

The Pituitary Is a Candidate Organ That Modulates Circulating Klotho Levels

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Context: The antiaging protein Klotho is shed and released into the blood stream (soluble Klotho). Growth hormone (GH) is considered an active Klotho regulator, because growth retardation is described in Klotho-deficient mice. The origin of circulating Klotho is, however, not fully understood.

Objectives: Our objective was to analyze a possible role of the pituitary in regulating soluble Klotho in patients with pituitary adenomas.

Patients, Design, and Setting: We analyzed serum levels of soluble Klotho, GH, and insulin-like growth factor 1 (IGF-1) from 21 consecutive patients in our center with pituitary tumor, 7 with GH-producing adenomas (GHomas), and 14 with non-GH-producing pituitary adenomas (non-GHomas), before and after endoscopic transsphenoidal surgery (eTSS).

Main Outcome Measure: Soluble Klotho levels were determined by ELISA with antihuman Klotho antibodies.

Results: Baseline soluble Klotho levels in all patients, those with GHoma and those with non-GHoma, were 542 (median) (interquartile range: 403, 652), 1083 (425, 1213), and 525 (399, 590), respectively. A drastic reduction in Klotho levels was identified in those with GHoma, accompanied by decreases in GH and IGF-1 levels, after eTSS. Interestingly, patients with non-GHoma had significant declines in soluble Klotho without any significant changes in GH levels. Moreover, an oral glucose tolerance test revealed that soluble Klotho levels decreased, whereas a paradoxical GH peak was observed after glucose intake in a patient with GHoma.

Conclusions: Our data suggest that the pituitary may be a key organ that regulates circulating Klotho concentrations, implying that the pituitary possibly controls circulating Klotho through GH-dependent and/or GH-independent mechanisms.

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Freeform/Key Words: pituitary, klotho, growth hormone, glucose tolerance

The antiaging gene klotho, encoding a 130-kDa transmembrane glycoprotein, was first identified in 1997 in a transgenic mouse strain whose mutation resulted in a syndrome resembling premature aging, which included shortened lifespan, growth retardation, vascular calcification, genital atrophy, emphysema, and osteomalacia [1]. Abundant evidence has accumulated that supports the association between klotho and an extended lifespan

Abbreviations: GH, growth hormone; GHoma, GH-producing adenoma; eTSS, endoscopic transsphenoidal surgery; IGF-1, insulin-like growth factor 1; IQR, interquartile range; non-GHoma, non-GH-producing adenoma; OGTT, oral glucose tolerance test.

[2, 3]. Klotho expression is decreased in aged brain white matter, indicating a role for klotho as a lifespan gene in the nervous system [4]. α -Klotho, lately known as one of the Klotho homologs, possesses aging-suppressing properties and has three primary isoforms: full-length transmembrane form (membrane Klotho), shed soluble form (soluble Klotho), and secreted truncated form that is produced by alternative splicing of klotho mRNA [5, 6]; this last form is considered the minor form of Klotho. Membrane Klotho is associated with fibroblast growth factor receptors [7, 8] to form coreceptors for the bone-derived phosphaturic hormone FGF23. Soluble Klotho is produced when the extracellular domain of membrane Klotho is shed from the cell surface into the blood, urine, and cerebrospinal fluid following proteolytic cleavage near the juxtamembrane region by the metalloproteinases ADAM10 and ADAM17 [9–13]. Following its release from the cell membrane, circulating soluble Klotho exerts its biological effects on distant organs or tissues. Interestingly, the atomic structure of the coreceptor complex that consists of shed Klotho, fibroblast growth factor receptor, and FGF23 has been revealed, indicating that shed Klotho is an enzyme-dependent active scaffold protein [14].

Gene and protein expression analyses indicate that Klotho expression in rodents and humans is relatively strong in the kidney, choroid plexus of the brain, pituitary, and parathyroid gland [15], whereas it is expressed to a lesser extent in areas such as the thyroid gland, pancreas, and sex organs [1, 16–18]. In contrast, β -Klotho, another Klotho homolog that modulates FGF21 [19], is overwhelmingly expressed in the liver, adipose tissue, and pancreas and acts as a critical targeting signal for FGF21 [20].

To be more specific regarding the antiaging property, the unique organ-specific expression and shedding process of Klotho may be the reason for human homeostasis, because Klotho plays a pivotal role in the lifespan. There is still the crucial issue of how soluble Klotho is regulated in the blood stream. The current study was carried out to focus on a possible key player, the pituitary, and to examine whether it is a candidate source of soluble Klotho. Our hypothesis was that pituitary cells including adenoma produce and release soluble Klotho into the blood stream. To address this intriguing issue clinically, we performed soluble Klotho measurements before and after endoscopic endonasal transsphenoidal surgery (eTSS) in patients with pituitary adenoma in our center to detect any postsurgical changes in soluble Klotho.

1. Subjects and Methods

The current study was a retrospective cohort study performed at Nagoya Daini Red Cross Hospital in Nagoya, Japan. The study design was in accordance with the ethical standards of the Declaration of Helsinki and approved by the regional institutional review board (Nagoya Daini Red Cross Hospital).

A. Patients

We collected data from 21 consecutive patients with pituitary tumors, 7 with growth hormone (GH)-producing adenoma (GHoma) and 14 with non-GH-producing pituitary adenoma (non-GHoma), who underwent eTSS at the Endoscopic Neurosurgery Center, Nagoya Daini Red Cross Hospital, between July 2016 and March 2017.

B. Blood Analysis

Routine blood biochemical analyses were performed at local laboratories according to current laboratory standards. Serum Klotho levels were determined by using a sandwich ELISA kit (Immuno-Biological Laboratories Co., Ltd., Gunma, Japan) using two antihuman monoclonal Klotho antibodies that recognize the extracellular domain of Klotho [21, 22].

C. Diagnosis

We reviewed pathological materials, including a full panel of pituitary hormone immunohistochemistry and MIB-1 (Ki-67), a cell proliferation marker, to confirm the diagnosis.

Pituitary adenomas were then categorized as either GHomas or non-GHomas [prolactinomas, ACTH-producing adenomas, or adenomas without hormone expression (nonfunctioning pituitary adenomas)]. One patient with GHoma underwent an oral glucose tolerance test (OGTT) before eTSS to identify any changes in plasma glucose, GH, and soluble Klotho levels.

D. Statistics

Continuous data are expressed as the mean (SD) or median [interquartile range (IQR)] depending on the distribution of continuous variables. Nonparametric tests were used where necessary. Analyses were performed with SPSS for Windows version 13.0 (IBM, Chicago, IL). All statistical tests were two sided, and P values <0.05 were considered to indicate significance.

2. Results

A. Baseline Characteristics

Table 1 presents the baseline characteristics in our patients who underwent eTSS for pituitary adenomas. The overall mean (SD) age was 54.3 (14.4). Patients with GHoma ($n = 7$) had a larger physique than those with non-GHoma [$n = 14$, height: 1.71 (0.140) vs 1.60 (0.0790) and weight: 74.7 (17.3) vs 65.2 (14.8), $*P < 0.01$]. Two had prolactinomas, one had ACTH-producing adenoma, and the rest of the patients with non-GHoma had nonfunctioning pituitary adenomas. Notably, patients with GHoma had higher GH, IGF-1, and Klotho levels than those with non-GHoma [5.9 (IQR, 2.7 to 9.2) vs 0.31 (0.030 to 0.73), 488 (396 to 742) vs 90.0 (57.0 to 114), 1083 (425 to 1213) vs 525 (399 to 590), respectively, $*P < 0.01$]. No statistically significant difference was detected in estimated glomerular filtration rate between patients with GHoma and non-GHoma.

B. Variables Before and After Endoscopic Pituitary Surgery

Serum Klotho levels decreased after eTSS in the overall patient sample [Fig. 1(a)]. GH and IGF-1 levels also decreased after eTSS [Fig. 1(b) and 1(c)]. Notably, upregulated circulating

Table 1. Baseline Characteristics

	Overall (n = 21)	GHoma (n = 7)	Non-GHoma (n = 14)
Age	54.3 (14.4)	61.3 (7.78)	50.8 (15.9)
Sex, n (male/female)	9/12	5/2	4/10
Height, m	1.64 (0.111)	1.71 (0.140)	1.60 (0.0790) ^a
Weight, kg	68.3 (15.9)	74.7 (17.3)	65.2 (14.8) ^a
ACTH, pg/mL	22.5 (18.0, 28.5)	25.9 (22.9, 31.0)	21.5 (15.3, 25.0)
Cortisol, µg/dL	10.9 (7.42, 16.1)	10.7 (6.98, 15.3)	11.1 (2.70, 16.2)
LH, mIU/mL	1.7 (0.88, 5.4)	3.6 (1.6, 7.2)	1.4 (0.70, 3.5)
FSH, mIU/mL	9.5 (3.6, 13)	15 (10, 27)	6.6 (3.2, 11)
TSH, µIU/mL	0.70 (0.50, 1.4)	0.66 (0.22, 0.71)	1.1 (0.57, 2.1)
FT3, pg/mL	2.7 (1.8, 3.0)	2.7 (1.9, 3.1)	2.3 (1.9, 2.8)
FT4, ng/dL	1.2 (0.92, 1.3)	1.3 (1.2, 1.5)	1.0 (0.85, 1.3)
GH, ng/mL	0.73 (0.13, 2.7)	5.9 (2.7, 9.2) ^a	0.31 (0.030, 0.73) ^a
IGF-1, ng/mL	114 (76.8, 396)	488 (396, 742) ^a	90.0 (57.0, 114) ^a
PRL, ng/mL	9.0 (4.9, 17)	11 (4.8, 34)	8.7 (5.5, 17)
Klotho, pg/mL	542 (403, 652)	1083 (425, 1213) ^a	525 (399, 590) ^a
eGFR, mL/min/1.73 m ²	80.5 (69.9, 92.8)	91.0 (76.7, 117)	80.4 (69.6, 86.5)

Data are expressed as the number, mean (SD), or median (IQR), depending on the distribution of continuous variables. Groups were compared using the Mann-Whitney U test.

Abbreviation: eGFR, estimated glomerular filtration rate; FT3, free triiodothyronine; FT4, free thyroxine, PRL, prolactin.

^a $P < 0.01$.

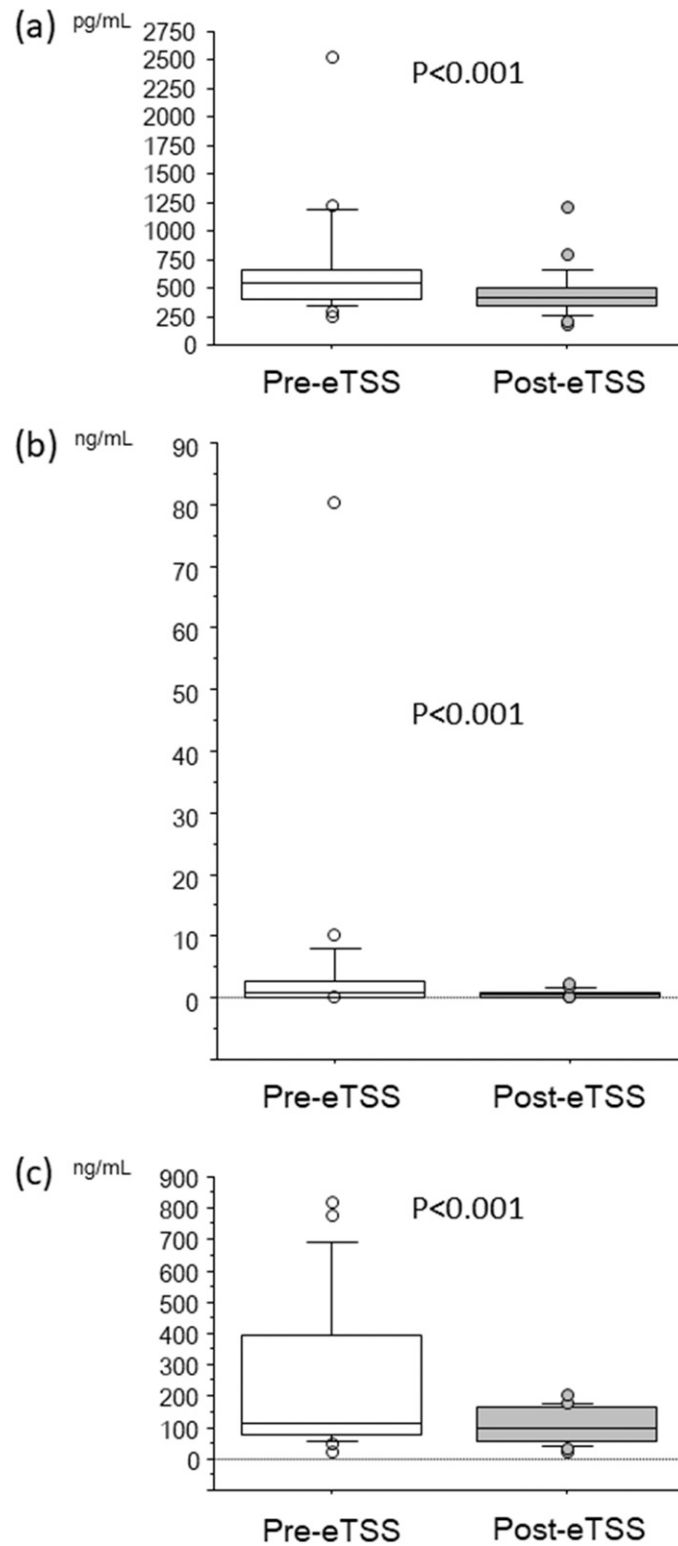


Figure 1. Box and whisker plots before (Pre) and after (Post) eTSS: (a) soluble Klotho, (b) GH, and (c) IGF-1 in all patients. The Wilcoxon signed-rank test identified a significant reduction in soluble Klotho, GH, and IGF-1 after eTSS.

Klotho levels showed a drastic reduction after eTSS in patients with GHoma [Fig. 2(a)]. GH and IGF-1 levels showed a robust decline after surgical GHoma resection in those patients [Fig. 2(b) and 2(c)].

Interestingly, soluble Klotho levels in patients with non-GHomas showed a statistically significant reduction after resection of pituitary adenoma [Fig. 3(a)], although no remarkable difference in GH levels was observed before or after the surgery [Fig. 3(b)], suggesting that circulating Klotho levels are regulated through both GH-dependent and GH-independent mechanisms. IGF-1 levels showed a borderline reduction after eTSS [Fig. 3(c), $P = 0.048$].

OGTT revealed a patient with GHoma had a markedly elevated fasting GH level. The GH levels throughout the test were not suppressed by glucose intake at all and were even increased after glucose intake, which was the absolute opposite of the effect that would normally be expected (Fig. 4). The fasting soluble Klotho level of the patient was quite elevated (1228 pg/mL), even compared with patients with GHomas. Circulating Klotho levels time-dependently decreased during glucose tolerance, although the serum Klotho level was maintained at a relatively high level 2 hours after glucose intake (1053 pg/mL).

3. Discussion

Circulating soluble Klotho has long been considered to be protective against kidney injury because recombinant Klotho administration restores kidney function [23]. However, a crucial issue is whether the kidney is the major organ that produces and secretes shed Klotho into the bloodstream. Regarding the secreted form, Klotho was not found to be related to kidney function and did not predict adverse renal outcomes in one cohort [24], but its relationship with kidney function has been reported in another study [25, 26]. The current study demonstrates that pituitary adenoma, whether it produces GH, is a possible source of the soluble form of Klotho because soluble Klotho levels declined after pituitary surgery, although a remarkable difference was observed between patients with GHomas and those with non-GHomas. Our data are consistent with the results that GH triggers endogenous soluble Klotho levels [27, 28]. Interestingly, GHomas as well as non-GHomas have been proven to show Klotho expression by immunohistochemistry [29], suggesting that non-GH-producing pituitary cells have an ability to produce Klotho. Strikingly, non-GHomas have stronger Klotho positivity than GHomas according to a semiquantitative analysis [29]. Our data from non-GHomas have indicated the clinical relevance of elevated soluble Klotho levels before eTSS because soluble Klotho levels were modestly reduced after the surgical resection of pituitary adenoma. These observations clearly show that the pituitary is related to soluble Klotho regulation. GH-producing cells are likely responsible for the upregulation of soluble Klotho levels because GHomas had markedly elevated circulating Klotho levels, which eventually showed a robust reduction after pituitary surgery. We encountered a patient with a GHoma who underwent an octreotide test before surgery. Serum Klotho and GH levels at baseline and 2, 4, 6, and 8 hours after a single injection of 50- μ g octreotide were 1861, 1821, 1800, 1800, and 1950 pg/mL and 33.0, 6.84, 11.8, 14.6, and 15.0 ng/mL, respectively. Apparently, treatment with somatostatin analog promptly reduces GH levels but suppresses soluble Klotho levels modestly and gradually. Other mediators might regulate circulating Klotho levels. However, the next question is whether elevated GH levels predominantly upregulate the shed form of Klotho or whether there are any other crucial mechanisms that regulate soluble Klotho. Our OGTT data imply some possibilities regarding the relation of GH and Klotho in the bloodstream. Markedly upregulated soluble Klotho levels in a patient with a GHoma (Fig. 4) steadily decreased after oral glucose administration, whereas GH levels increased during OGTT, which was completely different from the normal response. OGTT paradoxically triggered the GH reaction and steadily reduced soluble Klotho levels. GH is likely linked to elevated soluble Klotho levels, whereas oral glucose intake may be another regulator of circulating Klotho levels.

Pituitary cells, particularly GH-producing adenoma cells, may increase soluble Klotho levels, presumably via a GH-related mechanism, whereas oral glucose intake may negatively regulate Klotho even in the presence of robustly elevated GH levels in patients with GHomas. Given all our data, we would like to emphasize that certain pituitary cells and functions may

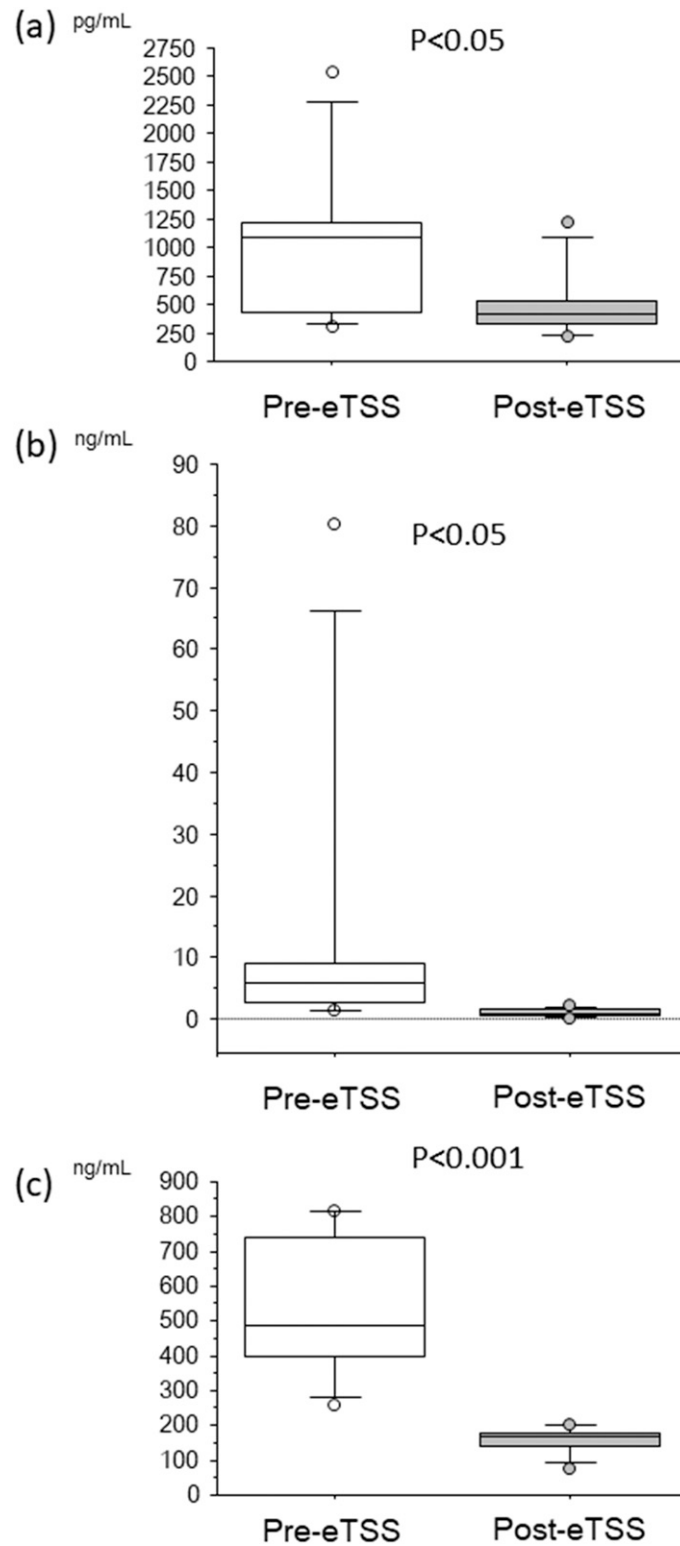


Figure 2. Box and whisker plots before (Pre) and after (Post) eTSS: (a) soluble Klotho, (b) GH, and (c) IGF-1 in patients with GHoma showing a robust decline in soluble Klotho, GH, and IGF-1 after eTSS.

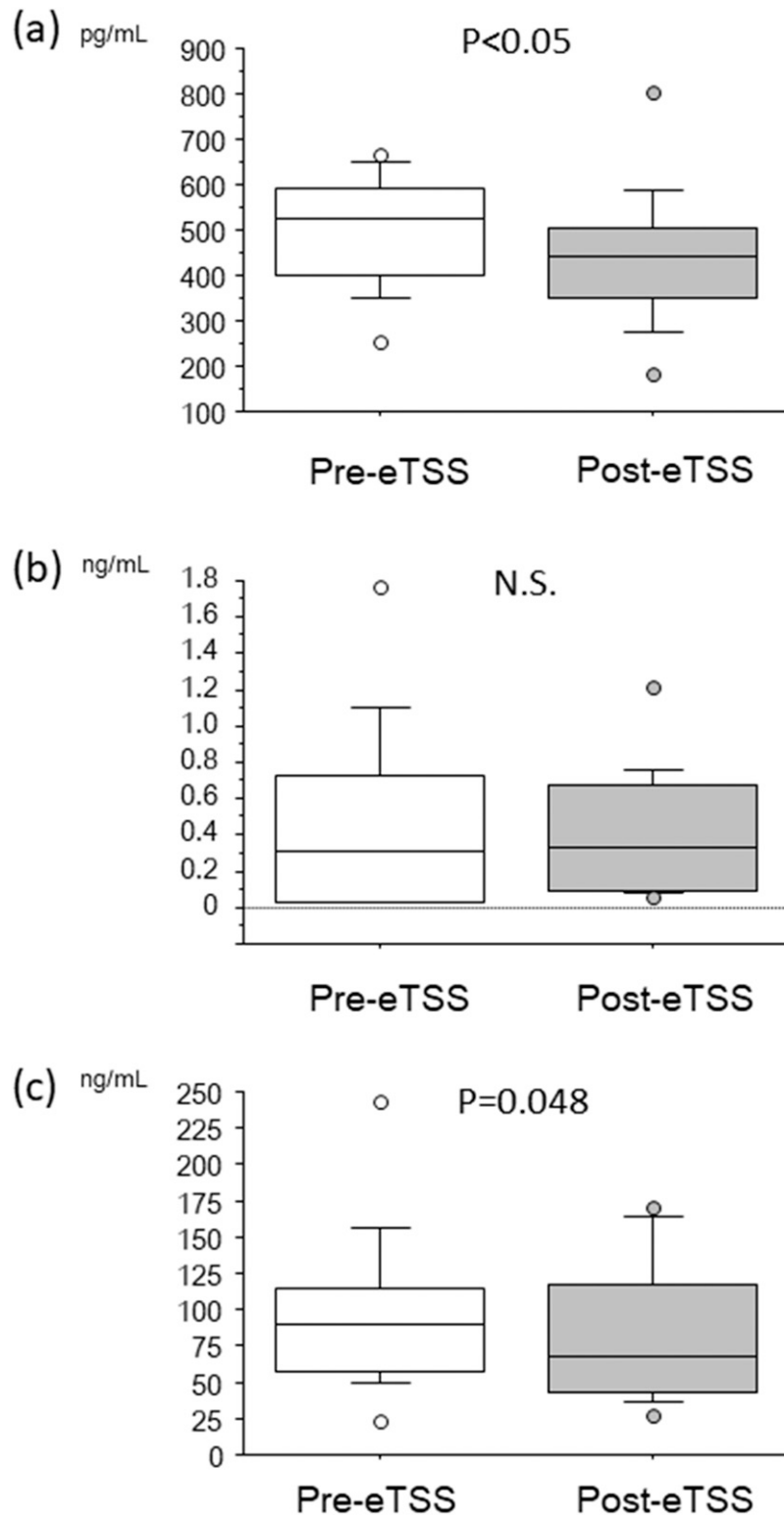


Figure 3. Box and whisker plots before (Pre) and after (Post) eTSS: (a) soluble Klotho, (b) GH, and (c) IGF-1 in patients with non-GHoma indicating a modest decrease in soluble Klotho and IGF-1 after eTSS, although no significant change in GH was observed. N.S., not significant.

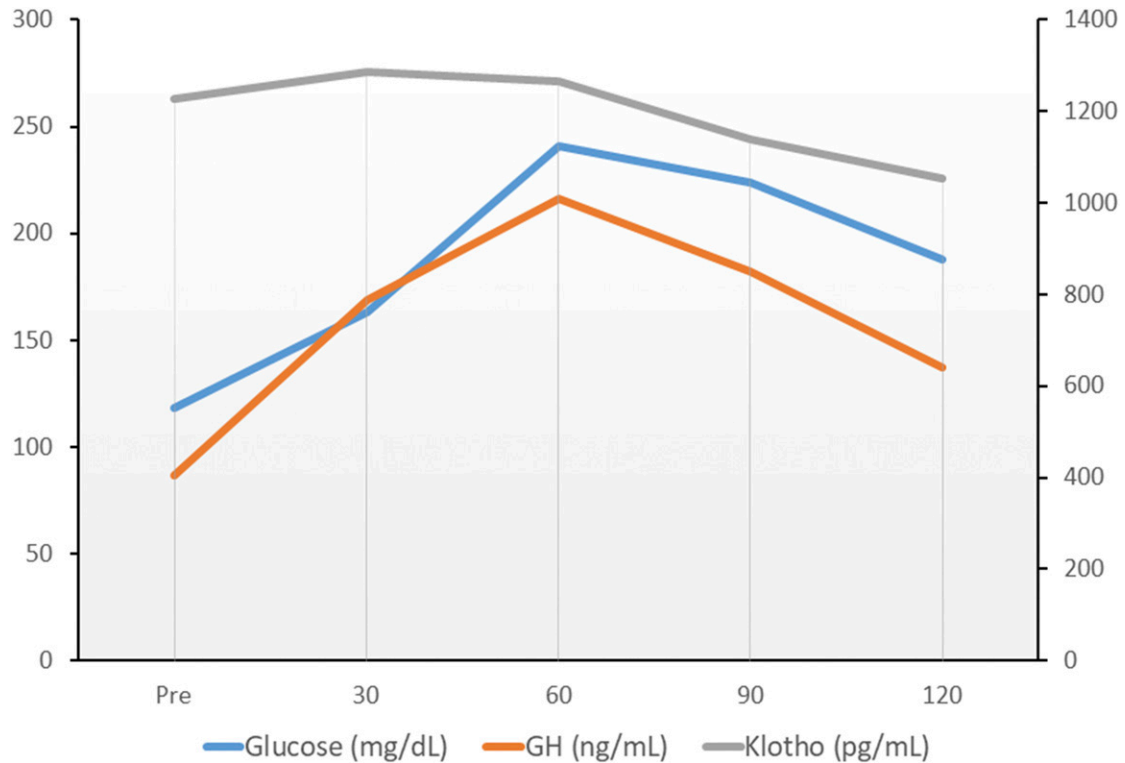


Figure 4. Result of a 75-g OGTT from a patient with GHoma before pituitary surgery. The paradoxical GH increase was remarkable, whereas impaired glucose tolerance was presented. Serum Klotho levels steadily decreased during the test.

affect soluble Klotho production and/or secretion, where both GH-dependent and GH-independent mechanisms underlie this antiaging hormone regulation, which deserves more attention in endocrinology.

This study has some limitations. First, we could not demonstrate that normal pituitary cells express Klotho and secrete soluble Klotho. Whether GH directly enhances shed Klotho expression and whether other energy balance mechanisms, including oral glucose intake, influence clinically relevant Klotho levels are also crucial issues. Second, there is a lack of direct evidence regarding Klotho expression in pituitary adenomas. Our unpublished preliminary results, which were obtained through quantitative polymerase chain reaction, that the normal mouse pituitary gland has modest klotho expression are encouraging for further studies. Interestingly enough, Klotho-deficient mice have been found to possess hypopituitarism-resembling phenotypes, including growth retardation, hypogonadism, and hypoglycemia [1]. Experimental animal models are needed to clarify normal pituitary function regarding Klotho expression.

In conclusion, the pituitary is the organ responsible for the release of shed Klotho into the blood stream, and pituitary adenomas potentially produce clinically relevant levels of soluble Klotho. The somatotrophic axis may play a key role in the underlying mechanism, although a GH-independent pathway is another possible compensatory player in the regulation of circulating soluble Klotho.

The authors have provided supplementary data at the designated repository storage site [30].

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