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Naturally occurring iodine in humic substances in drinking water in Denmark is bioavailable and determines population iodine intake

Stig Andersen[1,2]*, Klaus M. Pedersen[1], Finn Iversen[3], Steen Terpling[3], Peter Gustenhoff[1], Steffen B. Petersen[2] and Peter Laurberg[1]

[1]*Department of Endocrinology & Medicine, Aalborg Hospital, Aarhus University Hospital, Denmark*
[2]*Department of Life Science, Aalborg University, Denmark*
[3]*Skagen Hospital, Frederikshavn-Skagen Hospital, Skagen, Denmark*

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Iodine intake is important for thyroid function. Iodine content of natural waters is high in some areas and occurs bound in humic substances. Tap water is a major dietary source but bioavailability of organically bound iodine may be impaired. The objective was to assess if naturally occurring iodine bound in humic substances is bioavailable. Tap water was collected at Randers and Skagen waterworks and spot urine samples were collected from 430 long-term Randers and Skagen dwellers, who filled in a questionnaire. Tap water contained 2 μg/l elemental iodine in Randers and 140 μg/l iodine bound in humic substances in Skagen. Median (25; 75 percentile) urinary iodine excretion among Randers and Skagen dwellers not using iodine-containing supplements was 50 (37; 83) μg/24 h and 177 (137; 219) μg/24 h respectively ($P < 0{\cdot}001$). The fraction of samples with iodine below 100 μg/24 h was 85·0 % in Randers and 6·5 % in Skagen ($P < 0{\cdot}001$). Use of iodine-containing supplements increased urinary iodine by 60 μg/24 h ($P < 0{\cdot}001$). This decreased the number of samples with iodine below 100 μg/24 h to 67·3 % and 5·0 % respectively, but increased the number of samples with iodine above 300 μg/24 h to 2·4 % and 16·1 %. Bioavailability of iodine in humic substances in Skagen tap water was about 85 %. Iodine in natural waters may be elemental or found in humic substances. The fraction available suggests an importance of drinking water supply for population iodine intake, although this may not be adequate to estimate population iodine intake.

Iodine bioavailability: Humic substances: Drinking water: Population-based study

The iodine intake level influences thyroid function in a population and both high and low iodine intake levels are associated with increased risk of disease[1,2].

Dietary iodine content is decisive for iodine intake[3,4]. Water is a ubiquitous component of the diet and ground water is an important drinking water resource in many countries[5].

Tap water iodine content exhibits regional differences[5–7], which are associated with differences in population iodine intake levels[4,7,8]. Also, the iodine content of drinking water has been shown to influence the occurrence of thyroid disease in populations[6,9,10]. However, thyroid disease may persist despite iodine-replete diets[11,12]. This raises the question of iodine bioavailability.

The bioavailability of elemental iodine was demonstrated decades ago[13] and confirmed recently in a study that also demonstrated a reduced bioavailability of iodine in seaweed[14], as found by others[15].

Chemical analysis of natural waters has demonstrated variable amounts of humic substances[3,16–18], which bind iodine[3,19] to form iodo derivatives[20]. This may modify the biogeochemical behaviour of iodine[21] and a reduced uptake has been found in animals[22,23] and suggested in man[24,25]. Previous studies of tap water iodine content have looked into the association with thyroid disorders[6,25–28] and subsurface geology[25,28], while data on whether it is bioavailable are lacking.

We previously found that natural waters in Skagen had a high content of iodine[7], but that this was bound in humic substances[3]. In addition, we have identified a town with a low content of iodine in tap water, likely due to a different subsurface geochemistry.

We aimed to obtain data on the content of humic substances in tap water low in iodine compared with iodine-rich waters to evaluate the influence of iodine in humic substances in drinking water on the urinary iodine excretion levels on a population level and to assess if iodine bound in humic substances in natural waters is bioavailable.

Methods

Subsurface geology

The investigations were performed in two towns, Randers and Skagen, both situated on the peninsula of Jutland in Denmark. Skagen is situated in the northernmost part of Jutland, which still rises after deglaciation approximately 15 000 yeas ago. In combination with the apposition of sand by the North Sea to the northern tip of Jutland, this has built the Isthmus of

* **Corresponding author:** Stig Andersen, fax +45 9932 6857, email stiga@dadlnet.dk

Skagen. Consequently, the tap water from the waterworks of Skagen is derived from an aquifer source rock (buried sea floor) that contains marine deposits[3]. In contrast, Randers is situated on the phenoscandinavian brim with no uplift of land[5]. Thus, the waterworks of the two towns, although being situated relatively close, differ by aquifer source rock, which was the background for selection of these two areas for investigation.

Water samples

Water samples were collected on 3 separate days from both locations. Furthermore, Skagen tap water was collected at 2-month intervals for 6 months and once every year for 4 consecutive years from 1997 to 2000[3]. The iodine content was unaltered with time[3].

Skagen had a high iodine content of tap water (three to four times sea water level)[3] and Randers had a low iodine content of tap water.

Procedures and solutions

Tap water samples were collected in iodine-free polyethylene containers from the final tap before leaving the waterworks. Samples were kept dark at 4°C until freeze drying. Freeze-dried samples were stored in an oxygen-free environment until they were re-dissolved 1:10 in ultrapure water from a Mili-Q water purification system (Millipore Corporation, Billerica, MA, USA) for further analysis. Iodine content was unaffected by freeze drying when re-dissolved 1:1 (recovery 90–103 %; average 98 %).

HPLC size exclusion was performed on ÄKTApurifier™ (Amersham Pharmacia Biotech, Freiburg, Germany) using a Superose 12 HR 10/30 column (Amersham Pharmacia Biotech) as described in detail previously[3]. This is an agarose gel with exclusion limits from 1000 to 300 000 D (limits stated by supplier). Raw or resuspended tap water (500 μl) was added to the column after filtering through a 0·20 μm membrane (Minisart®; Sartorius, Göttingen, Germany) to eliminate particulate matter. Identical iodine concentrations were seen before and after filtering. Tris buffer 10 mM, pH 7·0 was eluent. Elution speed was 1 ml/min and pressure was 1·45–1·52 MPa. Absorbance at 280 nm was registered and effluent was collected in fractions of 1·5 ml. Experiments were carried out at 21°C and performed in triplicate.

Populations

Participants were men and women living in the towns of Randers or Skagen in Jutland, Denmark. Names and addresses were obtained from the national civil registration system, in which all individuals living in Denmark are recorded. In Randers, all men and women born in 1920 were invited to take part (*n* 483). Skagen had a smaller population and all individuals born between 1918 and 1923 were invited to participate (*n* 432).

The investigation took place at the local hospital (90 %) or, at request, as home visits (10 %) in 1997 and 1998, i.e. before the recent iodine fortification of salt in Denmark[29]. Information about medication and the use of iodine containing vitamin and mineral preparations was collected by a questionnaire. Among these long-time Skagen dwellers, 95 % had lived in the town for more than 10 years and 91 % for more than 30 years. Also, this population had retired and thus had the time to participate. In addition, the intake of water and other liquids was known[30].

The study was approved by the regional ethics committee for Nordjylland and Viborg County.

Urine

At the interview, a non-fasting spot urine sample was collected from all participants. Urine samples were frozen and stored at −20°C in iodine-free polyethylene containers for subsequent measurements of iodine and creatinine.

Iodine and creatinine determination

Iodine was determined by the Sandell-Kolthoff reaction modified after Wilson and van Zyl[31] as described previously[32,33]. The principle is evaporation and alkaline ashing of the sample followed by resuspension and measurement of iodine by the spectrophotometric detection of the catalytic role of iodine in the reduction of ceric ammonium sulfate in the presence of arsenious acid. For determination of iodine content, a 1·5 ml sample was used giving an analytical sensitivity of 1·0 μg/l. The intra-assay CVs were 9·2 % (interval 2–4 μg/l, *n* 8); 8·7 % (interval 5–9 μg/l, *n* 4); 4·2 % (interval 10–15 μg/l, *n* 4); 1·5 % (interval 15–50 μg/l, *n* 5). Urinary creatinine was determined by a kinetic Jaffé method[34].

Urinary iodine excretion was expressed in μg/l and as an estimate of the 24 h urinary iodine excretion by adjusting the iodine:creatinine ratio for the average 24 h urinary creatinine excretion in an age and gender matched group of Caucasians (men 0·95 g/24 h; women 0·7 g/24 h)[35,36].

Statistical analysis

The χ^2 test was used to compare frequency among populations. Mann Whitney U-test was used to compare the median iodine content of samples. Bartlett test for homogeneity of variance was used for comparing variances. Factors important to urinary iodine excretion were tested in a multivariate logistic regression model with urinary iodine excretion entered as dependent variable. Explanatory variables entered were gender, origin of tap water, use of iodine-containing supplements and the lifestyle factors smoking and alcohol use. A *P*-value of $<0·05$ was considered significant.

Results

In Skagen, the mean iodine content of raw water was 152·7 (±4·0) μg/l. It was 139·7 (±5·2) μg/l after water treatment consisting of aeration, sedimentation and chemical coagulation before sedimentation, adsorption in granular activated carbon contractors and tandem sand filtration. Thus, the extensive water treatment reduced the iodine content by 8·5 %. In Randers, the average tap water iodine content was 2·0 μg/l.

Fig. 1(b) shows Skagen tap water with a high content of iodine bound in macromolecules (Fig. 1(a)) previously found to be humic substances[3,17]. Randers tap water had a low iodine content (Fig. 1(d)) not bound in humic substances (Fig. 1(c)).

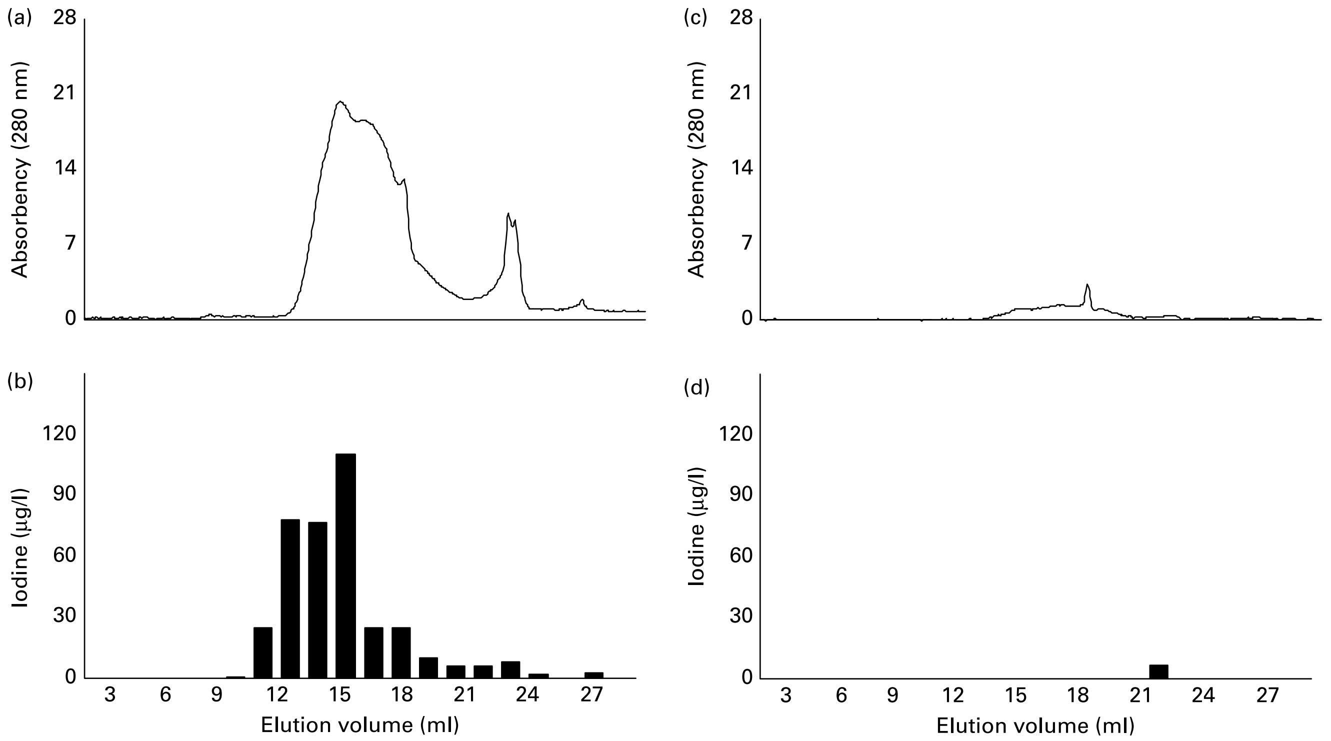

Fig. 1. HPLC size exclusion performed on preconcentrated tap water from Randers (left) and Skagen (right). Elution of iodine measured (b,d) and absorbency at 280 nm was registered (a,c). For details of subjects and Procedures, see Methods.

Table 1 shows the characteristics of the 430 participants from Randers and Skagen. The participation rate was 43·9 % in Randers and 50·5 % in Skagen. An equal number of long-term Randers- and Skagen-dwelling men and women participated from the two towns (χ^2; $P=0{\cdot}98$). More women (*n* 264) than men (*n* 166) participated in accordance with the demographic characteristics of the population aged 75 to 80 years. Iodine-containing supplements were taken more frequently in Randers than in Skagen (χ^2; $P=0{\cdot}003$).

Table 1. Descriptions of the participants from the two towns, Randers and Skagen, in Jutland, Denmark§

	Randers		Skagen	
	n	%	*n*	%
Gender				
Men	82	39	84	38
Women	130	61	134	62
Age	78 years		75-80 years	
Alcohol use*				
Regularly	32	16	34	16
Sometimes	149	73	137	64
Never	23	11	43	20
Smoker†				
Present	54	26	46	21
Past	81	39	95	44
Never	74	35	75	35
Use of supplements with iodine‡				
Daily	81	38	53	24
Sometimes	14	7	26	12
Never	116	55	139	64

* 12 no answer
† 5 no answer.
‡ 1 no answer.
§ For details of subjects and procedures, see Methods.

Urinary iodine excretion in participant groups is shown in Table 2. Urinary iodine excretion among participants not taking iodine-containing supplements was markedly lower in Randers than in Skagen (Mann–Whitney, $P_{\mathrm{between towns}} < 0{\cdot}001$). Participants with a daily use of iodine-containing supplements had a 66 µg/24 h (Randers) and 54 µg/24 h (Skagen) higher urinary iodine excretion, with no difference between towns (Mann–Whitney, $P=0{\cdot}88$). The fraction of urine samples with iodine excretion below 100 µg/24 h, i.e. the level set to discriminate iodine deficiency when investigating groups[37], was higher in Randers than in Skagen (Table 2, both in participants with no use of iodine-containing supplements and all participants: χ^2, $P<0{\cdot}001$). Conversely, the fraction of samples indicating more than adequate iodine intake (>200 µg/24 h) was clearly higher in Skagen (χ^2; $P<0{\cdot}001$), as was the fraction of samples indicating excessive iodine intake (>300 µg/24 h) (χ^2, $P<0{\cdot}001$), as can be seen in Table 2. Expressing the results in µg/l did not alter the results of comparisons.

Fig. 2 shows the distribution of estimated 24 h urinary iodine excretion among all Randers and Skagen dwellers. The difference was marked, even when blurred by the use of iodine-containing supplements, and it was also significant for both median (Mann–Whitney; $P<0{\cdot}001$) and dispersion (Bartlett test; $P<0{\cdot}01$) among supplement users.

The factors important for urinary iodine excretion (>100 µg/24 h) were origin of tap water (reference:Randers; OR 72; 95 % CI 33, 153) and use of iodine-containing vitamins (reference:no users; OR 6·4; 3·4, 12) when tested in a multivariate logistic regression model. This suggests that iodine in humic sub-

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Table 2. Urinary iodine excretion among participants from the two towns Randers and Skagen in Jutland, Denmark. The two towns have a different aquifer source rock, that caused different iodine content of drinking water: 2 µg/l in Randers and 140 µg/l in Skagen¶

	Randers *		Skagen†	
	µg/l median (25; 75 percentiles)	µg/24 h ‡ median (25; 75 percentiles)	µg/l median (25; 75 percentiles)	µg/24 h ‡ median (25; 75 percentiles)
Iodine excretion among:				
All participants	55 (36; 98)	74 (45; 118)	160 (126; 228)	184 (144; 246)
Use of supplements with iodine:				
Daily	94 (59; 148)	116 (83; 165)	200 (146; 266)	231 (180; 325)
Sometimes	65 (54; 97)	88 (49; 102)	163 (128; 216)	182 (162; 263)
Never	42 (28; 58)	50 (37; 83)	146 (116; 206)	177 (137; 219)
Never use supplements with iodine§:				
Men	45 (33; 63)	60 (38; 89)	167 (131; 231)	188 (158; 238)
Women	40 (25; 56)	48 (34; 80)	134 (106; 178)	160 (126; 205)
Samples from:	*n* (%)	*n* (%)	*n* (%)	*n* (%)
All participants:				
< 25 g/l or g/24 h	28 (13·5)	7 (3·4)	0 (0·0)	0 (0·0)
< 50 g/l or g/24 h	86 (41·3)	66 (32·2)	2 (0·9)	0 (0·0)
< 100 g/l or g/24 h	158 (76·0)	138 (67·3)	25 (11·5)	11 (5·0)
> 100 g/l or g/24 h	50 (24·0)	67 (32·7)	193 (88·5)	207 (95·0)
> 200 g/l or g/24 h	12 (5·8)	12 (5·9)	72 (33·0)	94 (43·1)
> 300 g/l or g/24 h	1 (0·5)	5 (2·4)	13 (6·0)	35 (16·1)
Never use supplements with iodine §‖:				
< 25 g/l or g/24 h	23 (20·0)	6 (5·6)	0 (0·0)	0 (0·0)
< 50 g/l or g/24 h	69 (60·0)	53 (49·1)	2 (1·4)	0 (0·0)
< 100 g/l or g/24 h	104 (90·4)	91 (84·3)	22 (15·8)	9 (6·9)
> 100 g/l or g/24 h	11 (9·6)	17 (15·7)	117 (84·2)	122 (93·1)
> 200 g/l or g/24 h	2 (1·7)	2 (1·9)	37 (26·6)	47 (35·9)
> 300 g/l or g/24 h	0 (0·0)	2 (1·9)	5 (3·6)	16 (12·2)

* 205 urine samples available.
† 218 urine samples available.
‡ Corrected for age and gender specific creatinine excretions (men 0·95 g/l; women 0·7 g/l).
§ Excluding also individuals treated with thyroxin (n 23).
‖ $n_{Randers}$ 108; n_{Skagen} 131.
¶ For details of subjects and procedures, see Methods.

stances in natural waters was absorbed. Gender may be important for iodine intake (reference:women; OR 1·8; 1·0, 3·3) while the life-style factors smoking (reference:no smoking; OR 1·4; 0·7, 2·7) and alcohol intake (reference:no alcohol; OR 1·0; 0·4, 2·5) were not.

The average creatinine concentration in spot urine samples was 856 mg/l in men and 556 mg/l in women, with no difference between towns (Mann-Whitney; men $P=0·54$; women $P=0·45$). This creatinine excretion suggests a 24 h urine volume of 1·26 litres in women and 1·11 litres in men. Weighted according to 264 women and 166 men, this equals an average of 1·2 litres in this population, in keeping with previous findings[30].

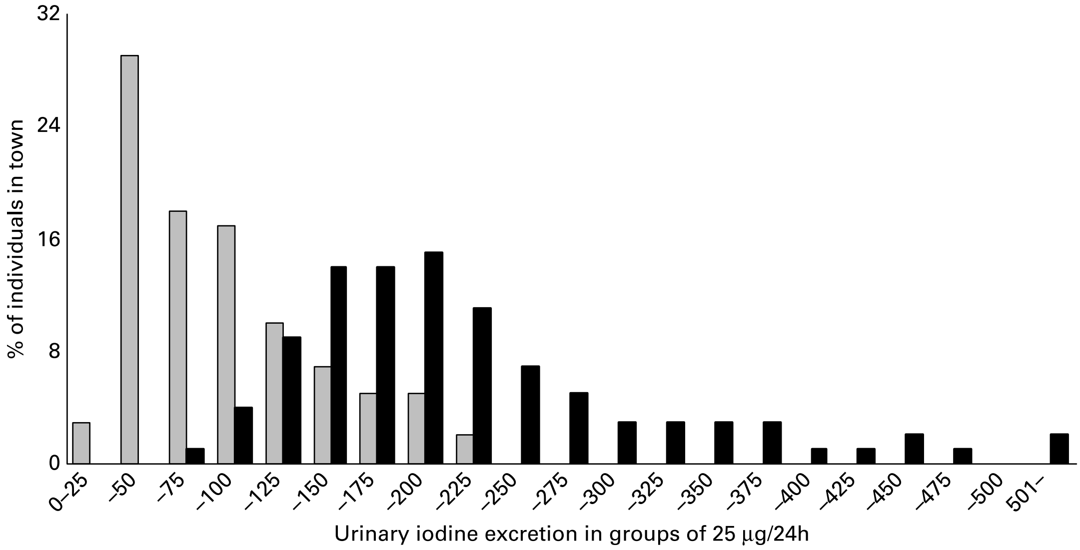

Fig. 2. Distribution of estimated 24 h urinary iodine excretion among old Randers and Skagen dwellers at intervals of 25 µg/24 h: Randers (□); Skagen (■). Dispersion differed between towns (Bartlett's test; $P < 0.01$) as did median (Mann–Whitney; $P < 0.001$). For details of subjects and procedures, see Methods.

Table 3 shows an estimate of the bioavailability of iodine from natural waters calculated from differences between Randers and Skagen. The difference in iodine content of drinking water was 138 µg/l. If all drinking water iodine was bioavailable, the difference in the contribution to iodine intake from tap water would be 166 µg/24 h (138 µg * 1·2 l/24 h), of which 90 % (149 µg) was excreted in the urine[13]. The difference in estimated 24 h iodine excretion in urine between Randers and Skagen dwellers was 127 µg/24 h. Hence, the fraction of iodine available can be calculated as 127 µg / 149 µg = 0·85, suggesting a bioavailability of 85 % (93 % and 79 % with a tap water intake of 1·1 and 1·3 litres respectively).

Discussion

Iodine, element no. 127, is involved in the cycle of organic matter in most surface environments. It has a biophilic nature and is abundant in marine environments, where sediments are particularly rich in iodine[14,38,39].

Northern Europe experienced several ice ages over the Quaternary period. Ice depressed the Earth's crust by up to several hundred metres. When the ice melted, the land rose after a delay. During this delay, sea flooded the deglaciated terrain. This was followed by an uplift exposing large areas of sea floor and marine deposits have been found up to 60 m above sea level[5].

The northern part of the peninsula of Jutland in western Denmark still rises and the most northern part, the Isthmus of Skagen, is less than 15 000 years old. The Skagen waterworks uses shallow wells. Thus, the aquifer source rock is marine sediments and Skagen tap water was previously shown to contain humic substances[17] with iodine[3]. The binding of iodine in organic matter[20] has been hypothesized to modify bioavailability of iodine in man[20,21] and thus the occurrence of thyroid disorders in a population[2].

Table 3. Estimated bioavailability of iodine bound in humic substances in natural waters, calculated iodine excretion among long-time Randers and Skagen dwellers not using iodine-containing supplements or thyroxine (*n* 239)**

	Randers		Skagen	
Tap water iodine (g/l)	2		140	*
Difference in tap water iodine (g/l)		138		†
Tap water ingestion (litre/24 h)	1·2		1·2	‡
Iodine intake from tap water (g/24 h)	2		168	§
Difference in expected total iodine excretion (g/24 h)		166		†
Difference in expected urinary iodine excretion (g/24 h)		149		‖
Urinary iodine excretion (g/24 h)	50		177	*
Difference in measured urinary iodine excretion (g/24 h)		127		†
Estimated bioavailability (%)		85		¶

* Measured.
† Calculated: Skagen ! Randers.
‡ Calculated from creatinine and references 7 and 30, and includes tap water, coffee, tea and flavoured water.
§ Calculated: difference in tap water iodine * tap water ingestion.
‖ Urinary iodine excretion is approximately 90 % total iodine excretion.
¶ Calculated from u-iodine-difference: measured/expected * 100.
** For details of subjects and procedures, see Methods.

It has been demonstrated that 90 % of elemental iodine disposed from the human body is excreted in the urine[13] and, when a steady state is present, iodine in urine is generally accepted as a measure used to estimate bioavailable iodine in the diet[11,14,15,23,24,40].

The availability of dietary iodine was found to be incomplete in mice[22] and rats[23]. In man, complete absorption of elemental iodine has been demonstrated[13]. Also, iodine added to salt showed a complete[41] or near-complete absorption[14]. A review of the bioavailability in man of naturally occurring iodine concluded that data on organically bound iodine were lacking[24]. The relevance of the question has since been emphasized by the finding of a missing relationship between iodine in the environment and endemicity of goitre, in the UK[12], on the continent[6,11] and in the USA[42].

About 10 % of disposable iodine is excreted with faeces[11,24]. Humic acids are absorbed in the gastrointestinal tract, where some enter the enterohepatic circulation[43]. Thus, iodine in the humic substances in drinking water may be retained in this circulation and excreted in faeces. We did not measure iodine in faeces, but this provides an explanation for a reduced bioavailability of iodine bound in humic substances.

In keeping with these considerations, we calculated that approximately 85 % of iodine bound in humic substances in Skagen tap water was bioavailable on a population level. Concentration of tap water during boiling or other food processing may lower this estimate. Hence, it is similar to the findings by Aquaron *et al.*[14] that between 60 and 85 % of the iodine in seaweed was bioavailable. The available fraction of iodine in solid foods varied markedly[15], while our previous finding that iodine in drinking water correlated with urinary iodine excretion across towns[7] suggested that the bioavailable fraction varies within certain limits between tap waters. This is in keeping with the present findings in tap water from Skagen.

There are limitations to our estimate of the bioavailability of iodine bound in humic substances. First, the volume of tap water ingested was from food tables for Denmark[30]. It was, however, in keeping with the intake estimated from population spot urine creatinine. Second, urinary iodine content varies considerably due to factors such as dilution and variations in diet. To overcome some of the variation caused by dilution, we used estimated 24 h urinary iodine excretion as the correction for age and gender specific creatinine excretion diminishes this variation and describes the actual iodine excretion more accurately[33,44–46]. Third, to overcome some of the dietary variations, we used differences in the iodine content of tap water and urine between towns in our calculations, as no systematic differences are known to exist between the populations in these two towns located in the same part of Denmark. Fourth, the Randers and Skagen dwellers differed in the use of iodine-containing supplements. Thus, individuals using iodine-containing supplements were excluded from the calculations in addition to thyroxin users. Hence, the difference in aquifer source rock for drinking water supply provided a method for estimating the bioavailability of naturally occurring iodine bound in humic substances in tap water on a population level. Still, a balance study including individuals with a fixed intake of organically bound iodine, i.e. Skagen tap water, is needed to detail the bioavailability.

The study was carried out just prior to the initiation of the Danish iodine supplementation programme[29] and the iodine excretion shows the iodine intake from the natural diet in Denmark. Living in Randers caused a low iodine intake level but is likely to have increased. Among Skagen dwellers, the iodine excretion was markedly higher and 36 % of samples were in the range of more than adequate iodine intake. Thus, even though living in Skagen provided a stable iodine intake within the recommended range for the majority of the population, more than one third of samples suggested an iodine excretion above adequate[37], which may adversely affect thyroid function[2]. An increase may be considered an adverse effect of iodine supplementation and a study of the occurrence of thyroid dysfunction in this population is warranted. Also, this should consider that humic substances may influence thyroid function[47].

The finding of high iodine excretion among Skagen dwellers is unique in Denmark and illustrates that iodine monitoring programmes, despite thoroughly considered design and keen efforts to portray population iodine status, may miss subgroups.

The use of iodine-containing supplements influenced iodine excretion markedly in individuals. It was associated with an equal increase in urinary iodine between towns. Thus, neither the humic substances nor the differences in intake of naturally occurring iodine seems to influence the availability of iodine from supplements.

In conclusion, iodine in humic substances was present in tap water from the Skagen aquifer only. This naturally occurring organically bound iodine influenced population iodine intake, the bioavailability was assessed to be about 85 %. The increase in iodine excretion associated with intake of iodine-containing supplements was unaffected by the humic substances and it reduced the number of samples in the range set to define iodine deficiency. Also, it increased the fraction of samples that indicate more than adequate and excessive iodine intake in Skagen with a high content of iodine in drinking water. This may be of concern and a study of thyroid function in this population is warranted.

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