

Biological sex influences the biodistribution and absorbed dose of ^{131}I (iodide) in mice

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Abstract

Females are generally more radiosensitive than males, but sex is rarely considered in biodistribution studies of radionuclides. ^{131}I is used clinically and may be released at nuclear accidents. The aim was to investigate differences in biodistribution and dosimetry of ^{131}I (iodide) between male and female mice. C57BL/6N mice received 170 kBq ^{131}I i.v. and were killed after 1 hour to 7 days. ^{131}I concentration was determined in various collected organs and tissues, and mean absorbed dose per injected activity, D/A_{inj} , was calculated. Results showed higher ^{131}I concentration and D/A_{inj} in most organs in females than males except for kidneys, where males received 4 times higher D/A_{inj} after 7 days. The D/A_{inj} in most organs in females was about up to a factor of two higher than for males. In conclusion, statistically significant differences in ^{131}I biodistribution between males and females were found in all tissues studied. The magnitude of the differences was tissue dependent. Biological sex should be treated as a variable in dose estimations and risk assessments after exposure to ^{131}I .

Introduction

Previously, most information of the human body and normal physiology was collected from young males, and many clinical studies also included preferably males [1–3]. Besides using single-sex cohorts, sex as a relevant factor has often been neglected in many biomedical studies. Today, subjects of both sexes are generally included in clinical studies. Further, the importance of studying effects related to sex is also highlighted[4].

Within radiation research, it is well known that the radiosensitivity and effects of exposure are influenced by several factors, including age, sex, and various genetic and lifestyle factors [5–10]. Females are considered more sensitive to ionising radiation than males, with higher risk of incidence of cancers of all types, and about 35% higher overall lethality risk [11]. In the Life Span Study (LSS) cohort of the Hiroshima and Nagasaki atomic bomb survivors (exposed to acute whole-body irradiation from photons and neutrons), the overall cancer excess risk was also higher in women than men [12, 13]. The cancer incidence and sex-dependence varied between tumour types.

The molecular mechanisms behind the sex-related effects on radiation response are largely unknown. Factors that have been proposed are differences in overall genetic risks, hormone and hormone receptor expression, regulation of X-chromosome specific genes, and anatomic differences such as lipid distribution [10, 14–16]. We recently demonstrated that sex impacts the proteomic expression profile of radiation-associated responses in murine blood plasma collected after whole body exposure [17]. Effects were pronounced for DNA damage and repair pathways and associated pro-survival cellular outcome. Biomarkers suggested from a single-sex cohort might not be relevant for the opposite sex, and biomarkers suggested from studies on both sexes might be less sensitive and specific than if sex is included as influencing factor.

The thyroid produces the iodine-containing thyroid hormones triiodothyronine (T3) and thyroxine (T4) that regulate important cellular mechanisms such as energy metabolism in the body [18, 19]. Iodide (I^-) is transported from the blood into the thyroid cells via the sodium-iodine symporter (NIS) [20, 21]. NIS activity is regulated mainly by thyroid stimulating hormone (TSH) that is released by the pituitary gland. Inside the follicular lumen of the thyroid, iodide is then oxidised and integrated into thyroglobulin to create T4 and T3 hormones [19]. The hormones are then secreted into the blood and transported to the various tissues in the body. The thyroid mainly produces T4, which is a prohormone that is subsequently de-iodinised to T3 in various tissues in the body [22].

Exposure to ^{131}I can occur from several sources. Radioactive iodine is frequently used in medicine, with ^{131}I as one of the most widely used radioiodine isotope in patients. Thyroid carcinoma and hyperthyroidism is diagnosed and treated by ^{131}I as iodide [23]. When using ^{131}I -labelled pharmaceuticals for diagnosis and treatment of other types of tumours, ^{131}I as iodide can be released by dehalogenation, e.g. for ^{131}I -metaiodo-benzylguanidine (MIBG) that is used for neuroblastomas, paragangliomas and pheochromocytomas [24]. Exposure to ^{131}I can also occur after nuclear accidents. Examples are the nuclear accidents that occurred in Chernobyl and Fukushima, where $1.8 \cdot 10^{18}$ and $1 \cdot 10^{17}$ Bq ^{131}I , respectively, were released to the environment [25]. The fallout from the Chernobyl accident resulted in an increased incidence of thyroid cancer in exposed children during the following years [25, 26]. Further, other thyroid and hormonal dysfunctions were observed in women regardless of age, while in men it was limited to those above 50 years of age [27].

Currently, the knowledge on sex-specific radiation-induced effects is primarily based on data from acute whole-body exposure. For internal exposure to radionuclides, data on sex-specific radiation effects is scarce. An essential step towards accurate dose-effect relations is to determine if there are sex-specific differences in biodistribution of the radionuclide or radiotracer, and if there are resulting dosimetric differences between the sexes. Such data are necessary as a basis for studies on radiobiological effects, which need to be compared with absorbed dose and dose rates for a proper evaluation.

The aim of this study was to compare the biodistribution of ^{131}I and absorbed dose to various major tissues in male and female normal mice.

Methods

Animal experiments

Seventy C57BL/6N mice (Charles River Laboratories International, Inc., Salzfeld, Germany) (35 males and 35 females; $n = 5/\text{group}$) 9–10 weeks of age were used in the study. After delivery, the mice were allowed to acclimate to the research facility for at least a week before study start. The body weight of the males was 24.3 ± 1.6 g and of the females 20.8 ± 0.75 g. Breeding chow, with $0.87 \mu\text{g/g}$ stable iodine concentration, and water was given *ad libitum* from arrival at the animal facility and throughout the

experiment. Animals were housed in individually ventilated cages, and standard laboratory day and night cycle was maintained during the experiments, i.e. dark from 6 pm to 6 am. The study was approved by the Ethical Committee for Animal Research at the University of Gothenburg (no. 146–2015), and all experiments performed were performed in accordance with this approval. All authors complied with the ARRIVE guidelines. It should be noted that biodistribution and dosimetric data for male mice are published in Andersson et al. 2020 [28].

Na^{131}I (GE Healthcare Braunschweig, Germany) was diluted with saline solution to desired activity concentration. Syringes (0.1–1 ml Braun syringes with 0.4x20 mm cannulas) were prepared with ^{131}I from stock solution, of which some were used to validate the activity in syringes. Syringes were weighed individually after preparation and after administration to determine the administered activity to each subject.

The mice were divided into groups ($n = 5/\text{group}$) and injected in the tail vein with $170 \pm 20 \text{ kBq } ^{131}\text{I}$, in 0.1 ml saline solution, at 8 am. Animals were anesthetized by intraperitoneal injection with sodium pentobarbital (APL, Sweden) and killed via cardiac puncture, at 1, 4, 8, 18, 24, 72 or 168 h after ^{131}I administration. The mice were dissected immediately to collect blood samples and the heart, kidneys, liver, lungs, intestines (small and large), salivary glands, spleen, stomach and thyroid & throat. The thyroid was carefully separated from the throat and surrounding muscles and connective tissues. The sample of the small intestine was collected from the upper part, and the large intestine from the lower part. The contents in stomach and intestine samples were removed and analysed separately from the respective tissues. All samples were weighed.

Radioactivity measurements

A CRC-15R dose calibrator ion chamber, produced by Capintec, IA, USA, was used to measure ^{131}I activity in stock solutions for preparation of syringes. Injected activity was determined using 4–5 control syringes for each stock solution.

^{131}I activity in tissue samples and control syringes was measured using two 1480 Wizard® 3" NaI(Tl) gamma counters (Wallac Oy, Turku, Finland), with an energy window of 260–430 keV. Measurements in gamma counters were made with at least 900 counts over background, and obtained signal was corrected for background radiation and dead time loss. The gamma counters were cross calibrated with the ion chamber.

Biodistribution data

Activity concentration in tissues, $C_{\text{tissue}}(t)$, was calculated as percentage of administered activity of ^{131}I per organ mass (%IA/g)

$$C_{tissue}(t) = \frac{A_{tissue}(t)}{A_{inj} \bullet m_{tissue}} \bullet 100\%$$

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,

where $A_{tissue}(t)$ is the measured activity in the tissue at time t , decay-corrected to the time of ^{131}I administration ($t = 0$), A_{inj} is the administered activity at $t = 0$ and m_{tissue} is the weight of the tissue.

Dosimetric estimations

Mean absorbed dose, $D_{tissue}(t)$, was estimated using the MIRD formalism [29]:

$$D_{tissue}(t) = \tilde{C}_{tissue}(t) \bullet E \bullet \phi_{tissue}$$

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where $\tilde{C}_{tissue}(t)$ is the time-integrated activity concentration in the tissue (target organ) at time t , E is the energy emitted per decay, and ϕ_{tissue} is the absorbed fraction of the emitted energy in the target organ. Only dose contributions from electrons were considered, and E was set to 191 keV/Bq [30]. Absorbed fractions for tissues were taken from literature [31], with the exception for blood, carcass and salivary gland, where the self-absorbed fraction was assumed to be 1. The content in stomach and intestines was not included in the dose estimation for these tissues.

The time-integrated activity concentration $\tilde{C}_{tissue}(t)$ was calculated using the trapezoidal method between the various time-points in the activity concentration data, but in this case not decay corrected. The initial activity concentration was assumed to be zero at $t = 0$ for all tissue samples except for blood, where the initial activity was considered to be the administered activity.

Statistical analyses

Statistical significance of differences between the male and female mice was determined by performing a Welch's unequal variance t-test. Probabilities above 95% ($p < 0.05$) were considered statistically significant.

Results

Sex differences in ^{131}I biodistribution

The ^{131}I activity, $A_{\text{tissue}}(t)$, in all entire organs together with that in thyroid & throat and carcass was determined at time points from 1 h to 7 d after administration (Table 1, Fig. 1, Suppl. Figure 1). Statistically significant differences between the sexes were found in all organs and tissues except for thyroid & throat. In general, the values were lower in males, with the exception of kidneys (18 h-7 d) and stomach (18 h). In most tissues the activity was highest at 1 h followed by a fast decrease during the first 18 h. Exceptions were found for thyroid, salivary glands, and thyroid & throat. Maximal mean activity in thyroid and thyroid & throat occurred at the 18 h time-point for both sexes. In salivary glands, the maximal mean activity occurred at 4 h for females and 1 h for males.

Table 1

The ^{131}I activity, $A_{\text{tissue}}(t)$ (given as %IA), corrected for physical decay in different samples (organs, tissues and content in stomach and large intestine (LI), and small intestine (SI)) from male (m) and female (f) mice at 1 hour to 7 days after injection of 170 kBq ^{131}I . Data are given as the mean (SEM). **Bold** indicates statistically significant differences between the sexes.

Sample	$A_{\text{tissue}} [\%IA]$						
	1 hour	4 hours	8 hours	18 hours	24 hours	72 hours	168 hours
Carcass (m)	38 (2)	11 (1)	6.8 (0.2)	1.8 (0.1)	1.4 (0.3)	0.86 (0.06)	0.52 (0.06)
Carcass (f)	43 (2)	30 (2)	18 (2)	2.2 (0.3)	2.1 (0.2)	1.0 (0.1)	0.80 (0.08)
Heart (m)	0.17 (0.01)	0.041 (0.004)	0.035 (0.006)	0.0081 (0.0008)	0.0039 (0.0002)	0.0031 (0.0002)	0.0013 (0.0001)
Heart (f)	0.15 (0.01)	0.11 (0.01)	0.052 (0.004)	0.0090 (0.0004)	0.0078 (0.0010)	0.0044 (0.0004)	0.0011 (0.0001)
Kidneys (m)	0.75 (0.04)	0.18 (0.01)	0.13 (0.01)	0.047 (0.003)	0.074 (0.010)	0.38 (0.02)	0.24 (0.04)
Kidneys (f)	0.57 (0.05)	0.44 (0.07)	0.22 (0.02)	0.026 (0.002)	0.024 (0.003)	0.026 (0.002)	0.0061 (0.0005)
Liver (m)	2.0 (0.1)	0.52 (0.04)	0.38 (0.03)	0.13 (0.01)	0.11 (0.01)	0.13 (0.01)	0.039 (0.003)
Liver (f)	1.8 (0.1)	1.2 (0.1)	0.69 (0.08)	0.18 (0.02)	0.14 (0.02)	0.12 (0.01)	0.036 (0.003)
Lungs (m)	0.49 (0.03)	0.14 (0.02)	0.097 (0.005)	0.020 (0.009)	0.012 (0.008)	0.0087 (0.0004)	0.0036 (0.0005)
Lungs (f)	0.59 (0.02)	0.39 (0.03)	0.20 (0.02)	0.027 (0.002)	0.020 (0.002)	0.013 (0.001)	0.0036 (0.0003)
Salivary glands (m)	1.5 (0.2)	0.89 (0.15)	0.58 (0.09)	0.12 (0.01)	0.026 (0.004)	0.0098 (0.0009)	0.0036 (0.0007)
Salivary glands (f)	1.5 (0.1)	2.0 (0.4)	0.71 (0.09)	0.027 (0.005)	0.022 (0.005)	0.012 (0.001)	0.0037 (0.0008)
Spleen (m)	0.16 (0.01)	0.047 (0.003)	0.031 (0.002)	0.0059 (0.0006)	0.0036 (0.0005)	0.0020 (0.0002)	0.0008 (0.0001)
Spleen (f)	0.26 (0.01)	0.22 (0.04)	0.099 (0.008)	0.010 (0.001)	0.0071 (0.0007)	0.0048 (0.0004)	0.0010 (0.0001)
Stomach (m)	4.7 (0.8)	1.2 (0.3)	0.80 (0.16)	0.13 (0.02)	0.028 (0.005)	0.010 (0.002)	0.0030 (0.0004)
Stomach (f)	4.6 (0.4)	3.7 (0.3)	1.6 (0.3)	0.072 (0.006)	0.066 (0.015)	0.023 (0.002)	0.0055 (0.0001)

Sample	$A_{\text{tissue}} [\%IA]$						
	1 hour	4 hours	8 hours	18 hours	24 hours	72 hours	168 hours
Thyroid (m)	0.85 (0.34)	0.52 (0.31)	2.7 (0.9)	7.2 (1.0)	2.8 (1.4)	0.77 (0.50)	0.84 (0.19)
Thyroid (f)	2.5 (0.4)	4.3 (0.7)	5.9 (0.8)	12 (1)	5.8 (1.5)	4.6 (0.2)	0.96 (0.25)
Thyroid & throat (m)	4.6 (0.7)	5.2 (0.7)	8.3 (0.7)	11 (1)	7.4 (0.3)	4.2 (0.6)	1.4 (0.2)
Thyroid & throat (f)	3.4 (0.4)	5.4 (0.8)	7.8 (0.8)	15 (2)	7.1 (1.4)	5.0 (0.3)	1.2 (0.2)

The ^{131}I activity concentration, $C_{\text{tissue}}(t)$, in organs and tissues together with the content in large intestine, small intestine and stomach was determined at several time-points from 1 h to 7 d after administration (Table 2, Figs. 1–2, Suppl. Figure 1). Statistically significant differences between the sexes were found for all organs, tissues and gastrointestinal contents. Altogether, the values for lower for males, with the exception for blood (7 d), kidneys (18 h-7 d), large intestinal content (7 d), salivary gland (18 h) and stomach (18 h). In general, statistically significant differences between sexes were found at, at least, three time points in all but one of the tissues. The thyroid presented statistically significant differences at only two time points, 24 h and 72 h. In total, statistically significant differences between male and female mice were demonstrated in 72 out of 112 observations (64%) for the tissues and sample types studied.

Table 2

The ^{131}I activity concentration, $C_{\text{tissue}}(t)$, (given as %IA/g), corrected for physical decay in different samples (organs, tissues and content in stomach and large intestine (LI), and small intestine (SI)) from male (m) and female (f) mice at 1 hour to 7 days after injection of 170 kBq ^{131}I . Data are given as the mean (SEM). **Bold** indicates statistically significant differences between the sexes. *Italics* indicates the mean sample volume when parts of organs, tissues and gastrointestinal contents were measured.

Sample	Mass [g]	$C_{\text{tissue}}(t)$ [%IA/g]						
		1 hour	4 hours	8 hours	18 hours	24 hours	72 hours	168 hours
Blood (m)	0.46	4.3 (0.5)	1.2 (0.2)	0.78 (0.07)	0.14 (0.01)	0.057 (0.007)	0.056 (0.003)	0.020 (0.002)
Blood (f)	0.63	4.4 (0.3)	2.8 (0.2)	1.5 (0.1)	0.12 (0.01)	0.083 (0.004)	0.072 (0.004)	0.014 (0.001)
Carcass (m)	19 (1)	2.1 (0.1)	0.57 (0.06)	0.35 (0.02)	0.095 (0.004)	0.076 (0.018)	0.046 (0.003)	0.028 (0.004)
Carcass (f)	16 (1)	2.8 (0.2)	2.0 (0.1)	1.1 (0.1)	0.14 (0.01)	0.13 (0.01)	0.064 (0.002)	0.049 (0.005)
Heart (m)	0.10 (0.01)	1.8 (0.1)	0.42 (0.05)	0.31 (0.03)	0.075 (0.003)	0.038 (0.003)	0.031 (0.002)	0.012 (0.002)
Heart (f)	0.095 (0.002)	1.7 (0.1)	1.2 (0.1)	0.59 (0.05)	0.088 (0.004)	0.069 (0.008)	0.044 (0.001)	0.012 (0.001)
Kidneys (m)	0.28 (0.01)	3.0 (0.2)	0.74 (0.09)	0.48 (0.03)	0.17 (0.01)	0.26 (0.04)	1.4 (0.1)	0.79 (0.15)
Kidneys (f)	0.22 (0.01)	3.0 (0.2)	2.0 (0.2)	1.1 (0.1)	0.11 (0.01)	0.11 (0.02)	0.11 (0.01)	0.028 (0.003)
LI (m)	0.082	2.6 (0.2)	0.83 (0.08)	0.58 (0.04)	0.12 (0.02)	0.05 (0.01)	0.033 (0.002)	0.014 (0.003)
LI (f)	0.088	3.2 (0.2)	2.2 (0.1)	1.3 (0.3)	0.09 (0.01)	0.11 (0.01)	0.056 (0.008)	0.019 (0.003)
LI content (m)	0.064	2.8 (0.7)	3.5 (0.6)	3.0 (0.4)	0.45 (0.07)	0.23 (0.03)	0.16 (0.03)	0.060 (0.004)
LI content (f)	0.064	2.2 (0.3)	2.0 (0.6)	7.1 (1.0)	0.78 (0.14)	0.29 (0.03)	0.13 (0.02)	0.036 (0.002)
Liver (m)	1.2 (0.1)	1.7 (0.1)	0.45 (0.06)	0.34 (0.03)	0.12 (0.01)	0.1 (0.01)	0.10 (0.01)	0.035 (0.003)
Liver (f)	0.96 (0.02)	1.9 (0.1)	1.4 (0.1)	0.75 (0.08)	0.18 (0.02)	0.15 (0.02)	0.13 (0.01)	0.034 (0.003)
Lungs (m)	0.14 (0.01)	3.9 (0.3)	1.0 (0.1)	0.67 (0.05)	0.16 (0.01)	0.08 (0.01)	0.062 (0.004)	0.023 (0.002)

Sample	Mass [g]	C _{tissue} (t) [%IA/g]						
		1 hour	4 hours	8 hours	18 hours	24 hours	72 hours	168 hours
Lungs (f)	0.14 (0.01)	4.3 (0.3)	2.8 (0.2)	1.4 (0.1)	0.21 (0.02)	0.13 (0.02)	0.093 (0.004)	0.028 (0.001)
Salivary glands (m)	0.15 (0.01)	12 (2)	6.4 (1.1)	4.1 (0.6)	0.79 (0.06)	0.18 (0.02)	0.065 (0.005)	0.022 (0.003)
Salivary glands (f)	0.098 (0.003)	17 (1)	24 (4)	7.1 (0.7)	0.29 (0.04)	0.22 (0.05)	0.11 (0.00)	0.034 (0.007)
SI (m)	0.17	2.9 (0.2)	0.98 (0.06)	0.67 (0.10)	0.25 (0.01)	0.087 (0.003)	0.048 (0.003)	0.021 (0.001)
SI (f)	0.14*	3.5 (0.3)	3.0 (0.4)	1.9 (0.1)	0.39 (0.03)	0.14 (0.02)	0.065 (0.004)	0.022 (0.006)
SI content (m)	0.093	5.7 (0.5)	1.8 (0.3)	1.8 (0.3)	0.30 (0.02)	0.14 (0.01)	0.12 (0.01)	0.035 (0.004)
SI content (f)	0.065	6.6 (1.0)	8.0 (1.0)	3.4 (0.4)	0.41 (0.04)	0.28 (0.04)	0.15 (0.04)	0.036 (0.008)
Spleen (m)	0.065 (0.002)	2.7 (0.2)	0.72 (0.11)	0.45 (0.02)	0.10 (0.01)	0.06 (0.01)	0.035 (0.004)	0.011 (0.001)
Spleen (f)	0.075 (0.002)	4.0 (0.3)	2.8 (0.2)	1.4 (0.1)	0.14 (0.01)	0.1 (0.01)	0.054 (0.003)	0.014 (0.001)
Stomach (m)	0.15 (0.01)	28 (5)	7.7 (1.7)	5.1 (0.7)	0.86 (0.07)	0.21 (0.03)	0.071 (0.007)	0.024 (0.002)
Stomach (f)	0.17 (0.01)	28 (2)	23 (2)	7.9 (1.5)	0.49 (0.04)	0.39 (0.05)	0.12 (0.01)	0.033 (0.006)
Stomach content (m)	0.11	78 (10)	21 (3)	14 (2)	1.6 (0.3)	0.40 (0.08)	0.11 (0.02)	0.048 (0.010)
Stomach content (f)	0.12	140 (11)	58 (9)	20 (1)	0.9 (0.24)	1.2 (0.2)	0.31 (0.06)	0.066 (0.012)
Thyroid (m)	0.0022 (0.0002)	1500 (600)	280 (150)	2200 (1100)	5800 (1400)	1100 (500)	480 (350)	330 (70)
Thyroid (f)	0.0021 (0.0002)	1500 (200)	2500 (820)	2800 (300)	6000 (1700)	3900 (700)	2700 (400)	490 (110)
Thyroid & throat (m)	0.038	93 (15)	110 (20)	240 (30)	840 (80)	230 (30)	74 (10)	95 (13)
Thyroid & throat (f)	0.016	170 (30)	360 (100)	370 (40)	1200 (200)	430 (60)	390 (20)	100 (20)

For most tissues, the activity concentration was highest at 1 h followed by a fast decrease during the first 18 h. Exceptions were found for thyroid, salivary glands and small intestine (SI). Maximal mean activity concentration in salivary glands occurred after 4 h for females but at 1 h for males.

The highest activity concentration was found in thyroid, with maximal values after 18 h for both sexes (Table 2, Fig. 1). After 24 and 72 h the activity concentration was statistically significant higher in females than males. The absolute ^{131}I activity in thyroid showed similar curve pattern as the activity concentration, but the absolute activity was much higher in females than males. For thyroid & throat no statistically significant differences between sexes were found for ^{131}I activity in the sample but occurred for activity concentration (Suppl. Figure 1).

For the kidneys from males, the activity concentration curve showed a fast monotonic decrease during the first 18 h, followed by an increase with a local maximum after 72 h. For females, no such local maximum was found, and the initial monotonic decrease was somewhat slower (Fig. 2B). Statistically significant differences between sexes were found at all time-points except for 1 h.

Sex differences in absorbed doses

Estimated mean absorbed dose per unit injected activity, D/A_{inj} , to various organs and tissues from male and female mice are presented for different time points after administration (Table 3, Fig. 3). Females received a higher D/A_{inj} in all organs except for the kidneys; in contrast, males received the highest D/A_{inj} to kidneys of 20 mGy/MBq compared with 5 mGy/MBq for the female mice during the first 7 days. For thyroid the corresponding values were 13 Gy/MBq for male and 38 Gy/MBq for female mice.

Table 3

Mean absorbed dose per unit injected activity, D/A_{inj} (mGy/MBq), to various organs and tissues at different time points after administration of 170 kBq ^{131}I in male (m) and female (f) mice. Data are given as mean (SEM), n = 5. LI, large intestine; SI, small intestine.

Tissue	D(t) [mGy/MBq]						
	1 hour	4 hours	8 hours	18 hours	24 hours	72 hours	168 hours
Blood (m)	27 (1)	36 (1)	40 (1)	45 (1)	46 (1)	49 (1)	51 (1)
Blood (f)	28 (1)	40 (1)	49 (1)	57 (1)	58 (1)	62 (1)	65 (1)
Carcass (m)	1.1 (0.1)	5.4 (0.1)	7.5 (0.1)	11 (1)	12 (1)	16 (1)	19 (1)
Carcass (f)	1.5 (0.1)	9.3 (0.1)	16 (1)	24 (1)	26 (1)	34 (1)	42 (1)
Heart (m)	0.90 (0.07)	4.2 (0.2)	5.8 (0.3)	8.0 (0.4)	8.4 (0.4)	9.9 (0.4)	12 (1)
Heart (f)	0.88 (0.06)	5.4 (0.3)	9.1 (0.4)	13 (1)	13 (1)	16 (1)	18 (1)
Kidneys (m)	1.4 (0.1)	6.4 (0.3)	8.7 (0.3)	12 (1)	13 (1)	45 (2)	120 (10)
Kidneys (f)	1.3 (0.1)	8.3 (0.6)	14 (1)	20 (1)	21 (1)	25 (1)	30 (1)
LI (m)	1.4 (0.2)	6.7 (1.1)	9.6 (1.5)	13 (2)	14 (2)	15 (2)	17 (3)
LI (f)	1.7 (0.2)	10 (1)	17 (2)	25 (3)	25 (3)	29 (4)	32 (4)
Liver (m)	0.93 (0.06)	4.4 (0.2)	6.1 (0.2)	8.4 (0.3)	9.0 (0.3)	13 (1)	19 (1)
Liver (f)	1.0 (0.1)	6.1 (0.2)	10 (1)	15 (1)	16 (1)	22 (1)	28 (1)
Lungs (m)	1.7 (0.1)	8.4 (0.4)	12 (1)	16 (1)	16 (1)	20 (1)	23 (1)
Lungs (f)	2.1 (0.1)	13 (1)	21 (1)	29 (1)	30 (1)	35 (1)	39 (1)
Salivary gland (m)	5.7 (0.6)	33 (3)	54 (4)	80 (5)	83 (5)	88 (5)	92 (5)
Salivary gland (f)	8.4 (0.8)	67 (6)	130 (10)	170 (10)	170 (10)	180 (10)	180 (10)
SI (m)	1.6 (0.1)	8 (1)	11 (1)	16 (1)	17 (1)	20 (1)	23 (1)

Tissue	D(t) [mGy/MBq]						
	1 hour	4 hours	8 hours	18 hours	24 hours	72 hours	168 hours
SI (f)	1.9 (0.2)	12 (1)	23 (2)	35 (2)	36 (2)	41 (2)	44 (2)
Spleen (m)	1.6 (0.1)	7.6 (0.4)	11 (1)	14 (1)	14 (1)	16 (1)	18 (1)
Spleen (f)	2.0 (0.1)	13 (1)	22 (1)	30 (2)	31 (2)	34 (2)	37 (2)
Stomach (m)	16 (3)	79 (9)	110 (10)	140 (10)	140 (10)	150 (10)	150 (10)
Stomach (f)	14 (1)	88 (5)	150 (10)	200 (10)	200 (10)	210 (10)	220 (10)
Thyroid (m)	160 (60)	910 (270)	3200 (800)	20000 (3000)	31000 (4000)	59000 (13000)	78000 (16000)
Thyroid (f)	490 (100)	4400 (600)	12000 (1000)	45000 (5000)	65000 (6000)	148000 (17000)	220000 (20000)

Discussion

This work demonstrated differences in biodistribution of ^{131}I (as iodide) between male and female mice. Overall, statistically significant differences between the sexes were found for about 60% of all investigated tissues and time points. The vast majority of tissues exhibited significant differences for at least three of the seven observed time points. The differences in biodistribution translated to sex-related differences in absorbed doses to tissues after exposure to ^{131}I .

Overall, female mice had somewhat higher ^{131}I activity concentration in almost all organs compared with male mice. For most organs and tissues, the initial concentration was similar 1 h after injection, but thereafter the retention was higher in females with statistically significant differences during two or more following measurements. The exception was stomach with higher value in males than females after 18 hours and the kidneys with higher values in males from 18 hours (further discussed below). Reasons for the sex-dependent differences in biodistribution can be assumed to be differences in body size, diurnal variations in iodine biokinetics, differences in intake of stable iodine, and physiological and hormonal differences [32].

In this study, the body weight of female mice was ca 20% lower than of male mice. However, differences in body size do not explain the results as it would require equal values of percental uptake in the tissues (%IA), and an equal difference for individual organ masses for males and females; neither was observed in this study. Furthermore, the ^{131}I concentration was up to more than 3 times higher in females for most tissue types indicating a non-linear relation to body weight.

We have previously demonstrated in male mice of the same strain that the biodistribution and absorbed dose to tissues varies depending on the time of the day for ^{131}I injection, where the mice showed a higher retention in activity concentration after injection at 12 pm, lasting up to 18 h after administration, compared with mice administered at 8 am and 4 pm [28]. Similar data were also found in another mouse strain [33]. However, in the present study, all mice were injected at the same time of day to preclude above confounder in the study design. Hypothetically, there may be a diurnal difference in iodine metabolism between male and female mice underlying these data, but these factors have not yet been investigated in conjunction to the best of our knowledge.

It is well known that high intake of stable iodine reduces the uptake of radioactive iodine in the thyroid [33], and probably in other NIS-expressing tissues. Due to the difference in body weight, male mice consume more food. The mice in the present study were given food *ad libitum*, in agreement with standard laboratory care. It was therefore not possible to monitor how much food, and hence stable iodine, each mouse had ingested during the day. The number of stable iodine atoms (^{127}I) available in a daily serving of the breeding chow are about 75,000 times the number of ^{131}I atoms injected (assuming a daily intake of 3 gram of breeding chow and 170 kBq of administered ^{131}I). Hence, a 20% higher stable iodine intake in males does not explain the general higher retention in females.

Thus, likely explanations for the sex-related differences would be physiological and hormonal sex differences that affect iodine metabolism. Intracellular uptake of iodide requires an active transport over the cell membrane mediated via NIS, also called SLC5A5. Beside expression by the thyrocytes, NIS is also known to be expressed by cells primarily in the salivary gland and stomach, but also in small intestine, colon, mammary gland, ovary, testis, kidney, lung, and in many other tissue types, although the function is still unknown in these tissues [21, 34–37]. NIS is regulated in the thyroid, the mammary glands and the ovaries by different transcription factors and hormones, especially estrogens, and the regulation seems to differ by tissue type.

To the best of our knowledge, the influence of sex hormones or factors linked to the X chromosome on biodistribution of iodine have not been studied. Female and male sex hormones are not only involved in reproduction but are also involved in other physiological functions. Previous studies have demonstrated that sex hormone receptor signalling influences immune functions, not only in humans but also in other mammals [38]. It is also known that sex hormones, especially estrogens, influence thyrocytes and may be an important factor that explain the higher incidence of thyroid cancer and diseases in females [39]. These hormonal functions might alter systemic effects related to iodine metabolism and should be investigated in follow-up studies. Future research should include measurements of hormonal levels and try to elucidate the influence of these hormones. Such studies need to consider the variations in sex hormone levels in females during the estrous cycle.

As expected, the ^{131}I concentration was highest in the thyroid. This organ is small in mice, and therefore difficult to dissect with removal of adjacent connective tissue and muscle and without losing thyroid tissue. There is also a risk of larger uncertainty in mass determination. Therefore, the ^{131}I activity in the

thyroid & throat was first measured and then in the thyroid. Furthermore, the absolute ^{131}I activity in thyroid is also shown. The finding of no statistically significant differences between the sexes in the activity in thyroid & throat but in activity concentration can to some extent be explained by larger tissue samples for males although the activity concentration in surrounding tissues should be very much lower than in the thyroid. Differences in sample dissection accuracy could, however, not explain the sex differences in ^{131}I concentration in thyroid, since the thyroid mass was very similar between the sexes. Nevertheless, we noticed a large interindividual difference in ^{131}I concentration in the thyroid in general, a fact that has been reported previously both in mice and rats [28, 40, 41]. In the present study we also obtained about twice as high coefficient of variation (CV) in males compared with the females.

Iodide is primarily excreted by the kidneys, which demonstrated a pronounced sex differential in ^{131}I biodistribution. Male mice showed higher activity concentration from 18 hours onward and a distinctly different curve behaviour compared with females. A local maximum was found 72 h after ^{131}I administration in males, while females showed a monotonic decreasing activity concentration over time. Similar curve behaviour for males was also demonstrated in a previous study on male mice of the same strain [28]. Higher NIS expression in male renal tissue may explain increased ^{131}I retention and activity concentration 3 days after administration. The higher retention in most tissues in females resulted in up to two times higher mean absorbed dose per injected activity after 7 days in these tissues compared to those in males, with the largest differences in thyroid, salivary glands, spleen and carcass. For kidneys, the corresponding value was approximately 4 times higher in males compared with females after 7 days.

Age is another important factor in conjunction with sex specificity. The mice in the present study were 9–10 weeks old corresponding to the onset of adulthood in humans [42]. Mice attain puberty at the mean age of about 42 days, and the age of pairing (mating) is about 6–8 weeks for both female and male mice [42]. Since there are physiological, hormonal, and metabolic changes with age, it is reasonable to assume that biodistribution data for young adult mice could deviate from that of older mice. Age should therefore be considered as another experimental variable when obtaining biodistribution data and subsequent absorbed dose estimations after exposure.

We have previously demonstrated age and sex bias in radiation response to irradiation of mice and rats, and found that age influenced the protein expression more than sex effects [17, 43, 44]. Conclusions from those studies were, e.g., that effective radiation biomarker candidates are probably also specific for sex and age. In another study on rats aged 5 or 17 weeks, we found distinct age-dependent differences in regulation of gene and protein expression in thyroid and blood in rats aged 5 weeks or 17 weeks, 3–9 months after injection of 50 kBq ^{131}I [43, 45]. Although age seems to have a larger impact on absorbed dose to a majority of tissues and radiobiological responses, the effect size of sex-specific differences is relevant and should be considered in preclinical study designs and translational efforts.

Conclusion

The study demonstrated sex specificity of the ^{131}I biodistribution and biokinetic profiles, and resulting differences in absorbed dose to various tissues, notably for the thyroid, kidneys and salivary glands. Thus, data from both females and males should be collected and evaluated for sex-dependent effects in biokinetic and dosimetric in vivo studies of radionuclides or radiopharmaceuticals. Ultimately, this approach may guide future efforts towards personalized nuclear medicine and radiation protection.

Declarations

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ME: Data collection, Conceptualization, Investigation, Funding acquisition, Writing - original draft, Writing - reviewing & editing;

CY: Data collection, Conceptualization, Investigation, Writing - reviewing & editing;

ES: Data, collection, Conceptualization, Investigation, Writing - reviewing & editing;

JS: Conceptualization, Supervision, Writing - reviewing & editing;

BL: Conceptualization, Supervision, Writing - reviewing & editing;

EFA; Conceptualization, Funding acquisition, Supervision, Writing - reviewing & editing;

All authors have read and approved this version of the article. It should be noted that biodistribution and dosimetric data for male mice are published in Andersson et al. 2020 [28] (Andersson, C. K., Elvborn, M., Spetz, J. K. E., Langen, B. & Forssell-Aronsson, E. B. Biodistribution of (131)I in mice is influenced by circadian variations. *Sci Rep* **10**, 15541 (2020). <https://doi.org/10.1038/s41598-020-72180-7>).

Ethics declarations

Competing interests

The authors declare no competing interests.

Ethics approval

This study was approved by the Ethical Committee for Animal Research at the University of Gothenburg (no. 146-2015) and was performed according to the Committee's guidelines and regulations. We declare that all authors followed the ARRIVE guidelines.

Data availability statement

All results from the study are presented in the paper. All raw data are available by request from the corresponding author.

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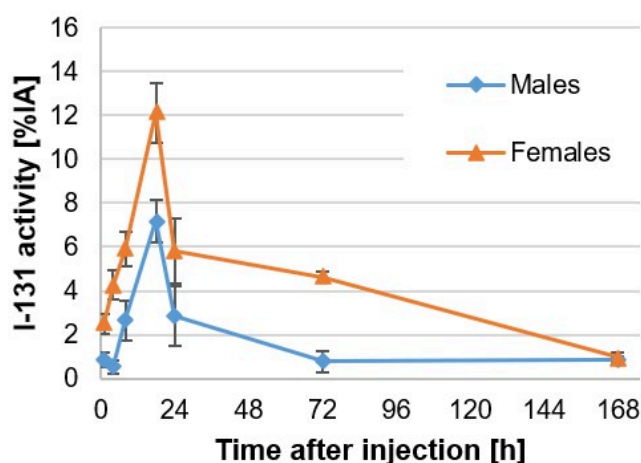
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Figures

A Thyroid (%IA)



B Thyroid (%IA/g)

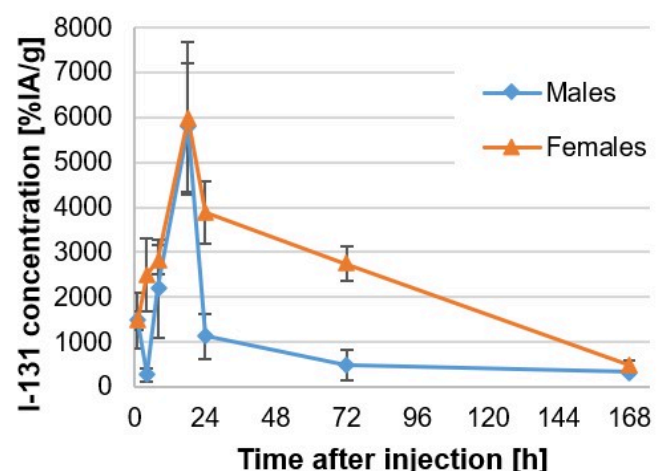


Figure 1

Biodistribution data on ^{131}I in thyroid for male and female mice after injection of 170 kBq ^{131}I as iodide. A: Activity in thyroid, given as percent of injected activity (%IA), B: Activity concentration in thyroid, given

as percent of injected activity per gram tissue (%IA/g). Error bars display SEM; n=5. Statistically significant differences were found at 1, 4, 8, 18 and 72 hours (h) for activity in thyroid (panel A) and at 24 and 72 h for activity concentration in thyroid (panel B).

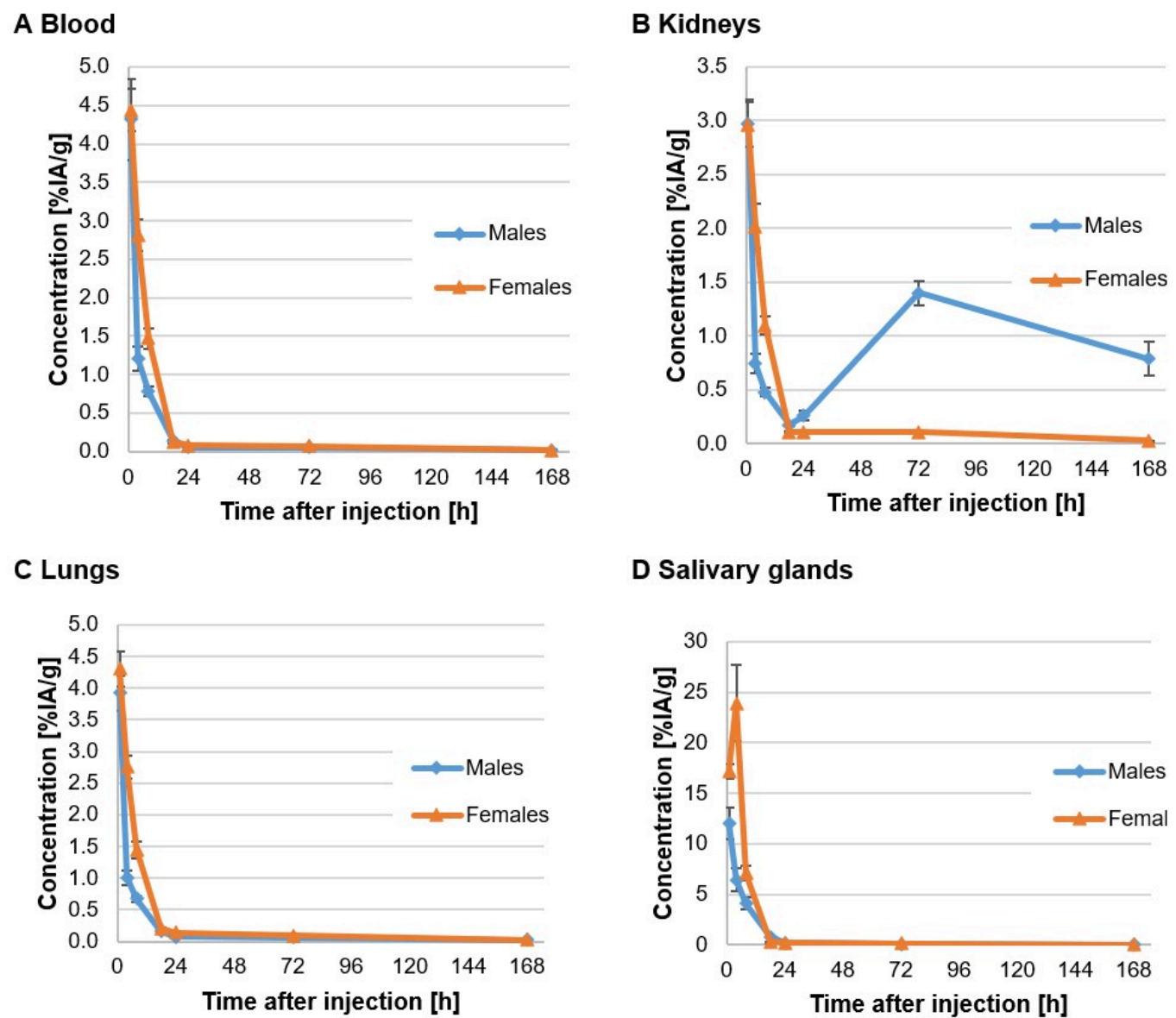
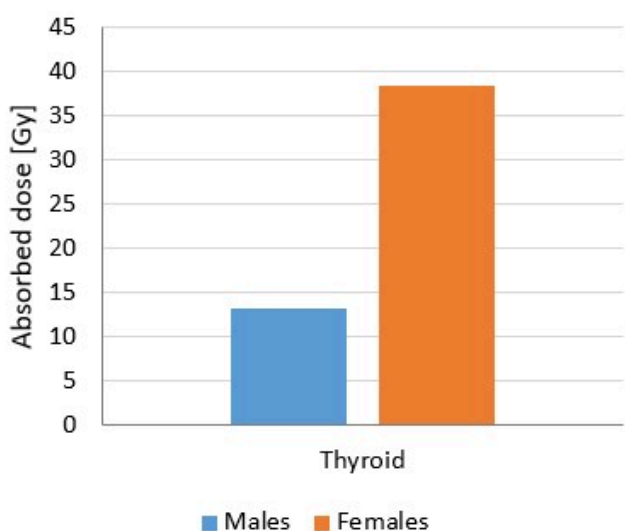
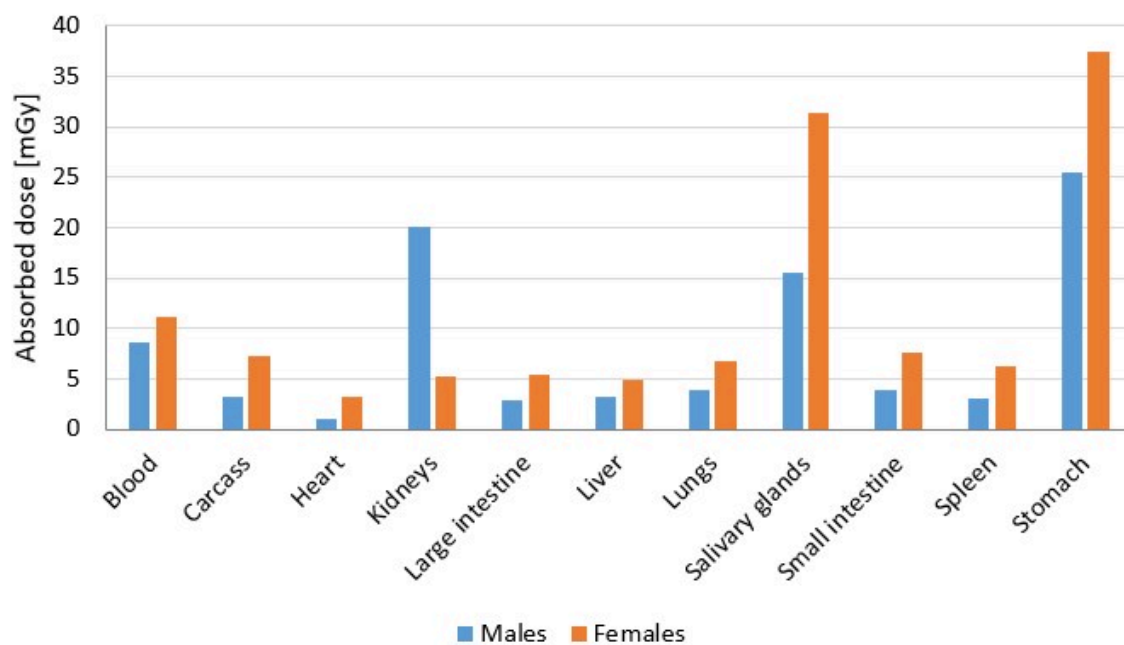


Figure 2

^{131}I activity concentration given as percent of injected activity per tissue mass (%IA/g) in (A) blood, (B) kidneys, (C) lungs and (D) salivary glands for male and female mice after injection of 170 kBq ^{131}I as iodide. Error bars display SEM; n=5. Statistically significant differences between sexes in activity concentration were found at 4, 8, 24, 72 and 168 hours (h) for blood (panel A), at 4, 8, 18, 24, 72 and 168 h for kidneys (panel B), at 4, 8, 24, and 72 h for lungs (panel C), and at 1, 4, 8, 18 and 72 h for salivary glands (panel D).

A**B****Figure 3**

Total mean absorbed dose to (A) thyroid and (B) other major organs during the first 7 days after injection of 170 kBq ^{131}I in male and female mice.

Supplementary Files

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