Eur J Nutr (2013) 52:161–168
DOI 10.1007/s00394-011-0298-9

ORIGINAL CONTRIBUTION

Effects of varying dietary iodine supplementation levels as iodide or iodate on thyroid status as well as mRNA expression and enzyme activity of antioxidative enzymes in tissues of grower/finisher pigs

Qimeng Li · Christiane Mair · Karl Schedle · Isabella Hellmayr · Wilhelm Windisch

Received: 17 November 2011 / Accepted: 22 December 2011 / Published online: 7 January 2012

Abstract
Purpose The objective of this study was to investigate the influence of high dietary iodine supply and different iodine sources on thyroid status and oxidative stress in target tissues of the thyroid hormones in fattening pigs.
Methods Eighty castrates (body weight: 33.3 ± 0.4 kg) were randomly allotted into five different treatments: The control diet contained 150 μg I/kg as KI, the other feeding groups were supplemented with 4,000 μg I/kg (as KI and KIO_3) and 10,000 μg I/kg (as KI and KIO_3), respectively. The mRNA expression levels of sodium/iodide symporter (NIS) and key antioxidant enzymes (Cu/Zn SOD, CAT, GPx) were analyzed in thyroid gland, liver, kidney, muscle, and adipose tissue sampled during slaughter. Furthermore, antioxidant enzyme activities and the effect on lipid peroxidation (MDA) were determined in liver and muscle.
Results In thyroid gland, a significant downregulation of NIS and Cu/Zn SOD mRNA expression was observed in high-iodine groups. In liver, a source effect on the mRNA expression of Cu/Zn SOD between KI and KIO_3 at 4,000 μg I/kg was shown. In contrast, not SOD but GPx activity was affected by iodine source with strongest downregulation in high KIO_3 group. In muscle, GPx activity was affected by both iodine source and dose, showing stronger downregulation in KI groups. In kidney and adipose tissue, oxidative stress parameters showed no or only unsystematic changes. However, variation in iodine supply had no effect on MDA concentrations.
Conclusions NIS expression was significantly decreased with increased iodine supplementation, which is to ensure the thyroid gland function. However, the alleviating effect of iodine supplementation observed in antioxidant enzyme mRNA expression and activity did not reflect on the lipid peroxide level.

Keywords Iodide · Iodate · NIS · Oxidative stress · Fattening pig

Q. Li · C. Mair (✉) · K. Schedle · I. Hellmayr · W. Windisch
Institute of Animal Nutrition, Products, and Nutrition Physiology (APN), Department for Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences, Muthgasse 11, 1190 Vienna, Austria
e-mail: christiane.mair@boku.ac.at

Introduction

The essential trace element iodine is necessary for the synthesis and metabolism of the thyroid hormones (T_3 and T_4) in humans and animals [1]. Although the iodine deficiency disorders (IDDs) are still a worldwide public health problem for both humans and animals, the toxicity of iodine excess has been demonstrated in vitro and in vivo [2–4]. Excessive iodine intake leads to thyroiditis, goiter, hypo- and/or hyperthyroidism [5], which may result from high-iodine-containing sea food or food of animal origin with high-iodine carryover rates from feed to food, like milk and eggs.

Oxidative stress is an imbalance between pro-oxidants and antioxidants in a biological system [6]. Reactive oxygen species (ROS) can induce oxidation and cause membrane damage, enzyme inactivation, and DNA damage [7]. To protect from ROS, there are antioxidants with enzymatic and non-enzymatic components. The former include superoxide dismutase (SOD), which detoxifies the superoxide anion, catalase (CAT) and the GSH peroxidase system, peroxiredoxins, which inactivate H_2O_2, a member of the ROS family, and glutathione peroxidase (GPx), whose function is to detoxify cellular peroxides [6]. The balance

between pro-oxidants and antioxidants determines the susceptibility of particular tissues to lipid oxidation. Malondialdehyde (MDA) is one of the metabolic products of lipid peroxidation (LPO), which is generated by reaction of lipid oxidation–induced free radicals in tissues with susceptible lipids [8].

Active transport of iodide into the thyroid gland at the basolateral membrane is mediated by the sodium/iodide symporter (NIS). In the thyroid gland, the oxidation from iodide (KI) to iodine (I_2) by the enzyme thyroid peroxidase (TPO) in the presence of H_2O_2 is the critical step for the thyroid hormone biosynthesis [9]. This oxidation process of iodide in thyroid gland can reduce the damaging potential of H_2O_2. Thus, iodine might be considered to act as an inorganic antioxidant [10].

On the other hand, previous data provide information that iodine also has pro-oxidant activities. Iodine has been found to increase reactive oxygen species (ROS) and lipid peroxidation levels in several cell systems through increasing thyroid hormones production [11].

Until today, only few experiments have been conducted to study the effect of high-iodine levels in animal feed on LPO and the antioxidant defense system in pigs, which is an important model for both livestock and human beings.

Thus, the study aimed at assessing the effect of the high-iodine intake up to 10,000 μg I/kg, which is the maximum permitted dietary content according to the feed law of the European Union (EU-UL) [12] as well as different iodine sources on the metabolism in the thyroid gland of pigs. Furthermore, it should be investigated whether high dietary iodine supplementation, as iodide or iodate, may affect oxidative stress in selected tissues, by measuring the antioxidant enzyme mRNA levels and activities, as well as the LPO levels.

Materials and Methods

Animals and diet

Austrian cross-breed castrated male growing pigs [$n = 80$; OEHYB: (Large White × Landrace) × Piétrain] with a mean initial body weight (BW) of 33.3 ± 0.4 kg were randomly allotted to five feeding groups: a control group (150 μg I/kg), two potassium iodide (KI) groups (4,000 μg I/kg and 10,000 μg I/kg), and two potassium iodate (KIO_3) groups (4,000 μg I/kg and 10,000 μg I/kg). In detail, the diet of the control group contained 20 μg/kg native iodine from natural feed stuffs and a KI supplementation of 130 μg I/kg, according to the GfE recommendations [13]. The grower (12.7 MJ metabolizable energy/kg, 15.7% crude protein, as-fed basis) and finisher (12.7 MJ metabolizable energy/kg, 12.7% crude protein, as-fed basis) diets were based on maize, soybean extracts, barley and peas. Animals were kept at the Austrian Pig Testing Facility (Streitdorf, Austria) in 10 pens with eight animals each and were allowed ad libitum access to water and feed. For detailed description of feeding trial and housing, see Li et al. [14].

Sampling

Animals were slaughtered under standardized conditions at an individual body weight of 115 ± 0.3 kg. The samples were excised immediately after slaughtering. Different tissue pieces from thyroid gland, kidney (marrow), liver (left lobe of the liver), mere muscle tissue (M. rectus abdominis) and abdominal adipose tissue for mRNA level assay were collected in cryogenic plastic tubes (2 mL, Biozym, Germany) and immediately snap-frozen in liquid nitrogen. The samples from liver and abdominal muscle tissue for antioxidant enzyme activities and lipid peroxide measurements were immediately vacuumed in plastic bags and snap-frozen as well. All samples were stored at −80 °C until further processing.

Gene expression analysis by one step RT-qPCR

MIQE-guidelines were applied for gene expression analysis [15]. Tissues were homogenized in TriFast™ (Peqlab, Germany) reagent using a Precellys 24 homogenizer (PeqLab, Germany). Total RNA was then extracted by the phenol/chloroform procedure according to the protocol of Li et al. [16]. RNA concentration was measured at 260 nm and purity of RNA assessed by the OD_{260}/OD_{280} ratio using a UV spectrophotometer (NanoDrop Technologies, USA). The RNA integrity was checked by a microfluidic capillary gel electrophoresis applying the Experion system (Bio-Rad Laboratories, USA) with Experion software 3.2. RNA was stored at −80 °C prior to use. RT-qPCR was performed in duplicate with the Corbett Rotor-Gene™ Q (Corbett, Australia) using the QuantiFast™ SYBR® Green RT-qPCR Kit (Qiagen, Germany). Primers (Table 1) were obtained from literature [17–19], and beta-actin and Histone H3 were used as reference genes. The RT-qPCR cycling conditions were set as follows: 10 min at 50 °C, 5 min at 95 °C, followed by 40 cycles of 10 s at 95 °C and 30 s at 60 °C. To verify the consistency of the amplicon, a melting point analysis was performed.

Antioxidant enzyme activity measurement

Tissue samples from liver and muscle were homogenized 1:10 (w/v) (1:5 (w/v) for SOD and GPx in muscle) with the respective concentrated potassium phosphate–buffered solutions (50 mM K_2HPO_4, 50 mM KH_2PO_4, 1 mM

Table 1 Specific primers used for real-time quantitative PCR

Gene	Accession no		Primer sequences	Tm	Product length [bp]
Histone H3 sus	NM_213930	For	5′-actggctacaaaagccgctc-3′	60 °C	232
		Rev	5′-acttgcctcctgcaaagcac-3′		
beta-actin sus	U07786	For	5′-gagaagctctgctacgtcgc-3′	60 °C	263
		Rev	5′-ccagacagcaccgtgttggc-3′		
NIS sus	AJ 487855.1	For	5′-ctctcctggcagggcatatct-3′	60 °C	187
		Rev	5′-gctgagggtgccgctgta-3′		
TNF-alpha sus	X57321	For	5′-cccccagaaggaagagtttc-3′	60 °C	255
		Rev	5′-ttggcccctgaagaggac-3′		
Cu/Zn SOD sus	AF396674.1	For	5′-caggtcctcacttcaatcc-3′	60 °C	255
		Rev	5′-ccaaacgacttccagcat-3′		
CAT sus	NM_214301.1	For	5′-cgaaggcgaaggtgtttg-3′	60 °C	195
		Rev	5′-agtgtgcgatccatatcc-3′		
GPx sus	NM_214201.1	For	5′-cacaacggtgcgggacta-3′	60 °C	326
		Rev	5′-cattgcgacacactggagac-3′		

EDTA, 1% Triton X-100) using an Ultra Turrax T25 model homogenizer (IKA®Werke GmbH and Co. KG., Germany). In the tissue homogenates, total SOD was determined according to the method of Marklund and Marklund [20] using inhibition of pyrogallol auto-oxidation in a basic buffer. Sodium cyanide NaCN (1 mM) was added to assay the Mn SOD activity, and the Cu/Zn SOD activity was calculated from the difference of total SOD and Mn SOD. The CAT activity was measured by the method of Beers and Sizer [21], based on the decomposition of H_2O_2 per minute. Furthermore, the activity of GPx was determined by measuring the reduction of GSH, coupled to a NADPH oxidation, according to the method of Lawrence and Burk [22].

Protein content of the homogenates was determined using the Quick Start Bradford protein assay (Bio-Rad, USA), according to the manufacturer′s instructions. SOD, GPx, and CAT actives were expressed in terms of units/mg protein.

Measurement of lipid peroxides

Lipid peroxides were analyzed measuring the production of thiobarbituric acid–reactive substances (TBA-RS) applying the method of Ohkawa et al. [23] with minor modifications. Briefly, the samples of 0.5 g obtained from entire frozen (−80 °C) liver or muscle were homogenized using the Ultra Turrax T25 model homogenizer with 4.5 mL of 1.15% ice-cold KCl buffer and 75 µL of 0.3% butylated hydroxytoluene (BHT, in ethanol). The 10% homogenate (200 µL for liver and 400 µL for muscle samples, respectively) was added to the reaction mixture containing 0.2 mL of 8.1% sodium dodecyl sulfate (SDS), 1.5 mL of 20% acetic acid (adjusted to pH 3.5 with NaOH), 1.5 mL of 0.8% aqueous 2-thiobarbituric acid (TBA) as well as 0.6 and 0.4 mL, respectively, of double distilled water (ddH_2O). The homogenate and reagents were heated for 60 min using a Dry-block at 100 °C. After cooling on ice, 5 mL n-butanol (SIGMA-ALDRICH®, Germany) for liver or 2.5 mL n-butanol for muscle was added applying thorough mixing. After centrifugation at 6000*g* for 10 min, the organic upper layer was removed and its color intensity of the pink staining was determined at 532 nm by a Hitachi U-1500 spectrophotometer (Metrohm INULA GmbH, Germany). The MDA content was calculated using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} [24]. The TBA-RS values are expressed as µmol of MDA per kilogram of wet tissue.

Data analysis

The quantification cycle (Cq) and single run efficiencies (E) were used for calculation of the relative expression ratios between control and treated groups using the efficiency-corrected quantification model [25]. The Statistical Analysis Software System package 9.1.3 (SAS Institute, Cary, NC) was used to determine the statistically significant differences ($p < 0.05$) between the iodine doses and sources as well as their interaction in the present study. Results were given as mean ± SEM (gene expression) and least square means ± SEM (enzyme activity and LPO). In case of significant interaction of the factors source and dose, the Student–Newman–Keuls test was used to define differences between the individual groups in gene expression analyses, whereas Tukey–Kramer test was applied for the parameters enzyme activity and lipid peroxidation. A p value of <0.05 was taken to indicate statistical significance, the level of high significance was defined at $p < 0.01$, and trends were considered at $p < 0.10$.

Results

mRNA expression rates

RNA quality and integrity were checked by the RNA quality indicator (RQI) value for each eukaryotic RNA sample run on an Experion RNA Stdsens analysis chip, which yields a number between 10 (intact RNA) and one (highly degraded RNA). In this study, samples of different tissues showed RQIs of 9 on average.

In thyroid gland tissue, high-iodine supplementation levels downregulated mRNA expression of NIS and Cu/Zn SOD with high significance ($p < 0.01$) and significantly ($p < 0.05$), respectively, irrespective of iodine source (Table 2).

In kidney, different supply levels and sources of iodine were without statistically significant effect on analyzed mRNA expression levels except for a tendency ($p < 0.1$) of NIS to be upregulated with high-iodine intake.

Not only a significant source influence ($p < 0.05$), but also a significant interaction ($p < 0.05$) was shown for Cu/Zn SOD mRNA expression in liver. Thus, in animals fed 4,000 μg I/kg as KI the Cu/Zn SOD mRNA expression in liver was significantly ($p < 0.05$) upregulated in comparison with animals fed 4,000 μg I/kg as KIO_3.

The mRNA expression profiles in muscle tissue did not show any response to the changes in amounts and sources of dietary iodine.

Significant interactions ($p < 0.05$) between source and dose were also observed for Cu/Zn SOD and CAT mRNA expressions in abdominal adipose tissue. Consequently, the Cu/Zn SOD mRNA expression in animals fed 4,000 μg I/kg as KIO_3 was significantly ($p < 0.05$) upregulated in comparison with animals fed 4,000 μg I/kg as KI and animals fed 10,000 μg I/kg as KIO_3 in abdominal adipose tissue. Furthermore, CAT mRNA expression in animals fed 10,000 μg I/kg as KIO_3 was significantly ($p < 0.05$) downregulated compared to other groups.

Antioxidant enzyme activities and TBA-RS production

A significant source influence ($p < 0.05$) on GPx activity in both liver and muscle was observed, and at the same

Table 2 Relative mRNA expression of selected enzymes in thyroid gland, kidney, liver, muscle, and adipose tissue compared with that of control (=treatment 1)

Treatment	1	2	3	4	5	SEM	*p* values		
Source Supplementation level (μg I/kg)	Native/KI 20/130	KI 4,000	KIO_3 4,000	KI 10,000	KIO_3 10,000		Source	Dose	Source × Dose
Thyroid gland									
NIS	1.00	0.19	0.19	0.20	0.08	0.06	0.552	**<0.0001**	0.533
TNF-alpha	1.00	0.74	0.84	0.87	0.59	0.07	0.530	0.606	0.194
Cu/Zn SOD	1.00	0.73	0.49	0.47	0.54	0.05	0.404	**0.015**	0.133
CAT	1.00	0.99	0.74	0.73	0.55	0.06	0.079	0.143	0.791
Kidney									
NIS	1.00	1.41	0.95	3.17	1.27	0.30	0.087	0.088	0.294
Cu/Zn SOD	1.00	1.18	1.16	0.87	1.36	0.09	0.252	0.961	0.217
CAT	1.00	0.88	1.10	1.20	0.80	0.10	0.713	0.255	0.205
GPx	1.00	0.83	0.78	0.98	1.39	0.11	0.443	0.989	0.336
Liver									
Cu/Zn SOD	1.00^{ab}	1.30^{a}	0.43^{b}	0.68^{ab}	0.64^{ab}	0.09	**0.024**	0.586	**0.039**
CAT	1.00	1.06	1.20	0.80	0.85	0.84	0.730	0.532	0.864
GPx	1.00	2.28	3.65	1.77	1.72	0.31	0.329	0.098	0.296
Muscle									
Cu/Zn SOD	1.00	0.65	0.99	1.09	0.81	0.10	0.912	0.781	0.187
CAT	1.00	0.46	0.84	1.02	0.81	0.11	0.736	0.377	0.233
GPx	1.00	0.44	0.60	1.05	0.96	0.11	0.888	0.126	0.621
Adipose tissue									
Cu/Zn SOD	1.00^{ab}	0.51^{b}	1.23^{a}	0.70^{ab}	0.54^{b}	0.09	0.121	0.202	**0.027**
CAT	1.00^{a}	0.86^{a}	0.93^{a}	1.00^{a}	0.32^{b}	0.07	0.329	0.143	**0.023**
GPx	1.00	0.85	1.04	1.09	1.43	0.09	0.283	0.120	0.708

Bold values denote significant *p* values ($p < 0.05$)

[a,b] Means within rows without a common superscript differ significantly ($p < 0.05$)

time, a significant dose effect ($p < 0.05$) on GPx activity was also shown in muscle, with distinctly lower enzyme activity in KI groups. Dietary iodine supplementation did not affect ($p > 0.05$) the concentration of the activities of total SOD, Mn SOD, and Cu/Zn SOD as well as CAT and TBA-RS in both liver and muscle of fattening pigs (Table 3).

Discussion

Thyroid gland

The data of the present pig study showed a significantly ($p < 0.01$) downregulated NIS mRNA expression rate in thyroid gland already starting at 4,000 μg I/kg diet, irrespective of iodine source. Additionally, previous results revealed that the dose-dependent increase in thyroid iodine tissue content (+122% of control level at 10,000 μg I/kg feed) was smaller compared to the target tissues of thyroid hormones (+131 to +522%) including kidney, liver, muscle, and adipose tissue [14], thus reflecting the regulatory effect of NIS. Hence, iodine concentration of 4,000 μg I/kg in feed, which is far below the maximum supplementation level allowed in fattening pigs [26], was high enough to decrease NIS gene expression at the transcriptional level. However, the expression of NIS is differentially regulated, as it occurs not only at a transcriptional, but also at a translational and post-translational level [27]. A discrepancy is often observed between NIS mRNA and protein levels [28, 29]. The level of NIS protein was not checked in this study, because it was stated in other studies that the suppression of NIS protein required lower iodine concentrations than the suppression of NIS mRNA [30, 31]. As stated above, thyroid gland tissue showed a 122% increase in iodine content from control to 10,000 μg I/kg feed in the present study. However, since no significant differences in the concentration of the hormones T_3 and T_4 were observed in our study [14], it may be hypothesized that thyroid cells may be capable of accumulating a high concentration of iodine without adverse effects on the thyroid hormone production.

Additionally, significantly decreased Cu/Zn SOD mRNA was shown in thyroid gland with increased iodine supplementation. SOD plays a dual role in the thyroid gland, firstly, as a strategic antioxidant enzyme to protect the thyroid cell against ROS, and secondly, as a H_2O_2 cooperator for the thyroid hormone synthesis [32]. It has been reported that thyroid H_2O_2 is produced by a membrane-bound NADPH oxidase system of the apical membrane without O_2^- generation [32, 33]. In mammalian cells, H_2O_2 was usually converted from O_2^- by SOD, an antioxidative enzyme which also exists in thyroid cell [34–36]. However, no O_2^- was detected in the medium of the intact thyroid cell by Nakamura et al. [32], and a hypothesis from him was reported that the production of O_2^- was inside the plasma membrane, O_2^- would be converted by SOD to H_2O_2, and then these H_2O_2 would diffuse through the plasma membrane.

In the present study, decreases in both SOD and NIS mRNA expressions at the same time were determined, which may add to an increase in oxidative potential and

Table 3 Effect of dietary iodine supplementation on antioxidant enzyme activities and TBA-RS content in liver and muscle of growing pigs

Treatment	1	2	3	4	5	SEM	*p* values		
Source	native/KI	KI	KIO_3	KI	KIO_3		Source	Dose	Source × Dose
Supplementation level (μg I/kg)	20/130	4,000	4,000	10,000	10,000				
Liver									
SOD (U/mg protein)	14.87	15.64	15.26	17.03	16.51	0.41	0.625	0.160	0.939
Mn SOD (U/mg protein)	2.99	3.37	3.45	3.40	3.15	0.10	0.697	0.272	0.467
Cu/Zn SOD (U/mg protein)	11.88	12.27	11.81	13.63	13.35	0.37	0.659	0.129	0.914
CAT (U/mg protein)	760.42	806.10	885.74	947.49	896.06	33.23	0.851	0.266	0.384
GPx (U/μg protein)	140.99	144.42	140.31	138.80	115.37	3.00	**0.030**	0.055	0.125
TBA-RS (μmol/kg wet tissue)	362.50	372.12	368.09	368.52	345.37	7.30	0.420	0.678	0.570
Muscle									
SOD (U/mg protein)	2.36	1.99	2.04	2.15	2.45	0.55	0.462	0.268	0.591
Mn SOD (U/mg protein)	1.33	1.43	1.20	1.34	1.97	0.21	0.431	0.402	0.096
Cu/Zn SOD (U/mg protein)	0.99	0.69	1.04	1.06	0.64	0.40	0.881	0.891	0.067
CAT (U/mg protein)	8.86	7.90	8.13	8.69	8.64	0.24	0.871	0.334	0.800
GPx (U/μg protein)	14.02	10.49	13.41	10.51	13.23	0.53	**0.021**	**0.032**	0.936
TBA-RS (μmol/kg wet tissue)	52.03	46.74	46.23	53.69	52.71	1.54	0.832	0.143	0.947

Bold values denote significant *p* values ($p < 0.05$)

hypothyroidisms in thyroid gland during long-term excessive iodine diet. However, the tumor necrosis factor-alpha (TNF-alpha) mRNA expression rate was checked to investigate the signs for an inflammatory potential of high dietary iodine levels on the thyroid gland. Results showed that there was no difference between control group and high-iodine diet groups for TNF-alpha expression. These findings confirmed previous observations that pigs were more tolerant concerning iodine excess than other farm animals [37]. Joanta et al. [38] reported that excessive iodine intake had a pro-oxidant effect in the thyroid gland of rats. However, one question from the author′s experiment remained unanswered, whether the thyroid hormone production would be increased by excessive iodine during a short period of time. The current study suggests that an iodine supplementation up to 10,000 μg I/kg does not impair the function of thyroid gland and thyroid hormone biosynthesis, at least in growing pigs.

Kidney

Iodine is mainly excreted as iodide via the kidneys [39]; thus, besides the thyroid gland, the highest iodine concentration was observed in the kidney of pig in the present study, as reported elsewhere [17]. In the previous data, NIS transcripts and protein have already been detected in a broad variety of tissues, including the salivary glands, gastric mucosa, and the lactating mammary gland. However, NIS is clearly differently regulated in each tissue [30]. Whether the main iodide clearance pathway in kidney is through NIS still remains to be elucidated. Possible mechanisms have been suggested through glomerular filtration, tubular secretion, and reabsorption [30]. The studies of Vayre et al. [40], Smanik et al. [41], and Lacroix et al. [42] found no NIS expression by RT-qPCR and immunohistochemistry in human, nevertheless, Spitzweg [9] detected full-length human NIS (hNIS) mRNA expression by RT-PCR and Southern hybridization in human and functional NIS protein expression in human kidney epithelial cell line derived from Wilms tumor. Variable levels of hNIS gene expression in different kidney segments could be the reason for these contrasting data [9]. In the present study, NIS mRNA expression levels were detected and tended ($p < 0.10$) to be upregulated due to increasing dietary iodine concentrations in kidney of pig, with more distinct effects for KI than for KIO_3 ($p < 0.10$). This observation suggests that orientation of the NIS transport system within the kidney tissue is positioned in a way that favors the excretion of iodine from the blood toward the urine rather than re-absorption from the primary urine back into the body. Of course, more research is needed to assess the NIS function in kidney.

Liver

Oxygen-derived free radicals are generated in many biological processes, and their generally toxic nature has resulted in the evolutionary development of defense mechanisms [39]. Intracellular antioxidant enzymes are considered to play a major role against ROS generated in vivo during oxidative stress. Among them, GPx could reduce H_2O_2 to water and oxygen and could detoxify organic hydroperoxides (ROOH) using reduced glutathione as a cofactor [43, 44]. As stated above, not only a significant ($p < 0.05$) reduction on Cu/Zn SOD mRNA expression rate due to increased iodine concentration, but also a significant iodine sources effect on gene expression of Cu/Zn SOD between animals fed with 4,000 μg I/kg as KI and animals fed with 4,000 μg I/kg as KIO_3 was observed in liver. Besides the thyroid gland and kidney, the highest iodine content was determined in liver from this study [14]. Hence, the antioxidant enzymes activities were primarily investigated in liver in the current study. In experiments with pigs, Vazquez-Medina et al. [45] reported double higher SOD activity in liver of the control group, whereas CAT and GPx activity levels were much higher than in this study. The mean levels of total SOD, Mn SOD, Cu/Zn SOD, and CAT activity of treatment groups increased with higher iodine supplementation, although these results were not significant. Since SOD and CAT are coupled enzymes, the similar diet effects on its activity are comprehensible.

Comparing the results obtained from gene expression with enzyme activity, the Cu/Zn SOD enzyme activity in the liver of pig was not significantly affected by dietary iodine supply despite the changes in gene expression in the present study. In contrast, GPx mRNA expression levels remained unaffected by the high-iodine diet in liver, nevertheless a significant ($p < 0.05$) source influence on its enzyme activity levels was determined. These results demonstrate that a simple relationship does not always exist between the antioxidant enzymes mRNA levels and its activities in tissues [46], probably due to further modulations of translational and/or posttranslational processes following transcription. Yuan et al. [46] reported similar findings between antioxidant enzymes mRNA levels and enzyme activity in the developing guinea pig lung. From these results, one could deduce that post-transcriptional control mechanisms also play an important role in the regulation of antioxidant enzymes.

Lipid peroxidation in liver showed comparable values between control and the groups with increasing iodine contents. In the study of Joanta et al. [38], an increased lipid peroxide level and CAT activity in liver was shown in rats, an incident, which was interpreted as a pro-oxidant effect by excessive iodide diet. However, the changes in hepatic enzyme activity did not seem to be distinct enough

to affect the lipid peroxide concentration in liver in this study. From reports by Joanta et al. [38], it could be hypothesized that high content of iodine may lead to oxidative stress directly or indirectly in liver, although the mode of the action is still unknown. However, as our results showed no distinct negative effects of iodine supplementation, iodine concentrations up to 10,000 μg I/kg were obviously not high enough to cause oxidative stress in fattening pigs.

Muscle and adipose tissue

There were only small amounts of iodine stored in the muscle (120–360 μg I/kg dry matter) and abdominal adipose tissue (8–20 μg I/kg dry matter) in the present study [14]. Hence, no changes were found in muscle in antioxidant enzyme mRNA expression and enzyme activity levels as well as in the level of lipid oxidation, except the GPx activity was affected by both source and dose of iodine diet. In abdominal adipose tissue, interactions by iodine supply did show significance in gene expression for Cu/Zn SOD between animals fed with 4,000 μg I/kg as KI and animals fed with 4,000 μg I/kg as KIO_3; however, these alterations were unsystematic. Therefore, it might be hypothesized that iodine source was of subordinate relevance compared to the effect of overall dietary iodine supply in adipose tissue.

Conclusion

The results of the present study show that an iodine concentration of up to 10,000 μg I/kg feed, which is still within the legal range in a diet for fattening pigs in Europe, is high enough to provoke changes in NIS expression in the thyroid gland. At the same time, the antioxidant potential of SOD against ROS in thyroid gland may be reduced due to the downregulation of SOD gene expression. Furthermore, the differences, mostly downregulations, in the gene expression rates of antioxidative enzymes have also been observed in selected target tissues of the thyroid hormones. In addition, GPx enzyme activity in liver and muscle was influenced by iodine supplementation. However, it remains unclear whether the reductions in antioxidative enzymes can be considered as an unfavorable side effect of excessive iodine entailing an increased oxidative pressure on the body or a consequence of a protective effect of high dietary iodine levels on oxidative stress. Therefore, definite modes of actions are still to be elucidated; however, even high-iodine concentrations up to 10,000 μg I/kg do not seem to cause oxidative stress in thyroid gland, liver, and muscle tissues.

References

1. Thilly CH, Vanderpas JB, Bebe N, Ntambue K, Contempre B, Swennen B, Moreno-Reyes R, Bourdoux P, Delange F (1992) Iodine deficiency, other trace elements, and goitrogenic factors in the etiopathogeny of iodine deficiency disorders (IDD). Biol Trace Elem Res 32:229–243
2. Mahmoud I, Colin I, Many MC, Denef JF (1986) Direct toxic effect of iodide in excess on iodine-deficient thyroid glands: epithelial necrosis and inflammation associated with lipofuscin accumulation. Exp Mol Pathol 44:259–271
3. Belshaw BE, Becker DV (1973) Necrosis of follicular cells and discharge of thyroidal iodine induced by administering iodide to iodine-deficient dogs. J Clin Endocrinol Metab 36:466–474
4. Takasu N, Handa Y, Kawaoi A, Shimizu Y, Yamada T (1985) Effects of iodide on thyroid follicle structure and electrophysiological potentials of cultured thyroid cells. Endocrinology 117:71–76
5. Pennington JA (1990) A review of iodine toxicity reports. J Am Diet Assoc 90:1571–1581
6. Halliwell B (1999) Antioxidant defence mechanisms: from the beginning to the end (of the beginning). Free Radic Res 31:261–272
7. Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J (2004) Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem 266:37–56
8. Zhan XA, Wang M, Xu ZR, Li WF, Li JX (2006) Effects of fluoride on hepatic antioxidant system and transcription of Cu/Zn SOD gene in young pigs. J Trace Elem Med Biol 20:83–87
9. Spitzweg C, Dutton CM, Castro MR, Bergert ER, Goellner JR, Heufelder AE, Morris JC (2001) Expression of the sodium iodide symporter in human kidney. Kidney Int 59:1013–1023
10. Venturi S (2001) Is there a role for iodine in breast diseases? Breast 10:379–382
11. Stayner LT, Dankovic DA, Lemen RA (1996) Occupational exposure to chrysotile asbestos and cancer risk: a review of the amphibole hypothesis. Am J Public Health 86:179–186
12. European Union (2005) Commission Regulation (EC) of 8 September 2005 amending the Conditions for Authorisation of a Number of Feed Additives belonging to the Group of Trace Elements. The Commission of the European Communities
13. GfE (2008) communications of the committee for requirement standards of the society of nutrition physiology: prediction of metabolisable energy of compound feeds for pigs. DLG Verlag, Frankfurt/Main
14. Li Q, Mair C, Schedle K, Hammerl S, Schodl K, Windisch W (2011) Effect of iodine source and dose on growth, iodine content in tissue and plasma thyroid hormones in fattening pigs. Euro J Nutrition. doi:10.1007/s00394-011-0247-7. in press
15. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT (2009) The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem 55:611–622
16. Li Q, Domig KJ, Ettle T, Windisch W, Mair C, Karl S (2011) Evaluation of potential reference genes for relative quantification by RT-qPCR in different porcine tissues derived from feeding studies. Int J Mol Sci 12:1727–1734
17. Xu CL, Wang YZ, Guo J, Liu JX, Feng J (2007) Comparison of age-related differences in expression of antioxidant enzyme mRNA and activity in various tissues of pigs. Comp Biochem Physiol B Biochem Mol Biol 147:445–451
18. Norden MM, Larsson F, Tedelind S, Carlsson T, Lundh C, Forssell-Aronsson E, Nilsson M (2007) Down-regulation of the sodium/iodide symporter explains 131I-induced thyroid stunning. Cancer Res 67:7512–7517

19. Schedle K, Pfaffl MW, Plitzner C, Meyer HH, Windisch W (2008) Effect of insoluble fibre on intestinal morphology and mRNA expression pattern of inflammatory, cell cycle and growth marker genes in a piglet model. Arch Anim Nutr 62:427–438
20. Marklund S, Marklund G (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 47:469–474
21. Beers RF Jr, Sizer IW (1954) Sulfide inhibition of catalase. Science 120:32–33
22. Lawrence RA, Burk RF (1978) Species, tissue and subcellular distribution of non Se-dependent glutathione peroxidase activity. J Nutr 108:211–215
23. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358
24. Buege JA, Aust SD (1978) Microsomal lipid peroxidation. Methods Enzymol 52:302–310
25. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29:e45
26. EFSA (2005) Opinion of the scientific panel on additives and products or substances used in animal feed on the request from the commission on the use of iodine in feeding stuffs. EFSA J 168:1–42
27. Dohan O, De la Vieja A, Paroder V, Riedel C, Artani M, Reed M, Ginter CS, Carrasco N (2003) The sodium/iodide Symporter (NIS): characterization, regulation, and medical significance. Endocr Rev 24:48–77
28. Suzuki K, Mori A, Saito J, Moriyama E, Ullianich L, Kohn LD (1999) Follicular thyroglobulin suppresses iodide uptake by suppressing expression of the sodium/iodide symporter gene. Endocrinology 140:5422–5430
29. Kogai T, Endo T, Saito T, Miyazaki A, Kawaguchi A, Onaya T (1997) Regulation by thyroid-stimulating hormone of sodium/iodide symporter gene expression and protein levels in FRTL-5 cells. Endocrinology 138:2227–2232
30. Eng PH, Cardona GR, Previti MC, Chin WW, Braverman LE (2001) Regulation of the sodium iodide symporter by iodide in FRTL-5 cells. Eur J Endocrinol 144:139–144
31. Suzuki K, Kimura H, Wu H, Kudo N, Kim WB, Suzuki S, Yoshida A, Caturegli P, Kohn LD Excess iodide decreases transcription of NIS and VEGF genes in rat FRTL-5 thyroid cells. Biochem Biophys Res Commun 393:286–290
32. Nakamura Y, Makino R, Tanaka T, Ishimura Y, Ohtaki S (1991) Mechanism of H2O2 production in porcine thyroid cells: evidence for intermediary formation of superoxide anion by NADPH-dependent H2O2-generating machinery. Biochemistry 30:4880–4886
33. Sugawara M, Sugawara Y, Wen K, Giulivi C (2002) Generation of oxygen free radicals in thyroid cells and inhibition of thyroid peroxidase. Exp Biol Med (Maywood) 227:141–146
34. Marklund SL, Westman NG, Lundgren E, Roos G (1982) Copper- and zinc-containing superoxide dismutase, manganese-containing superoxide dismutase, catalase, and glutathione peroxidase in normal and neoplastic human cell lines and normal human tissues. Cancer Res 42:1955–1961
35. Marklund SL (1984) Extracellular superoxide dismutase and other superoxide dismutase isoenzymes in tissues from nine mammalian species. Biochem J 222:649–655
36. Sugawara M, Kita T, Lee ED, Takamatsu J, Hagen GA, Kuma K, Medeiros-Neto GA (1988) Deficiency of superoxide dismutase in endemic goiter tissue. J Clin Endocrinol Metab 67:1156–1161
37. Schone F, Rajendram R (2009) Iodine in farm animals. In: Preedy V, Burrow G, Watson R (eds) Comprehensive handbook of iodine. Elsevier Academic Press, Oxford
38. Joanta AE, Filip A, Clichici S, Andrei S, Daicoviciu D (2006) Iodide excess exerts oxidative stress in some target tissues of the thyroid hormones. Acta Physiol Hung 93:347–359
39. McCord JM (1985) Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med 312:159–163
40. Vayre L, Sabourin JC, Caillou B, Ducreux M, Schlumberger M, Bidart JM (1999) Immunohistochemical analysis of Na+/I− symporter distribution in human extra-thyroidal tissues. Eur J Endocrinol 141:382–386
41. Smanik PA, Ryu KY, Theil KS, Mazzaferri EL, Jhiang SM (1997) Expression, exon-intron organization, and chromosome mapping of the human sodium iodide symporter. Endocrinology 138:3555–3558
42. Lacroix L, Mian C, Caillou B, Talbot M, Filetti S, Schlumberger M, Bidart JM (2001) Na(+)/I(-) symporter and Pendred syndrome gene and protein expressions in human extra-thyroidal tissues. Eur J Endocrinol 144:297–302
43. Foster LH, Sumar S (1997) Selenium in health and disease: a review. Crit Rev Food Sci Nutr 37:211–228
44. Arthur JR, Bermano G, Mitchell JH, Hesketh JE (1996) Regulation of selenoprotein gene expression and thyroid hormone metabolism. Biochem Soc Trans 24:384–388
45. Vazquez-Medina JP, Zenteno-Savin T, Elsner R (2006) Antioxidant enzymes in ringed seal tissues: potential protection against dive-associated ischemia/reperfusion. Comp Biochem Physiol C Toxicol Pharmacol 142:198–204
46. Yuan HT, Bingle CD, Kelly FJ (1996) Differential patterns of antioxidant enzyme mRNA expression in guinea pig lung and liver during development. Biochim Biophys Acta 1305:163–171