SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Investigation of metabolic parameters in endocrine disorders

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Head of the **Examination Committee**:	Éva Remenyik PhD Dsc
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The Examination takes place at the Meeting Room of Building A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen

Debrecen, August 28., 2025. at 09:00 AM

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The PhD Defense takes place at the Lecture Hall of Building A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen

Debrecen, August 28, 2025. at 11:00 AM

Introduction

Nowadays, thanks to the developing laboratory background and rapidly advancing imaging technologies, more and more endocrinological diseases are being diagnosed. Early diagnosis is essential, as definitive treatment as soon as possible can avoid or minimise complications arising from the disease course. In our work, we have investigated metabolic parameters that may not only help in diagnosis but may also be useful as predictive factors and in monitoring disease progression, and may open up new therapeutic options in treatment protocols.

Insulinoma, although relatively rare, is the most common neuroendocrine tumour of the pancreas. The time gap between the onset of clinical symptoms and diagnosis is often many years, and during this time the health of insulinoma patients continues to deteriorate due to hypoglycaemic attacks which mainly affect the autonomic nervous system . Obesity is also a very common feature of the pathology, resulting in metabolic dysregulation, generalised atherosclerosis and ultimately cardiovascular diseases. Early recognition of insulinoma is difficult for several reasons. On the one hand, clinical symptoms are often nonspecific and relatively easy to confuse with other pathologies, and on the other hand, the solid pancreatic tumour itself is small, which makes diagnostic imaging very difficult. Surgical treatment of insulinoma is curative, but the most possibly accurate preoperative localisation of the tumour as accurately is essential. Nowadays, with the spread of newer, more up-to-date imaging techniques, selective intra-arterial calcium stimulation CT angiography (arterial stimulation venous sampling, ASVS) has been somewhat overshadowed, however it continues to play an important role in the diagnosis of insulinoma. ASVS scan not only provides a morphological image of the actual lesion, but also delivers very important information about the hormone-producing function of the tumour. By detecting and measuring the abnormal insulin excess released by calcium stimulation during the test, we can determine the location of the small insulinoma in the pancreas, identify the involved pancreatic third of the artery supply area , and decide whether the abnormal insulin release is caused by insulinoma or nesidioblastosis (hyperinsulinemic hypoglycaemia caused by diffuse beta cell hypertrophy) in the background of the lesion causing endogenous hyperinsulinemia. While insulinoma requires surgery,

nesidioblastosis is best treated with drug therapy. Newer diagnostic techniques, became available in the recent decade, are often costly and difficult to access, in some of the cases have limited diagnostic value and do not provide functional information, so the role of ASVS remains significant.

Fibroblast growth factor 21 (FGF21) is a peptide hormone playing a key role in the regulation of carbohydrate and lipid metabolism and body weight. Its multi-organ benefits protect against atherosclerosis and fatty liver disease, and anti-inflammatory effects have also been observed in type 2 diabetes (type 2 diabetes mellitus, T2DM). Its blood levels are elevated in the presence of various metabolic abnormalities, and it can be used to predict the development of metabolic diseases such as diabetes mellitus. This is very important of prevention, as it can draw attention to the deteriorating metabolic status before the appearance of complications, when with an early initiation of appropriate therapeutic measures and lifestyle changes, the appearance of cardiovascular complications and the development of cardiovascular diseases can be slowed down or avoided. It is well known that the thyroid gland plays an important role in the regulation of metabolism. Although animal studies have found correlations between FGF21 levels and thyroid function, to date only a limited number of human studies have been conducted and their results are controversial. Hashimoto's thyroiditis (HT) is the most common organ-specific autoimmune disease and the most common cause of hypothyroidism. It has also been associated with obesity, atherosclerosis, several cardiovascular diseases and the development of malignant thyroid tumours. To date, the relationship of serum FGF21 levels, thyroid function and various metabolic parameters in adult Hashimoto's thyroiditis patients has not been investigated. In our opinion, by mapping these relationships in more detail, we can better understand the details of metabolic dysregulation in HT patients, for developing new therapeutic designs to avoid more severe metabolic dysregulation and to prevent the development of later complications, like obesity, atherosclerosis, cardiovascular disease, thus ensuring a higher quality of life and a longer life expectancy in this patient population.

Literature review

Onset, incidence and prevalence of insulinoma

Neuroendocrine tumours (NETs) are a rare, heterogeneous group of tumours that most commonly develop in the gastroenteropancreatic (GEP) tract or lung. Overexpression of somatostatin receptors (SSTR), mainly subtype 2, is observed on the cell membrane of these tumours. In recent years, the incidence and prevalence of NETs have skyrocketed, partly because of early detection due to advances in technology and due to longer life expectancy thanks to increasingly advanced treatments.

Pancreatic neuroendocrine tumours (pNETs) are tumours that develop from pancreatic neuroendocrine cells. Their specialness lies in the ability of certain pancreatic neuroendocrine tumours to produce and secrete neuropeptides and hormones which leads to the development of pathologies with different clinical symptoms, such as Zollinger-Ellison syndrome, which is associated with gastrin overproduction, or insulinoma, which is associated with hyperinsulinemia.

Insulinoma is a rare pancreatic beta cell tumour with an incidence of 1-4/1 million/year. However, despite its rare occurrence, it is the most common neuroendocrine tumour of the pancreas. Most insulinomas are sporadic in occurrence, although in 5-10% of cases they may present as part of a multiple endocrine neoplasia (MEN) syndrome. Typical clinical signs tend to appear early, usually before the age of 30.

Early detection and appropriate treatment of cases associated with endocrine tumor syndromes is even more important, as 16-25% of insulinoma cases with MEN-1 syndrome are malignant, so early detection can significantly improve the patient's life expectancy. 87-95% of cases insulinomas are benign and solitary in location, allowing effective and rapid surgical therapy.

Clinical picture and laboratory diagnosis of insulinoma

The accurate diagnosis of insulinoma is often delayed and tipically comes many years after the onset of clinical symptoms. The vegetative and neuroglycopenic symptoms that dominate the clinical picture occur in the case of a hyperinsulinemic hypoglycemia. Among neuroglycopenic symptoms, the most revealing are confusion, blurred vision, headache, convulsions and altered state of consciousness, while the vegetative symptoms are sweating, weakness, palpitations, sense of hunger and various paraesthesias.

In many cases, detection is delayed, and the presence of diverse neurological and psychiatric symptoms may precede an accurate diagnosis by years. A persistent hypoglycaemic coma may lead to temporary or permanent brain damage, which is why early diagnosis is crucial to prevent serious neurological complications. Weight gain due to significant and frequent sense of hunger following hyperinsulinaemia is also a common feature of the syndrome, observed in 39-50% of cases.

Hypoglycaemia with endogenous hyperinsulinaemia should be confirmed by all means. The possibility of insulinoma should be considered primarily in the presence of Whipple's triad. Whipple's triad is present when a documented (plasma glucose <2.5 mmol/l) hypoglycaemic episode with clinical signs occurs, which resolves immediately on glucose administration . The diagnosis emerging from Whipple's triad can be confirmed by the so-called fasting test. Concurrent beta-cell polypeptide values (insulin >4 μU/ml, C-peptide >0.2 nmol/l and proinsulin >5 pmol/l) measured during hypoglycaemia during a 72 hours fasting are diagnostic in 99% of cases.

Location of insulinomas and diagnostic imaging options

Surgical removal of the tumour is curative in most cases. With the available conventional imaging techniques, tumour localisation is often difficult, as insulinomas are often less than 2 cm in diameter. In patients with MEN-1 syndrome, insulinomas are usually between 1-3 cm in size and multifocal in location. Insulinomas can develop anywhere within the pancreas, but an extrapancreatic location is also possible, though very rare (<2%), and finding a tumour in an unusual location is challenging.

The main aim of surgery is to minimise endocrine and exocrine loss of function of the pancreas, thus preserving residual pancreatic function. However, this sparing, parenchyma-preserving partial pancreatectomy or tumour enucleation can only be performed after an adequate preoperative localisation of the tumour.

In some case, the diagnostic identification rate of traditional non-invasive imaging techniques such as abdominal ultrasound (9-63%), contrast-enhanced CT (63-94%) and MRI (60-90%) may not be sufficient.

Neuroendocrine cells communicate with target cells through the secretion of various peptides via stimulatory or inhibitory receptors on the cells. Of these, the most important are

somatostatin receptors, with 5 subtypes (SSTR1-5) known in humans. In many cases, several different receptor subtypes are present in the same cells, but their expression levels differ between normal and tumour cell types. The distribution of SSTRs is different in differing neurondocrine tumours, depending mainly on the type and degree of differentiation of the tumour. Among neuroendocrine tumours located in the pancreas, insulinomas express SSTR2 in 70%, SSTR1 in 60%, SST3 in 35% and SSTR4 in 3%.

Somatostatin receptor scintigraphy (SRS) is a nuclear imaging technique which uses a radiolabelled somatostatin analogue molecule to identify the NET expressing somatostatin receptor. Following receptor-ligand binding, the radiolabelled molecule emits gamma radiation, thus the tracer molecule and its distribution can be visualised using a gamma camera, localising the tumour. In insulinoma cases, somatostatin receptor scintigraphy has a sensitivity of 47-60%.

The endoscopic US technique where the ultrasound probe is inserted into the duodenum, getting closer to the pancreas than transdermal abdominal US, can achieve a sensitivity of up to 92.6%, but currently the highest localisation rate for insulinomas is achieved with 68Ga-Exendin- 4 PET/CT, which has an outstanding hit rate of 97.7%. A major limitation of the latter technique is its limited availability and extremely high price.

Invasive procedures, such as endoscopic ultrasound or selective intra-arterial calcium stimulation (ASVS), may significantly improve the success rate, however, the success rate of these tests depends largely on the technical equipment level of the centre and the skill of the examining physician.

Preoperative localisation is also of paramount importance because in 9-23% of cases, the tumour cannot be found intraoperatively with neither inspection nor palpation.

The definition of nesidioblastosis

Endogenous hyperinsulinaemic hypoglycaemia may also be associated with a condition of another pancreatic origin called nesidioblastosis. The difficulty of diagnosing it lies in the fact that its clinical symptoms are virtually identical to those described in insulinoma.

The term itself was first used by Laidlaw, in 1938, to describe the disease as a hyperplasia of islet cells of the pancreas, dysplasia of endocrine cells and ductoinsular proliferation .

The aetiology of nesidioblastosis in adults is still unclear, with some studies suggesting a link between gastric bypass surgery and the development of nesidioblastosis. Hormonal changes after surgery, especially glucagon-like peptide-1 (GLP-1), may affect the proliferation of pancreatic beta cells.

The selective intra-arterial calcium stimulation angiography technique investigated in the present study is based on the observation that parenteral calcium induces a high insulin release in overactive pancreatic beta cells, a phenomenon not observed in physiologically functioning beta cells.

During the ASVS examination, the main arteries supplying the pancreas, the gastroduodenal artery, the superior mesenteric artery and the lienal artery are stimulated with calcium administered through a catheter, and blood is drawn several times from the right hepatic vein. The advantage of the technique is that, in addition to the localisation of the insulinoma, it provides additional functional information to the morphological image, increasing sensitivity to 62.5%-100% and specificity to 89.2% .

The role of FGF21 in metabolism

Fibroblast growth factor 21 (FGF21), synthesised mainly in the liver, is a peptide hormone that acts in both paracrine and endocrine way. It plays an important role in regulating the body's energy balance and body weight.

In mouse studies, FGF21 has been shown to reduce blood glucose, triglyceride levels and promote weight loss. Furthermore, high serum levels of FGF21 also prevented weight gain in mice on a high-fat diet. In human studies, beneficial effects have been demonstrated in dyslipidaemia and fatty liver disease.

In humans, FGF21 plays a role in macronutrient uptake, acts as a hunger hormone and a stress-secreted hepatokine, and functions as a postprandial regulator of metabolic processes. FGF21 is also assumed to play a protective role in non-alcoholic fatty liver disease and its inflammatory form.

Mechanism of action of FGF21

On target organs, FGF21 binds to tyrosine kinase-type receptors of the FGF receptor (FGFR) superfamily and activates them via its obligate coreceptor βKlotho (KLB), an FGFR-binding transmembrane protein.

Free fatty acids (FFA) increase FGF21 levels through activation of peroxisome proliferator-activated receptor alpha (PPAR-α), whereas in starvation, FGF21 inhibits lipolysis and free fatty acid formation. This connection suggests the presence of a very important regulatory system that fine-tunes the communication between adipose tissue and the liver.

In addition to its metabolic effects, FGF21 is also thought to have anti-inflammatory effects observed in type 2 diabetic patients and patients with diabetic peripheral neuropathy, and also appears to have beneficial effects on the hypothalamic inflammatory process associated with obesity.

FGF21 circulating in the blood also has an indirect immunomodulatory effect by affecting the uptake of glucose by activated monocytes.

The connection between FGF21 and chronic metabolic diseases

Elevated FGF21 levels are observed in patients with obesity, dyslipidaemia, metabolic syndrome, diabetes mellitus, fatty liver disease of non-alcoholic origin, coronary artery disease, atherosclerosis, acute myocardial infarction, diabetic nephropathy, and arterial hypertension.

FGF21 levels increase with the increase of blood glucose, which has been shown in human studies to predict the development of diabetes and may be associated with early stages of nephropathy in type 2 diabetic patients. These findings suggest that FGF21 may be an early indicator of the development of metabolic diseases. The higher levels of FGF21 in the aforementioned chronic diseases may be part of an adaptive process whereby the depletion of protective mechanisms leads to an FGF21-resistant state when the desired beneficial effects of FGF21 on the target organs are no longer present.

The relationship between FGF21 and thyroid function

The above-mentioned fine-tuning effect of FGF21 on metabolism has already drawn attention to a possible link between FGF21 and thyroid function. Hamster studies have demonstrated that an upregulation of the enzyme deiodinase-2 (DIO2) is observed in the

hypothalamus and interscapular brown adipose tissue following the administration of FGF21 infusion. The DIO2 enzyme converts thyroxine (T4) to its biologically active form, triiodothyronine (T3). High expression of DIO2 is observed in hypothalamic tanycytes, and the consequently altered hypothalamic T3 concentration affects appetite and energy use. In animal studies, T3 treatment via a PPAR-dependent pathway increased hepatic expression of FGF21, whereas administration of FGF21 decreased thyroid hormone levels, suggesting a reciprocal relationship between FGF21 and thyroid function .

Although animal studies suggest a high probability of strong association between FGF21 and thyroid function, there are only limited and inconclusive human studies where elevated FGF21 levels were found in both hypo- and hyperthyroidism.

Hashimoto's thyroiditis (HT) is an organ-specific autoimmune disease and the most common cause of hypothyroidism, with a prevalence of 5.8-14.2%. HT has been associated with various cardiovascular diseases, atherosclerosis, obesity, and malignant thyroid lesions .

Based on literature data, no study has been performed yet to investigate the relationship of FGF21 levels with various metabolic and thyroid parameters in a group of adult patients with Hashimoto's thyroiditis.

Objectives

Use of ASVS in the diagnosis of insulinoma

In our retrospective study, we investigated the efficacy of ASVS in hypoglycaemic patients with hyperinsulinemia where conventional imaging techniques failed to indentify the source of abnormal insulin excess.

We had the following objectives during our study:

a./ To determine the sensitivity of ASVS in hypoglycaemic patients with endogenous hyperinsulinemia and compare the results with literature data.

b./ Observation of the dynamics of the insulin excess.

c./ Comparison of insulin excess in patients with insulinoma and nesidioblastosis.

d./ Comparison of ASVS examination with newer diagnostic techniques based on the latest available literature.

e./ Monitoring the complication rate of ASVS.

f./ Follow-up of patients after the examination and evaluation of treatment success.

g/ Assessing the present-day usefulness of ASVS.

FGF21 determination in a group of patients with Hashimoto's thyroiditis

To date, the relationship of FGF21 levels with metabolic and thyroid parameters has not been investigated in adult patients with Hashimoto's thyroiditis.

In our study, we set out to investigate the following:

a./ To research and evaluate the correlations between FGF21 levels and thyroid hormones (TSH, FT3, FT4) and compare the results with literature data.

b./ Search for correlations between FGF21 levels and thyroid function in patients with Hashimoto's thyroiditis.

c./ Comparison of FGF21 levels with various metabolic parameters and other factors affecting metabolism, such as BMI, triglyceride levels, LDL-C, HDL-C, hsCRP, age.

d./ To compare the metabolic parameters and FGF21 levels of an adequately substituated with levothyroxin Hashimoto's thyroiditis patient group and a control group with no thyroid disorders, to evaluate results and draw conclusions.

e./ Based on the results, to search for new therapeutic options in the prevention and treatment of comorbidities associated with metabolic disorders in Hashimoto's thyroiditis.

Patient data and methods

Patient data and methods in the ASVS examination of our patients with endogenous hyperinsulinemic hypoglycaemia

Patient groups

We retrospectively analysed the data and outcomes of 9 patients treated at the Department of Internal Medicine, Endocrinology Division, University of Debrecen between 2006 and 2021. All patients included in the study were showing insulinoma-type symptoms and the diagnosis was confirmed by the necessary laboratory tests. Hypoglycaemia induced by endogenous hyperinsulinaemia was confirmed by elevated insulin and C-peptide levels (insulin >4 μU/ml, C-peptide >0.2 nmol/l) measured during a documented hypoglycaemic episode (blood glucose <2.5 mmol/l) with clinical symptoms during a 72-h fasting test.

The mean age of the patients was 45 ± 25 years with a male predominance ratio of 7:2. We included patients where conventional imaging methods such as abdominal ultrasound, CT or MRI scans failed to find an underlying cause of hyperinsulinemia. Patients with a clear history of iatrogenic hypoglycaemia caused by antidiabetic drugs or with a history of diabetes mellitus were excluded.

Implementation and evaluation of the ASVS examination

During selective angiography, after the puncture of the right femoral artery, the bigger arteries supplying the pancreas were catheterized separately, one after the other; first the gastroduodenal artery, then the superior mesenteric artery, and finally the splenic artery. 4 ml of 10% calcium gluconate solution was injected into each artery. The sampling catheter was introduced through the right femoral vein into the right hepatic vein.

Before and 30, 60 and 120 seconds after selective calcium stimulation of the arteries supplying the examined pancreatic region, samples were taken via a catheter from the right hepatic vein. We used the the highest measured insulin level from the samples for comparison. An increase of more than 1.5-fold from baseline was considered as significant, which also confirmed the location of the insulinoma in the pancreas.

Insulin levels were measured from blood samples by the Institute of Laboratory Medicine, University of Debrecen using the chemiluminescent immunoassay (CLIA) technique with a Liaison XL analyzer (Diasorin Inc, Stillwater, MN, USA).

Analysis of FGF21 levels in patients treated with Hashimoto's thyroiditis

Patient groups

We examined the potential link between serum FGF21 levels and lipid and thyroid parameters in our patients with Hashimoto's thyroiditis.

80 patients with HT of Caucasian race (75 women and 5 men; mean age 47 ± 13 years, mean duration of disease 6.7 ± 4.5 years) and 82 Caucasian controls (n=82, 76 women and 6 men, mean age 46 ± 14 years) were included in the study by the endocrinology practice of Department of Endocrinology, Institute of Internal Medicine, University of Debrecen, Hungary.

The group of patients with Hashimoto's thyroiditis received adequate doses of levothyroxine substitution for hypothyroidism, with an average daily thyroxine dose of 1.16 μg/t per body weight kg (interquartile range, IQR: 0.85-1.47). Pregnant women, patients with malignancies or diabetes mellitus and other autoimmune diseases were excluded.

Members of the control group were matched for age, sex and BMI, had no history of thyroid disease, and had their TSH, fT4, and fT3 levels within the normal range. The study met the criteria of the ethics committe. All patients and control group members consented to the study.

Blood collection techniques and methods for evaluating the results

Venous blood samples were collected into Vacutainer® serum separation tubes and EDTA-anticoagulated tubes (BD-Belliver Industrial Estate, Belliver Way, Roborough, Plymouth PL6 7BP, UK) and centrifuged after one hour of coagulation, in compliance with the local clinical protocol. Plasma and serum were separated by centrifugation at 2200×g for 10 minutes, aliquoted and stored at -80 °C for later analysis.

Serum FGF21 levels were measured by enzyme-linked immunosorbent assay (ELISA) in compliance with manufacturer's instructions (Fibroblast Growth Factor 21 Human ELISA Kit, BioVendor Laboratorni Medicina a.s., Brno, Czech Republic). Serum thyroid hormone levels (free thyroxine-fT4, free triiodothyronine-fT3) and thyroid-stimulating hormone (TSH) levels were determined by electrochemiluminescence immunoassay (FT4 G2 Elecsys, FT3 Elecsys, TSH Elecsys, Roche Diagnostics GmbH, Mannheim, Germany). Reference values for fT4 were 12-22 pmol/L, for fT3 2.4-6.3 pmol/L and for TSH 0.3-4.2 mU/L. Concentration of thyroid peroxidase autoantibody (aTPO) was measured by chemiluminescence immunoassay (LIAISON®-Anti-TPO, DiaSorin S.p.A., Saluggia, Italy). Serum levels of high-sensitivity C-reactive protein (hsCRP) were determined

by immunoturbidimetry; triglyceride, total cholesterol, low-density lipoprotein cholesterol (LDL-C), non-high-density lipoprotein cholesterol (non-HDL-C) and high-density lipoprotein cholesterol (HDL-C) were measured by enzymatic colorimetric assays; glucose was determined by hexokinase kinetic enzymatic assay using a Cobas c600 automated analyser (Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analyses

Statistical analyses were performed by using STATISTICA v.14 software, and graphs were created using Graphpad Prism v.8. The distribution of continuous variables was checked with Kolmogorov-Smirnov test. For comparisons between groups, Student's two-sample t-test was used with normally distributed data, and results are presented as mean ± standard deviation (SD). Data with asymmetric distribution were compared using the Mann-Whitney U-test, where the median and the 25th and 75th percentiles were reported. A Chi-square test was used to assess the relationship between categorical variables. Pearson correlation was used to analyze the relationships between continuous variables, and non-normally distributed variables were transformed for correlation using logarithm transformation. Multiple linear regression was performed using the logarithm of FGF21 as dependent variable in HT and control groups separately. The variables correlating with FGF21 in each group were included in the multiple regression models based on univariate analysis. P values below 0.05 were considered statistically significant.

Results

Our results after ASVS examination

In our retrospective study, 9 patients with endogenous hyperinsulinemic hypoglycemia underwent selective calcium stimulation angiography (ASVS).

Conventional imaging techniques such as abdominal ultrasound, CT and MRI scans did not find any suspected neuroendocrine tumour in the pancreas in any of the patients. 2 patients also underwent octreotide scintigraphy with negative results. As our study covered a relatively long period of time, no endoscopic US (EUS) was performed as this kind of technique was not available in our institution at that time.

In five patients, the exact source of the significant insulin excess was identified by comparing insulin levels in blood samples taken from the hepatic vein. In most cases, the measure of insulin levels reached their peak concentration as early as 30 seconds after calcium stimulation, then a slow downward trend could be observed from the measurements in the later samples.

Clinical symptoms ceased and laboratory abnormalities resolved after surgery in 4 of the patients. We lost one patient with severe comorbidities due to postoperative complications. Histopathological examination and local lymph node metastases confirmed malignant insulinoma in this case. In the remaining 4 patients, blood samples taken from the right hepatic vein showed nearly equally elevated insulin levels after selective calcium stimulation of the different major arteries supplying pancreas. This finding is suggestive of a pathology associated with the hyperplasia of pancreatic beta cells, called nesidioblastosis. In these cases, no surgery was performed, except in one case of partial pancreatectomy followed by drug treatment. Clinical symptoms resolved with a combined drug therapy of calcium antagonists and diazoxide.

In all of our patients with insulinoma, selective calcium stimulation angiography (ASVS) proved to be of diagnostic value and a great help in the more accurate localization of the insulinoma before surgery, and also provided useful information on neuroendocrine tumors hormone production capacity.

Our results after measuring FGF21 levels

Measured serum FGF21 levels were significantly lower in the Hashimoto's thyroiditis patient group than in the age- and BMI-matched control group. Levels of TSH and Ft4 were higher in the HT group, while Ft3 levels were lower compared to the control group.

The serum hs-CRP, total cholesterol, LDL-C, HDL-C and triglyceride levels showed no significant difference between the two groups, while blood glucose levels were slightly higher in the HT group, but overall, the aforementioned laboratory parameters all fell within the normal range**.**

FGF21 showed a significant positive correlation with age, and the levels of triglyceride, total cholesterol, and LDL-C in both study populations. In the HT patient group, FGF21 was positively correlated with BMI and negatively correlated with HDL-C. In addition, FGF21

showed significant correlations with fT4, TSH and hsCRP, while no clear correlation was found between FGF21 and BMI in healthy subjects. Furthermore, no significant correlation was found between thyroid function (ft4 and TSH) and FGF21 levels in the Hashimoto's thyroiditis patient group.

The correlations between FGF21 and the other researched parameters were further analysed using multiple linear regression analysis. In the HT patient group, the model included age, BMI, triglyceride, LDL-C, and HDL-C values. According to this analysis, LDL-C level was the best predictor of the expected serum FGF21 level (standardized $\beta = 0.225$ (0.109); $p = 0.043$) in the HT patient group. Furthermore, another model including age, TSH, FT4, triglyceride, LDL-C, and hsCRP values found ft4 to be the strongest independent predictor of FGF21 level in the control group (standardized $\beta = -0.270$ (0.120); $p = 0.027$).

Discussion

Insulinoma is the most common functional neuroendocrine tumour of the pancreas and the most common cause of hypoglycaemia associated with endogenous hyperinsulinaemia.

Preventing neurological damage is key, so efforts should be made to localise the insulinoma as soon as possible. In most cases, surgical removal is curative, and preoperative localisation is very important to facilitate surgery, however, as most such tumours are less than 2 cm in diameter, their accurate localisation is often difficult. The rate of recovery from insulinoma after surgical resolution is excellent, with a 5-year disease-free survival rate of 100% after surgery, thanks to the relative rarity of these malignant tumours.

Nesidioblastosis is characterized by the hyperplasia and hypertrophy of the beta cells of the pancreas. Except in extremely rare focal cases, which can be treated surgically, most cases are treated with medication. The hyperplasia of pancreatic islet cells is often difficult to confirm with conventional imaging techniques, and ASVS is an extremely useful diagnostic technique in these cases as well. Following selective calcium stimulation of the arteries supplying the given pancreatic region, the rate of insulin elevation measured in blood samples from the right hepatic vein during ASVS is significantly higher in insulinoma than in nesidioblastosis. The rise in insulin levels was very similar in our two cases of nesidioblastosis.

Endoscopic ultrasound technique (EUS) is a relatively new procedure. It is a minimally invasive technique capable of identifying tumour lesions of less than 2 cm in diameter with high sensitivity and specificity. However, its sensitivity can vary from 40% to 92.6% depending on the pancreatic location of the tumour. Furthermore, the effectiveness of the examination is strongly influenced by the expertise of the examining physician, and is not suitable for the detection of distant metastases. In addition, the lack of hyperechogenic lesions significantly limits the usefulness of this examination in nesidioblastosis cases.

ASVS also provides information about the hormonal activity of the lesion, helping in localising it more accurately, which is essential for the success of the surgical intervention, thus significantly reducing the number of reoperations.

In our study, a diffuse insulin outflow could be detected by ASVS in 4 out of 9 patients. In these patients who were considered to be nesidioblastosis cases, medication improved their condition and surgical intervention could be avoided, except in one case who became asymptomatic after drug administration following the surgery.

In a study published by Morera et al, ASVS showed a sensitivity of 90.9% in tumour localisation, the sensitivity of the ASVS technique was close to that of a combination of intraoperative palpation and intraoperative ultrasound (IOUS).

Although octreotide scintigraphy is reported to have a sensitivity of 47-60%, the octreotide scintigraphy available in our centre could not detect the actual insulinoma in either of our two cases, probably because of the low expression of SSTR2.

Several studies of 10-20 patients have reported an overall sensitivity of around 90% for ASVS. A large, comprehensive meta-analysis of 339 patient outcomes found 93% sensitivity and 86% specificity .

Cases where no solid abnormality was found by conventional imaging and similarly elevated insulin levels were detected by ASVS after selective calcium stimulation of more than one pancreas artery were considered to be nesidioblastosis. No tumour was found in these patients during surgical exploration either. Furthermore, after conservative medication treatment, hypoglycemic incidents resolved, which also supported the diagnosis of nesidioblastosis.

Of course, this procedure also has its limitations and risks. Due to the invasive nature of the procedure, several mechanical complications associated with punctuation may occur, such

as damage to the vessel wall, haemorrhage, haematoma formation. In addition, false negative and false positive results may occur due to technical errors or anatomical variations in the blood vessels, although the overall occurrence of complications is very low with experienced operators and well-equipped centres. In a study of data from 17 patients, no complications were reported after ASVS. This correlates well with our findings, as none of our 9 patients had complications during or after the procedure.

Newer imaging techniques have recently emerged, such as octreotide scans using somatostatin receptors, 68Ga-DOTATATE positron emission tomography (PET) or 68Ga-DOTATOC PET, which have significantly improved the detection, tracking and follow-up of neuroendocrine tumours.

A recent study showed that Exendin-4 PET/CT was more effective than both 68Ga-DOTATATE PET/CT and 18F-FDG PET/CT in localising insulinomas, especially in small, G2-type tumours. Exendin-4 is a molecular marker targeting the glucagon-like peptide-1 receptor (GLP-1R), which is expressed in highest amounts on beta islet cells of insulinomas, and therefore has a very high sensitivity and specificity for identifying insulinoma. Its sensitivity can go as high as 97.7%, which is superior to any other technique. 68Ga-NOTA-exendin-4 PET/CT is currently the most sensitive imaging technique for preoperative localisation of insulinomas with a sensitivity of 97.7%.

However, these newer methods also have limitations in detecting insulinomas. The expression of GLP-1R in nesidioblastosis is higher than in normal pancreatic tissue but lower than in insulinoma, which can also be a limitation of this method.

Molecular imaging is an emerging and promising imaging technique for the detection of insulinomas, but a significant proportion of insulinomas do not express somatostatin receptors. Overexpression of GLP-1R is observed in 93% of insulinoma cases, in which cases the GLP-1 PET/CT hit rate is consequently much higher. However, GLP-1R overexpression in malignant or metastatic cases is only 36%, making this technique less informative in these much rarer cases with a significantly worse prognosis. In addition, metastatic inulinomas often lack GLP-1 receptor expression and often show SST2 receptor overexpression, SRS scan detection rate has a 73% in these cases. In a systematic review of 6222 cases between 1960 and 2011, ASVS found the exact location of the tumour in 84.7% of patients with insulinoma, with an average sensitivity of 89.2%.

ASVS may also provide additional functional information in cases with MEN-1 syndrome where multiple neuroendocrine tumours are present in the pancreas and a distinction between hormone-producing and non hormone-producing tumours is required.

There are different views on the most appropriate method to localise the tumour; the diagnostic procedures available vary from centre to centre and the success can depend greatly on the competence and experience of the centre.

Our institute is a regional centre for neuroendocrine tumours, which explains the high rate of localisation of insulinomas, and additionally, the recognition and successful treatment of cases with nesidioblastosis. This confirms the importance of treating rare neuroendocrine tumours, such as insulinoma, in centres, to avoid pancreatic insufficiency resulting from subsequent endocrine and exocrine dysfunction and to promote postoperative recovery. Currently, the best option is pancreas-sparing surgery, for which knowing the exact location of the tumour is essential.

The most recent European Neuroendocrine Tumor Society (ENETS) recommendation suggests that minimally invasive surgery is recommended for the first-line treatment of insulinomas which were successfully localized preoperatively; based on the reported results, laparoscopic procedures are considered to be particularly safe and effective .

The number of cases of nesidioblastosis is constantly increasing, we also identified a relatively high number of cases of nesidioblastosis among our hyperinsulinemic patients. Surgical intervention is ineffective in nesidioblastosis and after surgery complete remission is unattainable. Preoperative screening is essential to avoid unnecessary surgeries.

Diazoxide reduces insulin secretion and increases glycolysis through its indirect action on beta cells. The administration of calcium-antagonists can also be effective in many cases. Long-acting somatostatin analogues (SSAs: octreotide, lanreotide and pasireotide) may protect against hypoglycaemia in cases where insulinoma tumour cells express somatostatin receptor subtype 2. In malignant, metastatic insulinoma cases, a combination of peptide receptor radionuclide therapy (PRRT) with 177Lu-DOTATATE and everolimus may be considered, especially in more progressive cases where hypoglycaemia is refractory to somatostatin analogue treatment.

The possible connection between FGF21 and thyroid function has not yet been studied in detail.

In a previous study, Bonde and his team found normal FGF21 levels in the samples taken from 20 hyperthyroid patients. Xiao et al. observed elevated FGF21 levels in a large population of patients with Graves-Basedow's disease with manifest hyperthyroidism, which started to decline after reaching euthyroid status. They established an independent association of FGF21 levels with hyperthyroidism. FT3 and FT4 values in hyperthyroid patients showed a clear correlation with FGF21 levels. In another study, Bande and his team found similarly elevated levels of FGF21 in hyperthyroidism. In a study led by Lee, euthyroid individuals were compared to individuals with subclinical and manifest hypothyroidism, and elevated FGF21 levels were found in hypothyroid patients, regardless of BMI values or lipid- or carbohydrate metabolism. Wang and his colleagues reported conflicting results in subclinical and manifest hypothyroidism. A decrease in FGF21 levels was found in hypothyroidism, followed by a clear increase after levothyroxine substitution. Changes in FGF21 levels correlated well with elevations in FT3 and FT4 levels. Fu and their team found no association between FGF21 and TSH levels in healthy subjects. They also found no significant difference in FGF21 levels between groups with and without anti-thyroid antibodies.

The conflicting data on the association between FGF21 and thyroid function may also be due to liver injury-induced FGF21 elevation, which is often attributed to hyperthyroidism, as published by Xiao and his team.

FGF21 levels may also be elevated in subclinical hypothyroidism, which can be explained by an adaptive mechanism related to the associated obesity. The contradictory results may be explained by different inclusion criteria, different ages of patients, different TSH cut-off values, different technical designs or different FGF21 ELISA kits used. However there is not much literature data available, as only a small number of studies have addressed the possible relationship between FGF21 and thyroid function so far.

In our study, we aimed to select a group of HT patients that is representative of a real-life HT patient population substituted with levothyroxine. Our study patients and the control group were matched for known metabolically impairing parameters such as age and BMI.

In our study, we found significantly lower serum FGF21 levels in the HT patient group compared to the age- and BMI matched control group. Our results are consistent with the results of Wang and his team who found lower FGF21 levels in manifest hypothyroidism.

To our knowledge, we are the first to investigate in detail the relationship between thyroid function and FGF21 levels in a cohort of adult HT patients.

We found a positive correlation between BMI and FGF21 levels in the HT patient group, furthermore, a positive correlation was found between TSH and FGF21 serum levels and, inversely, between Ft4 and FGF21 levels in the control group, however no such correlations were found in the HT patient group.

The differences observed between the thyroid function and FGF21 levels suggest that there may still be some difference between Hashimoto's thyroiditis patients in the state of euthyroidism, adequately substituted with levothyroxine, and healthy individuals, namely in the effect of thyroid hormone on their metabolism, which may contribute significantly to the observed metabolic abnormalities and the development of obesity in HT patients.

Our statistical analysis showed that LDL-C was the predictor of expected FGF21 levels in HT patients, whereas FT4 levels were the best predictor of FGF21 in the control group.

These results also supports the theory that the regulatory function of thyroid hormone is impaired in patients with HT. Our results suggest that, despite adequate levothyroxine substitution, a malfunction of metabolic control mechanisms may persist in patients with HT.

Since no significant correlation was found between FGF21 and FT3 levels, supplementing the levothyroxine substitution with triiodothyronine would probably not help the FGF21-related metabolic issues in these patients. The usefulness of this treatment is further limited by the short half-life of DIO2 enzyme, which is further reduced in the presence of its physiological substrate, T4. Our results may suggest a dysfunction between the thyroid gland and the adipokine regulation of the nucleus arcuatus, in which FGF21 may be playing a role.

FGF21 may also be associated with other thyroid diseases, as higher FGF21 levels have already been associated with the aggressiveness of papillary thyroid cancer and may also predict the emergence of orbitopathy in Graves' disease.

Our results may explain, albeit only partially, the cause of the clinical observation that we can only achieve modest success in weight reduction or lipid reduction even in adequately substituted Hashimoto's thyroiditis patients. Therefore, new strategies might be necessary to reduce metabolic risk, specifically designed for this patient population.

Because of the proven metabolic benefits of FGF21, there have been several attempts for its therapeutic use, particularly in obesity-related comorbidities such as type 2 diabetes and dyslipidemias. Unfortunately, native FGF21 is not suitable for therapeutic use due to its short half-life and its proteolytic cleavage by serum proteases. Long-acting analogues coupled to

polyethylene glycol (PEG) polymers or immunoglobulins or even FGF21 receptor agonists, including bispecific monoclonal antibodies that bind to the FGFR1-KLB complex or ultimately avymers that activate FGFR1 and KLB may be the subjects for future drug development in this direction.

Nowadays, glucagon-like peptide-1 receptor agonists (GLP-1RA), which are also well known for their beneficial lipid-lowering effects, have become the first-line of treatment for achieving persistent weight loss. However, these agents only take FGF21-lowering effects in combination with exercise. The complex metabolic effects of GLP-1RA therapy should be investigated in Hashimoto's thyroiditis patient group to map the exact effects of GLP-1RA treatment in this patient population.

It should also be mentioned that one limitation of our study is the relatively low number of patients.

The evaluation of FGF21 levels in patients with Hashimoto's thyroiditis combined with obesity, metabolic syndrome and cardiovascular disease is particularly important in order to clarify the impact of lower FGF21 levels on the incidence of the above mentioned comorbidities.

In the future, it would be worthwhile to study HT patients who do not have manifested metabolic disorders or cardiovascular co-morbidities yet, in order to explore the role of FGF21 in the development of various cardiovascular diseases and metabolic abnormalities.

Our HT patients have been followed and treated for many years, which is definitely one of the strengths of our study.

Summary

Although ASVS has been somewhat overshadowed recently, it still has its role in the diagnosis of pancreatic neuroendocrine tumours, even besides the newer, emerging and promising molecular imaging techniques. The main advantage of this examination method is that, in addition to the morphological picture, it provides very useful functional information on the hormone production of the lesions. Abnormally elevated insulin levels following the selective calcium stimulation of each major arteries supplying the pancreas can be used to

localise the origin of insulin excess. With this additional functional information, we can differentiate between the localized insulinoma cases and nesidioblastosis cases. This is of paramount importance for planning the therapy, as surgery is curative in insulinoma cases, whereas in nesidioblastosis, as shown in one of our patients, surgery wasn't curative, in which case medication is the proper therapeutic step which causes regression in clinical symptoms.

In our study, in patients with endogenous hyperinsulinemic hypoglycemia where conventional imaging techniques failed to clarify the etiology, ASVS confirmed the origin of insulin excess in all patients. Benign insulinoma was confirmed in 4 patients, malignant insulinoma in 1 patient and nesidioblastosis in the remaining 4 patients. This 100% finding rate correlates well with literature data, where finding rates of 90-100% were reported for most studies. In most cases, the peak of insulin flux was observed in the 30 s post-stimulation sample and the rate of insulin flux was significantly higher in insulinoma cases than in nesidioblastosis cases. These results correspond well to literature data. Cases with benign insulinoma got asymptomatic after surgery. We lost our patient with malignant insulinoma due to postoperative complications. Of the cases with nesidioblastosis, 3 patients did not undergo surgery and became asymptomatic with conservative therapy, while one patient underwent surgery but still had no symptomatic remission, and then became asymptomatic with medication. No complications occurred during our study. Although the number of patients included in the study was relatively low, which is a limitation of the study, our results suggest that ASVS is still an effective diagnostic technique in cases where non-invasive imaging techniques fail to find the origin of insulin excess. This is also in line with the recent ENETS recommendation. A limitation of our study is that many of the currently available testing procedures (EUS, IOUS, various radionuclide procedures) were not used or were used only partially and therefore no comprehensive comparison of these procedures has been concluded. Accurate localisation of insulinomas is essential for successful surgical intervention leading to a complete cure. ASVS remains a reliable tool for localising insulinomas, providing additional useful functional information about the nature of the tumour which are not delivered or only partially by other newer, more costly, difficult to obtain imaging techniques.

In our further study, we investigated the possible role of FGF21 in a group of HT patients. FGF21 is a peptide hormone of particular importance in the regulation of metabolism and body weight. It has a number of beneficial effects on several organs, a positive effect on metabolism through complex regulatory mechanisms and, in general, provides protection against fatty liver disease, atherosclerosis, obesity, and thus against cardiovascular diseases which constitute the

diseases with the highest mortality in our days. Its elevated serum levels, presumably due to a depleting compensatory mechanism, correlate well with various metabolic disorders and diseases, and thus FGF21 levels may be predictive of the development of diabetes mellitus, for instance.

However, the relationship between FGF21 levels and thyroid hormones reported in animal experiments has not yet been clearly demonstrated in human studies, and the small number of studies published are reporting data which are often conflicting. To date, the relationship of serum FGF21 with various metabolic parameters and thyroid function in adult patients with Hashimoto's thyroiditis has not been investigated.

In our results, FGF21 levels were significantly lower in the HT patient group compared to the control group. However, we have found no significant correlation between FGF21 levels and thyroid function in HT patients, which we attribute generally to the change of metabolic risk.

A positive correlation has been found between FGF21 levels and BMI in the HT patient group. Our results suggest that the observed correlations between FGF21 levels and thyroid function are different in the two groups studied, suggesting that despite adequate thyroxine substitution, thyroid hormone regulation is impaired in patients with HT. Our results suggest that, despite adequately substituted HT, metabolic abnormalities leading to obesity and various metabolic and cardiovascular diseases may persist despite the treatment.

Our results confirms the theory that triiodothyronine treatment is usually not effective. Further examination of FGF21 and other metabolites and their role in the regulation of metabolism is necessary. Further drug development trials are expected in the future.

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List of publications related to the dissertation

1. Berta, E., **Halmi, S.**, Molnár, I., Hutkai, D., Csiha, S., Bhattoa, H. P., Lőrincz, H., Somodi, S., Katkó, M., Harangi, M., Paragh, G., Nagy, E. V., Bodor, M.: Low Serum Fibroblast Growth Factor 21 Level and Its Altered Regulation by Thyroid Hormones in Patients with Hashimoto's Thyroiditis on Levothyroxine Substitution.
Metabolites. 14 (10), 1-10, 2024.
DOI: http://dx.doi.org/10.3390/metabo14100565
IF: 3.4 (2023)

2. **Halmi, S.**, Berta, E., Diószegi, Á., Sira, L., Fülöp, P., Nagy, E. V., Győry, F., Kanyári, Z., Tóth, J., Bhattoa, H. P., Bodor, M.: Single center experience in localization of insulinoma by selective intraarterial calcium stimulation angiography: a case series of 15 years.
Front Endocrinol (Lausanne). 15, 1-7, 2024.
DOI: http://dx.doi.org/10.3389/fendo.2024.1305958
IF: 3.9 (2023)

List of other publications

3. Csiha, S., Molnár, I., **Halmi, S.**, Hutkai, D., Lőrincz, H., Somodi, S., Katkó, M., Harangi, M., Paragh, G., Nagy, E. V., Berta, E., Bodor, M.: Advanced glycation end products and their soluble receptor (sRAGE) in patients with Hashimoto's thyroiditis on levothyroxine substitution.
Front. Endocrinol. 14, 1-9, 2023.
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IF: 3.9

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4. Gál, K., Veres, K., **Halmi, S.**, Bozoki-Beke, K., Fekete, K., Homoki, J., Gálné Remenyik, J., Baráth, B., Varga, Á., Németh, N., Soltész, P.: The effect of rheopheresis treatment on the cytokine profile in diabetic foot syndrome with hyperviscosity in the aspect of clinical changes: a preliminary study.
Clin. Hemorheol. Microcirc. 80 (2), 117-125, 2022.
DOI: http://dx.doi.org/10.3233/CH-211188
IF: 2.1

5. Szilágyi, B., Fejes, Z., Rusznyák, Á., Fenyvesi, F., Pócsi, M., **Halmi, S.**, Griger, Z., Kunapuli, S. P., Kappelmayer, J., Nagy, B. J.: Platelet Microparticles Enriched in miR-223 Reduce ICAM-1-Dependent Vascular Inflammation in Septic Conditions.
Front. Physiol. 12, 1-14, 2021.
DOI: http://dx.doi.org/10.3389/fphys.2021.658524
IF: 4.755

6. Berta, E., Lengyel, I., **Halmi, S.**, Zrínyi, M., Erdei, A., Harangi, M., Páll, D., Nagy, E. V., Bodor, M.: Hypertension in thyroid disorders.
Front Endocrinol (Lausanne). 10, 1-11, 2019.
DOI: http://dx.doi.org/10.3389/fendo.2019.00482
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