Elastographic and morphological testicular changes in hypothyroidism – an experimental study

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Abstract

Introduction: Methimazole-induced hypothyroidism is a clinical problem in the treatment of hyperthyroidism in people and animals and is an example of metabolic disease that can lead to fertility disorders and can give elastographic testicular changes. **Material and Methods:** Ultrasound elastography using the Esaote MyLab Twice ultrasound system and a morphological examination of testes were performed in seven methimazole-administered (group E) and seven healthy rats (group C). **Results**: The elasticity ratio of strains in the scrotal wall of the near-field test area to testicular tissue (ELX-T-RAT) and hardness percentage of strained tissues in the defined area of a testicle (ELX-T%HRD) in group E were statistically significantly lower than in group C. The degree of spermatogenesis was statistically significantly higher in group E than in group C and similarly seminiferous tubule diameters in group E were statistically significantly higher than in group C. Body weight and testicular weight in group E were statistically significantly lower than in group C. **Conclusion:** Changes in the elastographical parameters of testes may result from disorders secondary to hypothyroidism. The usefulness of elastography is noteworthy in the case of evaluation of testis function in patients with some metabolic disorders.

Keywords: rats, testes, elastography, hypothyroidism, Johnsen score, fertility.

Introduction

Every tissue has mechanical properties such as elasticity and hardness that can be assessed with external pressure, which is the basis for ultrasonic elastography (18). This is a specific kind of palpation useful in detecting pathological changes which are located in organs that lie deep under the skin, depending on the properties of soft tissues such as amount of fat, water, collagen, and elastin fibres or cytoarchitecture. In the course of many pathological metabolic conditions, these properties change and can influence the biophysical features of organs. Iatrogenic hypothyroidism, as a major clinical problem especially in people, cats, and other animal species which are treated with methimazole or other antithyroid drugs, influences numerous functions (3, 4, 10–12, 16). Naturally occurring hypothyroidism, as a systemic disease, leads to pituitary and testicular damage, especially during hypogonadotropic hypogonadism; however, other reasons for these conditions, such as testicular functional changes, have been found (7, 8, 16, 24). Therefore, it probably differs from iatrogenic thyroid gland insufficiency, in that the spectrum of adverse effects of an antithyroid drug depends on the kind of medicine, its dose, exposure time, and the stage of life when treatment was conducted (3, 26). The need for use of this medicine is usually in adult subjects, and the evaluation of this treatment for its effects on testicular biophysical features, structure, spermatogenesis, and their reciprocal relationships in mature males is crucial from the clinical point of view. Current data about testicular sonographic elastography concern, for instance, neoplastic masses or painful inflammatory and ischaemic conditions, but not secondary properties of tissues resulting from metabolic diseases such as hypothyroidism (9). In relation to

spermatogenesis, elastographic parameters have been analysed; however, the influence of methimazole-induced hypothyroidism on male fertility still seems to be unclear (26, 27).

The objective of this study was to investigate the influence of methimazole-induced hypothyroidism in rats on elastographic testicular changes and its relation to microscopic testicular structure and spermatogenesis.

Material and Methods

Animals. Wistar male rats (220–260 g) were randomly divided into two groups: C (the control group) with healthy males (n = 7) and E (the experimental group) with hypothyroid males, receiving methimazole (n = 7). The rats were kept in an air-conditioned room with average humidity of 45%–47%, temperature of 22–23°C, and 12/12 h light cycle. The rats underwent a 14-day adaptation prior to the experiment. Then, they were fed a commercial diet for laboratory animals (Agropol, Poland). Rats from the control group had access to tap water *ad libitum*. The rats from the experimental group were given 0.05% methimazole (Sigma-Aldrich, USA) water solution, administered *ad libitum* in a dose that was very effective in inducing hypothyroidism (11). Fresh solution was prepared daily. In rats from the experimental group, hypothyroidism was confirmed by the measurement of the serum total thyroxine (TT4) concentration by ELISA (Cloud-Clone Corp., Katy, TX, USA), before the end of the experiment. On day 58 of the experiment, blood samples (0.5 mL) were collected from the lateral tail vein and allowed to clot. Next, these samples were centrifuged to obtain serum. In group E, the concentration of TT4 was statistically significantly lower (6.8 nmol/L ± 0.9) than in group C (72.7 nmol/L ± 6.4). After 60 days of the experiment, the animals underwent further procedures.

Testicular ultrasound elastography. Ultrasound elastography was performed in animals in dorsal recumbency under anaesthesia which was induced with ketamine (80 mg/kg of b.w. i.m.). The scrotum was clipped and alcohol was applied to the skin followed by acoustic coupling gel. Only one sonographer performed all ultrasound and elastography examinations. B-mode and elastography images of the left and right testicles were obtained with the Esaote MyLab Twice ultrasound system with a linear array 4–13 MHz transducer and ElaXto ML T20 and ML T20B software (Esaote Group, Italy). Machine settings were adjusted to optimise B-mode image acquisition for each animal including gain, depth, focus, and frequency. Elastography was carried out with minimal hand pressure on the scrotum. Ultrasound images were obtained with the use of a split screen with a B-mode image on the left side and elastography image on the right side. The amplitude of elastic deformation of scanned testicles was coded on the screen with a colour scale. The most deformed tissues were displayed in red (SOFT), tissues of the highest stiffness were displayed in blue (HARD), and tissues of

intermediate stiffness were displayed in green. Only images whose manual oscillation quality was relatively high (at least four out of six indicators displayed by ElaXto software) were interpreted. The images were stored in the ultrasound system memory. Regions of interest (ROI) were marked in an ultrasound image in B-mode presentation, separately for each testicle in longitudinal section. In the elastographic images of the testicles, ROIs were marked by hand-tracing gonadal parenchyma, with the exception of tunica regions. For strain ratios, ROI also included a hand-drawn outline of the scrotal wall visible in near-field of scans. The assessment of tissue strain ratios and percentages of elasticity of strained tissues were produced with the manufacturer's software. To assess the homogeneity of the area, the parameters were determined as follows: ELX-T-RAT is the ratio between strains in the scrotal wall of the near-field test area (Z1) and testicular tissue (Z2); ELX-T% HRD is the hardness percentage of strained tissues in the defined area of a testicle with area expressed in mm^2; ELX-T% SFT is the percentage measurement of strained tissue softness in parenchyma with the area expressed in mm^2; and ElaXto Histogram is the mean value of elasticity of strained points of parenchyma with the area expressed in mm^2 (values derived from the computer-generated histogram of points distribution with different elasticities). The results from both testicles were averaged in each individual case, and each elastographic parameter was averaged from three different measurements.

Morphological analysis of testicles. Immediately after euthanasia using pentobarbital natrium (Morbital, Biowet, Poland), the left and right testicles were taken without epididymis. After measuring testicular weight (TW), which was expressed as the mean value of right and left testicle weights, the right testicle was preserved in 10% neutral buffered formalin and submitted for standard histological processing. Sections 4 μm thick were stained with haematoxylin and eosin (HE) and examined under an Olympus BX63 light microscope (Olympus Corporation, Japan). All morphological studies were made using a blank test method, and slides were decoded when the results were obtained.

The slides were also analysed microscopically for evaluation of spermatogenesis according to the Johnsen score (JS) (15). Spermatogenesis was classified using a scale from 1 to 10 where these scores correspond to these criteria: score 10 – full spermatogenesis; score 9 – slightly impaired spermatogenesis, many late spermatids, disorganised epithelium; score 8 – less than five spermatozoa per tubule, late spermatids; score 7 – no spermatozoa, no spermatosis; score 6 – no spermatozoa, no late spermatids, few early spermatids; score 5 – no spermatozoa or spermatids, many spermatocytes; score 4 – no spermatozoa or spermatids, few spermatocytes; score 3 – spermatogonia only; score 2 – no germinal cells, Sertoli cells only; and score 1 – no seminiferous epithelium. The average JS was calculated from 100 seminiferous tubules.

Seminiferous tubule diameter (STD) was analysed with the use of the same preparations under the same microscope with Olympus cellSens software (Olympus Corporation, Japan) for Microsoft Windows 10 Pro. For each tubule, the average diameter was calculated from two perpendicular measurements if the difference between them did not exceed 30 μm (to avoid oval tubules). For every animal, this parameter was calculated as the average of 10 different tubules.

Statistical analysis. All values are presented as means ±SD. Statistical analysis was performed using Statistica software (version 10.0) (StatSoft (now Tibco), USA) and the Mann–Whitney U test. The differences between mean values were considered as statistically significant at P ≤ 0.05. Correlations were calculated with the Spearman rank method.

Results

The results of elastography of testes, spermatogenesis, seminiferous tubules diameter, and measurement of body weight and testicular weight are presented in Table 1.

The elasticity ratio of strains in the scrotal wall of the near-field test area to testicular tissue (ELX-T-RAT) was lower in group E (Fig. 1) than in group C and was statistically significant in its difference (P = 0.008). Similarly, the hardness percentage of strained tissues in the defined area of a testicle given in mm^2 (ELX-T%HRD) was lower in group E (Fig. 2) than in group C and was likewise a statistically significant difference (P = 0.043). Both the percentage of strained soft tissue of the testicle with the area expressed in mm^2 (ELX-T%SFT) and the mean value of elasticity of strained points of testicular parenchyma with the area expressed in mm^2 (ElaXto Histogram) in group E (Figs 2 and 3) were lower in group E than in group C, but did not differ significantly.

The JS defining the degree of spermatogenesis was higher in group E than in group C and provided another statistically significant difference. The seminiferous tubule diameter (STD) in group E was also higher (Fig. 4) than in group C and was also statistically significant (P = 0.003). Histopathological examination of testicles from both groups did not reveal any other lesions.

Rat body weight (BW) in group E was surpassed by that of group C and this difference was statistically significant (P = 0.0007). Testicular weight (TW) in group E did not equal that of group C and differed statistically significantly (P=0.038).

In group E, positive correlations between ELX-T-RAT and TW (rho = 0.47), BW and TW (rho = 0.68), and ELX-T%SFT and ElaXto Histogram (rho = 0.71) were found. Negative correlations between ELX-T%SFT and ELX-T-RAT (rho = −0.83); ELX-T%SFT and STD (rho = −0.58); JS and BW (rho = −0.38); JS and ELX-T-RAT (rho = −0.61); and JS and ELX-T%HRD (rho = −0.63) were noted. Only the correlation between ELX-T%SFT and ELX-T-RAT was statistically significant.
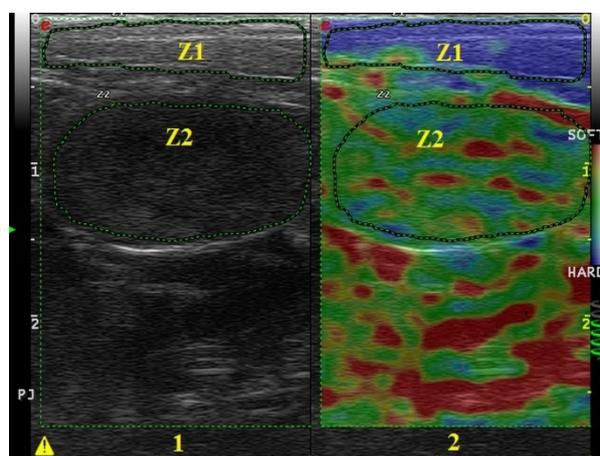

Fig. 1. Measurement of ELX-T-RAT in rat testicle from group E. Z1 – scrotal wall of the near-field test area and Z2 – testicular tissue – regions of interest (ROI). 1 – B-mode presentation, 2 – colour elastographic image

Table 1. Results of elastographic findings, spermatogenesis, seminiferous tubules diameter, body weight, and testicular weight in groups E and C

| Parameter | Group | | | |
| | E | | C | |
	x	SD	x	SD
ELX-T-RAT	1.08*	0.09	1.34	0.18
ELX-T%HRD	22.87*	3.87	28.48	4.58
ELX-T%SFT	72.51	10.41	73.38	3.03
ElaXto Histogram	61.42	2.69	63.56	1.93
JS	9.63*	0.05	9.21	0.32
STD	365.40*	31.98	325.86	10.35
BW	382.33*	28.61	653.50	76.79
TW	1.78*	0.12	1.90	0.14

x – arithmetic mean, SD – standard deviation, ELX-T-RAT – elasticity ratio of strains in the scrotal wall of the near-field test area (Z1) to testicular tissue (Z2); ELX-T% HRD – hardness percentage of strained tissues in the defined area of the testis with area expressed in mm^2; ELX-T% SFT – percentage measurement of strained tissue softness in gonadal parenchyma with the area expressed in mm^2; ElaXto Histogram – mean value of elasticity of strained points of gonadal parenchyma with the area expressed in mm^2; JS – Johnsen score; STD – seminiferous tubules diameter (μm); BW – body weight (g); TW – testicular weight (g); * – statistically significant differences in comparison with control group (P ≤ 0.05)
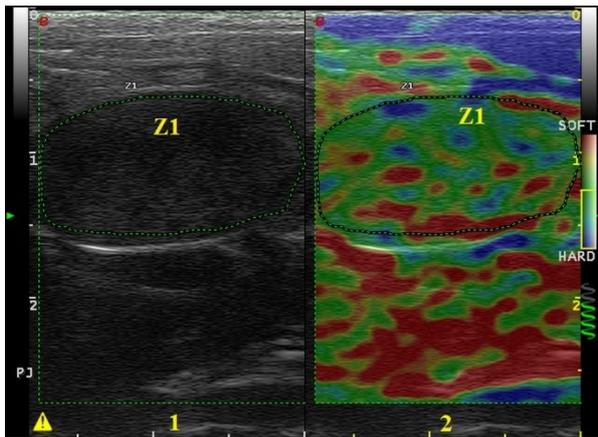
Fig. 2. Measurement of ELX-T% HRD and ELX-T% SFT in rat testicle from group E. Z1 – testicular tissue – is the region of interest (ROI). 1 – B-mode presentation, 2 – colour elastographic image
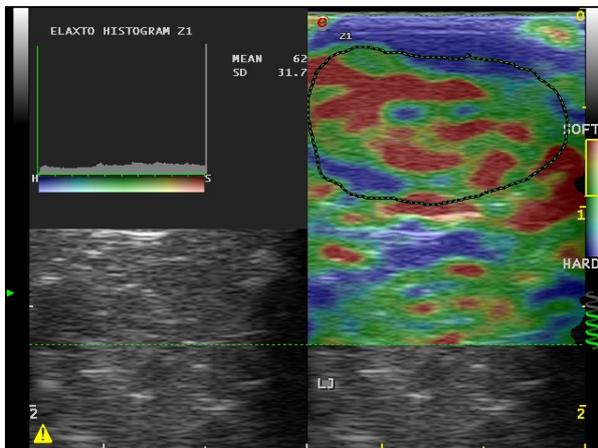

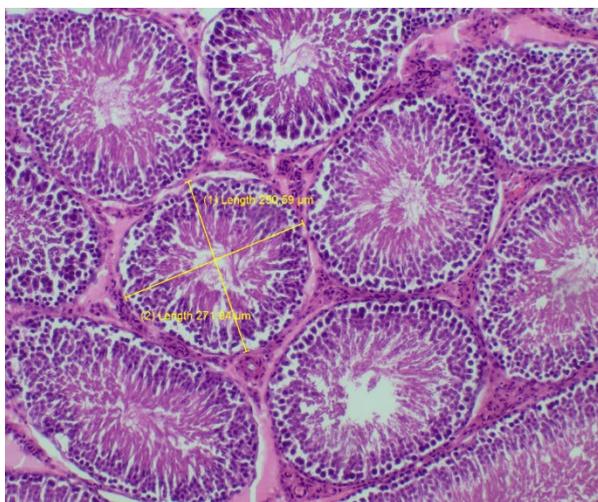**Fig. 3.** ElaXto Histogram in rat testicle from group E

Fig. 4. Measurement of seminiferous tubule diameter in rat testicle from group E. H.E. 100×

Discussion

Reduction or complete inhibition of spermatogenesis leads to infertility in males and, among many factors responsible for this disorder, iatrogenic factors are significant, including drugs (6, 23). Their impact on the function of the testes can be both direct and indirect.

Methimazole may act in such a way and lead to hypothyroidism in certain cases, in the absence of appropriate monitoring of hyperthyroidism treatment. As with many other medications, the exact mechanism of its effects on male fertility has not been explained. On the one hand, it may seem that the effect on fertility of methimazole-induced hypothyroidism has little clinical importance because methimazole is usually prescribed in older aged humans and animals. On the other hand, in humans, the disease may even involve deterioration of sexual performance, which significantly lowers the quality of life (24).

The conducted study did not reveal any histopathological changes in the testes in the experimental group as compared with the control group. The effect of methimazole on the function and structure of the testes was previously reported and considerable side effects were present, however, doses of the drug were much higher than recommended in clinical practice and used in our studies and previous experiments (10–12). Ai *et al.* (1) administered doses of 25 mg/kg of methimazole to rats, although only for five consecutive days. While thyroid hormone concentrations in blood decreased in the experimental group as compared with the control group, the animals' weight increased. In our experiment, the body weight of individuals in the experimental group was lower than in the control group and was statistically significant, but the experiment lasted for 60 days because it aimed to simulate conditions of chronic use of this medicine in clinical practice and the duration of the spermatogenesis cycle (5). In addition, we found reduction of weight of the testes in the experimental group against the control group. This contradicts the statement that spermatogenesis is positively correlated with the volume of the testis, despite the fact that seminiferous tubules constitute 70%–80% of its mass (27). Ai *et al.* (1) also pointed out that in sick animals there was a decrease in diameter of seminiferous tubules and a significant reduction in the number of spermatogonia, primary spermatocytes, spermatozoids, Leydig cells, and Sertoli cells, which suggested a relationship with degeneration and inhibition of proliferation of reproductive cells. According to the authors, the obtained results were similar to those by Tahmaz *et al*. (25), however, the latter induced hypothyroidism through administration of a completely different antithyroid drug, propylthiouracil, for 15 days. Therefore, the changes in the morphology of the testes observed by Ai *et al*. (1) were most likely, mainly caused by methimazole as a chemical substance and not only as a drug inducing hypothyroidism. Similar conclusions can be drawn from the research by Okdach (20) who studied the effects of administration of 0.1% solution of methimazole in drinking water for 30 days in rats. He found the following histological changes in the testes in the experimental group: the loss of the most advanced cell type of spermatogenesis and the appearance in a few tubules of some spermatids with the beginning of elongation, mostly without condensation.

Exfoliation of germ cells was frequently observed in the seminiferous epithelium, and some tubules showed severe reduction of spermatogenesis. In hypothyroid rats tubule diameter decreased significantly. However, in this study, the concentration of the medication was double the concentration used in our studies, which could have affected the results. With regards to the results obtained by other authors (1), the data we obtained are surprising. We found that the diameter of seminiferous tubules in animals with metimazole-induced hypothyroidism was significantly higher than in the control group. Similarly, the JS was statistically significantly higher in animals treated with methimazole, but in view of the fact that the results in groups E and C showed a similarly high level, we assumed that the induced condition did not affect the maturation of the reproductive cells and testicular function, provided that the experiment lasted for 60 days. This was despite an increase in flexibility of the testes. Our earlier data suggested that methimazole significantly modulates the production of reactive oxygen species, or stimulates the mechanisms of anti-free radical defence and that this is one of the protective actions of this medicine, whereas oxidative stress is an important cause of testicular damage and infertility in males (10, 22).

Similarly to ultrasound imaging of the testes, elastosonography of these organs in B-mode can be a useful tool in diagnosis of infertility in males; however, there is a lack of extensive studies in this area (21). It is quick and non-invasive and capable of increasingly higher sensitivity in view of the rapid technological progress in the fields of manufacturing ultrasound systems and creating advanced software for processing and analysis of obtained images. Normal testes have a homogenous structure of high stiffness, and even small lesions, both focal or diffuse, can be detected with the use of elastography. So far, this examination has been repeatedly used in diagnosis and monitoring of treatment of proliferative and inflammatory lesions in testicular parenchyma, the assessment of retained testis, testicular torsion, cysts, haematoma, scarring or ischaemia (2, 13, 14). However, all studies carried out so far pertained to incremental lesions in the testicular area; but did not take systemic conditions into account. These sonographic investigations excluded metabolic conditions, which could affect elastographic parameters, especially with regard to secondary fertility impairment and the intensification of spermatogenesis. In similar research carried out by Zhang et al. (27), a correlation between elastographic parameters of the testis and amelioration of spermatogenesis was determined with the use of the histological JS after testicular torsion in rabbits. It was expected that changes in tissue stiffness depend on changes in its morphology and structure. Similarly to our experiment, the results obtained after conducting elastography correlated with the degree of spermatogenesis in the case of testicular torsion, however, the testicle exhibited significant histopathological lesions such as

hyperaemia, oedema, infiltration of inflammatory cells, blood vessel swelling and degeneration, necrosis, and scaling of germ cells, which discontinued spermatogenesis. It was assumed that elastography can be used for reliable quantitative assessment of testicular tissue hardness in relation to various aspects of changes in spermatogenesis and may be implemented in further clinical evaluation. Unfortunately, there is no analysis of spermatogenesis in the intact testis on the opposite side, which would relate to the results we obtained to a greater extent, as that testis could be affected by numerous general factors. The values of elastographic parameters may be affected by stiffness of seminiferous tubules and by changes in vascular flow. It is possible that changes in the studied elastographic parameters also depend on the increase in tissue water content in the course of hypothyroidism (19). However, the reasons for this are not clear, as in the aspect of hypothyroidism which suggested elastography be conducted, the studies focused exclusively on the affected thyroid gland responsible for the condition. There are no available analyses of other organs, and, therefore, our research is likely to be the first in this area. Elastography generally represents a new diagnostic method and studies related to it are relatively few (17).

To summarise, we state that chronic administration of methimazole leading to hypothyroidism does not cause fertility impairment as a result of this iatrogenic endocrinopathy but may be associated with the effect of methimazole *per se,* depending on the dose. Specific changes in the elastographical parameters of testicular parenchyma may be present in a patient, and such changes may not necessarily be grounded in primary pathological conditions of this organ. They may result exclusively from secondary disorders related to hypothyroidism. Therefore, the usefulness of elastography is noteworthy for evaluation of function of the testes or other organs in patients with metabolic disorders by comparing the results with the results of a histopathological examination of testes, which remains the gold diagnostic standard. Our research, however, concerns only laboratory animals, therefore, before applying any of the results obtained, clinical analyses of men and males of target animal species, particularly cats, are necessary.

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