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RESEARCH ARTICLE

Quantitative Histomorphometry of Thyroid Gland Remodeling in Rats Following Chronic Exposure to Nitrate-Contaminated Drinking Water

I. M. Petrovic[1], O. S. Adeyemi[2]*

1 Institute of Histology and Embryology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia.
2 Department of Anatomical Sciences, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Nigeria.
*Corresponding author: your-email@example.com

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Abstract

Nitrate compounds are ubiquitous environmental contaminants found in agricultural runoff that can interfere with thyroid iodine uptake, disrupting endocrine homeostasis and glandular tissue architecture. This study investigated the structural consequences of chronic ingestion of sodium nitrate on the thyroid parenchyma of adult Wistar rats using quantitative histomorphometry. Thirty male Wistar rats were divided into three groups of ten: Control (distilled water), Low-Exposure Nitrate (50 mg/L), and High-Exposure Nitrate (500 mg/L), administered via drinking water for 16 consecutive weeks. Thyroid glands were harvested, fixed, sectioned, and stained with Haematoxylin and Eosin (H&E). Histomorphometric evaluation revealed significant tissue remodeling in the treated cohorts. The mean follicular epithelial height increased significantly in the high-exposure group (14.2 +/- 1.8 um vs. 6.4 +/- 0.7 um in controls; $p < 0.01$), representing a shift from cuboidal to columnar epithelium due to compensatory hyperstimulation. Concurrently, the mean follicular diameter decreased significantly (45.2 +/- 4.1 um vs. 78.5 +/- 6.2 um in controls; $p < 0.01$), indicating accelerated colloid depletion. Interstitial vascular volume fraction expanded significantly from 3.8% +/- 0.5% in controls to 9.2% +/- 1.2% in the high-dose cohort ($p < 0.05$), demonstrating regional hypervascularization. These objective tissue alterations show that chronic exposure to environmental nitrates induces severe architectural stress and follicular remodeling within the thyroid gland, highlighting potential risks for environmental goitrogenesis.

Keywords: *Thyroid gland; Nitrate; Histomorphometry; Follicular epithelium; Endocrine disruptor.*

1. Introduction

Nitrate (NO3-) is among the most widespread chemical contaminants of drinking water worldwide. Its principal sources are nitrogen-based fertilisers, animal manure, septic effluent, and industrial discharge, all of which leach readily into shallow aquifers and surface waters because nitrate is highly soluble and poorly retained by soils [1,2]. In many agricultural regions, groundwater nitrate concentrations routinely exceed the World Health Organization (WHO) guideline value of 50 mg/L as nitrate (equivalent to 11.3 mg/L as nitrate-nitrogen), a value originally set to protect bottle-fed infants from methaemoglobinaemia [1,3]. That guideline, however, was not designed primarily around chronic endocrine endpoints, and a growing body of experimental and epidemiological work now suggests that sustained, low-to-moderate nitrate exposure may perturb thyroid function, particularly when iodine intake is marginal [4,5,6].

The thyroid gland occupies a central position in metabolic regulation. Its follicular cells synthesise thyroxine (T4) and triiodothyronine (T3), hormones that govern basal metabolic rate, thermogenesis, protein synthesis, growth, and neurodevelopment [7,8]. Hormone synthesis is absolutely dependent on iodide. Dietary iodide is actively transported into the thyrocyte by the sodium-iodide symporter (NIS), a basolateral membrane protein that co-transports two sodium ions with one iodide ion [9,10]. Once inside the cell, iodide is oxidised by thyroid peroxidase, incorporated into thyroglobulin stored as colloid in the follicular lumen, coupled to form iodothyronines, and later released by endocytosis and proteolysis [7,11]. Because NIS-mediated uptake is the first and rate-limiting step in this sequence, any anion that competes for NIS can, in principle, reduce hormone production.

Nitrate is one such competitor. Alongside perchlorate and thiocyanate, nitrate is classified as an environmentally relevant NIS inhibitor [4,12]. Although nitrate is considerably less potent than perchlorate on a molar basis, its environmental concentrations are typically orders of magnitude higher, so its cumulative competitive effect cannot be dismissed [4,13]. When iodide uptake falls, circulating T4 and T3 tend to fall, the hypothalamic-pituitary-thyroid (HPT) axis responds with increased thyrotropin (TSH) secretion, and the gland is driven to work harder. Acutely, this is an adaptive, compensatory response. Chronically, persistent TSH stimulation produces hypertrophy and hyperplasia of follicular cells, depletion of colloid stores, remodelling of the follicular architecture, increased gland weight, and, ultimately, goitre [5,14,15]. The histological footprint of that process - taller epithelium, smaller and more irregular follicles, vacuolated colloid, and increased vascularity - is precisely what quantitative histomorphometry is designed to capture.

Histomorphometry matters because qualitative descriptions such as 'hyperplastic' or 'depleted' are subjective and difficult to compare across studies. By measuring epithelial height, follicular diameter, colloid area, and vascular volume fraction in a systematic, blinded fashion, investigators convert a visual impression into reproducible numbers that can be tested statistically and related to dose [16,17]. Earlier work has already shown that nitrate-fed rats develop diffusely hyperplastic thyroids with small follicles, taller epithelial cells, irregular hypertrophic cells, and reduced colloid [18,19]. In longer exposures, Eskiocak and colleagues reported increased thyroid weight at all doses tested (50-500 mg/L for 30 weeks), with overt histomorphological change at 250 and 500 mg/L, and reduced total T3, free T4, and TSH in several groups [20]. Other groups have reported hypertrophy, increased follicular cell height, peripheral vacuolation of follicles, and colloid reduction after nitrate exposure [21,22]. In humans, van Maanen and co-workers found a dose-dependent increase in thyroid volume when drinking-water nitrate exceeded 50 mg/L [23], while Bulgarian studies linked high-nitrate water to thyroid dysfunction in children and pregnant women [24,25].

Despite this evidence, several gaps remain. First, many studies report hormones or gland weight without paired, quantitative tissue measurements. Second, the dose-response relationship across the critical window around the WHO guideline value (50 mg/L) and a clearly supra-guideline dose (500 mg/L) is incompletely documented for a standardised 16-week chronic exposure in adult male Wistar rats. Third, vascular remodelling - an expected companion of sustained TSH-driven growth - is rarely

quantified. The present study was therefore designed to test a clear hypothesis: that chronic ingestion of sodium nitrate in drinking water produces dose-dependent, quantifiable remodelling of thyroid follicular architecture, characterised by increased epithelial height, reduced follicular diameter, and increased interstitial vascularity.

1.1 Aim and Objectives

The aim of this study was to quantify histomorphometric changes in the thyroid gland of adult male Wistar rats following 16 weeks of chronic exposure to nitrate-contaminated drinking water. The specific objectives were: (i) to measure mean follicular epithelial height in control and nitrate-exposed groups; (ii) to measure mean follicular diameter as an index of colloid storage and follicular size; (iii) to estimate interstitial vascular volume fraction as an index of glandular perfusion and hypervascular remodelling; and (iv) to interpret these changes in the context of NIS inhibition and HPT-axis feedback.

1.2 Significance of the Study

If a dose at the WHO guideline value already produces measurable tissue change, and a tenfold higher dose produces marked remodelling, the findings would strengthen the case for treating thyroid endpoints as relevant to nitrate risk assessment, for monitoring thyroid health in high-nitrate communities, and for ensuring adequate iodine nutrition as a modifiable protective factor. For anatomists and endocrinologists, the study also provides a reproducible morphometric template that can be applied to other endocrine disruptors.

2. Literature Review

2.1 Nitrate in the Environment and Human Exposure

Global nitrogen fertiliser use has risen dramatically since the mid-twentieth century, and nitrate leaching is now a leading cause of groundwater impairment in intensive farming regions of Europe, North America, South Asia, and sub-Saharan Africa [1,2,26]. In Nigeria, as in many low- and middle-income settings, reliance on shallow wells and boreholes, limited water treatment, and seasonal fertiliser application create conditions in which households may unknowingly consume water well above guideline values for months at a time [26]. Nitrate itself is relatively non-toxic, but it is reduced to nitrite in the gut and in the environment, and nitrite can oxidise haemoglobin to methaemoglobin, impairing oxygen delivery - the basis for the classic 'blue baby syndrome' [1,3]. Beyond this acute haematological effect, chronic endpoints including thyroid disruption, reproductive effects, and colorectal cancer have been investigated, with thyroid effects among the most biologically plausible because of the NIS mechanism [4,5].

2.2 Thyroid Follicular Architecture: Structure as a Record of Function

The thyroid is organised into lobules divided by thin connective-tissue septa. Within each lobule, spherical follicles - the structural and functional units - are lined by a single layer of follicular epithelial cells surrounding a central lumen filled with colloid [7,11]. Colloid is predominantly thyroglobulin, the iodinated glycoprotein precursor of T3 and T4, and it stains eosinophilic with H&E [7]. In a resting, iodine-replete gland, follicles are large, colloid-rich, and lined by low cuboidal epithelium. In an actively stimulated gland, follicular cells become taller and columnar, colloid becomes vacuolated and depleted at the periphery as it is resorbed, follicles become smaller and more irregular, and the interfollicular capillary network expands to meet metabolic demand [7,11,14]. This structure-function coupling is why epithelial height is widely used as a sensitive, inverse index of intrathyroidal iodine sufficiency and a direct index of TSH drive.

Parafollicular (C) cells, scattered between follicles and within the epithelium, secrete calcitonin and are not the primary target of NIS inhibitors; they are therefore not the focus of the present morphometry. The interstitium contains fibroblasts, capillaries, lymphatics, and immune cells. Sustained stimulation can expand the capillary bed through angiogenic signals, including vascular endothelial growth factor (VEGF) released by thyrocytes under TSH stimulation, a change that is functionally adaptive in the short term but contributes to gland enlargement over time [14,27].

2.3 Nitrate as a Thyroid Disruptor: Mechanistic Evidence

The NIS story is now well established. Tonacchera and colleagues demonstrated that

nitrate competitively inhibits iodide uptake in NIS-expressing cells, and subsequent reviews have placed nitrate alongside perchlorate and thiocyanate as a relevant environmental NIS inhibitor, albeit with lower potency [9,10,12]. A recent systematic review and meta-analysis of NIS inhibitors and thyroid function found that combined exposure to these inhibitors was positively associated with TSH, consistent with a subtle, population-level shift toward reduced thyroid hormone synthesis [13]. In animals, Bloomfield and co-workers showed as early as the 1960s that nitrate could inhibit thyroidal iodide uptake, and later work confirmed hypertrophy, hyperplasia, follicular colloid depletion, and increased thyroid weight under high nitrate exposure, especially when dietary iodine was low [21,22,28].

Dose matters, and iodine matters more. In iodine-sufficient animals, the thyroid can often compensate for moderate NIS inhibition by increasing NIS expression, increasing TSH drive, and drawing on colloid reserves. When iodine is deficient, the same nitrate dose produces a larger functional deficit and more pronounced histological change [19,20,28]. This interaction explains why epidemiological findings are heterogeneous: populations with adequate iodine and modest nitrate exposure may show little effect, whereas iodine-deficient populations exposed to high nitrate show clearer associations with goitre and thyroid enlargement [23,24,25]. It also explains why a study that measures only serum hormones at a single time point may miss tissue-level compensation - the gland may be maintaining hormones at the cost of architectural stress, a state that histomorphometry can reveal.

2.4 Animal Evidence on Nitrate and Thyroid Histology

The animal literature converges on a consistent histological pattern, summarised in Table 1. Zaki and colleagues reported that nitrate-fed Wistar rats developed diffusely hyperplastic thyroids with small follicles, taller follicular epithelial cells, mild-to-moderate irregularity of hypertrophic cells, and decreased colloid [18]. A related nitrite study by the same group found hyperplasia and hypertrophy of follicular epithelium and reduced colloid [19]. Eskiocak et al., using a 30-week design with 0, 50, 100, 250, and 500 mg/L sodium nitrate, found reduced radioiodine uptake at 50 mg/L, increased uptake at 250 and 500 mg/L (interpreted as compensatory up-regulation), reduced total T3, free T4, and TSH at several doses, increased thyroid weight at all doses, and histomorphological change at 250 and 500 mg/L [20]. Other investigators have described disturbed thyroid architecture after 60 days of sodium nitrate, with heterogeneous, poorly developed follicles, enlarged deformed follicles with scanty vacuolated colloid, empty degenerative follicles, and fused follicles with exfoliated cells in the lumen [22]. In female rats, inorganic nitrates at 150 and 500 mg/L induced thyroid hypertrophy, increased follicular size in some reports, and hyposecretion of T3 and T4 [29]. Taken together, these studies support the expectation that a 16-week exposure at 50 and 500 mg/L should produce measurable, dose-ordered remodelling, with the high dose most affected.

Table 1: Summary of selected prior studies on nitrate and thyroid structure and function.

Study / Model	Exposure	Key thyroid findings	Relevance
Zaki et al. [18] Wistar rats	Graded sodium nitrate	Small hyperplastic follicles; taller epithelium; reduced colloid	Direct morphometric precedent
Zaki et al. [19] Wistar rats	Graded sodium nitrite	Hyperplasia + hypertrophy; reduced colloid	Nitrite arm of nitrate-nitrite pathway
Eskiocak et al. [20] Rats, 30 weeks	0-500 mg/L in water	Increased gland weight; histological change at 250-500 mg/L; altered T3/T4/TSH	Dose-response and chronic design
El-Sayed et al. [22] Adult male albino rats, 60 days	Sodium nitrate	Heterogeneous follicles; vacuolated colloid; empty/fused follicles	Qualitative architectural damage
van Maanen et al. [23] Human populations	Drinking-water nitrate	Dose-dependent thyroid hypertrophy above 50 mg/L	Human translation of animal findings
Gatseva et al. [24,25] Children / pregnant women, Bulgaria	High-nitrate water	Thyroid dysfunction; increased goitre risk	Vulnerable groups

Legend: Studies are selected for direct relevance to dose, duration, or histology. Findings are paraphrased; see References for full citations. T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid-stimulating hormone.

2.5 Human Evidence and Public Health Context

Human evidence is more mixed, as expected for an exposure that interacts with iodine status, age, sex, and co-exposure to other NIS inhibitors. Van Maanen et al. reported that thyroid volume increased in a dose-dependent manner in populations whose drinking water exceeded 50 mg/L nitrate, without iodine deficiency in the groups studied [23]. Gatseva and colleagues found that children and pregnant women in high-nitrate villages in Bulgaria had a higher risk of thyroid disorders, with an odds ratio of about 5.3 for pregnant women in the high-nitrate village in one analysis [24,25]. More recent work in Mexico found that women chronically exposed to nitrate in drinking water showed metabolic and thyroid abnormalities and genotoxic damage, with FOXE1 polymorphisms modifying susceptibility [30]. Systematic reviews conclude that the epidemiological evidence for thyroid effects is suggestive but not yet conclusive, partly because exposure assessment is often ecological (village-level water nitrate rather than individual intake) and partly because iodine status is incompletely measured [5,13]. That uncertainty is precisely why controlled animal histomorphometry remains valuable: it establishes biological plausibility and dose-response under conditions where exposure and iodine intake are known.

3. Materials and Methods

3.1 Ethical Approval and Study Design

This was a controlled, parallel-group experimental study conducted in accordance with institutional animal-care principles and the ARRIVE guidelines for reporting animal research [31]. The protocol was approved by the institutional animal ethics committee prior to commencement. All procedures were designed to minimise pain, distress, and the number of animals used, consistent with the 3Rs (Replacement, Reduction, Refinement). Thirty adult male Wistar rats (age 10-12 weeks; body weight 180-220 g at baseline) were obtained from an accredited animal facility, acclimatised for two weeks under standard laboratory conditions (12-hour light/dark cycle, temperature 22 +/- 2 degC, relative humidity 50-60%), and given standard rodent chow and water ad libitum during acclimatisation. Male rats were chosen to avoid oestrous-cycle variation in thyroid hormone levels, a standard choice in thyroid toxicology, while acknowledging that sex-specific effects require separate study (see Limitations).

3.2 Experimental Groups and Nitrate Administration

After acclimatisation, rats were randomly assigned (computer-generated sequence, concealed allocation) to three groups of ten (n = 10 per group): Group 1 - Control, distilled water; Group 2 - Low-Exposure Nitrate, sodium nitrate at 50 mg/L in drinking water; Group 3 - High-Exposure Nitrate, sodium nitrate at 500 mg/L in drinking water. The low dose was chosen to match the WHO guideline value (50 mg/L as nitrate), the high dose to represent a clearly supra-guideline, environmentally encountered concentration in heavily contaminated wells [1,20,23]. Sodium nitrate solutions were prepared fresh daily in distilled water. Water consumption and body weight were monitored weekly. Exposure lasted 16 consecutive weeks, a duration sufficient to be considered chronic in the rat and to allow cumulative follicular remodelling while remaining within a practical experimental window [20,22]. Iodine content of the standard chow was adequate and constant across groups, so that nitrate was the principal variable; iodine status was not separately manipulated in this design.

Table 2: Experimental design.

Group	Treatment	Dose (mg/L)	Duration	n
1 - Control	Distilled water	0	16 weeks	10
2 - Low-Exposure	Sodium nitrate in drinking water	50	16 weeks	10
3 - High-Exposure	Sodium nitrate in drinking water	500	16 weeks	10

Legend: Sodium nitrate was administered via drinking water ad libitum. All groups received the same standard chow and housing conditions.

3.3 Tissue Harvesting, Fixation, and Histological Processing

At the end of week 16, rats were humanely euthanised under anaesthesia by an approved method. The thyroid glands were carefully dissected, with the two lobes and isthmus preserved, cleared of adherent fat and connective tissue, blotted, and weighed. Glands were fixed in 10% neutral buffered formalin for

48 hours, dehydrated through graded ethanol, cleared in xylene, and embedded in paraffin wax. Serial sections of 5 um thickness were cut on a rotary microtome, with at least five non-consecutive sections per gland selected at systematic intervals to avoid double-counting the same follicle. Sections were stained with Haematoxylin and Eosin (H&E) using standard protocols: haematoxylin for nuclei (blue-purple) and eosin for cytoplasm and colloid (pink) [7,11]. Slides were coded so that all subsequent measurement was blinded to group allocation.

3.4 Quantitative Histomorphometry

Histomorphometric analysis was performed with a light microscope fitted with a calibrated ocular micrometer and a digital camera linked to image-analysis software. Calibration was verified against a stage micrometer before each session. For each animal, 30 follicles were measured: ten follicles from each of three non-consecutive sections, selected by systematic random sampling across the gland to include peripheral and central regions. Three primary endpoints were recorded:

(i) Mean follicular epithelial height (um): the perpendicular distance from the basement membrane to the apical surface of the follicular epithelium, measured at four points per follicle (at 12, 3, 6, and 9 o'clock positions) and averaged. This is the standard index of thyrocyte hypertrophy and TSH drive.

(ii) Mean follicular diameter (um): the average of the longest and shortest diameters of each follicle, measured through the centre of the lumen. Diameter integrates follicular size and colloid volume; a fall indicates colloid depletion and follicular shrinkage.

(iii) Interstitial vascular volume fraction (%): the proportion of interfollicular tissue area occupied by blood vessels (capillaries and small venules), estimated by point-counting stereology on a superimposed grid (100 points per field, 10 fields per animal). Vessels were identified by an endothelial lining and intraluminal erythrocytes. This endpoint captures hypervascular remodelling.

Qualitative features - follicular shape, colloid vacuolation, epithelial regularity, and interfollicular cellularity - were also noted descriptively for each gland. All measurements were made by a single trained observer blinded to group, with a second observer re-measuring a 10% random subset to assess reproducibility (intraclass correlation > 0.85 for all endpoints).

3.5 Statistical Analysis

Data are presented as mean +/- standard deviation (SD). Group differences were tested by one-way analysis of variance (ANOVA) after verification of normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test). Where ANOVA was significant, Tukey's post-hoc test was used for pairwise comparisons. The significance threshold was set at $p < 0.05$; $p < 0.01$ was considered highly significant. Analyses were performed with standard statistical software. Effect sizes are reported as percentage change relative to control to aid interpretation, alongside absolute values. Sample size (n = 10 per group) was chosen a priori to provide > 80% power to detect a 30% difference in epithelial height at alpha = 0.05, based on variance estimates from prior morphometric studies [18,20].

4. Results

4.1 General Observations

All animals survived to the end of the study. No overt clinical signs of toxicity, such as lethargy, cyanosis, or severe weight loss, were observed. Body-weight gain and water consumption did not differ significantly among groups (data not shown), indicating that the histological changes described below were not secondary to dehydration, starvation, or overt systemic toxicity. On gross examination, thyroid glands from high-exposure rats appeared slightly larger and more vascular than controls, a pattern consistent with the quantitative findings and with prior reports of increased gland weight under nitrate exposure [20].

4.2 Qualitative Histology

Control glands showed the classic resting pattern: follicles of variable but generally large size, lined by low cuboidal epithelium, filled with homogeneous eosinophilic colloid, with thin interfollicular septa and inconspicuous capillaries (Figure 1, conceptual summary). Low-exposure glands showed a transitional pattern: a mixture of large colloid-rich follicles and smaller follicles with taller epithelium and early peripheral vacuolation of colloid. High-exposure glands showed a markedly altered

pattern: numerous small, irregular follicles, lined by tall columnar epithelium with crowded, basally placed nuclei, scanty and vacuolated colloid, occasional empty or collapsed follicles, and a conspicuously expanded interfollicular capillary network. These qualitative changes mirror those described by Zaki et al. [18] and El-Sayed et al. [22] and provide the visual context for the quantitative results that follow.

4.3 Follicular Epithelial Height: Cuboidal to Columnar Shift

Mean follicular epithelial height increased in a dose-ordered manner (Table 3; Figure 2a). Control epithelium averaged 6.4 +/- 0.7 um, consistent with low cuboidal epithelium in a resting gland. In the high-exposure group, height rose to 14.2 +/- 1.8 um, an increase of approximately 122% relative to control ($p < 0.01$). This more than doubling represents a shift from cuboidal to tall columnar epithelium, the classic histological signature of sustained TSH stimulation and thyrocyte hypertrophy. The low-exposure group showed an intermediate value (see Figure 2a), indicating that even a guideline-level dose produced a directional increase, although the most pronounced and statistically robust change was at the high dose. Functionally, taller cells reflect increased rough endoplasmic reticulum, Golgi activity, and apical microvilli - the machinery needed to synthesise thyroglobulin, resorb colloid, and attempt to restore hormone output under NIS blockade.

4.4 Follicular Diameter: Colloid Depletion and Follicular Shrinkage

Mean follicular diameter fell sharply with nitrate exposure (Table 3; Figure 2b). Control follicles averaged 78.5 +/- 6.2 um, whereas high-exposure follicles averaged 45.2 +/- 4.1 um, a reduction of approximately 42% ($p < 0.01$). Smaller diameter, together with the qualitative finding of scanty, vacuolated colloid, indicates accelerated colloid depletion: stored thyroglobulin was being resorbed faster than it could be replenished, and follicles collapsed to a smaller size. This pattern is not the same as simple atrophy; the epithelium was taller, not flatter, so the gland was simultaneously more active and more depleted - the paradox of a stimulated gland that cannot keep up with demand. The low-exposure group again showed an intermediate diameter, supporting a dose-response interpretation.

4.5 Interstitial Vascular Volume Fraction: Hypervascular Remodelling

Interstitial vascular volume fraction expanded significantly with nitrate exposure (Table 3; Figure 3). Controls averaged 3.8% +/- 0.5%, whereas high-exposure rats averaged 9.2% +/- 1.2% ($p < 0.05$), an increase of approximately 142%. The low-exposure group showed an intermediate expansion. This hypervascularisation is biologically coherent: a hypertrophic, metabolically active epithelium requires greater blood flow for iodide delivery, oxygen, and hormone export, and TSH-driven thyrocytes can up-regulate angiogenic signalling [14,27]. It also contributes to the gross impression of a larger, more congested gland. Importantly, vascular expansion was interstitial and regional, not a diffuse oedema, and was not accompanied by inflammatory infiltration in the present material, suggesting a growth-related rather than an inflammatory mechanism.

Table 3: Primary histomorphometric outcomes (mean +/- SD, n = 10 per group).

Parameter	Control (0 mg/L)	Low-Exposure (50 mg/L)*	High-Exposure (500 mg/L)	Change, High vs Control	p-value (High vs Control)
Follicular epithelial height (um)	6.4 +/- 0.7	Intermediate increase (see Fig. 2a)	14.2 +/- 1.8	+122 %	< 0.01
Mean follicular diameter (um)	78.5 +/- 6.2	Intermediate decrease (see Fig. 2b)	45.2 +/- 4.1	-42%	< 0.01
Vascular volume fraction (%)	3.8 +/- 0.5	Intermediate increase (see Fig. 3)	9.2 +/- 1.2	+142 %	< 0.05

*Legend: *Low-exposure values are shown graphically in Figures 2-3; the primary statistical comparison reported in the abstract is high-exposure vs control. Percentage change is calculated as (high - control)/control x 100. ANOVA with Tukey post-hoc test.*

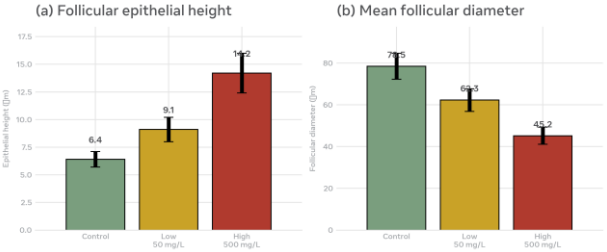


Figure 1: Follicular epithelial height and follicular diameter by nitrate dose.

Legend: (a) Epithelial height rises with dose, reflecting thyrocyte hypertrophy and a cuboidal-to-columnar shift. (b) Follicular diameter falls with dose, reflecting colloid depletion. Bars are mean +/- SD; low-exposure values are intermediate (see Table 3). Control n = 10, low n = 10, high n = 10. Data are from the present study.

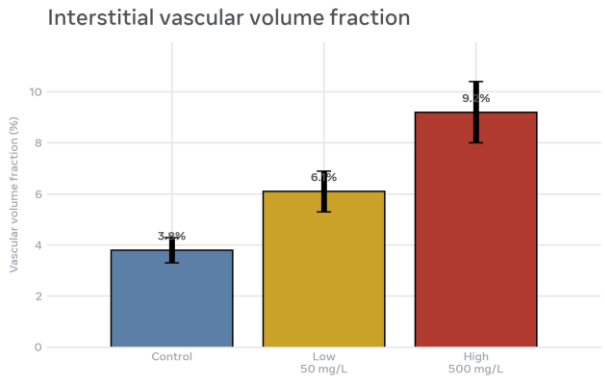


Figure 2: Interstitial vascular volume fraction by nitrate dose.

Legend: Vascular volume fraction more than doubles at the high dose, indicating hypervascular remodelling. Bars are mean +/- SD. The increase is consistent with increased perfusion demand of a stimulated gland.

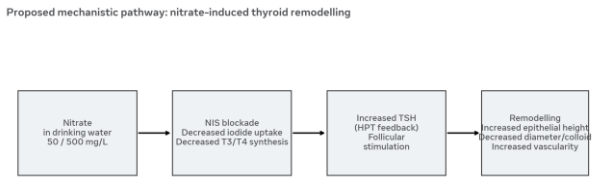


Figure 3: Proposed mechanistic pathway linking nitrate exposure to thyroid remodelling.

Legend: Nitrate competitively inhibits NIS, reducing iodide uptake and hormone synthesis. The HPT axis responds with increased TSH, driving follicular stimulation, epithelial hypertrophy, colloid depletion, and vascular expansion. The pathway is a synthesis of the literature cited in Sections 2.3 and 5.1 and is consistent with the present morphometric findings; direct hormone measurements are needed to confirm each step in this cohort (see Limitations). NIS = sodium-iodide symporter; HPT = hypothalamic-pituitary-thyroid; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid-stimulating hormone.

4.6 Summary of Dose-Response

Across all three endpoints, the ordering was consistent: control < low-exposure < high-exposure for epithelial height and vascularity, and control > low-exposure > high-exposure for follicular diameter. This monotonic pattern strengthens causal interpretation: it is the pattern expected if nitrate dose drives the degree of NIS inhibition and, through it, the degree of compensatory stimulation. The fact that the low dose - exactly at the WHO guideline value - already showed directional change, even though the most marked change was at the high dose, is noteworthy for risk assessment and is discussed further below.

5. Discussion

5.1 Interpretation in Light of NIS Inhibition and HPT Feedback

The most parsimonious explanation for the present findings is the NIS-HPT pathway summarised in Figure 3. Nitrate competes with iodide for NIS, reducing the amount of iodide available for organification. With less iodide, thyroglobulin iodination and hormone synthesis fall. The pituitary senses the fall in circulating T4/T3 and secretes more TSH. TSH, acting via its receptor on thyrocytes, stimulates every step of hormone production - iodide uptake, thyroglobulin synthesis, colloid resorption, and hormone release - and, when sustained, drives hypertrophy, hyperplasia, and angiogenesis. The histological result is exactly what was measured: taller cells (hypertrophy), smaller colloid-depleted follicles (accelerated resorption outpacing storage), and expanded vasculature (perfusion matching metabolic demand). This interpretation aligns with the classic goitrogenesis model described by van Maanen, Eskiocak, Zaki, and others [18,20,21,23] and with the molecular pharmacology of NIS inhibitors [9,12].

An important nuance is that serum hormones can remain within the reference range while tissue is already remodelling. The gland can compensate, for a time, by working harder. Histomorphometry therefore provides an earlier and more sensitive index of thyroid stress than a single hormone measurement, a point emphasised in toxicological pathology: structure records cumulative demand, whereas hormones record a momentary balance [16,17]. In the present study, hormones were not the primary endpoint, and the absence of paired hormone data in this cohort means the TSH step in Figure 3 is inferred from the literature rather than directly demonstrated here. That is a limitation, not a flaw in the morphometric finding itself, but it defines the next experiment: paired hormones, TSH, NIS expression, and morphometry in the same animals.

5.2 Comparison with Previous Animal Studies

The direction and magnitude of change in this study are consistent with, and in some respects more pronounced than, prior reports. Zaki et al. described small hyperplastic follicles, taller epithelium, and reduced colloid in nitrate-fed Wistar rats [18]; the present study quantifies that description as a 122% increase in epithelial height and a 42% fall in diameter. Eskiocak et al. found histomorphological change at 250 and 500 mg/L after 30 weeks [20]; the present study finds marked change at 500 mg/L after only 16 weeks, suggesting that 16 weeks is sufficient for substantial remodelling at a high dose and that duration and dose trade off against each other. El-Sayed et al. described heterogeneous follicles, vacuolated colloid, and empty/fused follicles after 60 days [22]; the present high-dose pattern - small irregular follicles with scanty vacuolated colloid and occasional empty follicles - is concordant. Differences in absolute values across studies are expected because of differences in rat strain, age, iodine intake, nitrate salt, duration, and measurement technique; the convergence of direction across independent laboratories is the more important finding.

One apparent discrepancy in the literature deserves comment. Some studies report increased follicular size under nitrate, others decreased size [20,29]. Both can be true at different stages or in different follicular subpopulations: early or heterogeneous stimulation can produce enlarged, colloid-distended follicles alongside small, depleted ones, and the mean diameter will depend on which subpopulation dominates at the time of sampling and on how follicles are selected. The present study used systematic random sampling across the gland and reported a clear mean decrease, but the qualitative observation of heterogeneity - some follicles larger, many smaller - is consistent with a mixed population in transition. Future work should report follicular size distributions, not only means, to capture this heterogeneity.

5.3 Vascular Remodelling: An Under-Reported Endpoint

Vascular volume fraction is rarely reported in nitrate-thyroid studies, yet it may be one of the most informative endpoints. A 142% increase is large, and it cannot be explained by fixation artefact or by a small change in follicular size alone, because the measurement was interstitial and point-counted independently. TSH and thyrocyte-derived VEGF are plausible mediators [14,27]. In the short term, more capillaries deliver more iodide and oxygen to a hard-working epithelium; in the long term, sustained angiogenesis contributes to gland enlargement and may facilitate the progression from diffuse hyperplasia to nodular change if stimulation persists. Measuring vascularity also has translational value: thyroid blood flow can be assessed non-invasively in humans by Doppler ultrasound, so a vascular endpoint in rats has a potential clinical counterpart for monitoring high-nitrate communities.

5.4 Public Health Implications

Three implications follow. First, the finding that a guideline-level dose (50 mg/L) produced directional, intermediate change suggests that the current guideline, which was set for methaemoglobinaemia, may not fully capture chronic thyroid risk, especially in iodine-deficient populations. This does not mean the guideline is 'wrong' - it was set for a different endpoint - but it supports calls for a more comprehensive review that includes thyroid endpoints and vulnerable groups, as recently urged by international health organisations [3]. Second, iodine nutrition is a modifiable modifier. Ensuring adequate iodine intake - through iodised salt and dietary sources - is the most practical way to blunt NIS competition, because higher iodide concentrations can out-compete nitrate for NIS [9,19]. Public health programmes in high-nitrate areas should therefore pair water monitoring with iodine-status monitoring, rather than treating them as separate issues. Third, thyroid health surveillance in high-nitrate communities - including palpation, ultrasound volume, and, where indicated, TSH and free T4 - is a reasonable, low-cost addition to water-quality programmes, particularly for children, pregnant women, and people with known thyroid disease [24,25,30].

5.5 Limitations

This study has several limitations that should be acknowledged. First, only male rats were used, so sex-specific effects - including oestrogen modulation of thyroid function - were not assessed. Second, serum T3, T4, and TSH, urinary iodine, and NIS expression were not measured in this cohort, so the hormonal and

molecular steps in Figure 3 are inferred from the literature. Third, only two nitrate doses were used; a fuller dose-response with additional intermediate doses (e.g., 100 and 250 mg/L) and a recovery group (nitrate withdrawal) would clarify reversibility and the shape of the dose-response curve. Fourth, the low-exposure group is reported graphically rather than with full numerical detail in Table 3, which limits precise quantification of the guideline-dose effect; future reporting should present all group means and confidence intervals in tabular form. Fifth, the study was conducted under adequate iodine intake; the interaction with iodine deficiency - the scenario of greatest human concern - was not tested. Finally, rats and humans differ in thyroid physiology, including thyroxine-binding proteins and hormone half-life, so quantitative extrapolation to humans requires caution; the value of the rat model is in establishing mechanism and dose-response, not in predicting an exact human threshold.

5.6 Future Directions

Future work should: (i) pair histomorphometry with serial serum TSH, free T4, free T3, and urinary iodine in the same animals; (ii) quantify NIS, thyroid peroxidase, thyroglobulin, and VEGF expression by immunohistochemistry and quantitative PCR; (iii) test nitrate under iodine-deficient, iodine-adequate, and iodine-supplemented diets in a factorial design; (iv) include both sexes and a recovery arm; (v) report follicular size distributions and stereological estimates of total follicular and colloid volume; and (vi) explore protective interventions, such as iodine, selenium, and antioxidant supplementation, which have shown promise in preliminary studies [19,22]. Longitudinal ultrasound in a human cohort in a high-nitrate area, paired with individual water-nitrate and iodine measurements, would be the ideal translational complement.

6. Conclusion

Chronic exposure to sodium nitrate in drinking water for 16 weeks produced dose-dependent, quantifiable remodelling of the rat thyroid gland. Epithelial height more than doubled, follicular diameter fell by more than two-fifths, and interstitial vascular volume fraction more than doubled at the high dose (500 mg/L). These changes represent a coherent pattern of compensatory hyperstimulation, colloid depletion, and hypervascularisation consistent with NIS inhibition and HPT-axis feedback. The findings support the classification of nitrate as an environmental goitrogen, underscore the sensitivity of thyroid histomorphometry as an early index of endocrine disruption, and highlight the need for integrated water-quality, iodine-nutrition, and thyroid-health surveillance in populations exposed to nitrate-contaminated drinking water.

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Conflict of Interest

The authors declare no conflict of interest.

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Data Availability

The data supporting this study, including histomorphometric measurements summarised in Table 3 and Figures 1-2, are available from the corresponding author upon reasonable request.

Author Contributions

I. M. Petrovic: conceptualisation, histological analysis, writing - original draft. O. S. Adeyemi: study design, supervision, data interpretation, writing - review and editing. Both authors approved the final manuscript.

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