ISSN 1517-6932
ISSN ON LINE 2447-181X

# ENDOCRINOLOGIA & DIABETES CLÍNICA E EXPERIMENTAL

FACULDADE EVANGÉLICA MACKENZIE DO PARANÁ (FEMPAR)
HOSPITAL UNIVERSITÁRIO EVANGÉLICO MACKENZIE DE CURITIBA

VOL. 19 - NUMBER 4 JAN/FEB/MAR/APR.2023

EDITORIAL

# *Will actor Chris Hemsworth ("Thor") really have dementia from Alzheimer disease?*

## Chris Hemsworth Discovers Genetic Propensity

When the 39-year-old Australian actor, Chris Hemsworth, best known in the movies for the role of “Thor”, announced in December 2022, a break in his career, the world was perplexed and shocked. The reason, according to him, was that the doctors found that he carries two copies of the ApoE4 gene, one inherited from his mother and the other one from his father, which renders him eight to ten times more likely to develop Alzheimer's disease than people that do not carry both of the copies of the aforementioned gene.

The actor emphasized that he was not diagnosed with Alzheimer's, but he was warned about his genetic propensity, so he decided to stop filming to dedicate himself to his family and take the preventive measures against this terrible and incurable disease.

This case comes to rekindle the discussion on how to prevent and what to do in the face of this disastrous disease, that causes so much harm to its bearer and also to their family members.

The major concern is knowing that there is a demented person in the world every three seconds and that this number almost doubles every 20 years, as this is a degenerative, progressive disease, with accentuated cognitive deficits, personality changes, and that it significantly interferes with the personal, social and work activities, turning the patient into, in a more advanced stage, totally dependent on a 24-hour care.

That are several types of dementia, with Alzheimer's disease being the most prevalent type, usually more than 50% of all of them. Other common types are: vascular (8%), fronto- temporal (6%), combined (10%), potential reversible (8%) and others such as Lewy corpuscles and Parkinson's disease, in fewer percentages, but no less harmful and with similar symptoms.

Science is incessantly looking for a drug or vaccine that can cure or prevent this awful disease and unfortunately, it hasn't succeeded yet. After many frustrating and costly attempts, what we have so far, is a small group of medications, which work symptomatically, and may even give the patient a longer period of lucidity, but not leading to a cure. So, the most important thing we can really do is to avoid risk factors and follow preventive measures.

We can list the most important risk factors for dementia, which are:

a) Non-modifiable: age and family background.

b) Modifiable: diabetes, hypertension, obesity, physical inactivity, smoking, depression, low education, hearing loss (deafness), excessive alcohol consumption, head trauma, environmental pollution, social and family isolation.

The most important preventive measures are: physical exercises, cognitive reserve (neuroplasticity), education, constant mental activity with new learning, social interaction, avoiding isolation and good nutrition.

The unanswered question is: *Is there any test that can detect early, with certainty, if the person will have the dementia of Alzheimer's Disease*?

Even though this disease may begin decades before its initial manifestations, with the gradual deposition of a protein called beta amyloid that starts to form plaques in the brain and gradually destroys synapses and neurons, the answer is that, unfortunately, there is still no test capable of show with absolute accuracy that the person will have this type of dementia. The diagnosis is essentially clinical, but a series of routine blood tests can initially be performed, such as blood glucose, complete blood count, vitamin B12, cholesterol, kidney, liver and thyroid functions, which can also rule out other possible causes of potentially reversible dementia.

In further research, series of tests may point to possible signs of this disease; such potential Alzheimer´s markers or biomarkers are:

1) Biomarkers in the CSF (cerebrospinal fluid), for the detection of Beta Amyloid, Total Tau and Phospho Tau proteins (which are responsible in large part for Alzheimer´s);

2) Imaging markers, such as MRI (Magnetic Resonance), and others carried out in larger centers such as: SPECT, FDG-PET, PIB-PET and a few more that are gradually being implemented in Brazil;

3) Genetic biomarkers, such as: PSEN1, PSEN2, PPA and Apolipoprotein (APO) E and its alleles (E2, E3, E4).

Despite the enormous potential for the use of biomarkers in clinical practice, this is still not a reality. None of these tests are conclusive and definitive for Alzheimer's dementia, and should only be used in research, mainly due to the lack of standardization for CSF dosages, with false positive and false negative results. Studies show that these types of tests generate more concern and anxiety than benefits. If the patient has any memory lapse, he may think that he is already developing this fateful disease, which can lead to depression.

For Hemsworth, the fact of being an APO E4 carrier may increase the risk of the disease, but it is not a definitive diagnosis and his concern is certainly due to the fact that those who inherited only one of the copies with the E4 variant have two to three times more chance of developing Alzheimer's disease, while in his case, people who inherited both copies with the E4 variant, the risk is 10 times greater compared to the population without this gene.

The uncertainty of the APO E4 genetic mutation is also due to two important aspects: the first is that more or less 50% of people suffering from Alzheimer's do not have this gene. Therefore, although it increases the risk, it is not a condition for the development of the disease.

The second aspect is that this allele is also found in 10% to 15% of the population. The majority of people who have the allele, either one or two, will not develop the disease.

Whether Chris Hemsworth's decision to leave his glorious career at an young age was right or not is an intimate and very private matter, but he could have take into account epigenetics as the fact of being active, working, memorizing texts and interpreting is already a great protective factor against this dementia. On the other hand, what he is doing and publicizing is very important, which is to take out risk factors and always prioritize the aforementioned preventive measures, which are what we have confirmed when trying to avoid this ruthless disease.

Luiz Antonio da Silva Sá
Specialist in Internal Medicine, Geriatrics and Gerontology
Professor at Faculdade Evangélica Mackenzie do Paraná (FEMPAR) - Brazil

**References**

- World Health Organization (WHO). (20 de março, 2021) Dementia: a public health priority. W.H.O
- Krysinska K, Sachdev PS, Breitner J, Kivipelto M, Kukull W, Brodaty H. Dementia registries around the globe and their applications: A systematic review. Alzheimer's Dement 2017;13:1031-47.
- Ministério da Saúde. Alzheimer: o que é, causas, sintomas, tratamento, diagnóstico e prevenção. Disponível em: https://saude.gov.br/saude-de-a-z/alzheimer.
- Alzheimer's Association – Brasil; Alzheimer Disease International;- Associação Brasileira de Alzheimer;
- Verdullas, R. A.; Ferreira, M.; Nogueira, V. O. Dificuldades enfrentadas pelo cuidador familiar mediante o paciente com mal de Alzheimer em fase avançada. Revista Saúde Coletiva. Rio de Janeiro, v. 50, n. 8, p. 109-113, maio 2011.
- Brasil. Ministério da Saúde. Protocolo Clínico e Diretrizes Terapêuticas: doença de Alzheimer. 2014.
- Pinto, Ânderson de Vasconcelos et al. Tratamento farmacológico da doença de Alzheimer em idosos. Congresso Internacional de Envelhecimento Humano, 2017. Disponível em:http://www.editorarealize. com.br/ revistas/cieh/ trabalhos/ TRABALHO_EV075_MD2_SA3_ID1221_11092017200451.
- Alzheimer's Association (AA). (2020). 2020 Alzheimer's disease facts and figures. Alzheimer's & Dementia, 16, 391-460.
- J.L. Molinuevo et al. / Alzheimer's & Dementia - (2014) 1-10 The clinical use of cerebrospinal fluid biomarker testing for Alzheimer's disease diagnosis: A consensus paper from the Alzheimer's Biomarkers Standardization Initiative.
- The cerebrospinal fluid "Alzheimer profile": Easily said, but what does it mean? DUITS F.H DOI: https://doi.org/10.1016/j.jalz.2013.12.023.
- Mattsson N, Zetterberg H, Hansson O, Andreasen N, Parnetti L, Jonsson M et al. CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment. JAMA.2009;302(4):385-93.
- Kang, J.-H. et al. The Alzheimer's Disease Neuroimaging Initiative 2 Biomarker Core: A review of progress and plans. Alzheimer's & Dementia, v. 11, n. 7, p. 772–791, jul. 2015.
- Fridman, C. et al. Alterações genéticas na doença de Alzheimer. Archives of Clinical Psychiatry (São Paulo), v. 31, n. 1, p. 19–25, 2004.

Websites
• www.alz.org/br/demencia-alzheimer-brasil.asp
• abraz.org.br
• Alzheimer's Association
• The Alzheimer's Society
• Institute of Neurological Disorders and Stroke Alzheimer's Disease Information

# Endocrinol. diabetes clín. exp. - VOL.XIX - NUM. 4

Endocrinology & Diabetes - Clinical and Experimental is a journal of open access that publishes case reports, original article, reviews with new insights in pathogenesis, physiology and metabolism of hormone secretion, cellular mechanisms and tissue action. This journal belongs to the Discipline of Endocrinology and Metabolism of Faculdade Evangélica Mackenzie do Paraná and Service of Endocrinology and Diabetes - Diabetes Unit - Hospital Universitário Evangélico Mackenzie, Curitiba - Brazil



Endocrinologia & Diabetes Clínica e Experimental
Disciplina de Endocrinologia e Metabologia da Faculdade Evangélica Mackenzie, Serviço de Endocrinologia e Diabetes do Hospital Universitário Evangélico Mackenzie. – v.19, nº 4 – Curitiba:
FEMPAR/HUEM, 2000
p.2358- 2387: il.; 29cm

Quadrimestral
ISSN 1517-6932
ISSN on line 2447-181X

1.Endocrinologia – Periódicos. 2. Saúde – Periódicos. I. Faculdade Evangélica Mackenzie do Paraná. II. Faculdade Evangélica Mackenzie.

CDD 616.4
CDU 612.34

# Contents

**EDITORIAL**.....*2359*

**MINI REVIEW AND CASE REPORT**

**Pancreatic cancer ant type 3c diabetes**

*Pancreatic adenocarcinoma is an exocrine malignant tumor which is responsible for > 90% of all pancreatic cancers*.....*2363*

**ORIGINAL ARTICLES**

**Halogens as potential thyroid disruptors – *in sílico* simulation and mathematical model for triggering autoimmune thyroiditis.**

*The halogens are the non-metallic chemical elements belonging to group 17 of the Periodic Table, namely: fluorine, chlorine, bromine, iodine, astate, and teness*.....*2370*

**"Sonohistology": ultrasound characterization of liver tissue in the diagnosis of hepatic steatosis**

*"Sonohistology," the sonographic characterization of "sonography tissue", can be defined as the correlation between the subjective qualitative parameters of ultrasound and the objective quantitative histological parameters of liver tissue*.....*2374*

**TOPICS IN MEDICAL CLINIC**

**B12 vitamin deficiency in metformin users**

*The use of metformin in patients with type diabetes (DM) 2 is related to the development of vitamin B12 deficiency*.....*2379*

**Evaluation of feet function in axial spondyloarthritis: a cross sectional study using the foot and ankle outcome score**

*To study the foot involvement in a sample of Brazilian patients with axial spondylarthritis*.....*2382*

**Our Cover:** Thor (actor Chris Hemsworth)

**Source:** Google Images

# MINI REVIEW AND CASE REPORT
# PANCREATIC CANCER AND TYPE 3c DIABETES

## *CANCER PANCREÁTICO E DIABETES TIPO 3C*

VICTOR COIMBRA ZEQUIM*
MATHEUS SCHICK*
RICARDO RIBEIRO GAMA**
MIRNALUCI PAULINO RIBEIRO GAMA*

**Key words:** Pancreatic neoplasms Adenocarcinoma, Pancreas, Diabetes mellitus.
**Descritores:** Neoplasma de pâncreas, Adenocarcinoma, Pâncreas, Diabetes mellitus.

***Abstract***

Pancreatic adenocarcinoma (ductal and its variants) is an exocrine malignant tumor which is responsible for > 90% of all pancreatic cancers. It most commonly affects patients above 55 years old. The worldwide age-standardized annual incidence is 5.5 per 100,000 in men and 4 per 100,000 in women. Major risk factors include tobacco use, older age, genetic predisposition and family history, obesity, chronic pancreatitis, and preexisting diabetes. Activation of *KRAS* oncogene is present in > 90% of tumors; inactivation of tumor suppressor gene *CDKN2A* in > 90%, *TP53* in 70% and *SMAD4* in about 50%. The prognostic of pancreatic adenocarcinoma is very poor, with only 15-20% of patients with possible cure and resection at diagnostics, and with 5-year overall survival of less than 10%. **Endocrinol diabetes clin exp 2023 / 2363 - 2369.**

***Resumo***

O adenocarcinoma pancreático (ductal e suas variantes) é um tumor maligno exócrino responsável por > 90% de todos os cânceres pancreáticos. Afeta mais comumente pacientes acima de 55 anos. A incidência anual padronizada por idade em todo o mundo é de 5,5 por 100.000 em homens e 4 por 100,00 em mulheres. Os principais fatores de risco incluem tabagismo, idade avançada, predisposição genética e história familiar, obesidade, pancreatite crônica e diabetes preexistente. A ativação do oncogene KRAS está presente em > 90% dos tumores; inativação do gene supressor de tumor CDKN2A em > 90%, TP53 em 70% e SMAD4 em cerca de 50%. O prognóstico do adenocarcinoma pancreático é muito ruim, com apenas 15-20% dos pacientes com possível cura e ressecção no momento do diagnóstico e com sobrevida global em 5 anos inferior a 10%. **Endocrinol diabetes clin exp 2023 / 2363 - 2369.**

**INTRODUCTION**

Pancreatic cancer (PC), ductal and its variants is one of the most serious forms of human cancer and is almost always lethal, mainly due to the difficulty of an early diagnosis. The prognostic of pancreatic adenocarcinoma is very poor, with only 15-20% of patients with possible cure and resection at diagnostics, and with 5-year overall survival of less than 10%. However, its incidence is unusual, affecting 5-17 per 100,000 worldwide. CA19-9 is the only biomarker routinely used for early diagnosis with low specificity and should not be used as the only test for screening this disease. Therefore, in daily medical practice, there is no reliable test for early diagnosis (1).

Type 2 diabetes (DM2) is the most prevalent type, accounting for about 90-95% of all subtypes of DM in the whole world. DM2 is associated with a higher body mass index (BMI), insulin resistance (IR) and hyperinsulinemia. Hyperinsulinemia and obesity are associated with several types of tumors such: breast, liver, lung, endometrium, colorectal and pancreatic cancer (2,3). Diabetes associated with pancreatic disease is denominated Type 3c diabetes where both the endocrine and exocrine pancreas are involved (3). It is important to be aware that individuals with no family history of diabetes (DM), over 50 years of age with a recent diagnosis of diabetes and who become insulin dependent within 6 months, should have an abdominal imaging exam and a CA 19- 9 test.

The tumor takes up to 20 years to be diagnosed through diabetes, symptoms and image exams. Usually, hyperglycemia occurs 6 months to 3 years before diagnosis. Risk factors for PC are age greater than 60 years, male gender, cigarette smoking (potent carcinogen for PA), gene mutations, diabetes, obesity and chronic pancreatitis (3).

**CASE REPORT**

N.S., female, 81 years old, white, whose previous past history included diabetes mellitus with onset 3 years prior to this report, insulin-dependent, besides hypothyroidism, systemic arterial hypertension and unspecified hepatitis after miscarriage 50 years ago. Former smoker for 30 years with an estimated smoking load of 15 pack/years, with no history of alcoholism. The patient referred caesarean as the only previous surgery. No family history of neoplasms, but mother and brother had a diagnosis of type 2 diabetes.

She was admitted to the Hospital Universitário Evangélico Mackenzie (HUEM) in February 2023 due to generalized pruritus and jaundice seen in previous care at a Basic Health Unit.

The patient reported that she had hyporexia for more than a month, having lost 20 kg in the last three months and a week ago it would have started with generalized pruritus.

In the initial evaluation at our service, the patient was jaundiced (3+/4), with a flat, flaccid abdomen, preserved bowel sounds and pain on palpation of the hypogastrium, left iliac fossa and right hypochondrium, with a palpable mass in the right hypochondrium. Inpatient had hyperglycemia needing insulin (capilar glycemia media was fast 187mg/dl, before lunch 244mg/dl ,two hours after lunch 284mg/dl, before dinner 197 mg/dl and bed time 290mg/dl), and insulin therapy basal bolus (NPH insulin with regular insulin at breakfast, lunch and dinner with corrections) was started. She remained during hospitalization with low doses of NPH insulin in the morning (10 units) and bedtime (10units).

The patient brought previous external laboratory tests, carried out 10 days before admission to the hospital, which already showed changes in liver function (Gamma-GT: 295U/L; Alkaline Phosphatase: 793U/L; TGO: 313U/L; TGP: 491 U/L and other isolated changes such as urea (52.5mg/dL) and 9% HbA1C.

For the investigation, new laboratory tests were requested, which showed worsening of liver function : Gamma-GT 601.5U/L (RV woman 38mg/dl), Alkaline Phosphatase 1806U/L (RV 40-150U/L), oxalacetic transaminase 353;U/L (RV 5-40 U/L) pyruvic transaminase 459U/L (RV 7-56U/L), a total bilirubin of 12.21mg/dl (RV 0.2-1.20mg/dl), direct bilirubin 6 .27mg/dl (RV 0.1-0.4mg/dl)

*Department of Endocrinology and Diabetes – Diabetes Unit - Hospital Universitário Evangélico Mackenzie Curitiba - PR – Brazil
**Department of Head and Neck Surgery - Hospital do Câncer – Barretos – São Paulo - Brazil
E-mail:mirnaluci.gama@yahoo.com

and indirect bilirubin 5.94mg/dl (RV 0.1-0.7mg/dl), urea 72.3 mg/dl (RV 10-45mg/dl), urobilinogen 3+, a hyperkalemia 5.71mEq/L (RV 3.5-5.0 MEq/L), lipase 34U/L (RV <95U/L) and amylase 53.2U/L (RV <110U/L), vitamin D 8.7ng/mL (RV > 20ng/mL), phosphorus 3 .67mg/dL (RV 2,5-4,5mg/dL),magnesium 2.38mg/dL(RV 2.0 - 2.6 mg/dL),calcium 7.46mg/dL (RV 8,8-10,6mg/dL), PTH 58pg/mL (RV 12-88pg/ml), CA 19-9 less than 2.0 U/mL (RV < 37U/ml) in two dosages, CEA 12.10ng/mL (RV < 5.0ng/ml) in two dosages and alpha-fetoprotein 3.7ng/mL (RV < 8.0ng/ml).

A CT scan of the abdomen and pelvis was performed (02/23/23), which showed the following alterations: pancreas with heterogeneous expansive formation on its head, with irregular contours and imprecise limits, with heterogeneous enhancement by the contrast, with well-defined hypodense (liquefied? necrotic?) formations in between, with the largest tumor dimension measuring 3.5 centimeters and dilation of the main pancreatic duct and upstream bile ducts. Liver, spleen, kidneys and adrenal glands were normal. Hyperdistended gallbladder, without clear anomalous content. Other pancreatic portions tapered and of usual morphology. Several endophytic hypodense images scattered throughout the cortices, without contrast enhancement, the largest in the upper pole measuring 1.5 cm. Absence of lymph node enlargement and free fluid in the cavity. The diagnostic hypothesis was pancreatic head neoplasia and palliative surgical treatment was indicated. Chest tomography for staging without signs of secondary involvement in the lungs or pleura.

The patient was submitted on 03/01/2023 to biliodigestive anastomosis (choledoco-jejunum) with cholecystectomy. Anatomopathological product of cholecystectomy showed signs of chronic cholecystitis and cholesterolosis. She evolved postoperatively with a biliary fistula, with later resolution of the same. She was discharged with insulin with day blood glucose average 158mg/dl with satisfactory symptom control.

The patient returned to the emergency room on 03/28/2023 due to a complaint of abdominal pain, colic-like, worse in the hypogastrium, episodes of vomiting and hyporexia, and diarrhea with a yellowish appearance, without fever.

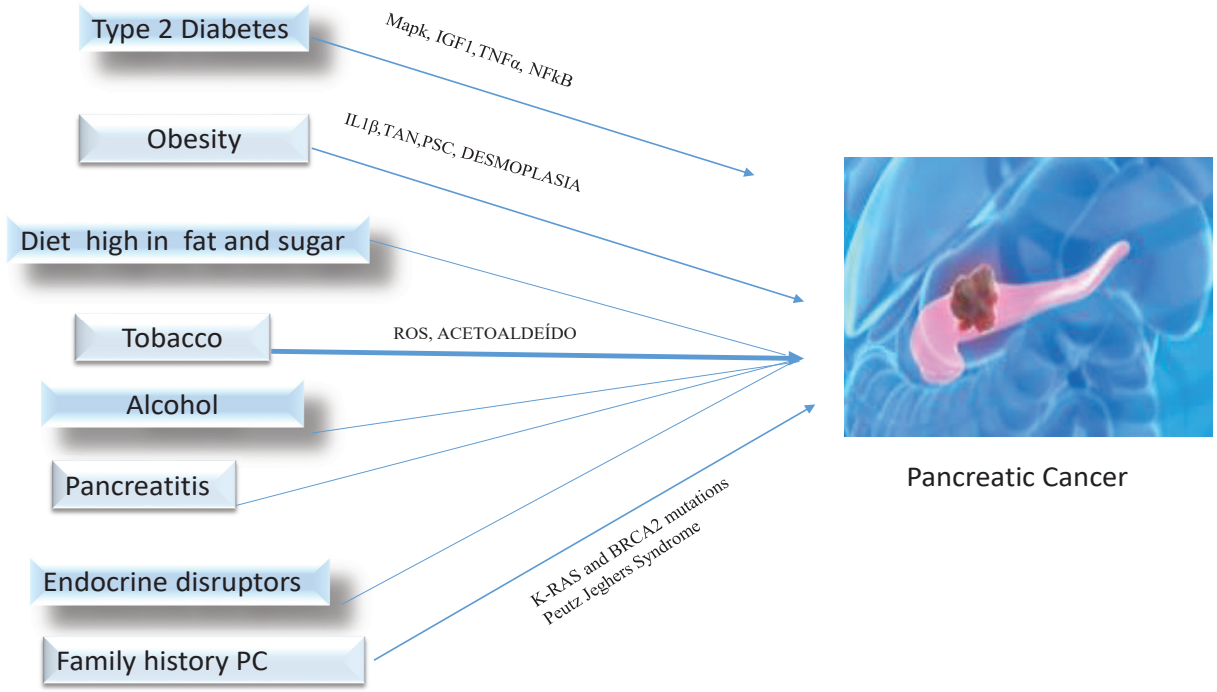
A CT abdomen and pelvis scan performed on 03/31/2023 showed signs of surgical manipulation in the right hypochondrium, noting densification of superficial soft tissues and drainage in the middle of the vesicular fossa. Pancreas showing heterogeneous expansive formation on its head, with irregular contours and imprecise limits, with heterogeneous enhancement by the contrast medium, with well-defined hypodense (liquefied/necrotic) formations in between, the largest measuring 4.0 cm. It determines dilations of the main pancreatic duct and bile ducts upstream, as well as atrophy of the rest of the pancreatic parenchyma. Suspicious aspects for primary neoplasia. Liver with normal dimensions, regular contours and preserved attenuation. Pervious portal and hepatic veins. Cholecystectomy.

The patient and family refuse further surgical treatment and chemotherapy being referred to palliative care.

| Blood tests | Reference | 10-days prior | Admission |
|---|---|---|---|
| Gamma-GT | 5-27U/L | 295U/L | 601.5U/L |
| Alkaline Phosphatase | 36-150U/L | 793U/L; | 1806U/L |
| TGO | <35U/L | 313U/L | 353U/L |
| TGP | <35U/L | 491 U/L | 459U/L |
| Urea | 16-40mg/dL | 52.5mg/dL | 72.3mg/dL |

| Blood tests | Reference | 10-days prior | Admission |
|---|---|---|---|
| HbA1c | <5,7% | 9% | - |
| Lipase | 160U/L | - | 34U/L |
| Amylase | <110U/L | - | 53.2U/L |
| CA 19-9 | <37U/mL | - | < 2.0 U/mL |
| CEA | <3ng/mL | - | 12.10ng/mL |
| Total Billirubin | 0,3-1,1mg/dL | - | 12.21mg/dL |
| Magnesium | 1,6-2,6mg/dL | - | 2.38mg/dL |
| Calcium | 8,8-10,4mg/dL | - | 7.46mg/dL |
| Vitamin D | 30-100ng/dL | - | 8.7ng/mL |
| PTH | 10-65pg/mL | - | 58pg/mL |
| Alpha-fetoprotein | <8,1ng/mL | - | 3.7ng/mL |

**Fig 1** Main risk factors and their molecular mechanisms involved in PC




MAPK: mitogen-activated protein kinase; IGF-1: insulin-like growth factor 1; IL-6: interleukin-6; TNF-α: tumor necrosis factor-alpha; VEGF: vascular endothelial growth factor; NF-κB: nuclear factor kappa B; IL-1β: interleukin-1 beta; TAN: tumor-associated neutrophil; PSC: pancreatic stellate cells; ADH1: alcohol dehydrogenase 1;ALDH2: alcohol dehydrogenase 2; ROS: reactive oxygen species, K-RAS: Kirsten rat sarcoma virus gene; BRCA2: breast cancer gene 2
Adapted ref 3

## OBESITY

Obesity is considered worldwide a pandemic disease. This is due to changes in lifestyle as physical inactivity, intake of diet high in fat and sugar associated with alcohol and tobacco. Obesity, results of heavy fatty acids mobilization that increase triglycerides storage in peripheral adipose tissue (2). The increase of triglycerides in adipocytes causes limitation of cell expansion with inflammatory reaction with secretion of the tumor necrosis factor-alpha (TNF-α), interleukins, adipocytokines, macrophage invasion or its local secretion, fibrosis and finally apoptosis of adipocytes. Thus, excess of circulating triglycerides accumulate in the omentum, liver, kidneys, pericardium, heart and pancreas. This is the visceral fat risk factor for insulin resistance, cardiovascular disease and malignant tumors (4).

## THE ROLE OF INSULIN RESISTANCE, HYPERINSULINISM AND HYPERGLYCEMIA IN THE DEVELOPMENT OF PC

Hyperinsulinism is responsible for stimulating insulin-like growth factor receptor (IGF1) via serine phosphorylation of insulin receptor substrates (IRS1, IRS2), activating intracellular targets through mammalian target of rapamycin and phosphatidyl--inositol-3 kinase (mTOR /PI3), Studies have shown the role of hyperinsulinism, by activating IGF1 receptors, in tumor growth, malignancy and metastases (5). Hyperinsulinism increase IGFI by inhibition of hepatic production of IGF1 binding proteins (IGF1BPs). Insulin itself is mitogenic and its receptors are highly responsive to cell growth factors. Cancer cells are avid for glucose which confirms tumor growth during hyperglycemia, however the involvement of defined tumor genes is still unknow (3).

## ADIPONECTIN AND LEPTIN: THE ADIPOCYTOKINES OF ADIPOSE TISSUE

### *Adiponectin*

Adiponectin (AdipoQ) protects the beta cell from apoptosis with increased insulin secretion and gene expression. AdipoQ is decreased in obesity and diabetes. It binds to receptor (AdipoR) activating intracellular AKT, MAPK and AMPK pathways. It takes action on several tissues to control energy homeostasis and insulin sensitivity regulating carbohydrate and lipid metabolism through the adenosine monophosphate -activated protein kinase (AMPK) pathway (5). The inhibitory action of AdipoQ on cell proliferation is achieved by decreasing AKT and beta catenin levels on breast, colon and prostate tumors (5,6).

The molecular mechanism by which AdipoQ levels inhibit tumor progression is still unknown; probably its action is achieved by several mechanisms:

a- Action via phosphorylation of insulin receptors, which down-regulates insulin/IGF-1 signaling;

b- Decrease of inflammatory expression of cytokines that inhibit NF-κB activation;

c- Action in AMPK pathway to activate p53 tumor suppressor gene;

d- Promoting cancer cell apoptosis via peroxisome proliferator-activated receptor gamma (PPARγ) activation and inhibiting angiogenesis (5,7,8,9).

It is possible that the tumor-suppressing role of adiponectin in PC is by inhibiting cell proliferation and inducing apoptosis (5,10). Based on these observations, we can expect an adiponectin agonist drug as a possible therapy for PC (11).

### *Leptin*

The first adipokine detected in 1993 in the white adipose tissue was named Leptin. It controls food intake, energy expenditure via activation of the satiety center and inhibition of the hunger center, both located in the hypothalamus. Its full action depends on the size of the adipose tissue (5,12). Leptin with

hypoxia inducible factor- 1 (HIF1) are over expressed in tumor pancreatic cells. The leptin receptor also regulates its own expression through hypoxia inducible factor-1, resulting in pancreatic cancer cell survival, being this an unknown mechanism (9). The positive feedback between leptin and HF1, activates JAK2/STAT3 pathway, which leads to matrix metalloproteinase-13 activation and pancreatic cancer metastasis. The association LEP-HIF1 is responsible for poor prognosis, decreased survival and increased metastasis in PC (5,13).

## INFLAMMATION AND PANCREATIC CANCER

Hyperglycemia and hyperinsulinism are inflammatory states that reduce beta cell function and risk factors for the development of pancreatic cancer. Pathway activation of NFκB and signal transducer and activation of transcription 3 (STAT3) stimulates cell proliferation and inhibitors of programmed cell death. Hyperglycemia produces large number of reactive oxygen species (ROS) and reduces the activity of antioxidant enzymes to promote mitosis and stimulate cell proliferation (14,15). Hyperglycemia with its inflammatory action alters the phenotype of ductal cells by decreasing the action of E cadherin, responsible for inhibiting tumor growth and metastases (14,16).

Obesity also causes tissue inflammation and that is a fertile microenvironment for pancreatic tumor. Mesenteric adipose tissue adjacent to the pancreas has a proinflammatory response to a high-fat, high-calorie diet that could stimulates the progression of pancreatic intraepithelial neoplasia, precursor of pancreatic cancer (17,18).

## THE ROLE OF GUT MICROBIOTA

Fat caloric diet, environmental factors and gut microbioma contribute to the development of cancer in liver and pancreas due to changing a gut-liver/pancreas axis.

The disbalance between *Firmicutes and Bacteroidetes*, and gram negative bacteria have an important role in obesity and diabetes (5,19). Lipopolysaccharide (LPS) secreted by Gram-negative binds to toll like receptors and CD14 receptors on monocytes, macrophages and neutrophils inducing inflammation which lead to decreased intestinal tight junction proteins increasing the entrance of LPS to circulation. The binding of LPS to up-regulated receptors (CD14 or TLRs) on immune cells, induces tumor cell proliferation and cancer cell invasion (5,20).

Gut microbiota process fermentation of the starch, in large gut, releases short-chain fatty acids (acetate, butyrate and propionate). Butyrate is the most important short –chain fatty acid in the gut. It promotes b-cell development, proliferation, differentiation and function and inhibits apoptosis. Butyrate degradation in small intestine is avoided by good microbiome and brings numerous health benefits decreasing circulatory glucose levels, body weight, and inflammation without causing any side effects (5,21). Studies have shown that intervention in gut microbiota could be a novel immunotherapeutic strategy (22).

## GENETIC INFLUENCE IN DIABETES AND PC

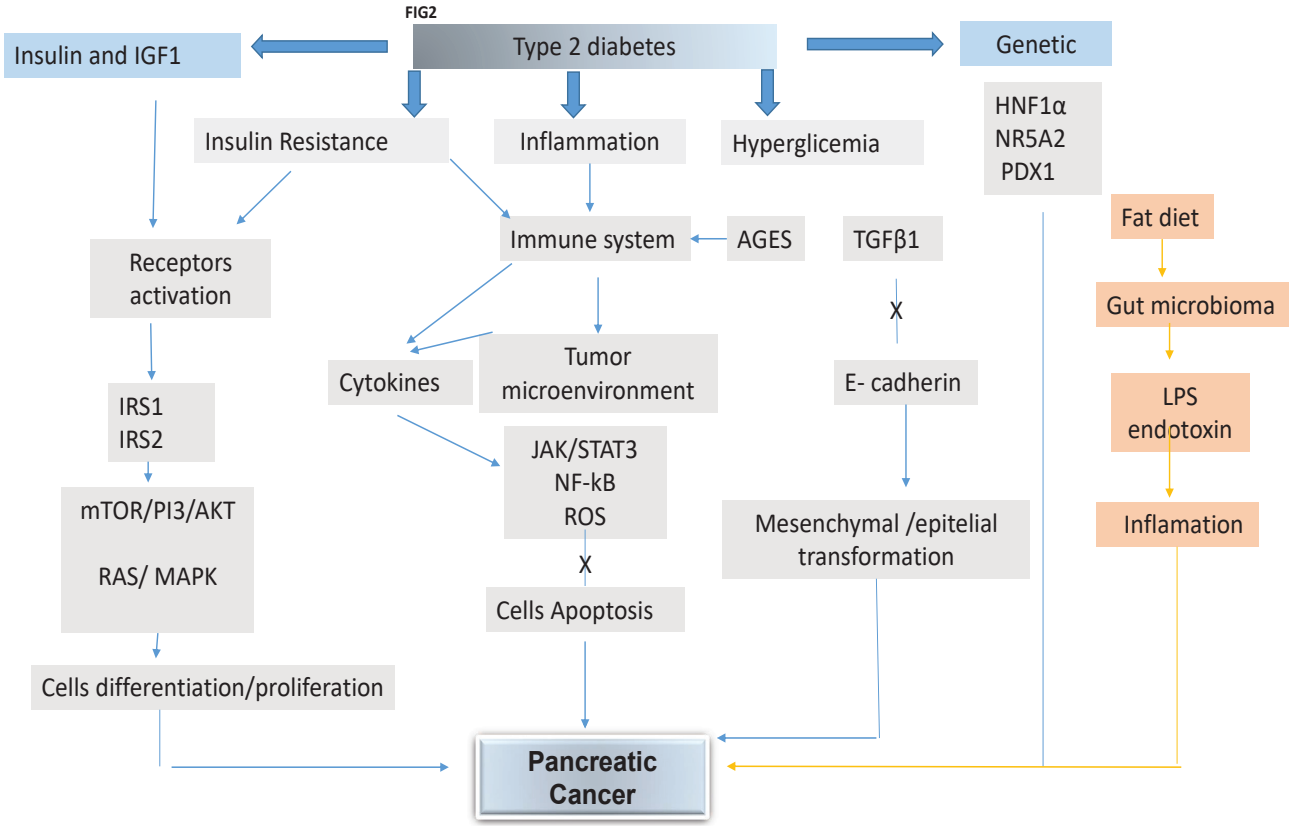
Activation of *KRAS* oncogene is present in > 90% of tumors; inactivation of tumor suppressor gene *CDKN2A* in > 90%, *TP53* in 70% and *SMAD4* in about 50%.

Some pancreatic developmental genes, such as NR5A2, PDX1, and HNF1A, are susceptibility factors for PC in T2DM. Gene mutations in PDX1 and HNF1A lead to different types of monogenic diabetes in young people. PDX1 and HNF1A are also associated with an increased risk of association T2DM and PC (14,23).

## HEPATIC INSULIN RESISTANCE

Patients with type 3c diabetes have hepatic insulin resistance and high glucose production secondary to pancreatic resection, chronic pancreatitis, pancreatic ductal adenocarcinoma and cystic fibrosis. Pancreatic polypeptide (PP) regulates the expression of hepatic insulin receptors, insulin sensitivity and its deficiency is common in type 3c. Some studies show that insulin sensitivity in liver was improved by 72 h of subcutaneous infusion of pancreatic polypeptide (24,25).

## THE RELATIONSHIP BETWEEN TYPE 2 DIABETES MELLITUS AND PANCREATIC CANCER



AGEs, advanced glycation end products; AMPK, adenosine monophosphate protein-activated kinase; IGF-1, insulin-like growth factor-1; ; IRS1--IRS2, insulin receptor substrate1,2; LKB, liver kinase B; ; LPS,- lipopolysaccharides; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor kappa B; PI3K, phosphatidyl inositol-3 kinase; STAT3, signal transducer and activator of transcription 3; TGF-β1, transforming growth factor-β1; TME, tumor microenvironment; PDX1, pancreatic and duodenal homeobox-1; HNF1A, HNF1 Homeobox A; UCP2, uncoupling protein 2. Adapted ref 14.

## PANCREATOGENIC DIABETES OR TYPE 3c DIABETES

The perfect interaction between the exocrine and endocrine pancreas remains unknown.

Secondary diabetes diseases linked to changes in exocrine function is called pancreatogenic diabetes or currently 3c diabetes (1,2,18).

The pathophysiology of T3C DM involves pancreatic glandular inflammation and irreversible fibrotic damage leading to islet cell apoptosis with loss of polypeptide pancreatic (PP) in the early stages of disease which does not happen in the other main types of diabetes. Decreased exocrine function causes malabsorption of nutrients and decreased production of incretins and insulin post-prandial. Replacement of pancreatic enzymes restores the incretin-insulin axis early in the disease. Total loss of beta-cell function only occurs after loss of exocrine function (1,2,18,25).

## CAUSES OF TYPE 3C DIABETES (25)

***Congenital or acquired with absence of islets function***

Pancreatic agenesis

Total Pancreatectomy

***Acquired with partial absence of islets function (transient or permanent)***

Chronic pancreatitis, tropical pancreatitis

Partial pancreatectomy

Severe pancreatitis

Cystic fibrosis

Haemochromatosis

Transient hyperglycemia for two weeks during acute pancreatitis (can be persistent)

***Paraneoplastic***

Pancreatic ductal adenocarcinoma

Differential diagnosis between the most common types of diabetes (adapted refs 18,26)

| | Type 1 DM | Type 2 | Type 3c DM |
|---|---|---|---|
| Ketoacidosis | Common | Rare | Rare |
| Hypoglicemia | Common | Rare | Common |
| Peripheral IR | Normal /decreased | Increased | Normal/decreased |
| Hepatic IR | Normal/increased | Increased | Normal /increased |
| Insulin | Low/absent | High | Low |
| Glucagon | Normal/high | Normal/high | Low |
| PP | Normal /Low | Normal/high | Low/absent |
| GLP1 | Normal | Variable | Variable |
| GIP | Normal/Low | Variable | Low |
| Triggers | Autoimmunity | Age/obesity | Tumor/ pancreatitis |
| Age onset | Childhood/adolescents | Adulthood | Any |

(IR insulin resistance, PP polypeptide pancreatic, GLP1 glucagon like peptide1, GIP glucose dependent insulinotropic polypeptide)

## DIAGNOSTIC CRITERIA FOR T3CDM (18,27)

***Major criteria*** (all criteria must be present)

Presence of exocrine pancreatic insufficiency (monoclonal fecal elastase 1).

Pathological pancreatic imaging; Endoscopic Ultrasound, Magnetic Resonance, or Computed Tomography.

Negative for T1DM and autoimmune markers.

***Minor criteria***

Decreased b-cell function (detected by HOMA-B beta cell function, C- peptide or C-peptide/glucose ratio).

Low insulin resistance (showed by HOMA of insulin resistance).

Decreased secretion of GIP or PP.

Decreased levels of lipid soluble vitamins (A, D, E, or K).

## BIOMARKERS FROM PC IN DIABETICS

***Carbohydrate Antigen 19-9 (CA19-9)***

CA19-9 secreted by cancer cells in patients with new onset diabetes may be a good predictor for PC. However, diseases that cause pancreatic inflammation can increase Ca 19-9 (14,28). CA19-9 measure can be useful and cost-effective in the first 2 years of diabetes to detect small cancer lesions that cannot be found on imaging tests (14,28,29).

***Vanin 1***

Vascular non-inflammatory molecule-1 (VNN1) is a enzyme expressed in many organs. It is responsible for increased oxidative stress (ROS) and inflammation in the tumor microenvironment. Overexpression of VNN1 in tumor tissues can

decrease glutathione concentration aggravating paraneoplastic islet dysfunction (14,30).

***Circulating microRNA***

Recently, circulating microRNAs (miR-483-5p, miR-19a, miR-29a, miR-20a, miR-24, miR-25) are noninvasive biomarkers for the early detection of PC. Pancreatic cells release a large amount of RNA into the bloodstream and serum levels of microRNA could be able to distinguish PC-related DM from type 2 diabetes (14,30)

## HYPOGLICEMIC DRUGS AND THEIR IMPACT IN PANCREATIC CANCER

***Metformin***

Metformin is the first choice drug to treat type 2 diabetes. Studies have shown that metformin has influence in the risk of pancreatic cancer and tumor growth and possibly can increase survival when combined with chemotherapy in small tumors (14).

*Mechanisms of metformin in* PC

1- Decreased hyperinsulinism with consequent reduction in hepatic glucose production. The molecular target of metformin in liver is the inhibition of mitochondrial glycerophosphate dehydrogenase enzyme resulting in an altered hepatocellular redox state, reducing conversion of lactate and glycerol to glucose, and decreasing hepatic gluconeogenesis (14,18).

2- Activation of liver kinase B1 (LKB1) and adenosine monophosphate protein-activated kinase (AMPK) pathway, to promote cell energy production, inhibition of mitochondrial oxidative stress, inhibition of tumor growth – via mTOR (14,31). Studies found that metformin has best action in reducing proliferation and inducing apoptosis of pancreatic cancer cells, in euglycemic state (14,32).

**Sulphonylureas**

Sulfonylureas stimulate insulin release by closing potassium channels, beta cell depolarization and opening calcium channels. It causes hyperinsulinism and induces weight gain, therefore are associated with increased risk for CP (18)

**Incretin-based therapy**

GLP-1 stimulates insulin secretion through activation of the GLP-1 receptor in beta cells, increasing the production of cAMP. It also increases insulin mRNA stability via upregulation of the transcription factor PDX-1 and, in rodents, promotes b-cell growth and survival. Studies have shown that both GLP1 agonists and DPP4 inhibitors stimulate GLP1 receptors on pancreatic exocrine cells and may increase the risk for pancreatitis and PC (14,18,33). Currently, researchers have reached no conclusions about the risk of incretin-based therapy and pancreatic cancer (33).

**Sodium-Glucose Cotransporter 2 Inhibitors (SGLT2i)**

SGLT2i are a new class of drugs involved in the treatment of type 2 DM with recognized benefit in the treatment of diabetic chronic kidney disease as well as heart failure with reduced or preserved ejection fraction. SGLT2i control blood glucose by inhibiting sodium-glucose co-transport in the renal tubules and increasing the excretion of urinary glucose (18). Study found that SGLT2 is expressed in PC and that SGLT2i blocks glucose uptake and reduces tumor growth and survival in a xenograft model of PC. These findings suggest that SGLT2i may be useful for cancer therapy (34,35).

**Thiazolidinediones**

Thiazolidinediones are gamma PPAR and alpha PPAR agonists. These medication act by regulating glucose and fat metabolism and decreasing insulin resistance. They promote weight gain and fluid retention and are not indicated in patients at risk of heart failure There is no proof of direct action of this drug on the risk for PC (14,18).

**Insulin**

Insulin is the oldest treatment for diabetes and is indicated as the only treatment for DM1 and DM2 with loss of insulin secretion or with intense insulin resistance.

Insulin promotes the proliferation and glucose utilization of PC cells by activating ERK and PI3K and by increasing the expression of MMP-2, causing migration and invasion of PC. Insulin also induces phosphorylation of ERK and PI3K/Akt, which indicates that stimulation of Ras/Raf/MAPK and PI3K/Akt pathways could increase tumorigenesis and development of metastasis (36). It is important to be aware that diabetics who take insulin for a long time, are at PC risk (14).

## CONCLUSION

Pancreatic cancer is a malignant disease of late diagnosis and with very little chance of survival for more than 5 years. Attention to patients over 50 years of age, smokers, those with no family history of diabetes, obese, who have been losing weight for some time, and who suddenly become dependent on insulin. The risk of an obese patient to have pancreatic cancer is explained by the action of hyperinsulinism, hyperglycemia and the production of inflammatory adipokines by adipose tissue.

The true link between PC and diabetes is still not well known. The question is whether cancer is the cause of glucose metabolism disturbance by affecting the endocrine pancreas or whether diabetes is a paraneoplastic disease.

Understanding the pathophysiology of diabetes in pancreatic cancer is important for definition and distinction of different types of diabetes to classify it as a disease caused by the tumor or a paraneoplastic disease, or even if cancer would be caused by type 2 diabetes. Genetic mechanisms of disease, physiological differences in β-cell function in various type 3c diabetes, insulin sensitivity, immune cells and the role of gut microbiota can help differentiate type 3c diabetes from the much more prevalent type 2 diabetes.

Future studies are needed in order to develop diagnostic criteria using new onset type 3c diabetes for early detection and treatment of PC aiming a better survival and quality of life for patients with this highly lethal disease.

**References**


1. Oldfield L, Evans A, Rao RG, Jenkinson C, Purewal T, Psarelli EE, et al. Blood levels of adiponectin and IL-1Ra distinguish type 3c from type 2 diabetes: Implications for earlier pancreatic cancer detection in new-onset diabetes. **EBioMedicine**. 2022;75:103802.
2. Hart PA, Bellin MD, Andersen DK, Bradley D, Cruz-Monserrate Z, Forsmark CE, et al. Type 3c (pancreatogenic) diabetes mellitus secondary to chronic pancreatitis and pancreatic cancer. **Lancet Gastroenterol Hepatol**. 2016;1(3):226-37.
3. George S, Jean-Baptiste W, Yusuf Ali A, et al. (June 24, 2022) The Role of Type 2 Diabetes in Pancreatic Cancer. **Cureus** 14(6): e26288. DOI 10.7759/cureus.26288
4. Kai Sun, Christine M Kusminski, Philipp E Scherer. Adipose tissue remodeling and obesity. **J Clin Invest**. 2011; Jun;121(6):2094-101.
5. Ramesh Pothuraju, Satyanarayana Rachagani Wade M. Junke, Sanjib Chaudhary, Viswanathan Saraswath,Sukhwinder Kaur and Surinder K. Batra Pancreatic cancer associated with obesity and diabetes: an alternative approach for its targeting **J Exp Clin Cancer Res**. 2018 Dec 19;37(1):319.
6. Bub JD, Miyazaki T, Iwamoto Y. Adiponectin as a growth inhibitor in prostate cancer cells. **Biochem Biophys Res Commun**. 2006; 340(4):1158–66.
7. Wijesekara N, Krishnamurthy M, Bhattacharjee A, Suhail A, Sweeney G, Wheeler. Adiponectin-induced ERK and Akt phosphorylation protects against pancreatic beta cell apoptosis and increases insulin gene expression and secretion. **J Biol Chem** 2010 Oct 29;285(44):33623-31
8. Bao B, Wang Z, Li Y, Kong D, Ali S, Banerjee S, et al. The complexities of obesity and diabetes with the development and progression of pancreatic cancer. **Biochim Biophys Acta**. 2011;1815(2):135–46.
9. Vansaun MN. Molecular pathways: adiponectin and leptin signaling in cancer. **Clin Cancer Res**. 2013;19 (8):1926–32.

10. Kato M, Watabe K, Tsujii M, Funahashi T, Shimomura I, Takehara T.Adiponectin inhibits murine pancreatic cancer growth. **Dig Dis Sci**. 2014; 59(6):1192–6.
11. Messaggio F, Mendonsa AM, Castellanos J, Nagathihalli NS, Gorden L, Merchant NB, et al. Adiponectin receptor agonists inhibit leptin induced pSTAT3 and in vivo pancreatic tumor growth. **Oncotarget**. 2017;8 (49):85378–91.
12. Tamori Y, Kasuga M. Glypican-4 is a new corner of adipokines working as insulin sensitizer. **J Diabetes Investig**. 2013;4 (3):250–1.
13. Fan Y, Gan Y, Shen Y, Cai X, Song Y, Zhao F, et al. Leptin signaling enhances cell invasion and promotes the metastasis of human pancreatic cancer via increasing MMP-13 production. **Oncotarget**. 2015;6 (18):16120–34.
14. Duan X, Wang W, Pan Q, Guo L: Type 2 diabetes mellitus intersects with pancreatic cancer diagnosis and development. **Front Oncol**. 2021, 11:730038
15. Li W. Hyperglycemia as a Mechanism of Pancreatic Cancer Metastasis. **Front Biosci** 2012; 17(1):1761.
16. Rahn S, Zimmermann V, Viol F, Knaack H, Stemmer K, Peters L, et al. Diabetes as Risk Factor for Pancreatic Cancer: Hyperglycemia Promotes Epithelial-Mesenchymal-Transition and Stem Cell Properties in Pancreatic Ductal Epithelial Cells. **Cancer Lett** (2018) 415:129–50.
17. Hertzer KM, Xu M, Moro A, et al. Robust early inflammation of the peripancreatic visceral adipose tissue during diet-induced obesity in the KrasG12D model of pancreatic cancer. **Pancreas** 2016;45:458–465
18. Dana K. Andersen, Murray Korc, Gloria M. Petersen, Guido Eibl, Donghui Li, Michael R. Rickel .Suresh T. Chari, and James L. Abbruzzese. Diabetes, Pancreatogenic Diabetes, and Pancreatic Cancer. **Diabetes** 2017;66:1103–1 110
19. Goyert SM, Ferrero E, Rettig WJ, Yenamandra AK, Obata F, Le Beau MM. The CD14 monocyte differentiation antigen maps to a region encoding growth factors and receptors. **Science**. 1988;239 (4839):497–500.
20. Haziot A, Chen S, Ferrero E, Low MG, Silber R, Goyert SM. The monocyte differentiation antigen, CD14, is anchored to the cell membrane by a phosphatidylinositol linkage. **J Immunol**. 1988;141 (2):547–52.
21. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. **Diabetes**. 2008;57(6):1470–81.
22. Sethi V, Kurtom S, Tarique M, Lavania S, Malchiodi Z, Hellmund L, et al. Gut microbiota promotes tumor growth in mice by modulating immune response. **Gastroenterology**. 2018;155(1):33–7 e6.
23. Spaninger E, Potočnik U, Bren U. Molecular Dynamics Simulations Predict That rSNP Located in the HNF-1a Gene Promotor Region Linked With MODY3 and Hepatocellular Carcinoma Promotes Stronger Binding of the HNF-4a Transcription Factor. **Biomolecules** (2020) 10(12):1700
24. Goldstein JA, Kirwin JD, Seymour NE, Trachtenberg JE, Rademaker EA, Andersen DK. Reversal of in vitro hepatic insulin resistance in chronic pancreatitis by pancreatic polypeptide in the rat. **Surgery**. 1989; 106:1128–32.
25. Phil A Hart, Melena D Bellin, Dana K Andersen, David Bradley, Zobeida Cruz-Monserrate, Christopher E Forsmark, Mark O Goodarzi, Aida Habtezion et als .Type 3c (pancreatogenic) diabetes mellitus secondary to chronic pancreatitis and pancreatic cancer **Lancet Gastroenterol Hepatol**. 2016 November ; 1(3): 226–237
26. Cui Y, Andersen DK. Pancreatogenic diabetes: special considerations for management. Pancreatology 2011;11:279–294
27. Ewald N, Bretzel RG. Diabetes mellitus secondary to pancreatic diseases (type 3c) are we neglecting an important disease? **Eur J Intern Med** 2013;24:203–206
28. Illés D, Terzin V, Holzinger G, Kosár K, Róka R, Zsóri G, et al. New-Onset Type 2 Diabetes Mellitus – A High-Risk Group Suitable for the Screening of Pancreatic Cancer? **Pancreatology** 2016; 16(2):266–71
29. Choe JW, Kim HJ, Kim JS, Cha J, Joo MK, Lee BJ, et al. Usefulness of CA 19–9 for Pancreatic Cancer Screening in Patients With New-Onset Diabetes. **Hepatob Pancreat Dis** (2018) 17(3):263–8.
30. Dai X, Pang W, Zhou Y, Yao W, Xia L, Wang C, et al. Altered Profile of Serum microRNAs in Pancreatic Cancer-Associated New-Onset Diabetes Mellitus. **J Diabetes** (2016) 8(3):422–33
31. De Souza A, Khawaja KI, Masud F, Saif MW. Metformin and Pancreatic Cancer: Is There a Role? **Cancer Chemoth Pharm** (2016) 77(2):235–42.
32. Ma M, Ma C, Li P, Ma C, Ping F, Li W, et al. Low Glucose Enhanced Metformin's Inhibitory Effect on Pancreatic Cancer Cells by Suppressing Glycolysis and Inducing Energy Stress via Up-Regulation of miR-210-5p. **Cell Cycle** (Georgetown Tex) 2020; 19(17):2168–81
33. Gale EAM. Response to Comment on: Butler Et al. A Critical Analysis of the Clinical Use of Incretin-Based Therapies: Are the GLP-1 Therapies Safe? **Diabetes Care** 2013; 36:2118–252
34. Scafoglio C, Hirayama BA, Kepe V, Liu J, Ghezzi C, Satyamurthy N, et al. Functional Expression of Sodium-Glucose Transporters in Cancer. **Proc Natl Acad Sci** 2015; 112(30):E4111–9.
35. Tang H, Yang K, Li X, Song Y, Han J. Pancreatic Safety of Sodium-Glucose Cotransporter 2 Inhibitors in Patients With Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis. **Pharmacoepidem Dr S** 2020; 29 (2):161–72.
36. Wang G, Yin L, Peng Y, Gao Y, Gao H, Zhang J, et al. Insulin Promotes Invasion and Migration of KRASG12D Mutant HPNE Cells by Upregulating MMP-2 Gelatinolytic Activity via ERK- and PI3K-Dependent Signalling. **Cell Proliferat** 2019; 2(3):e12575

Received in: 10-04-2023
Reviewed in: 19-04-2023
Accepted in: 24-04-2023
Conflict of interests: none
Correspondence: Mirnaluci Paulino Ribeiro Gama
Rua Carlos Augusto Cornelsen 280 - Bom Retiro - Curitiba - PR - Brazil
CEP: 80520-560

# ORIGINAL ARTICLE
# HALOGENS AS POTENTIAL THYROID DISRUPTORS – *IN SÍLICO* SIMULATION AND MATHEMATICAL MODEL FOR TRIGGERING AUTOIMMUNE THYROIDITIS

## *HALOGÊNIOS COMO POTENCIAIS DISRUPTORES DA TIRÓIDE - SIMULAÇÃO COMPUTACIONAL E MODELO MATEMÁTICO PARA DESENCADEAMENTO DA TIROIDITE AUTOIMUNE*

[1,5]LUIS JESUINO DE OLIVEIRA ANDRADE
[2]LUÍS MATOS DE OLIVEIRA
[3]ALCINA MARIA VINHAES BITTENCOURT
[4]LUISA CORREIA MATOS DE OLIVEIRA
[5]GABRIELA CORREIA MATOS DE OLIVEIRA

**Key words:** Halogens, Thyroid disruptors, Autoimmune thyroiditis, Mathematical model.
**Descritores:** Halogêneos, Disruptor da tireoide, Tiroidite autoimune, Modelo matemático.

***Abstract***

**Introduction:** The halogens are the non-metallic chemical elements belonging to group 17 of the Periodic Table, namely: fluorine, chlorine, bromine, iodine, astate, and teness. Halogens are biologically atypical components, however are frequent as replacement in the binders of the thyroid hormones and inhibitors, binding precisely to nucleic acids and proteins. **Objective:** Simulate in *sílico* and through a mathematical model the interactions between the ionic changes in the thyroxine (T4) molecule in the process of autoimmunity induction. **Methods:** We used an online application to simulate the docking of fluorine, chlorine, and bromine in the T4 molecule in place of iodine. A hypothetical-deductive mathematical model was assembled to evaluate halogen substitution in the T4 molecule and immune system and its correlation with the development of autoimmune thyroiditis. **Results:** Simulation of the coupling of fluorine, chlorine and bromine, instead of iodine, to T4 were successful using the induced fit docking program. Positioning of each halogen ion in replacing the iodine at position 5 of T4 was achieved. The mathematical model used demonstrated that the change of the halogen ion in the T4 molecule has been shown to be the trigger for the autoimmune trigger of thyroiditis. **Conclusion:** The findings from this study suggest that halogens of lower atomic weight than iodine may act as a trigger for the onset of autoimmune thyroiditis. **Endocrinol diabetes clin exp 2022 / 2370 - 2373.**

***Resumo***

**Introdução:** Os halogênios são os elementos químicos não metálicos pertencentes ao grupo 17 da Tabela Periódica, a saber: flúor, cloro, bromo, iodo, astate e teness. Os halogênios são componentes biologicamente atípicos, porém são frequentes como substitutos nos ligantes dos hormônios e inibidores da tireoide, ligando-se precisamente aos ácidos nucléicos e proteínas. **Objetivo:** Simular computacionalmente e através de um modelo matemático as interações entre as mudanças iônicas na molécula de tiroxina (T4) no processo de indução de autoimunidade. **Métodos:** Utilizamos um aplicativo on-line para simular o acoplamento do flúor, cloro e bromo na molécula T4 em lugar do iodo. Um modelo matemático hipotético-dedutivo foi montado para avaliar a substituição de halogênio na molécula T4 e no sistema imunológico e sua correlação com o desenvolvimento da tireoidite autoimune. **Resultados:** A simulação do acoplamento do flúor, cloro e bromo, em lugar do iodo, ao T4 foi bem sucedida utilizando o programa de acoplamento induzido. Foi obtido o posicionamento de cada íon halogênio na substituição do iodo na posição 5 do T4. O modelo matemático utilizado demonstrou que a mudança do íon halogênio na molécula T4 demonstrou ser o gatilho para o desencadeamento da tireoidite autoimune. **Conclusão:** Os resultados deste estudo sugerem que os halogênios de peso atômico inferior ao iodo podem atuar como gatilho para o desencadeamento da tireoidite autoimune. **Endocrinol diabetes clin exp 2023 / 2370 - 2373.**

**INTRODUCTION**

The halogens are the non-metallic chemical elements belonging to group 17 of the Periodic Table, namely: fluorine, chlorine, bromine, iodine, astate, and teness. Halogens are biologically atypical components, however are frequent as replacement in the binders of the thyroid hormones and inhibitors, binding precisely to nucleic acids and proteins (1).

The thyroid synthesizes through iodination of tyrosine by thyroglobulin the pro-hormone thyroxine (T4) and the active hormone triiodothyronine (T3), which is secreted by thyroid stimulating hormone (TSH) via feed-back. The T4 after its synthesis is transported and activated in the target cells by iodothyronine deiodinase, depending on their metabolic needs (2).

The first step in the biosynthesis of T4 is the filtration of iodine from the blood. However, this filtration by the thyroid is not only limited to iodine, but also to all halogens. Halogen link to the iodothyronine deiodinases that regulate thyroid hormones is a feasible mechanism for thyroid disruption, as a function of the theory density functional theory, where the halogen bonding interactions with thyroid hormones is in the halogen bonding strength with respect to the molecular weight of the halogen (3). Thus, the molecular mimicry of naphthyl-based deiodinase will exhibit high activity via halogen bonding (4).

The halogens (fluorine, chlorine, bromine) are considered competitive inhibitors of the iodine binding sites within the thyroid, and in the thyroid gland the biological action of the halogens are similar to the biological action of iodine. Due to the lower atomic weight of fluorine, chlorine, bromine, they will displace the higher atomic weight iodine influencing in availabi-

[1]Health Department State University of Santa Cruz - Ilhéus – Bahia – Brazil.
[2]Escola Bahiana de Medicina e Saúde Pública – Salvador – Bahia – Brazil.
[3]Medical School - Universidade Federal da Bahia – Salvador – Bahia – Brazil.
[4]Ecole Supériuere des Sciences et Technologies de l'Ingénie de Nancy – Polytech Nancy - Frace.
[5]Gell Clinic - Itabuna – Bahia – Brazil.
E-mail: luis_jesuino@yahoo.com.br

lity of iodine and its uptake by the thyroid as well as competing with the thyroid TSH receptors with alteration in thyroid hormone production as well as the production of antithyroid antibodies (5). Furthermore, halogens can replace the iodine in position 5 of both T3 and T4 without loss of thyroid hormonal activity, leading to enlarged thyroid glands even when iodine intake is sufficient (6,7).

We applied an *in silico* simulation of molecular docking between the halogens fluorine, chlorine, bromine and the T4 by replacing the iodine at position 5 of T4 with fluorine, chlorine and bromine and applied a mathematical model to simulate the different situations of the interactions between the ion change in the molecule in the process of inducing autoimmunity.

## MATERIAL AND METHODS

The structure two-dimensional and three-dimensional of T4 was retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/compound/DL-Thyroxine). The two-dimensional and three-dimensional structures of T4 are demonstrated in Figure 1.

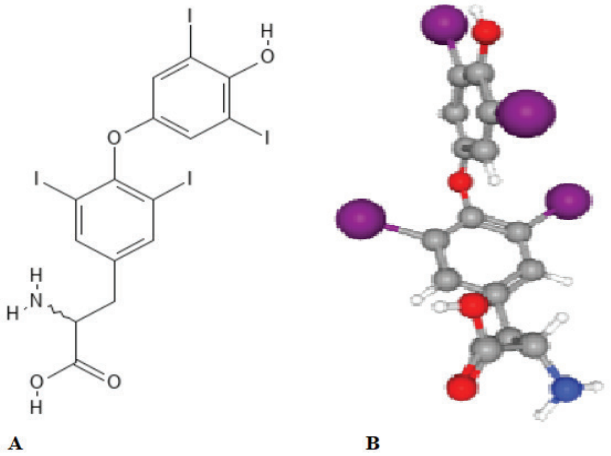


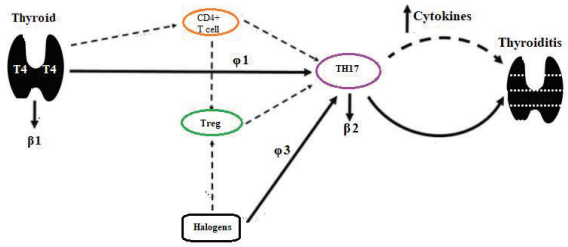

**Figure 1.** The structure two-dimensional (A) and three-dimensional (B) of T4.

The Schrodinger suite was used to simulate the docking of fluorine, chlorine, and bromine (https://www.schrodinger.com/suites/Schro%CC%88dinger).

A hypothetical-deductive mathematical model was assembled to evaluate the substitution of halogens in the T4 molecule and the immune system and its correlation with the development of autoimmune thyroiditis.

We devised a theoretical diagram of cellular activity to describe the correlation between the docking of halogens in the T4 molecule (**Fig. 2**).

**Figure 2.** Simulation model of autoimmune thyroiditis with halogen disruptor.




The hypothetical-deductive mathematical model suggested for the autoimmune thyroiditis trigger (8), elaborated as a function of the immune system activity, is expressed by the following equation:

$$TH'_{17}(t) = \frac{\varphi 1 \; T(t)}{1+T(t)} \mathrm{TH}_{(17)}(t) + \frac{1}{Treg} \frac{\varphi 3 \; H(t)}{1+H(t)} \mathrm{TH}_{17}(t) - \beta_2$$

Where: TH = T helper cell, and Treg = regulatory T cell

TH17 (t) = TH17 lymphocytes concentration - cells/mL;

φ1 = TH17 lymphocyte differentiation rate - %;

T(t) =Thyrocytes concentration - cells/mL;

Treg = Treg lymphocyte concentration - cells/mL;

φ3 = Maximum contribution rate of halogens to TH17 lymphocyte - %;

H(t) = halogens - mcg/mL;

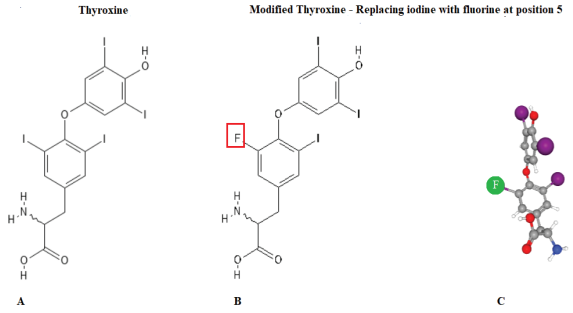
β2 = TH17 mortality rate lymphocyte predation rate over thyrocytes - %.

According to Resolution CNS 510/2016 (Brazil), our study was not required to be approved by the ethics committee due to the fact that the research aimed at deepening the theoretical understanding of a situation that arises spontaneously and contingently in professional practice

## RESULTS

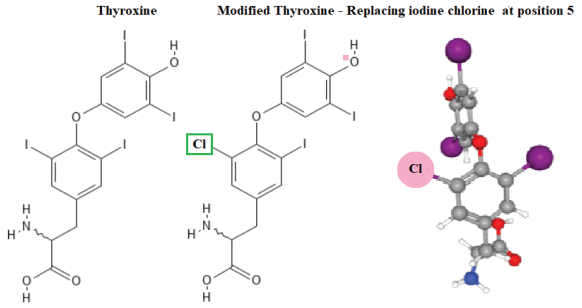
Simulation of the coupling of fluorine, chlorine and bromine, instead of iodine, to T4 were successful using the induced fit docking program. Positioning of each halogen ion in replacing the iodine at position 5 of T4 was achieved (**Figures 3 to 5**).

**Figure 3.** Thyroxine and substitution of iodine for fluorine at position 5.



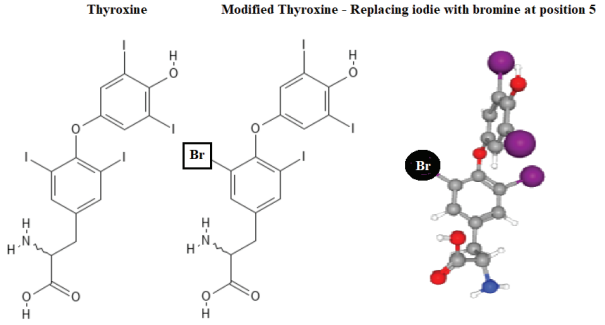

**Source:** *In silico* simulation.

**Figure 4.** Thyroxine and substitution of iodine for chlorine at position 5.




**Source:** *In silico* simulation.

**Figure 5.** . Thyroxine and substitution of iodine for bromine at position 5

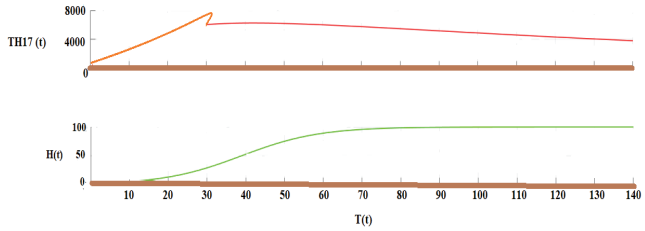



**Source:** *In silico* simulation.

In this hypothetical-deductive mathematical model that we present, we associate 2 indicator criteria of autoimmune thyroiditis, the proliferation of TH17 lymphocytes and the modification of the T4 molecule with the replacement of the iodine ion by fluorine, chlorine or bromine. We evaluated the sensitivity of the Treg and φ 3 correlations, thus assessing the relationship between halogen modification of the T4 molecule and TH17 lymphocyte differentiation (**Figure 2**).

The results of the deterministic model simulations, evaluated by the equation, assume that the rise in TH17 lymphocytes is influenced by modification of the T4 molecule (**Figure 6**).

**Figure 6.** Numerical simulation of the hypothetical-deductive mathematical model parameters.




**Source**: Research result

The green line shows the halogens, the red line represent the growth of the TH17 lymphocytes, and the brown line corresponds to the development of thyrocytes. In the simulation scenario presented in the graphics, it is assumed that the value of φ1 = 0.04, φ3 = 0.01, Treg = 0.8, and β2 = 0.04.

## DISCUSSION

Halogens have attracted attention for some time now because of their potential as endocrine disruptor. We evaluated the fluorine, chlorine and bromine as potential triggers of autoimmune thyroiditis through computer simulation, docking a halogen ion at position 5 of the T4 molecule in place of the iodine ion. In addition, we applied a hypothetical-deductive mathematical model where the replacement of the iodine ion in the T4 molecule by another halogen in the immune system and its interface with the development of autoimmune thyroiditis was evaluated. Thus, we demonstrate that halogenation at the lateral position of the T4 molecule can produce an alteration in thyroid activity.

Halogens do not interfere with the thyroid gland's ability to synthesize thyroid hormone when the amount of iodine in the blood is abundant. However, if the iodine pool is low and the pool of the other lower atomic weight halogens is high in the blood there will be a change in hormone synthesis with a reduction of several parameters in thyroid biological activity (9). Studies have shown that the thyroid has an attraction not only to iodine, but also to the other halogens (10). Studies using the halogens have demonstrated the thyroid's ability to accumulate fluorine, chlorine and bromine in a higher concentration than in other body tissues (11).

In the thyroid hormones the iodine atom generates a bond in T4 that will play key roles in the recognition of these hormones. The binding of halogens in the thyroid hormone molecule stabilizes the molecular interactions that modify the structure of T4 (12).

The halogen elements are like to iodine structurally, competing for uptake and utilization, and can replace each other in physiological reactions. Competition between iodine and the other halogens in binding to T4 at position 5 is a potential thyroid disruptor mechanism, and this happens when iodine is in lesser amounts than fluorine, bromine or iodine (13). The synthesis of T4 takes place exclusively in the thyroid, and the iodine in the T4 molecule can be replaced in its aromatic rings, which in turn are linked by a diphenylether moiety, adopting different configurations that allow different intra- and intermolecular interactions (14). In our study, we performed a crystallographic evaluation of the T4 molecule with the replacement of the iodine ion by other halogens of lower atomic weight than iodine in the T4 molecule.

The triggering of autoimmune thyroiditis depends on genetics and an endogenous or exogenous trigger through molecular mimicry (15). Halogen derivatives can increase thyroiodin's antigenicity, where an epitope of T4 with iodine replaced by another halogen of lower atomic weight becomes an active autoantigen (16).

Halogens have been added as disinfectants in drinking water treatment plants (17). In recent decades endocrine dysfunctions secondary to environmental pollution have been studied, including the impact of halogens on thyroid function (18). The consequences of halogens on human physiology have been described in the literature. Thus, the effects of high intake of chlorine and fluorine through drinking water and environmental pollution as a thyroid disruptor in both animals and humans have been studied, with reports of thyroid dysfunction (19, 10).

Mathematical models coupled with experimental evaluations have furthered the understanding of physiological concepts (20). Mathematical models coupled with experimental evaluations have furthered the understanding of physiological concepts. In mathematical models concerning the thyroid, the fundamental question is whether there is a mathematical model that only addresses factors from which values are readily acquired based on the assessment of thyroid function, i.e. a model suitable for evaluating physiological and pathological states of the thyroid. However, mathematical models for evaluating thyroid pathologies have been insufficient (21). In this study we present a hypothetical-deductive mathematical model, based on the study by Salazar-Viedma M et al. (8), in which we associate 2 factors that indicate the presence of autoimmune thyroiditis, TH17 lymphocytes and modification of the T4 molecule with the replacement of ion iodine by lower atomic weight halogens (fluorine, chlorine, and bromine). In the study by Salazar-Viedma M et al. (8), a mathematical model was generated to simulate the various scenarios of the relationship between thyrocytes, the gut microbiota and the immune system in triggering autoimmune thyroiditis. Yang et al. formulated a thyroid regulation model with their pathologies and subsequently specified the parameters for patients with autoimmune thyroid diseases (Graves' disease and Hashimoto's thyroiditis) and euthyroid individuals, and the simulation results of the autoimmune thyroiditis model demonstrated that the levels of antithyroid antibodies can be a major determinant of thyroid function (20). Since there are no similar studies in the literature, our results could not be compared.

Despite of the limitations, our study provides evidence of autoimmune thyroiditis triggered due to alteration by halogens with atomic weight less than iodine in the T4 molecule.

## CONCLUSION

The findings from this study suggest that halogens of lower atomic weight than iodine may act as a trigger for the onset of autoimmune thyroiditis, as well as the use of a suitable mathematical model can predict risk for autoimmune thyroid disease.

### References


1. Ho PS. Biomolecular halogen bonds.**Top Curr Chem**. 2015;358:241-76.
2. Parmentier M, Libert F, Maenhaut C, Lefort A, Gérard C, Perret J, et al. Molecular cloning of the thyrotropin receptor. **Science** 1989;246 (4937):1620-2.
3. Clark T, Hennemann M, Murray JS, Politzer P. Halogen bonding: the sigma-hole. Proceedings of "Modeling interactions in biomolecules II", Prague, September 5th-9th, 2005. **J Mol Model** 2007;13(2):291-6.
4. Manna D, Mugesh G. Regioselective deiodination of thyroxine by iodothyronine deiodinase mimics: an unusual mechanistic pathway involving cooperative chalcogen and halogen bonding. **J Am Chem Soc**. 2012;134 (9):4269-79.

5. Zhao W, Zhu H, Yu Z, Aoki K, Misumi J, Zhang X. Long-term Effects of Various Iodine and Fluorine Doses on the Thyroid and Fluorosis in Mice. **Endocr Regul**. 1998;32 (2):63-70.
6. Baker DH. Iodine toxicity and its amelioration. **Exp Biol Med** (Maywood) 2004; 229(6):473-8.
7. Velický J, Titlbach M, Dusková J, Vobecký M, Strbák V, Raska I. Potassium bromide and the thyroid gland of the rat: morphology and immunohistochemistry, RIA and INAA analysis. **Ann Anat**. 1997;179 (5):421-31.
8. Salazar-Viedma M, Vergaño-Salazar JG, Pastenes L, D'Afonseca V. Simulation Model for Hashimoto Autoimmune Thyroiditis Disease. **Endocrinology** 2021;162(12):bqab190.
9. Bürgi H, Siebenhüner L, Miloni E. Fluorine and thyroid gland function: a review of the literature. **Klin Wochenschr**. 1984;62 (12):564-9.
10. Marsan ES, Bayse CA. Halogen-Bonding Interactions of Polybrominated Diphenyl Ethers and Thyroid Hormone Derivatives: A Potential Mechanism for the Inhibition of Iodothyronine Deiodinase. **Chemistry** 2017;23(27):6625-6633.
11. Lisco G, De Tullio A, Giagulli VA, De Pergola G, Triggiani V. Interference on Iodine Uptake and Human Thyroid Function by Perchlorate-Contaminated Water and Food. **Nutrients** 2020 Jun 4;12(6):1669.
12. Lévesque D, Beaudoin JD, Roy S, Perreault JP. In vitro selection and characterization of RNA aptamers binding thyroxine hormone. **Biochem J**. 2007;403 (1):129-38.
13. Hill KL, Mortensen ÅK, Teclechiel D, Willmore WG, Sylte I, Jenssen BM, et al. In Vitro and in Silico Competitive Binding of Brominated Polyphenyl Ether Contaminants with Human and Gull Thyroid Hormone Transport Proteins. **Environ Sci Technol**. 2018;52 (3):1533-1541.
14. Schweizer U, Steegborn C. Thyroid hormones--From Crystal Packing to Activity to Reactivity. **Angew Chem Int Ed Engl.** 2015;54 (44):12856-8.
15. Oldstone MB. Molecular mimicry: its evolution from concept to mechanism as a cause of autoimmune diseases. **Monoclon Antib Immunodiagn Immunother**. 2014; 33 (3):158-65.
16. Kolypetri P, Noel NA, Carayanniotis KA, Carayanniotis G. Iodine content of thyroglobulin in Nod.H2h4 mice developing iodine-accelerated autoimmune thyroiditis. **Hormones** (Athens) 2010;9 (2):151-60.
17. Liu J, Sayes CM, Sharma VK, Li Y, Zhang X. Addition of lemon before boiling chlorinated tap water: A strategy to control halogenated disinfection byproducts. **Chemospher** 2021; 263:127954.
18. Kheradpisheh Z, Mirzaei M, Mahvi AH, Mokhtari M, Azizi R, Fallahzadeh H, et al. Impact of Drinking Water Fluoride on Human Thyroid Hormones: A Case- Control **Study Sci Rep** 2018;8 (1):2674.
19. Liu JL, Han X, Zhang J, Wang HJ, Zhou MX, Li SW, et al. Total organic halogen in two drinking water supply systems: Occurrence, variations, and relationship with trihalomethanes. **Chemosphere** 2022;288(Pt 2):132541.
20. Yang B, Tang X, Haller MJ, Schatz DA, Rong L. A unified mathematical model of thyroid hormone regulation and implication for personalized treatment of thyroid disorders. **J Theor Biol**. 2021;528:110853.

Luis Jesuino de Oliveira Andrade
https://orcid.org/0000-0002-7714-0330
Luís Matos de Oliveira
https://orcid.org/0000-0003-4854-6910
Alcina Maria Vinhaes Bittencourt
https://orcid.org/0000-0003-0506-9210
uisa Correia Matos de Oliveira
https://orcid.org/0000-0001-6128-4885
Gabriela Correia Matos de Oliveira
https://orcid.org/0000-0002-8042-0261

Received in: 13-03-2023
Accepted in: 27-03-2023
Conflict of interest: none
Address for correspondence: Luís Jesuíno de Oliveira Andrade
Campus Soane Nazaré de Andrade, Rod. Jorge Amado, Km 16 - Salobrinho, Ilhéus – BA - Brazil
CEP: 45662-900

# ORIGINAL ARTICLE
# "SONOHISTOLOGY": ULTRASOUND CHARACTERIZATION OF LIVER TISSUE IN THE DIAGNOSIS OF HEPATIC STEATOSIS

## *"SONOHISTOLOGIA": CARACTERIZAÇÃO ULTRASSONOGRÁFICA DO TECIDO HEPÁTICO NO DIAGNÓSTICO DE ESTEATOSE HEPÁTICA*

[1]LUÍS JESUINO DE OLIVEIRA ANDRADE
[2]GABRIELA CORREIA MATOS DE OLIVEIRA
[3]ALCINA MARIA VINHAES BITTENCOURT
[4]LUÍS MATOS DE OLIVEIRA

**Key words:** Hepatic steatosis, Ultrasonography, Histology.
**Descritores:** Esteatose hepática, Ultrassonografia, Histologia.

***Abstract***

**Introduction:** "Sonohistology," the sonographic characterization of "sonography tissue", can be defined as the correlation between the subjective qualitative parameters of ultrasound and the objective quantitative histological parameters of liver tissue. **Objective:** Correlate sonographic imaging and histological aspects in hepatic steatosis. **Material and Methods:** Liver biopsy a slide of cases in which hepatic steatosis was classified as mild, moderate and severe was evaluated to determine the number of fat cells per microscopic field (mm2) from the histological sections. The number of fat cells per mm2 was correlated with the degree of hepatic steatosis suggested on ultrasound examination. **Results:** The mean number of fat cells per mm2 was 50 cells in mild steatosis, 85 fat cells in moderate steatosis, and more than 150 fat cells in severe steatosis. A significant correlation was observed between the increase in echogenicity and the number of fat cells. This means that the greater the number of fat cells in the liver, the greater the proportion of perpendicularly incident sound waves that are reflected back to the transducer. In normal liver tissue, a greater dispersion of the sound wave occurred. **Conclusion:** The main interface of echo reflection in steatotic liver tissue is the boundary between the normal hepatocyte and fatty infiltration. **Endocrinol diabetes clin exp 2023 / 2374 - 2378.**

***Resumo***

**Introdução:** A "sonohistologia" na caracterização do "tecido ultrassonográfico" para o diagnóstico de infiltração gordurosa hepática pode ser definida como a correlação entre parâmetros ultrassonográficos qualitativos subjetivos e parâmetros histológicos quantitativos objetivos do tecido hepático. **Objetivo:** Correlacionar aspectos de imagem ultrassonográfica com aspectos histológicos na esteatose hepática. **Material e Métodos:** Lâminas de biópsia hepática com esteatose hepática classificada como leve, moderada e grave foram avaliadas pela determinação do número de células adiposas por campo microscópico (mm2) de cortes histológicos. O número de células de gordura por mm2 foi correlacionado com o grau de esteatose hepática sugerido pelo exame ultrassonográfico. **Resultados:** O número médio de células adiposas por mm2 foi de 50 células na esteatose leve, 85 células adiposas na esteatose moderada e mais de 150 células adiposas na esteatose grave. Foi observada uma correlação significativa entre o aumento da ecogenicidade e o número de células adiposas, o que significa que quanto maior o número de células adiposas no fígado, maior a proporção de ondas sonoras incidentes perpendicularmente refletidas de volta para o transdutor (hiperecogenicidade), enquanto no tecido hepático normal ocorreu uma maior dispersão da onda sonora (hipoecogenicidade). **Conclusão:** A principal interface de reflexão do eco no tecido hepático esteatótico é o limite entre o hepatócito normal e a infiltração gordurosa. **Endocrinol diabetes clin exp 2023 / 2374 - 2378.**

**INTRODUCTION**

Hepatic fatty infiltration is defined as when more than 5% of hepatocytes have large fat vacuoles. It is estimated that nonalcoholic fatty infiltration of the liver affects about 1 billion people worldwide, comprising about 19-46% of liver diseases occurring in the Western world (1,2).

Liver biopsy, despite possible harm, is considered to be the current gold standard in diagnosing hepatic steatosis .3 Hepatic fatty infiltration is classified histologically according to the percentage of large or medium fat droplets in the hepatocyte: less than 5% is normal, between 5-33% is mild steatosis (Grade 1), between 34-66% is moderate steatosis (Grade 2), and greater than 66% is severe steatosis (Grade 3) (4,5).

Imaging methods are commonly used for the evaluation of hepatic steatosis, and ultrasonography is the initial imaging method used in the identification and classification of hepatic fatty infiltration because it allows for the subjective assessment of the degree of fatty infiltration in the liver (6). Studies have demonstrated that ultrasonography presents quite a variable sensitivity and specificity, which limits this method in the diagnosis of this disease (7). However, a meta-analysis has demonstrated that ultrasonography is an accurate and reliable imaging method in the identification of moderate and severe hepatic steatosis when compared to histology (8). Hepatic fatty infiltration is classified sonographically into: Normal (Grade 0) when the hepatic echographic texture is normal; Mild (Grade 1) when a level, diffuse, non-attenuating increase in hepatic echogenicity is observed with normal visualization of the intrahepatic vessels and diaphragm; Moderate (Grade 2) when a moderate increase is observed with attenuation of the acoustic beam, with impaired visualization of the diaphragm and hepatic vascularization; Severe (Grade 3) when a severe and diffuse increase in hepatic echogenicity is observed, without visualization of the intrahepatic

[1]Health Department State University of Santa Cruz, Ilhéus – Bahia – Brazil
[2]Gell Clinic - Itabuna – Bahia – Brazil
[3]Medical School - Universidade Federal da Bahia – Salvador – Bahia – Brazil.
[4]Escola de Medicina e Saúde Pública - Salvador –Bahia –Brazil
E-mail: luis_jesuino@yahoo.com.br

vessels and posterior liver contours (9).

Therefore, ultrasonography in assessing the degree of hepatic fatty infiltration would be a useful noninvasive method in predicting liver histology, especially in cases where biopsy is contraindicated. The aim of this study was to compare the degree of echogenicity of hepatic fatty infiltration on two--dimensional ultrasonography with the histological degree of hepatic fatty infiltration based on the number of fat cells per histological mm².

## MATERIAL AND METHODS

Photographs of liver biopsy slides with normal liver, mild, moderate, and severe steatosis were obtained from a specialized pathological anatomy laboratory. The number of fat cells per microscopic field (mm2) was evaluated in the histological sections. The number of fat cells per mm2 was then correlated with the degree of hepatic steatosis suggested by ultrasound examination.

Estimation of the degree of hepatic fat infiltration by two--dimensional ultrasonography was obtained using ultrasound features that included brightness, contrast between the liver and the adjacent kidney, acoustic beam attenuation, the visualization of intrahepatic vessels, and the posterior contours of the hepatic lobe.

In accordance with CNS Resolution 510/2016, the study did not require ethics committee submission as the research aimed to further the theoretical understanding of pathologies in clinical practice.

## RESULTS

A significant correlation occurred between echogenicity and the number of fat cells. The greater the number of fat cells, the greater the proportion of sound waves incident perpendicularly, reflecting back to the transducer as hyperechogenicity.

Using basic physical principles of ultrasonography (10), we present a combination of parameters for analyzing low and high echogenicity (**Table 1**). Sound reflection results from the difference in acoustic impedance at the boundary between two media, while sonic energy reduction results from sound absorption and sound dispersion.

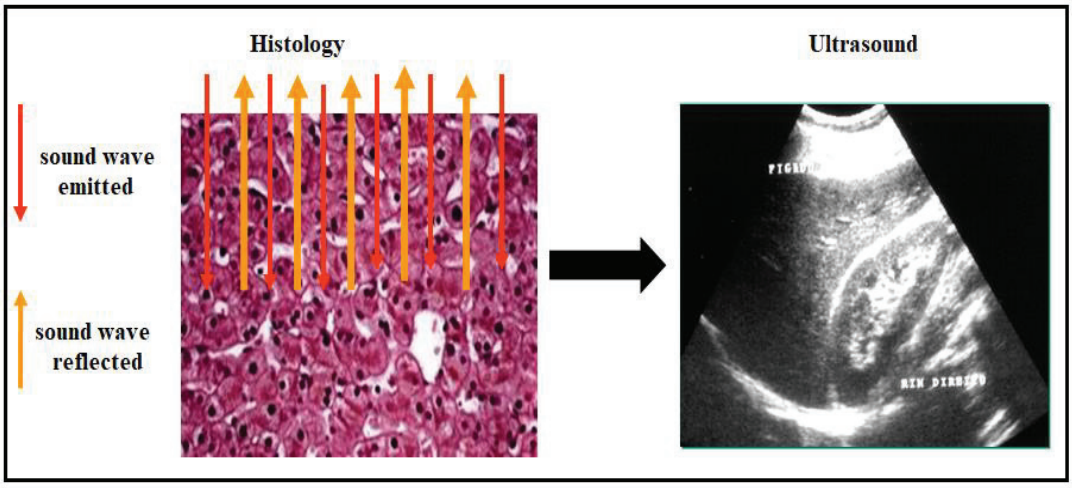
**Table 1**. Physical parameters of echogenicity

| | Difference of acoustic impedance at interfaces | Scattering | Sound absorption |
|---|---|---|---|
| **High ecogenicity** | High | Low | Low |
| **Low echogenicity** | Low | High | High |

There was a significant correlation between echogenicity and the number of fat cells, meaning that the higher the number of fat cells, the higher the proportion of sound waves that reflect back perpendicularly to the transducer as hyperechogenicity. In normal liver tissue, there is greater dispersion of the sound wave, resulting in hypoechogenicity.

We illustrate ultrasonography and histology of a normal liver, with an ultrasound longitudinal section demonstrating liver tissue of normal echogenicity (**Figure 1**).

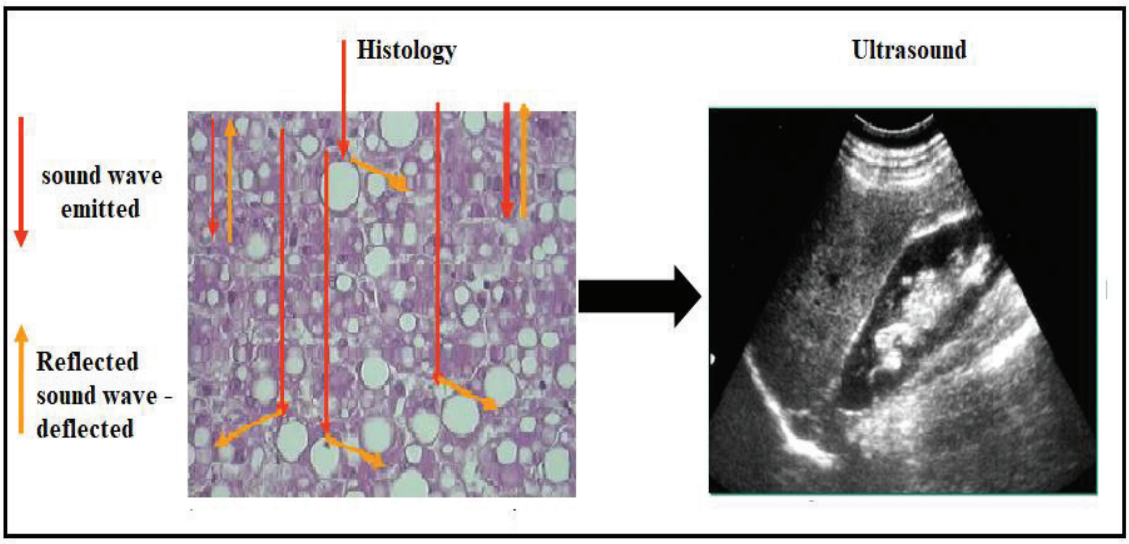
**Figure 1**. Ultrasonography and histology of a normal liver.




Source: search result

In the histological evaluation of mild hepatic fatty infiltration, the mean number of fat cells per microscopic mm2 was 50 cells (Figure 2).

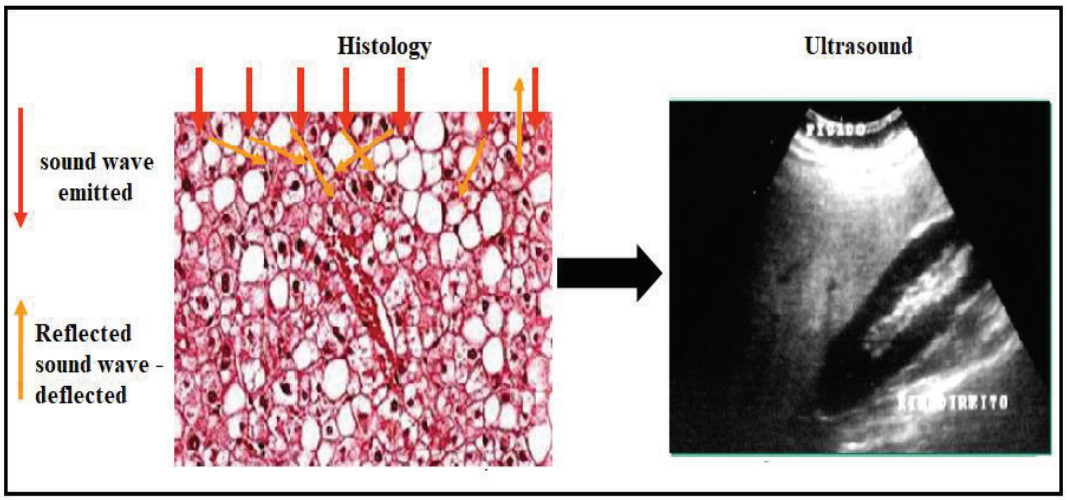
**Figure 2**. Ultrasonography and histology of the mild hepatic fatty infiltration




Source: search result

In moderate hepatic fatty infiltration, the histological evaluation revealed a mean number of fat cells per microscopic mm2 of 85 cells (**Figure 3**).

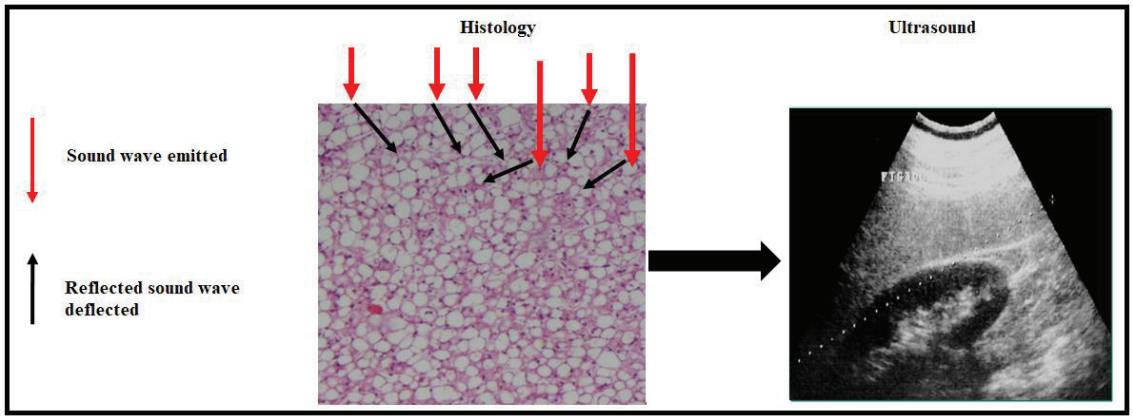
**Figure 3.** Ultrasonography and histology of the moderate hepatic fatty infiltration.




Source: search result

In severe hepatic fatty infiltration, the histological evaluation indicated a mean number of fat cells per microscopic mm2 > 150 cells (**Figure 4**).

**Figure 4.** Ultrasonography and histology of the severe hepatic fatty infiltration.




Source: search result

## DISCUSSION

Our results demonstrate that the degree of hepatic echogenicity on ultrasound shows a quantitative correlation to the histologic severity of hepatic fatty infiltration, based on the number of fat cells present per mm2 on histologic sections. This allows for the “sonohistological” characterization of nonalcoholic fatty liver disease. Thus, our study describes the sonographic characteristics of nonalcoholic fatty liver disease and its histological diagnosis based on the number of fat cells found in liver biopsies, thus characterizing the sonohistological diagnosis or "sonohistology."

Histological evaluation is considered the "gold standard" for diagnosing pathology. However, the quality of B-mode ultrasound imaging has significantly increased the resolution of images, allowing for ultrasound tissue characterization based on the evaluation of statistical local tissue data. This has given rise to the term "sonohistology" (11).

"Sonohistology" in ultrasonographic characterization of liver tissue in the diagnosis of hepatic fat infiltration can be defined as the correlation between the subjective qualitative parameters of ultrasonography and the objective quantitative histological parameters of liver tissue.

Liver biopsy is considered the standard for diagnosing hepatic fat infiltration when compared to other non-invasive methods such as imaging and serological methods. However, the guidelines of the American Association for the Study of Liver Disease recommend liver biopsy only for patients who have "benefited" from it (12). In specialized centers, most liver biopsies are performed under ultrasound guidance, and the knowledge of the examining pathologist is of fundamental importance for the diagnosis of hepatic fat infiltration (13). A diagnosis of nonalcoholic fatty liver disease requires more than 5% macrovesicular steatosis (14).

The histological classification of hepatic steatosis uses a semi-quantitative scale based on the number of hepatocytes with microscopically evident cytoplasmic fatty droplets. However, sampling errors may induce heterogeneity in the histological diagnosis of hepatic steatosis (15).

In our study, we used the number of fat cells per mm2 instead of the gold standard method that depends on the percentage of hepatocytes with fat deposition. We did this to compare with the presence of fat greater than 5% of hepatocytes (14), and thus to objectively determine the subjective echographic pattern of hepatic fatty infiltration.

Several imaging studies have been performed to characterize the tissue of hepatic fatty infiltration, such as dual-energy computed tomography and magnetic resonance imaging using neural networks, in addition to ultrasound (16-18). We used the physical principles of ultrasound to analyze normal tissue that is characterized histologically by higher cellularity, translated on ultrasound by an average echogenicity slightly more hypoechogenic than the spleen and brighter than the adjacent kidney. Thus, in normal liver tissue, there is a greater dispersion of the sound wave (hypoechogenicity).

Non-alcoholic fatty liver disease is the most common pathology found in the liver today (19). Carefully applied ultrasonography has been found to be a good diagnostic method. Liver tissue with fat infiltration is characterized by increased echogenicity compared to the echogenicity of the adjacent spleen and kidney, due to the percentage of fat cells present in this tissue (6). With this, there is an attenuation of the ultrasound beam with reduced sound intensity through the liver tissue changing the absorption and dispersion of sound with consequent divergence of the acoustic beam. The attenuation of sound in the insonated structure reduces the visualization of details of the liver structures (20). Hepatic fatty infiltration is categorized based on the percentage of fat within the hepatocytes: grade 0 (healthy, <5%), grade 1 (mild, 5%-33%), grade 2 (moderate, 34%-66%), and grade 3 (severe, >66%) (21). Our study observed that greater number of fat cells, the greater the proportion of sound waves that incur perpendicularly and are reflected back to the transducer (hyperecogenicity). Thus, correlating the study by Brunt EM et al.(21) with our findings, a percentage of 5%-33% fat in the hepatocyte corresponds to an average of 50 adipocytes per mm2, between 34%-66% fat in the hepatocyte corresponds to an average of 85 adipocytes per mm2, and more than 66% of fat in the hepatocyte corresponds to more than 150 adipocytes per mm2 on the histological slides evaluated.

Quantitative ultrasound is under development as a technique programmed to reduce the subjectivity of the sonographer, based on the attenuation coefficient and backscatter coefficient, and thereby lead to a more accurate diagnosis and classification of hepatic steatosis (22). In our study, using a combination of parameters to analyze the variation of echogenicity, we observed a correlation between the number of fat cells and the proportion of perpendicularly incident sound waves resulting in increased echogenicity and thus classified as hepatic steatosis, unlike normal liver tissue where there is a greater dispersion of the sonicated wave resulting in reduced echogenicity.

## CONCLUSION

Based on this study, we can consider the echogenicity of the liver tissue as a measure of its cellular content. Normal echogenicity demonstrates the existence of normal hepatocytes with normal homogeneous echographic patterns, thus ruling out other pathologies such as fatty infiltration. Thus, the main echo reflection interface in steatotic liver tissue is the boundary between normal hepatocytes and fatty infiltration.

### References


1. Jennison E, Patel J, Scorletti E, Byrne CD. Diagnosis and management of non-alcoholic fatty liver disease. **Postgrad Med J**. 2019;95(1124):314-22.
2. Castera L, Friedrich-Rust M, Loomba R. Noninvasive Assessment of Liver Disease in Patients With Nonalcoholic Fatty Liver Disease. **Gastroenterology**. 2019;156(5):1264-1281.e4.
3. Idilman IS, Ozdeniz I, Karcaaltincaba M. Hepatic Steatosis: Etiology, Patterns, and Quantification. **Semin Ultrasound CT MR**. 2016;37(6):501-10.
4. Bedossa P, Poitou C, Veyrie N, Bouillot JL, Basdevant A, Paradis V, et al. Histopathological algorithm and scoring system for evaluation of liver lesions in morbidly obese patients. **Hepatology**. 2012;56(5):1751–9.
5. Bedossa P. Pathology of non-alcoholic fatty liver disease. **Liver Int**. 2017;37(Suppl 1):85–9.
6. Ferraioli G, Soares Monteiro LB. Ultrasound-based techniques for the diagnosis of liver steatosis. **World J Gastroenterol**. 2019;25(40):6053-62.
7. Makhija N, Vikram NK, Srivastava DN, Madhusudhan KS. Role of Diffusion-Weighted Magnetic Resonance Imaging in the Diagnosis and Grading of Hepatic Steatosis in Patients With Non-alcoholic Fatty Liver Disease: Comparison With Ultrasonography and Magnetic Resonance Spectroscopy. **J Clin Exp Hepatol**. 2021;11(6):654-60.
8. Hernaez R, Lazo M, Bonekamp S, Kamel I, Brancati FL, Guallar E, et al. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. **Hepatology**. 2011;54(3):1082-90.
9. Hamaguchi M, Kojima T, Itoh Y, Harano Y, Fujii K, Nakajima T, et al. The severity of ultrasonographic findings in nonalcoholic fatty liver disease reflects the metabolic syndrome and visceral fat accumulation. **Am J Gastroenterol**. 2007;102(12):2708-15.
10. Chivers RC. Tissue characterization. **Ultrasound Med Biol**. 1981;7(1):1-20.
11. Scheipers U, König K, Sommerfeld HJ, Garcia-Schürmann M, Senge T, Ermert H. Sonohistology - ultrasonic tissue characterization for prostate cancer diagnostics. **Cancer Biomark**. 2008;4(4-5):227-50.
12. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. **Gastroenterology**. 2012;142(7):1592-609..

13. Nalbantoglu IL, Brunt EM. Role of liver biopsy in nonalcoholic fatty liver disease. **World J Gastroenterol**. 2014;20(27):9026-37.
14. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. **Hepatology**. 2005;41(6):1313-21.
15. Arun J, Jhala N, Lazenby AJ, Clements R, Abrams GA. Influence of liver biopsy heterogeneity and diagnosis of nonalcoholic steatohepatitis in subjects undergoing gastric bypass. **Obes Surg**. 2007;17(2):155-61..
16. Lamb P, Sahani DV, Fuentes-Orrego JM, Patino M, Ghosh A, Mendonça PR. Stratification of patients with liver fibrosis using dual-energy CT. **IEEE Trans Med Imaging**. 2015;34(3):807-15.
17. Guo D, Qiu T, Bian J, Kang W, Zhang L. A computer-aided diagnostic system to discriminate SPIO-enhanced magnetic resonance hepatocellular carcinoma by a neural network classifier. **Comput Med Imaging Graph**. 2009;33(8):588-92.
18. Acharya UR, Sree SV, Ribeiro R, Krishnamurthi G, Marinho RT, Sanches J, et al. Data mining framework for fatty liver disease classification in ultrasound: a hybrid feature extraction paradigm. **Med Phys**. 2012;39(7):4255-64.
19. Younossi ZM. Non-alcoholic fatty liver disease - A global public health perspective. **J Hepatol**. 2019;70(3):531-44.
20. Wu M, Sharma PG, Grajo JR. The Echogenic Liver: Steatosis and Beyond. **Ultrasound Q**. 2020;37(4):308-14.
21. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. **Am J Gastroenterol**. 1999;94(9):2467-74.
22. Paige JS, Bernstein GS, Heba E, Costa EAC, Fereirra M, Wolfson T, et al. A pilot comparative study of quantitative ultrasound, conventional ultrasound, and MRI for predicting histology-determined steatosis grade in adult nonalcoholic fatty liver disease. **AJR Am J Roentgenol**. 2017;208(5):W168-W177.

Received in: 07-03-2023
Accepted in: 27-03-2023

Conflict of interest: The authors declare that they have no conflicts of interest in relation to this article.
Luís Jesuino de Oliveira Andrade
https://orcid.org/0000-0002-7714-0330
Gabriela Correia Matos de Oliveira
https://orcid.org/0000-0002-8042-0261
Alcina Maria Vinhaes Bittencourt
https://orcid.org/0000-0003-0506-9210
Luís Matos de Oliveira
https://orcid.org/0000-0003-4854-6910

Corresponding Author: Luís Jesuino de Oliveira Andrade
Departamento de Ciências da Saúde
Universidade Estadual de Santa Cruz – UESC – Ilhéus – Bahia,

# ORIGINAL ARTICLE
# TOPICS IN CLINICAL MEDICINE
# B12 VITAMIN DEFICIENCY IN METFORMIN USERS.
## *DEFICIÊNCIA DE VITAMINA B12 EM USUÁRIOS DE METFORMINA.*

EMILLY ALMEIDA MAZON*
THELMA L SKARE**

**Key words:** Metformin, Diabetes mellitus, Vitamin B12
**Descritores:** Metformina; Diabetes mellitus; Vitamina B12

***Abstract***

**Introduction:** The use of metformin in patients with type diabetes (DM) 2 is related to the development of vitamin B12 deficiency, and the prevalence found of this deficiency in Brazil ranges from 6.9% to 32.8%. **Objectives:** To investigate the prevalence of vitamin B12 deficiency and its associated factors in patients with type 2 diabetes mellitus using metformin. **Material and Methods:** A retrospective study was conducted in patients with DM2 using metformin with vitamin B12 dosage. Metformin dose data, data on other medications in use, time of diabetes, and complications such as retinopathy, nephropathy and polyneuropathy were collected. **Results:** The prevalence of B12 deficiency in type 2 diabetic patients using metformin was 26.8%. The deficiency found was not associated with the daily dose of metformin or time of diabetes diagnosis, or with the use of H2 antihistamines, proton pump inhibitors, antiaggregant, or calcium channel blockers. No relationship was found with retinopathy, nephropathy, or diabetic polyneuropathy. **Conclusion:** Vitamin B12 deficiency is frequent in the population with DM2 using metformin. There was no association between vitamin B12 deficiency and the variables investigated. **Endocrinol diabetes clin exp 2023 / 2379 - 2381.**

***Resumo***

**Introdução:** O uso de metformina em pacientes com diabetes tipo (DM) 2 está relacionado com o desenvolvimento de deficiência de vitamina B12, a qual tem uma prevalência que varia de 6,9% a 32,8% no Brasil. Objetivos: Pesquisar a prevalência de deficiência de vitamina B12 e seus fatores associados em pacientes com diabetes mellitus tipo 2 em uso de metformina. **Material e Métodos:** Foi realizado um estudo retrospectivo em pacientes com DM2 em uso de metformina com dosagem de vitamina B12. Coletaram-se dados de dose de metformina, outras medicações em uso, tempo de diabetes, presença de complicações como retinopatia, nefropatia e polineuropatia. **Resultados:** A prevalência de deficiência de B12 em pacientes diabéticos tipo 2 em uso de metformina foi de 26,8%. A deficiência encontrada não se associou com a dose diária da metformina ou tempo de diagnóstico da diabetes nem com uso de anti-histamínicos H2, inibidores de bomba de prótons, anti-agregantes, ou bloqueadores dos canais de cálcio. Também não se encontrou associação com retinopatia, nefropatia e polineuropatia diabética. **Conclusão:** A deficiência de vitamina B12 é frequente na população com DM-2 em uso de metformina. Não houve associação entre deficiência de vitamina B12 com nenhuma das variáveis investigadas. **Endocrinol diabetes clin exp 2023 / 2379 - 2381.**

### INTRODUCTION

Diabetes mellitus defines a disease of abnormal carbohydrate metabolism that are characterized by hyperglycemia. It is linked to a relative or absolute impairment in insulin secretion, along with variable degrees of peripheral resistance to the action of insulin. The chronically elevated glucose levels in the blood are responsible for the macro and microvascular complications that include ischemic cardiopathy, retinopathy, nephropathy and neuropathy among others (1).

According to the Brazilian, American and European Societies of Diabetes, the first treatment choice is metformin (MTF). This drug improves patient's prognosis decreasing insulin resistance, decreasing LDLc, triglycerides and leading to a small weight loss. However, its use is associated to B12 deficiency (2).

B12 vitamin or cobalamin is an enzymatic cofactor found in food of animal origin such as red meat, eggs and dairy products. To be absorbed, in the terminal ileum, the presence of intrinsic factor that is produced by parietal cells from stomach, is needed (3). The B12 functions are related to the fatty acids and amino acids metabolism, and DNA synthesis; this vitamin plays an important role in erythropoiesis and functioning of the nervous system (4).

The consequences of vitamin B12 deficiency include neurological and hematological damage, such as gait instability, autonomic dysfunction, anemia, and neuropathy, which can be confused with peripheral diabetic neuropathy. (5)

The main causes of vitamin B12 deficiency are autoimmune diseases, malabsorption disorders, and food insufficiency. (5) The MTF related B12 deficiency is caused by the interference of this drug in the membrane of the cells that are responsible for the absorption of vitamin B12 in the terminal ileum (3).

This study aimed to determine the prevalence of vitamin B12 deficiency in patients with Type 2 DM using MTF in a sample of the local population, as well as to look for factors associated with the development of this deficiency that could allow prompt clinical identification of such patients.

### MATERIAL AND METHODS

This is a retrospective study made through the analysis of the medical records of patients of an Endocrinology Service of a tertiary hospital in Curitiba, PR and treated between January 2019 and December 2020. In this outpatient clinic, the dosage of B12 is routinely requested in patients with DM using MTF.

Data collection was initiated after approval of the project by the Research Ethics Committee and included patients diagnosed with DM type 2 undergoing treatment with MTF for at least 12 months, of both sexes and older than 18 years.

Exclusion criteria were: diagnosis of type 1 diabetes, interruptions in the MTF treatment or MTF treatment for less than 12 months, history of gastrectomy, vegetarians, individuals with recent vitamin B12 replacement, history of diseases with malabsorption, diagnosis of pernicious anemia and hypothyroidism, and lack of B12 dosage in the patient's medical records.

After literature review, the variables chosen to be collected were: age, gender, time of DM type 2, time of metformin use, metformin dose, serum vitamin B12 and creatinine levels, use

*Faculdade Evangélica Mackenzie do Paraná (FEMPAR) Brazil
**Discipline of Rheumatology - Faculdade Evangélica Mackenzie do Paraná (FEMPAR) Brazil
E-mail: tskare@onda.com.br

of PPI (proton pump inhibitor), use of H2 antihistamines (H2 antihistamines or H2 receptor antagonists), use of calcium channel blockers, use of platelet anti-aggregators, peripheral neuropathy, history of retinopathy, polyneuropathy, or nephropathy.

The criteria for diagnosing comorbidities included:

• Nephropathy: patients with detectable albuminuria on at least two occasions, or at least two records of glomerular filtration rates estimated by CKD-epi formula below 60 mL/min/1.73 m2.

• Diabetic retinopathy: diagnosis established by the ophthalmologist;

• Polyneuropathy: if anomalies in at least two tests at clinical evaluation (such as thermal sensitivity, monofilament, Achilles reflex or vibratory perception), suggestive symptomatology (paraesthesias, hyposensitivity, pain without other identifiable cause) or ENMG (electroneuromyography) tests.

Patients were considered to have vitamin B12 deficiency when they had values below 200 pg/mL. Patients with vitamin B12 values between 200 and 300 pg/mL were classified as patients with possible vitamin B12 deficiency, and patients with values above 300 pg/mL were considered without vitamin B12 deficiency.

The data obtained were entered was submitted to statistical analysis. The chi-square test was used to compare nominal variables. Kruskal-Wallis test was used to compare numerical variables. The significance adopted was 5%.

## RESULTS

Between January 2019 and December 2020, data were collected from 250 medical records that met the inclusion criteria: 70.4% (n = 176) females and 29.6% (n = 74) males. Patients had a median age of 64 (57 - 61) years; the median time of diagnosis of DM type 2 was 12 (7 - 20) years, and the median daily dose of metformin was 2000 (1000 - 2000) mg/day.

Regarding the comorbidities, the prevalence obtained was: nephropathy - 21.6%, retinopathy - 24.8%, and polyneuropathy - 14.0%. About the use of medications, platelet anti-aggregating agents were used in 43.6% of patients, use of calcium channel blockers in 21.6%, and of proton pump inhibitors or H2 antihistamines in 30.4%. Most patients using platelets anti-aggregants used acetylsalicylic acid, while most patients using proton pump inhibitors or H2 antihistaminic were using omeprazole.

In this sample, 67 (26.8%) had B12 deficiency; 89 (35.6%) had probable B12 deficiency and 94 (37.6%) had normal values.

The comparison of individuals with normal values, deficiency and probable disability is found in Table 1. In this table, it is observed that when the groups of patients with and without B12 deficiency were compared, none of the variables presented statistical significance. However, it was found that the group with disabilities had a higher age and shorter time of diagnosis of DM-2 when compared to the group without disability, although it did not reach statistical significance.

The group without deficiency presented a lower daily dose of metformin when compared to the group with disabilities, also without reaching statistical significance. Regarding therapy, it was observed that the group with B12 deficiency showed a non-significant tendency to use PPI or H2 antihistamines, calcium channel blockers, and platelet anti-aggregants when compared to the group without deficiency.

**TABLE 1** - Clinical, epidemiological and treatment comparison of patients with type 2 diabetes mellitus according to B12 serum levels

| | Total (n = 250) | No deficiency (n = 94) | Probable deficiency (n = 89) | With deficiency (n = 67) | P |
|---|---|---|---|---|---|
| Serum B12 (pg/mL) | 257 (192 - 340) | 365 (332 - 428) | 241 (223-264) | 161 (139-185) | <0.0001 |
| Sex (female) | 70.4% | 71.3% | 67.4% | 73.1% | 0.72 |
| Age (years) | 64 (57-71) | 64 (56-83) | 64 (57-71) | 67 (56-71) | 0.82 |
| Disease duration (years) | 12 (7-20) | 12 (8-18) | 12 (7.7-22) | 10.5 (5-20) | 0.63 |
| Dose of MTF mg/dia | 2000 (1000-2000) | 1700 (1000-3400) | 2000 (1000-2000) | 2000 (1000-2000) | 0.52 |
| Creatinine mg/dL | 0.80 (0.67-0.95) | 0.79 (0.68-0.94) | 0.84 (0.69-1.04) | 0.75 (0.64-0.91) | 0.72 |
| Retinopathy (n) | 24.8% | 29.8% | 22.5% | 20.9% | 0.35 |
| Polineuropathy (n) | 14.0% | 14.9% | 14.6% | 11.9% | 0.84 |
| Nephropathy (n) | 21.6% | 17.0% | 28.1% | 19.4% | 0.16 |
| Anti -H2 or proton bomb blockers use | 30.4% | 25.5% | 32.6% | 34.3% | 0.41 |
| Calcium channel blockers use | 21.6% | 16.0% | 24.7% | 25.4% | 0.24 |
| Platelets anti agregant use | 43.6% | 35.1% | 46.1% | 52.2% | 0.08 |

N=number

## DISCUSSION

In the present study, a high prevalence of vitamin B12 deficiency was found in patients with DM type 2 using metformin. The percentage of patients with deficiency and possible vitamin B12 deficiency were, respectively, 26.8% and 35.6%. Previous studies that investigated the prevalence of vitamin B12 deficiency in diabetic patients using metformin showed very heterogeneous results, ranging from 3.3% to 32.8% (6-15). In Brazil, in three studies conducted on vitamin B12 deficiency in diabetic patients using metformin, the prevalence ranged from 6.9% to 32.8% (6,12,13). It is understood that these degrees of deficiency vary according to the geographic region studied, since the diet may influence these results. In another study, conducted in the same city of present, in a sample with a similar size of patients (n = 290) and using the same cutoff point to characterize vitamin B12 deficiency (<200 ng/mL), the prevalence found was 32.8%, moderately higher than the prevalence found today(13). A possible explanation is that in this other study it was not considered diseases with malabsorption as a criterion for excluding the sample.

At present, no statistically significant relationship was found between vitamin B12 deficiency and the variables surveyed. In the literature, it is observed that the main variables that were related to vitamin B12 deficiency were the time of metformin use, (6,7) daily metformin dose, (10,11) time of diabetes and the use of proton bomb blockers. (8,12) However, these findings are not universal.

Regarding comorbidities, De Groot-Kamphluis et al. (9) found a significant association between metformin use and the prevalence of peripheral neuropathy, but studies by Bello et al. (15) did not find this association. It is necessary to consider that diabetes neuropathy can be confused with B12 deficiency neuropathy, bringing a bias in the interpretation of these data.

The recommendations for screening for vitamin B12 deficiency with the use of metformin by the Brazilian Diabetes Society (SBD) have been recently updated. Until the 2019/2020 edition of the SBD, the recommendation was that the follow-up of this patient should be individualized, leaving it up to the physician to choose how to perform it, since the frequency of vitamin B12 dosage to prevent the development of this deficiency with the use of metformin was not clear in the literature. However, as of the 2021 edition, the SBD recommendation states that "vitamin B12 levels should be evaluated annually after 4 years of metformin onset due to the risk of deficiency, and replaced if necessary" (16).

One of the limitations of this study was the lack of the time variable of metformin use – since almost no medical records contained this information. This is important since the duration of metformin treatment was one of the main variables that demonstrated a relationship with the development of vitamin B12 deficiency in the literature(10,11). This fact is probably because this information is not routinely investigated during the data collection in the anamnesis. This information would be beneficial for the prevention of vitamin B12 deficiency in patients using metformin, as the consequences of this deficiency may be irreversible.

Other limitations include the fact that, due to the nature of this study, it was not possible to establish a causal relationship between the use of metformin and the development of deficiency. Indirect markers of vitamin B12 deficiency (methylmalonic acid and homocysteine) were also not collected, which would be useful to exclude false positives and false negatives among the disabled. Another limitation is in relation to the justification for the investigation of vitamin B12 levels in these patients. This may have been performed after clinical suspicion, which may create a bias that selects patients with previous suspicion of vitamin B12 deficiency.

## CONCLUSION

The prevalence of vitamin B12 deficiency in the local MTF user population is 26.8%. The deficiency was not associated with daily dose of MTF and time of diagnosis of DM type 2. In this sample, B12 deficiency could not be associated with H2 antihistamines, proton pump inhibitors, anti-aggregator, and calcium channel blockers use. The prevalence of B12 deficiency was the same in patients with and without diabetic retinopathy, polyneuropathy, and nephropathy.

### References


1. Gross JL, Silveiro SP, Camargo JL, Reichelt A, de Azevedo, M. Diabetes Melito: Diagnóstico, Classificação e Avaliação do Controle Glicêmico. **Arq Bras Endocrinol Metab**. 2002; 46(1:16-26.
2. Sociedade Brasileira De Diabetes. (Brasil). **Diretrizes da Sociedade Brasileira de Diabetes** 2019-2020. 1. ed. São Paulo: Clannad.2019 (1): 38. Disponivel em https://portaldeboaspraticas.iff.fiocruz.br/biblioteca/diretrizes-da-sociedade-brasileira-de-diabetes-2019-2020/. Capturado em setembro de 2022.
3. Paniz C, Grotto D, Schmitt GC, Valentin J, Schott KL, Pomblum VJ, Garcia SC. Fisiopatologia da deficiência de vitamina B12 e seu diagnóstico laboratorial. **J. Bras. Patol. Med. Lab**. 2005;41(5): 323-324.
4. Ahmed MA. Metformin and Vitamin B12 Deficiency: where do we stand? **J Pharm Pharm Sci**. 2016;19(3):382-398.
5. Hvas A, Nexo E. Diagnosis and treatment of vitamin B12 deficiency--an update. **Haematologica**. 2006; 91 (11):1506-1512.
6. Nervo M, Lubini A, Raimundo FV, Faulhaber GA, Leite C, Fischer LM, et al. Vitamin B12 in metformin-treated diabetic patients: a cross-sectional study in Brazil. **Rev Assoc Med Bras**. (1992). 2011; 57(1):46-9.
7. Reinstatler L, Qi YP, Williamson RS, Garn JV, Oakley GP Jr Association of biochemical $B_{12}$ deficiency with metformin therapy and vitamin $B_{12}$ supplements: the National Health and Nutrition Examination Survey, 1999-2006. Diabetes Care. 2012; 35 (2): 327-333.
8. Longo SL, Ryan JM; Sheehan KB; Reid DJ, Conley MP, Bouwmeester, CJ. Avaliação do monitoramento da vitamina B12 em pacientes em uso de metformina em ambulatórios urbanos. **Pharmacy Pract**. (Granada), 2019; 17(3): 1499-1503.
9. de Groot-Kamphuis DM, van Dijk PR, Groenier KH, Houweling ST, Bilo HJ, Kleefstra N. Vitamin B12 deficiency and the lack of its consequences in type 2 diabetes patients using metformin. **Neth J Med**. 2013;71(7):386-90.
10. Kang D, Yun JS, Ko SH, Lim TS, Ahn YB, Park YM, Ko SH.Higher prevalence of metformin-induced vitamin b 12 deficiency in sulfonylurea combination compared with insulin combination in patients with type 2 diabetes: A cross-sectional study. **PLoS One**. 2014;9(10):e109878.
11. Ko SH, Ko SH, Ahn YB, Song KH, Han KD, Park YM, Ko SH, Kim HS. Association of vitamin B12 deficiency and metformin use in patients with type 2 diabetes. **J Korean Med Sci**. 2014;29(7):965-72
12. Damião CP, Rodrigues AO, Pinheiro MF, Cruz RA Filho, Cardoso GP, Taboada GF, et al. Prevalence of vitamin B12 deficiency in type 2 diabetic patients using metformin: a cross-sectional study. **Sao Paulo Med J.** 2016;134(6):473-479.
13. Nazário AR, Brittes KU, Haliski, LB, Mizobuchi, LS, Polonio RR. Prevalência da deficiência de vitamina B12 em indivíduos portadores de diabetes mellitus tipo 2 em uso de metformina. **Rev. Soc. Bras. Clín. Méd**.2018; 16(2): 99-103,
14. Martin D, Thaker J, Shreve M, Lamerato L, Budzynska K. Assessment of vitamin B12 deficiency and B12 screening trends for patients on metformin: a retrospective cohort case review. **BMJ Nutr Prev Health**. 2021;4(1):30-35.
15. Tavares Bello C, Capitão RM, Sequeira Duarte J, Azinheira J, Vasconcelos C. Défice de vitamina B12 na diabetes mellitus tipo 2. **Acta Med Port**. 2017;30(10):719-726
16. Silva Filho R, Albuquerque L, Cavalcanti S, Tambascia M. Tratamento farmacológico da hiperglicemia no DM2. **Diretriz Oficial da Sociedade Brasileira de Diabetes** (2022). ISBN: 978-65-5941-622-6. Disponível em https://diretriz.diabetes.org.br/tratamento-farmacologico-da-hiperglicemia-no-dm2/.



Received in: 04-04-2023
Reviewed in: 06-04-2023
Accepted in: 10-04-2023
Conflict of interest: none


Adress for correspondence: Thelma L Skare
Al. Primavera, 69 - CEP 81210-060 - Mossunguê - Curitiba - PR - Brazil

# TOPICS IN CLINICAL MEDICINE
# ORIGINAL ARTICLE
# EVALUATION OF FEET FUNCTION IN AXIAL SPONDYLOARTHRITIS: A CROSS SECTIONAL STUDY USING THE FOOT AND ANKLE OUTCOME SCORE

## *AVALIAÇÃO DA FUNÇÃO DOS PÉS EM ESPONDILOARTRITIS AXIAIS: UM ESTUDO TRANSVERSAL UTILIZANDO O "FOOT AND ANKLE OUTCOME SCORE".*

[1]OHANA IVANSKI DÓRIA DE VASCONCELOS
[1]YNARAY BELTRÃO BRANDÃO DOS SANTOS
[2,3]ANA PAULA BECKHAUSER
[2]JULIANA SIMIONI
[3]THELMA SKARE

**Key words:** Foot, Axial spondylarthritis, Quality of life
**Descritores:** Pé, Espondilartrite axial, Qualidade de vida

***Abstract***

**Aim:** To study the foot involvement in a sample of Brazilian patients with axial spondylarthritis. **Material and Methods:** The Brazilian version of the Foot and Ankle Outcome Score (FAOS) was applied to 57 axial spondylarthritis patients. Clinical profile was obtained as well as indexes of disease activity by BASDAI (Bath Ankylosing Spondylitis Disease Activity Index), ASDAS (Ankylosing Spondylitis Disease Activity Score)-ESR (erythrocyte sedimentation rate), ASDAS-CRP (C reactive protein) and function by BASFI (Bath Ankylosing Spondylitis Functional Score). **Results:** The most common complaints were difficulties with daily activities followed by pain. The clinical profile showed an inverse association of FAOS with presence of uveitis (P=. 0.009). Activity indexes correlated with FAOS (r=-0.51, p=0.01 for ASDAS-ESR; r=-0.51 with p=0.0004 for ASDAS-CRP and r=-0.66, p<0.0001 with BASDAI). The BASFI also showed a negative correlation (r=0.70; p<0.0001). **Conclusions:** The main foot complaints in axial spondylarthritis are difficulties in daily activities followed by pain. The FAOS correlated negatively with disease activity and general functioning. **Endocrinol diabetes clin exp 2023 / 2382 - 2385.**

***Resumo***

**Introdução:** Os pés podem ser envolvidos nas espondiloartrites axiais causando dor e dificuldades para a marcha. **Objetivo:** Estudar o envolvimento dos pés numa amostra de pacientes brasileiros com espondiloartrites axiais. **Material e Métodos:** A versão brasileira da Foot and Ankle Outcome Score (FAOS) foi aplicada a 57 pacientes com espondiloartrite axial. Obteve-se o perfil clínico, bem como os índices de atividade da doença medidos pelo BASDAI (Bath Ankylosing Spondylitis Disease Activity Index), ASDAS (Ankylosing Spondylitis Disease Activity Score)-VHS (velocidade de hemossedimentação), ASDAS-PCR (proteína C reativa) e função através do BASFI (Bath Ankylosing Spondylitis Functional Index). **Resultados:** As queixas mais comum foram as de dificuldades com as atividades diárias seguidas por dor. O perfil clínico mostrou uma associação inversa de FAOS com a presença de uveíte (P=. 0.009). Índices de atividade correlacionaram-se com FAOS (r=-0,51, p=0,01 para ASDAS-ESR; r=-0,51 com p=0,0004 para ASDAS-CRP e r=-0,66, p<0,0001 com BASDAI). O BASFI também apresentou uma correlação negativa com FAOS (r=0,70; p<0,0001). **Conclusões:** As principais queixas dos pés em espondiloartrite axial são dificuldades nas atividades diárias seguidas por dor. O questionário FAOS correlacionou-se negativamente com a atividade da doença e o funcionamento geral**. Endocrinol diabetes clin exp 2023 / 2382 - 2285.**

## INTRODUCTION

Axial spondyloarthritis are a group of chronic inflammatory rheumatic disease characterized by inflammatory low back pain and radiological findings of sacroiliitis, tendonitis, enthesitis, and extra articular manifestations such as anterior uveitis (1). Genetic factor such as the presence of HLA B27 and ambiental factors are known to play a role on its appearance;(1) mechanical strain favors the localization of inflammatory process (2). So, lower limbs are commonly affected in these patients. A study by Aggarwal et al (3). showed that Achilles tendon is the second most affected site of enthesitis after the chondro-sternal junction and that the ankle is the second most common site for peripheral joint disease after the knee.

Good foot function is important to patients to preserve autonomy and quality of life. So, the frequent involvement of the feet seen in axial spondylarthritis may cause pain and deformities leading to patient's limitations in daily functioning and increasing the risk of falls. In this context the study of foot function and the possible associations of its involvement with other disease variables are important to formulate strategies to minimize this problem.

In this study, a sample of Brazilian patients with axial spondylarthritis was analyzed aiming to know the degree of alterations in the feet function and its relationship with diseases' epidemiological and clinical aspects.

## MATERIAL AND METHODS

**Ethical Issues.** This study was approved by the local Committee of Ethics in Research under protocol 4.377.879. All participants signed consent.

**Sample.** It was form by a convenience sample of axial spondylarthritis patients from a single rheumatology outpatient clinic that came for regular consultations during one year. They were included according to appointment order and willingness

[1]Faculdade Evangélica Mackenzie, Curitiba, PR. Brazil
[2]Spondyloarthitis Outpatient Clinic. Rheumatology Unit from Hospital Universitário Evangélico Mackenzie Curitiba. PR. Brazil
[3]Discipline of Rheumatology Faculdade Evangélica Mackenzie do PR, Curitiba, PR.Brazil
E email- tskare@onda.com.br

to participate on the study. Individuals with disease onset prior to 16 years of age, any other associated rheumatic disease, neurologic or orthopedic problems were excluded. To be included the participants should fulfilled the 2009 ASAS classification criteria for axial spondylarthritis (4).

**Data collection. It included:**

**a) Epidemiological data:** age, sex, ethnic background, tobacco and alcohol use and age at disease onset.

**b) Clinical data:** presence of uveitis, peripheral arthritis, HLA B27, comorbidities, disease activity measured by BASDAI (Bath Ankylosing Spondylitis Disease Activity Index), ASDAS (Ankylosing Spondylitis Disease Activity Score) ESR (erythrocyte sedimentation rate) and ASDAS CRP (C reactive protein).

BASDAI is a 6 items questionnaire that ranges from 0 (no disease activity) to 10 (worst scenario) that considers pain in the spine, peripheral pain and pain in the enthesis, fatigue and morning stiffness (4).

ASDAS is a composite measurement of disease activity that uses either ESR or CRP and total back pain, patient's global score of disease activity, peripheral pain/swelling and duration of morning stiffness. Values under 1.3 are considered as disease inactivity; between 1.3 and under 2.1 as low disease activity, between 2.1 and lower than 3.5 as high disease activity and 3.5 or more as very high disease activity (4).

**c) Feet function questionnaires:** the tool Foot and Ankle Outcome Score or FAOS that assesses how foot pain impacts on patients' quality of life was used. FAOS has been translated and validated for Portuguese language (5).

The FAOS questionnaire is self-applicable and consists of 42 questions divided into five subscales: pain, other symptoms, activities of daily living, sports and recreations and quality of life in relation to the ankle and foot. The last week is considered when the questionnaire is answered. Each question has standard options with a score from 0 to 4. A total score is calculated for each subscale, where 100 indicates no symptoms and 0 indicates extreme symptoms (5).

**d) BASFI or Bath Ankylosing spondylitis functional score** - It measures the general function of the patient with AS through a 10 item self-applicable visual scales (0 - 10 cm) anchored by the descriptors "easy" and "impossible." All questions are related to the patient's ability to perform daily activities. The total score is calculated by the arithmetic mean of the individual scores. The final score of zero means absence of functional impairment and 10 the worst scenario (4).

**Statistical analysis.** Mann Whitney tests was used to analyze values of FAOS according to epidemiological and clinical variables. Correlation studies of numerical variables (age, disease duration, activities indexes and BASFI) with FFI and FAOS were done by Spearman test. To judge data distribution, the Shapiro-Wilk test was used. The adopted significance was 5%. Tests were performed using GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com.

## RESULTS

**A) Description of studied sample.**

The sample had 57 patients. The epidemiological and clinical characteristics and activity indexes of this sample are on Table1. This table shows that according to median values of ASDAS (but not BASDAI), the sample had moderated to high disease activity.

**TABLE 1** - Main characteristics of studied sample: 57 patients with axial spondyloarthritis

| | |
|---|---|
| Male/female sex | 33 (57.8%) /24 (42.1%) |
| Mean age - (years) | 50.13±10.98 |
| Mean age at diagnosis - (years) | 37.97±11.14 |
| Mean disease duration - (years) | 10.46±7.46 |
| Ethnic background | |
| Euro descendants | 37 (64.9%) |
| Afro descendants | 20 (35.0%) |
| HLA B27(*) (n) | 37 (75.5%) |
| Uveitis (n) | 26 (45.6%) |
| Enthesitis | 28 (49.1%) |
| Dactilitis | 8 (14.0%) |
| Peripheral arthritis | 19 (33.3%) |
| Activity index | |
| Median ASDAS VHS (IQR) | 2.2 (1.72-3.6) |
| Median ASDAS PCR (IQR) | 2.1 (1.5-2.9) |
| Median BASDAI (IQR) | 3.8 (2.0-5.35) |
| Median BASFI (IQR) | 3.7 (1.42-6.25) |
| Comorbidities | |
| Arterial hypertension | 16 (28.0%) |
| Dyslipidemia | 13 (22.8%) |
| Diabetes | 6 ( 10.5%) |

*(*) data on 49 patients. ASDAS= Ankylosing Spondylitis Disease Activity Score; ESR= erythrocyte sedimentation rate; CRP= C reactive protein; BASDAI= Bath Ankylosing Spondylitis Disease Activity Index; BASFI= Bath Ankylosing Spondylitis Functional Index; n= number; IQR= interquartile range.*

**B) Study of FAOS in relation to spondylarthritis clinical variables:**

**Table 2** shows the comparison of FAOS values according to clinical and epidemiological variables. An association of good feet function and presence of uveitis was noted.

**TABLE 2:** Study of FAOS (Foot and Ankle Outcome Score) according to clinical and epidemiological variables in 57 patients with axial spondyloarthritis.

| | FAOS median values with the variable | FAOS median values without the variable | P |
|---|---|---|---|
| Male gender | 88.0 (59.9-99) | 73.6 (64.0-91.0) | 0.32 |
| Euro descendants | 88.0 (67.0-98.0) | 69.0(58.7-96.5) | 0.10 |
| HLA-B27 | 86.0 (60.5-98.0) | 76.5(61.5-91.0) | 0.55 |
| Uveitis | 92.0 (78.5-99.0) | 69.0 (59.0-88.0) | **0.009** |
| Enthesitis | 72.5 (57.2-93.5) | 88.0 (68.0-99.0) | 0.10 |
| Dactilitis | 61.0 (55.0-78.2) | 86.0 (62.5-98.0) | 0.09 |
| Peripheral arthritis | 67.0 (58.0-92.0) | 88.0 (66.5-98.0) | 0.10 |

*OBS- Between brackets- interquartile range; Values scored in %. FAOS- ranges from 0-100 with 100 meaning good performance.*

The correlation studies of FAOS with activity indexes, age and disease duration are on **Table 3.** A good correlation of foot function with inflammatory index and general function was found.

**Table 3 –** Correlation studies of FAOS (Foot and Ankle Outcome Score) with activity indexes, BASFI, age and disease duration.

| | R | 95% Confidence interval | P |
|---|---|---|---|
| Age | -0.11 | -0.38 to 0.16 | 0.40 |
| Disease duration | 0.13 | -0.15 to 0.40 | 0.33 |
| ASDAS ESR | -0.39 | -0.62 to -0.09 | 0.01 |
| ASDAS CRP | -0.51 | -0.70 to 0.24 | 0.0004 |
| BASDAI | -0.66 | -0.79 to-0.48 | <0.0001 |
| BASFI | -0.70 | -0.81 to 0.53 | <0.0001 |

*ASDAS= Ankylosing Spondylitis Disease Activity Score, ESR= erythrocyte sedimentation rate, CRP= C reactive protein, BASDAI= Bath Ankylosing Spondylitis Disease Activity Index; BASFI= Bath Ankylosing Spondylitis Functional Index.*

The study results of FAOS study according to the different domains is on **Figure 1.**

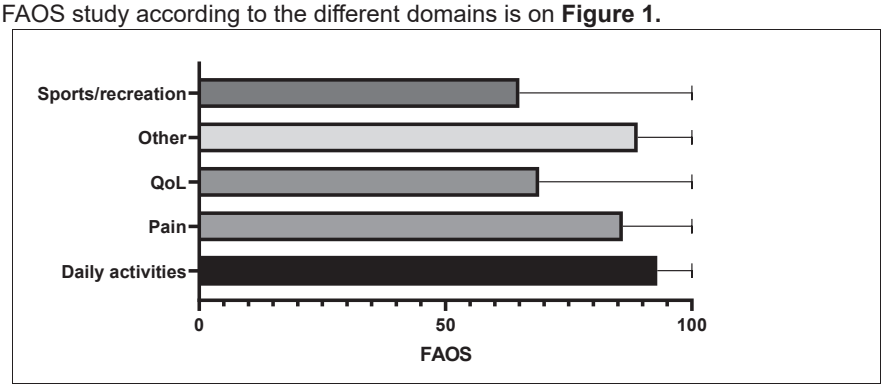



**Figure 1 - Performance of FAOS domains in 57 patients with axial spondylarthritis.**

*The lowest values were on Sports/recreation (median 65%) followed by quality of life (median 69%) and the highest values were on daily activities (median value of 93%) followed by others (median value of 89%). OBS- others=stiffness, edema, dorsal and plantar flexion, creaking and locking).*

## DISCUSSION

This study showed that a high proportion of patients with axial spondyloarthritis have feet complaints mainly those related to daily activities. It also showed that these complaints correlated with disease activity indexes and with general function.

The foot has 31 joints and it is separated in three chief regions: forefoot (phalanges and metatarsals), mid foot (cuboid, navicular, and cuneiforms) and hind foot (talus and calcaneus). It has a great number of periarticular structures such as fascia and entheses whose inflammatory involvement followed by new bone formation is one of the main mechanisms of structural damage in spondylarthritis (6). The foot, with the pelvis, is the structure that bears most weight answering for load distribution through lower limbs, spine, foot arches and tarsal areas. Mechanical stress induces inflammation in spondyloarthritis (1,2). The lower limb involvement may lead to gait and equilibrium impairments - with imbalance and increased risk of falls (7-9). Furthermore, mid foot involvement, also called tarsitis, causes pain and swelling in the mid foot; The inflammatory involvement of the ankle, Achilles tendon and plantar fascia may progress, in severe cases, to ankylosing tarsitis (1). In this study we did not individualize the involved foot structure and this is one its limitation.

Not disease duration neither age, but inflammatory activity correlated with foot complaints presently. To treat this type of involvement efficiently and to decrease foot problems in axial spondylarthritis, it should be considered that the pathophysiologic mechanism of entheseal inflammation has some particularities. The discovery that IL-23-responsive population of T cells and the description of a cluster of cytokine-dependent lymphoid cells (ILCs) participating in the entheseal inflammatory process suggest a contribution of the innate immune cells (10,11). Conventional DMARDs such as methotrexate or sulphasalazine are not effective (10); NSAIDs continue to be the first-line therapy. Other choices considered effective are local I infiltrations with glucocorticoids, use of anti TNF, anti-IL 17, and JAK inhibitors (9,10,12). However, enthesitis treatment is difficult and remains a challenge.

Impairment of daily activities according to FAOS was the most common complaint in the current study. A study by Koka et al. (6) in individuals with ankylosing spondylitis from Turkey observed that disability also the most common complaint in their sample; they also verify correlation of foot function impairment with inflammatory activity, as presently.

This study is limited by the low number of included patients, by its cross-sectional design, and lack of image studies that would allow the recognition of involved anatomic structures. However, it highlights that foot involvement is frequent in axial spondylarthritis and that causes pain and difficulties in daily living. It also shows a good correlation with disease's activity by the three used instruments (ASDAS -ESR, ASDAS -CRP and BASDAI) showing that the inflammatory control is an important measure to be taken to reduce this problem.

## CONCLUSION

This study shows that foot involvement is common in axial spondylarthritis; that, in this context, the main complaint is impairment in daily activities and that the foot involvement is related to inflammatory activity.

### References


1. Romero-López JP, Elewaut D, Pacheco-Tena C, Burgos-Vargas R. Inflammatory foot involvement in spondyloarthritis: From tarsitis to ankylosing tarsitis. **Front Med** (Lausanne). 2021; 8:730273. doi: 10.3389/fmed.2021.730273. eCollection 2021.
2. Debusschere K, Cambré I, Gracey E, Elewaut D. Born to run: the paradox of biomechanical force in spondyloarthritis from an evolutionary perspective. **Best Pract Res Clin Rheumatol**. 2017; 31:887–94. doi: 10.1016/j.berh.2018.07.011.
3. Aggarwal R, Malaviya AN. Clinical characteristics of patients with ankylosing spondylitis in India. **Clin Rheumatol.** 2009; 28: 1199-205. doi: 10.1007/s10067-009-1227-7.
4. Sieper J, Rudwaleit M, Baraliakos X, Brandt J, Braun J, Burgos-Vargas R, et al. The Assessment of Spondyloarthritis International Society (ASAS) handbook: a guide to assess spondyloarthritis. **Ann Rheum Dis**. 2009; 68 Suppl 2:ii1-44. doi: 10.1136/ard.2008.104018.
5. Imoto AM, Peccin S, Rodrigues R, Mizusaki JM. Translation, cultural adaptation and validation of FOOT and ankle outcome score (FAOS) questionnaire into Portuguese. **Acta Ort. Bras**. 2008;17(4):232-5. doi.org/10.1590/S1413-78522009000400008.
6. Koca TT, Göğebakan H, Koçyiğit BF, Nacitarhan V, Yildir CZ. Foot functions in ankylosing spondylitis. **Clin Rheumatol**. 2019; 38:1083-1088. doi: 10.1007/s10067-018-4386-6.
7. Woodburn J, Hyslop E, Barn R, McInnes IB, Turner DE. Achilles tendon biomechanics in psoriatic arthritis patients with ultrasound proven enthesitis. **Scand J Rheumatol.** 2013; 42:299302. doi: 10.3109/03009742.2012.747626.
8. Carter K, Walmsley S, Oliffe M, Hassett G, Turner DE. Increased falls risk in people with psoriatic arthritis-related foot problems: a novel finding. **Rheumatology** (Oxford). 2021; 60:976-977. doi: 10.1093/rheumatology/keaa654.
9. Mickle KJ, Munro BJ, Lord SR, Menz HB, Steele JR. Foot pain, plantar pressures, and falls in older people: a prospective study. **J Am Geriatr Soc.** 2010; 58: 193640. doi: 10.1111/j.1532-5415.2010.03061.x.
10. Watad A, Cuthbert RJ, Amital H, McGonagle D. Enthesitis: Much more than focal insertion point inflammation. **Curr Rheumatol Rep**. 2018; 20:41. doi: 10.1007/s11926-018-0751-3.
11. Spits H, Artis D, Colonna M, Diefenbach A, Di Santo JP, Eberl G, et al. Innate lymphoid cells—a proposal for uniform nomenclature. B. 2013; 13:145–9. doi: 10.1038/nri3365.
12. Ebihara S, Date F, Dong Y, Ono M. Interleukin-17 is a critical target for the treatment of ankylosing enthesitis and psoriasis-like dermatitis in mice. **Autoimmunity**. 2015;48 (4):259–66. doi: 10.3109/08916934.2014.976630.
13. Sakkas LI, Alexiou I, Simopoulou T, Vlychou M. Enthesitis in psoriatic arthritis. **Semin Arthritis Rheum.** 2013; 43: 325–34. doi: 10.1016/j.semarthrit.2017.12.008.



Received in: 02-03-2023
Accepted in: 06-03-2023
Conflict of interests: none
Funding: none


Correspondence address: Thelma L Skare.
Travessa Luiz Leitner, 50. Curitiba, PR.
CEP-80730-000

# Instructions for the publication of the Journal Endocrinology & Diabetes Clinical and Experimental

## The journal follows the International Committee of Medical Journal Editors

**01** All the manuscripts will be published in English. The journal accepts original articles, preliminary notes, case reports, review articles, updates and letters to editor. There a topic dedicate to internal medicine linking endocrinology and medical clinic. The journal strongly encourages on line submissions of manuscripts. Those should be accompanied by a title, keywords and an abstract in English for the purposes of international registration. Abstracts in other languages may also be attached.

**02** The articles received by the Editor will be analyzed with the Assistance of the Editorial Board. Minor changes to "copy desk" can be effective with the purpose of standardizing the articles, without substantial changes in original text.

**03** Manuscripts can be sent on CD or via on line to publicacao@revistaendocrino.com. The text should be typed on pages containing 20 to 24 rows and rows with 70 to 75 spaces, with the objective of enabling the diagramming the calculation of space required for each article. The word processor used must be either Microsoft Windows compatible program (Word, Write etc.).

**05** The article must have title, full name of the authors; quote from site (full address) where out performed the work; full titles of authors, key words (or "keywords") without exceeding a limit of 250 words; introduction; material or material and methods or description of the case; results; discussion and/or comments (when applicable); conclusions (when applicable); summary (summary in English), consisting in the correct version of the summary, not exceeding 250 words; references (as quoted below in item 08) in alphabetical order; the accompanying illustrations must follow appropriate rules, described in item 07.

**06** Illustrations are of figures and graphs referred to in Arabic numerals (example: fig. 3, graph 7), in the form of ink drawings photographs ECG EEG etc. When possible must be submitted in original form. The illustrations will be accepted only allow good reproduction. Should not be glued in the middle of the article text and it must be attached with the respective legends typed on the bottom of the same (one sheet for each illustration). Must take care to number each illustration on the back of the same and indicate the correct place where should be introduced. Tables and frames are specified in Arabic numerals, consisting always the respective title, accurately. Tables and frames without its description in the text and are intended to summarize the article. The units used to express the results (m, g, g/100 ml, etc.) will appear at the top of each column. It will be up to the Editor to judge excessive illustrations (figures, tables, graphs, tables etc.), deleting the redundant.

**07** The references must follow the alphabetical order or the order of appearance in the text. Showing them all authors cited in the text. It must be contain: name of author, name of the journal abbreviated in accordance with the criteria used in the Index Medicus (www.nlm.nih.gov/tsd/serials/lji.html). Papers accepted but not yet published may be included in the references. You should avoid using as reference poster or free themes from conferences unless they are of high relevance. Articles published online may be cited in the references and should bear the name of the site as well as the date of access.Chapter of Book: Ruch, TC.Somatic Sensation. In Ruch T C et al. Neurophysiology. Philadelphia Saunders 1963; 330-332 Journal article: R.W.G Gruessner, Sutherland D.E.R, Najarian j. S, et al. Solitary pancreas transplantation for non uremic patients with insulin-dependent diabetes mellitus labile. Transplantation 1997; 64: 1572-77.

**08** The names of drugs cited in the text (names of fantasy, officers, patented, and acronyms of chemical research) shall comply with corresponding regulations of the World Health Organization, according to rules summarised by KOROLKOVAS, a.-Regulatory Editorial Nomenclature-Names of drugs (Drug Nomen--clature). Rev. Bras. Clin. Terap. 5: 1976 (February).

**09** The authors will receive ten copies of the issue in which their work was published (for reprints), which will be sent directly to the place where the work performed. Reprints must be ordered and previously combined with the Commercial Direction.

**10** The manuscripts that don't fit the standards or that does not suit the needs of the journal editorials may be forwarded to the authors to carry out the necessary adjustments that will be indicated in the personal letter from the Editor. Will be mentioned the dates of receipt and approval of work for publication, in order to safeguard the interests of the author's priority. In the case of re-routing of work to adapt to our rules for publication, the date cited is always receive the first forwarding of work. The content of the articles is the responsibility of the authors. The link between the author (s) and pharmaceutical laboratories, as well as another source that is generating resources must always be quoted by author (s). The copyright of the manuscripts are of the magazine in question.

**11** Will be given top priority in the publication of articles and/or notes that they concerned about matters directly or indirectly related to the basic purpose of the journal Endocrinology & Diabetes Clinical and Experimental


ENDOCRINOLOGIA & DIABETES CLÍNICA E EXPERIMENTAL
FEMPAR - HUEM - CURITIBA - BRASIL