RESEARCH ARTICLE

Excess dietary iodine differentially affects thyroid gene expression in diabetes, thyroiditis-prone versus -resistant BioBreeding (BB) rats

Eleonora Swist[1], QiXuan Chen[1], Cunye Qiao[2], Don Caldwell[3], Heidi Gruber[1] and Kylie A. Scoggan[1,4]

[1] Nutrition Research Division, Food Directorate, Health Products and Food Branch, Health Canada, Banting Research Centre, Ottawa, Ontario, Canada
[2] Bureau of Food Surveillance and Science Integration, Food Directorate, Health Products and Food Branch, Health Canada, Banting Research Centre, Ottawa, Ontario, Canada
[3] Scientific Services Division, Food Directorate, Health Products and Food Branch, Health Canada, Banting Research Centre, Ottawa, Ontario, Canada
[4] Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Ontario, Canada

Scope: To identify genes involved in the susceptibility to iodine-induced autoimmune thyroiditis.
Methods and results: Diabetes, thyroiditis-prone (BBdp) and -resistant (BBc) rats were fed either a control or a high-iodine diet for 9 wk. Excess iodine intake increased the incidence of insulitis and thyroiditis in BBdp rats. BBdp rats fed the high-iodine diet that did not develop thyroiditis had higher mRNA levels of *Fabp4*, *Cidec*, *perilipin*, *Pparγ* and *Slc36a2* than BBdp rats fed the control diet and BBc rats fed either the control or the high-iodine diet. BBdp rats fed the high-iodine diet that did develop thyroiditis had higher mRNA levels of *Cidec*, *Icam1*, *Ifitm1*, and *Slpi* than BBdp rats fed the control diet and BBc rats fed either the control or the high-iodine diet. BBdp rats that did develop thyroiditis had lower mRNA levels of *Fabp4*, *perilipin* and *Slc36a2* but higher mRNA levels of *Icam1*, *Ifitm1* and *Slpi* than BBdp that did not develop thyroiditis. Excess dietary iodine also increased the protein levels of Fabp4, Cidec and perilipin in BBdp rats.
Conclusion: Differential expression of thyroid genes in BBdp versus BBc rats caused by excess dietary iodine may be implicated in autoimmune thyroiditis and insulitis pathogenesis.

Received: May 3, 2011
Revised: June 29, 2011
Accepted: July 4, 2011

Keywords:
Autoimmune thyroiditis / BB rats / Gene expression / Insulitis / Iodine

1 Introduction

Autoimmune thyroiditis, also known as Hashimoto's thyroiditis or chronic lymphocytic thyroiditis, is an organ-specific inflammatory disease that occurs when lymphocytes infiltrate the thyroid gland leading to impaired thyroid function resulting in hypothyroidism [1]. Both genetic and environmental factors, especially excessive intake of iodine, have been shown to contribute to the incidence and severity of autoimmune thyroiditis [2, 3].

Correspondence: Dr. Kylie A. Scoggan, Research Scientist, Nutrition Research Division, Food Directorate, HPFB, 2203E Banting Research Centre, 251 Sir Frederick Banting Driveway, Ottawa, ON, K1A 0K9, Canada
E-mail: kylie.scoggan@hc-sc.gc.ca
Fax: +1-613-941-6182

Abbreviations: ANOVA, analysis of variance; **BB rats,** BioBreeding rats; **BBc rats,** diabetes, thyroiditis-resistant BB rats; **BBdp rats,** diabetes, thyroiditis-prone BB rats; ***Cidec,*** cell death-inducing DFFA-like effector c; ***Fabp4,*** fatty acid binding protein 4; **GOI,** gene of interest; ***Hprt,*** hypoxanthine–guanine phosphoribosyltransferase; ***Icam1,*** intercellular adhesion molecule 1; ***Ifitm1,*** interferon-induced transmembrane protein 1; ***Kcnj13,*** potassium inwardly-rectifying channel, subfamily J, member 13; **OCT,** optimal cutting temperature; **real-time qPCR,** real-time quantitative PCR; ***Slc36a2,*** solute carrier family 36, member 2; ***Slpi,*** secretory leukocyte peptidase inhibitor; **T3,** triiodothyronine; **T4,** tetraiodothyronine or thyroxine; **TSH,** thyroid-stimulating hormone

© 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Iodine is an essential trace element required for normal thyroid function. The thyroid gland requires iodine to produce thyroid hormones, triiodothyronine (T3) and tetraiodothyronine (T4), which regulate body's basal metabolic rate. The synthesis and secretion of these hormones are regulated by the release of thyroid-stimulating hormone (TSH) from the pituitary gland in a negative feedback loop. Individuals with Hashimoto's thyroiditis who develop hypothyroidism have reduced serum levels of T3 and T4 hormones and increased levels of TSH. Although relatively large amounts of iodine were considered well tolerated [4] and several physiological mechanisms assure homeostasis of hormone secretion in the presence of widely varying iodine intakes [5], animal studies have demonstrated that high levels of dietary iodine lead to an increased incidence of autoimmune thyroiditis in susceptible diabetes-prone rats [6], hamsters [7], mice [8] and chickens [9]. Recently, long-term monitoring of the iodine fortification programs in Denmark [10], Greece [11], China [12] and Turkey [13] has also demonstrated that a high iodine intake increases the prevalence of autoimmune thyroiditis and/or hypothyroidism in humans. As such, potential health concerns regarding widespread consumption of excess iodine for susceptible individuals exist. Therefore, it is important to understand the underlying mechanisms of adverse effects of excess iodine intake to identify early markers of disease progression.

The mechanisms by which iodine leads to lymphocyte infiltration into the thyroid remain unclear. Previous in vitro studies in rat thyroid follicular cell line PCC13 and in cultured human thyroid follicles have shown that high iodine concentrations alter the expression of dozens of thyrocyte genes [14, 15]. Sharma et al. [16] demonstrated that iodine can directly stimulate the expression of intercellular adhesion molecule-1 (ICAM-1) on cultured thyrocytes of NOD.H2^{h4} mice, a model for autoimmune thyroiditis [8, 17], suggesting that excess dietary iodine may be affecting the thyroid itself. We speculated that iodine may directly alter the expression of other thyrocyte-specific genes that result in the promotion of the autoimmune process leading to autoimmune thyroiditis. Similar to Hashimoto's thyroiditis in humans, the diabetes, thyroiditis-prone BioBreeding (BBdp) rat strain has a predisposition to autoimmune diseases and spontaneously develops thyroiditis and/or type 1 diabetes [18]. In this study, we have identified thyroid genes that were differentially regulated by excess dietary iodine between BBdp and thyroiditis-resistant BB (BBc, the control) rat strains to identify genes which may be specifically involved in the susceptibility to iodine-induced autoimmune thyroiditis.

2 Materials and methods

2.1 Experimental diets

Two diets (Table 1) were prepared according to the AIN-93G recommendations for rodent diets [19]. The AIN-93G-MX contains 0.01 g/kg potassium iodate and was used to provide the recommended level of iodine, 0.2 mg iodine/kg diet, in the control diet. AIN-93G-MX-High iodine (Harlan Teklad, Madison, WI, USA), a modification of the AIN-93G-MX that contains 2.409 g/kg potassium iodate, was used to provide 50 mg iodine/kg diet in the high-iodine diet. Diets were made in pelleted form by the Animal Resources Division of Health Canada and stored at 4°C. The iodine content of the diets was analyzed using acid digestion followed by colorimetric analysis according to a modified method by Fischer et al. [20]. The final iodine content of the diets was 0.4 mg/kg in the control diet and 48 mg/kg in the high-iodine diet.

2.2 Animals

The animal protocol (2005-033) was approved by the Institutional Animal Care Committee at Health Canada and all animal handling and care followed the guidelines of the Canadian Council for Animal Care. Thyroiditis is more common in women than men therefore only female rats were used in this study. Thirty female BBdp rats (23 days old) and 30 age- and body weight-matched female control BBc rats of the same genetic strain were obtained from the Animal Resources Division of Health Canada (Ottawa, Canada). The rats were block randomized according to body weight into two groups and fed purified diets containing either the recommended (0.2 mg/kg) or high (50 mg/kg) levels of iodine (Table 1) for 9 wk. These iodine levels and time frame were chosen since previous studies demonstrated that administration of iodine through drinking water (approximately 10 mg/day) for 8 wk significantly increased the incidence of autoimmune lymphocytic thyroiditis in 30-day-old female BB/Worcester rats [6]. Fresh pellets were provided once every 4 days when food consumption and

Table 1. Composition (g/kg diet) of test diets[a)]

Ingredient	Control diet (0.2 mg iodine/kg diet)	High-iodine diet (50 mg iodine/kg diet)
Casein[b)]	200.0	200.0
Soybean oil	100.0	100.0
Cornstarch	499.5	499.5
Mineral mix (93G-MX)	35.0	0.0
Mineral mix (93G-MX-high iodine)[c)]	0.0	35.0
Vitamin mix (93-VX)	10.0	10.0
Miscellaneous[d)]	155.514	155.514

a) Based on the AIN-93G diet [19].
b) Vitamin-free test casein (Harlan Teklad, Madison, WI, USA).
c) Modification of AIN-93G-MX. Designed to supply 50 mg iodine/kg diet when included in the diet at 35 g/kg (3.5%) (Harlan Teklad).
d) Granulated sugar (100.0 g/kg), cellulose (alphacel) (50.0 g/kg), L-cystine (3.0 g/kg), choline bitartrate (2.5 g/kg) and *tert*-butylhydroquinone (0.014 g/kg).

© 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

body weights were recorded. Distilled pure water was changed daily. The onset of diabetes was monitored daily after 50 days of age by estimating the levels of glucose in the urine with TesTape (Eli Lilly, Indianapolis, IN, USA). After 9 wk on the test diets, 12 h food-deprived rats were euthanized via exsanguination from the abdominal aorta while under 3% isoflurane anesthesia. Blood was collected in serum separation tubes (BD, Oakville, ON, USA) and centrifuged at $1000 \times g$ for 10 min at 4°C to obtain serum for the analysis of thyroid hormones. Each thyroid lobe was dissected out and placed into its own plastic tissue mold containing optimal cutting temperature (OCT) compound and subsequently placed into a polystyrene weigh dish and allowed to freeze while floating on liquid nitrogen. These tissues were stored at −80°C for future histological and/or gene expression profiling analyses. Pancreas tissue was placed in 10% neutral buffered formalin for future histological analyses. Blood and tissues were not collected for one BBdp rat fed the control diet that died prior to completion of the 9-wk feeding phase.

2.3 Histology of the pancreas and thyroid

The pancreas was fixed in formalin and embedded in paraffin. Histological sections (5 μm) were prepared and stained with Meyer's hematoxylin–eosin. The frozen thyroids were cut at 8 μm in a cryostat set at −20°C. Cut sections were placed on superfrost plus coated slides (Fisher Scientific, Ottawa, ON, Canada) and stained with hematoxylin–eosin. Three sections from each rat were graded blindly. A score of 1–5 was used to describe the degree of inflammation [21]. For pancreas, grade 1 indicated no inflammation or within normal limits; grade 2 indicated mild inflammation; grade 3 indicated diffuse involvement; grade 4 indicated moderate infiltration; grade 5 indicated major invasion of islets by infiltrating leukocytes. For thyroid, grade 1 indicated no inflammation and morphology within normal limits; grade 2 indicated $<10\%$ of the thyroid infiltrated by inflammatory cells; grade 3 indicated 10–20% infiltration; grade 4 indicated 30–50% infiltration; grade 5 indicated greater than 50% infiltration.

2.4 Serum thyroid hormone analyses

Serum TSH levels were measured using a commercially available radioimmunoassay (RIA; American Laboratory Products (ALPCO) Diagnostics, Salem, NH, USA) as per the manufacturer's recommended conditions. Commercially available ELISA kits were used to measure serum total T3 and T4 (Alpha Diagnostic International, San Antonio, TX, USA) and free T3 and T4 (ALPCO Diagnostics, Salem, NH, USA). The washing steps were performed using the Skanwasher 300 version B plate washer (Skatron Instruments, Lier, Norway). The optical densities were measured using a Spectramax Plus 384 microplate reader (Molecular Devices, Union City, CA, USA) and the results were calculated from standard curves using Softmax Pro 4.7.1 (San Diego, CA, USA).

2.5 RNA isolation

Frozen thyroid tissue embedded in OCT compound was placed in 25 mL of RLT buffer (Qiagen) and teased free of OCT compound using forceps. Thyroid tissue was then placed in 3 mL of RLT buffer to wash away any remaining OCT compound and finally homogenized in a new aliquot of 600 μL of RLT buffer using a glass tissue grinder. Total RNA was isolated, purified and DNase I treated using RNeasy Mini kits (Qiagen, Mississauga, ON, Canada). Purified RNA was quantified using a NanoDrop Spectrophotometer (ThermoScientific, Wilmington, DE, USA). RNA concentrations of 50 ng/μL were prepared in RNase-free water. The RNA integrity of each sample was verified using an Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany).

2.6 Microarray hybridization, image acquisition and statistical analysis

High-quality thyroid RNA samples from five groups ($n = 5$/group) were labeled, hybridized to Affymetrix rat gene 1.0 ST arrays (Santa Clara, CA, USA), washed and scanned at the Ontario Genomics Innovation Centre StemCore Microarray Facility (Ottawa, Ontario, Canada) following the manufacturers' specifications. Briefly, RNA samples were amplified using the WT expression method (Ambion, Austin, TX, USA), and the generated cDNA targets were labeled using the Terminal labeling protocol (Affymetrix). Targets were hybridized to the Genechip Rat Gene 1.0 ST array in cartridge format (Affymetrix). The arrays were washed and stained with streptavidin–phycoerythrin conjugate using an automated protocol on the Genechip Fluidics Station 450 and scanned with the Genechip Scanner 3000 7G (Affymetrix). The five groups were: (i) BBc rats fed the control diet; (ii) BBc rats fed the high-iodine diet; (iii) BBdp rats fed the control diet; (iv) BBdp rats fed the high-iodine diet that did not develop thyroiditis; and (v) BBdp rats fed the high-iodine diet that did develop thyroiditis.

Affymetrix microarray data were initially analyzed for quality using the R package [22]. One array was excluded from the analyses due to poor data quality. The remaining microarray data were then analyzed using GeneSpring GX 9 software (Agilent Technologies, Santa Clara, CA, USA) with RatExonExpression technology and Robust Multichip Average (RMA) as the summarization algorithm. The baseline was set to the median of all samples, and the data were divided into the five previously mentioned treatment/disease groups in the study. The data were then filtered

© 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

eliminating all probes with unreliable signal intensities, either below the 20th or above the 100th percentile and by retaining only those entities that were within the 20–100 cut-off range for all the values in at least one out of the five conditions. Statistical analysis of the filtered microarray data was performed using a one-way analysis of variance (ANOVA) with a *p*-value cut-off of 0.05. Tukey's honestly significant differences (HSD) test was used as the post-hoc test with an asymptotic *p*-value computation and Benjamini–Hochberg method for multiple testing corrections.

2.7 Real-time quantitative PCR (real-time qPCR)

Based on the microarray data analysis, several genes that were greater than twofold ($p \leq 0.05$) differentially regulated by excess dietary iodine were selected for analysis by real-time qPCR. Procedures for cDNA synthesis and real-time qPCR were as previously described [23]. Briefly, total RNA (0.5 μg) was reverse transcribed to synthesize cDNA with Retroscript Kit (Ambion). Real-time qPCR was performed on a Mx4000 Multiplex Quantitative PCR System using the Brilliant SYBR Green QPCR Core Reagent Kit (Stratagene, La Jolla, CA, USA) and previously published primers for hypoxanthine–guanine phosphoribosyltransferase (*Hprt*) [24] or newly designed primers for several other genes (Table 2). For each experiment, a non-template reaction and a no-RT reaction were included as negative controls. The specificity of the PCR amplifications was confirmed by dissociation curves analysis of the products as well as by size verification of the amplicons in a conventional agarose gel.

The relative amounts of each gene-of-interest (GOI) were normalized to *Hprt* expression levels in the thyroid as an endogenous internal standard. A serial dilution of a standard was run on each plate for each GOI, as well as *Hprt*. These standard curves were used to calculate the relative levels of mRNA for each gene. Normalized values (GOI/*Hprt*) were then calibrated to the control group, namely control rats (BBc) fed the control diet (set as 1.0).

2.8 Protein isolation and Western blot analyses

Protein was extracted from rat thyroids using the Qiagen AllPrep (tissue) protocol (Qiagen) with the following modifications. Thyroids were dislodged from their OCT molds by submersing the mold in 25 mL of RLT buffer. The thyroids were transferred to fresh RLT buffer (600 μL) and then homogenized for 30 s in glass tissue grinders (size 21) and the collected homogenate centrifuged at $16\,000 \times g$ for 3 min. The protein pellets were re-suspended in 5% SDS buffer (pH 9.2) and heated to 95°C for 10 min. Protein concentrations were determined using the BCA method [25]. Forty micrograms of each protein sample was mixed with SDS sample buffer (50 mmol/L Tris-HCl, pH 6.8, 10% glycerol v/v, 2% SDS w/v, 144 mmol/L 2-mercaptoethanol, 0.008% bromophenol blue w/v final), separated over an 8–16% Tris-glycine gradient gel (Invitrogen, Burlington, Canada) and electroblotted onto PVDF Immun-Blot transfer membranes (Bio-Rad, Mississauga, ON, Canada). The membranes were blocked overnight at 4°C in TBS-Tween (20 mmol/L Tris, 500 mmol/L NaCl, 0.1% Tween-20 v/v, pH 7.5) containing 5% w/v non-fat dry milk (Bio-Rad). The membranes were then probed with an antibody against fatty acid binding protein 4 (Fabp4; sc-18661, Santa Cruz Biotechnology, CA, USA), perilipin (sc-67164, Santa Cruz Biotechnology) or cell death-inducing DFFA-like effector c (Cidec; NB-100-430, Novus Biologicals, Littleton, CO, USA) overnight at 4°C at a final concentration of 4.0, 4.0 and 10 mg/L, respectively, in TBS-Tween supplemented with 5% non-fat dry milk. The membranes were washed and incubated with either a goat anti-mouse or goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (Bio-Rad). Enhanced chemiluminescence was visualized using the Chemi Genius 2 Bio Imaging System (PerkinElmer, Woodbridge, Canada) following the addition of Super-Signal West Dura Extended Duration Substrate (ThermoScientific, Rockford, IL, USA) to the membranes. The membranes were stripped of antibodies using the Western blot recycling kit (Alpha Diagnostic International) following manufacturers' instructions and probed with an antibody against β-actin (Ab8227-50, Abcam, Cambridge, MA, USA) overnight at a final concentration of 0.5 mg/L. Protein–antibody complexes were detected after incubation with a goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (Bio-Rad) as described above.

2.9 Statistical analysis

Statistical analysis of the microarray data was described above. The Wilcoxon rank sum test was used to determine the statistical significance for the difference of the incidence of thyroiditis and insulitis among groups. All other data were presented as the means ± SD. Data were evaluated for equality of variance before statistical analysis. Variables with skewed distribution were logarithmically or square root transformed. Statistical analyses were performed using Statistica 8.0 software (StatSoft, Tulsa, OK, USA). One-way ANOVA followed by post hoc Duncan's test was used to determine the diet effects and compare differences among group means. Two-way ANOVA was used to determine the effects of strain (two levels: BBc and BBdp), diet (two levels: control and high iodine), and their interactions and comparisons between groups were made by Duncan's test. The non-parametric Kruskal–Wallis test, followed by pairwise Wilcoxon rank sum tests, was employed if the data failed to satisfy equality of variance. Differences were considered significant when $p \leq 0.05$.

© 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Table 2. Identified function and primer sequences of genes selected for real-time quantitative PCR (rat)

Gene	Forward primer (5′→3′); reverse primer (5′→3′)	Amplicon size (bp)	NCBI GenBank	Identified function
Fabp4	AGAAGTGGGATGGAAAGTCGACCA CACGCCTTTCATGACACATTCCAC	83	NM_053365	Intracellular lipid-binding protein; mediates fatty acid trafficking [28, 29] and inflammatory pathway [30]
Cidec	CGGAAGGTTCGCAAAGGCATCAT TGTGCCATCTTCCTCCAACACCA	117	NM_001024333	Lipid droplet-associated protein; links to the development of metabolic disorders [34–36]
Perilipin	ATATCTCCTGCCACCAGACAAGGT GCATGGTGTGTCGAGAAAGAGTGT	137	NM_013094	Lipid droplet-associated protein; plays a role in lipid metabolism [38–40]
Pparγ	AATGCCATCAGGTTTGGGCGAATG ATACAAATGCTTTGCCAGGGCTCG	126	NM_013124	Ligand-activated transcript factor; mediates expression of genes involved in lipid metabolism [38, 41]
Icam1	TGGTGAAGTCTGTCAAACGGGAGA AGGTGTAAATGGACGCCACGATCA	186	NM_012967	Cell adhesion molecule; ligand for leukocyte adhesion molecule LFA-1 [16, 42]
Ifitm1	TGGGACCCTAGCACCAAATTGAGA GTTCCTTGGGCATCTCTTGGCTTT	144	NM_001106314	Interferon-induced transmembrane protein; involved in signal transduction of anti-inflammation and anti-proliferation activities mediated by interferons [44, 45]
Slpi	GCCCAGTGCCTTAAACGTGAGAAA TCTTCACTGGTCCACGAATGGGAA	130	NM_053372	Secretory leukocyte peptidase inhibitor; regulates immune functions [43]
Slc36a2	TGCTGTGAGCTTCTTCCTCATCGT CGTTTCGTTCTTGTGGCAGCTGAT	133	NM_139339	Regulates pH-dependent, Na(+)-independent uptake of glycine, proline and α(methyl)aminoisobutyric acid [46]
Kcnj13	GCAGTTGTAGCTCACAAAGATGGCA TGATAGAGTACAGCAGAGACCCGAA	104	NM_053608	Potassium ion transport [48]
Hprt	GCGAAAGTGGAAAAGCCAAGT GCCACATCAACAGGACTCTTGTAG	76	NM_012583	House-keeping gene used in this study [24]

© 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

3 Results

3.1 Body weight, food intake and food efficiency of the rats

Body weight of the animals increased steadily during the 9-wk feeding phase of the study. BBdp rats had higher initial body weight ($p = 0.0038$) but less weight gain ($p = 0.0074$) and less food intake ($p = 0.0488$) than BBc rats; however, there were no effects of diet on body weight gain, total food intake and food efficiency ($p > 0.05$) (data not shown).

3.2 Excess dietary iodine increased the incidence and severity of thyroiditis and insulitis in BBdp rats

The incidence and severity of thyroiditis and insulitis in BB rats are shown in Table 3 and Fig. 1. There was no incidence of thyroiditis nor insultitis in BBc rats fed either the recommended or high levels of iodine. Figure 1A and C represents the normal structures of pancreas and thyroid from BBc rats, respectively. Compared with the control diet, the excess iodine diet increased the incidence (60% (9/15) versus 28.6% (4/14), Table 3) ($p = 0.043$) and severity (Fig. 1B) of insulitis in BBdp rats. Similarly, the excess iodine diet increased the incidence (33.3% (5/15) versus 7.1% (1/14), Table 3) and severity (Fig. 1D) of thyroiditis in BBdp rats; however, the increased incidence only achieved statistical significance for the left thyroid lobe ($p = 0.025$) and not for the right thyroid lobe ($p = 0.14$) nor for the combined thyroid lobes ($p = 0.083$). The percentage of rats with thyroiditis that also demonstrated insulitis was 100%. The percentage of rats with insulitis that also demonstrated thyroiditis was 25% (1/4) for BBdp rats fed the 0.2 mg I/kg diet and 60% (5/9) for BBdp rats fed the 50 mg I/kg diet (Table 3). The thyroid lesions were characterized by focal to multifocal interstitial and occasionally intrafollicular, infiltrations of lymphoid cells (Fig. 1D). The pancreas lesions were characterized by variable non-suppurative inflammation centered on islets, with the infiltrating leukocytes being mainly lymphocytes (Fig. 1B).

3.3 Excess dietary iodine decreased serum-free T4 and increased serum TSH levels

Compared with BBc rats, BBdp rats had lower levels of serum-free T3 ($p < 0.0001$) and T4 ($p = 0.0009$), as well as higher levels of serum TSH ($p = 0.005$; Table 4). The high-iodine diet decreased serum-free T4 hormone levels (overall diet effect, $p = 0.048$) and increased serum TSH levels (overall diet effect, $p = 0.044$) in both rat strains when compared with the control diet. Serum total T3 and T4 hormone levels were not statistically different among the four groups.

Table 3. Incidence and severity of thyroiditis and insulitis of rats fed diets containing either recommended or high-iodine levels for 9 wk

Rat strain	Diet	Thyroiditis											Insulitis					
		Left lobe					Right lobe					Overall incidence	1	2	3	4	5	Overall incidence
		1	2	3	4	5	1	2	3	4	5							
BBc	Control (0.2 mg I/kg)	15	0	0	0	0	15	0	0	0	0	0/15	15	0	0	0	0	0/15
	High iodine (50.0 mg I/kg)	14[a)]	0	0	0	0	15	0	0	0	0	0/15	15	0	0	0	0	0/15
BBdp	Control (0.2 mg I/kg)	13[a)]	0	0	0	0	13	1	0	0	0	1/14	10	2	2	0	0	4/14
	High iodine (50.0 mg I/kg)	10	2	2	1	0	11	0	2	0	2	5/15[b)]	6	2	3	4	0	9/15[c)]

a) Left thyroid lobe missing from one rat.
b) Compared with BBdp rats fed the control diet, $p = 0.025$ (left thyroid lobe only); $p = 0.14$ (right thyroid lobe only); $p = 0.083$ (combined left and right thyroid lobes).
c) Compared with BBdp rats fed the control diet, $p = 0.043$.

© 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

3.4 Different thyroid gene expression profiles were obtained due to different dietary iodine levels, rat strains and disease states

Microarray analysis revealed that different thyroid gene expression profiles were obtained due to different dietary iodine levels. Three hundred thirty genes (15 genes ≥ 2.0-fold) were differentially regulated by the high-iodine diet in BBc rats ($p \leq 0.05$; Table 5). Compared with BBdp rats fed the control diet, 399 genes (13 genes ≥ 2.0-fold) were differentially regulated by the high-iodine diet in BBdp rats that did not develop thyroiditis and 1997 (480 genes ≥ 2.0-fold) in BBdp rats that did develop thyroiditis ($p \leq 0.05$). Differential gene expression was also observed due to strain differences. Five hundred twenty genes (58 genes ≥ 2.0-fold) were differentially expressed between BBc and BBdp rats on the control diet ($p \leq 0.05$). Eight hundred seventy-six genes (57 genes ≥ 2.0-fold) were differentially expressed by the high-iodine diet between BBc and BBdp rats without thyroiditis ($p \leq 0.05$). Differential gene expression was also observed due to disease state. One thousand five hundred seven genes (407 genes ≥ 2.0-fold) were differentially expressed between BBdp rats fed the high-iodine diet that did not develop thyroiditis compared with rats that did develop thyroiditis ($p \leq 0.05$). Many of these latter gene expression differences are most likely due to genes from infiltrating lymphocytes as a result of the presence of thyroiditis. Genes that were differentially regulated by the high-iodine diet in BBc versus BBdp rats were further investigated by real-time qPCR, as these genes may represent candidate thyroid-specific genes involved in iodine-induced thyroiditis.

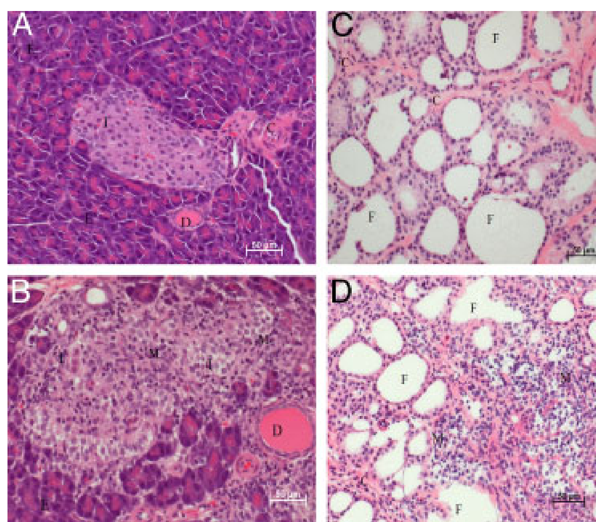

Figure 1. Representative hematoxylin and eosin-stained histology sections of the pancreas and thyroid at 200 × magnification. (A) BBc rat, normal pancreas. (B) BBdp rat with insulitis. (C) BBc rat, normal thyroid. (D) BBdp rat with thyroiditis. C, interfollicular connective tissue; D, duct; E, exocrine cells; F, follicular lumen; I, islets of Langerhans; M, infiltrating mononuclear cells.

3.5 Excess dietary iodine differentially affected specific thyroid mRNA expression levels in BBdp versus BBc rats

Real-time qPCR analysis demonstrated that BBdp rats fed a high-iodine diet that did not develop thyroiditis had higher mRNA levels of *Fabp4* (2.3-fold), *Cidec* (2.7-fold), *perilipin* (2.7-fold), *Pparγ* (1.7-fold) and solute carrier family 36, member 2 (*Slc36a2*; 2.7-fold) than BBdp rats fed the control diet ($p \leq 0.05$, Table 6). They also had higher mRNA levels of these five genes than BBc rats fed the control diet or the high-iodine diet ($p \leq 0.05$). BBdp rats fed the high-iodine diet that did develop thyroiditis had higher mRNA levels of *Cidec*, *Icam1*, interferon-induced transmembrane protein 1 (*Ifitm1*) and secretory leukocyte peptidase inhibitor (*Slpi*) than BBdp rats fed the control diet and BBc rats fed the control diet or the high-iodine diet ($p \leq 0.05$). Interestingly, in response to iodine treatment, BBdp rats that did develop thyroiditis had lower mRNA levels of *Fabp4* (0.66-fold), *perilipin* (0.64-fold) and *Slc36a2* (0.66-fold) but higher mRNA levels of *Icam1* (2.0-fold), *Ifitm1* (5.8-fold) and *Slpi* (39.0-fold) than BBdp that did not develop thyroiditis ($p \leq 0.05$). In addition, the high-iodine diet down-regulated potassium inwardly rectifying channel, subfamily J, member 13 (*Kcnj13*) mRNA expression in both BBc and BBdp rats ($p \leq 0.05$). BBdp rats fed the control diet had lower mRNA levels of *Icam1* (0.74-fold) and *Kcnj13* (0.64-fold) than BBc rats fed the control diet ($p \leq 0.05$).

Table 4. Serum thyroid hormone concentrations of rats fed diets containing either recommended or high-iodine levels for 9 wk

	BBc rats		BBdp rats		*p*-Value (two-way ANOVA)		
	0.2 mg I/kg ($n = 15$)	50 mg I/kg ($n = 15$)	0.2 mg I/kg ($n = 14$)	50 mg I/kg ($n = 15$)	Strain	Diet	Strain × diet
Free T3 (ng/L)	4.3 ± 0.8^{b}	4.2 ± 0.9^{b}	3.0 ± 0.7^{a}	3.1 ± 0.9^{a}	< 0.0001	0.977	0.598
Total T3 (ng/L)	678.8 ± 99.4	671.3 ± 85.6	668.7 ± 125.9	644.0 ± 148.5	0.542	0.600	0.778
Free T4 (ng/L)	18.6 ± 3.0^{c}	16.9 ± 4.6^{bc}	15.4 ± 4.0^{ab}	13.4 ± 2.7^{a}	0.0009	0.048	0.888
Total T4 (μg/L)	52.6 ± 25.3	52.8 ± 13.7	53.1 ± 6.8	48.4 ± 12.2	0.644	0.594	0.563
TSH (μg/L)	0.92 ± 0.17^{a}	0.99 ± 0.28^{a}	1.06 ± 0.31^{a}	1.33 ± 0.42^{b}	0.005	0.044	0.219

Values are presented as mean ± SD. Means in a row not sharing a superscript letter are significantly different ($p \leq 0.05$, Duncan's test).

© 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Table 5. Number of genes found to be differentially expressed in thyroids of BBc or BBdp rats fed the control or the high-iodine diet for 9 wk ($n = 4$ or 5) as measured by rat gene 1.0 ST array analysis

Group name	BBc rats fed the high-iodine diet (BBcH)	BBdp rats fed the control diet (BBdpL)	BBdp rats fed the high-iodine diet, without thyroiditis (BBdpH)	BBdp rats fed the high-iodine diet, with thyroiditis
BBc, control diet	330 (15)	520 (58)	1122 (152)	2517 (608)
BBcH		661 (68)	876 (57)	2141 (427)
BBdpL			399 (13)	1997 (480)
BBdpH				1507 (407)

Numbers in parentheses represent number of genes with ≥ 2.0-fold differential expression ($p \leq 0.05$).

Table 6. Expression of selected genes in thyroids of BBc or BBdp rats fed the control or the high-iodine diet for 9 wk as measured by real-time qPCR

Gene	BBc rats		BBdp rats			*p*-Value (one-way ANOVA)
	Control diet ($n = 5$)	High-iodine diet ($n = 5$)	Control diet ($n = 5$)	High-iodine diet (no-thyroiditis) ($n = 5$)	High-iodine diet (thyroiditis) ($n = 4$)	
Fabp4	1.00 ± 0.59^{a}	1.18 ± 0.36^{ab}	1.37 ± 0.56^{ab}	3.13 ± 1.11^{c}	2.07 ± 0.51^{b}	0.0006
Cidec	1.00 ± 0.68^{a}	1.36 ± 0.43^{a}	1.64 ± 0.69^{a}	4.37 ± 1.54^{b}	2.81 ± 0.57^{b}	<0.0001*
Perilipin	1.00 ± 0.81^{a}	1.50 ± 0.53^{ab}	1.56 ± 0.71^{ab}	4.24 ± 1.61^{c}	2.70 ± 0.77^{b}	0.0003
Pparγ	1.00 ± 0.43^{a}	1.16 ± 0.24^{a}	1.33 ± 0.36^{a}	2.26 ± 0.74^{b}	1.67 ± 0.40^{ab}	0.0039
Icam1	1.00 ± 0.15^{b}	0.89 ± 0.16^{ab}	0.74 ± 0.12^{a}	0.70 ± 0.09^{a}	1.37 ± 0.35^{c}	0.0003
Ifitm1	1.00 ± 0.09^{a}	1.66 ± 0.29^{b}	0.59 ± 0.19^{a}	0.75 ± 0.17^{a}	4.33 ± 0.96^{c}	<0.0001
Slpi	1.00 ± 0.21^{a}	2.05 ± 0.61^{b}	0.70 ± 0.28^{a}	1.05 ± 0.27^{a}	40.9 ± 20.7^{c}	<0.0001**
Slc36a2	1.00 ± 0.89^{a}	1.47 ± 0.50^{a}	2.35 ± 1.32^{ab}	6.31 ± 2.57^{c}	4.16 ± 1.59^{b}	0.0002
Kcnj13	1.00 ± 0.25^{c}	0.24 ± 0.04^{a}	0.64 ± 0.22^{b}	0.24 ± 0.04^{a}	0.20 ± 0.04^{a}	<0.0001

Values are presented as mean ± SD. Means in a row not sharing a superscript letter are significantly different ($p \leq 0.05$, Duncan's test).
*Square root transformation prior to one-way ANOVA. **Logarithmic transformation prior to one-way ANOVA.

3.6 Excess dietary iodine up-regulated thyroid protein expression levels of Fabp4, Cidec and perilipin in BBdp rats

Western blot analyses determined that thyroid protein expression of Fabp4, Cidec and perilipin was up-regulated in BBdp rats fed the high-iodine diet compared with the other groups ($p \leq 0.05$, Fig. 2).

4 Discussion

As expected, weanling BBdp rats fed excess dietary iodine for 9 wk showed an increased incidence of autoimmune thyroiditis. Our results are in agreement with those from Allen et al. [6] who reported that 30-day-old BBdp rats treated with 0.05% NaI in drinking water for 8 wk showed a significant increase in the incidence of autoimmune lymphocytic thyroiditis. Interestingly, our findings showed for the first time that excess dietary iodine also increased the incidence and severity of insulitis in BBdp rats and that BBc rats are resistant to developing thyroiditis and insulitis even at extreme intakes of dietary iodine. These results reinforce the genetic influence of these autoimmune diseases. Hartoft-Nielsen et al., however, demonstrated that sodium iodide administered neonatally by injection reduced the incidence of type 1 diabetes and insulitis [26] as well as autoimmune thyroiditis [27] in BB/Worcester rats. The reasons for this discrepancy are not apparent and might be related to factors such as differences in the timing of the treatment (neonates versus weanlings), the method of iodine treatment (injected versus oral), different rat strains, dose and duration of treatment.

In this study, we measured serum hormone levels as an indicator of thyroid function. Serum levels of free T3 and T4 were lower in BBdp rats compared with BBc rats, whereas serum TSH levels were higher in BBdp rats than BBc rats (Table 4). Excess dietary iodine decreased serum-free T4 levels and increased serum TSH levels in both rat strains, but to a slightly greater extent in BBdp rats. These responses are consistent with serum hormone changes associated with Hashimoto's thyroiditis, suggesting that excess dietary iodine contributes to the incidence and severity of this disease in BBdp rats.

In this paper, we also report for the first time that a high-iodine diet increased mRNA and protein levels of Fabp4 (also known as adipocyte protein aP2, A-FABP) in BBdp rats but not in BBc rats. The Fabp4 gene has been shown to play

© 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

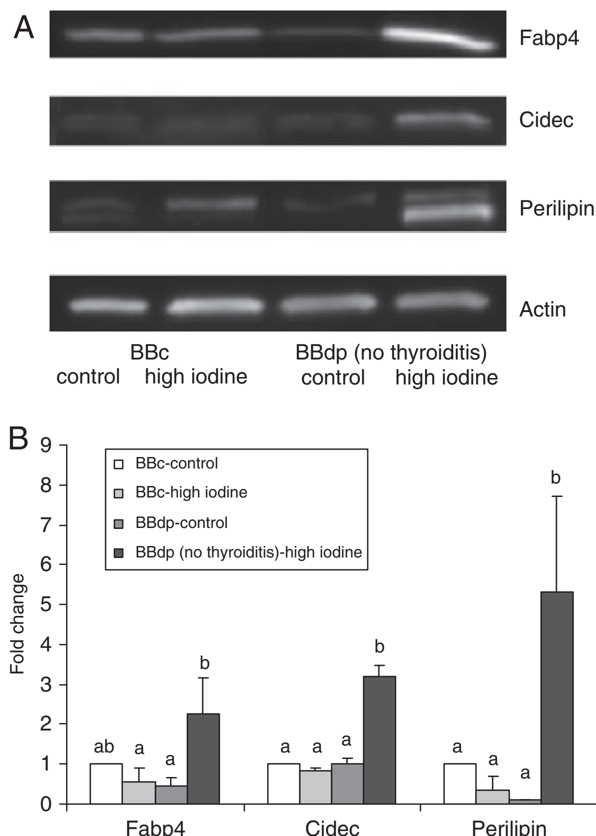

Figure 2. Excess dietary iodine up-regulated Fabp4, Cidec and perilipin protein expression in thyroids of BBdp rats. (A) Western blot analyses. (B) Bar graph depicting specific protein expression normalized to β-actin protein expression. Values are presented as mean $\pm$ SD. Means in a panel not sharing a superscript letter are significantly different ($p \leq 0.05$, Duncan's test).

an important role on plasma lipid levels, insulin sensitivity and coronary heart disease risk [28, 29]. Recent studies demonstrated that Fabp4-deficient macrophages not only altered cholesterol trafficking but also reduced IkB kinase and NF-κB activity leading to reduced expression of cyclooxygenase-2 and inducible nitric-oxide synthase as well as impaired production of inflammatory cytokines, suggesting that Fabp4 simultaneously regulates metabolic and inflammatory pathways [30]. Reynolds et al. also proposed that metabolic–inflammatory pathway cross-regulation by Fabps contributes to adaptive immune responses and subsequent autoimmune inflammation [31]. His study demonstrated that mice deficient for Fabp4 were protected from the development of experimental autoimmune encephalomyelitis, exhibiting reduced clinical symptoms of disease, reduced inflammatory cytokine mRNA expression, reduced leukocyte infiltration into CNS tissue and impaired inflammatory T-cell activity. Shum et al. also found that infiltration of leukocytes, especially eosinophils, into the airways was highly dependent on Fabp4 function and that Fabp4 was acting locally within the lung, most likely at bronchial epithelial cells, as the site of action of Fabp4 in allergic airway inflammation [32]. Therefore, in the present study, the increased expression of thyroid Fabp4 in thyroiditis-prone BB rats fed high levels of iodine may be a contributing factor to the pathogenesis of autoimmune disease, although we cannot rule out the possibility that up-regulation of Fabp4 may be due to an effect of the disease process. In either scenario, Fabp4 may be a potential early biomarker for disease progression.

Our results also demonstrated that high levels of iodine increased mRNA and protein levels of Cidec (also known as fat-specific protein 27, Fsp27) and perilipin in BBdp rats but not in BBc rats. Cidec/Fsp27 was reported to induce cell death in earlier studies [33] and to increase lipid droplet size and enhance the accumulation of lipids, resulting in the development of metabolic disorders including obesity and diabetes in recent studies [34–36]. In addition, $Fsp^{-/-}$ mice showed a dramatic lean phenotype with lower adiposity, decreased plasma lipid levels and were resistant to diet-induced obesity and liver steatosis [37]. Cidec/Fsp27 was also found to co-localize with perilipin [38], a canonical lipid-droplet resident protein involved in controlling access of lipases to the neutral lipid core of the droplet [39]. Pinnick et al. [40] used perilipin protein as a marker of adipocytes and reported a positive correlation between pancreatic triglyceride content and perilipin-positive adipocytes in mice fed a high-fat diet. The authors proposed that high levels of lipids damage islet cells of the pancreas and eventually contribute to islet dysfunction and diabetes development. Therefore, the up-regulation of Cidec/Fsp27 and perilipin in BBdp rats fed high levels of iodine in this study suggests that excess dietary iodine may accelerate impaired glucose uptake rates and reduced insulin sensitivity in BBdp rats, which are consistent with the increased incidence and severity of insulitis in BBdp rats fed high levels of iodine.

PPARγ is a lipid-activated transcription factor regulating the expression of numerous genes involved in lipid metabolism and inflammatory responses, including Fabp4 [41], Cidec and perilipin [38]. Our results indicated similar responses of these four genes to the high-iodine diet in which the expression of these genes remained unaltered in BBc rats but up-regulated in BBdp rats, supporting the concept that these genes play important roles in the disease process.

ICAM-1 is a cell adhesion molecule involved in the early stages of an inflammatory response. Bonita et al. [42] reported in 2002 that excess iodine ingestion in NOD.H2^{h4} mice up-regulated Icam1 protein expression in both areas of the thyroid gland with and without cellular infiltration. Sharma et al. demonstrated in 2005 that iodine can directly stimulate the expression of Icam-1 on cultured thyrocytes of NOD.H2^{h4} mice [16]. In agreement, our present study demonstrated that BBdp rats fed the high-iodine diet that developed thyroiditis had higher *Icam1* mRNA expression than the other groups (Table 6). Although we cannot

© 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

determine from this result whether *Icam1* mRNA expression increased directly in thyrocytes or increased due to infiltration of lymphocytes expressing *Icam1*, it is believed that *Icam1* plays an important role in disease progression.

Our present study also demonstrated for the first time that the high-iodine diet dramatically increased the mRNA levels of *Ifitm1* (5.8-fold) and *Slpi* (39.0-fold) in BBdp rats that did develop thyroiditis but not in BBdp rats that did not develop thyroiditis, when compared with BBdp rats fed a control diet. *Slpi* was proposed to display anti-protease/anti-inflammatory activities [43], and *Ifitm1* was proposed to be involved in signal transduction, anti-inflammation and anti-proliferation activities mediated by interferons in the immune system [44, 45]. The role of these two genes in the progression of autoimmune thyroid disease is not clear; it is plausible that they play compensatory roles to try to reduce the inflammatory response. For example, in response to high iodine, thyroids of BBc rats were able to up-regulate mRNA levels of *Slip* and *Ifitm1* to try to prevent an immune response. Thyroids of BBdp rats, however, did not appear to be able to up-regulate expression levels of *Slip*, *Ifitm1* and *Icam1*. However, their mRNA levels were significantly increased in BBdp rats that developed thyroiditis suggesting that these gene changes might have been due to the infiltrated lymphocytes, which express these genes to try to combat autoimmune responses.

Our study also showed that the high-iodine diet increased mRNA levels of *Slc36a2*, a proton-coupled amino acid transporter [46], in BBdp rats, but not in BBc rats when compared with the control diet. Up-regulation of *Slc36a2* was also reported in the endothelium of diabetic mice [47], suggesting utilization of amino acids as alternative energy substrates. In addition, BBdp rats fed the control diet had lower mRNA levels of *Kcnj13* (0.64-fold), a potassium transporter [48], than BBc rats fed the control diet, and the high-iodine diet down-regulated *Kcnj13* mRNA expression in both BBc and BBdp rats. The roles of *Slc36a2* and *Kcnj13* in the progression of autoimmune thyroid disease require further investigation.

In conclusion, chronic high levels of dietary iodine increased the incidence of insulitis and thyroiditis in diabetes, thyroiditis-prone BB rats. Excess iodine intake also altered mRNA and protein levels of numerous thyroid genes involved in lipid droplet movement and inflammatory activities in BBdp rats but not in BBc rats. These results suggest that alterations in the expression of these genes may contribute to the progression of iodine-induced autoimmune thyroid disease. In addition, the gene expression changes observed in thyroids from BBdp rats that have not yet developed thyroiditis may represent early thyrocyte gene expression changes in the development of iodine-induced thyroiditis, whereas gene expression changes observed in thyroids from animals with thyroiditis may be due to thyrocytes and/or infiltrating lymphocytes and/or the disease process.

The authors thank Dr. Rudi Mueller for insightful discussions, Pascale Bellon-Gagnon and Sonia Abbas for technical support and Stephen Hayward for statistical support. They also thank the members of the Animal Resources Division of Health Canada for their technical assistance with the animal phase of this study. This research was funded by Health Canada and Agricultural Policy Framework.

The authors have declared no conflict of interest.

5 References

[1] Burek, C. L., Talor, M. V., Environmental triggers of autoimmune thyroiditis. *J. Autoimmun.* 2009, *33*, 183–189.

[2] Laurberg, P., Cerqueira, C., Ovesen, L., Rasmussen, L. B. et al., Iodine intake as a determinant of thyroid disorders in populations. *Best Pract. Res. Clin. Endocrinol. Metab.* 2010, *24*, 13–27.

[3] Tomer, Y., Genetic susceptibility to autoimmune thyroid disease: past, present, and future. *Thyroid* 2010, *20*, 715–725.

[4] Pennington, J. A., A review of iodine toxicity reports. *J. Am. Diet. Assoc.* 1990, *90*, 1571–1581.

[5] Bürgi, H., Iodine excess. *Best Pract. Res. Clin. Endocrinol. Metab.* 2010, *24*, 107–115.

[6] Allen, E. M., Appel, M. C., Braverman, L. E., The effect of iodide ingestion on the development of spontaneous lymphocytic thyroiditis in the diabetes-prone BB/W rat. *Endocrinology* 1986, *118*, 1977–1981.

[7] Follis, R. H., Jr., Further observations on thyroiditis and colloid accumulation in hyperplastic thyroid glands of hamsters receiving excess iodine. *Lab. Invest.* 1964, *13*, 1590–1599.

[8] Rasooly, L., Burek, C. L., Rose, N. R., Iodine-induced autoimmune thyroiditis in NOD.H2^{h4} mice. *Clin. Immunol. Immunopathol.* 1996, *81*, 287–292.

[9] Bagchi, N., Brown, T. R., Urdanivia, E., Sundick, R. S., Induction of autoimmune thyroiditis in chickens by dietary iodine. *Science* 1985, *230*, 325–327.

[10] Pedersen, I. B., Laurberg, P., Knudsen, N., Jørgensen, T. et al., An increased incidence of overt hypothyroidism after iodine fortification of salt in Denmark: a prospective population study. *J. Clin. Endocrinol. Metab.* 2007, *92*, 3122–3127.

[11] Fountoulakis, S., Philippou, G., Tsatsoulis, A., The role of iodine in the evolution of thyroid disease in Greece: From endemic goiter to thyroid autoimmunity. *Hormones (Athens)* 2007, *6*, 25–35.

[12] Teng, W., Shan, Z., Teng, X., Guan, H. et al., Effect of iodine intake on thyroid diseases in China. *N. Engl. J. Med.* 2006, *354*, 2783–7293.

[13] Bastemir, M., Emral, R., Erdogan, G., Gullu, S., High prevalence of thyroid dysfunction and autoimmune thyroiditis in adolescents after elimination of iodine deficiency

© 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

in the Eastern Black Sea Region of Turkey. *Thyroid* 2006, *16*, 1265–1271.

[14] Yamazaki, K., Yamada, E., Kanaji, Y., Yanagisawa, T. et al., Genes regulated by thyrotropin and iodide in cultured human thyroid follicles: analysis by cDNA microarray. *Thyroid* 2003, *13*, 149–158.

[15] Leoni, S. G., Galante, P. A., Ricarte-Filho, J. C. M., Kimura, E. T., Differential gene expression analysis of iodide-treated rat thyroid follicular cell line PCC13. *Genomics* 2008, *91*, 356–366.

[16] Sharma, R. B., Alegria, J. D., Talor, M. V., Rose, N. R. et al., Iodine and IFN-gamma synergistically enhance intercellular adhesion molecule 1 expression on NOD.H2^{h4} mouse thyrocytes. *J. Immunol.* 2005, *174*, 7740–7745.

[17] Braley-Mullen, H., Sharp, G. C., Medling, B., Tang, H., Spontaneous autoimmune thyroiditis in NOD.H-2h4 mice. *J. Autoimmunol.* 1999, *12*, 157–165.

[18] Wright, J. R., Senhauser, D. A., Yates, H. A., Sharma, H. M., Thibert, P., Spontaneous thyroiditis in BB Wistar diabetic rats. *Vet. Pathol.* 1983, *20*, 522–530.

[19] Reeves, P. G., Components of the AIN-93 diets as improvements in the AIN-76A Diet. *J. Nutr.* 1997, *127*, 838S–841S.

[20] Fischer, P. W., L'Abbé, M. R., Giroux, A., Colorimetric determination of total iodine in foods by iodide-catalyzed reduction of Ce+4. *J. Assoc. Off. Anal. Chem.* 1986, *69*, 687–689.

[21] Hoorfar, J., Scott, F. W., Cloutier, H. E., Dietary plant materials and development of diabetes in the BB rat. *J. Nutr.* 1991, *121*, 908–916.

[22] R Development Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria 2005.

[23] Scoggan, K. A., Gruber, H., Chen, Q., Plouffe, L. J. et al., Increased incorporation of dietary plant sterols and cholesterol correlates with decreased expression of hepatic and intestinal Abcg5 and Abcg8 in diabetic BB rats. *J. Nutr. Biochem.* 2009, *20*, 177–186.

[24] Hillebrands, J. L., Whalen, B., Visser, J. T., Koning, J. et al., A regulatory CD4+T cell subset in the BB rat model of autoimmune diabetes expresses neither CD25 nor Foxp3. *J. Immunol.* 2006, *177*, 7820–7832.

[25] Stoscheck, C. M., Quantitation of protein. *Methods Enzymol.* 1990, *182*, 50–68.

[26] Hartoft-Nielsen, M. L., Rasmussen, A. K., Bock, T., Feldt-Rasmussen, U. et al., Iodine and tri-iodo-thyronine reduce the incidence of type 1 diabetes mellitus in the autoimmune prone BB rats. *Autoimmunity* 2009, *42*, 131–138.

[27] Hartoft-Nielsen, M. L., Rasmussen, A. K., Kaas, A., Feldt-Rasmussen, U., Buschard, K., Neonatal stimulation of the thyroid gland with iodine or suppression during adolescence with triiodothyronine changes the prevalence of autoimmune thyroiditis in BB rats. *Eur. J. Endocrinol.* 2004, *151*, 375–382.

[28] Brouillette, C., Bosse, Y., Perusse, L., Gaudet, D., Vohl, M.-C., Effect of liver fatty acid binding protein (FABP) T94A missense mutation on plasma lipoprotein responsiveness to treatment with fenofibrate. *J. Hum. Genet.* 2004, *49*, 424–432.

[29] Tuncman, G., Erbay, E., Hom, X., De Vivo, I. et al., A genetic variant at the fatty acid-binding protein aP2 locus reduces the risk for hypertriglyceridemia, type 2 diabetes, and cardiovascular disease. *Proc. Natl. Acad. Sci. USA* 2006, *103*, 6970–6975.

[30] Makowski, L., Brittingham, K. C., Reynolds, J. M., Suttles, J., Hotamisligil, G. S., The fatty acid-binding protein, aP2, coordinates macrophage cholesterol trafficking and inflammatory activity. *J. Biol. Chem.* 2005, *280*, 12888–12895.

[31] Reynolds, J. M., Liu, Q., Brittingham, K. C., Liu, Y. et al., Deficiency of fatty acid-binding proteins in mice confers protection from development of experimental autoimmune encephalomyelitis. *J. Immunol.* 2007, *179*, 313–321.

[32] Shum, B. O. V., Mackay, C. R., Gorgun, C. Z., Frost, M. J. et al., The adipocyte fatty acid-binding protein aP2 is required in allergic airway inflammation. *J. Clin. Invest.* 2006, *116*, 2183–2192.

[33] Liang, L., Zhao, M., Xu, Z., Yokoyama, K. K., Li, T., Molecular cloning and characterization of CIDE-3, a novel member of the cell-death-inducing DNA-fragmentation-factor (DFF45)-like effector family. *Biochem. J.* 2003, *370*, 195–203.

[34] Kim, J. Y., Liu, K., Zhou, S., Tillison, K. et al., Assessment of fat-specific protein 27 in the adipocyte lineage suggests a dual role for FSP27 in adipocyte metabolism and cell death. *Am. J. Physiol. Endocrinol. Metab.* 2008, *294*, E654–E667.

[35] Puri, V., Konda, S., Ranjit, S., Aouadi, M. et al. Fat-specific protein 27, a novel lipid droplet protein that enhances triglyceride storage. *J. Biol. Chem.* 2007, *282*, 34213–34218.

[36] Matsusue, K., A physiological role for fat specific protein 27/cell death-inducing DFF45-like effector C in adipose and liver. *Biol. Pharm. Bull.* 2010, *33*, 346–350.

[37] Gong, J., Sun, Z., Li, P., CIDE proteins and metabolic disorders. *Curr. Opin. Lipidol.* 2009, *20*, 121–126.

[38] Puri, V., Ranjit, S., Konda, S., Nicoloro, S. M. et al., Cidea is associated with lipid droplets and insulin sensitivity in humans. *Proc. Natl. Acad. Sci. USA* 2008, *105*, 7833–7838.

[39] Bickel, P. E., Tansey, J. T., Welte, M. A., PAT proteins, an ancient family of lipid droplet proteins that regulate cellular lipid stores. *Biochim. Biophys. Acta* 2009, *1791*, 419–440.

[40] Pinnick, K. E., Collins, S. C., Londos, C., Gauguier, D. et al., Pancreatic ectopic fat is characterized by adipocyte infiltration and altered lipid composition. *Obesity (Silver Spring)* 2008, *16*, 522–530.

[41] Szanto, A., Balint, B. L., Nagy, Z. S., Barta, E. et al., STAT6 transcription factor is a facilitator of the nuclear receptor PPARγ-regulated gene expression in macrophages and dendritic cells. *Immunity* 2010, *33*, 699–712.

[42] Bonita, R. E., Rose, N. R., Rasooly, L., Caturegli, P., Burek, C. L., Adhesion molecules as susceptibility factors in spontaneous autoimmune thyroiditis in the NOD-H2h4 mouse. *Exp. Mol. Pathol.* 2002, *73*, 155–163.

© 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

[43] Sallenave, J. M., Secretory leukocyte protease inhibitor and elafin/trappin-2: versatile mucosal antimicrobials and regulators of immunity. *Am. J. Respir. Cell Mol. Biol.* 2010, *42*, 635–643.

[44] Yu, F., Ng, S. S., Chow, B. K., Sze, J. et al., Knockdown of interferon-induced transmembrane protein 1 (IFITM1) inhibits proliferation, migration, and invasion of glioma cells. *J. Neurooncol.* 2011 *103*, 187–195.

[45] Deblandre, G. A., Marinx, O. P., Evans, S. S., Majjaj, S. et al., Expression cloning of an interferon-inducible 17-kDa membrane protein implicated in the control of cell growth. *J. Biol. Chem.* 1995, *270*, 23860–23866.

[46] Bermingham, J. R., Jr., Pennington, J., Organization and expression of the SLC36 cluster of amino acid transporter genes. *Mamm. Genome* 2004, *15*, 114–125.

[47] Maresh, J. G., Shohet, R. V., In vivo endothelial gene regulation in diabetes. *Cardiovasc. Diabetol.* 2008, *19*, 7–8.

[48] Döring, F., Karschin, A., Genomic structure and promoter analysis of the rat kir7.1 potassium channel gene (Kcnj13). *FEBS Lett.* 2000, *483*, 93–98.

© 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim