Phase 1 enzymes by SF, which enhances the detoxification of carcinogens [1, 10]. Following studies suggest that SF offers protection against tumor development during the “postinitiation” phase by controlling cell proliferation, differentiation, apoptosis, cell cycle, angiogenesis, and metastasis [1, 11]. During the past few years, SF has been shown to target cancer stem cells through direct or indirect influence on the self-renewal pathways of cancer stem cells in a number of studies [12–15].

SF is converted from glucoraphanin, the major glucosinolate in broccoli and broccoli sprouts, by myrosinase, a β-thioglucosidase [4]. Broccoli sprouts contain approximately 20 times more glucoraphanin than mature broccoli, which represents 74% of all glucosinolates in the sprouts [16]. Myrosinase is physically separated from glucosinolates in the

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intact plant cells. Disruption of the plant during harvesting, processing, and chewing leads to loss of cellular compartmentalization and subsequent mixing of glucoraphanin and myrosinase to produce SF [17, 18]. Epithiospecifier protein (ESP), another protein naturally occurring in broccoli and broccoli sprouts, directs hydrolysis of glucoraphanin toward SF nitrile [19]. While SF has been shown to possess cancer chemopreventive properties, SF nitrile has not.

Glucoraphanin is abundant in broccoli and broccoli sprouts, however, the processing of these vegetables often results in decreased intake of SF. Cooking procedures that inactivate plant myrosinase and ESP were shown to significantly reduce the bioavailability of SF [20], although the conversion of glucoraphanin to SF can be also mediated by the microflora in human gastrointestinal tract [21]. In addition, SF is relatively thermolabile [22, 23]. It was shown to rapidly degrade when the temperature was above 60°C [23]. Glucoraphanin in broccoli sprouts, on the other hand, is relatively stable to heat. It was suggested that thermal degradation is unlikely to be a major cause of glucosinolate loss when cooking time is less than 10 min [24]. The loss of glucoraphanin during cooking is primarily due to the leaching into the cooking water [25].

Therefore, the two main objectives of this study are: (i) to use different methods to develop three broccoli sprout preparations and to compare their ability to deliver SF in vivo; and (ii) to study the pharmacokinetics and for the first time to build a physiologically based pharmacokinetic (PBPK) model of SF in mice after oral administration of the broccoli sprout preparations. This study will provide useful information to optimize dose regimen of broccoli sprout preparation for cancer chemoprevention studies in the mouse and help make prediction for human studies in the future.

2 Materials and methods

2.1 Chemicals

SF and sulforaphane glutathione conjugate (SF-GSH) were purchased from LKT Laboratories (St. Paul, MN). Glucoraphanin was obtained from Cfm Oskar Tropitzsch (Marktredwitz, Germany). Sinigrin hydrate and myrosinase (thioglucosidase) were from Sigma-Aldrich (St. Louis, MO). ACN and methanol of HPLC-grade were purchased from Fisher Scientific (Pittsburgh, PA). Ammonium acetate (HPLC-grade) and formic acid (spectroscopic grade) were purchased from Sigma-Aldrich.

2.2 Broccoli sprout preparations

Broccoli sprouts were purchased from a local Whole Foods Market (Ann Arbor, MI). The procedure of making broccoli sprout preparations is described below and illustrated in Fig. 1. Fresh broccoli sprouts were quick-frozen in liquid nitrogen, and freeze-dried for 48 h. The dried sprouts were then ground into fine powder with pestle and mortar to get “fresh preparation” (FR preparation).

To obtain “steamed preparation” (ST preparation), fresh broccoli sprouts were quick-steamed for 10 min over boiling water, frozen in liquid nitrogen, freeze-dried for 48 h, and then ground into fine powder. The length of steaming was determined based on several studies that had been done to characterize the thermal stability of myrosinase and ESP in intact cruciferous vegetables [26, 27]. The activity of broccoli myrosinase was shown to be reduced by more than 95% after a 10 min treatment at 70°C [27]. The activity of ESP (which is more heat-labile than myrosinase) in broccoli and broccoli sprouts was completely eliminated after the vegetables had been heated at 70°C for 5 min or 60°C for 10 min [26].

The ST powder (3 g) was incubated with approximately 5 units of myrosinase in water at 37°C for 4 h, with occasional stirring. The mixture was then frozen in liquid nitrogen and freeze-dried for 3 days to get SM preparation (steamed preparation followed by myrosinase treatment). The three preparations were stored at −80°C until used.

2.3 Determination of glucoraphanin in broccoli sprout preparations

The procedure for measurement of glucoraphanin in broccoli sprout preparations was modified from previously reported method described by Tian et al. [28]. Briefly, 50 mg of sprout preparation was sonicated for 30 min at 70°C in 1 mL of 70% aqueous methanol (1 mM sinigrin as internal standard). After cooling in an ice bath, the supernatant of the extracts was collected by centrifugation at 4000 rpm for 10 min. The extraction procedure was repeated three times and the supernatants were combined to ensure the recovery. The 450 µL of each extract was transferred to a glass vial and dried under nitrogen gas. The dried samples were reconstituted in 500 µL water, filtered through 0.2 µm nylon filters, and diluted 1000-fold in ACN (containing 5 mM ammonium acetate) for analysis.
All mass spectra were obtained using a 3200 Q-Trap linear ion trap quadrupole mass spectrometer (Applied Biosystems, Carlsbad, CA) coupled to an Agilent 1200 Series HPLC system (Agilent Technologies, Santa Clara, CA). HPLC separation was performed on a Luna 3-µm HILIC column (50 × 2.00 mm, 3 micron; Phenomenex, Torrance, CA). Mobile phase A (ACN/water 50%/40% containing 5 mM ammonium acetate) was first kept at 0% for 2.5 min, then increased linearly to 50% in 2.5 min, returned to 100% B (ACN containing 5 mM ammonium acetate) and maintained for 5 min. The flow rate was 400 µL/min. Negative ion MS/MS was conducted to detect glucoraphanin and sinigrin, respectively. A dwell time of 200 ms was used for each transition. Data acquisition and quantitation were performed using Analyst software version 1.4.2 (Applied Biosystems).

2.4 Determination of SF in broccoli sprout preparations

The procedure for measurement of SF in broccoli sprout preparations was modified from a previously reported method [29]. Briefly, 50 mg of sprout preparation was extracted with 1 mL of ethyl acetate (1 mM sinigrin as internal standard) by vortexing for 1 min and sonicating in ice bath for 10 min. After centrifugation at 4000 rpm for 10 min, the organic layer was collected. The extraction procedure was repeated three times, the organic phases were combined and dried under nitrogen gas. The dried samples were reconstituted in 1 mL of a mixture of mobile phase A (water containing 5 mM ammonium acetate and 0.1% formic acid) and B (ACN containing 0.1% ammonium acetate) (50:50), sonicated in ice bath for 10 min, filtered through 0.2 µm nylon filters, and diluted 1000-fold for analysis.

HPLC separation was performed on an Agilent ZORBAX 5-µm extend-C18 column (50 × 2.1 mm) (Agilent Technologies). Mobile phase A (water containing 5 mM ammonium acetate and 0.1% formic acid) was first kept at 95% for 1 min, then decreased to 10% and maintained for 2 min, returned to 5% B (ACN containing 0.1% ammonium acetate) and maintained for 3 min. The flow rate was 400 µL/min. Positive ion MS/MS was conducted to detect SF. The mass spectrometric conditions were as follows: source temperature, 400°C; curtain gas (CUR), 30 psi; ionspray voltage (IS), 5500 V; desolvation gas temperature (TEM), 400°C; ion source gas 1 (GS1), 60 psi; ion source gas 2 (GS2), 40 psi; collision gas (CAD), high; entrance potential (EP), 4 eV; collision energy (CE), 15 eV. The transition 178 > 114 was used to detect SF, and a dwell time of 200 ms was used for the transition.

2.5 Collection of animal samples

All the animal experiments were performed under the guidelines of the University Committee on Use and Care of Animals (UCUCA) at University of Michigan. Female CD-1 mice (20–25 g in body weight) were purchased from Charles River Laboratories (Wilmington, MA), and randomly separated into groups (three mice per time point for each broccoli sprout preparation). Broccoli sprout preparations were suspended in PBS solution on ice, and administered to the mice through oral gavage (2.5 mg/g body weight). Blood samples were collected into heparinized tubes from each mouse by terminal cardiac puncture at pre dose and 0.5, 1, 2, 4, 6, 8, 12, and 24 h post dose. The plasma fraction was immediately separated by centrifugation at 2000 × g for 10 min at 4°C and stored at −80°C until analysis. Tissue samples, including heart, liver, kidney, lung, muscle, and mammary fatpad were immediately dissected, weighed, snap-frozen and ground with mortar and pestle in liquid nitrogen, and then stored at −80°C until analysis.

To collect urine and feces, mice (n = 5/group) were placed in metabolic cages (Harvard Apparatus, South Natick, MA) and dosed with broccoli sprout preparations (2.5 mg/g body wt) via oral gavage. Pooled urine and feces samples were collected at pre dose and 1, 2, 4, 6, 8, 12, and 24 h post dose, and stored at −80°C until analysis.

2.6 Determination of SF and SF-GSH in animal samples

Tissue samples and feces were homogenized using a tissue homogenizer (Tissuemiser Homogenizer, Fisher Scientific, Pittsburgh, PA). Plasma, tissue homogenate, urine, or feces homogenate (50 µL) was extracted with 400 µL methanol containing 0.5% formic acid (1 mM sinigrin as internal standard) and vortexed for 4 min, as described previously [30]. The supernatant was collected after centrifugation at 13 000 rpm for 5 min. The 450 µL of supernatant was transferred to a clean centrifuge tube and dried. The dried sample was reconstituted in 200 µL of mixture of mobile phase A (water containing 0.1% formic acid and 5 mM ammonium acetate) and B (ACN containing 0.1% formic acid), mixed for 3 min, and centrifuged at 13 000 rpm for 5 min. The supernatant was transferred to HPLC vial for analysis. Similarly to above, an Applied Biosystems 3200 Q-Trap linear ion trap quadrupole mass spectrometer coupled to an Agilent 1200 Series HPLC system was used for the quantification of SF and SF-GSH. The transition 485.3 > 179 was used to detect SF-GSH.

2.7 Kinetic analysis and PBPK modeling

The plasma concentration data were analyzed by noncompartmental pharmacokinetic model using WinNonlin software
A whole body PBPK model was built with blood and tissue concentration-time data after oral administration of SM broccoli sprout preparation (2.5 mg/g body weight) using ADAPT 5 software [31]. In this model, the whole body of the mouse is composed of seven tissue compartments and one blood compartment; a “rest-of-body” compartment is incorporated to represent all the unsampled tissues (depicted in Fig. 4). The amount of SF entering the blood was calculated with the bioavailability (F%) published by Lin et al. [32]. For each individual organ, as the equation shows, change in amount of drug in the tissue is equal to the difference between rate in and rate out.

\[
\frac{dC_T}{dt} = Q \cdot C_{in} - Q \cdot C_{out} = Q \cdot (C_{in} - C_T) / k_p
\]

The physiological parameters were either obtained from literature [33–35] or determined by experiment and listed in Table 3. The parameters obtained from individual organ models were used as initial estimates for the whole body model. The initial estimates of partitioning coefficient were determined by the ratio of the area under concentration curves (AUC) between tissue and blood. The AUC values were calculated with noncompartmental analysis by WinNonlin software (Pharsight Corp.). All equations were solved simultaneously with the maximum likelihood estimator in the ADAPT 5 software [31].

### 2.8 Statistical analysis

Statistical differences were determined using two-tailed Student t-test. Data are presented as mean ± SD (n ≥ 3).

### 3 Results and discussion

#### 3.1 Development of broccoli sprout preparations

Although glucoraphanin is abundant in broccoli and broccoli sprouts, the processing of broccoli/broccoli sprouts often results in the loss of SF. This is attributed to several factors—the presence of ESP in the plant, inactivation of plant myrosinase by cooking, the loss of glucoraphanin in the cooking water, and the low stability of SF at high temperature. ESP directs hydrolysis of glucoraphanin toward SF nitrile, which does not have cancer chemopreventive properties [19]. However, cooking processes that inactivate plant ESP and myrosinase significantly reduce the bioavailability of SF up to threefold [20], indicating the incomplete conversion of glucoraphanin to SF by microflora in human gastrointestinal tract. In addition, glucoraphanin is easily leached into the cooking water during preparation [25]. Thus, steam cooking has minimal effects on glucosinolates of broccoli florets compared to high-pressure boiling, conventional boiling, and microwave cooking [25]. Furthermore, SF once formed is relatively unstable at high temperature [22, 23].

Therefore, we developed three different broccoli sprout preparations and measured the content of SF and glucoraphanin in these preparations, as described in Section Materials and methods and illustrated in Fig. 1. They are freeze-dried fresh broccoli sprouts (FR preparation), quick-steamed, freeze-dried broccoli sprouts (ST preparation), and a SF-enriched SM preparation. We used a two-step method to generate the SM preparation. First, we quick-steamed broccoli sprouts to inactive myrosinase and ESP in the plant and thus minimize the hydrolysis of glucoraphanin. The length of steaming was determined based on several studies that had been done to characterize the thermal stability of myrosinase and ESP in intact cruciferous vegetables [26, 27]. Then, we converted glucoraphanin to SF by adding back myrosinase. This product was then freeze-dried immediately to preserve the high level of SF.

The retention of SF and glucoraphanin in the broccoli sprout preparations was largely affected by the way the preparation was made (Table 1). ST preparation preserved the highest level of glucoraphanin, while SM preparation contained the largest amount of SF among all the three preparations. The glucoraphanin content of ST preparation (22.74 ± 1.33 mg/g dry wt.) was approximately 12- and 253-fold higher than that of FR (1.94 ± 0.10 mg/g dry wt.) and SM (0.09 ± 0.01 mg/g dry wt.), respectively. As expected, the glucoraphanin in SM preparation was converted to SF by incubation with myrosinase. The SF content of SM was 4.25 ± 0.15 mg/g dry wt, which is approximately 11 and 5 times higher than FR (0.39 ± 0.04 mg/g dry wt.) and ST (0.92 ± 0.05 mg/g dry wt.), respectively. Low content of both SF and glucoraphanin in FR preparation suggests that glucoraphanin might be converted to SF nitrile during the processing and storage. The low stability of SF might also contribute to this observation.

<table>
<thead>
<tr>
<th>Broccoli sprout preparations</th>
<th>Glucoraphanin (mg/g dry wt.)</th>
<th>SF (mg/g dry wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steamed + myrosinase (SM)</td>
<td>0.09 ± 0.01</td>
<td>4.25 ± 0.15</td>
</tr>
<tr>
<td>Steamed (ST)</td>
<td>22.74 ± 1.33</td>
<td>0.92 ± 0.05</td>
</tr>
<tr>
<td>Fresh (FR)</td>
<td>1.94 ± 0.10</td>
<td>0.39 ± 0.04</td>
</tr>
</tbody>
</table>

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3.2 Kinetic study of SF and SF-GSH after oral administration of the broccoli sprout preparations in mice

Next, we evaluated these broccoli sprout preparations for their ability to deliver SF in mouse plasma after oral administration and examined the in vivo pharmacokinetics and tissue distribution of SF. Each preparation was administered into CD-1 mice by oral gavage, and the plasma and tissue samples were collected at 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 h post dose. Previous studies have shown that after absorption SF is quickly conjugated to glutathione to form SF-GSH conjugates, which are the major means of transport of SF throughout the body [36], therefore, we also measured SF-GSH in plasma.

The plasma concentration-time profiles of SF and SF-GSH are shown in Fig. 2. Oral administration of all three preparations led to an immediate increase in the levels of SF and SF-GSH in plasma, indicating a rapid oral absorption of SF. SF was shown to efficiently and rapidly absorbed in human subjects given a single dose of broccoli extract, with peak plasma concentration occurring 1 h after feeding and a half life of 1.77 ± 0.13 h [1, 37, 38]. Similarly, in our study, following oral administration of broccoli sprout preparations, the greatest plasma concentration we measured occurred at 0.5 h.

Notably, we observed a “secondary peak” at the time point of 2 h after oral administration of ST preparation, suggesting that it took approximately 2 h for the conversion of glucoraphanin to SF by gut microflora. Lai et al. reported that introduction of glucoraphanin (150 μmol/kg body weight) directly into the cecum resulted in a significant rise of isothiocyanates (free SF plus glutathione metabolites) in the mesenteric plasma by 120 min [39]. Our observation of the secondary peak at 2 h is consistent with this finding [39].

A summary of pharmacokinetic parameters is listed in Table 2. The highest plasma concentration of SF was 6.0 and 2.6 times higher when SM preparation (337 ng/mL) was administered, compared to FR (56 ng/mL) and ST (130 ng/mL) preparations, respectively. The AUC0-last of SM preparation (856 ng × h/mL) was 8.1 and 2.1 times higher, compared to FR (106 ng × h/mL) and ST (399 ng × h/mL) preparations, respectively. Similarly, the AUC0-∞ in mice after oral administration of SM preparation (864 ng × h/mL) was 7.6 and 2.1 times higher, compared to FR (113 ng × h/mL) and ST (402 ng × h/mL) preparations, respectively. The AUC value reflects the overall exposure. Likewise, for SF-GSH

Table 2. Pharmacokinetic parameters of SF and SF-GSH after a single oral administration of 2.5 mg/g body weight of broccoli sprout preparations in mice

<table>
<thead>
<tr>
<th>Broccoli sprout preparations</th>
<th>SF</th>
<th>SF-GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SM</td>
<td>ST</td>
</tr>
<tr>
<td>AUC0-∞ (ng × h/mL)</td>
<td>864</td>
<td>402</td>
</tr>
<tr>
<td>AUC0-last (ng × h/mL)</td>
<td>856</td>
<td>399</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>337</td>
<td>130</td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>4.35</td>
<td>3.82</td>
</tr>
</tbody>
</table>
conjugate, the Cmax of SM group (1090 ng/mL) was 8.3 and 4.0 times higher than the Cmax of FR (132 ng/mL) and ST (272 ng/mL) groups. The AUC in mice after oral administration of SM preparation (AUC0-last: 2290 ng × h/mL; AUC0-∞: 2290 ng × h/mL) was 11.9 and 2.6 times higher, in comparison to FR (AUC0-last: 191 ng × h/mL; AUC0-∞: 193 ng × h/mL) and ST (AUC0-last: 866 ng × h/mL; AUC0-∞: 867 ng × h/mL) preparations, respectively. Collectively, these data demonstrate that SM preparation can deliver the highest amount of SF and SF-GSH into plasma.

3.3 Physiologically based pharmacokinetic modeling

Given that the plasma levels of SF and SF-GSH were very low for FR preparation, we only evaluated tissue distribution for ST and SM groups. As shown in Fig. 3, levels of SF in liver, kidney, lung, heart, muscle, and mammary fatpad tissues were highest at 0.5 h after administration of SM preparation, reaching 449.97 ± 90.45, 235.26 ± 33.84, 90.49 ± 14.34, 58.55 ± 10.73, 34.90 ± 11.47, and 19.40 ± 6.41 ng/g tissue, respectively, and decreased thereafter. Similarly, the greatest tissue concentrations of SF-GSH we measured occurred at 0.5 h (1857.97 ± 288.10, 51.85 ± 5.08, 536.30 ± 118.33, 190.76 ± 63.30, and 61.33 ± 2.16 ng/g tissue for liver, kidney, lung, heart, and muscle, respectively). SF-GSH was under LOD in mammary fatpad. Our data have shown that tissue levels of SF reached the highest at 0.5 h after dosing, indicating that SF was quickly distributed throughout the mouse body after consumption of broccoli sprout preparations, which is in agreement with previous studies [37].

A PBPK model was developed to describe the concentration-time profiles in different tissues. PBPK models are mathematical models derived from the anatomical and

![Figure 3](image-url)
Figure 4. Whole body PBPK model for sulforaphane following an oral dose. The whole body of the mouse is composed of seven tissue compartments and one blood compartment; a “rest-of-body” compartment is incorporated to represent all the unsampled tissues.

physiological structure of the organism studied [40]. They are used to characterize the pharmacokinetics of drugs within the body in relation to blood flows, tissue volumes, routes of administration, and other factors. PBPK modeling offers several advantages. It helps to gain insight of the pharmacokinetic behavior of the compound, in animals, in different organs that could otherwise never be assessed in humans [40, 41]. The whole body PBPK model was constructed as shown in Fig. 4 to describe the SF disposition in plasma, lung, heart, kidney, muscle, fatpad, and liver. Physiological parameters used in the model are reported in Table 3. They were either obtained from literature [33–35] or determined by experiment.

The model simulated plasma and tissue concentration–time profiles of SF following a single dose of 2.5 mg/g body weight of SM broccoli sprout preparation in mice. Data points represent observed concentrations (mean ± SD, n = 3) and lines are PBPK model-simulated profiles.

4 Concluding remarks

In conclusion, as we hypothesized, the SF-rich broccoli sprout preparation (SM) generated by the two-step procedure contained the highest level of SF and produced the highest plasma concentration of SF. The freeze-dried broccoli sprouts with plant enzyme activities (FR) resulted in the lowest plasma concentration. The pharmacokinetic analysis and biodistribution indicate that SF was well and rapidly absorbed and distributed to various tissues following oral administration of our broccoli sprout preparations in the mice. The PBPK model for SF can be utilized to guide the dose regimen design for cancer chemoprevention efficacy studies in mice and could be very helpful to determine efficacious dose in human after extrapolation.

Table 3. Physiological parameters used in the whole body model in mice

<table>
<thead>
<tr>
<th></th>
<th>Total Volume (mL)</th>
<th>Organ blood flow (% cardiac output)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole body</td>
<td>23.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
</tr>
<tr>
<td>Blood</td>
<td>1.230&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100</td>
</tr>
<tr>
<td>Lung</td>
<td>0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100</td>
</tr>
<tr>
<td>Liver</td>
<td>1.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fatpad</td>
<td>0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.11&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Heart</td>
<td>0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Muscle</td>
<td>9.638&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rest of the body</td>
<td>10.272&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Determined by averaging the experimental data for each organ and assuming a density of 1 g/mL except for bone (1.5 g/mL); average body weight = 25.1 g.
<sup>b</sup> Values obtained from Brown et al. [42].
<sup>c</sup> Values obtained from Wang et al. [43], cardiac output = 51.36 mL/min/100 g body weight.

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The authors have declared no conflict of interest.

5 References


