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sumário

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& METABOLOGIA

BRAZILIAN ARCHIVES OF ENDOCRINOLOGY AND METABOLISM

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Bariatric surgery – An update for the endocrinologist

Cirurgia bariátrica – Uma atualização para o endocrinologista

Marcio C. Mancini[1]

ABSTRACT

Obesity is a major public health problem, is associated with increased rates of mortality risk and of developing several comorbidities, and lessens life expectancy. Bariatric surgery is the most effective treatment for morbidly obese patients, reducing risk of developing new comorbidities, health care utilization and mortality. The establishment of centers of excellence with interdisciplinary staff in bariatric surgery has been reducing operative mortality in the course of time, improving surgical safety and quality. The endocrinologist is part of the interdisciplinary team. The aim of this review is to provide endocrinologists, physicians and health care providers crucial elements of good clinical practice in the management of morbidly obese bariatric surgical candidates. This information includes formal indications and contraindications for bariatric operations, description of usual bariatric and metabolic operations as well as endoscopic treatments, preoperative assessments including psychological, metabolic and cardiorespiratory evaluation and postoperative dietary staged meal progression and nutritional supplementation follow-up with micronutrient deficiencies monitoring, surgical complications, suspension of medications in type 2 diabetic patients, dumping syndrome and hypoglycemia. Arq Bras Endocrinol Metab. 2014;58(9):875-88

Keywords

Bariatric surgery; Roux-en-Y gastric bypass; biliopancreatic diversions; obesity; weight loss

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RESUMO

A obesidade é um problema de saúde pública, está associada com aumento do risco de mortalidade e de desenvolver diversas comorbidades e diminui a expectativa de vida. A cirurgia bariátrica é o tratamento mais eficaz para pacientes com obesidade mórbida, reduzindo o desenvolvimento de novas comorbidades, a utilização dos cuidados de saúde e a mortalidade. A criação de centros de excelência com equipes interdisciplinares em cirurgia bariátrica vem reduzindo a mortalidade operatória no decorrer do tempo, melhorando a segurança e a qualidade cirúrgica. O endocrinologista faz parte da equipe interdisciplinar. O objetivo desta revisão é fornecer aos endocrinologistas, médicos e prestadores de cuidados de saúde elementos cruciais de boas práticas clínicas no tratamento de pacientes com obesidade mórbida candidatos à cirurgia bariátrica. Essas informações incluem indicações formais e contraindicações para as operações bariátricas, descrição das operações bariátricas e metabólicas habituais, bem como tratamentos endoscópicos, avaliação pré-operatória, incluindo avaliação cardiorrespiratória psicológica, metabólica e no pós-operatório, dieta com refeições progressivamente estagiadas e seguimento com suplementação nutricional e monitoramento de deficiências de micronutrientes, complicações cirúrgicas, suspensão de medicamentos em pacientes diabéticos tipo 2, síndrome de dumping e hipoglicemia. Arq Bras Endocrinol Metab. 2014;58(9):875-88

Descritores

Cirurgia bariátrica; bypass gástrico em Y de Roux; desvios biliopancreáticos; obesidade; perda de peso

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INTRODUCTION

Obesity is a major public health problem (1). The past three decades have seen an astonishing increase in obesity rates, and it is usually assumed that environmental changes are the main causes, although genetic bases can undoubtedly be involved (2). In 2008, according to the World Health Organization, 1.4 billion adults were overweight and 500 million adults worldwide were obese. It has been projected that 2/3 of the world's population could be overweight (2.2/3.3 billion) or obese (1.1/3.3 billion) by 2030 (2). Obesity is common and is associated with increased rates of mortality and morbidity (3,4). Excess weight radically elevates an individual's risk of developing a quantity of non-communicable diseases, such as type 2 diabetes, hypertension, dyslipidemia, sleep apnea, non-alcoholic steatohepatitis, stroke, cancer, coronary heart disease, osteoarthritis, gallbladder disease, as well as respiratory complications, infertility, reduced quality of life and psychological difficulties (3-5). Obesity appears to lessen life expectancy markedly, especially among individuals in younger age groups adults (6), and the lifetime of morbidly obese subjects is decreased by an estimated 5-20 years depending on gender, age and race (1,7).

Bariatric surgery has demonstrated to be the most effective method of treatment of the morbidly obese patients (1,7,8). The long-term studies provide evidence of substantial reduction in: risk of developing new comorbidities, health care utilization and mortality in bariatric surgery subjects (7,9,10). Bariatric operations are a recognized and fundamental part in the treatment of morbid obesity (7,8). The aim of this review is to provide endocrinologists, physicians and health care providers crucial elements of good clinical practice in the management of morbidly obese patients. Standard search strategy was used to retrieve international journal articles from PubMed database within the last 10 years. The interventions of interest were bariatric operations and the outcomes were improvement in comorbidities, weight loss and adverse effects.

PREOPERATIVE PHASE

The interdisciplinary team consists of the following specialists experienced in obesity management and bariatric surgery: endocrinologist or specialized physician, bariatric surgeon, nutritionist, psychiatrist or psychologist, anesthetist, nurse, social worker (11). The bariatric procedures should be done at interdisciplinary obesity management centers with properly qualified staff and adequate equipment. The bariatric surgeon's skill is a crucial issue. It is not prudent to practice bariatric techniques on a sporadic basis (12).

The formal indications for bariatric operations are: 1) patients in age groups from 18 years to 65 years (in some guidelines the limit is 60 years) (11). Over 65 years, a specific evaluation considering surgical and anesthetic risk, presence of co-morbidities, life expectancy, benefits of weight loss, and age-limitations such as esophagic dismotility and osteoposoris. In elderly patients the objective of the operation is mainly to improve quality of life (8). 2) Body mass index (BMI) greater than or equal to 40 kg/m^2 or 35 kg/m^2 with one or more severe obese related co-morbidities (in which surgically induced weight loss is likely to improve the condition). 3) The patients should have failed to lose weight or to maintain weight loss despite appropriate medical care (11).

In the 2013 AACE, TOS, and ASMBS Medical Guidelines for Clinical Practice for the Perioperative Nutritional, Metabolic, and Nonsurgical Support of the Bariatric Surgery Patient (2013 AACE-TOS-ASMBS CPG) among these more severe obesity-related co-morbidities were included type 2 diabetes, hypertension, hyperlipidemia, obstructive sleep apnea (OSA), obesity-hypoventilation syndrome (OHS), Pickwickian syndrome (a combination of OSA and OHS), nonalcoholic fatty liver disease (NAFLD) or nonalcoholic steatohepatitis (NASH), pseudotumor cerebri, gastroesophageal reflux disease (GERD), asthma, venous stasis disease, severe urinary incontinence, debilitating arthritis, and considerably impaired quality of life, also could be included among debilitating conditions allowing to be offered a bariatric procedure (13). In the 2013 CPG, patients with BMI of 30-34.9 kg/m^2 with diabetes or metabolic syndrome were also included to be offered a bariatric procedure, although current evidence is limited by the number of subjects studied and lack of long term data demonstrating net benefit. This new position in the 2013 Guidelines has emerged because it has become hugely ostensible that bariatric surgery exerts a robust and beneficial effect on type 2 diabetes, leading to high rates of remission and reduction in diabetes-related deaths. Further, the mechanisms that lead to the improvement of type 2 diabetes seem to be not fully dependent of the reduction of food intake and

bodyweight (*e.g.*, reduction in ghrelin levels, increase in adiponectin and glucagon-like peptide-1 levels) (13). The International Diabetes Federation allows bariatric surgery acceptable for subjects with a BMI greater than or equal to 30 kg/m² and type 2 diabetes who have failed to respond accordingly to lifestyle modifications and at least two oral antidiabetic medications (14).

In some countries (like Brazil) the patient should have be in the BMI greater than or equal to 35 or 40 for at least two years (except if a severe co-morbidity is present or if BMI is greater than or equal to 50) (15). Surgery is guaranteed in patients who had a previous BMI above the limit, exhibited a considerable weight loss in a medical therapy, but are below the minimum indication weight for surgery (11).

Recently the inferior limit in Brazil was lowered from 18 to 16 years if the Z-score BMI is greater than or equal +4 and the epiphyseal growth plate is already closed when there is an agreement between the legal guardian and the multidisciplinary team. Below 16 years of age, an ethical committee must be consulted after cautious attention, in exceptional cases such as genetic syndromes (*e.g.* Prader-Willi) (15,16).

The main contraindications for bariatric surgery are the absence of clinical treatment or management, the existence of active non-stabilized psychiatric illnesses such as major depression and psychotic disorders, alcohol and/or drug active dependencies, diseases threatening life in the short term, and patients incapable to care for themselves without family or social support (11).

In the preoperative phase, it is of crucial importance to evaluate the patient motivation and disposition to adhere to follow-up programs, certifying that the patient is entirely informed on the benefits and risks of the surgical choices and also the need of lifetime follow-up, as well as the likely limited results of the operations. In this context, the evaluation should include a psychological and social examination to assess expectation and motivation, and also life conditions and social support network (11).

It is also important to clarify the dietary, behavioral and lifestyle changes mandatory after the operation. Depending on the scheduled procedure and clinical status of the patient, the patient should undertake additional assessment for metabolic and endocrine disorders, sleep apnea and cardiopulmonary function, gastroesophagic disorders, bone density and bone composition (11). The presence of eating pathologies such as binge eating disorder (BED) increase the risk of lower weight loss and weight regain after bariatric surgery (11) and also of developing other eating disorder patterns such as post-surgical refusal to eat (17).

OPERATIONS TECHNIQUES

The usual bariatric and metabolic operations that are presently offered for patients needing weight loss and/or metabolic control are: adjustable gastric banding (AGB), sleeve gastrectomy (SG), Roux-en-Y gastric bypass (RYGB), biliopancreatic diversion/Scopinaro (BPD-S) and BPD/duodenal switch (BPD-DS) (Figure 1). At this time, there is still insufficient evidence to generalize in favor of one bariatric surgical procedure for the morbidly obese population. Physicians should exercise caution when recommending BPD-S, BPD-DS, or related procedures because of the greater associated nutritional risks related to the increased length of bypassed small intestine. A laparoscopic procedure should be considered as the desirable method to the operation in bariatric surgery, as long as no contraindications for the laparoscopic approach exist. The most common performed procedures in Brazil are RYGB and SG. AGB is less performed nowadays due to weight regain and less weight loss and BPD due to nutritional risks (13).

The RYGB comprises the construction of a small gastric pouch that is then connected to a distal segment of small intestine (alimentary limb, length ~100 cm). The remnant of the stomach is left *in situ* but is disconnected from the food stream. It reconnects with the alimentary limb at the jejuno jejuno anastomosis (biliopancreatic or digestive limb, length ~60 cm). The restrictive component is based on the small pouch as well as the narrow aperture connecting the gastric pouch to the jejunum. The RYGB can be performed placing a band above the gastro jejuno anastomosis (banded gastric bypass) to restrict the inside width to around one centimeter, although most surgeons do not use this procedure. The malabsortive component is marginal at best. Early complications associated to the procedure are reported to be leakage, bleeding pulmonary embolus, and gastrojejunal strictures and late complications include internal hernias, ulcers, vitamin deficiencies, and anemia (12,13) (Figure 1).

The SG involves removing 80% of the stomach, leaving behind only a "sleeve" of the stomach (18) (Figure 1).

The AGB is placed around the top portion of the stomach to reduce stomach size restricting the volume of ingested solid food. The band itself is made of silicone and is connected via plastic tubing to a port implanted in the abdominal wall. The quantity of fluid injected into the port gradually increases the restriction on the stomach. Patients must have frequent follow-ups with their physicians to titrate the volume placed into the port. No alteration of the anatomy is required, and thus the procedure is completely reversible. The mortality is 0.05%. Complications include band erosions, esophageal dilatation, port infections, band slips, reoperations and port problems (13) (Figure 1).

BPD-S is a malabsortive procedure is which a distal gastrectomy and a Roux-en-Y configuration are created with a short common limb. The alimentary limb is 250 cm length and the common limb is only 50 cm. The stomach links straight to the ileum. The duodenal switch (BPD-DS) procedure is similar to the Scopinaro technique, but the gastrectomy is vertical, not distal, a duodenoileal anastomosis is performed to enhance absorption of minerals, to preserve the pilorum and the common limb is a little bit longer (75-100 cm). BPD can cause significant complications, such as hypovitaminosis, hypoalbuminemia, severe metabolic bone disease, and cirrhosis (13) (Figure 1).

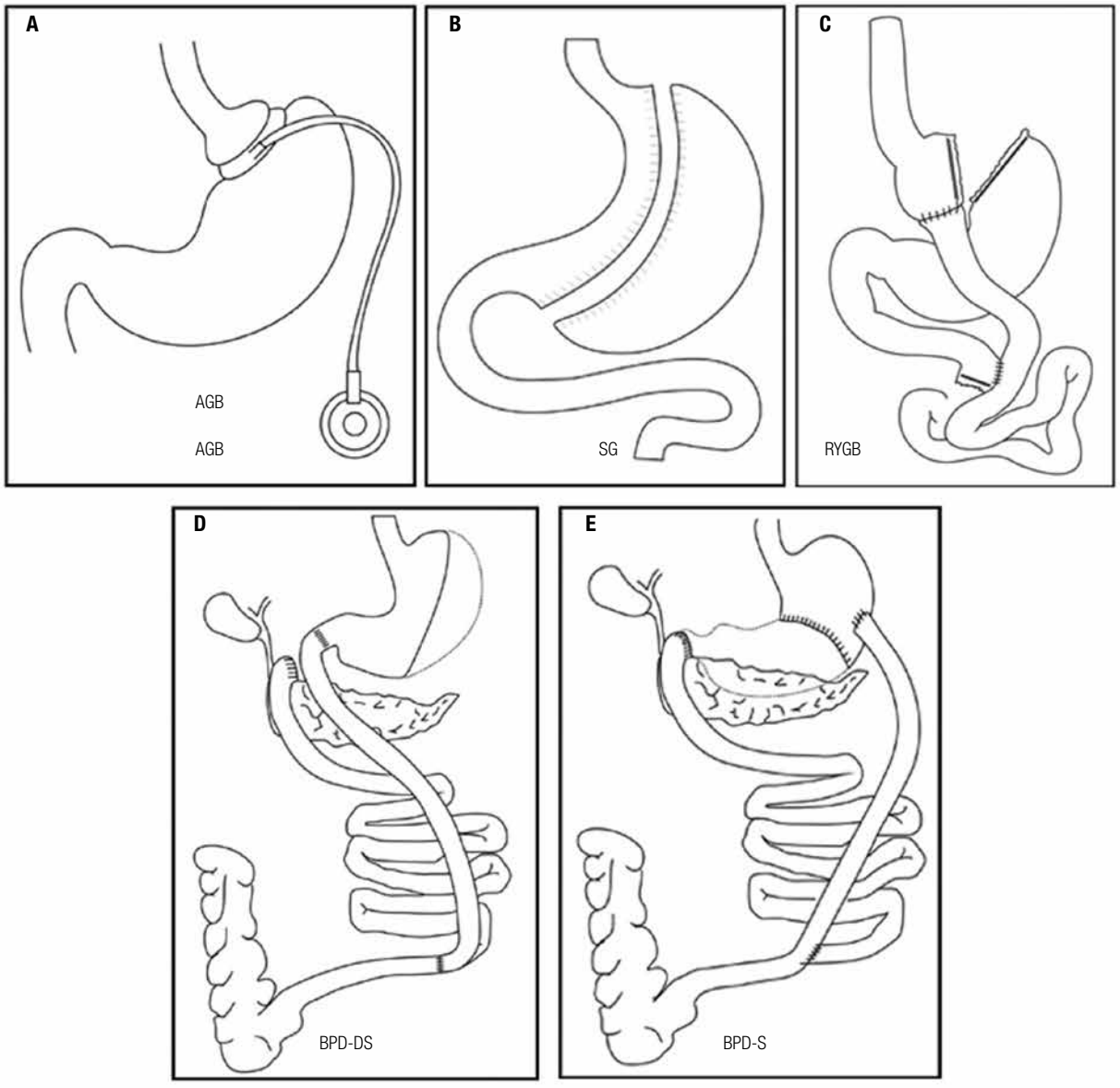

Figure 1. Usual bariatricoperations: (**A**) adjustable gastric banding (AGB), (**B**) sleeve gastrectomy (SG), (**C**) Roux-en-Y gastric bypass (RYGB), (**D**) BPD/duodenal switch (BPD-DS), and (**E**) biliopancreatic diversion/Scopinaro (BPD-S).

Other surgical techniques have been proposed as bariatric and/or metabolic procedures: duodenojejunal bypass (sleeve gastrectomy with alimentary limb of 150 cm and biliopancreatic limb of 100 cm), omentectomy, ileal interposition with sleeve gastrectomy, sleeve gastrectomy with omentectomy and jejunectomy, bipartition of the gastintestinal transit (ileus brought directly to the stomach without occluding the duodenum and an anastomosis between the jejunum and ileum below brings the segments together again). Although some of these procedures are highly promising, they are currently considered experimental (19).

Intragastric balloon treatment is an endoscopic treatment. The balloon is placed inside the stomach for six months and inflated with saline solution, promoting a weight loss of 5-9 kg/m² with improvement of obesity-related comorbidities. A regain of weight in a 1-year follow-up period of 25-40% and a failure rate of 15% in studies that defined a successful weight loss have to be considered. Gastrointestinal complications, mainly esophagitis, nausea and vomiting were present in more than 5% of patients, leading to intolerance and resulting in its removal in 7%. Balloons deflated in 8%, and some of these patients had to be operated. Intragastric balloon treatment can be a valuable method in selected patients, such as super obese patients preoperatively who need to lose weight to minimize risks before undergoing bariatric surgery, but nonresponse, intolerance and weight regain have to be taken into account (20).

With the concept that the exclusion of the proximal segment of the small intestine (biliopancreatic limb of RYGB), an endoscopic single, minimally invasive device for temporary duodenal exclusion (EndoBarrier) was developed, consisting of a metal anchoring system with tiny hooks and a sleeve made of a polymer about 60 cm long which prevents contact of food with the biliary and pancreatic secretions until the initial segment of the jejunum (Figure 2). This device, which has been studied for up to one year in obese diabetic patients by our group in the Hospital das Clínicas, was effective in reducing weight, hemoglobin A1c and weight control in type 2 diabetes (21) and is already being used in several European countries and Chile (in Brazil is in process of scrutiny by National Health Surveillance Agency – Anvisa). Possible mechanisms of action include malabsorption of calories, abnormal gastrointestinal motility and modulation of gastrointestinal neurohormonal signaling (21,22).

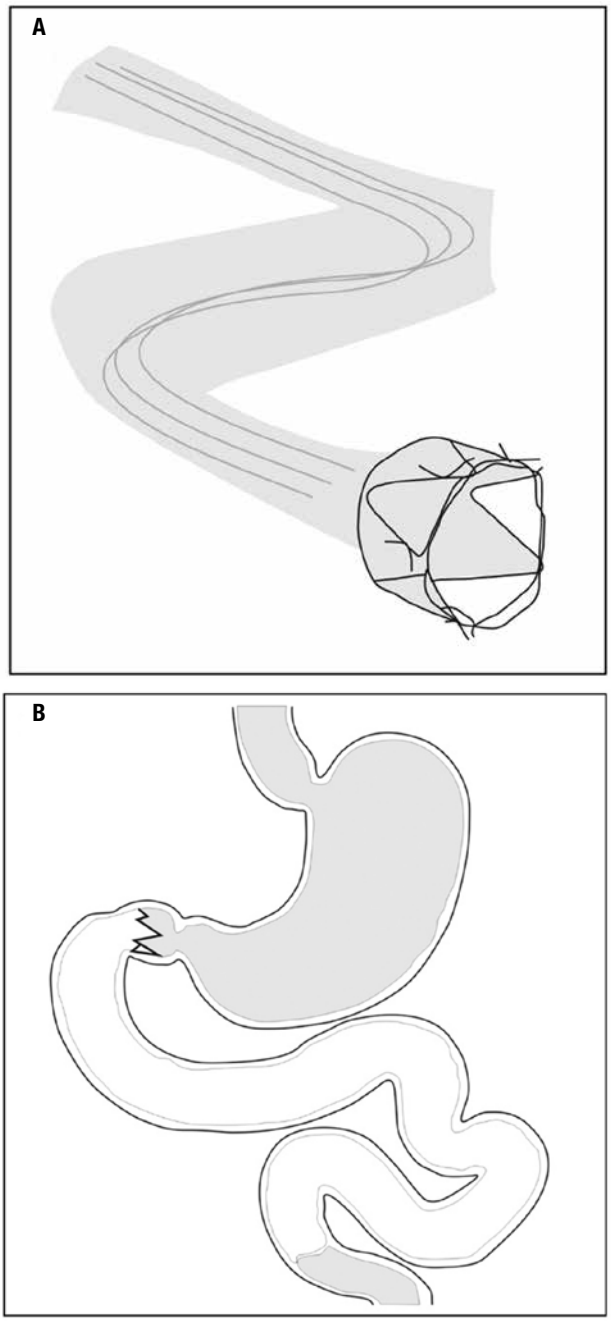

Figure 2. Endoscopic device for temporary duodenal exclusion (EndoBarrier) consisting of a metal anchoring system with tiny hooks and a sleeve made of a polymer 60 cm long (**A**) which prevents contact of food with the biliary and pancreatic secretions until the initial part of the jejunum (**B**).

PREOPERATIVE EVALUATION AND POSTOPERATIVE IMMEDIATE FOLLOW-UP

Morbid obesity is a permanent illness. The treating physician is in charge for the follow-up not only before, but mainly after the operation. Reasonably, the treatment outcome is significantly reliant on, among other factors, on patient agreement with long-term follow-up.

The patients should be instructed on the overall importance of the intake of adequate quantity of protein to avoid disproportionate lean body mass loss, avoidance of ingestion of intense sweets to prevent dumping syndrome, particularly after RYGB, and better use of crushed and/or rapid release medication. Type 2 diabetic (T2DM) patients, should have anti-diabetic medication and/or insulin adjusted with no delays post-operatively in order to minimize risks of hypoglycaemia. Most patients can have the medications suspended during the first or second post-operative days. The criteria for assessment of the effect of bariatric surgery on remission of T2DM are summarized in table 1 (11).

Table 1. The criteria for assessment of the effect of bariatric surgery on remission of type 2 *diabetes mellitus* (1)

	Partial remission	Complete remission	Prolonged remission
Fasting blood glucose	100-125 mg/dL	< 100 mg/dL	< 100 mg/dL
HbA1c	> 6%, but < 6.5%	< 6%	< 6%
No active pharmacologic therapy or procedures	At least 1-year	At least 1-year	At least 5-year

RYGB surgery is associated with durable remission of T2M (23) in many but not all severely obese diabetic adults and about one third experiences a relapse within 5 years of initial remission. The median duration of remission was 8.3 years. Significant predictors of complete remission and relapse were poor preoperative glycemic control, insulin use, and longer diabetes duration. Weight trajectories after surgery were significantly different for never remitters, relapsers, and durable remitters (24). Failure of resolution of the diabetes can also result from lack of patient compliance, inadequate weight loss, longstanding uncontrolled diabetes, or when the diabetes is actually a latent autoimune diabetes of the adult (LADA) or a type 1 (25).

Other criteria were suggested for the evaluation of the influence of bariatric surgery on optimization of metabolic status of some other co-morbid situations, for instance, total cholesterol < 4 mmol/l (< 154.67 mg/dL), LDL-cholesterol < 2 mmol/l (< 77.34 mg/dL), triglycerides < 2.2 mmol/l (194.86 mg/dL), blood pressure < 135/85 mmHg, > 15% weight loss, or lowering of HbA1c by > 20% (11).

Women candidates for bariatric surgery should avoid pregnancy in the preparatory period and for at least 12 to 18 months postoperatively. Women who become pregnant after bariatric operations should be correctly recommended and supervised for suitable weight gain, dietary, nutritional supplementation, and fetal conditions. Pregnancies post-LAGB should have band adjustments if necessary. Laboratory screenings should include iron folate, vitamin B12, calcium and fat soluble vitamins every trimester. Women after RYGB, BPD-S or BPD-DS should be counseled on non-oral contraceptive choices (13). To detect gestational diabetes, alternative paths like fasting and 2 h postprandial glycaemia have to be used after RYGB, BPD-S or BPD-DS or if the patient reports dumping complications. Women with polycystic ovary syndrome should be firmly counseled that their fertility status might be improved postoperatively. Estrogen replacement should be discontinued for at least three weeks before bariatric surgery to reduce the risks for postoperative thromboembolic phenomena (26).

Noninvasive cardiac testing is determined on the basis of the individual risk factors and findings on history and physical examination. If the patient has an active potentially unstable or unstable cardiac condition (that dramatically increase the risk of cardiac morbidity and mortality) such as unstable coronary syndromes (unstable or severe [class III or IV] angina, recent myocardial infarction [less than 30 days]), decompensated heart failure (New York Hear Association class IV or worsening or new onset heart failure), significant arrhythmias (atrioventricular block [AVB] of high grade, 3rd degree AVB; known recently symptomatic bradycardia, ventricular tachycardia, symptomatic ventricular arrhythmia, supraventricular arrhythmias (including atrial fibrillation) with rapid ventricular response (heart rate > 100 bpm); severe valvular disease (severe aortic stenosis, symptomatic mitral stenosis). In the presence of some of these active cardiac conditions, the patient should be referred to a cardiologist to assess the need for correction of the condition before the proposed surgical procedure (27).

By the way, if there is no active cardiac condition, we can proceed with the evaluation. Then, we should assess the functional capacity of the patients. In most of the cases, the determination of the functional capacity of the patients can be performed by simple and worthy questions. A patient who cannot perform his daily activities (dressing, bathing alone) because of cardiac symptoms obviously has a low functional capacity. The patient who can play a game of football or swim-

ming practice has a great functional capacity. In a very practical way Freeman & Gibbons suggest two simple questions to determine the functional capacity of the individual: "Do you walk four blocks without stopping for limiting symptoms?" and "Are you able to climb two flights of stairs without stopping for limiting symptoms?" The affirmative answers to these questions confirm adequate functional capacity for patients, freeing the patient to surgery without further investigation. These activities correspond to an exercise tolerance of 4-5 metabolic equivalents (METs), which is typically equivalent to the physiological stress of most non cardiac surgery requiring general anesthesia. On the other hand, in the negative of these issues can confirm poor functional capacity and improved clinical investigation of the patient response is required. However, when evaluating obese individuals, particularly those with morbid obesity, it is very difficult to predict the functional capacity only through clinical history. The presence of pulmonary disease, osteoarthritis and poor physical conditioning make evaluation of cardiovascular symptoms quite difficult (27,28).

Frequently, it can be impossible to assess the functional capacity of the patient. Then, we evaluate the cardiac risk index. The following conditions are considered situations of increased cardiac risk: history of ischemic heart disease (prior myocardial infarction, prior positive stress test; typical angina, Q waves on the ECG; angioplasty, use of nitrates), history of congestive heart failure, past of acute pulmonary edema, presence of B3; crackles in both lung bases; evidence of heart failure on a chest radiograph; history of prior cerebrovascular disease; *diabetes mellitus* for more than five years, evidence of microvascular complications; renal impairment (creatinine > 2 mg/dL), multiple cardiovascular risk factors (three or more of the following conditions: hypertension, high LDL, decreased HDL, smoking, glucose intolerance or diabetes) (27).

If the patient has not any of these six indicators of cardiac risk, he may proceed with the planned operation, without further cardiac testing. In these cases, the anticipated risk of perioperative cardiac events is approximately 0.5%. It is generally agreed that beta-blockers should be continued in patients who undergo surgery and who have used the therapy to treat angina, arrhythmias, and hypertension. Interruption of beta-blockers in some patients can lead to recurrent angina, rebound hypertension and atrial fibrillation in postoperative period when the patient is particularly vulnerable to an additional physiological stress. Patients who previously were not in use of beta-blockers and feature two conditions of increased cardiac risk may benefit from the use of this medication. In the presence of three or more risk factors the use of beta-blockers aiming at reducing heart rate is mandatory. The dose should be titrated individually, aiming to become a resting heart rate < 65 bpm. The dose increase should be gradual and this should be done at least one month before the procedure. The patient should be followed closely in the perioperative period to avoid the use of excessive doses, since inadequate doses are associated with symptomatic hypotension and bradycardia. The POISE study (Perioperative Ischemic Evaluation) where the blocker was started immediately before non cardiac surgery caused an increase in hypotension and bradyarrhythmia, and higher mortality at 30 days postoperatively. Therefore, a gradual titration is essential in the preoperative period for the benefit of beta-blocker (29).

Regarding non-invasive cardiac testing, they should only be considered if the test result has the potential to modify the conduct. As the blocker will be established in patients with increased cardiac risk, the rationale for the non-invasive test is to find out which patients should undergo a procedure of coronary revascularization before bariatric surgery. The objective of the preoperative evaluation is to minimize the risks precipitated by surgery and not necessarily accurately diagnose the heart condition. Consequently, non-invasive tests would be limited to a small number of circumstances, such as a patient with previous myocardial infarction, stable angina and low functional capacity. Non-invasive cardiac tests most commonly used include echocardiography or myocardial perfusion study, both after exercise or pharmacological stress. The exercise tests are limited in large obese because of their weight or orthopedic problems. In these situations it is preferable to use pharmacological stress. The choice will depend on the test and availability service experience. Obesity can reduce the accuracy of perfusion test with thallium-201 or technetium-99. Attenuation correction is needed to improve specificity in patients with BMI > 30 kg/m^2. Stress echocardiography may have the quality of the image limited due to obesity. The use of transesophageal technique offers superior image quality, but its safety has been questioned in obese patients. Few studies conducted evaluation of preoperative risk using transesophageal echocardiography stress in morbidly obese patients (30). Those with positive findings on non-invasive tests should be re-

ferred to a cardiologist, who then analyzes the need for angiography and/or coronary revascularization before the operation (27,28).

In the respiratory system assessment, it is important to recognize the following conditions: lung diseases associated with obesity (such as obstructive sleep apnea syndrome [OSAS] and obesity hypoventilation syndrome [OHS]) and intrinsic lung diseases that may be aggravated by obesity (such as chronic obstructive pulmonary disease, asthma, pulmonary fibrosis, pulmonary hypertension and chronic pulmonary embolism). Abdominal obesity causes reduced lung volumes, reduces chest wall compliance and increases the total demand of the body for oxygen. Moreover, obesity-related diseases such as type 2 diabetes, may lead to neuropathic and vascular damage, compromising the function of the respiratory muscles and pharyngeal dilator muscles. In OHS, patients have a restrictive pattern in the evaluation of pulmonary function due to decreased compliance of the chest wall. The most common is a reduction in total lung capacity as a result of reduced expiratory residual volume and functional residual volume, leading to an abnormal distribution of ventilation and worsening of gas exchange. Added to this, there is a limitation of the expiratory flow, with an increased work of diaphragmatic muscles. Patients with OHS are at increased risk because of the greater severity of clinical manifestations. These patients, therefore, should be carefully evaluated before surgery (31).

The most common respiratory disease associated with obesity is OSAS. Obesity can predispose to OSAS due to the accumulation of fat around the oropharynx and the muscles of the pharynx, changing the geometry of the airways, resulting in increased extraluminal pressure and prone to collapse. The measurement of the neck circumference is an imperative anthropometric measure and may be adjusted according to the presence of other symptoms and signs (Table 2). The assessment of daytime sleepiness can be done using the Epworth Sleepiness Scale (ESS) (32) (Table 3). The Berlin questionnaire is another mean to identify patients with OSAS (33).

Less common, but probably underdiagnosed is the OHS, defined as the development of hypercapnia ($PaCO_2$ > 45 mmHg) during wakefulness in obese individuals (BMI > 30 kg/m²) in the absence of other reasons for hypoventilation (*e.g.*, chest deformity, coexisting pulmonary or neuromuscular disease, etc.). These patients share many clinical features with patients with OSAS (and many have both diseases), but the OHS is more severe and is associated with increased morbidity and mortality, usually related to cardiac and respiratory failure. In our experience, in the Obesity Clinic of the Hospital das Clínicas, the measurement of oxygen saturation allows us to identify patients with higher risk of OHS and/or OSAS, being abnormal (SpO2 < 95%) in approximately 33% of our obese ambulatory patients (BMI > 30 kg/m²). OHS patients not receiving noninvasive ventilatory support (NIV: continuous positive air pressure CPAP, or oxygen therapy) during treatment have a mortality of 23% at 18 months and 46% in seven years, compared to 3% and 22% respectively in those receiving NIV support, which should be established preferably before bariatric surgery (34).

The initial respiratory evaluation of patients before bariatric surgery includes, beyond history and detailed physical examination, measurement of adjusted neck circumference (Table 2), ESS (Table 3), chest radiography, and measurement of SpO_2. Patients with excessive daytime sleepiness (ESS >10) and/or adjusted neck circumference > 43 cm ideally should undergo polysom-

Table 2. Adjusted neck circumference and clinical probability of OSAS

If the patient	
Is hypertensive	add 4 cm
Is an habitual snorer	add 3 cm
reporting choking or suffocation most nights	add 3 cm
Total	. . . . . cm
Clinical probability	
Less than 43 cm	Low
From 43 to 48 cm	Intermediate
Greater than 48 cm	High

Table 3. Epworth sleepness scale

Condition			**Chance of dozing**
Sitting reading			
Watching TV			
Sitting inactive in a public place (*e.g.* waiting room, theater)			
As a passenger in a car for 1 hour without stopping			
Laying to rest after lunch if circumstances permit			
Sitting and talking to someone			
Sitting in a quiet place after lunch without alcohol			
In a car stopped for a few minutes due to traffic			
Score		**Results**	
0	No chance of dozing	0-10	Normal
1	Low chance of dozing	11-12	Borderline
2	Moderate chance of dozing	> 12	Abnormal

nography, even when the SpO2 is normal. If it is not possible to perform polysomnography or if OSAS is diagnosed, respiratory therapy with CPAP at night is indicated. Patients with SpO2 < 95% and/or bicarbonate in venous blood > 27 mEq/L (in the absence of an oximeter, assessment of venous bicarbonate levelscan be used instead) should undergo arterial blood gas analysis to assess the $PaCO_2$. If confirmed the diagnosis of OHS, continuous oxygen therapy is indicated. At that time, it is extremely important to rule out the presence of active intrinsic lung disease through medical history, chest radiography, echocardiography, and spirometry. The severity of the nocturnal hypoxemia is a useful tool to suspect OHS when interpreting the polysomnogram as patients with OHS spend more than 50% of total sleep time with SpO_2 below 90%. These patients, as well as smokers, should be referred to a pulmonologist, who will assess the severity of the disease and surgical risk. In this group of patients are those with respiratory disease associated with elevated pulmonary arterial pressure, in which should be evaluated together with the endocrinologist, the potential reduction of pulmonary hypertension with weight loss, to judge whether surgery is feasible for the improvement of the patient. It is mandatory cessation of smoking at least two months before surgery. Likewise, smoking should be avoided after surgery given the increased risk for of poor wound healing and anastomotic ulcer (31,34).

A careful and detailed evaluation of the upper airway of the obese patient is required prior to elective intubation and even mask ventilation can be difficult. The incidence of difficult intubation by anesthetists is around 13%, especially in patients with Mallampati score III and IV. These problems are caused by the presence of fatty deposits in the face, malar region, chest, tongue, and the short neck with excess soft tissue, palate, pharynx and upper and anterior larynx. Furthermore, there may be restriction of mouth opening and limitations of flexion and extension of the cervical spine. Equipment for cricothyroidotomy and transtracheal ventilation should always be available. The examination of these patients should include an analysis of the head and neck region, including flexion, extension and lateral rotation of the neck, jaw mobility and mouth opening, inspection of the oropharynx, teeth and patency of the nostrils. It is essential to enquiry the patient about former difficulties in ventilation or intubation (28).

Gastrointestinal complaints should be evaluated before bariatric surgery with imaging studies, endoscopy, and screening for the presence of *Helicobacter pylori*. Abdominal ultrasound and a viral hepatitis screen are recommended to evaluate symptomatic biliary disease and if liver function tests are elevated (8,13).

In patients with a history of gout, prophylactic treatment for gouty attacks should be considered before bariatric surgery, because active weight loss has been identified as an important risk factor for hyperuricemia and acute gouty attacks (35).

A psychological assessment is mandatory for all patients before bariatric surgery. Patients should undertake an assessment of their capability to incorporate nutritional and behavioral changes after the operation. If there is an identified or suspected psychiatric disease, or substance abuse, or addiction, a proper mental evaluation with a psychiatrics before the surgical procedure must be done. After surgery, mainly RYGB, high-risk individuals should eliminate alcohol intake because of reduced alcohol metabolism and enhanced risk of alcohol dependence. Every patient should undergo a proper nutritional assessment, including micronutrient measurements, before bariatric procedures. A consultation for postoperative meal initiation and staged meal progression must be conducted by a nutritionist who is familiar with the postoperative bariatric diet. Furthermore, patients should be advised to masticate small bites of food carefully before swallowing, and also follow values of healthy eating, including a minimal protein intake of 1.0 to 1.5 g/kg ideal body weight per day, eliminating intense sweets after RYGB to reduce symptoms of the dumping syndrome, as well as after any bariatric technique to decrease energy ingestion (13,26).

Extended-release medications should be avoided; as an alternative, crushed or liquid rapid-release medications should be used to maximize absorption in postoperative phase (13).

Minimal regular nutritional supplementation for patients with RYGB, SG and BPD should contain an adult multivitamin plus mineral supplement (containing folic acid, iron and thiamine) at least 1200 mg of elemental calcium (in diet and if possible as calcium citrate supplement in divided doses, because citrate is less dependent of the acid for its absorption than carbonate), at least 3000 international units daily of vitamin D (or 20000 international units weekly to prevent or minimize secondary hyperparathyroidism), and vitamin B12 (intramuscular, or orally, if determined to be sufficiently absorbed) as required to conserve vitamin

B12 levels in the normal range (at least above 400 pg/mL). Vitamin B12 levels also should be checked in every patient in the baseline and postoperatively annually in those procedures that exclude the lower part of the stomach (*e.g.*, SG, RYGB). Oral supplementation with crystalline vitamin B12 at a dosage of 1000 µg daily or more may be used to maintain normal vitamin B12 levels. Some countries have available intranasal and sublingual vitamin B12. We are used to prescribe intramuscular B12 supplementation, 1000 µg/month to 5000 µg every 6 months if B12 sufficiency cannot be maintained using oral (5000 µg tablet daily) or intranasal routes (13). Total iron should be at least 45 mg provided by multivitamins and/or additional supplements (In table 4 is displayed the minimal regular nutritional supplementation for patients after RYGB). Minimal daily nutritional supplementation for patients with AGB should be the same, however, without iron and vitamin B12. Liquids should be consumed slowly, if possible at least 30 minutes after meals to avoid gastrointestinal complaints, and in satisfactory volumes to sustain satisfactory hydration (at least 1.5 liters daily) (36).

Levels of homocysteine, red blood cell folate and methylmalonic acid can also be used as biochemical and functional markers to maintain folic acid and vitamin B12 within target ranges. Vitamin D supplementation as high as 6000 IU daily is harmless and obligatory in several post bariatric surgery subjects to reach target levels (13).

Micronutrient deficiencies should be treated with the respective micronutrient (8,36). In cases of severe vitamin D malabsorption, oral doses of vitamin D2 or D3 may need to be as high as 50,000 international units 1 to 3 times weekly to daily and more refractory cases may require simultaneous oral administration of calcitriol (1,25-dihydroxyvitamin D). Assessment should comprise serum parathyroid hormone (PTH), total calcium, phosphorus, 25-hydroxyvitamin D, and 24-hour urine calcium levels (13).

Table 4. Minimal regular nutritional supplement for patients after Roux-en-Y gastric bypass

Component	Via
Multivitamin plus minerals tablets	Oral
Calcium citrate > 1,200 mg/day, divided doses	Oral
Vitamin D3 > 3000 IU/day, or > 20000 IU/week*	Oral
Vitamin B12 5000 ug tablets, or 1000, 5000 or 15000 ug vial*	Oral, or intramuscular
Iron (sulfate, fumarate, hydroxide, gluconate) > 45 mg/day**	Oral

* As needed to maintain normal range levels; ** As needed to maintain normal hematocrit and ferritin range levels.

In patients who have undertaken RYGB, and BPDs, bone density measurements with use of axial (spine and hip) dual-energy x-ray absorptiometry (DXA) may be indicated to monitor for osteopenia or osteoporosis at baseline and around 2 years. Although new but little available larger machines can accommodate up to 204 kg, forearm bone density measurements remain a reasonable option for preoperative screening and post operative surveillance. Bisphosphonates may be considered in patients with osteoporosis after appropriate treatment with calcium and vitamin D. Concerns generally occur about adequate oral absorption and possible anastomotic ulceration with orally administered bisphosphonates; therefore, intravenously bisphosphonates should be preferred. The recommended dosages of bisphosphonates include zoledronic acid, 5 mg intravenous once a year, or ibandronate, 3 mg intravenous every 3 months. If the concerns above are irrelevant, oral bisphosphonate administration can be delivered (alendronate 70 mg/week; risedronate 35 mg/week or 150 mg/month; or ibandronate 150 mg/month) (13).

Iron levels should be monitored in all bariatric surgery patients and anemia deserves assessment of alimentary insufficiencies, and appropriate causes. Treatment routines include oral ferrous sulfate, fumarate, hydroxide or gluconate to provide up to 150-200 mg of elemental iron daily. Intravenous iron infusion may be needed for patients with severe intolerance to oral iron or refractory deficiency due to severe iron malabsorption (13).

Folic acid supplementation in the dosage of 400 µg daily usually is part of the mineral-multivitamin supplementation, but should be administered in all women of reproduction age to diminish the risk of fetal neural tube defect (13).

After malabsorptive procedures such as BPDs, nutritional anemias might comprise deficits in copper, selenium, and zinc. Selenium levels should be monitored in patients with a malabsorptive bariatric surgical procedure who have inexplicable anemia, diarrhea, cardiomyopathy, or bone disease. Screening for zinc deficit should be considered after BPDs and should be consistently supplemented. Zinc deficiency should be considered in patients with hair loss, pica, dysgeusia, or in male patients with hypogonadism or erectile dysfunction. Copper supplementation in the dosage of 2 mg daily should be part of the multivitamin-mineral supplementation. Routine copper screening is not indicated after bariatric surgery but should be appraised in patients with anemia, neutropenia, myeloneuropathy and impaired wound

healing. In severe deficiency, treatment can be initiated with intravenous copper. Patients being treated for zinc deficit or using supplemental zinc for hair loss should receive 1 mg of copper for each 8 to 15 mg of zinc as zinc replacement can cause copper deficit (13).

Thiamine is part of routine multivitamin supplementation and screening is only necessary in the scenario of post bariatric surgery patients with fast weight loss, prolonged vomiting, alcohol abuse, neuropathy, encephalopathy, or heart failure. Thiamine deficiency (suspected or established), if severe, should be treated with intravenous thiamine, 500 mg daily, for 5 days, followed by 250 mg daily until resolution of symptoms. Less severe cases can be treated with intravenous thiamine, 100 mg daily for 1 or 2 weeks (13).

In patients with type 2 diabetes, fasting blood glucose concentrations and hemoglobin A1c should be determined occasionally. Preprandial, 2-hour postprandial, and bedtime reflectance meter glucose determinations in the home setting should be requested at the discretion of the physician caring for the patient and if symptoms of hypoglycemia occur. In patients with diabetes, the use of insulin secretagogues (sulfonylureas and meglitinides) should be stopped and insulin dosages should be adjusted or discontinued postoperatively to reduce the hazard of hypoglycemia. Metformin may be sustained postoperatively until persistent clinical resolution of diabetes is demonstrated by normalized glycemic goals, when antidiabetic medications should be withheld. Metformin associated to incretin-based therapies should be considered in patients not getting glycemic targets (13).

The requirement of antihypertensive and lipid-lowering medications should be periodically assessed. The effect of weight loss on blood pressure and dyslipidemia is variable, and drugs should not be stopped unless clearly indicated (37).

Deep venous thrombosis (DVT) and pulmonary embolism are frightening complications after bariatric surgery. Prophylaxis against DVT is recommended for every patient, including subcutaneously administered unfractionated heparin or low-molecular-weight heparin given within 24 hours after bariatric surgery sequential compression devices and early ambulation. Gastrointestinal bleeding can occur in the lines or stapling or anastomoses being manifested as melena. It is usually self-limited, and it should be evaluated the possibility of stopping the prophylaxis against DVT (13).

Our group some years ago documented that severe obesity is associated with increased TSH levels and subclinical hypothyroidism, and that following bariatric surgery and weight loss, TSH levels decrease. In short, obesity corpulence appears to be associated with TSH elevation in the absence of a primary thyroid disease (38).

The regularity and frequency of follow up depends on the bariatric procedure performed and the severity of the co-morbidities. For instance, after AGB, recurrent band adjustments and visits to the dietitian are of imperative importance for maximal weight loss. Significant regain of weight or failure to lose weight due to decreased patient adherence with lifestyle modification, development of maladaptive eating behaviors, or inadequate band restriction. In other patients, without complete resolution of type 2 diabetes, dyslipidemia, or hypertension, continued surveillance and management these conditions should assure frequent follow up (13).

Interventions should contain a multidisciplinary approach, including dietary change, physical activity, behavioral modification with frequent follow up; and the if appropriate, pharmacologic therapy and (if necessary, in selected cases) surgical revision. Routine metabolic and nutritional monitoring is recommended after all bariatric surgical procedures. Visits should be more frequent in the first two years, and can be judiciously less recurrent at the discretion of the physician caring for the patient according to the examination (13).

Patients who have undertaken RYGB, BPD/Scopinaro, or BPD/DS and who present with postprandial hypoglycaemic symptoms, evidence for lowered blood glucose simultaneous with signs and symptoms should first be advised on dietary changes (low sugar regimens, regular mealtime). Continuous glucose monitoring system can give a clue in patients with questions regarding sporadic hypoglycemia. Sporadic reflectance meter glucose determinations in the home setting should be requested to certificate and these subjects should be submitted to an evaluation to differentiate non insulinoma pancreatogenous hypoglycemia syndrome (NIPHS) from factitious or iatrogenic causes, dumping syndrome, and insulinoma. In these patients, second-line medical therapeutic strategies can be considered, including acarbose, calcium channel antagonists (verapamyl), octreotide and diazoxide (11,13,39,40).

Hygienic plastic and body-contouring operation can be executed after bariatric surgery to handle excess skin that prejudices asepsis, causes distress, and is muti-

lating. This surgery is best performed after stabilization of the weight (41).

Reversal of the bariatric surgeries is very rare. In a retrospective review, there were found 13 reversals among 2,825 bariatric surgeries, including primary and revisional operations. The main rationale for reversal of bariatric procedures were intractable vomiting or diarrhea, substance and alcohol abuse, and severe metabolic complications, but reversal could be obviated in about 50% of patients with patient education and adequate follow up (42).

SURGICAL COMPLICATIONS

Marginal ulcer at the gastrojejunal anastomosis in RYGB and erosion of the band in the banded RYGB (can cause pain, weight gain or obstruction) are causes of later gastrointestinal bleeding postoperatively. The silicone band of the AGB can move distally, which is called slippage, causing vomiting, dysphagia, heartburn or halitosis. The diagnosis is confirmed by endoscopy or contrast radiography showing a dilated gastric pouch with asymmetric air-fluid level and a diverted band. The band of the banded RYGB can also slide causing stenosis due to be displaced from its usual position or due to be too tight, leading to conditions of difficulty of gastric emptying, vomiting and food intolerance, esophagitis, and dilated pouch. The treatment may be to remove the band or endoscopic dilatation (13,26).

GI barium contrast studies or computed tomography can be indicated to assess suspected anastomotic leaks. Exploratory laparotomy/laparoscopy is warranted if the suspicion for anastomotic leaks is extremely high (*e.g.*, persistent tachycardia, tachypnea, hypoxia, fever, elevated C-reactive protein levels) in spite of a negative study. SG leaks are feared complications due to be leaks of high pressure, taking much longer to close than RYGB leaks, and being causes of prolonged hospitalization, and high morbidity. Overall SG leaks occur at the angle of Hiss, due to excessive narrowing of the gastrectomyat the *incisura angularis*, which can raise the pressure, initiating the leak (13,43).

Ultrasound is the best feasible method and is conventionally used to assess gallstone formation in the post bariatric operated patient. Ursodeoxycholic acid (300-1200 mg/d) significantly reduces gallstone formation after bariatric surgery. In patients that already have gallbladder disease, with cholecystolithiasis, there is no consensus. Most surgeons prefer to perform the bariatric surgery first, not to increase the surgical time, reducing the risk of complications. Then, after some months, the cholecystectomy is performed in better patient's conditions. Others, depending on the surgical conditions, make the two procedures (bariatric surgery and cholecystectomy) at the same time. There are some who prefer first remove the gallbladder and, in a second time perform the bariatric surgery (11,44).

Obesity can be associated with impairments of cutaneous wound healing, wound failure and fascial dehiscence, and postsurgical complications after open bariatrical operations result in unplanned hospitalizations as well as readmissions. Fortunately, nowadays, most of the surgeries have been performed by through videolaparoscopy so that this complication has been less common (45).

The risk of rhabdomyolysis rises with BMI > 55-60 kg/m^2 particularly in extensive and prolonged surgical procedures. The risk of rhabdomyolysis has declined in recent years due to advances in anesthesia, in the degree of muscular relaxation, use of adequate padding at pressure points during bariatric surgery, due to the recommendation of preoperative weight loss in patients at high risk and technical advances that allowed reduction of surgical time. Nevertheless, screening creatine kinase levels are justified in these higher risk individuals, urine output should be supervised, and adequate hydration is warranted (11).

Likewise, preoperative weight loss can reduce liver volume improving technical aspects of the operation in patients with an enlarged fatty liver, and also be useful to improve comorbidities, such as reach reasonable preoperative glycemic targets in uncontrolled diabetic patients (13).

If the patient has gastrointestinal symptoms suggestive of stricture or foreign body (*e.g.*, suture, staple), endoscopy is the chosen technique because it can be diagnostic and also therapeutic (dilation of the stricture and foreign body removal) (13).

Non steroidal antiinflammatory medications should be absolutely avoided after bariatric surgery, because they have been associated with increase in ulcerations and/or perforations in the gastrojejunal anastomosis and substitute pain drugs should be used. In the presence of anastomotic ulcers, they should be treated with H2 receptor blockers, proton pump inhibitors, sucralfate, and if *H. pylori* is identified, triple therapy to include antibiotics, bismuth, and proton pump inhibitors (37).

Symptomatic hernias that supervene after bariatric surgery need rapid surgical assessment. Subjects with abrupt onset, severe cramping periumbilical discomfort or repeated episodes of abdominal pain after RYGB or BPDs surgeries must be evaluated with an abdominal CT scan to exclude the complication of an internal hernia with a bowel obstruction. Exploratory laparotomy or laparoscopy is indicated in patients who are suspected of having an internal hernia since this potentially life-threatening complication can be missed with upper gastrointestinal x-ray studies (13).

Enteric hyperoxaluria and kidney stones of calcium oxalate development can occur after RYGB and BPDs and are linked to fat malabsorption, which fixes to calcium, increasing free oxalate that is absorbed in the gut. Therapeutic approaches to manage hyperoxaluria include sufficient hydration, decreasing oxalate dietary consumption, and calcium citrate supplementation (13).

The mortality associated with bariatric surgery can be higher in patients with BMI above 60 kg/m^2, with morbid obesity for more than five years, over 40 years old, with more than three significant comorbidities such as diabetes, sleep apnea, lung and heart diseases, and Classification of the American Society of Anesthesiology (ASA) 3 and 4 (46).

Nonetheless, RYGB has been improved and amended in the course of time, making it safer than it was 20 or 30 years ago, thus, reducing operative mortality that is now lower for laparoscopic RYGB than for a cholecystectomy, being lower than 0.2%. The establishment of centers of excellence in bariatric surgery was associated with lower rates of reoperations and complications. Such policies, associated to laparoscopic and robotic surgery, which provide more accuracy, better training and preparation of the staff and surgeons may become a powerful tool to improve surgical safety and quality (47,48).

In conclusion, bariatric surgery is the most effective method of treatment of the morbidly obese patients, with robust evidence of reduction in the incidence of new comorbidities, in the prevalence of pre-existing disorders, as well as in mortality, provided that the procedure is followed by an interdisciplinary experienced staff and in a qualified obesity management center.

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Brown adipose tissue: what have we learned since its recent identification in human adults

Tecido adiposo marrom: o que aprendemos desde sua recente identificação em humanos adultos

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ABSTRACT

Brown adipose tissue, an essential organ for thermoregulation in small and hibernating mammals due to its mitochondrial uncoupling capacity, was until recently considered to be present in humans only in newborns. The identification of brown adipose tissue in adult humans since the development and use of positron emission tomography marked with 18-fluorodeoxyglucose (PET-FDG) has raised a series of doubts and questions about its real importance in our metabolism. In this review, we will discuss what we have learnt since its identification in humans as well as both new and old concepts, some of which have been marginalized for decades, such as diet-induced thermogenesis. Arq Bras Endocrinol Metab. 2014;58(9):889-99

Keywords

Brown adipose tissue; obesity; thermogenesis; UCP-1; energy expenditure

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RESUMO

O tecido adiposo marrom, órgão essencial para a termorregulação de animais hibernantes e pequenos devido à sua capacidade desacopladora, era até poucos anos considerado presente apenas em recém-nascidos na espécie humana. A identificação do tecido adiposo marrom em adultos com o desenvolvimento e uso da tomografia de emissão de pósitron marcado com 18-fluorodesoxiglicose (PET-FDG) gerou questões sobre sua real importância para nosso metabolismo. Nesta revisão, discutiremos o que aprendemos nesse tempo, assim como conceitos antigos e novos, alguns marginalizados por décadas, como a termogênese induzida por dieta. Arq Bras Endocrinol Metab. 2014;58(9):889-99

Descritores

Tecido adiposo marrom; obesidade; termogênese; UCP-1; diabetes; PET-FDG

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INTRODUCTION

The first description of brown adipose tissue dates from 1551 when Gessner described, in an anatomy book, the tissue as being "nec pinguitudo nec caro", which means "neither fat nor flesh" (1). However, it has only been recognized as a mammal specific thermogenic organ, essential for mammalian thermoregulation, for less than half a century (2). It was believed, for the second half of the 20th Century and the beginning of the 21st, to only be present in the human newborn and that it began to involute throughout childhood (2,3). Although some indirect evidence had led a few authors to postulate its presence before (1,4-9), it was only when positron emission tomography marked with 18-fluorodeoxyglucose (PET-FDG), a functional imaging method that evaluates areas of greater metabolic activity, started being used more frequently in the follow-up of some cancers, that brown adipose tissue was identified in at least one subgroup of the adult human population (2,10-14). This amazing discovery was of great interest to medical researchers in the field and to the hypothesis that the presence or absence of brown adipose tissue could be a causal effect in common non-transmissible diseases such as obesity and type 2 diabetes, and could also be a potential therapeutic target since thermogenesis wastes extra energy.

What is brown adipose tissue?

Being present uniquely in mammals, as already mentioned, the main function of brown adipose tissue is thermoregulation via non-shivering thermogenesis (1). Thermogenesis occurs due to a unique and specific enzyme, called UCP-1 (Uncoupling Protein-1), which uncouples ATP energy production in the mitochondria, generating heat instead (1,15). UCP-1 is a unique protein that promotes proton leakage across the inner mitochondrial membrane, reducing its transmembrane electrochemical proton gradient, and therefore producing non shivering thermogenesis via heat production (1,15). UCP-1 is a marker of thermogenic adipocytes (not only the classic brown but also the recently discovered beige adipocytes that will later be discussed) and it is believed to be the only protein physiologically capable of inducing non-shivering thermogenesis, as UCP-1 knock-out mice can only produce heat by shivering (2,16,17).

Brown adipose tissue is activated by sympathetic noradrenergic stimuli, mainly via β-3 receptors, although other receptors can activate it too (2,18). Cold is the main physiological stimuli to this noradrenergic activation, as mammals need to maintain their thermoneutrality, but it has long been speculated that it may also be activated by food (2,17,19).

When brown adipose tissue is analyzed by microscopy, cells are seen with multiple fat droplets and multiple mitochondria, positively-expressing UCP-1, with a central nucleus (2,17,20,21). Classical white adipocytes present a single large fat droplet and fewer mitochondria which points to its storage function (21).

Although the total mass of brown adipose tissue in mammals is small, past research has already demonstrated that its activation could quadruple the energy expenditure (EE) of an animal, as an increase in tissue perfusion occurs (15,21-23). Not only the fat stored in its lipid droplets are used to generate heat, but also free fatty acids and glucose from systemic circulation, exponentially increasing its thermogenic capacity (15). This is of particular importance to hibernating animals, as there is an urgent need to increase body temperature after microarousals which occur during hibernation (2,21,22). During hibernation, its metabolism presents under hypothermia and it must achieve thermoregulation as quick as possible during microarousals or after the end of the hibernation period (2,21). Only a high-capacity thermogenic tissue would be able to achieve adequate short term thermoregulation (24).

In addition to hibernators, the importance of brown adipose tissue to small mammals, which have a greater body surface area in proportion to their internal volume, is well recognized (2,24,25). This greater area increases heat loss to the environment and costs more energy, used by brown adipose tissue, to maintain thermoregulation. Human newborns possess a high area/volume ratio, and it has long been documented that brown adipose tissue is present in our early life (2,23,26-28). However, with the progressive reduction of surface area in relation to internal volume, the energy cost of thermoregulation is decreased and it was believed that brown adipose tissue suffered progressive involution until its total disappearance during early childhood (23). The invention and then popularization of PET-FDG changed this view however, as it was hypothesized that at least a fraction of humans still present brown adipose tissue in adult life, as the detection, in a fraction of exams, of areas of metabolic activity raised the possibility of there being unnoticed thermogenic fat (15,23,29).

PET-FDG: the "denouncer" of brown adipose tissue

PET-FDG is a functional exam characterized by its ability to detect metabolically active areas that uptake FDG, a glucose radioisotope (23,30,31). It began being used in the 90s, mainly in oncology, to detect tumors and metastases, which generally have a high metabolic rate and therefore high glucose uptake. The heart and brain are also consistently detected in a PET-FDG scan, organs with long recognized marked glucose utilization, even during fasting (31). Unexpectedly, however, a small fraction of PET exams detected bilateral symmetrical FDG uptake areas in supraclavicular, cervical and parasternal regions that could not easily be interpreted as tumors, due to the described characteristics (7,29,30,32). Anatomically, the highly active areas were within areas of fat attenuation on computerized tomography, suggesting metabolically active fat. The high proportion of images with such characteristics on PET-FDG of pheochromocytoma diagnosed individuals, with catecholamine-producing tumors known to be of intense noradrenergic activation, raised suspicions that these areas were actually unrecognized brown adipose tissue, which had proliferated under chronic noradrenergic stimuli (7,29,33,34).

The interest from endocrinologists and metabolism experts rose in 2007 after the publication of an article which showed compelling evidence, from a functional

point of view, that these mysterious areas detected by PET-FDG scans were brown adipose tissue (29). However, just two years later, with the publication of three articles in the same edition of the New England Journal of Medicine, the scientific community accepted that at least a fraction of human adults possess brown adipose tissue (12,13,14,35). We will review those articles shortly (Table 1).

Cypess and cols. analyzed 3640 PET-FDGs taken to detect neoplasms and detected uptake areas suggestive of BAT in 7.5% of women and 3.1% of men (12). Age, body mass index (in older subjects), outdoor temperature and fasting glucose were inversely related to PET positive imaging, and the use of beta-blockers was associated with a lower probability of uptake. However, after multivariate analysis, fasting glucose and BMI lost their statistical significance, but BMI continued to have a significant negative relationship with uptake in older individuals.

Van Marken Lichtenbelt and cols., concomitantly, opted to perform PET-FDGs in 24 individuals after acute cold exposure (16 degrees Celsius for two hours), using light clothes and avoiding shivering (13). The authors observed 96% of positive images and also demonstrated an inverse relationship between the level of uptake measured in kilobecquerels and BMI or body fat mass. The single individual that presented negative uptake after cold exposure was obese. Tissues obtained from positive uptake regions expressed UCP-1.

Table 1. Summary of findings of the three New England Journal of Medicine articles from 2009 that confirmed to scientific community the presence of brown adipose tissue in adult humans

Van Marken Lichtenbelt and cols. (13)	Virtanen and cols. (14)	Cypess and cols. (12)
BAT prevalence on FDG-PET in 96% of individuals after acute cold exposure	Cold induced PET-FDG uptake was 15 times greater in paracervical and supraclavicular adipose tissue in five subjects	Positive scans seen in 7.5% of women and 3.1% of men
Significantly lower activity in overweight and obese individuals (the only PET negative subject was obese)	Mathematical analysis from one subject suggests a 4.1 kg decrease in body weight over the course of 1-year if BAT is active	Probability of detection inversely related to outdoor temperature, years of age, BMI in older adults and beta blocker use
Higher activity has a significant direct positive correlation with resting metabolic rate and correlates negatively with BMI and body fat mass	Biopsy specimens of three subjects were collected and demonstrated messenger RNA and protein levels of UCP-1, as well as other brown fat markers (such as PGC1α, DIO2, PRDM16 and ADRB3) and morphologic assessment found evidence of multilocular fat cells	Probability of detection inversely related to fasting glycemia in univariate analysis, but not significant in multivariate analysis

The last paper in the aforementioned edition of the New England Journal, by Virtanen and cols., analyzed tissue biopsy from PET positive areas visualized in three young individuals and detected positive expression of UCP-1, confirming it as being brown adipose tissue (14). Considering that only 10% of energy utilization by BAT is from glucose and 90% from fatty acids, the Finnish group calculated by mathematical analysis based on FDG uptake in these individuals, a 7% increase in energy expenditure, which corresponds to 4.1 kilograms a year.

Neither of the studies was designed to assess causal-effect relations between BMI and the presence or absence of BAT, leading to innumerous hypotheses. One hypothesis being that as obese people have greater fat protection they may feel less cold, in turn meaning they need less BAT activation. Another possibility, which should be investigated further, is that the absence of brown adipose tissue could be, at least partially, related to weight gain and worsening of blood glucose levels in a subgroup of individuals.

Is brown adipose tissue metabolically important in humans?

For such a fascinating hypothesis to be plausible, brown adipose tissue would have to be metabolically important in humans. What evidence exists to support this?

Initially, after first PET-FDG imaging, it was hypothesized that brown adipose tissue was a residual organ, similar to other organs that frequently do not involute during embryology and development. Data from the Van Marken Lichtenbelt study demonstrated nearly 100% captation with cold stimulation and turned out this hypothesis less probable (13).

The total brown adipose tissue mass in humans is no larger than 60 to 100 grams, a minute fraction of white adipose tissue mass, even in lean individuals (23). However, as already pointed out, the perfusion of BAT increases substantially during noradrenergic stimulation, leading to a high peripheral uptake of glucose and fatty acids, which makes BAT an energy demanding tissue as well as also being an important regulator of

glucose homeostasis (2,22,23,35,36). It has an insulin independent glucose uptake capacity and helps the clearance of free fatty acids, which are closely related to insulin resistance (35,36).

Some studies have tried to evaluate the energy expenditure increase after BAT activation, and variances between 5 and 77% over basal levels have been reported (23). As previously described, Virtanen and cols. suggested a 7% increase in basal metabolism (14). Orava and cols. found a similar 8% increase after cold exposure, but when the analysis was done only in PET-FDG positive individuals, the increase climbed to 22% (35). Ouellet and cols. opted to measure energy expenditure by indirect calorimetry, before and after cold exposure in an 18°C water filled shirt (37). The percentage increase in this study reached 77% after cold exposure. Yoneshiro and cols., divided PET-FDG positive and negative in regard to BAT activation, and found a stimulated 25% increase in energy expenditure in the positive group, corresponding to 358 kcal (38). The adipostat hypothesis, however, teaches us that an energy expenditure increase should necessarily lead to a parallel increase in energy intake to avoid weight variations (39,40). To better understand what happens to energy balance in a situation of increased energy expenditure due to thermogenesis, animal studies should be evaluated, as very little data on humans is available.

Cannon and Nedergaard demonstrated, several years ago, increased vO2 and energy intake in animals exposed to lower temperatures; but, although the feeding increase was proportional to O2 consumption, the cold living animals are lighter than their counterparts (39). However, several authors, based on different results in different experiments, still believe that any increase in energy expenditure will be readily compensated by increased food intake, and that isolated BAT activation will not result in weight loss (17,25,41,42). Ravussin and cols. elegantly suggested that BAT activation combined with an anorexic drug could induce weight loss synergy by dissociating the increased energy expenditure with that of food intake, but the results of his studies combining acute intermittent cold exposure with AM251, an endocannabinoid receptor antagonist, did not show any synergy nor weight loss in the group exposed to cold that did not receive the substance (42).

Yoneshiro and cols, who analyzed differences between BAT-positive and BAT-negative individuals based on their FDG-PET uptake, reported that BAT-positive subjects did not gain weight with age (43). On the other hand, BAT-negative subjects, as is common in humans, had increased BMI, total body fat mass and abdominal fat mass, supporting the notion that brown adipose tissue helps protect against weight gain and the development of obesity.

Gadea and cols. recently reported a case of a rare brown adipose tissue tumor, called hibernoma, in a 68-year old woman who had lost 10 kg in 6 months (44). She gained 15 kg in one-year after surgery, but a decrease in energy expenditure was intriguingly not reported after resection and an increase in food intake was observed. The description of this case suggests, although obviously with all the limitations of evaluating a single case, that brown adipose tissue is capable of inducing weight loss, at least when highly present and stimulated. Another situation that deserves a mention is hyperthyroidism. Although a comprehensive review about the relation of brown adipose tissue and thyroid hormones is beyond the scope of this review, Lahesmaa and cols. have shown that overt hyperthyroidism, which frequently leads to significant weight loss, is associated with a threefold greater BAT glucose uptake, with increased energy expenditure and greater use of lipids as an energy substrate (45). The relationship between brown adipose tissue and thyroid hormones is, however, known for decades (2,46-50).

The real significance of brown adipose tissue depots in human metabolism, as seen, is still highly speculative. An old concept, resurrected by studies on brown adipose tissue that could also have an important impact on energy balance, is diet-induced thermogenesis, which will be reviewed in the next section.

Diet-induced thermogenesis and metabolic inefficiency

The components of daily energy expenditure are basal metabolic rate, the energy cost of physical activity and the thermic effect of food (51). Food thermogenesis is classically considered as the energy cost of digestion and calorie storage. Another concept however, of diet-induced thermogenesis related to energy wasting was hypothesized decades ago, with a close relation to brown adipose tissue, although it was poorly accepted and few studies have tried going down this path until recently (17,19).

In 1979 Rothwell and Stock demonstrated, in a seminal study published in Nature which today has more than 1,000 citations, that in rats chronically fed with a "cafeteria-diet" consisting of a high energy value diet,

rich in fat and carbohydrates and protein-poor, there was a disproportional increase in energy expenditure that could not be attributed to the energy cost of food alone (19). The rats gained less weight than predicted, and it was suggested that they wasted part of the ingested energy in the form of heat. In accordance with these findings, the rats had higher rectal and interscapular temperatures in the postprandial period. After sacrifice, tissue analysis confirmed a 260% increase in brown adipose tissue mass compared with control rats fed with a standard chow, suggesting that the dissipated energy derived from brown adipose tissue recruitment and activation.

Despite the impact of the study, diet-induced thermogenesis was almost forgotten, at least by human physiologists. However, in 1999, Stock himself compiled a series of overfeeding studies in humans which intended to evaluate interindividual responses to weight gain based on possible differences between fat mass and fat free mass percentages (52). Using the law of thermodynamics Stock calculated that between 30 and 45 kJ/kg should be required to cause a one kilogram increase in body weight. However, some individuals presented values as high as 100 kJ/kg to gain one kilogram. The only possibility, according to the author, is that this large variation is due to metabolic inefficiency and thermogenesis. Recently, Wijers and cols. found an individual linear correlation between the energy cost of a 1 kg weight gain and the cold-induced energy expenditure, suggesting that the same mechanism which leads to metabolic inefficiency is involved in cold-induced thermogenesis, probably brown adipose tissue (53).

A recent study, assessing post-prandial PET-FDG, with all the interpretation caveats that it causes due to post-prandial muscle glucose uptake, suggested that compared with thermoneutrality, there is BAT glucose uptake after a hypercaloric and hyperproteic diet, similar to that which occurs after cold activation (54). Nevertheless, another recent study questioned the significance of diet-induced thermogenesis, after demonstrating that chronically overfed individuals (200% overfeeding) did not increase their BAT glucose uptake when PET-FDG scans done before and after the overfeeding period were compared (55). The post-overfeeding PET-FDG was performed four hours after the meal using a low carbohydrate diet. Timing and composition of the diet may have obscured the results.

Interestingly, the finding that individuals with constitutional thinness (CT), even at thermoneutral conditions, had a 16.7 times greater glucose uptake in BAT compared with normal weight control individuals (56) deserves mentioning. In contrast, women with anorexia nervosa, with a similar weight to CT individuals, had almost zero BAT glucose uptake, suggesting BAT has a role in metabolic inefficiency in this uncommon but fascinating subgroup of people.

Although it might be difficult to imagine an evolutionary advantage of metabolic inefficiency, as it is commonly accepted that obesity is the result of a thrifty phenotype adapted to store energy in a food scarce environment, a possible explanation would be that it is active in low protein diets and thermogenesis would be important to keep the animal constantly seeking protein sources (2,17,52). In fact, it has been demonstrated in humans that low protein diets are associated with greater metabolic inefficiency, as well as high-protein diets leading to high thermogenesis, the latter mainly due to the high cost of digestion, metabolization and storage of this macronutrient (52,57-59).

In spite of being a highly controversial concept, and highly refuted by some (60,61), the concept that brown adipose tissue may dissipate excess calories as heat and make weight gain difficult in some individuals deserves further investigation and could lead to the possible discovery of drugs which activate these mechanisms. Another debate which remains open for discussion is why some individuals would have more or less BAT than their counterparts.

What is the real prevalence of brown adipose tissue? Concepts of recruitment and activation

As highlighted previously, in 2009 Cypess and cols. found 7.5% of women and 3.1% of men to be BAT-positive in ambient temperatures (12). Similar results were found in previous studies done under similar conditions, with small variations (23). After acute cold exposure, van Marken Lichtenbelt and cols., in the Netherlands, encountered 96% of BAT-positive individuals (only one obese man was BAT-negative) (13), however this number dropped to 40% in a Japanese population (38). In Brazil, there are no published studies on this subject, but unpublished data suggests even lower levels. Table 2 highlights differences in BAT uptake in different populations, in ambient temperature and after cold exposure (12,13,14,23,30,38,62-67) What could the reason be for such differences considering similar protocols?

Table 2. Prevalence of positive PET-FDG uptake in different populations from different countries

Ambient temperature	Acute cold exposure
USA (Cohade, and cols.) – 13.7% (winter), 4.1% (rest of the year)	Netherlands (von Marken Lichtenbelt, and cols.) – 97%
USA (Yeung, and cols.) – 3.7% (neck fat)	Finland (Virtanen, and cols.) – 100%
USA (Cypess, and cols.) – 7.5% (women), 3.1% (men)	Japan (Saito, and cols.) – 53% (young individuals)
UK (Au-Yong, and cols.) – 7.2% (winter), 2.5% (summer)	Japan (Yoneshiro, and cols.) – 46%
Canada (Ouellet, and cols.) – 6.8%	
Australia (Lee, and cols.) – 8.5%	
Germany (Stefan, and cols.) – 3.05%	

To better understand this difference, we should return to physiology and studies in mice. If an animal lives under total thermoneutrality and is acutely exposed to cold, its first response is to shiver to protect internal temperature (2,68). At that moment the animal does not possess brown adipose tissue ready to be activated and defend its temperature. As cold exposure is maintained, the animal starts to recruit BAT and activate it, decreasing shivering (although muscular resistance also improves to allow muscles to shiver without fatigue). As recruitment reaches its maximum, the animal stops shivering and all heat production comes from BAT and its UCP-1 mitochondrial uncoupling. After returning to thermoneutrality, the animal retains its recruited BAT, inactive, but ready to be activated in case of a new cold exposure. This is demonstrated by injecting norepinephrine before and after recruitment. The energy expenditure increase after recruitment is substantial, proving that BAT was recruited, ready to become active, different to what occurs before when there is no BAT recruited to be rapidly activated after acute norepinephrine stimulus (2,69-72).

This is a fundamental concept because it helps to explain why there is such a population variance in PET-FDG uptake – daily chronic cold exposure probably recruits BAT, which can become more active after an acute reduction in ambient temperature. This hypothesis was confirmed by two groups that found greater BAT activation after chronic and daily cold exposure, as well as an increase in energy expenditure during acute cold exposure after chronic stimulation versus acute cold exposure in the beginning of the experiment (73,74).

A higher percentage of individuals, as we might expect based on this hypothesis, present BAT-positive PET-FDG in winter (62,63,67), even when ambient temperature is controlled (63). Another fascinating theory that helps to explain this difference is that photoperiod could also interfere with BAT recruitment (63). As the days start to get shorter, in anticipation of winter, BAT may slowly be recruited, in order to have sufficient levels ready for when the cold starts, and preventing body thermoneutrality from being reliant upon shivering. Melatonin is a possible player in this process, as previous studies in hibernators generally demonstrate higher BAT mass in melatonin supplemented animals (75) and, in a recent study, a higher body temperature measured by infrared thermography (which will be discussed further) was seen in mice given a melatonin rich 10 mg/kg supplement (76).

As the concept of recruitment has been explained, the next question is how this recruitment occurs.

Where does BAT come from? The difference between brown and beige adipocytes

Research on pre-adipocytes and progenitor cells suggest that BAT, as well as white adipose tissue, originates from mesoderm, but from distinct regions (77-79). Classic brown adipose tissue, as it is known, has its origins from engrailed-1-expressing cells in the central dermomyotome and from myogenic factor 5 (Myf-5) positive precursors. This myf-5 + lineage is the same for myocytes, but different from the lineage of white adipose tissue, derived from myf5 – cells from lateral mesoderm. It was therefore believed that in order to recruit new BAT, it would be necessary to start from initial pre-adipocyte differentiation and pass through a series of steps before developing mature brown adipocytes.

This particular view has changed since the discovery that cells present in areas of WAT, after specific stimulus (such as chronic cold exposure or norepinephrine), may be able to express UCP-1, and achieve thermogenesis (79-81). Would WAT be able to transdifferentiate in BAT? These cells, apparently white while non-stimulated, were called beige or "brite" (from brown-in-white) cells and their study developed rapidly.

The first doubt, already mentioned in the last paragraph was: any white cell could, under specific stimulus, express UCP-1 or only a specific cell lineage? This question has not been clearly solved, as different groups advocate different ideas (17,82), but recent evidence points more towards a specific lineage derived from myf5 – cells (77,80,81,83,84). Wu and cols. observed

a distinct gene expression between classic white adipocytes, unable to express UCP-1 after stimulus, and beige adipocytes (81). This same study also suggested that almost all adult human UCP-1 positive cells derived from beige adipocytes, questioning again whether humans possesses classic brown adipose tissue at all. Controversy increases with the study by Lidell and cols., which unequivocally demonstrates classic brown adipocytes in the interscapular region of newborns (expressing a specific gene called ZIC1) (85) The much smaller frequence of ZIC1 expression in other thermogenic areas (as periadrenal and supraclavicular) of newborns suggested that beige adipocytes were responsible for thermogenesis in these areas. As these articles are very recent, it is too early to draw any definitive conclusions about the real origins of all of our UCP-1 positive cells. From a clinical perspective, the main implication in differentiating both tissues would be on possible therapeutics derived from this knowledge (23,86,87). While it seems too slow and energy costing to differentiate embrionary cells through mature adipocytes, beige adipocytes, already present mixed with white adipocytes, can express UCP-1 much quicker. Research on therapeutics that permit UCP-1 expression in beige cells, increasing energy expenditure, appears to be an excellent choice.

Is it possible to pharmacologically stimulate brown adipose tissue?

After the great increase in BAT studies, the next step is the study of pathways and drugs that could lead to its differentiation, proliferation and activation, as already mentioned. A complete review of therapeutic possibilities is beyond the scope of this overview article and it has been well discussed by Bonet and cols. (86), and Broeders and cols., brilliantly reviews endogenous ways to stimulate brown adipose tissue (88). The pharmacological stimulation of brown adipose tissue will be a potential target for weight loss drugs, or as proposed by some authors, for weight maintenance drugs after weight loss (17,86,88). As the potential to increase energy expenditure could be limited to achieve great weight loss in short-term, it could, on the other hand, mitigate or totally counteract the physiological decrease in energy expenditure that follows weight loss (17).

Two possibilities, linked with distinct pathways more than any drug in particular, will be briefly discussed. The first is capsaicin, which has already been studied in humans, by a Japanese group. In a series of studies Yoneshiro and cols. found that capsaicin is able to activate previously recruited BAT (89). This group drew such a conclusion by observing that energy expenditure increased after the ingestion of capsaicin in the group that had previously presented a positive PET-FDG glucose uptake. In the PET-FDG negative group, this was not observed. In another study, the same group recruited BAT after two weeks of chronic daily cold exposure in PET-FDG negative individuals (PET-FDG done under acute cold exposure) and they observed an increase in energy expenditure after the cold habituation compared with basal state (74). As a conclusion, capsaicin seems to be able to activate an already recruited brown (or beige) adipocyte.

More useful, however, would be finding drugs able of "browning" apparent white adipocytes. A much celebrated discovery, the hormone irisin, may be able to do that physiologically (90). Discovered by Bruce Spiegelman's group, this hormone is produced by skeletal muscle after FNDC5 protein cleavage, in response to physical activity and its main function would be exactly the browning of apparent white cells, being partially responsible for the positive metabolic effects of exercise. The hormone was isolated in mice but the same group also detected circulating levels of this hormone in humans. A great debate is ongoing, after some previous findings questioned the relevance of the hormone (91,92). Raschke suggested that humans are unable to cleave FDNC5 and the detected irisin may just be laboratory interferences and even exogenous irisin administration in human cells does not appear to have the expected result. Independently of the future relevance of irisin in humans, "browning" drugs appear as potential therapeutic targets (86). As irisin is an endogenous substance, finding other potential endogenous browning hormones or substances seems like an excellent option, as recently reviewed (87). Our group started to evaluate BAT recruitment with circadian rhythms, believing in the possible role of melatonin, as previously mentioned (75). Since hypothalamic regulation of BAT is now being clearly recognized, hypothalamic effectors, such as orexin, also appear to have therapeutic potential (17,93).

Past studies with sibutramine and more recent ones with intracerebral injection of GLP-1, in animals, suggest that both drugs could activate BAT (94-96). Although this does not change clinical prescription, the

presence or absence of BAT could be a possible explanation for the sometimes incomprehensible interindividual variability in weight loss with both sibutramine and GLP-1 agonists (97,98).

Does brown adipose tissue play a role in diabetes, independently of weight loss?

Various physiological mechanisms previously described support the hypothesis that BAT regulates glucose homeostasis. Examples are the increased energy expenditure and possible weight loss seen in animal models and an increase in the uptake of free fatty acids (a major player in insulin resistance) as well as the uptake of insulin independent glucose when there is greater BAT perfusion (99). Clinical data supports this theory as BAT uptake is inversely related to fasting blood glucose levels, as already mentioned.

A recent review explores these possible mechanisms to exhaustion and suggests different ones, such as *batokines*, substances produced by BAT, like BMP8B, FGF21 and PTGDS, which can be implicated in improving insulin sensitivity and even insulin secretion by pancreatic beta-cells (99).

There are some studies with brown adipose tissue transplantation which demonstrate improvement in glucose homeostasis. Stanford and cols. transplanted BAT from male donor mice into the visceral cavity of age and sex matched recipient mice and found improved glucose tolerance and insulin sensitivity as well as a complete reversal of high-fat induced insulin resistance (100). Gunawardana and cols. studied mice with streptozocin induced diabetes, a model of type 1 diabetes, which also presented a significant improvement in glycemic response after BAT transplantation, supporting an insulin independent effect (101).

Therefore, evidence linking brown adipose tissue (or a lack of) with type 2 diabetes is emerging and should be an important field of research over the next few years.

New imaging methods for the detection of brown adipose tissue

The difficulties of studying BAT derive in part from the detection methods. PET-FDG was fundamental to the identification of BAT in human adults, but it is an expensive method, involving ionizing radiation, and depends on activation to be detected (17). As only 10% of BAT uptake comes from glucose, the sensitivity of a mainly glucose uptake method can be low (15).

New imaging methods are therefore being proposed to substitute PET-FDG in research and even clinical practice (102). Validation studies of magnetic resonance have already been done in animals, with promising results and good capacity for detecting brown depots within white adipose tissue (103-106). Pilot studies have also been performed in humans and suggest a good sensitivity with the additional advantage of detecting even non active tissue (although, logically, it must have at least been recruited) (106,107).

Another viable option is infrared thermography (IT), a non-invasive and simple method, which evaluates body temperature in different tissues by image (a method long used in civil construction and in medical conditions such as pain and cancer) (108,109). The feasibility of obtaining results with IT makes it a promising and useful method, as demonstrated by a letter from Lee and Ho, which describes an increase in temperature in BAT correspondent areas after cold exposure and meals in humans (110). Studies in children with infrared thermography have also found promising results (111).

Dual energy computerized tomography (DECT) appears to be a new option, as recently reviewed by Borga and cols (102).

In conclusion, the study of brown adipose tissue grew enormously after human adult detection by FDG-PET, becoming an intense research field not only in general biology but also in medicine. Most are recent discoveries and therefore need further confirmation and validation, but there is already a vast amount of data that puts BAT as an emerging protagonist in the fields of obesity and diabetes.

A better understanding of the differences between classic brown and beige adipocytes, and between recruitment and activation, may lead to a great impulse in the development of diabetes and obesity drugs that act through BAT. Browning apparent white adipocytes, and making them express UCP-1 and generating heat seems easier, under specific stimulus, than differentiating new preadipocytes from classic brown cells.

The idea that part of interindividual weight gain can be explained by greater diet-induced thermogenesis, or metabolic inefficiency, is also fascinating and could help to change some obesity (and constitutional thinness) paradigms and misconceptions. Unfortunately, the vast majority of clinical practitioners still see obesity as a minor condition associated to individual choices, despite decades of obesity research pointing in a different direction.

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Estudo do polimorfismo G54D do gene *MBL2* no diabetes melito gestacional

Study of polymorphism G54D of MBL2 *gene in gestational* diabetes mellitus

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RESUMO

Objetivo: Analisar a influência da associação do polimorfismo G54D (rs1800450) do gene *MBL2* no diabetes melito gestacional (DMG) quanto à necessidade de tratamento complementar e ocorrência de recém-nascidos grandes para a idade gestacional. **Sujeitos e métodos:** Cento e cinco pacientes com DMG segundo parâmetro da OMS (Organização Mundial da Saúde) foram avaliadas no período de novembro de 2010 a outubro de 2012. As gestantes foram divididas em dois grupos correspondentes à presença (n = 37) ou à ausência (n = 68) do alelo mutante. As variantes do polimorfismo G54D foram identificadas por meio da técnica de polimorfismos de comprimentos de fragmentos de restrição (RFLP). Parâmetros antropométricos e bioquímicos da mãe e do recém-nascido (RN) e a necessidade de terapia complementar associada à dietoterapia foram avaliados como desfechos primários. **Resultados:** Das pacientes analisadas, 35,2% carregavam pelo menos um alelo mutante do polimorfismo G54D. Os dois grupos não apresentaram diferença significativa quanto a ganho de peso, paridade, idade, índice de massa corporal e idade gestacional de chegada à maternidade. Os grupos de pacientes portadoras ou não do alelo mutante não diferiram quanto à necessidade de tratamento complementar à dietoterapia (16,2% *vs.* 26,7%) respectivamente e à ocorrência de recém-nascidos grandes para a idade gestacional (24,3% *vs.* 13,2%). **Conclusão:** Nossos dados demonstraram que o polimorfismo G54D do gene *MBL2* não teve efeito sobre a necessidade de tratamento complementar acrescido à dietoterapia e à ocorrência de recém-nascidos grandes para a idade gestacional na população estudada. Arq Bras Endocrinol Metab. 2014;58(9):900-5

Descritores
MBL2; polimorfismo de nucleotídeo único; diabetes melito gestacional

ABSTRACT

Objective: To assess the association of the G54D (rs1800450) polymorphism of the gene *MBL2* in the gestational *diabetes mellitus* with the need for additional treatment and the occurrence of large newborns for the gestational age. **Subjects and methods:** One hundred and five patients recruited in Joinville – Brazil were evaluated between November 2010 and October 2012. Pregnant women were divided in two groups correspondents to the presence (n = 37) or absence (n = 68) of the mutant allele. The variants of the polymorphism G54D were identified by restriction fragment lengths polymorphisms (RFLP). Anthropometric and biochemical parameters of the mother and the newborn, and the necessity of additional therapy associated with diet were assessed as the primary outcomes. **Results:** Thirty-five point two percent of the evaluated patients carried at least one mutated allele of G54D polymorphism. There were no significant differences in weight gain, parity, age, body mass index and gestational age of arrival at maternity between the two groups. The groups of patients with or without the mutated allele did not differ in the need for additional treatment associated with diet (16.2% *vs.* 26.7%) respectively and with the occurrence of large newborns for gestational age (24.3% *vs.* 13.2%). **Conclusion:** Our data showed that the polymorphism G54D of the gene *MBL2* had no effect in the need for additional treatment associated with the diet-based therapy and in the occurrence of large newborns for gestational age in the studied population. Arq Bras Endocrinol Metab. 2014;58(9):900-5

Keywords
MBL2; single nucleotide polymorphism; gestational *diabetes mellitus*

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INTRODUÇÃO

O diabetes melito gestacional (DMG) é conceituado como o aparecimento de um grau variável de intolerância à glicose diagnosticada pela primeira vez durante a gestação e que pode ou não persistir após o parto (1,2). Ocorre em 7% das gestações, podendo variar de 1% a 14% dependendo da população estudada (3,4).

DMG é um distúrbio heterogêneo no qual vários fatores genéticos e ambientais podem estar envolvidos. Duas características marcantes na gravidez são a hiperinsulinemia e a resistência à insulina, que podem predispor a paciente ao desenvolvimento do DMG. No diabetes, tem-se um estado de inflamação crônica subclínica no tecido adiposo, músculos e fígado, caracterizado pela produção anormal de citocinas e mediadores pró-inflamatórios (5-7).

A proteína lectina de ligação à manose (MBL) tem capacidade de ativar a cascata do complemento e estimular a fagocitose. Adicionalmente, a MBL também inibe a liberação de fator de necrose tumoral alfa (TNF-α). Então, a deficiência de MBL na gestação favorece uma resposta inflamatória prolongada e sustentada, favorecendo a atividade do TNF-α e outras citocinas pró-inflamatórias como as interleucinas IL-1β, IL-6, IL-8 e IL-12 que, por sua vez, participam das vias moleculares de resistência à insulina (8-10).

Há dados conflitantes sobre diabetes tipo 1 e a deficiência de MBL. No estudo de Araujo e cols. (11), demonstrou-se que a deficiência de MBL está associada a aumento do risco de desenvolver diabetes na infância e adolescência, resistência à insulina e à obesidade. Já no estudo de Bouwman e cols. (12), verificou-se o aumento sérico de MBL nos pacientes diabéticos tipo 1.

A MBL é uma proteína sintetizada no fígado, considerada um componente importante do sistema imune inato, cujo nível sérico é determinado geneticamente (13). São conhecidos três alelos mutantes principais no éxon 1 do gene *MBL2* associados à deficiência de MBL e implicados na redução da funcionalidade da proteína R52C (polimorfismo rs5030737), G54D (rs1800450) e G57E (rs1800451). A ocorrência dos alelos mutantes, em homozigose ou heterozigose, determina o fenótipo correspondente à deficiência de MBL (13,14).

No presente estudo trabalhamos com a hipótese de que a deficiência de MBL poderia estar envolvida no aparecimento de algum grau de resistência à insulina. Portanto, o objetivo do presente estudo foi verificar a prevalência das variantes do polimorfismo G54D do gene *MBL2* nas gestantes apresentando DMG. Avaliamos também a relação dos genótipos com parâmetros antropométricos da mãe e do recém-nascido (RN), assim como a necessidade de terapia complementar à dieta.

MATERIAIS E MÉTODOS

Sujeitos e variáveis clínico-laboratoriais

Estudo de coorte prospectivo com recrutamento consecutivo de participantes, entre novembro de 2010 a outubro de 2012, na Maternidade Darcy Vargas (MDV) em Joinville, SC. Participaram do estudo gestantes diagnosticadas com DMG, conforme os critérios da OMS (15).

Dividiram-se as gestantes em dois grupos, correspondentes à presença ou à ausência do alelo mutante. As gestantes apresentavam idade mínima de 18 anos, idade gestacional entre 20 e 32 semanas (calculada a partir da primeira ultrassonografia realizada pela paciente) e gestação única. Na primeira consulta de pré-natal, foram verificados a massa corporal, a estatura e o índice de massa corporal (IMC), que foi calculado pela divisão da massa corporal pela altura ao quadrado. Consideraram-se como sobrepeso os resultados obtidos acima de 25 kg/cm² até 29,9 kg/cm² e obesidade acima de 30 kg/cm². Foram avaliados o ganho de peso durante a gestação, a necessidade de tratamento complementar e o tipo de tratamento (hipoglicemiante oral ou insulina). Foram excluídas as gestantes diagnosticadas com doença hipertensiva específica da gravidez (DHEG) (n = 3) e uma gestante que teve aborto hidrópico.

Em todas as gestantes, após o jejum de 8 a 12 horas, foram coletadas as amostras de sangue em veia antecubital. As variáveis avaliadas foram: curva glicêmica com 75 gramas de glicose entre a 24ª e 28ª semanas de gravidez, glicemia de jejum no diagnóstico e glicemia após o tratamento, hemoglobina glicada (HbA1c) após tratamento e glicemia pós-prandial. As mensurações das glicemias foram realizadas por meio do método de química seca no aparelho Vitros Fusion 5.1. Foi coletado 1 mL de sangue, no mesmo local no momento das coletas da rotina de acompanhamento pré-natal dessas pacientes, sem necessidade de uma punção venosa específica. O sangue foi encaminhado para o Laboratório de Biologia Molecular da Universidade da Região de Joinville (Univille), onde foi processado e analisado para a pesquisa do polimorfismo G54D do gene *MBL2*.

Os dados avaliados dos recém-nascidos foram: peso ao nascer, sendo considerados recém-nascidos grandes

para a idade gestacional quando peso acima do percentil 90 em curvas de crescimento ou macrossomia se peso superior a 4.000 g (16), ocorrência de hipoglicemia (glicemia capilar < 40 mg/dL) e APGAR de primeiro e quinto minutos.

Todas as gestantes foram acompanhadas no serviço de alto risco da MDV, pela mesma equipe multidisciplinar, composta por médicos, nutricionista, enfermeiras e fisioterapeuta. As gestantes consentiram à participação por meio de assinatura do Termo de Consentimento Livre e Esclarecido específico. A pesquisa foi aprovada pelo Comitê de Ética em Pesquisa da Univille – processo 203/2011.

O tamanho da amostra foi calculado estimando que na MDV sejam atendidas, em média, 500 gestantes diagnosticadas com DMG por ano. A presença da deficiência da MBL na população geral é estimada em, aproximadamente, 10% (10). Os outros dois desfechos primários selecionados (ocorrência de recém-nascido grande para a idade gestacional e necessidade de terapia complementar) apresentam frequências estimadas de 30% (17). Uma vez que se estabeleceu comparar a diferença das frequências de necessidade de implementação de terapia complementar entre as gestantes portadoras e não portadoras do alelo mutante do polimorfismo G54D, então a frequência estimada é de 3% (30% x 10%). Considerando o intervalo de confiança (IC) da prevalência estimada de 1,5 a 4,5%, chega-se a uma amostra de 109 (80% de nível de confiança).

Análises genotípicas

As amostras de sangue periférico (300 µL) foram submetidas à extração do DNA genômico utilizando-se os procedimentos recomendados pelo fabricante do *kit Genomic DNA Extraction Kit* (Real Biotech Corporation, Taiwan).

Um segmento do gene *MBL2*, correspondente ao éxon 1 e parte da região 5' não traduzida, foi amplificado via reação em cadeia da polimerase (PCR) com emprego dos iniciadores GAGGCTTAGACCTATGGGGCTAG e CAGGCAGTTTCCTCTGGAAGG (18). As reações (50 µL) foram preparadas em cabine de uso específico e continham 50-500 ng DNA, 200 µM dNTP's, 1,5 mM $MgCl_2$, 1 U Platinum Taq® DNA Polimerase (Invitrogen, EUA), tampão de reação (Invitrogen), 50 pmol iniciadores e água grau PCR. A termociclagem foi realizada segundo as informações do produtor em aparelho XP Cycler (Bioer Technology Co., Japão).

Os produtos gerados na PCR (*amplicons*) foram submetidos à digestão pela endonuclease BanI (New England Biolabs, EUA), com sítio de reconhecimento específico correspondente a GGYRCC, a **37ºC** durante duas horas, conforme recomendações do fabricante. Os padrões de restrição obtidos foram verificados por meio de eletroforese (100V/2h) em gel de agarose a 1%, contendo 0,5 µg/mL de brometo de etídeo, e documentados digitalmente sob luz ultravioleta (MiniBis-Pro, DNR Bio-Imaging Systems Ltda., Israel). São previstos três padrões de restrição: para o genótipo heterozigoto (A/B) são esperados três fragmentos distintos (83, 1034 e 1117 pares de base; pb), para o genótipo homozigoto selvagem (A/A) preveem-se dois fragmentos (83 e 1034 pb) e para o genótipo homozigoto mutante (B/B) espera-se observar apenas o segmento de 1117 pb.

Análises estatísticas

Inicialmente, todas as variáveis foram analisadas descritivamente. Para as variáveis contínuas (quantitativas), a análise foi realizada por meio do cálculo de médias e desvios-padrão. Para as variáveis categóricas (qualitativas), calcularam-se frequências absolutas e relativas.

Para a análise da hipótese de igualdade entre as médias dos grupos, foi utilizado o teste *t*. Como a normalidade foi rejeitada, utilizou-se o teste não paramétrico de Mann-Whitney. O teste de normalidade utilizado foi Kolmogorov-Smirnov.

Para se testar a homogeneidade dos grupos em relação às proporções, foi utilizado o teste Qui-quadrado ou o teste exato de Fisher. Ficou estabelecido nível de significância menor que 0,05.

Os cálculos estatísticos foram realizados utilizando-se o *software* SPSS versão 11.0.

RESULTADOS

O polimorfismo G54D no éxon 1 do gene *MBL2* foi investigado em um grupo de 109 pacientes com DMG. Foram excluídas três gestantes que desenvolveram doença hipertensiva específica da gravidez (DHEG) e uma que teve aborto hidrópico, resultando em 105 pacientes efetivamente consideradas no presente estudo. As gestantes foram divididas em dois subgrupos correspondentes à presença (n = 37) ou à ausência (n = 68) do alelo mutado.

O perfil epidemiológico dos subgrupos foi comparado quanto ao ganho de peso, paridade, idade, IMC e idade gestacional de chegada à maternidade, não sen-

do observadas diferenças estatisticamente significantes (Tabela 1). No tocante à antropometria e à composição corporal das gestantes, identificou-se excesso de peso na maioria da população estudada. No subgrupo das pacientes portadoras do genótipo selvagem, 63,2% apresentavam sobrepeso, com um IMC médio de 26,5 kg/m², enquanto 62,2% das pacientes portadoras do alelo mutado apresentavam sobrepeso, com um IMC médio de 26,3 kg/m².

Tabela 1. Perfil epidemiológico da população (médias e desvio-padrão)

Gene *MBL2*	Alelo selvagem N = 68	Alelo raro N = 37	*P*
Idade (anos)	30,6 (6,2)	30,6 (6,0)	0,830*
Idade gestacional chegada (semanas)	28,1 (5,6)	26,1 (6,0)	0,380†
Gestações (número)	2,8 (1,9)	2,5 (1,5)	0,842†
IMC‡ (kg/m²)	26,5 (5,2)	26,2 (3,3)	0,520*
Ganho peso (kg)	11,3 (6,3)	10,4 (9,1)	0,923*

* Teste *t* Student; † Teste de Mann-Whitney; ‡ Índice de massa corporal.

Das 105 mulheres com DMG em que as variantes do polimorfismo G54D do gene *MBL2* foram estudadas, 37 (35,2%) apresentavam ao menos um alelo mutado, sendo que quatro (3,8%) apresentavam o genótipo AA e 33 (31,4%), o genótipo AG. Portanto, o genótipo GG (64,8%) e o alelo G (79,5%) mostraram-se os mais prevalentes na população estudada (Tabela 2). Os genótipos estavam em equilíbrio de Hardy-Weinberg.

De forma equivalente não foram encontradas diferenças significantes quanto aos resultados laboratoriais de glicemia em jejum no diagnóstico, glicemia duas horas após curva glicêmica com 75 gramas de glicose e HbA1c, assim como glicemias de jejum e pós-prandial com tratamento, conforme apresentado na tabela 3.

Tabela 2. Frequências genotípica e alélica relativas ao códon 54 do gene *MBL2* e necessidade de terapia complementar

	Somente dieta	Dieta e terapia complementar	*P*	OR
	[n (%)]	[n (%)]		(95% CI)
Genótipo				
MBL2 selvagem	40 (38,1)	28 (26,7)	0,7	1,21
MBL2 mutado	20 (19,0)	17 (16,2)		
G54D				
GG	40 (38,1)	28 (26,7)		
AG	17 (16,2)	16 (15,2)		
AA	3 (2,9)	1 (0,9)		
Alelo				
G	97 (46,2)	72 (34,3)		
A	23 (10,9)	18 (8,6)	1	0,948

OR: *odds ratio*.

Houve necessidade de tratamento complementar com metformina para 13 (35,1%) pacientes que apresentavam o alelo mutado do polimorfismo G54D e para 20 (29,4%) das pacientes com o genótipo selvagem ($p = 0,537$). A terapêutica com metformina não foi suficiente, sendo realizada complementação com insulinoterapia em 8 (11,8%) das gestantes que estavam no grupo do genótipo selvagem e 5 (13,5%) no grupo com o alelo mutado ($p = 0,475$).

Os recém-nascidos (RNs) das mães que apresentavam o alelo mutado do polimorfismo G54D geraram infantes mais pesados, porém sem alcançar significância estatística (Tabela 4). Verificou-se a presença de RN grandes para a idade gestacional (GIG) em nove (9/37; 24,3%) das pacientes no grupo dispondo o alelo raro e apenas nove (9/68; 13,2%) no outro grupo.

Tabela 3. Resultados de parâmetros clínico-laboratoriais de acompanhamento do diabetes melito gestacional (médias e desvios-padrão)

Gene *MBL2*	G54D selvagem (GG) n = 68		G54D mutado (AG+AA) n = 37		*P*
Glicemia jejum no diagnóstico	87,6 mg/dL	(12,4)	85,36 mg/dL	(10,7)	0,156*
Glicemia 2 h após GTT no diagnóstico†	155,36 mg/dL	(17,2)	154,66 mg/dL	(12,3)	0,968*
Glicemia jejum com tratamento	85,86 mg/dL	(8,9)	85,96 mg/dL	(8,6)	0,239*
Glicemia pós-prandial com tratamento	114,96 mg/dL	(15,1)	114,46 mg/dL	(13,7)	0,488*
HbA1c‡	5,4%	(0,3)	5,6%	(0,5)	0,748*
Trat. somente dieta§	40		20		0,482‖
Trat. dieta e med.¶	28		17		0,482‖

* Teste *t* Student; † Glicemia 2 horas após curva glicêmica com 75 g de glicose; ‡ Hemoglobina glicada; § Tratamento somente com dieta; ‖ Teste Qui-quadrado de Fischer; ¶ Tratamento com dieta e medicação.

Tabela 4. Características do recém-nascido

Gene *MBL2*	G54D selvagem (GG) [n (%)]	G54D mutado (AG+AA) [n (%)]	*P*
	68 (64,8)	37 (35,2)	
PIG	4 (5,9)	2 (5,4)	0,212*
AIG	55 (80,9)	26 (70,3)	0,216*
GIG	9 (13,2)	9 (24,3)	0,149*
APGAR 1	7,7 (1,9)	8,6 (0,6)	0,326†
APGAR 2	8,9 (1,5)	9,3 (0,5)	0,940†

* Teste Qui-quadrado de Fischer; † Teste de Mann-Whitney; PIG: pequeno para idade gestacional; AIG: adequado para idade gestacional; GIG: grande para idade gestacional.

DISCUSSÃO

O DMG é uma doença complexa, sendo razoável acreditar na existência de vários mediadores para sua ocorrência. Nos RNs das mães que desenvolveram DMG, há maior probabilidade de macrossomia fetal, sendo este um fator predisponente à resistência à insulina, obesidade e diabetes tipo 2 na infância e no adulto (19,20). Para essas mães, há um risco de 10% ao ano de desenvolver diabetes tipo 2 no futuro (1).

A identificação de variantes genéticas que influenciam o DMG é um foco importante de pesquisas na atualidade com vistas a melhorar o entendimento dos mecanismos subjacentes à patogênese dessa desordem. A mutação G54D do gene *MBL2* está associada à diminuição dos níveis de MBL (21).

O primeiro relato sobre o risco aumentado para o desenvolvimento de DMG associado a uma mutação no gene *MBL2* foi realizado por Megia e cols. em 2004 (10). A mutação no códon 54 do éxon 1 é considerada a mutação mais frequente do gene *MBL2*. O polimorfismo G54D é comum na população caucasiana. No estudo de Ferraroni em 2011, a prevalência do genótipo AA foi de 5,1%, que é semelhante à europeia (4%) e à japonesa (5%). Em nosso estudo, foram identificados quatro pacientes (3,8%) com presença do genótipo AA (22).

A presença de ao menos um alelo mutado do polimorfismo G54D foi observada em 30% da população na maioria dos grupos étnicos avaliados (23,24). Em nosso estudo, 35,2% das pacientes analisadas carregavam o alelo mutado do polimorfismo G54D, enquanto 43,8% das gestantes diabéticas apresentavam o mesmo alelo no grupo de espanholas estudado por Megia e cols.

Na análise do peso das gestantes com DMG, observou-se que a maioria das pacientes de ambos os subgrupos apresentou excesso de peso. É reconhecido que a obesidade constitui um fator de risco significativo para o desenvolvimento de diabetes melito tipo 2 (DM2) após o parto (25,26). A identificação da alta frequência (62,7%) de sobrepeso é de suma importância, pois as mulheres com DMG, avaliadas no presente estudo, encontram-se sob elevado risco de desenvolver DM2 após a gestação. Tal fato indica um aspecto a ser considerado no planejamento da assistência durante e após a gestação, o que também foi apontado por outros autores, constituindo fator de risco modificável passível de intervenção e prevenção quanto à ocorrência de DM2 após DMG (27-29).

Há estudos que demonstraram que a presença do alelo mutado está associada à deficiência de MBL, ocorrência que favorece o desenvolvimento de diabetes gestacional mais severo (10). No estudo de Megia e cols., as gestantes portadoras do alelo mutado necessitaram mais de insulina em seu tratamento complementar à dietoterapia, o que não foi observado em nossa população estudada.

Os dados coletados e resultados de genotipagem demonstraram que o polimorfismo não esteve significantemente associado aos parâmetros do RN, inclusive peso ao nascimento e ocorrência de RN classificado como GIG. Enquanto alguns autores encontraram uma relação com aumento do risco para o parto prematuro e peso reduzido ao nascimento quando as pacientes apresentavam o alelo mutado do polimorfismo G54D (30), outros autores, como Megia e cols., demonstraram associação com maior peso ao nascimento (10).

Considerando os resultados aqui apresentados, fazem-se necessários estudos adicionais para investigar a associação e o impacto do polimorfismo G54D do gene *MBL2* em relação ao DMG, visto que o número limitado de sujeitos alocados pode justificar a ausência de diferenças entre os subgrupos.

Declaração: os autores declaram não haver conflitos de interesse científico neste estudo.

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An international survey of screening and management of hypothyroidism during pregnancy in Latin America

Uma avaliação internacional do rastreio e manejo do hipotireoidismo durante a gestação na América Latina

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ABSTRACT

Objective: To determine how endocrinologists in Latin America deal with clinical case scenarios related to hypothyroidism and pregnancy. **Materials and methods:** In January 2013, we sent an electronic questionnaire on current practice relating to management of hypothyroidism in pregnancy to 856 members of the Latin American Thyroid Society (LATS) who manage pregnant patients with thyroid disease. Subsequently, we have analyzed responses from physician members. **Results:** Two hundred and ninety-three responders represent clinicians from 13 countries. All were directly involved in the management of maternal hypothyroidism and 90.7% were endocrinologists. The recommendation of a starting dose of L-thyoxine for a woman diagnosed with overt hypothyroidism in pregnancy, preconception management of euthyroid women with known thyroid autoimmunity and approach related to ovarian hyperstimulation in women with thyroid peroxidase antibodies were widely variable. For women with known hypothyroidism, 34.6% of responders would increase L-thyroxine dose by 30-50% as soon as pregnancy is confirmed. With regard to screening, 42.7% of responders perform universal evaluation and 70% recommend TSH < 2.5 mUI/L in the first trimester and TSH < 3 mUI/L in the second and third trimester as target results in known hypothyroid pregnant women. **Conclusion:** Deficiencies in diagnosis and management of hypothyroidism during pregnancy were observed in our survey, highlighting the need for improvement of specialist education and quality of care offered to patients with thyroid disease during pregnancy in Latin America. Arq Bras Endocrinol Metab. 2014;58(9):906-11

Keywords
Hypothyroidism; thyroid; pregnancy

RESUMO

Objetivo: Determinar, na América Latina, como os endocrinologistas lidam com cenários clínicos relacionados ao hipotireoidismo durante a gravidez. **Materiais e métodos:** Em Janeiro de 2013, foi enviado, para 856 membros da Sociedade Latino-Americana de Tireoide (LATS), um questionário eletrônico sobre práticas relacionadas ao manejo do hipotireoidismo durante a gestação. Subsequentemente, as respostas foram analisadas. **Resultados:** Duzentos e noventa e três médicos, de 13 países, responderam ao questionário. Todos estavam diretamente envolvidos no manejo de hipotireoidismo materno e 90,7% eram endocrinologistas. As recomendações de iniciar terapia com levotiroxina para uma mulher com hipotireoidismo franco durante a gravidez e o manejo na fase de pré-concepção de pacientes eutireoidianas com conhecida autoimunidade em hiperestimulação ovariana variaram amplamente. Para mulheres com hipotireoidismo conhecido, apenas 34,6% dos respondedores aumentariam a dose de levotiroxina em 30-50% assim que a gravidez fosse confirmada. Em relação ao rastreamento, 42,7% dos respondedores realizam avaliação universal. Setenta por cento recomendam TSH < 2,5 mUI/L no primeiro trimestre e TSH < 3 mUI/L no terceiro trimestre como alvos. **Conclusão:** Observamos problemas no diagnóstico e manejo do hipotireoidismo durante a gestação, enfatizando a necessidade, na América Latina, de melhoria na educação médica continuada em áreas como tireoiodopatias na gestação. Arq Bras Endocrinol Metab. 2014;58(9):906-11

Descritores
Hipotireoidismo; tireoide; gestação

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INTRODUCTION

Hypothyroidism complicates 2-3% of pregnancies, and up to 6,3% if the trimester-specific reference range is used (1). Over the past few years there has been a major expansion of our knowledge regarding thyroid disorders during pregnancy and its multiple adverse consequences, affecting pregnant women and their unborn children. The hypothyroid pregnant women have increased rates of miscarriage, preeclampsia, placental abruption, growth restriction, prematurity, stillbirths and their fetuses are at risk for impaired neurologic development (2,3). Although it is well known that overt hypothyroidism (OH) have deleterious impact on obstetrical outcomes, crucial data regarding treatment of subclinical hypothyroidism (SCH), euthyroid women positive for thyroperoxidase (TPO) and/or thyroglobulin (Tg) antibody (TAb+) and isolated maternal hypothyroxinemia is critically needed (4,5).

In 2010, significant variability in diagnostic and therapeutic management have been reported by a questionnaire which assessed management of hypothyroidism during pregnancy in Europe (6). However, even with novel prospective studies, many controversies still exist regarding the most appropriate approach for the L-T_4 dose adjustment in pregnancy (7,8). This study aims to analyze the management approach for hypothyroidism during pregnancy in Latin America and compare with data from Europe.

MATERIALS AND METHODS

The original questionnaire validated in the European survey was translated to Portuguese and Spanish by the authors (Appendix 1) (6). It was sent through e-mail to members of the Latin American Thyroid Society (LATS) who had their electronic addresses available (www.lats.org). In January 2013, 856 physicians living in Latin America with e-mail address available on LATS website were found. Four repeated messages were sent to these LATS members, in intervals of a couple of weeks. From the original 856 members of LATS, 180 (21%) did not receive the questionnaire by e-mail because their electronic addresses were incorrect. Results are given as frequencies, and chi-squared test was used to compare responses. A p-value < 0.05 was considered significant.

RESULTS

Characteristics of responders

Three hundred eleven responses were received. Nine were excluded because the responders were not involved with pregnancy care. The remaining 293 participants were retained for analyses and corresponded to 90% of endocrinologists; 0.3% nuclear medicine specialists; 4.2% surgeons; 2% of family practitioners; and 3.5% of others specialities, from thirteen countries. Most responses were from Brazil (31.7%), but there were also responders from, Mexico (25.0), Argentina (23.1%), Colombia (12.5%), Peru (2.2%), Chile (1.6%), Bolivia (1.0%), Uruguay and El Salvador (0.6%, both); other four countries (French Guyana, Paraguay, Venezuela, Guatemala) had one response each (1.2%).

Diagnosis and treatment of overt hypothyroidism during pregnancy

For a hypothyroid woman planning pregnancy (Case 1, Table 1), a majority of responders (53%) preferred increasing the L-T_4 dose only after performing biochemical tests while 34.6% have stated that this dose increase should be done immediately after pregnancy is suspected (Table 1). Indeed, in this setting, 6.7% recommended to increase the dose by two additional tablets weekly as soon as pregnancy is confirmed and 5.7% would increase the dose in the preconception period (Table 1). In Latin America, for women at preconception (Case 1, Table 1), L-T_4 adjustment have presented a trend of less recommendation than in Europe (34.6% *vs.* 44.4%, $p < 0.5$), and the responders presented a not significant more indication of thyroid function tests only after pregnancy confirmation for L-T_4 adjustment (53% *vs.* 43.2%, $p < 0.5$) if with we compare Europe and Latin America, respectively (Table 1).

For a woman with autoimmune hypothyroidism undergoing an assisted reproduction technology (Case 2, Table 1), a majority of responders (68%) preferred to adjust the L-T_4 dose following thyroid function tests immediately after the ovarian hyperstimulation, and this was higher but did not reach statistical significance when compared with previous results in Europe (57.1%, $p = 0.07$). Conversely, in Latin America, significantly less responders (1.8%) recommended to increase the L-T_4 just before the ovarian hyperstimulation or in-vitro fertilization (*vs.* 24.2% in the European survey, $p < 0.05$) (Table 1).

Table 1. Cases scenarios and comparison of hypothyroidism during pregnancy management in Europe and Latin America

Case 1. A 26 year old woman has autoimmune hypothyroidism and is euthyroid (prepregnancy TSH 2.4 mUI/L) on L-thyroxine replacement. She expresses her wish to become pregnant soon. What advice would you give?	**Responders, n (%)**	
	ETA	**LATS**
Increase the dose of L-thyroxine by 30-50% as soon as pregnancy is confirmed	72 (44.4)	98 (34.6)*
Increase the dose of L-thyroxine by two tablets per week as soon as pregnancy is confirmed	9 (5.6)	19 (6.7)
Check thyroid function tests as soon as pregnancy isconfirmed and increase the dose of L-thyroxine if necessary	70 (43.2)	150 (53.0)†
Increase L-thyroxine dose before pregnancy	11 (6.8)	16 (5.7)
Case 2. A 26 year old woman has autoimmune hypothyroidism and is euthyroid (TSH 1.8 mUI/L) on L-thyroxine replacement. She is infertile and should undergo an ovarian hyperstimulation before an IVF procedure. What advice would you give?		
Increase the dose of L-thyroxine by 30-50% as soon as pregnancy is confirmed	30 (18.6)	40 (14,1)
Immediately increase the dose of L-thyroxine before the hyperstimulation, to obtain a low normal TSH level	-	45 (15,9)
Check thyroid function tests after hyperstimulation and increase the dose of L-thyroxine if necessary	92 (57.1)	193 (68,2)§
Case 3. A 24 year old woman is 12 weeks pregnant and just been diagnosed with overt primary hypothyroidism (TSH 86 mUI/l). What dose of L-thyroxine would you initially start? Would you advise the above patient, who has been diagnosed with overt hypothyroidism in the late first trimester, to consider abortion? What tests would you use to monitor the dose of L-thyroxine during pregnancy? What are the target thyroid test results you aim to achieve with L-thyroxine replacement in pregnancy?		
Start on a small dose (*e.g.* 25-50 mcg daily)	10 (6.2)	25 (8.8)
Start on a full dose (*e.g.* 100-125 mcg daily)	74 (45.7)	116 (41.0)
Start on a dose based on pregnancy-adapted body weight	29 (17.9)	71 (25.1)
Start for a few days on a double dose (*e.g.* 200 mcg daily), then a dose based on pregnancy-adapted body-weight	40 (24.7)	54 (19.1)
Start on a dose based on pre-treatment TSH level	5 (3.1)	6 (2.1)
Other	2 (1.2)	11 (3.9)*

* Include: start on a small dose and progression to a full dose in 7 days (n = 6); start on a high full dose based (2.3 mcg/kg) on pregnancy body weight (n = 5).

† $p < 0,5$. § $p = 0, 07$.

In the setting of overt hypothyroidism (OH) diagnosed during first trimester (Case 3, Table 1), suggestions for starting regimes to initiate L-T_4 were widely variable (Table 1). Most responders judged full L-T_4 dose replacement necessary, either empirically (41%) or based on body weight (25.1%); conversely, 8.8% of responders felt that a high dose was unnecessary and preferred small starting dose (25 mcg/daily) (Table 1). There were no significant differences between LATS and ETA members responses, when choosing a starting low dose of L-thyroxine (8,8% *vs.* 6.2%), or starting the full dose (41% *vs.* 45.7%, $p = 0.5$) (Table 1) (6).

Seven different combinations of thyroid function tests were chosen to monitor the treatment but TSH associated with FT_4 was preferred by 54.3% of the physicians. The majority of responders (70%) have recommended to keep the TSH < 2.5 mUI/L in the first trimester and < 3 mUI/L in the second and third trimester; this was significantly higher compared with the last European survey (56.6%), $p < 0.05$. Only 7.3% of responders targeted to keep thyroid function tests within laboratory reference range (*vs.* 8.1% in Europe, $p = 0.6$) (6). Maternal OH during the first trimester have induced almost all the participants (96%) not to consider abortion in such a situation, but 4% would discuss or recommend abortion. In contrary, in Europe, 18% would recommend abortion or, at least, discuss this option (7%), $p < 0.05$.

Screening hypothyroidism in pregnancy

In Latin America, 38.4% (*vs.* 43% in Europe) of responders use universal screening strategy and 43% prefer a case-finding approach in high-risk groups. Surprisingly, 19.4% (*vs.* 17% in Europe) still reported absence of any screening strategy in their centers. The timing of screening was variable: 62.5% (*vs.* 67% in Europe) reported screening before conception, 24.5% (*vs.* 22% in Europe) at the time of the first prenatal visit and 10% (*vs.* 11% in Europe) had no strategy for this issue. Tests chosen for screening were highly variable and did not reached statistic difference if compared with Europe: TSH with TPO-Ab (47.5%); TSH alone (38.8%); TSH with FT_4 or TT_4 (33.7%); FT_4 (35%); FT_3 or TT_3 (9.4%); TT_4 (6.8%). When asked about retesting (double screening) in women whose initial screening was normal, 43.9% (*vs.* 53% in Europe; $p = 0,2$) would

recommend this in the presence of thyroid antibodies while 30% (*vs.* 31% in Europe) would not repeat.

Once screening has been performed, physicians have fundamentally addressed the decision of L-T_4 treatment based on different criteria: TSH above 2.5 mIU/L, 50.8%; TSH above the trimester-specific reference range, 37.7%; TSH above 5mIU/L, 20%; TSH above the population reference range, 11.2%; FT_4 bellow the trimester-specific reference range, 22.7%; and other criteria, 5%. There was inconsistency in responders' definition and management of maternal isolated hypothyroxinemia, with 59.5% (*vs.* 48% in Europe; p = 0,1) of physicians choosing follow-up without treatment and 36.8% (*vs.* 38% in Europe) recommending T_4 replacement.

The management was different in women with TSH level > 2.5 mUI/L (but < 5 mUI/L) with positive or negative thyroid antibodies when comparing answers from Latin America and Europe (Figure 1). Once TSH levels are between 2.5 to 5 mUI/L and TPO-Ab are detected, physicians have the following decision: treatment with L-T_4 (14.5% *vs.* 10% in Europe); close follow-up without treatment (81.4% *vs.* 73% in Europe; p = 0,06); no follow-up and/or treatment (4.1% *vs.* 17% in Europe; p = 0,4) (Figure 1). We could not find any significant difference for all management aspects between responders from countries in Latin America.

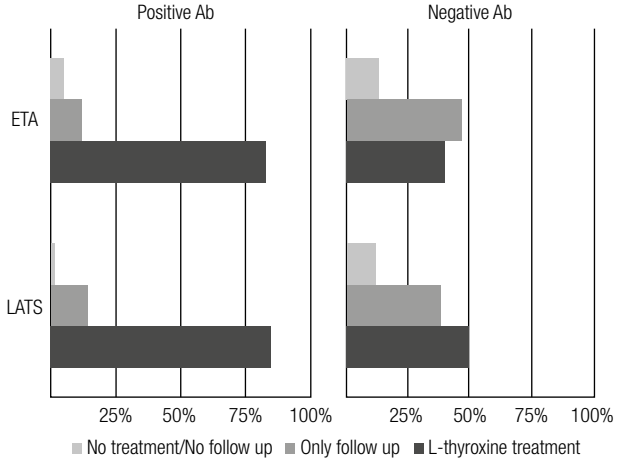

Figure 1. Management in pregnant women with TSH level > 2.5 mUI/L (but < 5 mUI/L) with positive or negative thyroid antibodies.

DISCUSSION

With regard to maternal OH diagnosed during pregnancy, the current guidelines provide recommendations to start treatment and normalize thyroid function as rapidly as possible (5,9). The Endocrine Society published in 2012 that when serum TSH is first checked during pregnancy, the average increments of L-T_4 needed are 75-100 mcg/d for those with a serum TSH above 20 mUI/L (5). In our study, practices regarding the L-T_4 starting dose were very variable, with only 41% reported starting full doses for this case. The publication of the Endocrine Society guidelines seems have not created a change in the clinical variability of decisions of LATS regarding maternal OH treatment once the responses are comparable with the Europe (5,6). Indeed, the vast majority of hypothyroid women are not managed by endocrinologists during pregnancy, and the published subspecialty guidelines related to thyroid in pregnancy might not reach the relevant audience and be ignored. The goal of L-T_4 treatment was to normalize maternal serum TSH values within the trimester-specific reference range for 16% of the responders and to a less than 2.5 mUI/L (first trimester) and 3.0 mUI/L (second and third trimester) in 70% of the participants (*vs.* 56.6% in Europe, p < 0.05). Perhaps the better management compared with the European answers are effect of the recent guidelines which have emphasized that a women with prior hypothyroidism should start their pregnancy with a serum TSH not higher than 2.5 mUI/L (5).

The guidelines of American Thyroid association states that treated hypothyroid patients who are newly pregnant should independently increase their dose of L-T_4 by 25-30% upon a missed menstrual cycle or positive home pregnancy test and notify their caregiver promptly (6). In contrary, in Latin America, only 6.7% (*vs.* 5.6% in Europe) of responders would recommend immediate adjustment in the treatment. The majority (53%) still prefers to perform thyroid function tests first and then make L-T_4 dose alterations. It is well known that the first prenatal visit in most centers typically does not occur until at least 8 weeks of gestation, and that the L-T_4 adjustments might not being addressed in a timely fashion for most patients. Even in an academic center, analyzing a cohort of 397 pregnant women followed since the first trimester with known hypothyroidism, TSH levels remained above the recommendations in 43% and 33%, during the first and second trimester, respectively (6). Data may reflect that management of pregnant women with both pre-existing as well as newly diagnosed hypothyroidism are suboptimal across Latin America, however it is not clear whether this is due lack knowledge or logistical problems.

Table 2. Utilized screening questions: pregnant with hypothyroidism

Routine screening; In your institution, who is primarily responsible for the management of hypothyroidism in pregnancy? Do you (or your institution) screen pregnant women for thyroid dysfunction? If you (or your institution) carry out *targeted screening* of high-risk pregnant women, which risk factors would you consider in classifying "high risk"? If you screen pregnant women for thyroid dysfunction, which tests do you use? When would you screen potentially pregnant women for thyroid dysfunction? If you screen pregnant women in the first trimester for thyroid dysfunction and if the initial screening test shows a TSH of 1.5 mUI/L, would you routinely test thyroid function again during the pregnancy? If you screen pregnant women for thyroid dysfunction, at when would you start L-thyroxine replacement?
SCH and isolated hypothyroxinemia management choice; If you screen pregnant women for thyroid dysfunction, would you treat or follow up with further tests (without treatment) the following conditions: a) Isolated hypothyroxinemia (low thyroid hormone levels with normal TSH); b) Isolated thyroid antibodies positive (with serum TSH < 2.5 mUI/L); c) TSH level > 2.5 mUI/L (but < 5 mUI/L) with positive thyroid antibodies; d) TSH level > 2.5 mUI/L (but < 5 mUI/L) with negative thyroid antibodies and no other thyroid disease; e) If you treat isolated hypothyroxinemia, how do you define isolated hypothyroxinemia?

In Latin America, counseling about the necessity for L-T_4 administration in TAb+ euthyroid women undergoing assisted reproductive procedures is more likely to be performed by obstetricians rather than endocrinologists. In infertile women, the prevalence of positivity for TPO-Ab is higher than fertile control and the pregnancy rate of those undergoing assisted reproduction is lower. In literature, there is not strong evidence to recommend L-T_4 therapy in TAb+ euthyroid women during assisted reproduction technology procedures (9). Negro and cols. have shown that L-T_4 treatment does not improve the delivery rate, suggesting that the autoimmunity rather than possible mild thyroid failure might be the etiology of the higher miscarriage rate. In Latin America, we have observed lower tendency to prescribe L-T_4 treatment prior ovarian hyperestimulation (16% *vs.* 24%, $p < 0.05$) than in Europe, but 68% of responders still recommended L-T_4 post-procedure (*vs.* 57.1%, $P = 0.07$).

Thyroid dysfunction routine screening of asymptomatic pregnant women has been controversial and universal screening seems not to be associated with improved obstetric outcomes. In Latin America, 42.7% of responders recommended universal TSH screening at the first trimester visit, while 38.4% performed targeted screening of women who are at risk for thyroid dysfunction. Interestingly, almost 18.8% of the responders affirmed not to perform any type of screening in their centers and this data is similar to obtained answers from European countries (17%) (6). The Endocrine Society guidelines could not reach agreement with regard to screening recommendations and both universal screening at ninth week of gestation or only in identified high risk patients were supported (5).

A key point is that the Endocrine Society guidelines has recommended L-T_4 replacement in women with SCH, mainly to avoid obstetrical outcomes (5). Assume an example of thyroid function testing in a high-risk population, in Latin America, the majority of responders used widely different criteria for indication of L-T_4, based on different TSH cut-off or FT4 levels. From a clinical perspective, these answers illustrate the difficulties inherent in any screening process followed by management decisions.

We conclude that the management of thyroid dysfunction during pregnancy could be more uniform and standardized among clinicians from LATS and presented quite different opinions from those of the European survey. In order to reach optimal impact in the medical community, the guidelines related to the care of thyroid disorders in pregnancy could be written both by and for endocrinologists, obstetricians, internists and family practitioners.

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Evaluation of percutaneous ethanol injections in benign thyroid nodules

Avaliação do tratamento com injeção percutânea com etanol em nódulos benignos de tireoide

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ABSTRACT

Objective: The objective of this study was to evaluate the efficacy and safety of percutaneous ethanol injection (PEI) in the treatment of benign thyroid nodules. **Subjects and methods:** We evaluated 120 patients with benign thyroid nodules. Patients underwent evaluation of serum TSH and free T4, cervical ultrasound, and thyroid scintigraphy (in those with suppressed TSH levels). The application of sterile ethanol 99% was guided by ultrasound, with the injected volume amounting to one-third of the nodule volume. Response was considered complete (reduction of 90%); partial (reduction between 50 and 90%); or none (reduction of < 50%). Autonomous nodules were evaluated for normalization of TSH levels. **Results:** Among the nodules studied, 30.8% were solid, 56.7% were mixed, 12.5% were cystic, and 21.6% were hyperfunctioning. The initial volume of the treated nodules ranged from 0.9 to 74.8 mL (mean 13.1 ± 12.4 mL). We performed 1-8 sessions of PEI, applying an average of 6.2 mL of ethanol for patient. After 2 years of follow-up, 17% of patients achieved a complete response (94% reduction); 53%, a partial response (70% reduction); and 30%, no response. A reduction in the volume of autonomous nodules was noted in 70% of cases, and 54% had a normalized value of TSH. The main side effect is local pain, lasting less than 24 hours in most cases. **Conclusion:** This study showed that PEI is a safe and effective procedure for treatment of benign, solid or mixed thyroid nodules. Most cases resulted in significant reduction in nodule volume, with normalization of thyroid function. Arq Bras Endocrinol Metab. 2014;58(9):912-7

Keywords

Nodule, thyroid, goiter; percutaneous ethanol injection; sclerotherapy

RESUMO

Objetivo: O objetivo deste estudo foi avaliar a eficácia e segurança da injeção percutânea de etanol (IPE) no tratamento de nódulos tireoidianos benignos. **Sujeitos e métodos:** Foram avaliados 120 pacientes com nódulos benignos de tireoide. Todos realizaram dosagens de TSH, T4 livre, ecografia cervical (US) e cintilografia de tireoide (em pacientes com TSH suprimido). A aplicação de etanol estéril a 99% foi guiada por US e o volume de etanol injetado correspondeu a um terço do volume nodular calculado. A resposta foi considerada completa (redução de 90%); parcial (redução entre 50 e 90%) ou ausência de resposta (redução menor que 50%). Nos nódulos autônomos, foi avaliada a normalização do TSH. **Resultados:** Entre os nódulos estudados, 30,8% eram sólidos, 56,7% eram mistos, 12,5% eram císticos e 21,6%, nódulos hiperfuncionantes. O volume inicial dos nódulos tratados variou de 0,9 a 74,8 mL (média 13,1 ± 12,4 mL). Foram realizadas de 1 a 8 sessões de IPE (média 2,8), com aplicação média de 6,2 mL de etanol por paciente. Após dois anos de seguimento, 17% dos pacientes obtiveram resposta completa (redução de 94%), 53% obtiveram resposta parcial (redução de 70%) e 30% não responderam. Houve redução de volume nos nódulos autônomos em 70% dos casos, e 54% normalizaram o valor do TSH. Os efeitos colaterais registrados foram decorrentes apenas do desconforto no local de aplicação. **Conclusão:** Este trabalho mostrou que a IPE é um procedimento seguro e eficaz para tratamento de nódulos benignos, sólidos ou mistos de tireoide. Na maioria dos casos, ocasiona redução do volume nodular, com melhora dos sintomas compressivos e normalização da função tireoidiana. Arq Bras Endocrinol Metab. 2014;58(9):912-7

Descritores

Nódulo; tireoide; bócio; injeção percutânea de etanol; escleroterapia

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INTRODUCTION

Thyroid nodules are often found in clinical practice. Epidemiological studies conducted in regions rich in iodine have shown that 4 to 7% of women and 1% of men have a palpable nodule (1-4). However, ultrasound studies show a prevalence much higher (reaching 68%), with a greater incidence in women and elderly people (1,5).

In the evaluation of thyroid nodules, in addition to the clinical and ultrasound findings, fine-needle aspiration (FNA) cytology is important for characterizing and defining treatment. In nodules with cytology suspicious or positive for malignancy, surgical resection is the appropriate treatment. The best treatment for benign nodules, however, remains uncertain, especially if they cause cosmetic problems or compressive symptoms (6).

The treatment of thyroid nodules by simple aspiration, thyroid hormone suppression, or sclerosing treatment with chemical agents, such as tetracycline, has shown unsatisfactory results (7,8). Ethanol has been used as a primary sclerosing agent (9) and can be considered a therapeutic option after excluding malignancy (6). Percutaneous ethanol injection (PEI) is currently the first choice treatment of cystic nodules relapsed after diagnostic evacuation. Its use for solid nodules is under debate and, generally, proposed only in case of poor surgical risk or in patients refusing surgical or radioiodine therapy. Despite some controversial results, PEI seems as effective as radioiodine in inducing partial or complete remission of autonomous nodules, with greater reduction in size and a low risk of recurrence and hypothyroidism (10). PEI is also an alternative for patients with nodular lesions with surgical contraindication or high surgical risk (9).

SUBJECTS AND METHODS

One hundred and twenty patients (mean age, 48.2 years, 94.2% female) with uninodular and multinodular goiter were selected between May 1998 and March 2010. All patients were ambulatory and followed at the Department of Endocrinology of the Hospital de Clinicas in Curitiba, Brazil. No patient had been previously treated with radioiodine, surgery, suppressive levothyroxine, or PEI. Before the first session, levels of thyroid-stimulating hormone (TSH) were measured by chemiluminescence (ARCHITECT; Abbott Laboratories, Lake Forest, IL) (benchmark, 0.35-4.94 mU/L) and free T4 chemiluminescence (ARCHITECT Free T4; Abbott Laboratories) (benchmark, 0.7-1.48 ng/dL). Patients who had suppressed TSH levels underwent thyroid scintigraphy (Pho/Dot Scanner; Nuclear-Chicago, Des Plaines, IL, USA), including those with toxic nodules. Patients also underwent thyroid ultrasound (SSD-500V, Hitachi Aloka Medical, Ltd, Tokyo, Japan) using a 7.5-MHz transducer. For measurement of the nodule, it was assumed that the nodule had an ellipsoid shape; thus, the following equation was used: volume = diameter of the nodule laterolateral × anteroposterior × longitudinal diameter × 0.52. Patients with solid and/or mixed nodules were included in the study, and malignancy was excluded in all patients by ultrasound-guided FNA biopsy of the dominant and/or suspected nodules.

For treatment, we used sterile ethanol 99% (Indústria Farmacêutica Rioquímica Ltda., São José do Rio Preto, Brazil). The PEI procedure was performed by two experienced operators in ultrasound and FNA, with one holding the transducer and the other performing the ethanol injection. Patients were informed about the procedure and positioned in dorsal decubitus with slight hyperextension of the neck. The insertion of the needle and ethanol injection was performed with the same ultrasound equipment used for the FNA, without anesthesia or sedation. A 23-gauge needle was inserted into the nodule, and sterile ethanol 99% was injected slowly. The ethanol volume applied amounted to one-third the size of the nodule. The distribution of ethanol was monitored in real time by the ultrasound equipment. In nodules with a cystic component, aspiration of the liquid contents was performed. The injection was stopped when ethanol completely filled the nodule or when the patient complained of severe neck pain. By the end of the procedure, each patient received a prescription of nonsteroidal anti-inflammatory for use in case of persistent pain.

Treatment efficacy was evaluated for nodule volume reduction and normalization of TSH levels (in autonomous nodules). Response was considered complete when the nodule volume decreased ≥ 90%; partial, if decreased between 50% and 90%; and none, if < 50%.

The tolerability of the treatment was assessed immediately after treatment and again during outpatient follow-up. Patients were asked about the presence or absence of cervical pain. Pain was classified as mild (limited to procedure), moderate (less than 24 hours) and severe (over 24 hours). Thyroid function was monitored in those with hyperthyroidism.

For statistical analysis, clinical, laboratory, and ultrasound data were entered in Microsoft Excel software

(Microsoft, Redmond, WA) and exported to SPSS version 17.0 software (IBM Corporation, Chicago, IL). Categorical variables were described by absolute frequency and relative frequency percentage and compared by χ^2 test or χ^2 test with Yates correction. For small samples, we used the Fisher exact test. *P* values < 0.05 was considered significant.

The research was approved by the Ethics Committee on Human Research of the Hospital de Clinicas, Federal University of Paraná. Patients were informed about the procedure and signed a consent form.

RESULTS

Before treatment, 94 (78%) patients had normal thyroid function, whereas 26 (22%) had hyperthyroidism. Patients with suppressed TSH levels underwent thyroid scintigraphy, which in all cases revealed an autonomous nodule.

Fifty-eight percent of patients had a single nodule; 42% of the patients had a multinodular goiter, and in these patients, treatment was only performed in the dominant nodule. Thirty-one percent of the nodules were solid, 57% were mixed, and 12% were cystic. Further, women comprised 94% of the study sample. Mean age was 48.5 ± 13.2 years.

Patients underwent a mean of 2.8 (range, 1-8) sessions each. The average volume of ethanol 99% applied was 6.2 mL for patient. Each patient had only one nodule treated (the dominant one in cases of multinodular goiter). The average volume of the nodules selected for initial treatment was 13.1 ± 12.4 mL. The final volume was 4.7 ± 5.6 mL, with a mean reduction of 60.4% ± 28.3% (P < 0.001).

In a follow-up ranging from 6 months to 11 years, 17% of patients achieved a complete response (volume reduction of 94%); 53%, a partial response (70% reduction); and 30%, no response (Figure 1). There was a reduction in the volume of autonomous nodules in 70% of cases. Analyzing the 26 patients with hyperfunctioning nodule, 54% achieved normalization of TSH (considering our reference values) (Figure 2A and 2B). No patient developed hypothyroidism after the procedure. Solid nodules were less likely to completely respond compared to mixed and cystic nodules (7.1% *vs.* 45%), with a relative risk of 1.67 (95% confidence interval, 0.92-3.05; P = 0.056) (Tabela 1). Hyperfunctioning nodules also showed a lower response rate (11.1%) than nonfunctioning nodules (40.4%), with a relative risk of 1.49 (95% confidence interval, 1.07-2.08; P = 0.09). There was no difference in the probability of response in the other parameters studied (i.e., sex, type of goiter [uninodular or multinodular], age, initial volume of the treated nodule [> 5 mL]) (Tabela 2). Among the patients analyzed, 25% did not report any pain, 17% reported mild pain, 32% reported moderate pain and 26% reported severe pain. When questioned only 1.7% of patients do not repeat the procedure because of pain. Other reported symptoms were pain radiating to the jaw, swelling and local hematoma, occurring in < 1% of cases. No patient required glucocorticoids.

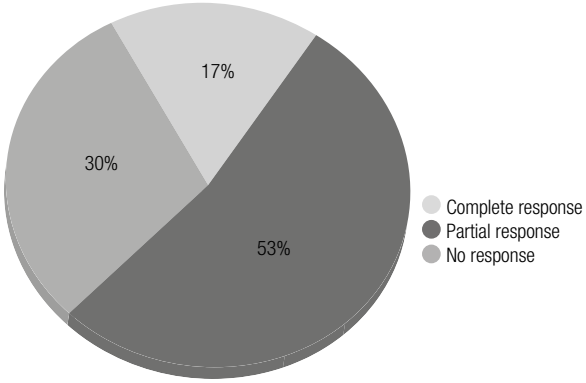

Figure 1. Response to sclerotherapy (n = 120).

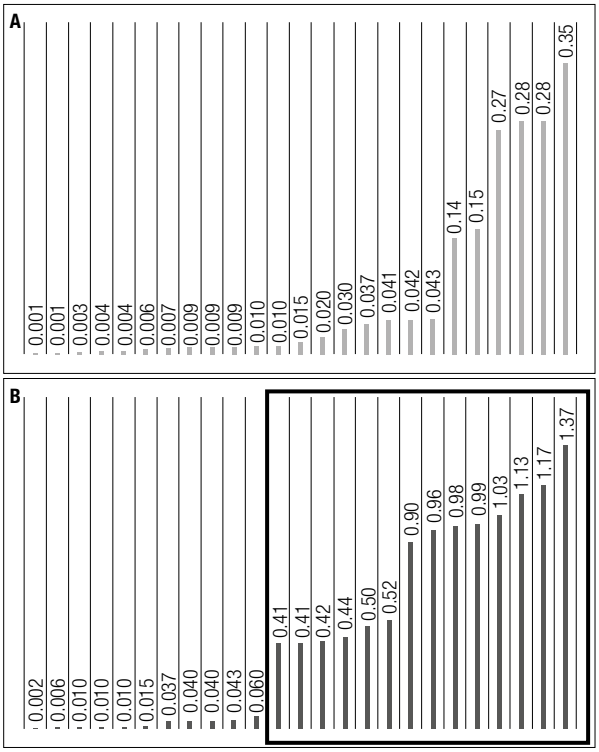

Figure 2. TSH levels (mUI/L) in patients with autonomous thyroid nodules. (**A**) TSH levels before treatment; (**B**) TSH levels after treatment. Inside the box are highlighted patients who normalized TSH levels with percutaneous ethanol injection.

Table 1. Changes in nodule volume with percutaneous ethanol injection treatment according to the nodule type

	Pre-treatment volume (mL)	Post-treatment volume (mL)	Reduction (%)
Solid	10.9 ± 10.7	5.4 ± 6.8	52.9
Mixed	14.5 ± 13.3	4.8 ± 5.1	63.0
Cystic	12.2 ± 12.3	2.6 ± 4.2	66.7

Table 2. Responses rates to percutaneous ethanol injection according patients and nodules characteristics

	Partial response		P value	Complete response		P value
Age	< 49 years	≥ 50 years		< 49 years	≥ 50 years	
	75.0%	65.6%	0.263	48.1%	24.1%	0.060
Gender	Female	Male		Female	Male	
	71.7%	42.9%	0.119	37.3%	20.0%	0.407
Type of goiter	Uninodular	Multinodular		Uninodular	Multinodular	
	65.7%	76.0%	0.225	33.3%	40.0%	0.617
Type of nodule	Solid	Cystic		Solid	Cystic	
	64.9%	66.7%	0.901	7.1%	44.4%	0.056
Initial volume	≥ 5 mL	< 5 mL		≥ 5 mL	< 5 mL	
	69.8%	70.6%	0.929	36.6%	33.3%	0.822

DISCUSSION

Currently, PEI is the first-line therapy in benign recurrent thyroid cysts. It has also been considered as an alternative therapy to surgery and radioiodine treatment in hyperfunctioning thyroid nodules. However, cost, efficacy and side effects disfavour PEI as opposed to radioactive iodine therapy. In solid nonfunctioning nodules, PEI appears to be effective in reducing volume and compressive symptoms, but there are no long-term follow-up studies assessing whether those results are sustained, and whether PEI improves quality of life. This study showed that PEI is an effective, simple, and safe method for treating benign nodules, reaching a mean volume reduction of 60% and controlling thyroid function in more than one-half of toxic nodule cases, in a follow-up for up to 11 years.

Sclerosing properties of ethanol have been known for many years, and PEI has been used to treat various benign and malignant lesions, including thyroid and parathyroid nodules; metastases from thyroid carcinoma; hepatic and intra-abdominal tumors (8,11-14). The mechanism of action of ethanol is related to cellular dehydration, coagulation necrosis, thrombosis, and vascular occlusion (15). In thyroid cysts, the application of ethanol leads to obstruction of vessels walls and prevention of recurrent bleeding and is proven to be more effective than single aspiration (11,16,17). Taking into account the changes caused by ethanol in thyroid tissue as well as fibrosis, hemorrhagic necrosis, and inflammation, reduced volume of nodules persists for some time after ethanol application, which justifies holding sessions every few months (18). Because the PEI technique uses ultrasound guidance, the nodule suffers the action of ethanol, but not the adjacent thyroid tissue, which explains the low rate of early and late clinical complications (15).

The treatment of patients in the present study with sclerotherapy showed complete response in 17% of cases, with volume reduction ≥ 90%, whereas 53% had a partial response with volume reduction between 50 and 70%. Approximately one-third of our patients did not respond to treatment, with volume reduction < 50%. Lima and cols. (4) selected 42 patients with nodular goiter or nodular hyperplasia and evaluated them after at least two sessions of PEI. Thyroid nodules were multiple (solid or cystic) in 52.4% of their patients, and single (solid, cystic, or mixed) in 47.6%. The mean reduction of nodules after injection of ethanol was 58.2% for single nodules and 60.8% for cystic nodules. In multiple nodules, the authors evaluated the reduction of all thyroid lobes and found a 52.4% reduction. The only side effects reported were discomfort at the application site. Bianchini and cols. (19) evaluated 50 patients (26 with solid nodules, 17 with cystic nodules, and 7 with autonomous nodules). After 1 year of treatment, solid nodules showed a 74% reduction in the initial volume, whereas cystic nodules decreased by 92% with no recur-

rences. In patients with autonomous nodules, euthyroidism occurred in five cases, whereas two maintained a subnormal TSH level.

In the present study, a reduction in the volume of autonomous nodules was seen in 70% of cases, and 53% of patients had normalized TSH values. In a multicenter study presented by Lippi and cols. (20), 429 patients with autonomous nodules underwent sclerotherapy. These patients underwent 2 to 12 sessions of PEI (mean, 4 sessions) with 2 to 50 mL ethanol administered in each patient (mean, 17 ± 9 mL). Euthyroidism was achieved in 66.5% of patients with toxic adenomas and in 83.4% with pretoxic adenomas after 12 months of follow-up. In all cases, there was a reduction in the volume of nodes, and those with a better response had an initial volume of < 15 mL. No patient had a recurrence or hypothyroidism at follow-up. Monzani and cols. (21) studied 77 patients with toxic nodules and observed a complete response (euthyroidism) in 60 (77.9%). Treatment failure occurred in 10 (13%) patients, all with an initial volume > 10 mL. Efficacy was similar in patients with uninodular and multinodular goiter. Recurrence of hyperthyroidism was not observed in any patient at the 5-year follow-up, and evolution to hypothyroidism occurred in only one case.

In the present sample, the ethanol dose calculated was 30% of nodular volume measured before the beginning of the session. Each patient had a mean of 2.8 PEI sessions, which agrees with the literature (4,22).

The main adverse effect associated with the procedure was pain at the injection site due to overflow of small amounts of ethanol in the subcutaneous tissue. Pain was discrete and transient in most cases. No cases of transient recurrent nerve palsy were reported in the medical charts. PEI is a safe procedure without serious complications, and immediate pain is the most common complication. Other complications can occur and are described in the literature as unilateral vocal paralysis (0.7%) (23), hematoma at the injection site (0.2-23%) (4,23), dysphonia (2.6-4.7%) (4,24), transitory hypotension (0.2%) (23), transitory thyrotoxicosis (3.2%) (8), and fibrosis complicating subsequent surgery (1.6%) (8). The main limitation of this study is the retrospective evaluation of data. Data were collected from medical records, which may reduce their reliability.

In conclusion, PEI appears to be an effective alternative method for the treatment of benign thyroid nodules. Ethanol treatment caused significant nodule reduction and normalization of thyroid function in most cases of hyperfunctioning nodules. Response was less likely to occur in solid non-functioning nodules. The reported complications were transitory and self-limited. Large prospective studies need to evaluate whether response is sustained, particularly for solid non-functioning nodules.

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The *TCF7L2* rs7903146 (C/T) polymorphism is associated with risk to type 2 *diabetes mellitus* in Southern-Brazil

O polimorfismo rs7903146 (C/T) no gene TCF7L2 *está associado com risco para o diabetes melito tipo 2 em uma população do sul do Brasil*

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ABSTRACT

Objective: The aim of this study was to investigate the association between the rs7903146 (C/T) polymorphism in the *TCF7L2* gene and type 2 *diabetes mellitus*, in a Southern-Brazilian population. **Materials and methods:** The *TCF7L2* rs7903146 polymorphism was genotyped in 953 type 2 diabetic patients and 535 non-diabetic subjects. All subjects were white. The polymorphism was genotyped by Real-Time PCR using TaqMan MGB probes (Life Technologies). Odds ratios (OR) and 95% confidence intervals (CI) were calculated for additive, recessive and dominant inheritance models. **Results:** Genotype and allele frequencies of the rs7903146 polymorphism differed significantly between type 2 diabetic patients and non-diabetic subjects (P = 0.001 and P = 0.0001, respectively). The frequency of the minor allele was 38% in type 2 diabetes group and 31% in non-diabetic subjects, and this allele was significantly associated with type 2 diabetes risk (OR = 1.42, 95% CI 1.15 – 1.76 for the dominant model of inheritance). Moreover, the T/T genotype was associated with a higher risk for type 2 diabetes (OR = 1.83, 95% CI 1.3-2.5) than the presence of only one copy of the T allele (OR = 1.31, 95% CI 1.1-1.6). Both results were adjusted for age and gender. **Conclusions:** Our results confirm the association between the *TCF7L2* rs7903146 polymorphism and increase risk for type 2 diabetes in Southern-Brazil. Arq Bras Endocrinol Metab. 2014;58(9):918-25

Keywords
Single nucleotide polymorphism; type 2 *diabetes mellitus*; transcription factor 7-like 2 (*TCF7L2*)

RESUMO

Objetivo: O objetivo deste estudo foi investigar a associação entre o polimorfismo rs7903146 (C/T) no gene *TCF7L2* e o diabetes melito tipo 2 em uma população do sul do Brasil. **Materiais e métodos:** O polimorfismo rs7903146 (C/T) no gene *TCF7L2* foi genotipado em 953 pacientes com diabetes melito tipo 2 e em 535 indivíduos não diabéticos. Todos os indivíduos estudados eram brancos. O polimorfismo foi genotipado por meio da técnica de PCR em tempo real, utilizando sondas TaqMan MGB (Life Technologies). A razão de chances e o intervalo de confiança de 95% foram calculados para os modelos de herança: aditivo, recessivo e dominante. **Resultados:** As frequências genotípicas e alélicas do polimorfismo rs7903146 diferiram significativamente entre os pacientes com diabetes melito tipo 2 e indivíduos não diabéticos (P = 0,001 e P = 0,0001, respectivamente). A frequência do menor alelo foi 38% no grupo dos pacientes com diabetes melito tipo 2 e 31% no grupo dos indivíduos não diabéticos, sendo esse alelo significativamente associado com risco para o diabetes melito tipo 2 (RC = 1,42; IC 95% 1,15 – 1,76 para o modelo de herança dominante). Do mesmo modo, o genótipo T/T foi associado com risco maior para o diabetes melito tipo 2 (RC = 1,83; IC 95% 1,3 – 2,5) do que a presença de apenas uma cópia do alelo T (RC = 1,31; IC 95% 1,1 – 1,6). Ambos os resultados foram ajustados para idade e gênero. **Conclusões:** Nossos resultados confirmam a associação entre o polimorfismo rs7903146 no gene *TCF7L2* e o risco para o diabetes melito tipo 2 em uma população do sul do Brasil. Arq Bras Endocrinol Metab. 2014;58(9):918-25

Descritores
Diabetes melito tipo 2; polimorfismos de DNA; fator de transcrição *7-like 2* (*TCF7L2*)

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INTRODUCTION

Type 2 *diabetes mellitus* (T2DM) is a heterogeneous group of disorders usually characterized by the incapability of pancreatic beta cells to increase insulin secretion to compensate the insulin resistance in peripheral tissues (1). T2DM is a multifactorial disease, and the susceptibility to it is determined by several genetic and environmental factors, in an orchestrated manner (2). The most likely explanation for the remarkable increase in T2DM prevalence observed over the past decades is changing patterns of diet, as well as lack of physical activity practice. However, it is supposed that these lifestyle changes may lead to T2DM, but only in the presence of genetic risk factors for this condition (2). For that reason, great effort has been made in an endeavor to identify genes associated with T2DM, and many loci associated with this disease have been uncovered by genetic association studies and genome-wide association scan (GWAS).

In this scenario, in early 2006, a large scale GWAS study reported that some single nucleotide polymorphisms (SNPs) in the *TCF7L2* gene were strongly associated with risk to T2DM in an Iceland case-control sample (3). The results presented in this study were substantial, with each additional copy of the risk allele being associated with an odds ratio (OR) of 1.5, and with an outstanding P value of 7.8×10^{-15} (3). Afterwards, this association has been replicated by numerous groups among different ethnicities, also with very low P values [reviewed in (4)]. Up to now, all of these studies firmly establish *TCF7L2* gene as the strongest genetic risk factor for T2DM (4-8). Amongst the *TCF7L2* variants, two intronic SNPs (rs12255372 and rs7903146) are the most closely associated with T2DM, and although both have showed a significant linkage disequilibrium (LD), the rs7903146 (C/T) variant seems to have the strongest effect in Caucasian populations (4,9).

The *TCF7L2* gene encodes a transcription factor involved in the Wnt signaling pathway, which plays an important role in pancreatic islet development and adipogenesis (10). TCF7L2 forms heterodimers with β-catenin, inducing the expression of various genes, including the insulinotropic hormone glucagon-like peptide 1 (*GLP-1*) gene, the *insulin* gene, and other genes that encode proteins involved in processing and exocytose of insulin granules (9,11-13). As GLP-1 and insulin play a key role in blood glucose homeostasis, it was hypothesized that *TCF7L2* variants may modify T2DM susceptibility by indirectly reducing GLP-1 secretion from enteroendocrine cells (14). On the other hand, as the Wnt pathway seems to be important for pancreas development during embryonic growth, it is also possible that the beta-cell mass, pancreatic beta-cell development and/or beta-cell function are also affected by this pathway (15). However, the exact molecular mechanism underlying the association of *TCF7L2* polymorphisms with T2DM remains to be enlightened (15,16).

Because the frequency of the *TCF7L2* rs7903146 T allele has been shown variable among different populations (17-23), which could influence the effect of this SNP on T2DM susceptibility, in the present study, we investigated the potential association of the *TCF7L2* rs7903146 (C/T) polymorphism with T2DM, in white Brazilian subjects. In addition, the genetic association of this SNP with T2DM has not yet been studied in Southern Brazil.

MATERIALS AND METHODS

Subjects

A total of 1,488 unrelated subjects were enrolled in this case-control study. The diabetic sample comprised 953 T2DM patients participating in a multicentre study that began recruiting patients in Southern Brazil in 2002. That project was originally designed to study risk factors associated with T2DM and its chronic complications. It included four Centers in teaching hospitals located in the Brazilian state of Rio Grande do Sul. A detailed description of that study can be found elsewhere (24). T2DM was diagnosed according to the American Diabetes Association criteria (25). The main characteristics of the T2DM patients were as follows: mean age was 59.3 ± 10.7 years, mean T2DM duration was 12.5 ± 9.5 years, mean age at T2DM diagnosis was 46.3 ± 11.4 years, mean glycated hemoglobin (HbA1c) was 7.7 ± 1.7%, and mean body mass index (BMI) was 28.7 ± 5.1 kg/m^2. Males comprised 51.0% of the sample, 71.0% of all patients had arterial hypertension (AH), and 34.2% had obesity.

The non-diabetic group comprised 535 healthy volunteers attending the blood donation facility at Hospital de Clínicas de Porto Alegre (Porto Alegre, Brazil; mean age = 44.0 ± 7.8 years; males = 51.0%). None of these subjects reported presence of diabetes or a family history of this disease. All subjects had Caucasian ancestry (mostly of Portuguese, Spanish, Italian and German descent), and were self defined as white.

A standard questionnaire was used to collect information regarding age, age at T2DM diagnosis, and drug treatment. All T2DM patients underwent physical examination and laboratory evaluations, as previously reported (26). BMI was calculated as weight (kg)/height square (meters). Presence of obesity was defined as BMI ≥ 30 kg/m^2. Office blood pressure (BP) was measured in sitting position, on the left arm, after a 5-min rest by a trained researcher, with a mercury sphygmomanometer. The mean of two measurements taken 1 min apart was used to calculate systolic and diastolic BP. Arterial hypertension (AH) was defined as BP levels higher than 140/90 mmHg at the initial visit and at two follow-up visits within 1 month of the initial visit, or if the presence of AH was previously registered on medical records.

Serum and plasma from T2DM patients were taken after a 12 hours of fasting for laboratory analyses (26). Plasma glucose levels were determined using the glucose oxidase method. HbA1c measurements were performed by different methods and results were traceable to the Diabetes Control and Complications Trial (DCCT) method by off-line calibration or through conversion formulae (27). Total plasma cholesterol, HDL cholesterol and triglycerides were assayed using enzymatic methods.

The information obtained from the study did not influence patients' diagnosis or treatment. The study protocol was approved by the Ethic Committee in Research from Hospital de Clínicas de Porto Alegre and all patients and non-diabetic subjects provided written informed consent.

Genotyping

DNA was extracted from peripheral blood leucocytes by a standardized salting-out procedure (28). *TCF7L2* rs7903146 (C/T) SNP was genotyped using primers and probes contained in the 40x Human Custom TaqMan Genotyping Assay (Life Technologies, Foster City, CA, USA), as previously reported (29). Sequences of primers and probes were: *TCF7L2*-5'CCTCAAAACCTAGCACAGCTGTTAT3' (forward primer), *TCF7L2*-5' TGAAAACTAAGGGTGCCTCATACG3' (reverse primer), *TCF7L2*-FAM-5' AAGCACTTTTTAGATATTATAT3'; *TCF7L2*-VIC-5' CTAAGCACTTTTTAGATACTATAT3'. Reactions were conducted in 96-well plates, in a total of 5 µL volume using 2 ng of genomic DNA, TaqMan Genotyping Master Mix 1x (Life Technologies) and Custom TaqMan Genotyping Assay 1x. Plates were then loaded in a real-time PCR thermal cycler (7500 Fast Real PCR System; Life Technologies) and heated for 10 min at 95°C, followed by 50 cycles of 95°C for 15s and 62°C for 1 min. The genotyping success rate was better than 95%, with a calculated error based on PCR duplicates of less than 1%.

Statistical analyses

Allelic frequencies were determined by gene counting and departures from the Hardy–Weinberg equilibrium (HWE) were verified using χ^2 tests. Clinical and laboratory characteristics were compared between groups by using One-way ANOVA, unpaired Student's *t* test or χ^2, as appropriate. Variables with normal distribution are presented as mean ± SD or percentage. Variables with skewed distribution were log-transformed before analyses and are shown as median (range).

The magnitude of associations of the *TCF7L2* rs7903146 SNP with T2DM were estimated using odds ratio (OR) with 95% confidence interval (CI). Multivariate logistic regression analyses were performed to assess the independent association of this SNP with T2DM, adjusting for age and gender. Moreover, in T2DM patients, logistic regression analysis was also performed using obesity as a dependent variable and age and gender as independent variables. Associations between the rs7903146 SNP and T2DM characteristics (continuous variables) were tested using general linear model (GLM) analyses, adjusting for covariates.

Results for which the P value was under 0.05 were considered statistically significant. Bonferroni correction was used to account for multiple comparisons. These statistical analyses were performed using SPSS version 18.0 (SPSS, Chicago, IL). Power calculations were done using the software PEPI, version 4.0, and showed that this study has a power of approximately 80% at a significance level of 0.05 to detect an OR of 1.4 or higher, for the presence of the minor allele.

RESULTS

Genotype and allele frequencies of rs7903146 (C/T) SNP in T2DM patients and non-diabetic subjects are shown in table 1. All genotypes were in agreement with those predicted by the HWE in non-diabetic subjects (P = 0.208). Both genotype and allele frequencies of the rs7903146 SNP were differently distributed between T2DM and non-diabetic subjects after Bonferroni corrections (P = 0.001 and P = 0.0001, respectively). Genotype frequencies of the rs7903146 SNP remained

significantly associated with T2DM after adjustment for age and gender (Table 1). In agreement with this data, the T allele was significantly associated with risk for T2DM under a dominant model of inheritance, adjusting for age and gender (OR = 1.42, 95% CI 1.15 – 1.76; P = 0.001). Moreover, homozygosis for the T allele was associated with a higher risk for T2DM (OR = 1.83, 95% CI 1.3-2.5, P = 0.001) than the presence of only one copy of this allele (OR = 1.31, 95% CI 1.1 – 1.6; P = 0.017), adjusting for age and gender.

Table 2 summarizes the clinical and laboratory data for T2DM patients according to the different genotypes of the rs7903146 SNP. There were no significant differences among the three rs7903146 SNP genotypes in terms of systolic and diastolic BP, BMI, total cholesterol, HDL cholesterol, LDL cholesterol, HbA1c,

Table 1. Genotype and allele frequencies of the *TCF7L2* rs7903146 (C/T) polymorphism in patients with type 2 *diabetes mellitus* (T2DM) and non-diabetic subjects

	T2DM patients (n = 953)	Non-diabetic subjects (n = 535)	Unadjusted P*	Adjusted OR (95% CI)/P†
Genotype				
C/C	382 (40.1%)	261 (48.8%)	0.001	1
C/T	415 (43.5%)	215 (40.2%)		1.31 (1.05 – 1.64)/0.017
T/T	156 (16.4%)	59 (11%)		1.83 (1.31 – 2.55)/0.001
Allele				
C	0.62	0.69	0.0001	
T	0.38	0.31		
Additive model				
C/C	382 (71.0%)	261 (81.6)	0.001	1
T/T	156 (29.0%)	59 (18.4)		1.807 (1.288 – 2.534)/0.01
Recessive model				
C/C-C/T	797 (83.6)	476 (88.9)	0.006	1
T/T	156 (16.4)	59 (11.1)		1.576 (1.146 – 2.175)/0.005
Dominant model				
C/C	382 (40.0)	261 (48.8)	0.001	1
C/T-T/T	571 (60.0)	274 (51.2)		1.424 (1.150 – 1.762)/0.001

Data are presented as number (%) or proportion. * P values were computed by χ^2 tests comparing T2DM patients and non-diabetic subjects. † Adjusted OR (95% CI)/P values were obtained from logistic regression analyses adjusting for age and gender. Only P values lower than the Bonferroni threshold (P = 0.010) were considered statistically significant.

Table 2. Clinical and laboratory characteristics of T2DM patients, broken down by presence of different *TCF7L2* rs7903146 (C/T) genotypes

	TCF7L2 rs7903146 (C/T) genotypes			F/P*	P†
	C/C (n = 382)	C/T (n = 415)	T/T (n = 156)		
Age (years)	58.2 ± 10.8	59.8 ± 10.8	60.3 ± 10.2	-	0.051
Age at diagnosis (years)	46.6 ± 11.9	46.8 ± 12.0	47.2 ± 12.0	-	0.881
Gender (% males)	47.6	48.4	50.2	-	0.857
HbA1c (%)[a]	7.7 ± 1.6	7.5 ± 1.6	8.3 ± 2.7	2.22/0.113	-
FPG (mmol/l)[a]	10.23 ± 4.18	9.69 ± 4.19	10.51 ± 3.74	2.44/0.088	-
Systolic BP (mmHg)[b]	136.74 ± 21.66	135.06 ± 20.76	137.7 ± 22.9	1.03/0.356	-
Diastolic BP (mmHg)[b]	83.3 ± 11.3	82.8 ± 13.6	84.3 ± 11.8	0.715/0.490	-
BMI (kg/m^2)[a]	29.05 ± 4.9	28.58 ± 5.6	28.25 ± 4.5	0.990/0.372	-
Total cholesterol (mmol/l)[c]	11.35 ± 2.66	10.20 ± 2.5	11.10 ± 2.66	0.371/0.691	-
HDL cholesterol (mmol/l)[c]	2.34 ± 0.64	2.48 ± 0.77	2.46 ± 0.69	1.057/0.349	-
LDL cholesterol (mmol/l)[c]	6.63 ± 2.57	6.75 ± 2.50	6.51 ± 2.15	0.347/0.707	-
Triglycerides (mmol/l)[c]	8.83 (2 – 75.5)	8.38 (1.94 – 79.9)	8.33 (2.33 – 46.7)	0.643/0.527	-
Diabetic nephropathy (%)	48.6	51.0	50.3	-	0.802
Diabetic retinopathy (%)	51.6	49.3	54.5	-	0.555

Data are mean ± SD, median (minimum-maximum values) or %. BMI: body mass index; BP: blood pressure; FPG: fasting plasma glucose; HbA1c: glycated hemoglobin; T2DM: type 2 *diabetes mellitus*. * F and P values obtained from general linear model univariate analyses, after adjustment for: [a] age and gender; [b] age, gender, use of medication for hypertension, and BMI; [c] age, gender and use of hypolipidemic medications. † P values were obtained from χ^2 tests or ANOVA, as appropriate. Only P values lower than the Bonferroni threshold (P = 0.0042) were considered statistically significant.

fasting plasma glucose or triglyceride levels, and prevalence of diabetic retinopathy or diabetic nephropathy, adjusting for covariables. It is worth mentioning that none of these variables attained statistical significance irrespective of whether recessive (C/C - C/T *vs.* T/T), dominant (C/C *vs.* C/T - T/T) or additive (C/C *vs.* T/T) models of inheritance were assumed for the T allele (data not shown).

DISCUSSION

TCF7L2 gene is considered one of the most important candidate genes for T2DM, playing a key role in blood-glucose homeostasis and beta-cell function (30). Following the initial report by Grant and cols. (3) showing that *TCF7L2* variants were strongly associated with T2DM risk, several other studies consistently replicated this association in different ethnicities [reviewed in (4)]. Among the *TCF7L2* variants, the rs7903146 (C/T) SNP seems to have the more robust effect in Caucasian populations (4,9). Here, we successfully replicated the association between the *TCF7L2* rs7903146 SNP and risk for T2DM in Caucasian-Brazilian subjects from Southern of Brazil, probably under an additive inheritance model given that the risk conferred by the T/T genotype was higher than that conferred by heterozygous genotype.

The consistency in the data showing the association between *TCF7L2* gene variants and risk for T2DM reported by many studies in different populations is believed to be a reliable indicative of a universal contribution of this gene to T2DM development (31), even though some studies have reported weak or no association with the disease, mainly in Asian populations (21,32-35). Thus, to date, around 10 meta-analyses evaluated the pooled effect of *TCF7L2* rs7903146 SNP in T2DM risk (5-8,31,36-40).

In 2009, Tong and cols. (5) published a large meta-analysis of 36 genetic association studies examining the association of T2DM with four *TCF7L2* polymorphisms (rs7903146, rs7901695, rs12255372 and rs11196205), totalizing 39,123 controls and 35,843 cases from different ethnicities. Results from the overall meta-analysis of the rs7903146 SNP showed that heterozygous carried just over a 1.4-fold increased risk for T2DM, while T/T homozygous carried near a 2.0-fold increase in T2DM risk, when compared with C/C homozygous. Moreover, the authors computed attributable risk (PAR) for the T/T-T/C genotypes of 16.9% for overall, 23.2% for Caucasians, 14.1% for North Europeans, 2.5% for East Asians, 17.9% for Indians, and 27.0% for Africans, suggesting that this polymorphism may contribute with approximately 1/5 of all T2DM risk in the globe, except for East Asians. The other three analyzed *TCF7L2* variants were also significantly associated with T2DM risk in different ethnicities, and the authors suggested that the rs7903143 and rs12255372 can be taken as reference loci for exploring T2DM susceptibility since they were associated with the highest pooled OR (5).

In 2012, the meta-analysis of Song and cols. (40) also confirmed the association of the rs7903146 SNPs with T2DM in 66 studies (OR = 1.41, 95% CI 1.37-1.46 for the T allele). Overall, they observed significant differences in the T allele frequencies of this SNP across different populations: this allele was quite common (0.16-0.48%) in all Caucasians, Africans and Hispanics except for Pima Indians, but less frequent (0.02-0.04) in all East Asian populations. Despite these differences in the frequencies of the rs7903146 SNP, they found similar association results across diverse ethnic groups. These authors also quantified the associations of the rs7903146 SNP with measures of beta-cell function among 35,052 non-diabetic subjects from 31 studies. In general, T allele carriers had significantly lower levels of fasting insulin and homeostasis model assessment of insulin secretion (HOMA-%B) and higher fasting glucose and 2h post-load glucose levels when compared to C/C homozygous.

Recently, Peng and cols. (6) conducted a comprehensive and updated meta-analysis regarding the association between *TCF7L2* variants and T2DM. Eight *TCF7L2* polymorphisms in 155 studies with 121,174 subjects (53,385 cases and 67,789 controls) were addressed in their meta-analysis. Significant associations were found between T2DM risk and rs7903146, rs12255372, rs11196205, rs7901695, rs7895340 and rs4506565 SNPs under an additive inheritance model. The highest pooled ORs were found for the rs7903146, rs12255372 and rs4506565 SNPs (OR = 1.39, 95% CI 1.34-1.45; OR = 1.33, 95% CI 1.27-1.40; and OR = 1.39, 95% CI 1.29-1.49; respectively), all of them in strong LD with each other across various populations. Subgroups analyses showed that no significant associations were found between the analyzed SNPs and T2DM in some ethnic groups as, for example, in American Pima Indians (6), showing the necessity of evaluating *TCF7L2* in different populations and ethnicities.

Three previous Brazilian studies have evaluated the association between the *TCF7L2* rs7903146 SNP and T2DM. Marquezine and cols. (41) evaluated the effect of the rs7903146 SNP on diabetes risk in 560 subjects with known coronary disease enrolled in the MASS II Trial and also in 1,449 residents from Vitoria, in Southeast Brazil. They confirmed the association between the rs7903146 SNP and T2DM risk (OR = 1.57 per T allele, 95% CI 1.16-2.11), but the inclusion of this SNP in an established clinical risk prediction score did not increase model accuracy. Franco and cols. (22) assessed whether the rs7903146 SNP could predict the development of glucose intolerance in Japanese-Brazilians in a population-based 7-year prospective study. In their study population, the T allele frequency was only 5%. No significant association was found between this SNP and glucose intolerance incidence; however, C/T genotype carriers had significantly lower insulin levels 2h after a 75-g glucose load than carriers of the C/C genotype. Barra and cols. (23) reported that the T/T genotype was significantly associated with T2DM risk (OR = 3.92, 95% CI 1.49-10.3 for the recessive model) in a small sample from the population of Brasilia, in the Central Western region of Brazil. Although they analyzed a sample of mixed ethnicity, the frequency of the T allele in their study was similar to that presented here.

The underlying mechanisms of action of *TCF7L2* variants in the etiology of T2DM is still uncertain given that all the *TCF7L2* SNPs identified so far are located in the intronic regions. Interestingly, none of the variants found in *TCF7L2* exons were associated with T2DM (30). Thus, it is necessary to clarify how the intronic variants affect *TCF7L2* gene expression. In this context, Lyssenko and cols. (42) found that rs7903146 T allele carriers exhibited a significant elevation of *TCF7L2* mRNA expression in human pancreatic islets, which was associated with impaired insulin secretion and incretin effects. Moreover, T allele carriers had enhanced rates of hepatic glucose production (42). Gaulton and cols. (43) reported that in human islets the chromatin state at the *TCF7L2* locus is more open in chromosomes carrying the rs7903146 T allele. They also created allele-specific luciferase reporter constructs and measured enhancer activity in two beta-cell lines (MIN6 and 832/13). Interestingly, T allele constructs showed significantly greater enhancer activity than the C allele. The authors concluded that the T allele affects T2DM susceptibility by altering *cis* regulation and local chromatin structure in human pancreatic islets. Palmer and cols. (44), through evaluation of tagging SNPs, showed that T2DM risk was limited to a 4.3-kb region in the *TCF7L2* gene, where is located the rs7903146 SNP. After sequencing this region in DNA from 96 African Americans, they reported that results of imputation, haplotype, and conditional analyses of the SNPs in this region were consistent with the rs7903146 SNP being the trait-defining variant. Thus, to date, both genetic and functional studies make a reliable case for a functional role for the rs7903146 SNP.

Some factors could have interfered with the findings of the present study. First, we cannot rule out the possibility of population stratification bias when analyzing our samples, even though only white subjects were studied and both T2DM patients and non-diabetic subjects were recruited from the same hospital, thus reducing the risk of false-positive/negative associations due to this bias. Second, we did not perform a replication of the observed association in another sample from Southern Brazil, although our data is in agreement with the majority of studies performed in different populations (5-8,31,36-40) and also with another Brazilian study (23). Third, we only analyzed one SNP in the *TCF7L2* gene. Therefore, even though the *TCF7L2* rs7903146 SNP seems to be one of the trait-defining variants (44), we can not exclude the possibility that another polymorphism in this gene could contribute to the T2DM pathogenesis in our population. Fourth, the presence of diabetes in the non-diabetic sample was only evaluated by questionnaire. Thus, a few number of undiagnosed T2DM subjects could be present in the non-diabetic sample. However, this is a conservative bias, which only could contribute to decrease the observed OR and not to give a false-positive association.

In conclusion, in Southern Brazil, the *TCF7L2* rs7903146 SNP is also associated with T2DM susceptibility. The risk conferred by the T/T genotype was higher than that of the heterozygous genotype, which is an indicative of an additive model of inheritance, and it is in agreement with the literature reported so far.

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The prevalence of the metabolic syndrome increases through the quartiles of thyroid stimulating hormone in a population-based sample of euthyroid subjects

Prevalência de síndrome metabólica aumenta em quartis de hormônio tireoestimulante em uma amostra de sujeitos eutiroides baseada na população

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ABSTRACT

Objective: The aim of the study was to assess the prevalence and characteristics of metabolic syndrome (MetS) and its elements in relation to TSH in euthyroid subjects. **Materials and methods:** In the cross-sectional study, 2,153 euthyroid adults, 47.2 ± 14.5 years (20-94) with no current antithyroid or thyroid replacement therapy were enrolled. All participants filled a questionnaire on past and current morbidities, medication and smoking. Body weight, height, waist circumference, serum TSH, glucose and lipids were measured. The subjects were stratified by quartiles of TSH (QTSH) and the prevalence of the MetS elements was calculated. MetS was determined by the IDF 2005 criteria. **Results:** Overweight prevalence was 37.2% (35.2-39.2), obesity in 25.1% (23.3-26.9), abdominal obesity – 61.4% (59.3-63.5), hypertension – 42.1% (38.9-43.1), diabetes/increased fasting glucose – 13.6% (12.1-15), low HDL-cholesterol – 27.6% (25.7-29.5), hypertriglyceridemia – 24.1% (22.3-25.9), MetS – 32.2% (30.2-34.2). MetS was more prevalent in the highest QTSH (34.9%, 30.9-38.9) than the lowest (27%, 23.3-30.9), $p < 0.001$, as were low HDL-C (32%, 28-35.9 *vs.* 25%, 21.3-28.7, $p < 0.001$) and hypertriglyceridemia (26.8%, 23-30.5 *vs.* 20.4%, 17-23.8, $p = 0.015$). Each QTSH increased the risk of MetS by 14%, $p < 0.001$, of hypertriglyceridemia by 20%, $p = 0.001$ and of low LDL-C by 9%, $p = 0.042$. Other significant factors for MetS were age, male gender and obesity. **Conclusion:** The prevalence of MetS increased with higher QTSH within the euthyroid range, mostly by an increase in the dyslipidemia. Arq Bras Endocrinol Metab. 2014;58(9):926-32

Keywords

Metabolic syndrome; thyroid function; euthyroidism; MetS; elements of the metabolic syndrome

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RESUMO

Objetivo: O objetivo deste estudo foi avaliar a prevalência e características da síndrome metabólica (MetS) e seus elementos em relação ao TSH em sujeitos eutireoides. **Materiais e métodos:** Foram analisados, em um estudo transversal, 2.153 adultos eutiroides, de 47,2 ± 14,5 anos (20-94) sem terapia antitiroidiana ou de reposição. Todos os participantes preencheram um questionário sobre doenças atuais e passadas, medicações e tabagismo. O peso corporal, altura, circunferência da cintura, TSH, glicose e lipídios séricos foram medidos. Os sujeitos foram estratificados em quartis de TSH (QTSH) e a prevalência dos elementos da MetS foram calculados. Os critérios da MetS foram determinados pela IDF 2005. **Resultados:** A prevalência de sobrepeso foi de 37,2% (35,2-39,2), de obesidade – 25,1% (23,3-26,9), obesidade abdominal – 61,4% (59,3-63,5), hipertensão – 42,1% (38,9-43,1), diabetes/aumento da glicose de jejum – 13,6% (12,1-15), baixo colesterol HDL – 27,6% (25,7-29,5), hipertrigliceridemia – 24,1% (22,3-25,9), MetS – 32,2% (30,2-34,2). A MetS foi mais prevalente no QTSH mais alto (34,9%; 30,9-38,9) do que no mais baixo (27%; 23,3-30,9), $p < 0,001$, assim como o baixo HDL-C (32%, 28-35,9 contra 25%, 21,3-28,7; $p < 0,001$) e hipertrigliceridemia (26,8%; 23-30,5 contra 20,4%, 17-23,8; $p = 0,015$). Cada QTSH aumentou o risco MetS em 14%, $p < 0,001$, de hipertrigliceridemia em 20%, $p = 0,001$ e de baixo LDL-C em 9%, $p = 0,042$. Outros fatores significativos para a MetS foram idade, sexo masculino e obesidade. **Conclusão:** A prevalência de MetS aumentou com um maior QTSH dentro da variação eutiroide, principalmente por aumento da dislipidemia. Arq Bras Endocrinol Metab. 2014;58(9):926-32

Descritores

Síndrome metabólica; função tiroidiana; eutiroidismo; MetS; elementos da síndrome metabólica

INTRODUCTION

The metabolic syndrome (MetS) is a cluster of cardiovascular risk factors that may co-occur in one subject and increase the cardiovascular disease (CVD) and *diabetes mellitus* type 2 (DM2) morbidity and mortality (1). While it is debated whether MetS is a single entity or an aggregate of separate phenomena, there is little doubt as to the detrimental effect of the metabolic syndrome on health. Most of the published data reveal a 1.5 to 3-fold increase in CV and all-cause mortality in the subjects with MetS (2,3). There are however many controversies related to the MetS, one of the primary issues being its definition. A number of attempts have been made to define the MetS through the years after it was put forward by Raven in 1988 (4). The elements that are included by most of them are insulin resistance, abdominal obesity, dyslipidemia and arterial hypertension. The separate definitions have stressed on different parts of the syndrome adding or subtracting elements over time. The first World Health Organization definition accentuated the insulin resistance as a key part of the syndrome. In 2001 the National Cholesterol Education Program Adult Treatment Panel III (NCEP:ATPIII) proposed a more balanced approach with an equal weight of the different elements (5). Later the International Diabetes Federation (IDF) prioritized the abdominal obesity as an obligatory element of the MetS (6). However it is still not possible to state that any definition is precise enough (7).

Irrespective of the differences in the diagnostic criteria, the prevalence of the MetS increases worldwide and it affects even young adults and children. As the pathogenesis of the syndrome is not entirely clear, there are multiple involved mechanisms such as genetic predisposition, adaptation to stress, nutritional and lifestyle factors and hormonal interactions. Several studies in the recent years have addressed the possible co-existence of multiple disorders and conditions that might affect CVD risk. Hypothyroidism is one of the most common endocrine disorders and numerous studies have found an association with atherosclerotic artery disease. The possible interplay between MetS and thyroid dysfunction has become a target of research as both conditions increase DVD risk. The existing data is however controversial. Uzunlulu and cols. for example found a 3-fold higher prevalence of subclinical hypothyroidism among subjects with the MetS (8). A study among a large group of Taiwanese elderly on the other hand failed to demonstrate such an association (9). As thyroid function among the population is a continuum without a biologically set cut-offs, an interesting question is whether there is a difference in the CVD risk within the laboratory reference range of TSH. Some links between thyroid function tests within the reference range and the metabolic profile have been described. Roef and cols. observed a positive association of TSH with arterial blood pressure and serum lipids in euthyroid subjects (10). Similar associations of TSH within the normal range have been found with insulin sensitivity and abdominal obesity (11). The direction of the association between TSH and some metabolic parameters such as body mass index (BMI) is not clear, though the existence of an association is beyond doubt (12). Two recent studies found an association of thyroid function tests in the euthyroid range with certain elements of the metabolic syndrome in young and separately in postmenopausal women (13,14).

The objective of the current study was to assess the prevalence of the metabolic syndrome and its elements in subjects with different TSH levels within the euthyroid range and to explore the associations between TSH and the other studied characteristics.

MATERIALS AND METHODS

The study was a part of the first population-based study of the prevalence of the most common endocrine disorders in Bulgaria. Two thousand four hundred and one adult subjects were recruited in six cities and the surrounding areas (Sofia, Plovdiv, Kurdjali, Veliko Tarnovo, Trojan, Sandanski). The participants were appointed randomly from the population registries. The sample size and age structure were planned in correspondence with the age structure of the population as determined by the latest census in 2005 (44.5% young, 35.5% middle-aged and 20% elderly). The study population has been described in more detail elsewhere (15).

The study was approved by the local Ethics committee at the University Hospital of Endocrinology in Sofia and all participants signed an informed consent. The participants filled a questionnaire, containing demographic data, current health status, medical history, family history for cardiovascular and thyroid disorders and diabetes, menstrual status for the females, current smoking. Body height, weight, waist circumference and sitting arterial blood pressure at the arm were measured. Increased waist circumference was defined after IDF recommendations if ≥ 80 cm for the females and ≥ 94 cm for the males.

The subjects were categorized according to BMI into normal-weight (BMI < 25 kg/m^2), overweight (BMI 25-29.9 kg/m^2) or obese (BMI ≥ 30 kg/m^2). Arterial hypertension was defined according to the NICE/BHS hypertension guideline (BP cut off is 140/90 mmHg).

Morning fasting blood was collected for serum TSH, lipids and fasting plasma glucose (FPG) measurement. Ultrasensitive TSH determination by a microparticulate immunoenzyme method (MEIA) at an automated analyzer AxSYM (ABBOTT, USA) was performed. The analytic sensitivity of the method was 0.01 μUI/ml. TSH reference range was 0,39-4,20 mUI/l. The subjects were stratified by quartiles of TSH (QTSH). The first quartile (Q1) ranged from 0.39 to 0.961 mU/l, the second (Q2) – from 0.962 to 1.356 mU/l, the third (Q3) – from 1,357 to 1.927 mU/l and the fourth – from 1.928 to 4.185 mU/l. Blood glucose was determined by an automated glucose-oxidase analyzer (Glucose Analyzer II, Beckman, USA) and all samples were processed by a single laboratory technician. The daily calibration and quality control was performed as per the manufacturer recommendations with a standard Presinorm (Roche) – glucose 4.9 ± 0.3 mmol/l and Presipath (Roche) – glucose 12.6 ± 0.5 mmol/l. Total cholesterol, HDL-cholesterol and triglycerides were determined by a direct enzymatic assay at an automated analyzer Cobas Mira Plus (ROCHE, Switzerland).

The metabolic syndrome was defined according to the IDF 2005 criteria as an increased waist circumference (for Caucasians ≥ 80 cm in the females and ≥ 94 cm in the males) plus at least two of the following traits: raised triglycerides ≥ 1.7 mmol/l, reduced HDL cholesterol < 1.03 mmol/l in males and < 1.29 mmol/l in females, raised arterial blood pressure ≥ 130 systolic or ≥ 85 diastolic (or antihypertensive treatment) and increased FPG (IFG) ≥ 5.6 mmol/l or diagnosed *diabetes mellitus* (6). Diabetes was defined after the WHO 1999 criteria: either as a previously known diabetes or fasting glucose ≥ 7.0 mmol/l, or a standard oral glucose tolerance test (at 120 minute measurement after a 75 g glucose load) ≥ 11.1 mmol/l, performed if FPG > 6.0 mmol/l.

Subjects with a history of thyroid dysfunction and current thyroid hormone or antithyroid drug therapy, or those with TSH levels above 4.2 mIU/l or below 0.39 mIU/l were excluded from further analysis.

Statistical analysis

The numerical data were presented as means and standard deviations if normally distributed or median and interquartile range if departing from the normal distribution. The category data were presented as proportions and 95% confidence intervals. Normality of distribution was assessed by the Kolmogorov-Smirnoff test. The empirical method was used for generating the TSH percentiles based on equal number of cases in each category. Categorical data and proportions were analyzed by Chi-square and Fisher's exact test. Numeric data were compared by a Student's t-test, ANOVA or Mann-Whitney rank analysis. Multiple correlations were done between the numerical variables. Univariate and multivariate binary logistic regression was applied to estimate the association of the studied characteristics with the metabolic syndrome, hypertension, dyslipidemia or diabetes prevalence. All tests were two-tailed and statistical significance was accepted at $\rho < 0.05$. The data were processed by SPSS for Windows v.13 (SPSS Inc, Chicago, Il).

RESULTS

Two thousand one hundred and fifty-three subjects complied with the criteria for inclusion in the study and were analyzed (152 subjects with hypothyroidism, 93 of them newly-diagnosed and 96 with hyperthyroidism, 75 of them newly-diagnosed were excluded). The major characteristics of the studied population and the interquartile range for the quartiles of TSH are presented in table 1. The metabolic syndrome and its elements, with the only exception of low HDL cholesterol, were more prevalent in the males (OR for MetS 1.6 (1.3-1.9) for the male gender). Overweight and obesity were also more common in the males.

There was no difference in the TSH levels between the subjects with and without metabolic syndrome (p = 0.22), with and without hypertension (p = 0.64) or with and without hypertriglyceridemia (p = 0.34). TSH was marginally higher in the subjects with low HDL-cholesterol than in those with normal HDL-C (1.33 [0.94-1.89] *vs.* 1.41 [0.99-2.04], p = 0.015). TSH did not correlate with the BMI (p = 0.17), the waist circumference (p = 0.59), total cholesterol (p = 0.33), HDL cholesterol (p = 0.52) or the triglycerides (p = 0.15). No significant differences were found in the serum lipid levels among the different quartiles of TSH (QTSH). Only the HDL-cholesterol in the males was marginally lower in Q4 as compared to Q1 (1.21 mmol/l, 1.17-1.25 *vs.* 1.29 mmol/l, 1.25-1.33,

p = 0.05). Triglycerides increased from Q1 to Q4 in the males (1.47, 1.34-1.60 to 1.79, 1.61-1.97, p = 0.061 for the trend).

The prevalence of the elements of the metabolic syndrome through the quartiles of TSH is presented in table 2. By far the most prevalent trait of the MetS throughout the quartiles of TSH was the increased waist circumference. Its prevalence however showed no dependence on the TSH. The prevalence of low HDL cholesterol increased with each quartile and the difference reached significance between Q1 (25%) and Q4 (32%). The prevalence of the cases with elevated triglyceride levels was also higher in the Q4 (26.8%) than Q1 (20.4%). The prevalence of the metabolic syndrome in Q2 (35%) and Q4 (34.9%) was higher than in Q1 (27%).

The unadjusted regression analysis showed that the risk of elevated triglyceride and low HDL levels increased through the quartiles of TSH (OR 1.2 (1.08-1.33), 0.001 and 1.12 (1.03-1.22), 0.007 respectively). The unadjusted risk was not significantly elevated for none of the other studied variables (MetS, arterial hypertension, diabetes, abdominal obesity). The summary of the regression analysis adjusted for age, gender and smoking for the metabolic syndrome, arterial hypertension, *diabetes mellitus*, abdominal obesity, low HDL and elevated triglycerides as dependent variables and the quartiles of TSH as an independent is presented in table 3. The adjusted for age, gender and smoking risk of a metabolic syndrome rose by 14% with each increase in the quartiles of TSH. After an additional adjustment for BMI however the association between

Table 1. Characteristics of the studied population. Where appropriate the female and male data are compared

Characteristic	Female N = 1,176	Male N = 977	P	Total N = 2,153
Age, years	48.3 (14.4)	45.9 (14.4)	< 0.001	47.2 (14.5)
BMI, kg/m^2	26.2 (5.5)	27.7 (4.3)	< 0.001	26.9 (5.1)
Waist circumference, cm	84.3 (13.1)	97.5 (11.4)	< 0.001	90.3 (14)
Overweight, %	30.7 (28-33.3)	44.5 (41.4-47.6)	< 0.001	37.2 (35.2-39.2)
Obese, %	22.4 (20-24.8)	28.4 (25.6-31.2)	< 0.001	25.1 (23.3-26.9)
Metabolic syndrome, %	29.6 (27-32.2)	35.3 (32-38.6)	< 0.001	32.2 (30.2-34.2)
Arterial hypertension, %	39 (36.2-41.8)	46 (42.9-49.1)	0.001	42.1 (38.9-43.1)
Diabetes and prediabetes, %	12.6 (10.7-14.5)	14.7 (12.5-16.9)	0.15	13.6 (12.1-15)
Isolated IFG, %	3.6 (2.5-4.7)	4.1 (2.9-5.3)	0.57	3.8 (3-4.6)
Low HDLc, %	31 (28.4-33.6)	23.5 (20.8-26.2)	< 0.001	27.6 (25.7-29.5)
Increased triglycerides, %	15.9 (13.8-18)	34 (31-37)	< 0.001	24.1 (22.3-25.9)
Abdominal obesity, %	59.6 (56.8-62.4)	63.6 (60.6-66.6)	0.061	61.4 (59.3-63.5)
Smokers, %	37.8 (35-40.6)	41.4 (38.3-44.5)	0.13	39.3 (37.2-41.4)
TSH, mIU/l	1.42 (0.99-2.1)	1.29 (0.92-1.74)	< 0.001	1.36 (0.96-1.93)
Q1 TSH (n = 540), mIU/l	0.77 (0.61-0.87)	0.75 (0.63-0.85)	-	0.76 (0.62-0.86)
Q2 TSH (n = 537), mIU/l	1.15 (1.06-1.25)	1.16 (1.05-1.25)	-	1.16 (1.05-1.25)
Q3 TSH (n = 538), mIU/l	1.58 (1.46-1.75)	1.58 (1.5-1.76)	-	1.58 (1.47-1.74)
Q4 TSH (n = 538), mIU/l	2.45 (2.16-2.89)	2.29 (2.1-2.7)	-	2.41 (2.12-2.83)

Table 2. Prevalence of the elements of the metabolic syndrome in the studied euthyroid subjects stratified by quartiles of TSH

Q	Abdominal obesity (%)	Low HDLc (%)	Elevated Tgl (%)	Hypertension (%)	Diabetes and pre-diab (%)	Met syndrome (%)
1-st	58.5 (54,3-62.7)	25 (21,3-28.7)	20.4 (17-23.8)	43.9 (39.7-48)	13 (10-15.8)	27 (23.3-30.9)
2-nd	63.7 (59.6-67.8)	25.7 (22-29.4)	25.9 (22.1-29.5)	41 (36.8-45.2)	16.4 (13.3-19.5)	35 (31-39)*
3-rd	61.2 (57-65.3)	27.9 (24.1-31.7)	23.4 (19.8-27)	42.2 (38-46.4)	13.9 (11-16.8)	31.6 (27.7-35.5)
4-th	62.5 (58.4-66.6)	32 (28-35.9)*	26.8 (23-30.5)**	41.3 (37.1-45.5)	11 (8.4-13.6)	34.9 (30.9-38.9)*
Total	61.4 (57.3-65.5)	27.6 (25.7-29.5)	24.1 (20.5-27.7)	42.1 (40-44.2)	13.6 (10.7-16.5)	32.2 (28.3-36.1)

All comparison is with Q1. * p < 0.001; ** p = 0.015.

TSH and MetS was reduced (7%, 95% CI -3 to 18, p = 0.15). The odds ratio for the MetS in overweight was 8.8, 95% CI 6.2-12.4 and in obesity – 23.4, 95% CI 19.9-40.6, as compared to normal-weight subjects.

Table 3. Odds ratios for the metabolic syndrome and its elements with each higher quartile of TSH in a multivariate logistic regression analysis adjusted by age, gender and smoking

Independent variable – Quartiles of TSH	Odds ratios (95% CI)	*p*-value
Metabolic syndrome	1.14 (1.04-1.26)	0.06
Abdominal obesity	1.08 (0.98-1.18)	0.11
Arterial hypertension	1.04 (0.94-1.14)	0.5
Diabetes and IFG	0.93 (0.81-1.06)	0.27
Elevated triglycerides	1.2 (1.08-1.33)	0.001
Low HDL cholesterol	1.09 (1.0-1.19)	0.042

Each increase by one quartile of TSH was associated with an increase in the prevalence of low HDL by 9% (0-19%) and of high triglycerides by 20% (8-33%).

The adjustment for BMI affected mildly the association of TSH with the hypertriglyceridemia (18%, 95% CI 5-31, p = 0.004).

DISCUSSION

The results of the current cross-sectional study demonstrated a significant association between the TSH within the reference range for euthyroidism and the metabolic syndrome. The prevalence of the metabolic syndrome increased in the higher quartiles if TSH. Furthermore TSH was positively associated with hypertriglyceridemia and to a lesser degree with the prevalence of low HDL-c. Similar observations have been reported by several other teams, who studied populations in different age groups. Waring and cols., Park and cols. and Heima and cols. for instance studied elderly groups (13,16,17). Oh and cols. reached somewhat similar conclusions in young women (18). Each one of those studies had a narrower age window of the target population in contrast to the wide age range of the current work (20-94 years). That may explain some differences between the published results. Among the elements of the metabolic syndrome only the lipids seemed to be associated with TSH in our cohort. The trend towards an increase in the cholesterol and triglyceride levels with increasing TSH was demonstrated by Canaris and cols. in the large Colorado study and by others thereafter (19). Pearce and cols. for example described a significant increase in total cholesterol, LDL-cholesterol and triglyceride levels in hypothyroid subjects (20). Despite the unequivocal overall increase in the levels of the lipids however, the latter authors demonstrated that a shift in the lipid particle sizes towards a less atherogenic profile also occurred. Their results puts up the question what the clinical significance of the hypothyroidism-induced dyslipidemia is. Furthermore, the cited observation was made in the circumstances of an acute overt hypothyroidism and it cannot be extrapolated to subclinical hypothyroidism or even less to euthyroid subjects. Though the Colorado study results are convincing, they are applicable throughout a large range of TSH values, but lose sensitivity if the data analysis is confined only within the euthyroid range. Such data from a large euthyroid population sample has been published by Asvold and cols. (21). They found a linear, statistically significant increase in the total cholesterol, LDL and triglyceride levels and a decrease in HDL-cholesterol over the normal TSH range. Stratification of the studied population by BMI however revealed a stronger association in the overweight and obese subgroup. It may seem that the association between thyroid function and lipid metabolism goes beyond the direct thyroid hormone/cell interaction, but is affected by factors that are by themselves related to CVD risk.

It has been hypothesized that in part the association between the MeTS and thyroid dysfunction might be mediated by an effect on insulin sensitivity. Bakker and cols. studied euthyroid non-diabetic subjects and provided evidence of the insulin sensitivity as an effect-modifying factor in the association between thyroid function and LDL-cholesterol (22). Moreover the effect was stronger in the subjects with insulin resistance than in those without, suggesting that low-normal thyroid function may be a risk factor in already high-CVD risk populations. In a larger group of type 2 diabetic subjects Chubb and cols. reached similar conclusions for serum cholesterol and triglyceride levels (23). They estimated further that the CVD risk in the diabetics doubles with the increase in the serum TSH levels between 1 and 7 mIU/l.

These data support the concept of a complex yet still not fully clear association between the thyroid and the MetS. We did not study the insulin sensitivity in our group, but found no correlation between the crude indirect markers of insulin resistance (waist circumference and glucose metabolism abnormalities) and the serum TSH. We did not find also an association with the BMI. Overweight and obesity though were a major

contributing factor for the MetS. There are many reports of a positive correlation between TSH and body fat – both subcutaneous and visceral. The direction of the association however is not fully understood and the mechanisms underlying it are still debated. On the one hand thyroid hormones and TSH alter the metabolic activity of the fat tissue (24). On the other hand a relative thyroid hormone resistant state in obesity has been proposed by some authors (25). Most probably there is a complex interplay between various mechanisms, as demonstrated by the observations of Muscogiuri and cols. (12). An evidence that the reality might be even more complex provide the recent findings of Lucas and cols. of a possible effect of smoking on the relationship between leptin and TSH (26).

There are several limitations to our study. First, the cross-sectional design does not permit cause-effect conclusions. To our best knowledge, similar longitudinal studies like the cited one are rare (27). Second, a determination of insulin sensitivity would throw more light on the discussed associations, but it could not be implemented in the current population-based study. Third, the study was performed in the winter season and a certain diet-induced bias in the serum lipid measurements cannot be ruled out. Also, the choice of the IDF definition may have introduced an overestimation of the MetS prevalence due to the high proportion of subjects with a larger waist circumference. No comparison between the different definitions has been made among the Bulgarian population to date and any assumption in this direction is speculative since local ethnic features may interfere.

As a conclusion, we observed a positive association between TSH in the euthyroid reference range and the prevalence of the metabolic syndrome. We failed to find an association of TSH with most of the elements of the MetS except the dyslipidemia, probably due to factors like the complexity of the interrelations or the impact of unaccounted confounders in the studied population. While there is an ample amount of data demonstrating an increased CVD risk in overt thyroid dysfunction, the clinical impact of the described by other authors and by us association of mild thyroid dysfunction or different thyroid hormone levels within the reference range with the MetS remains to be clarified.

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Thyroid nodules and thyroid cancer in Graves' disease

Nódulos tiroidianos e câncer de tiroide na doença de Graves

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ABSTRACT

Objective: The frequency of thyroid nodules accompanying Graves' disease and the risk of thyroid cancer in presence of accompanying nodules are controversial. The aim of this study was to evaluate the frequency of thyroid nodules and the risk of thyroid cancer in patients operated because of graves' disease. **Subjects and methods:** Five hundred and twenty-six patients in whom thyroidectomy was performed because of Graves' disease between 2006 and 2013 were evaluated retrospectively. Patients who had received radioactive iodine treatment and external irradiation treatment in the neck region and who had had thyroid surgery previously were not included in the study. **Results:** While accompanying thyroid nodule was present in 177 (33.6%) of 526 Graves' patients, thyroid nodule was absent in 349 (66.4%) patients. Forty-two (8%) patients had thyroid cancer. The rate of thyroid cancer was 5.4% (n = 19) in the Graves' patients who had no nodule, whereas it was 13% (n = 23) in the patients who had nodule. The risk of thyroid cancer increased significantly in presence of nodule (p = 0.003). Three patients had recurrence. No patient had distant metastasis. No patient died during the follow-up period. **Conclusions:** Especially Graves' patients who have been decided to be followed up should be evaluated carefully during the follow-up in terms of thyroid cancer which may accompany. Arq Bras Endocrinol Metab. 2014;58(9):933-8

Keywords
Graves' disease; thyroid nodules; thyroid cancer

RESUMO

Objetivo: A frequência da ocorrência de nódulos tiroidianos acompanhando a doença de Graves e o risco de câncer de tiroide na presença desses nódulos é controversa. O objetivo deste estudo foi avaliar a frequência de nódulos tiroidianos e o risco de câncer de tiroide em pacientes operados por doença de Graves. **Sujeitos e métodos:** Quinhentos e vinte e seis pacientes anteriormente submetidos à tiroidectomia por doença de Graves entre 2006 e 2013 foram avaliados retrospectivamente. Os pacientes que receberam tratamento com iodo radioativo e irradiação externa da região do pescoço e que anteriormente passaram por cirurgia de tiroide não foram incluídos no estudo. **Resultados:** Enquanto os nódulos de tiroide se apresentaram em 177 (33,6%) dos 526 pacientes com doença de Graves, eles estiveram ausentes em 349 (66,4%) pacientes. Um total de 42 (8%) dos pacientes teve câncer de tiroide. A ocorrência de câncer de tiroide foi 5,4% (n = 19) nos pacientes com doença de Graves que não apresentaram nódulos, e 13% (n = 23) nos pacientes com nódulos. O risco de câncer de tiroide aumentou significativamente na presença de nódulos (p = 0,003). Três pacientes apresentaram recidivas. Nenhum paciente apresentou metástase distante e nenhum paciente veio a óbito durante o período de acompanhamento. **Conclusões:** Pacientes com doença de Graves devem ser avaliados cuidadosamente no acompanhamento para a possível ocorrência de câncer de tiroide. Arq Bras Endocrinol Metab. 2014;58(9):933-8

Descritores
Doença de Graves; nódulos tiroidianos; câncer de tiroide

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INTRODUCTION

Thyroid nodules are frequently found in patients with Graves' disease, though the frequency varies depending on the method used (1-3). The prevalence of palpable thyroid nodule is 3-fold higher compared to the general population. In epidemiological studies, a higher thyroid nodule prevalence is found when thyroid ultrasonography is used for evaluation of thyroid morphology (2).

In addition, thyroid nodule develops in approximately half of the patients with Graves' disease during the follow-up (4). There is an increased risk of thyroid cancer in presence of these nodules (3).

While the rate of malignancy is approximately 5% in palpable thyroid nodules in the general population, it varies between 2.3% and 45.8% in patients with Graves' disease (2).

Moreover, there has been much controversy regarding biological behaviour of cancers in Graves' patients. While some authors have reported that thyroid cancers have a more agressive course (5,6), some others have reported the contrary (7,8). The aim of this study was to investigate the frequency of thyroid nodule in patients operated because of Graves' disease and the frequency of thyroid cancer in patients with and without nodule.

SUBJECTS AND METHODS

Five hundred and twenty-six patients who underwent thyroidectomy because of Graves' disease between 2006 and 2013 were included in the study. The diagnosis of Graves' disease was made with increased serum triiodothyronine (T3) and thyroxine (T4) levels and decreased thyroid stimulating hormone (TSH) levels and diffuse uptake on thyroid scintigraphy in patients who had a history and signs of hyperthyroidism. In most cases, the diagnosis was supported with increased thyroid stimulating antibody (TRAb) levels. Thyroid ultrasonography was performed in all patients. The patients were divided into two groups as the group with nodule and the group without nodule according to presence of thyroid nodule. Fine needle aspiration biopsy (FNAB) was performed in 172 of the patients who had thyroid nodule. Patients who had received radioactive iodine treatment and external irradiation treatment in the neck region and who had had thyroid surgery previously were not included in the study. The patients were treated with propylthiouracil or methimazole before surgery. The patients who required urgent surgery received Lugol solution for 7-10 days before surgery. The indications for surgery in Graves' patients included failure of antithyroid drug treatment and/or development of side effects due to these drugs, goitre which caused to compression symptoms in the trachea or esophagus, severe opthalmopathy, suspicious malignancy on FNAB and request of the patient. Total or near total thyroidectomy was performed in all patients.

RESULTS

Five hundred and twenty-six patients 352 (66.9%) of whom were female and 174 (33.1%) of whom were male were included in the study. The mean age of the patients was 41.2 ± 12.4 years (range 17-69 years). While thyroid nodule was present in 177 (33.6%) patients, 349 (66.4%) had no thyroid nodule. Among the patients who had thyroid nodule, 16 had solitary nodule and 161 had multiple nodules. The mean nodule diameter was 27.17 ± 14.10 mm (range 7.30-68.0 mm). One hundred and twenty (68.2%) of the patients who had thyroid nodule were female, whereas 57 (31.8%) were male and there was no statistically significant difference ($p > 0.05$) (Table 1). According to the largest nodule diameter, the thyroid nodule diameter was higher than 1 cm in 171 of 177 patients.

Table 1. Clinical characteristics of patients

Gender	**n**	**%**
Female	352	66.9
Male	174	33.1
Nodule status	**n**	**%**
Without nodule	349	66.4
With nodule	177	33.6
Solitary nodule	16	9.0
Multiple nodules	161	91.0
Female	120	68.2
Male	57	31.8

Thyroid cancer was found in 42 (8%) patients. All cancers had papillary origin; 33 (78.6%) of these were papillary microcarcinoma and 9 (21.4%) were papillary carcinoma. When the patients were divided into two groups as the group with thyroid nodule and the group without thyroid nodule, thyroid cancer was found in 13% of 177 patients (n = 23) who had thyroid nodule

and in 5.4% (n = 19) of 349 patients who had no thyroid nodule. This difference was statistically significant (p = 0.003). The risk of cancer increased markedly in presence of thyroid nodule. In our study, thyroid cancer was present in 2 (12.5%) of 16 patients who had a solitary nodule and in 21 (13%) of 161 patients who had multiple thyroid nodules.

A total of 17 patients had variant thyroid cancer 6 of whom had classical variant, 3 of whom had oncocytic variant and 2 of whom had tall cell variant. The mean age of 42 patients who were found to have cancer was 43.1 ± 12.4 years (range 20-69 years). The mean age was 44.8 ± 12.1 years (range 22-69 years) in women who had thyroid cancer and 40.2 ± 12.8 years (range 20-61 years) in men who had thyroid cancer. This difference was not statistically significant (p > 0,05). In the patients who were found to have thyroid cancer, the mean follow-up time between the diagnosis of Graves' disease and surgery was 3.95 ± 1.61 years (range 1-8 years) and the mean follow-up time after the diagnosis of thyroid cancer was 2.87 ± 1.61 years (range 0.5-7.50 years). Anti-Tg and Anti TPO were measured in nearly all patients with cancer and they were found to be positive in 52.5% of the patients and 48.8% of the patients, respectively (Table 2). FNAB was performed in 172 patients who had thyroid nodule and suspicious malignancy was present in 2 of them.

Table 2. Clinical characteristics of cancer patients

Characteristic		n	%
Gender	Male	16	38.1
	Female	26	61.9
Anti-Tg	Positive	21	52.5
	Negative	19	47.5
Anti-TPO	Positive	20	48.8
	Negative	21	51.2
Age (years)		43.1 ± 12.4	
Male		40.2 ± 12.8	
Female		44.8 ± 12.1	
Disease period[a] (years)		3.95 ± 1.61	
Cancer period[b] (years)		2.87 ± 1.61	

Data is expressed as mean ± SD; with the range presented in parenthesis a: the mean time between Graves' disease and operation. b: the mean follow-up time after the diagnosis of thyroid cancer.

Lymph node involvement was present in 3 (7.1%) of 42 patients who had thyroid cancer, thryoid capcule invasion was present in 6 (14.3%), invasion into the surrounding tissues was present in 4 (9.5%) and recurrence occured in 3 (7.1%). The tumor was multicentric in 5 patients. Cancer was present in both lobes in 5 patients. Three of 3 patients who had lymph node involvement, 3 of 6 patients who had thyroid capsule invasion and 2 of 4 patients who had invasion into the surrounding tissues had papillary thyroid cancer and the rest had micropapillary thyroid cancer (Table 3).

Table 3. Clinical characteristics of cancer patients

	n	%
Lymph node metastasis	3	7.1
PTC	3	100.0
MPTC	0	0.0
Mulicentricity	5	11.9
PTC	2	40.0
MPTC	3	60.0
Thyroid capsule invasion	6	14.3
PTC	3	50.0
MPTC	3	50.0
Surrounding tissue invasion	4	9.5
PTC	2	50.0
MPTC	2	50.0
Bilaterality	5	11.9
PTC	4	80.0
MPTC	1	20.0
Recurrence	3	7.1
PTC	2	66.6
MPTC	1	33.4

PTC: papillary thyroid cancer; MPTC: micropapillary thyroid cancer.

Recurrence occured in only one of 33 micropapillary thyroid cancers and in 2 of 9 papillary thyroid cancers. According to TNM staging, 37 patients had stage 1. Thirty-two of 33 micropapillary thyroid cancers were stage 1 and one was stage 2. Five of 9 papillary thyroid cancers were stage 1, 1 was stage 2, 2 were stage 4a and 1 was stage 4b (Table 4).

There was no statistically significant difference between the patients with thyroid cancer who showed and did not show lymph node invasion, thyroid capsule invasion, invasion into the surrounding tissues and recurrence in terms of age, TRAb titer and disease time (p > 0.05).

The cancer focus was in the parenchyma in 19 patients with toxic diffuse goitre. In addition, cancer was present in the parenchyma of 12 of the Graves' patients

who had nodule. Thus, the cancer focus was found in the parenchyma in 31 (73.8%) of 42 patients independent of accompanyment of thyroid nodule. Twenty-six cancers in the thyroid parancime were micropapillary. Mortality was not observed in any patient during the follow-up. Radioactive iodine treatment was given to 29 patients.

The data were assessed using SPSS 15.0 statistical package program. Chi-square and Fisher's exact test were used in assessment of the categorical data and Student's T-test and one-way variance analysis were used in assessment of the numerical data. p value of < 0,05 was considered statistically significant.

Table 4. TNM staging in cancer patients

TNM	n	%
Stage 1	37	88.0
Stage 2	2	4.8
Stage 4A	2	4.8
Stage 4B	1	2.4
Total	42	100.0

DISCUSSION

Thyroid nodule is observed with a higher rate in patients with Graves' disease compared to the general population. The prevalence of these nodules varies depending on the method used (examination, thyroid scintigraphy, thyroid ultrasonography or combinations of these). The prevalence is lower when only clinical examination and scintigraphy are performed compared to ultrasonography (9). Palpable thyroid nodules are observed with a rate of 5% in the general population and with a rate of approximately 15% in Graves' disease (1,10). When more sensitive echographic imaging is used, thyroid nodules are found more frequently in Graves' disease compared to the general population (10).

Cantalamessa and cols. found thyroid nodule with a diameter of 8 mm or larger in 33.6% of Graves' patients. When smaller lesions were included, the figure reached to 40.6% (4).

Kraimps and cols. could detect thyroid nodule with palpation only in 40 (28.6%) of 140 patients with thyroid nodule in their multicenter study which included 557 individuals. Scintigraphy revealed cold nodule in 54 patients (38.6%), whereas ultrasonography could detect 116 (82.9%) of these patients (3).

Kim and cols. detected thyroid nodule by palpation in 9.4% of the patients (23/245) and by thyroid ultrasonography in 35.1% of the patients (6/245) in a prospective study which they conducted with 245 Graves patients. Fifty percent of these patients (43/86) had solitary nodules and 50% (43/86) had multiple nodules (11).

Our center is a reference center and we initially perform thyroid ultrasonography in all thyroid patients referring to our department. In our series, we detected thyroid nodule in a total of 177 (33.6%) patients by thyroid ultrasonography; 16 of these patient had solitary nodule and 161 had multiple nodules. According to the size of the dominant nodule, 171 patients had a nodule larger than 1 cm.

Presence of these nodules increases the risk of thyroid cancer. However, the risk of differentiated thyroid cancer in Graves patients remains as a controversial issue. The malignancy rate in these nodules varies between 10% and 46% (10). This rate is approximately 5% in the general population (12). While the annual incidence of clinical thyroid cancer in the general euthyroid population has been reported to be 0.5-8/100.000, it is 175/100.000 in Graves disease (13).

Recently, Ren and cols. detected thyroid nodule in 22.7% of Graves patients (96/423) by ultrasonography. Twenty five of these patients (26%) had solitary nodule and 71 (74%) had multiple nodules. In the same study, the total incidence of thyroid cancer was 13.7% (58/423). While thyroid nodule accompanied to 46 of 58 patients, thyroid nodule was absent in 12 (3,6%) patients and the tumor was detected incidentally (14).

In our study, the rate of thyroid cancer in Graves' patients was 8% (42/526). One of the important points in our series was the high cancer rate in the parenchyma of the patients with Graves' disease. There was a cancer focus in the thyroid parenchyma in 31 (73.8%) of 42 patients. This finding is notable, because nearly all cancers reported in series of Graves' patients with coexisting carcinoma are in the thyroid nodule (1). Erbil and cols. (1) found that 67% (n = 12) of thyroid cancers found incidentally in Graves' patients were in the parenchyma outside the nodule. Ren and cols. (14) reported this rate to be 3.6% (n = 12). This shows that malignancy is not always related with nodule (12). If these patients were treated only with antithyroid and/or radioactive iodine, they would not have received an appropriate treatment because of accompanying thyroid cancer (15).

Thyroid cancers found in Graves' patients mostly have papillary origin (16). Many carcinomas related with Graves' disease are small and are found incidentally during surgery or postoperative pathological examination (17). In our study, all cancers detected in our study had papillary origin. Seventy-eight point six percent of these (33/42) were micropapillary thyroid cancer.

The pathogenic relationship between Graves' disease and thyroid cancer could not be understood clearly. Thyrotropin receptor antibodies TRAbs may possibly play a role in the initiation and progression of thyroid cancer. TRAbs and TSH activate the same intracellular pathway and both of them have mitogenic and antiapoptotic effects on thyroid follicular cells. TRAbs stimulate angiogenesis, which has a critical role in the growth and development of the tumor in the thyroid, by upregulating vascular endothelial growth factor and placental growth factor (2,10,13,18).

Biological behavior and optimal management of differentiated thyroid cancers accompanying Graves' disease are controversial (18). It has been reported that patients who have undergone thyroidectomy because of Graves' disease and who have small thyroid cancer (a diameter of 1 cm or smaller) have excellent prognosis and longer disease-free survival compared to patients who have small thyroid cancer without Graves' disease (19). Most of the patients in our series had small thyroid cancer. Thirty-seven of 42 patients had a TNM stage of 1. Thirty-two of 33 patients with micropapillary thyroid cancer had a TNM stage of 1 and recurrence was observed in only one of these patients. In addition, no patient died during the follow-up.

Currently, antithyroid drugs, radioactive iodine and surgery are used as current treatment methods in treatment of Graves' disease. Each method has its own side effects and success and failure rates. The advantage of surgical treatment is that it improves thyrotoxicosis faster compared to the other methods and provides effective treatment (20). The recommended operation for Graves' disease is total thyroidectomy. Less extensive surgery (subtotal thyroidectomy) carries a considerable risk of recurrent thyrotoxicosis (up to 30% of the patients) and revisional thyroid surgery is related with a high complication risk (21). Therefore, total or near total thyroidectomy was performed in all patients in our series. Radioactive iodine treatment following surgery was given to 29 of our patients who were found to have thyroid carcinoma.

Conclusively, the risk of cancer is considerably increased in Graves' patients in presence of accompanying nodule. Our study included the patients in whom surgery was performed because of Graves' disease. Patients who were given radioactive iodine or medical treatment for treatment of Graves' disease were not included in our study. Studies including these patients are needed to demonstrate the frequency of thyroid nodules and the risk of cancer clearly in Graves' patients. The risk of cancer in the thyroid parenchyma in Graves' patients should not be ignored. Long-term treatment and follow-up of these patients in whom surgery has not been performed should be pursued meticulously not only for providing and maintaining the euthryoid state, but also because of increased risk of thyroid cancer.

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Evaluation of cytopathological findings in thyroid nodules with macrocalcification: macrocalcification is not innocent as it seems

Avaliação dos achados citopatológicos em nódulos tiroidianos com macrocalcificações: elas não são tão inocentes como parecem

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ABSTRACT

Objective: Microcalcification is strongly correlated with papillary thyroid cancer. It is not clear whether macrocalcification is associated with malignancy. In this study, we aimed to assess the result of fine needle aspiration biopsies (FNAB) of thyroid nodules with macrocalcifications. **Subjects and methods:** We retrospectively evaluated 269 patients (907 nodules). Macrocalcifications were classified as eggshell and parenchymal macrocalcification. FNAB results were divided into four groups: benign, malignant, suspicious for malignancy, and non-diagnostic. **Results:** There were 79.9% female and 20.1% male and mean age was 56.9 years. Macrocalcification was detected in 46.3% nodules and 53.7% nodules had no macrocalcification. Parenchymal and eggshell macrocalcification were observed in 40.5% and 5.8% nodules, respectively. Cytologically, malignant and suspicious for malignancy rates were higher in nodules with macrocalcification compared to nodules without macrocalcification ($p = 0.004$ and $p = 0.003$, respectively). Benign and non-diagnostic cytology results were similar in two groups ($p > 0.05$). Nodules with eggshell calcification had higher rate of suspicious for malignancy and nodules with parenchymal macrocalcification had higher rates of malignant and suspicious for malignancy compared to those without macrocalcification ($p = 0.01$, $p = 0.003$ and $p = 0.007$, respectively). **Conclusions:** Our findings suggest that macrocalcifications are not always benign and are not associated with increased nondiagnostic FNAB results. Macrocalcification, particularly the parenchymal type should be taken into consideration. Arq Bras Endocrinol Metab. 2014;58(9):939-45

Keywords

Thyroid nodule; macrocalcification; malignancy; suspicious of malignancy

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RESUMO

Objetivo: A microcalcificação está fortemente correlacionada com o câncer papilar de tiroide. Não está claro se a macrocalcificação também está associada com malignidade. Neste estudo, nosso objetivo foi avaliar o resultado da biópsia de aspiração por agulha fina (FNAB) de nódulos tiroidianos com macrocalcificações. **Sujeitos e métodos:** Avaliamos retrospectivamente 269 pacientes (907 nódulos). As macrocalcificações foram classificadas como periféricas (casca de ovo) ou parenquimatosas (interna). Os resultados da FNAB foram divididos em quatro grupos citológicos: benignos, com malignidade, suspeita de malignidade e não diagnósticos. **Resultados:** Das amostras, 79,9% foram coletadas de mulheres e 20,1% de homens, e a idade média foi de 56,9 anos. A macrocalcificação foi detectada em 46,3% dos nódulos, e em 53,7% dos nódulos não havia macrocalcificação. A macrocalcificação parenquimatosa e periférica foi observada em 40,5% e 5,8% dos nódulos, respectivamente. Em termos citológicos, a malignidade e suspeita de malignidade foram mais comuns em nódulos com macrocalcificação em comparação com nódulos sem macrocalcificação ($p = 0,004$ e $p = 0,003$, respectivamente). Resultados benignos e não diagnósticos da citologia foram similares em ambos os grupos ($p > 0,05$). Os nódulos com calcificações periféricas apresentaram uma taxa maior de suspeita de malignidade e os nódulos com macrocalcificação parenquimatosa apresentaram taxas maiores de malignidade e suspeita de malignidade em comparação com nódulos sem macrocalcificação ($p = 0,01$, $p = 0,003$ e $p = 0,007$, respectivamente). **Conclusões:** Nossos achados sugerem que as macrocalcificações não são sempre benignas e esses nódulos não estão associados com maiores resultados não diagnósticos da FNAB. A macrocalcificação, particularmente do tipo parenquimatoso, deve ser levada em consideração. Arq Bras Endocrinol Metab. 2014;58(9):939-45

Descritores

Nódulo tiroidiano; macrocalcificação; malignidade; suspeita de malignidade

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INTRODUCTION

Thyroid nodules are commonly observed in the adult population and the incidence is increasing largely related with widespread use of Doppler ultrasonography (US) and other imaging techniques. Around 4-8% of thyroid nodules are found incidentally in asymptomatic adults, whereas 10-41% are detected by US (1). The majority of thyroid nodules are benign, with malignancy rates ranging from 9 to 13% in different studies (2,3). Thyroid US has an important role in the diagnosis of thyroid nodules because it is a simple, non-invasive, effective, and useful method. Nodules with a diameter of 2-3 milimeter (mm) can be detected by high resolution images. Also, vascularity can be determined by colour Doppler or power Doppler US. The use of US and US guided fine needle aspiration biopsy (FNAB) to assess thyroid nodules has reduced the number of unnecessary surgeries and increased the rate of diagnosis of thyroid cancer (4,5). The rate of accuracy of FNAB was reported up to 96%. Morphological features of nodules such as echogenicity, texture, margin regularity, presence of halo, presence and type of calcification can be assessed by high resolution US. Margin irregularity, hypoechogenicity and microcalcification were considered to be important risk factors for malignancy, however, size of thyroid nodule alone was not considered as a risk factor (6).

Thyroid nodular calcifications can be classified according to their diameter and location; calcifications < 2 mm and without acoustic shadow at posterior are microcalcifications, calcifications ≥ 2 mm and with posterior acoustic shadow are macrocalcifications, and calcifications surrounding the nodule are peripheral (eggshell) calcifications. Pathologically, microcalcification is a psammoma body that contains 10-200 μm, rough, smooth, bright, calcific aggregations (7). Large and irregular bordered macrocalcification can exist secondary to tumor necrosis and it can be seen in both benign and malignant nodules (2,8). Peripheral calcifications are believed to occur secondary to chronic degenerative changes.

Although, microcalcifications are known to be strongly associated with malignant nodules, the association of macrocalcifications with malignancy is controversial (8-15). Recent studies have revealed a relationship between macrocalcification and malignancy, particularly in papillary thyroid carcinomas (9,10,16-18). In addition, despite the general belief that peripheral macrocalcification indicates benign situations, it was shown that if it is irregular it can also be related with malignancy (8,19). Macrocalcification together with microcalcification in the same nodule or located in the middle of a hypoechoic nodule have a higher probability of malignancy (20).

The role of FNAB in thyroid nodules with macrocalcifications is unclear with 11 to 25% of the biopsies yielding false negative and 5 to 30% yielding non-diagnostic cytologies (19-22). Calcified lesions detected by USG have been reported to be the most common cause of insufficient FNAB sampling (23). In this study, we aimed to evaluate FNAB results of thyroid nodules with parenchymal and peripheral macrocalcifications. We also tried to find out the impact of macrocalcifications on nondiagnostic cytology results.

SUBJECTS AND METHODS

We retrospectively evaluated 907 nodules from 269 patients seen in our out-patient clinic. Patients > 15 years of age with nodular or multinodular goiter and macrocalcification in at least one nodule were included. Patients with a previous history of thyroid surgery, percutaneous invasive procedures for nodules, radiotherapy to head and neck region or radioactive iodine therapy were excluded from the study. Preoperative thyroid functions, thyroid autoantibodies, thyroid US findings and FNAB results were obtained from medical records. The study was approved by the local ethical committee in accordance with the ethical standarts of Helsinki declaration.

Blood samples were obtained between 08:00 to 10:00 in the morning from all patients. Serum sensitive thyrotrophin (TSH), free triiodothyronine (fT3), free thyroxine (fT4) and thyroid autoantibodies [antithyroid peroxidase antibody (anti-TPO) and anti-thyroglobulin antibody (anti-TGAb)] levels were measured with chemiluminescent immunoassay (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA, USA, and the UniCel DxI 800, Beckman Coulter, CA, USA). Normal levels were as follows; TSH: 0.4 - 4.0 uIU/mL, fT3: 1.57 - 4.71 pg/mL, fT4: 0.61 - 1.12 ng/dL, anti-TPO < 10 U/mL and anti-Tg < 30 U/mL.

US was performed with a color Doppler ultrasonography (FCW Tecnology Co., Ltd. Model: 796FDII Yung-ho City, Taipei, Taiwan) and a superficial probe (Esaote, Model No: LA523, 13 - 4, from 5.5 - 12.5 MHz) in all patients. Nodule location, diameters, volume, echogenicity (isoechoic, hypoechoic or hyperechoic), texture (solid, mixed or cystic), marginal

regularity (regular or irregular), presence of hypoechoic halo, presence and type of calcification (microcalcification, parenchymal macrocalcification, peripheral macrocalcification) and vascularization pattern were recorded for all nodules evaluated with FNAB. We defined calcifications < 2 mm as microcalcification and ≥ 2 mm in diameter and with an acoustic shadow as macrocalcification (Figure 1).

Thyroid FNAB was performed by an experienced clinician with 27-gauge needle and 20 mL syringe under US guidance. Each nodule was aspirated for 2 - 4 times and at least 4 - 6 preparations were obtained from each aspiration. Cytological assessment was conducted by an experienced cytopathologist. FNAB materials were air-dried and stained by May-Grunwald-Giemsa. The cytological diagnoses were classified as benign, non-diagnostic, suspicious for malignancy and malignant. FNAB results of nodules with parenchymal and peripheral macrocalcifications were compared with nodules not including macrocalcification in the same patient group.

All the data were analyzed with SPSS (Statistical Package of Social Science for Windows) 15.0. Descriptive statistics were expressed as mean ± standard deviation for continuous variables and as number of cases and percentage for nominal variables. Student's t test was used to compare differences between independent groups for continuous variables and Chi-square test was used to compare nominal variables. A p value < 0.05 was considered statistically significant.

RESULTS

There were 215 female (79.9%) and 54 (20.1%) male patients and the mean age was 56.9 ± 13.1 years (21 - 87 years). One hundred and sixty-one (60%) patients had multinodular goiter and 108 (40%) patients had solitary thyroid nodule. Macrocalcifications were observed in 420 (46.3%) nodules, and 487 (53.7%) nodules had no macrocalcification. Parenchymal and peripheral macrocalcifications were present in 367 (40.5%) and 53 (5.8%) of 907 nodules, respectively. Mean diameters of nodules with macrocalcification and without macrocalcificaiton were 23.92 ± 14.15 mm and 15.72 ± 7.53 mm, respectively (p < 0.001) (Table 1).

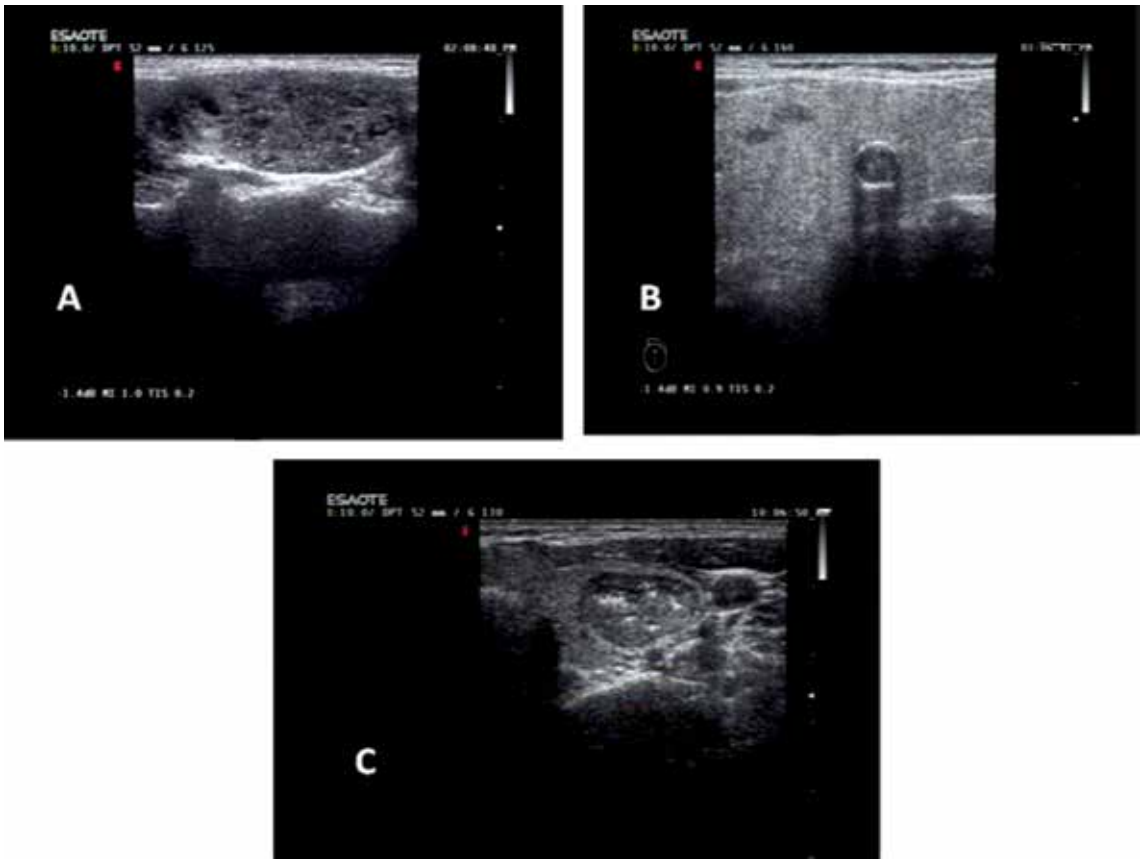

Figure 1. Thyroid nodule calcifications detected in ultrasonography. (**A**) Microcalcification, (**B**) peripheral (eggshell) macrocalcification, (**C**) parenchymal (internal) macrocalcification.

Table 1. Ultrasonography features and cytological results of thyroid nodules with and without macrocalcification

	Nodules with macrocalcification (n = 420) (%)	Nodules without macrocalcification (n = 487) (%)	p
Ultrasonography features			
Nodule diameter (mm)	**23.92 ± 14.15**	**15.72 ± 7.53**	**< 0.001**
Nodule volume (mL)	8.53 ± 13.21	2.25 ± 3.93	**< 0.001**
Presence of halo	97 (23.1)	111 (22.8)	0.914
Microcalcification	258 (61.4)	85 (17.5)	**< 0.001**
Margin regularity	141 (33.6)	171 (35.1)	0.626
Regular	279 (66.4)	316 (64.9)	
Irregular			
Echogenity			
Hypoechoic	136 (32.4)	217 (44.6)	
Isoechoic	281 (66.9)	266 (54.6)	**< 0.001**
Hyperechoic	3 (0.7)	4 (0.8)	
Texture			
Solid	49 (11.7)	243 (49.9)	
Cystic	1 (0.2)	10 (2.1)	**< 0.001**
Mixed	370 (88.1)	234 (48)	
Cytological result			
Benign	316 (75.2)	391 (80.3)	0.067
Malignant	15 (3.6)	4 (0.8)	**0.004**
Suspicious for malignancy	23 (5.5)	9 (1.9)	**0.003**
Non-diagnostic	66 (15.7)	83 (17)	0.590

Nodules with macrocalcification had significantly higher volume compared to nodules without macrocalcification. Ultrasonographically, rates of presence of hypoechoic halo and margin regularity were similar in two groups. Microcalcifications were observed more commonly in nodules with macrocalcification ($p < 0.001$). Thirty-two point four percent of nodules with macrocalcification and 44.6% of nodules without macrocalcification were hypoechoic ($p < 0.001$). In terms of texture, nodules with macrocalcification had a higher prevalence of solid-cystic mixed texture, while nodules without macrocalcification had a higher prevalence of solid texture (Table 1).

Cytological results of 420 nodules with macrocalcification were benign in 75.2%, non-diagnostic in 15.7%, suspicious for malignancy in 5.5% and malignant in 3.6%. Of the nodules without macrocalcification, 80.3% were benign, 0.8% malignant, 1.9% suspicious for malignancy, and 17% non-diagnostic (Table 1). Accordingly, the rates of suspicious for malignancy and malignant results were significantly higher in nodules with macrocalcification compared to nodules without macrocalcification ($p = 0.004$ and $p = 0.003$, respectively).

When we compared cytological results of nodules with peripheral macrocalcification and without macrocalcification, we found that the rate of suspicious for malignancy was higher in nodules with peripheral macrocalcification while rate of benign was higher in nodules without macrocalcification ($p = 0.01$and $p = 0.036$, respectively) (Table 2). Cytologically, 3.8% of nodules with parenchymal macrocalcification and 0.8% of nodules without macrocalcification were malignant ($p = 0.003$). Suspicious for malignancy rate was also higher in nodules with parenchymal macrocalcification compared to nodules without macrocalcification ($p = 0.007$) (Table 3). Although rate of nondiagnostic cytology was higher in nodules with peripheral macrocalcification, the difference was not statistically significant. In multiple logistic regression analysis, macrocalcification was found to be related with malignant cytology results independent from presence of microcalcification, irregular margins and absence of halo ($p = 0.008$).

The numbers and rate of the thyroid nodules with or without micro/macro-calcifications determined as suspicious for malignancy or malignant were shown in the table 4.

Table 2. Cytological results of thyroid nodules with peripheral macrocalcification and without macrocalcification

Cytological result	Nodules with peripheral macrocalcification (n = 53) (%)	Nodules without macrocalcification (n = 487) (%)	p
Benign	67.9	80.3	**0.036**
Malignant	1.9	0.8	0.442
Suspicious for malignancy	7.5	1.9	**0.010**
Non-diagnostic	22.7	17	0.309

Table 3. Cytological results of thyroid nodules with parenchymal macrocalcification and without macrocalcification

Cytological result	Nodules with parenchymal macrocalcification (n = 367) (%)	Nodules without macrocalcification (n = 487) (%)	p
Benign	76.3	80.3	0.159
Malignant	3.8	0.8	**0.003**
Suspicious for malignancy	5.2	1.9	**0.007**
Non-diagnostic	14.7	17	0.359

Table 4. The numbers and rate of the thyroid nodules with or without micro/macro-calcifications determined as suspicious for malignancy or malignant

	Suspicious for malignancy	Malignant
Nodules with macrocalcification/with microcalcification (n = 258)	16 (6.2%)	11 (4.3%)
Nodules with macrocalcification/ without microcalcification (n = 162)	7 (4.4%)	4 (2.5%)
Nodules without macrocalcification/ without microcalcification (n = 402)	7 (1.7%)	4 (1%)
Nodules without macrocalcification/with microcalcification (n = 85)	2 (2.3%)	0 (0%)

Histopathological results were available in 43 patients who underwent surgery for various reasons such as malignant or suspicous for malignancy cytology results, giant nodule, compression symptoms and suspicious US findings. There were 18 patients with malignant and 25 patients with benign histopathology. Ultrasonographically, micro and macrocalcification, particularly parenchymal macrocalcification were more prevalent in malignant nodules compared to benign nodules (Table 5).

Table 5. Preoperative calcification types in ultrasonography in patients with final histopathological results

Calcification type	Malignant (n = 18) (%)	Benign (n = 25) (%)	p
Microcalcification	14 (77.7)	12 (48)	**0.049**
Macrocalcification	13 (72.2)	10 (40)	**0.037**
Peripheral macrocalcification	2 (11.1)	2 (8)	0.473
Parenchymal macrocalcification	13 (72.2)	9 (36)	**0.019**
Without macrocalcification	2 (11.1)	11 (44)	**0.021**

DISCUSSION

Microcalcification in thyroid nodules is known to be associated with malignancy; however, the relationship between macrocalcification and malignancy is controversial. Large calcifications with irregular borders may occur secondary to tumor necrosis and may be present in benign and malignant nodules (7,18). Previously, peripheral calcification was thought to occur secondary to chronic degenerative changes and therefore indicate a benign status. However, recent studies have found that macrocalcifications including peripheral calcification might also be an indicator of thyroid nodule malignancy (9,10,16-18,24,25). In this study, we showed that cytologically malignant and suspicious for malignancy results are observed more frequently in nodules with parenchymal macrocalcification. Also, nodules with peripheral macrocalcification had a higher rate of suspicious for malignancy results.

Taki and cols. assessed preoperative US findings in 151 surgically resected thyroid nodules and found that 57 (38%) of nodules had calcification (14). Among 11 nodules with microcalcification, 9 (82%) were malignant and among 46 nodules with macrocalcification (intranodulary and peripheral) 22 (47.8%) were malignant. Additionally, malignancy was histologically identified in 6 (43%) of 14 nodules with peripheral calcification. The authors concluded that all calcification types may be associated with malignancy and nodules with macrocalcification should be examined thoroughly.

In previous studies, histopathologically proven malignancy rate of thyroid nodules with peripheral macrocalcification was reported to range between 18.5% to 70% with most of studies showing higher than 50% malignancy rate in these nodules (8-10,23). Majority of carcinomas were papillary type, with a few follicular carcinoma histopathologically. Even, anaplastic carcinoma was reported in nodules with peripheral macrocalcification which was blamed for insufficient FNAB result (26). Although there are some US criteria known to be associated with malignancy, it is difficult to apply these criteria for nodules with peripheral macrocalcification due to posterior shadowing and inability to interpret marginal regularity. This has led to search for additional criteria to indicate malignancy in these nodules. In the study by Park and cols., thickening and interruption of peripheral calcifications were suggested to be significant indicators of malignancy (11).

Ugurlu and cols. (27) retrospectively assessed the FNAB results of 1,004 patients with thyroid nodules and found that the risk of malignancy was greater in nodules containing microcalcification than those without calcification. However, presence of macrocalcification was not associated with increased risk of malignancy in FNAB compared to nodules without macrocalcification. These results are contrary to our

findings and those of some previous studies. We have observed cytologically higher malignant and suspicious for malignancy rates in nodules with macrocalcification compared to those without macrocalcification. Similarly, in a recent trial including 713 subcentimeter nodules, solid composition and macrocalcification in addition to hypoechogenicity, infiltrative margin, microcalcification, and taller-than-wide shape were found to be significantly associated with malignant cytology (28). The authors showed that including solid composition with or without macrocalcification improved the diagnostic performance in subcentimeter nodules for the identification of malignant lesions. Park and cols., investigated sonographic findings of 854 macrocalcified nodules and reported that 171 (20.8%) were nondiagnostic cytologically, 470 (55.0%) were benign (18 were confirmed by histopathology) and 179 (20.9%) were malignant histopathologically (29). In that study, the rates of nondiagnostic and suspicious for malignancy cytologies were similar with our findings. However, rate of malignancy was higher and rate of benign result was lower compared to our study. As the authors have mentioned as a limitation of their study, patients with benign findings at US had not undergone biopsy or surgery which might have resulted in relatively fewer benign nodules.

In contrary to some previous reports suggesting that the presence of calcification is significantly associated with non-diagnostic FNAB cytology (30), we found no difference in terms of non-diagnostic cytology between nodules with or without macrocalcification. This finding was also supported in a recent trial by Lee and cols. who retrospectively reviewed sonographic findings and histopathological results of 188 nodules with macrocalcification (23). They showed that 6.9% of nodules with macrocalcification was nondiagnostic cytologically and sensitivity, specificity, positive predictive value and negative predictive value of FNAB were all higher than 90% with a diagnostic accuracy of 96% in these nodules. The authors suggested that FNA of thyroid nodules with macrocalcification had a high diagnostic yield. In another study, ultrasonographic features of 1,195 nodules with inadequate cytology were evaluated prospectively and neither micro- nor macrocalcification was reported to be related with increased risk of inadequacy (31).

Our study has several limitations including the retrospective design and the fact that histopathological results were available only in a small percentage of patients who underwent surgery. Thus, we could not determine the exact effect of macrocalcification on false positivity or negativity of FNAB in nodules with macrocalcification.

In conclusion, peripheral and parenchymal macrocalcifications are associated with higher suspicious for malignancy and/or malignant results in FNAB. In addition to hypoechogenicity, marginal irregularity, absence of halo and vascularization pattern, the presence of macrocalcification in a nodule might be accepted as one of the suspicious US features. However, further studies including histopathological confirmation of these cytological findings are required to support this suggestion. Also, presence of macrocalcification is not related with increased nondiagnostic cytology in FNAB and should not prevent clinicians from making further assessments in case of nondiagnostic results.

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Redução da mobilidade funcional e da capacidade cognitiva no diabetes melito tipo 2

Reduction of functional mobility and cognitive capacity in type 2 diabetes mellitus

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RESUMO

Objetivos: Avaliar a mobilidade funcional e sua relação com a capacidade cognitiva em pacientes com diabetes tipo 2 (DM2) entre 50 e 65 anos de idade, e com menos de 10 anos de diagnóstico. **Materiais e métodos:** Estudo observacional, analítico e transversal envolvendo indivíduos não diabéticos e pacientes com DM2 com controle glicêmico inadequado, selecionados por amostra de conveniência. Em ambos os grupos, foram aplicados questionário estruturado, avaliação cognitiva com Miniexame do Estado Mental (MEEM) e teste do relógio (TDR), além da avaliação de mobilidade funcional pelo teste Timed Up & GO (TUG). **Resultados:** No TUG os pacientes com DM2 apresentaram tempo médio de 11,27 segundos *versus* 9,52 segundos nos controles (p = 0,013). A associação entre declínio cognitivo e dismobilidade foi positiva nos indivíduos com DM2 (p = 0,037). No subgrupo que apresentou dismobilidade e declínio cognitivo associados, 18% eram portadores de DM2 e 1,6% era do grupo sem DM2 (p < 0,01). **Conclusões:** Pacientes com DM2 apresentaram pior mobilidade funcional e desempenho cognitivo, favorecendo a hipótese de que o DM2 influencia a mobilidade funcional e capacidade cognitiva antes do aparecimento de complicações vasculares ou neuropáticas. Esses dados sugerem que a hiperglicemia é um fator agravante no desempenho de atividades que exijam funções mentais como atenção, orientação e memória de trabalho. Arq Bras Endocrinol Metab. 2014;58(9):946-52

Descritores
Diabetes tipo 2; cognição; acidentes por quedas; aptidão física

ABSTRACT

Objectives: The aim of the present study was to evaluate the functional mobility and its relationship to cognitive ability in patients with type 2 diabetes (T2DM), age between 50 and 65 years and under 10 years of diagnosis. **Materials and methods:** An observational, analytical and cross-sectional study, involving no diabetic and type 2 diabetic individuals with inadequate glycemic control, selected by convenience sampling. In both groups, were administered structured questionnaire and cognitive assessment with Mini-Mental State Examination (MMSE) and the clock drawing test (CDT), besides assessment of functional mobility by the Timed Up & Go (TUG). **Results:** In TUG, DM2 patients presented a mean time of 11.27 seconds *versus* 9.52 seconds (p = 0.013). The association between cognitive decline and decrease of mobility was positive in individuals with T2DM (p = 0.037). In the subgroup that showed decrease of mobility and associated cognitive decline, 18% were patients with DM2 and 1.6% were individuals without T2DM (p < 0.01). **Conclusions:** Patients with T2DM presented worse functional mobility and cognitive performance, supporting the hypothesis that DM2 influence functional mobility and cognitive ability, regardless of neuropathic or vascular complications. These data suggest that hyperglycemia is an aggravating factor in the performance of activities requiring mental functions such as attention, working memory and orientation. Arq Bras Endocrinol Metab. 2014;58(9):946-52

Keywords
Type 2 diabetes; cognitive decline; accidents from falls; physical fitness

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INTRODUÇÃO

O diabetes melito tipo 2 (DM2) exerce grande impacto na saúde pública em decorrência de suas complicações, comprometendo a qualidade de vida das populações por ele acometidas. Uma importante consequência do envelhecimento das pessoas com DM2 é a incapacidades física, especialmente a perda da mobilidade. A habilidade funcional se traduz como um conjunto de elementos como equilíbrio, marcha e coordenação. Assim, a dismobilidade torna esses pacientes mais dependentes, conduz à perda de massa muscular e reduz a expectativa de vida (1). Os fatores predisponentes frequentes em portadores de diabetes incluem: neuropatia periférica, comprometimento visual, redução da função renal, alterações autonômicas e uso de medicamentos (2-4). Outras características presentes no paciente com DM2 são o sedentarismo e o ganho de peso (1) que, aliados à dismobilidade, têm como consequência o maior risco de quedas, que aparecem como importantes causas de ferimento não fatal entre pessoas com mais de 50 anos (5). Portanto, a mobilidade funcional é um elemento central da qualidade da saúde, pois prediz o declínio funcional, o uso de serviços de saúde e a morbimortalidade (6).

Evidências apontam para a relação positiva entre DM2 e declínio cognitivo, o qual também está implicado na inabilidade motora, devido ao comprometimento da atenção, da memória e de respostas protetoras, apraxia, desorientação espacial e deterioração das funções executivas e motoras (marcha alterada, desequilíbrio, instabilidade postural, alteração de tônus muscular); além do fato de os pacientes com essas condições não poderem julgar adequadamente sua própria capacidade, tomando atitudes arriscadas que levam a acidentes (7). Os déficits cognitivos, quando somados à presença de hiperglicemia, também podem aumentar a incapacidade física, trazendo dificuldades na realização de atividades da vida diária (AVD). Entretanto, pouco se sabe sobre a relação entre distúrbio cognitivo e incapacidade física nos pacientes com DM2 (8). Estudos prévios sugerem que a alteração cognitivo-motora pode não apresentar uma relação linear com a glicemia, mas sim que pode haver um limite por volta de 270 mg/dL a partir do qual a função cognitivo-motora começaria a ser afetada (7). Embora se saiba a respeito da hipoglicemia e sua neuroglicopenia, um dos principais obstáculos para a investigação dos efeitos da hiperglicemia sobre o desempenho cognitivo-motor é a ausência de um mecanismo fisiológico claro que explique sua influência negativa sobre o funcionamento cerebral. A identificação de testes mais acurados para o estabelecimento de algoritmos práticos de mensuração da mobilidade funcional tem sido alvo de estudos recentes (9). Entre eles, o histórico sobre quedas (10), o teste Timed Up & Go (TUG) (11) e a avaliação da capacidade cognitiva com o Miniexame do Estado Mental (MEEM) se mostraram estratégias eficazes. Portanto, nosso estudo propõe avaliar pacientes com DM2 sem controle glicêmico adequado e comparar com controles não diabéticos em relação ao desempenho cognitivo e motor.

MATERIAIS E MÉTODOS

Trata-se de estudo observacional, analítico e transversal. A população em análise foi de 145 pessoas usuárias do serviço público municipal de saúde, sendo a amostra estudada composta por 118 pessoas, divididas em dois grupos distintos: grupo DM2, formado por 50 pacientes com DM2 e controle glicêmico inadequado, e grupo controle, com 68 indivíduos sem DM2.

A população com DM2 foi composta por homens e mulheres de 50 a 65 anos, com diagnóstico de DM2 confirmado pela pesquisa de resultados laboratoriais prévios nos prontuários e com tempo de diagnóstico inferior a 10 anos. Foram incluídos somente pacientes com glicemia de jejum maior de 200 mg/dL no dia da entrevista e realização dos testes cognitivos e da mobilidade funcional e com pelo menos uma glicemia de jejum maior de 150 mg/dL nos 6 meses que antecederam o estudo. Não incluímos hemoglobina glicada (HbA1C) como critério de inclusão, pois necessitávamos priorizar um exame que avaliasse o estado de hiperglicemia no momento da realização dos testes cognitivo-motores. E sabe-se que uma HbA1C elevada não exclui a possibilidade de grande variabilidade glicêmica. O grupo controle contou com homens e mulheres de mesma faixa etária, sem diabetes de qualquer etiologia, confirmado pela pesquisa de resultados laboratoriais prévios e com glicemia de jejum menor de 99 mg/dL no dia da entrevista. Os critérios de exclusão foram pacientes analfabetos, tabagistas, etilistas, usuários de drogas psicoativas, com distúrbios visuais ou auditivos incapacitantes, portadores de neuropatias, ou sequelas neurológicas de AVC, bem como pacientes que não concordaram com o termo de consentimento.

O tamanho da amostra foi calculado com base na prevalência de diabéticos no Brasil (12) e na prevalência de quedas de uma população idosa (38%) (13), com nível de confiança de 95% e erro tolerável de 5%,

para uma população de 22.049 indivíduos (homens e mulheres entre 50 e 65 anos residentes no município). A partir desse resultado, foram submetidos à pesquisa 145 pacientes. Após contato inicial com os pacientes (selecionados na sala de espera de um centro de diversas especialidades médicas), determinou-se a glicemia capilar de jejum por meio de glicosímetros. Os valores anteriores da glicemia (até 6 meses) para caracterização da hiperglicemia persistente (controle inadequado da doença) foram obtidos no prontuário do paciente. Foram excluídos 14 indivíduos com DM2 e 13 do grupo controle cujos dados laboratoriais estavam incompletos.

Foi aplicado um questionário padrão (características gerais) e realizadas medidas de peso e altura para cálculo do índice de massa corporal (IMC), que obedeceu à fórmula peso/altura (14). Para a avaliação da mobilidade funcional e da capacidade cognitiva, foram realizados os testes relacionados a seguir.

Metodologia de avaliação funcional

1) Verificação de ocorrência de quedas no último ano.

2) Foi requisitado ao paciente que levantasse de uma cadeira (de altura usual), sem apoio dos braços. Essa análise foi expressa em dificuldade ou não em realizar a tarefa (15).

3) Teste Timed Up & Go (TUG), método rápido, fácil e seguro para avaliação da mobilidade funcional (16). A verificação é realizada com uma cadeira, um cronômetro, fita métrica e uma ficha para anotações dos dados. Mede-se o tempo gasto para levantar de uma cadeira (com 45 cm de altura), andar uma distância de três metros, dar a volta, caminhar em direção à cadeira e sentar-se novamente. O paciente realiza primeiro o teste uma vez para se familiarizar e nenhuma ajuda é dada durante a sua realização (17). Considera-se que quanto maior o tempo gasto para realizar a atividade, mais limitada é a mobilidade (18). De modo geral, pacientes independentes e sem nenhuma alteração de equilíbrio demoram menos de 10 segundos. Por outro lado, pacientes dependentes demoram mais de 20 segundos. Pacientes que demoram de 10 a 20 segundos possuem certo grau de limitação, porém ainda são considerados independentes (19,20).

Metodologia de avaliação da função cognitiva

1) Miniexame do Estado Mental (MEEM): é um dos testes mais utilizados na prática clínica. Abrange seis áreas da cognição: linguagem, orientação, habilidade construtiva, atenção/cálculo, retenção e evocação. O escore máximo é de 30 pontos. São considerados como declínio cognitivo valores inferiores a 27 para pessoas com mais de 11 anos de escolaridade, inferiores a 22 para pessoas com menos de 11 anos de escolaridade e inferiores a 15 para analfabetos. Considerando que excluímos pacientes analfabetos, a pontuação corte para declínio cognitivo foi de 22 pontos (21).

2) Teste do Desenho do Relógio (TDR): reconhecido como teste de grande utilidade no diagnóstico e seguimento de demências. Pede-se ao paciente que desenhe um relógio marcando 11 horas e 10 minutos, sem tempo cronometrado. Todos os números devem estar representados (14,21). As notas variam de 0 (incapacidade absoluta de desenhar) a 5 (relógio perfeito).

A análise estatística foi realizada com auxílio do programa Statistical Package for the Social Sciences versão 17. Foram descritas as características quantitativas segundo presença ou não de DM2 com o uso de medidas resumo (média, desvio-padrão, mediana, mínimo e máximo) e comparadas pelo teste *t* de Student e, para as escalas MEEM e teste TUG, foram utilizados os testes Mann-Whitney. As características qualitativas foram descritas pelas de frequências absolutas e relativas e a existência de associação pelos testes qui-quadrado ou testes da razão de verossimilhanças. Foi criada uma regressão linear múltipla para explicar o resultado do teste TUG de acordo com as características dos pacientes, da escala MEEM e da presença ou não de DM2.

Este estudo obedeceu aos critérios de ética preconizados pela resolução nº 196/96 do Conselho Nacional de Saúde (CNS) do Ministério da Saúde e foi submetido à aprovação do Comitê de Ética em Pesquisa da Universidade Comunitária da Região de Chapecó (Unochapecó) antes de sua execução. Todos os participantes assinaram o termo de consentimento livre e esclarecido.

RESULTADOS

As características da população do estudo, de 118 indivíduos, sendo 50 com DM2 e 68 controles, são apresentadas nas tabelas 1 e 2.

A taxa de quedas no último ano foi de 42% entre pacientes com DM2 e 33,8% controles (p = 0,364). Entretanto, o número médio de quedas entre os grupos foi de 1,57 ± 1,07 nos DM2 e de 2,09 ± 1,97 nos controles (p = 0,058). A dificuldade em levantar da cadeira sem apoio foi presente em 22% dos pacientes com DM2 e 4,4% dos controles (p = 0,004).

Tabela 1. Características sociodemográficas da população em estudo (n = 118)

	DM2		Controles		Total		p
	n	%	n	%	n	%	
Sexo							**0,001**
Feminino	56	82,4	27	54,0	83	70,3	
Masculino	12	17,6	23	46,0	35	29,7	
Cor da pele							0,120
Não branca	11	16,2	14	28,0	25	21,2	
Branca	57	83,8	36	72,0	93	78,8	
Estado civil							0,076#
Casado	49	72,1	44	89,8	93	79,5	
Solteiro	2	2,9	0	0,0	2	1,7	
Viúvo	10	14,7	3	6,1	13	11,1	
Separado	7	10,3	2	4,1	9	7,7	
Escolaridade							0,746#
EF incompleto	50	73,5	38	76,0	88	74,6	
EF completo	9	13,2	7	14,0	16	13,6	
EM incompleto	3	4,4	3	6,0	6	5,1	
EM completo	6	8,8	2	4,0	8	6,8	
Queda no último ano							0,364
Não	45	66,2	29	58,0	74	62,7	
Sim	23	33,8	21	42,0	44	37,3	
Total	68	100	50	100	118	100	

Resultado do teste qui-quadrado # Resultado do teste da razão de verossimilhanças. DM2: diabetes melito tipo 2; EM: ensino médio; EF: ensino fundamental.

Tabela 2. Características antropométricas e laboratoriais da população em estudo (n = 118)

	Diabetes	Média	DP	Mediana	Mínimo	Máximo	N	p
Idade (anos)	Controle	56,21	4,31	55,5	50	65	68	0,056
	DM2	57,86	4,97	58	50	65	50	
Peso (kg)	Controle	71,71	13,28	71	45	101	62	**0,013**
	DM2	79,67	19,58	77,5	42	169	48	
Altura (m)	Controle	161,11	7,50	160	147	179	62	0,174
	DM2	163,33	9,50	165	132	181	48	
IMC	Controle	27,06	4,55	27	18	38	62	**0,034**
	DM2	29,48	7,20	28,5	19	59	48	
Glicemia capilar	Controle	81,89	10,17	82	56	100	37	**< 0,001**
	DM2	287,92	86,45	262,5	200	553	50	
Triglicerídeos (mg/dL)	Controle	138,58	72,75	142	36	364	33	**0,002**
	DM2	289,08	211,64	214,5	40	866	26	
HDL (mg/dL)	Controle	52,03	10,06	50	33	73	31	**0,006**
	DM2	43,82	10,17	44,5	23	61	22	
Colesterol T (mg/dL)	Controle	211,13	31,26	209,5	139	278	32	0,242
	DM2	228,73	69,84	227,5	124	463	26	

Resultados do teste *t* de Student. DM2: diabetes melito tipo 2; IMC: índice de massa corpórea; colesterol T: colesterol total.

O teste TUG foi realizado em 11,27 ± 3,2 segundos nos pacientes DM2, tempo superior aos controles, que foi de 9,52 ± 3,6 segundos (p = 0,001), (Tabela 3). Cerca de 30% e 52,9% dos pacientes DM2 e controles, respectivamente, levaram menos de 10 segundos para execução do teste (p = 0,013), evidenciando que a maior parte do grupo controle não tinha limitação funcional. Observamos que 22% dos pacientes DM2 e 57% dos controles não apresentaram limitação funcional (p < 0,01).

Tabela 3. Pontuação no MEEM e TUG nos pacientes com DM2 e sem DM2 (n = 118)

Testes funcionais	DM2	Média ± DP	Mediana	N	p
MEEM (pontos)	Controle	27,19 ± 2,4	28 (19-30)	68	0,001
	DM2	25,14 ± 3,4	26 (18-30)	50	
Teste TUG (tempo em segundos)	Controle	9,52 ± 3,6	9 (6-30)	56	0,001
	DM2	11,27 ± 3,2	10,5 (7-20)	44	

Teste Mann-Whitney. DM2: diabetes melito tipo 2; MEEM: Miniexame do Estado Mental. TUG: Timed Up and Go.

Em relação ao declínio cognitivo, avaliado pelo MEEM, os pacientes DM2 apresentaram mediana de 26 pontos (22 a 28), que foi inferior à dos controles, 28 pontos (25,5 a 29), p = 0,001 (Tabela 2). No TDR, a mediana de pontos foi semelhante entre o grupo DM2 2 (1-4) e controles 3 (1,5-4), (p = 0,121).

A avaliação da associação entre declínio cognitivo e dismobilidade foi positiva nos indivíduos com DM2: verificou-se pior mobilidade naqueles com maior declínio cognitivo (p = 0,037) utilizando regressão linear. A tabela 4 demonstra que 18% do grupo DM2 e 1,6% do grupo controle apresentaram dismobilidade e declínio cognitivo associados. No grupo que não apresentou dismobilidade e declínio cognitivo, 57,4% eram do grupo controle (p < 0,01).

Tabela 4. Avaliação da presença e ausência dos eventos dismobilidade e declínio cognitivo associadamente e sua correlação com DM2

	Controle n (%)	DM2 n (%)	p
Presença de dismobilidade e declínio cognitivo	1 (1,6)	11 (18,0)	**< 0,01**
Ausência de dismobilidade e declínio cognitivo	35 (57,4)	14 (22,9)	

RP: 15,84 (IC 95% 2,18 – 115,01).

DISCUSSÃO

Indivíduos com DM2 tiveram pior *performance* nos testes de avaliação funcional da mobilidade em relação aos controles. A população estudada, com idades entre 55 e 65 anos e predominantemente feminina, representa uma população que, mesmo independente da presença de DM2, já tem maior risco de fraturas em virtude da maior prevalência de osteoporose. Além disso, sabe-se que a população com DM2 pode apresentar maior risco de fraturas, pois as elevadas concentrações glicêmicas estão associadas ao acúmulo de produtos finais da glicação avançada (AGES) levando ao aumento da fragilidade óssea (22). Assim, a pior mobilidade funcional nesse subgrupo indica a importância desse fator na avaliação dos pacientes com DM2. Doenças osteomioarticulares têm relação com ocorrência de quedas, principalmente no tocante à redução da flexibilidade (23), mas fatores como neuropatia periférica, visão reduzida, alterações autonômicas e uso de medicamentos (2,3,1), bem como sedentarismo e o ganho de peso (1), são bastante prevalentes na população com DM2. Também foi importante, no presente estudo, o maior IMC do grupo com DM2. Tal característica pode estar envolvida na pior mobilidade verificada. Em estudo realizado com uma amostra representativa da população americana (15), 73,6% (IC 95% 70,2-76,9) dos pacientes com DM2 relataram mais dificuldades em realizar atividades rotineiras, incluindo levantar de uma cadeira sem apoio. Neste estudo, a redução da mobilidade funcional foi correlacionada com porcentagem de hemoglobina glicada e duração da doença.

Os pacientes com DM2 também tiveram pior desempenho no TUG do que o grupo controle (p < 0,05). A maior parte dos diabéticos (68%) situou-se no intervalo entre 10 e 20 segundos, caracterizado como médio risco de quedas, enquanto no grupo controle a maioria (52,9%) se enquadrou em menos de 10 segundos, com baixo risco de quedas. Em 2009, Cordeiro e cols. (20) avaliaram idosos (média de idade de 74,4 ± 5,9 anos) com DM2 utilizando o teste TUG. O tempo médio de execução foi de 15,7 ± 6,5 segundos, e a maioria dos pacientes (67,8%) levou um tempo entre 10 e 20 segundos e o restante (21,1%) levou mais de 20 segundos, mostrando que há piora importante desse aspecto em faixas etárias maiores.

Os pacientes com DM2 apresentaram ainda pior desempenho cognitivo avaliado pelo MEEM, com mediana de 26 pontos, contra 28 pontos para os controles (p = 0,01). Em estudo realizado com idosos de 70 anos

ou mais, dos quais 91% possuíam DM2, os testes de rastreio cognitivo (entre eles, o MEEM) caracterizaram como fatores preditivos de declínio cognitivo: avanço da idade, negligência no uso de anti-hipertensivos e insulina e baixo nível de escolaridade (24). No entanto, no presente estudo a mediana de idade foi de 57 anos, e não foi verificada correlação com baixa escolaridade, possibilitando dessa forma a avaliação da presença de DM2 como principal fator determinante do declínio cognitivo nesses indivíduos. O desenho do nosso estudo avaliando apenas indivíduos que apresentavam hiperglicemia, inclusive no momento da realização dos testes cognitivos, salienta não somente a influência do DM2 na capacidade cognitiva mesmo antes dos 65 anos, como também sugere a influência da presença de hiperglicemia no desempenho do grupo com DM2.

A presença de distúrbios cognitivos e mobilidade funcional teve correlação maior nos indivíduos com DM2, e foi evidenciada pior mobilidade naqueles indivíduos com maior declínio cognitivo. Carvalho e Coutinho (12), em estudo caso-controle com pacientes de 60 anos ou mais, internados por fratura secundária a quedas, encontraram associação positiva entre demência e a ocorrência de quedas (OR: 1,82), sendo que os pacientes com demência relataram um maior número de quedas no ano anterior. Da mesma forma, o nosso estudo mostrou correlação entre distúrbios cognitivos e mobilidade funcional. No entanto, avaliamos uma população menos idosa, o que favorece a hipótese de que o DM2 influencia a mobilidade funcional e a capacidade cognitiva antes do aparecimento de complicações vasculares ou neuropáticas e sugere que a hiperglicemia é um fator agravante no desempenho de atividades que exijam funções mentais como atenção, orientação e memória de trabalho.

A pior mobilidade funcional e o pior desempenho no MEEM dos pacientes com diabetes podem advir de mau controle glicêmico, sobreposto aos demais aspectos inerentes à doença. Contudo, poucos são os estudos relacionados ao assunto em pacientes com idade inferior a 65 anos, remetendo-nos à pesquisa também em indivíduos mais jovens a fim de melhorar a qualidade de vida destes. O conhecimento sobre prejuízos na capacidade cognitiva e na mobilidade funcional dos pacientes com DM2 em faixas etárias mais jovens permite traçar estratégias de prevenção de grande importância para os programas de saúde, alertando portadores da doença e seus familiares sobre sinais de incapacidades cognitivas e físicas.

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Marrow hypoplasia: a rare complication of untreated Grave's disease

Hipoplasia de medula óssea: uma rara complicação da doença de Graves não tratada

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SUMMARY

Atypical presentation forms of hyperthyroidism are always a challenge to the clinician. We present a female patient with the typical symptoms of thyrotoxicosis, without any thionamides treatment before, associated with pancytopenia, which recovered after euthyroidism state was achieved. Although the major cases of pancytopenia in Grave's disease are seen as a complication of antithyroid drugs (thioamides), in this case report the alteration in blood tests was associated with untreated hyperthyroidism. In the literature review, we found 19 case reports between 1981 to 2012, but it has been related to a hypercellular bone marrow with periferic destruction. Our case, however, is about a hypocellular bone marrow without fibrosis or fat tissue replacement, which proceeded with a periferic improvement following thyroid treatment. Although rare, pancytopenia, when present, may develop as an unusual and severe manifestation in untreated subjects. Arq Bras Endocrinol Metab. 2014;58(9):953-7

SUMÁRIO

Formas atípicas de apresentação do hipertireoidismo são sempre um desafio para o clínico. Apresentamos uma paciente do sexo feminino, com sintomas típicos de tireotoxicose associado a um quadro de pancitopenia sem nenhum tratamento prévio com tionamidas. A melhora da alteração hematológica ocorreu após recuperação do eutireoidismo. Embora a maioria dos casos de pancitopenia na doença de Graves seja uma complicação das drogas antitireoidianas (tionamidas), neste caso a alteração hematológica foi associada ao quadro de hipertireoidismo não tratado. Após uma revisão na literatura, encontramos 19 relatos de caso descritos no período de 1981 a 2012, nos quais o quadro de pancitopenia estava relacionado à hipercelularidade medular com destruição periférica das células sanguíneas. Nosso caso, entretanto, trata-se de uma pancitopenia com medula óssea hipocelular, sem infiltração por tecido adiposo ou fibrose, que evoluiu com melhora dos elementos do sangue periférico após tratamento do hipertireoidismo. Embora rara, a pancitopenia, quando presente, pode se manifestar como uma severa manifestação se não tratada a condição desencadeadora. Arq Bras Endocrinol Metab. 2014;58(9):953-7

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INTRODUCTION

Grave's disease (GD) usually presents with the several well-known symptoms and signs, but in some cases there is atypical manifestations of thyrotoxicosis, which include hematological alterations, as anemia. Pancytopenia is a rare, but a serious complication that physicians may come across.

Hyperthyroidism affects hematopoiesis in many ways, but its pathogenesis is still unclear. Both thyrotoxicosis and the underlying autoimmunity of GD may affect the production of blood cells. Imunological mechanisms are suggested to be involved, such as antineutrophil antibodies and antiplatelet antibodies, but the definitive ethiology remains uncertain. Neverthe-

less, a good response to hyperthyroidism treatment is almost guaranteed.

CASE REPORT

A 54 year-old female was referred to our hospital complaining of palpitations, hand tremor and a 23 kg weight loss in the last 5 months. The physical examination revealed thyroid (approximately double the normal size), irregular, firm, without lumps, sinus tachycardia (120 bpm) with holosystolic murmur in the mitral focus, hypertension (BP 160 x 100 mmHg) as well as moist and warm skin. There was no evidence of exophthalmos or pretibial myxedema. The patient was diagnosed with GD and stated never having been submitted to any previous treatment.

Besides the hyperthyroidism, her blood test results, at the time of the clinic visit included microcytic hypochromic anemia, thrombocytopenia, associated with neutrophilia (Table 1). We had her admitted for compensation of the thyroid disease and started treatment with Lithium – 900 mg/day. After 18 days of treatment with Lithium, she was submitted to radioiodine I-131 (15 mci).

An evaluation by the hematology department (HEMORIO) was asked in order to rule out other possible causes of pancytopenia. They requested laboratory tests and proceeded with aspirate and biopsy of bone marrow. Due to the worsening of neutropenia (Table 1) treatment with granulocyte stimulating factor (G-CSF) was initiated.

Subsequent results of laboratory tests (HIV and viral hepatitis serology, B12 vitamin, serum iron, ferritin,

Table 1. Evolution of blood tests (hematological values, thyroid hormones and TSH receptor antibodies – TRAb)

	First consult without any anti-thyroid medication	Started Lithium	With Lithium 900 mg/day	Before Therapeutic dose (09/26/12 – I131 15mci)	Started G-CSF		After ten days of G-CSF	Discharged	Started Propyl-thiouracil	Medications suspended	Started Levo-thyroxine
Laboratory	**08/22/12**	**09/06/12**	**09/10/12**	**09/24/12**	**10/04/12**	**10/07/12**	**10/14/12**	**10/17/12**	**10/24/12**	01/24/13	02/22/13
Ht/Hb RR:12 to 16g/dL /36 to 48%	32,2/9,5	30,6/9,5	30,8/9,5	27,6/8,5	28,9/8,7	26,3/7,8	30/9,4	32/10,3	28,9/9,1	42,3/12,8	42,0/12,1
MCV/MCH RR:80 to 96fL/27 to 33pg	72,2/21,3	69,5/21,6	68,8/21,2	68/20,9	68,6/20,7	64,3/21	69,4/21,8	68,8/22,2	70,7/22,2	74,3/22,5	80/25
WBC RR:4000 to 11000/mm^3	2100	1900	1700	1200	1000	**600**	7100	2600	2200	3400	4600
Neutrophils RR:1640 TO 7920/mm^3	**882**	**627**	**714**	**288**	**260**	**174**	**5396**	**1872**	**1122**	2040	2896
Platelets RR:150000 to 450000/mm^3	137000	136000	122000	103000	140000	106000	141000	149000	170000	179000	187000
TSH RR:0,3 to 5,0 µUI/mL	< 0,011	–	< 0,011	–	–	–	–	–	< 0,011	0,03	16,82
Free T4 RR: 0,8 to 1,9 ng/dL	6,9	5,9	5,1	–	3,4	2,9	2,6	2,4	2,9	0,6	0,3
TRAb RR:< 1,5 UI/L	**34**	–	–	–	–	–	–	–	**18,5**	–	–

Ht: hematocrit; Hb: hemoglobin; RR: reference range; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; WBC: white blood cell; TSH: thyroid stimulating hormone; TRAb: TSH receptor antibodies.

DHL, ANA, VHS, hemoglobin electrophoresis) (Table 2) and the total abdominal ultrasonography were normal. The bone marrow biopsy revealed hypocellularity without any infiltration or fibrous/fatty tissue replacement (Figures 1 and 2).

After thirty-eight days of lithium, twenty days of radioiodine I-131 (15 mci) and ten days of G-CSF associated with decreased levels of thyroid hormones, improvement of pancytopenia could be noticed (Table 1). The patient was discharged after a 42 days period of hospitalization with prescription for lithium (900 mg/day), and referral for our outpatient clinic.

She returned ten days after, as lab results were still abnormal (Table 1), propylthiouracil was added to the treatment with lithium. The decrease of thyroid hormones and complete improvement of pancytopenia finally occurred after two months of hospital discharge and the medications were therefore discontinued. Five months after the radioiodine treatment, she developed hypothyroidism. Levothyroxine was then initiated for the control of thyroid hormones.

Table 2. Laboratory tests

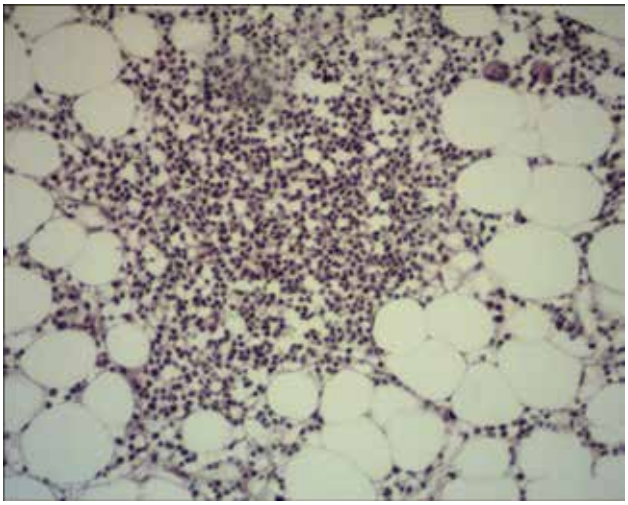
Laboratory	9/12/12
Anti-HIV	No reagent
Anti-HBs	No reagent
HBs Ag	No reagent
Anti-HBc	No reagent
Anti-HCV	No reagent
ANA	Negative
ERS (RR: 0 to 15 mm/hour)	2 mm/hora
LDH (RR: < 190 UI/l)	101 UI/l
B12 vitamin (RR: 180 to 914 pg/mL)	516 pg/mL
Serum iron RR: 37 to 150 µg/dL)	46 µg/dL
Ferritin (RR: 11 to 307 ng/mL)	198,2 ng/mL
Transferrin saturation	18% (RV: 20 to 50%)
Hemoglobin electrophoresis (Hg A1 RR: > 95%; Hg A2 RR: < 3,5%; Fetal Hg RR: < 0,5%)	Hg A1: 96,4%; Hg A2 3,4%; Hg Fetal 0,2%
Transferrin (RR: 200 to 360 mg/dL)	233 mg/dL

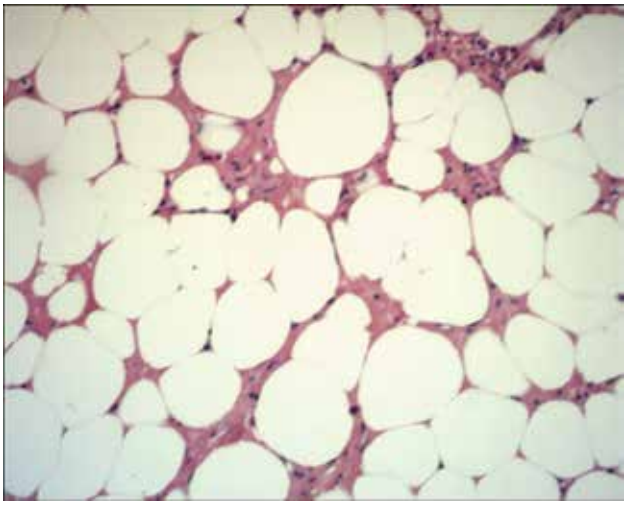
Anti-HBs: hepatitis B surface antibody; HBs Ag: hepatitis B surface antigen; Anti-HBc: hepatitis B core antibody; Anti-HCV: hepatitis C antibody; ANA: antinuclear antibodies; ERS: erythrocyte sedimentation rate; LDH: lactate dehydrogenase; RR: reference range.

DISCUSSION

Our patient presented with the classical signs and symptoms of hyperthyroidism due to diffuse toxic goiter (DTG), with the first lab results showing a pancytopenia that required investigation since she have never been submitted to any previous treatment. The American Thyroid Association as well as the American Association of Clinical Endocrinologists suggests that, prior to initiating antithyroid drug therapy (ATD), patients should have a complete blood count, and in case the patient has an absolute neutrophil count below 500/mm^3 ATD is not recommended (item 15 of reference 1).

Since the main cause of hematological alteration in hyperthyroidism is known to be the treatment with thionamides, this would be a relative contraindication. Although, in a systematic review about agranulocytosis induced by nonchemotherapy drugs, it was shown that propiltiouracil rarely causes agranulocytosis, and low doses of MMI are even less likely to do so (2).





Figures 1 and 2. Marrow biopsy revealed hypocellularity without any infiltration or fibrous/fatty tissue replacement.

Another study examined clinical features of hematopoietic disorders that occur after the administration of ATD. In a group of 50,385 patients analyzed in a Japanese hospital, only five developed pancytopenia after treatment for GD, and the majority patients who had been diagnosed with GD were treated with ATD. So, the incidence of pancytopenia in that study group can be estimated to have been minimal. In four of the patients in that study, pancytopenia was preceded by agranulocytosis. In addition, agranulocytosis and pancytopenia showed no manifest differences in their intervals of onset from the start of ATD administration. The authors suggest that these facts indicate, in this instance, these disorders could belong to the same disease with some probable overlap in their pathogenesis, only differing in severity. This would mean that ATD does damage hematopoietic stem cells. The pathogenic factors involved in erythropoietic organ disorders caused by ATD are diverse and require further research (3).

These evidences led us to choose the use of lithium as adjunct to RAI, so we could increase the radiation dose delivered to the thyroid (4). As seen in a retrospective cohort study that compared the efficacy of radioactive iodine therapy (RAI) given with or without concomitant lithium treatment in patients with newly diagnosed Graves' disease, patients treated with RAI plus lithium had a higher rate of recovery than those treated with RAI alone. In addition, patients treated with RAI and lithium were cured more rapidly than those treated with RAI alone. Treatment with lithium prevented serum free T4 increase after methimazole withdrawal and RAI therapy. At last, studies have shown that RAI is safe and except of transient or permanent iatrogenic hypothyroidism no significants outcomes have been reported (5). In a follow up study for 20 years, Ron and cols. didn't observe an increased mortality from cancer, including leukemia and hematological effects after low radioiodine therapy in GD. Cell killing, rather than mutation, should be the predominant effect (6,7).

Regarding the pancytopenia, our approach was to rule out other causes such as HIV, viral hepatitis, deficiency of vitamin B12 or iron, collagenosis, hemoglobinopathy and hypersplenism. With the hematological service support (HEMORIO), we got the marrow's biopsy and aspirate, which indicated that there was a hypocellularity without any infiltration or fibrous/fatty tissue replacement. This suggested that the pancytopenia was due to a complication of hyperthyroidism itself, since improvement in the hematological alterations occurred with euthyroid state.

It is well known that GD is an autoimmune disease associated with hyperthyroidism. Both thyrotoxicosis and the underlying autoimmunity of GD affect multiple tissues and their functions, including hematopoesis. Anemia is common, resembles the anemia of chronic disease, and it corrects promptly with return to the euthyroid state following treatment (8).

The pathogenesis of DTG has been linked to genetic factors such as HLA-DR3 and cytotoxic T-lymphocyte antigen-4 (CTLA-4) and to environmental factors such as stress, female sex steroids, and certain infections (9).

As already mentioned, pancytopenia is a rare but serious complication of thyrotoxicosis. We found 19 case reports in a literature review between 1981 to 2012, that made references to this relationship and all of them demonstrated reversible pancytopenia in response to hyperthyroidism's improvement. Single lineage abnormalities related to hyperthyroidism are more commonly reported than pancytopenia (5), *e.g.* anemia, and even in these cases it is demonstrated that after correction of hyperthyroidism an increase in hemoglobin values is detected, even in non-anemic patients (8,10).

In a very interesting case where the patient had a protracted period of pancytopenia prior to hyperthyroidism, therapy with anti-thyroid led to a sustained improvement in his blood cell levels. The authors suggest that thyroid hormones may have a direct effect on hematopoiesis at a stage previous to erythropoietic stem cell differentiation, disturbing maturation and differentiation of the pluripotent stem cells (11). However, the exact pathogenic mechanism is still unclear, since hyperthyroidism could affect hematopoiesis in many ways (although clinically important abnormalities are rare). Some causes are suggested: ineffective hematopoiesis caused by an excess of thyroid hormones; reduction in blood cell life span caused by hypersplenism imunological mechanisms, such as antineutrophil antibodies and antiplatelet antibodies; toxicity of thyroid hormone to bone marrow stem cells (12,13).

The major cases of pancytopenia in GD are seen as a complication of thioamides (14). When associated with untreated hyperthyroidism it has been related to a hypercellular bone marrow with periferic destruction (15). Our case, however, is about a hypocellular bone marrow without fibrosis or fat tissue replacement at the presentation of GD, which proceeded with a periferic improvement following thyroid treatment.

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Large thyroid cyst in a patient with congenital hypothyroidism

Grande cisto tiroidiano em paciente com hipotireoidismo congênito

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SUMMARY

Thyroid hormone biosynthetic defects are rare causes of congenital hypothyroidism. Although, initial presentations are usually diffuse goiter and hypothyroidism, subsequently they may develop thyroid nodules and or thyroid cancer. We describe a case of hypothyroidism due to dyshormonogenesis whose one of the previously solid nodules degenerates into a large cyst. A 22-year-old male was referred to our clinic for evaluation of enlarging thyroid nodule. Hypothyroidism was diagnosed in infancy, however due to poor compliance to treatment TSH values were elevated most of the times. When he was fifteen the first nodule was detected which was a solid cold nodule. Fine needle aspiration was in favor of benign follicular nodule. Seven years later we found a large multi nodular thyroid with a predominant large cyst corresponding to the previously detected solid nodule. 21^{cc} straw colored fluid was aspirated. Cytology was reported as benign cystic nodule. The patient underwent thyroidectomy and pathology confirmed a benign thyroid cyst. Although underreported thyroid dyshormonogenesis may progress to cystic degeneration. Taking into account the risk of malignancy and eventually cyst formation, we recommend more frequent evaluation in the face of nodule formation in these patients. Arq Bras Endocrinol Metab. 2014;58(9):958-61

SUMÁRIO

Os defeitos de biossíntese do hormônio tiroidiano são causas raras de hipotireoidismo congênito. Embora as apresentações iniciais sejam geralmente bócio difuso e hipotireoidismo, nódulos tiroidianos ou câncer de tiroide podem se desenvolver subsequentemente. Descrevemos aqui um caso de hipotireoidismo causado por disormonogênese e no qual um dos nódulos sólidos degenerou em um grande cisto. Um homem de 22 anos de idade foi encaminhado para nossa clínica para avaliação do aumento de um nódulo tiroidiano. O hipotireoidismo foi diagnosticado na infância. Entretanto, em razão da baixa conformidade ao tratamento, os valores de TSH estavam elevados na maior parte do tempo. Quando o paciente tinha 15 anos de idade, um primeiro nódulo sólido e frio foi detectado. A aspiração por agulha fina mostrou um nódulo folicular benigno. Sete anos depois encontramos múltiplos nódulos na tiroide e um grande cisto predominante que correspondia ao nódulo sólido anteriormente detectado. Foram aspirados 21^{cc} de fluido cor de palha. A citologia mostrou um nódulo cístico benigno. O paciente foi submetido à tiroidectomia e o exame histopatológico confirmou um cisto tiroidiano benigno. Embora não seja comumente relatada, a disormonogênese da tiroide pode progredir para a degeneração cística. Ao serem considerados o risco de malignidade e a eventual formação de cistos, recomendamos uma avaliação mais frequente da formação de nódulos nesses pacientes. Arq Bras Endocrinol Metab. 2014;58(9):958-61

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INTRODUCTION

Thyroid hormone biosynthetic defects are unusual causes of permanent congenital hypothyroidism account for about 10-15% of cases (1). The vast majority of cases are due to mutations in one of the genes encoding Na/I symporter (2), thyroid peroxidase (TPO) (3), pendrin (4), thyroglobulin (5) or dehalogenases (6). Although the patient typically presents with hypothyroidism and goiter, natural course of disease is poorly defined. In some patients despite adequate treatment, the goiter may worsen and even may become nodular years thereafter (7-10). However to date there is no

firm document considering cystic degeneration of these nodules. We present a case of congenital hypothyroidism that developed a large cyst in one of the previously detected solid thyroid nodules.

CASE REPORT

A 22 year old young man (JR) presented to our clinic due to slowly enlarging thyroid gland. He was diagnosed of congenital hypothyroidism in infancy; after that, underwent replacement therapy with levothyroxine (LT4). Review of his medical records revealed poor adherence to medication, with TSH values between 0.04 and 72 mU/L (Mean 11.2) over twenty years.

The patient was referred to endocrinology clinic on May 2005 for assessment of goiter, when sonography of thyroid gland disclosed a solid 20 × 19 mm solid nodule in right lobe and a small 10 × 7 mm solid nodule in left lobe. A thyroid scan using ^{99}TC one month after discontinuation of LT4 showed a cold nodule in right lobe and increased absorption of radiotracer in other parts of thyroid. Levothyroxine reinstituted after fine needle aspiration of cold nodule was reported as benign nodular goiter (July 2005).

He was referred to our clinic on June 2012 due to further enlargement of thyroid nodules. There was no history of radiation therapy or other coexistent diseases and family history was negative for thyroid diseases. On physical examination a well-developed young man with asymmetrically enlarged nodular thyroid and a 4.0 × 3.0 cm dominant nodule in right lobe without cervical adenopathy was noticed. TSH while receiving 175 µg LT4 daily was 5.5 mU/L (0.4 - 4.2). At the same time, thyroid sonography showed four iso-echo solid nodules of varying sizes, the largest measured 22 × 15 mm, in the left lobe and a cystic 44 × 31 mm nodule in the right lobe. In a search to determine the cause of hypothyroidism LT4 discontinued for four weeks then, serum TSH, thyroglobulin, antithyroglobulin antibody (Anti-Tg), antithyroid peroxidase antibody (Anti-TPO), antitissue transglutaminase (Anti-tTG) antibody and serum IgA were measured and a thyroid scan with ^{99}TC was obtained. While serum TSH was 37 mU/L, thyroglobulin was 30 ng/mL (20-50) and Anti-Tg, Anti-TPO and Anti-tTG were negative. Thyroid scan indicated a large cold nodule in right lobe and severe increased radiotracer uptake in other regions of both lobes including left nodules (Figure 1). Fine needle aspiration of right cyst yielded 21^{cc} straw colored fluid and cytological smears was reported as benign cystic goiter nodule.

In spite of recommendation for total thyroidectomy, the patient underwent right lobectomy and tissue examination confirmed diagnosis of simple thyroid cyst.

The patient advised to consume his medication regularly. Two months after surgery while taking 150 µg LT4 daily, he was euthyroid with a TSH of 1.4 mU/L.

The patient was discussed about the aims of this report and informed consent was provided.

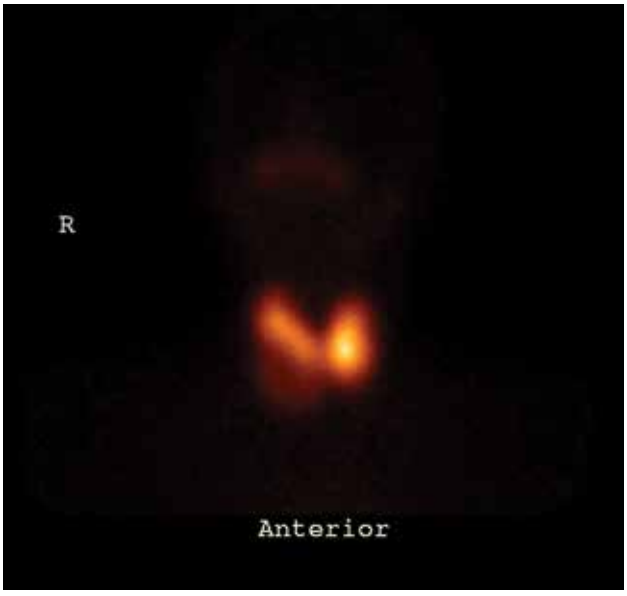

Figure 1. Large cold nodule in the right lobe along with high radioisotope uptake in other regions of thyroid.

DISCUSSION

Here, we describe a young man with goitrous congenital hypothyroidism that one of his nodules undergone cystic degeneration. According to inappropriately normal thyroglobulin in the presence of large goiter, high TSH with negative antithyroglobulin antibody, high uptake of radiotracer by thyroid, and no evidence of other defect, the most probable underlying abnormality is thyroglobulin synthesis defect. Typically, thyroglobulin synthesis abnormalities manifest with low thyroglobulin levels in the presence of high TSH (quantitative defect), however, in another subgroup thyroglobulin may be within normal values (qualitative defect) (11). Apart from the original abnormality, goiter with subsequent nodule formation has been reported in all types of thyroid dyshormonogenesis (7-10). Thyroid nodules were first detected in this patient when he was fifteen, however the size and number of nodules were increased progressively. Seven years later

when we visited him, there were multiple nodules of varying sizes and a large cyst corresponding to one of the previously discovered solid nodules.

Two main points should be considered according to findings in this patient; the first is an old question; what is the goal of treatment in these cases? While TSH has recognized as the major regulatory component of thyrocytes growth and differentiation, it is not surprising that higher TSH values may be accompanied by larger goiters and nodularity (11,12). Furthermore, as was shown by Chiesa and cols. the underlying mutation per se may play a synergistic role in goiter and nodule formation (7). Taken together it was suggested that TSH should be kept lower or even suppressed in this setting (9,10).

Second, since a great number of thyroid cysts are products of degeneration of solid nodules (13), it is conceivable why this patient developed a large cyst corresponding to his previously solid one. However it is largely unknown whether there is any association between changes in serum TSH and progression of nodular goiter to the cystic one, as in this case. To our knowledge this is the first case of goitrous congenital hypothyroidism whose solid nodule transformed into a large cyst. Therefore the natural course of thyroid biosynthetic defects usually begins with hypothyroidism and diffuse goiter, continues into nodule formation and eventually may terminate in cystic degeneration.

Another aspect of nodular disease in these patients is probable higher risk of malignancy. Again, not only elevated TSH levels (15,16) but also primary genetic abnormality including TPO (17-19) and thyroglobulin gene mutations may further prone these patients to thyroid cancer particularly in those with thyroglobulin gene mutations (20,21). In this regard, polymorphisms in the exon 10-12 cluster (22), biallelic p.R2223H mutation (19), activating mutation of V599E or K600E (20), g.IVS5 + 1G-- >A (23) and R-allele of Tg Q2511R (24) have been associated with higher risk of malignant transformation. Even non goitrous glands in patients with thyroid hormone biosynthetic defects may undergo malignant transformation (25). Owing to multiplicity of these nodules which causes the FNA difficult, higher risk of cancer, retrosternal extension, side effects of thyroid suppression and probable cystic degeneration which further complicates management; one should consider regular assessment and if indicated removing the "useless" thyroid particularly in the face of nodule formation.

In conclusion, thyroid nodules in the setting of dyshormonogenesis may further progress to cyst formation. To prevent more complications, we recommend more frequent follow ups and a lower threshold for thyroidectomy in these patients.

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A case of thyroid hormone resistance: a rare mutation

Caso de resistência aos hormônios tireóideos: mutação rara

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SUMMARY

Reduced sensitivity to thyroid hormones (RSTH) is a rare disease that affects about 3,000 individuals, belonging to about 1,000 families. It results from reduced intracellular action of thyroid hormones (TH) genetically determined and manifests as persistent hyperthyroxinemia with non-suppressed thyroid-stimulating hormone (TSH). We describe a 67-years old, Caucasian woman, with past history of subtotal thyroidectomy due to diffuse goiter, who presents with a recurrence of goiter. Although she is clinically euthyroid, laboratory evaluation shows persistent hyperthyroxinemia with non-suppressed TSH. Response to thyrotropin releasing hormone (TRH) test was normal and TSH concentrations were not suppressed during oral administration of suprafisiologic doses of levothyroxine (L-T4). Peripheral blood DNA was extracted from the patient and a mutation was found localized in cluster one, at codon 346 of the ligand binding domain of the THRB gene. The patient's son underwent thyroid function testing (TFT) and genetic study, both negative, suggesting a sporadic mutation. RSTH should be considered in all hyperthyroxinemic patients who are clinically euthyroid. Mutations interfering with three major steps required for TH action on target tissues have been, so far, identified (TR-β, TR-α, MCT8, SPB2). Each mutation is associated with a distinctive syndrome. Goal of management is to maintain a normal serum TSH level and a eumetabolic state and offer appropriate genetic counselling and prenatal diagnosis. Inappropriate treatment of eumetabolic patients results in hypothyroidism and need for TH replacement. Arq Bras Endocrinol Metab. 2014;58(9):962-6

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SUMÁRIO

A sensibilidade reduzida aos hormônios tiroidianos (RSTH) é uma doença rara que afeta cerca de 3.000 indivíduos em 1.000 famílias. Ela resulta de uma ação intracelular reduzida de hormônios tiroidianos (TH), é geneticamente determinada e se manifesta como hipertiroxinemia persistente com hormônio tireoestimulante (TSH) não suprimido. Descrevemos o caso de uma mulher caucasiana de 67 anos de idade com histórico de tiroidectomia subtotal por bócio difuso e que apresentou recorrência do bócio. Embora ela fosse clinicamente eutiroide, a avaliação laboratorial mostrou hipertiroxinemia persistente com TSH não suprimido. A resposta ao hormônio liberador da tireotrofina (TRH) foi normal e as concentrações de TSH não foram suprimidas durante a administração oral de doses suprafisiológicas de levotiroxina (L-T4). Foi extraído DNA de sangue periférico da paciente e encontrada uma mutação no *cluster* um do códon 346 do domínio de ligação do ligante do gene THRB. O filho da paciente foi submetido a um teste de função da tiroide e a um estudo genético, ambos negativos, o que sugeriu uma mutação esporádica. O RSTH deve ser considerado em todos os pacientes hipertiroxinêmicos que sejam clinicamente eutiroides. Foram identificadas, até hoje, mutações que interferem com os três passos principais necessários para a ação do TH sobre os tecidos-alvo (TR-β, TR-α, MCT8, SPB2). Cada mutação está associada com uma síndrome distinta. O objetivo do manejo é manter o nível sérico normal de TSH e um estado eumetabólico, além de se oferecer aconselhamento genético adequado e diagnóstico pré-natal. O tratamento inadequado de pacientes eumetabólicos leva ao hipotireoidismo e requer reposição de TH. Arq Bras Endocrinol Metab. 2014;58(9):962-6

INTRODUCTION

Reduced sensitivity to thyroid hormones (RSTH) is a rare disease, that affects about 3,000 individuals, belonging to about 1,000 families (1).

It results from reduced intracellular action of thyroid hormones (TH) genetically determined and manifests as persistent hyperthyroxinemia with non suppressed TSH (2,3).

Patients with RTH are usually euthyroid. Occasionally, they present signs and symptoms of thyrotoxicosis or, rarely, with hypothyroidism.

This condition is found with equal frequency in both genders and has wide geographic distribution having been reported in Caucasians, Africans, and Asians (3,4). The prevalence may vary among different ethnic groups. Familial occurrence of RTH has been documented in approximately 75% of cases (3,4).

Eighty-five percent of the patients harbour mutations in the thyroid receptor (TR) beta, and about one hundred of different mutations have been reported so far (3,4). Most patients with RTH present missense mutations located exclusively in ligand-binding domain and hinge domain, most often clustered in three hot spots (exon 8-10). Investigation failed to demonstrate correlation between the phenotype and location of mutations in the receptor gene.

The diagnosis requires a high degree of suspicion, and is often delayed or failed, involving inappropriate treatment and morbidity for the patients.

In this article, we present a case of resistance to thyroid hormones, in which genetic testing revealed a rare mutation.

In this context, we review the literature, and highlight "pitfalls" in the investigations and treatment of this rare syndrome.

CASE REPORT

We report a case of a 67-years old woman, Caucasian, with past history of subtotal thyroidectomy due to diffuse goiter, who presents with a recurrence of goiter, persistent elevation of serum levels of FT4 and FT3 and non-suppressed TSH (TSH – 10.1 uU/mL (0.35 – 5.50), FT3 – 6.21 pg/mL (2.3 – 4.2), FT4 – 2.3 ng/dL (0.80 – 2.0). She is clinically euthyroid. There is no family history of goiter or other thyroid abnormalities. TBG, anti-TPO and anti-TG were within normal range. Response to TRH test (200 ug) was normal. During oral administration of suprafisiologic doses of L – T4 (50, 100, 200 ug, each given for three days), TSH concentrations were not suppressed.

The goiter is well tolerated and the patient is euthyroid due to compensation of high levels of TSH, so no treatment was initiated.

Genomic DNA was isolated from peripheral blood. Exons 3-10 and 1-2 of THRB gene, including the flanking intronic sequences, were amplified by polymerase chain reaction (PCR) and screened by direct sequencing. Genetic study identified a heterozygous, missense mutation in exon 10, with c.1293G>A transition inducing a replacement of isoleucine for methionine (p.Ile431Met). The mutation is localized in cluster one, at codon 346 of the ligand binding domain of the THRB gene.

The patient's son underwent thyroid function testing (TFT) and genetic study, both negative, suggesting a sporadic mutation.

DISCUSSION

We described a rare case of RSTH syndrome associated with an infrequent mutation.

The patient has clinical presentation and pattern of thyroid function test (TFT) abnormalities suggestive of RTH syndrome, which was confirmed by genetic test. Reviewing literature, we found a single previous report of a South American women presenting the same mutation (5).

To our knowledge this is the first report of this specific mutations in Europe. Although, normally there is no correlation between phenotype and location of mutations in receptor gene, that woman was diagnosed with RTH at the age of 62 years old, and underwent inappropriate subtotal thyroidectomy due to goiter, similarly to our patient.

To avoid missing the diagnosis and inappropriate treatments, RSTH should be considered in all hyperthyroxinemic patients who are clinically euthyroid.

Mutations interfering with three major steps required for TH action on target tissues have been identified (TR-β, TR-α, MCT8, SPB2) so far (1,6-9). Each mutation is associated with a distinctive syndrome (Table 1). The TR-α gene defect was identified and reported only this year in two subjects (1,8,9).

RTH results from mutations of TR-β gene. Twenty-seven percent are *de-novo* mutations and 73% are inherited (3,4).

Table 1. Summary of thyroid abnormalities in the known syndromes of reduced sensitivity to thyroid hormone

	Inheritance	Gene	Gene product	FT4	FT3	rT3	TSH	Other manifestations
Resistance to TH (RTH)	Dominant except in one family	TR-β (85%)	TR-β Nuclear transcription factor of specific gene targets	↑↑	↑	↑↑	Normal or slight ↑	Eumetabolic *vs* Hypo and/or hyperthyroidism
		Unknown (15%)	Could be due to mosaism in a *denovo* mutation or a yet unidentified etiology (non-TR RTH)					
TH cell transpoter defect	X-linked	MCT8	Monocarboxylate transporter 8 (MCT8) Transports T3 into neurons of developing brain and TH into other tissues	↓	↑↑	↓	Normal or slight ↑	Sever psychomotor impairment
TH metabolism defect	Recessive	SBP2	Selenocysteine insertion sequence-binding protein (SBP2) epistatic to selenoprotein synthesis (including D1, D2, D3)	↑↑	↓	↑↑	Normal or slight ↑	Puberty delay infertility Probably underestimated (affected individuals are children)

↑: increased; ↓: decreased; TH: thyroid hormone; TR-β: thyroid hormone receptor beta.

In our case report, the patient's son underwent thyroid function testing (TFT) and genetic study, both negative, and there was no family history of thyroid disease, suggesting a sporadic mutation.

There are two different mutations, as described in figure 1.

The less common, described in only one family, causes RTH by deletion of all coding sequences of the TR-β gene and is inherited as an autosomal recessive trait (3,4). In addition to typical RTH syndrome, these individuals have severe deafness resulting in mutism, and monochromatic vision. Heterozygous individuals for the same mutation have no abnormalities. Although, some TH effects are absolutely isoform specific, TR-β and TR-α are interchangeable to a certain degree. There is no compensatory over expression of the single normal allele of TR-β gene nor that of the TR-α gene (3,4).

The most common form of RTH results from minor defects, in the ligand-binding domain or hinge domain of the TR-β gene, resulting in impaired T3 binding, attenuated interaction with the coactivator or delay in the corepressor release. Mutant TR-β gene (m TR-β) with impaired T3-bindind has a preserved DNA-binding domain and preserved ability to dimerize with homologous and heterologous partners, so it can interfere with the Wild Type TR (WT TR) function by occupying TH response elements (TRE) on target genes and by engaging WT TR-β in homodimerization, a phenomenon termed dominant negative effect (3,4,10). The reduced amount of wild type TR-β does not produce haploinsufficiency by itself. It requires the dominant negative effect to cause RTH. The dominant negative effect also explains why this trait is inherited in a dominant fashion (3,4,10).

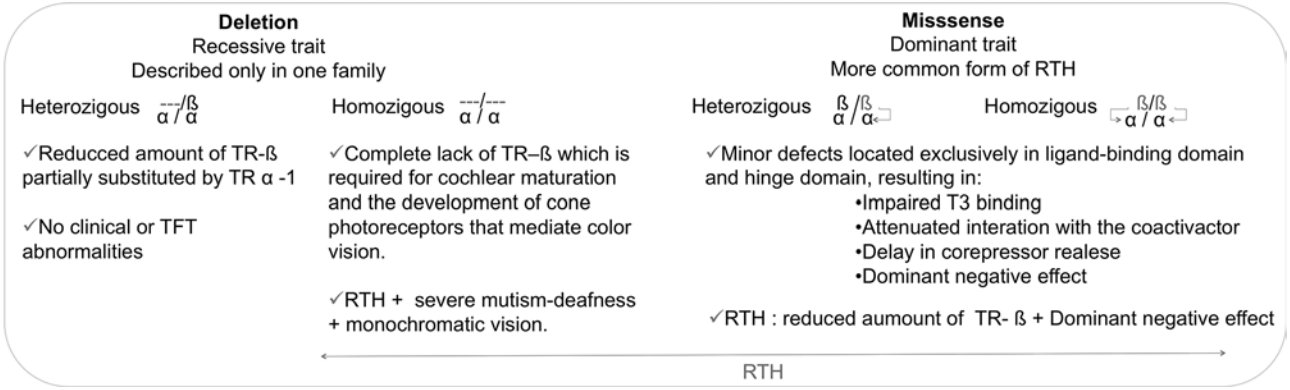

Figure 1. Resistance to thyroid hormone genotype.

Most patients with RTH, such as the patient described, present missense mutations located exclusively in ligand-binding domain and hinge domain, most often clustered in three hot spots (exon 8-10) (6).

Failure to identify a TR-β gene mutation in genomic DNA of subjects presenting the RTH phenotype could be due to mosaicism in a *de-novo* mutation or to a yet unidentified etiology of the syndrome (non-TR RTH) (4,6).

All tissues share reduced sensitivity to TH to variable extent. Differences between tissues are due to absolute and relative levels of TR-β and α expression during different stages of development. Inter and intra-familial variation is probably caused by genetically modulated cofactors (3,4,6).

Despite a past history of thyroidectomy, the patient described was euthyroid and presented with recurrence of a small, non obstructive goiter. In this cases, TSH hypersecretion and/or hyperactivity is compensatory, and patients shouldn't receive treatment (11,12).

When goiter isn't well tolerated it has been shown that treatment with supraphysiological doses of L-T_3, given as a single dose every other day, is successful in reducing goiter size, without causing side effects (11,12). This is the treatment of choice, considering that postoperative recurrence of goiter is the rule. The L-T_3 dose must be adjusted in increments until TSH and TG are suppressed and reduction of goiter size is observed.

Discordance of resistance to TH between pituitary and other body tissues, leads to hypo and/or hyperthyroidism.

Hypothyroidism should be managed with individualized dose of TH guided by reduction of serum TSH concentration to normal (when TSH is increased) or markers of TH action (when TSH is within normal range) (11).

Parameters representative of peripheral TH action are for example: cholesterol, sex hormone-binding globulin (SHBG), angiotensin converting enzyme (ACE), carboxyterminal crosslinked telopeptide of type I collagen (ICTP), soluble interleukine-2 receptor (sIL-2r), and osteocalcin (4).

Those who, due to misdiagnosis, have undergone ablative therapy or present concomitant autoimmune disease, and as a consequence have limited thyroid reserve, will be managed as RTH presenting hypothyroidism.

In hyperthyroidism, first line is symptomatic treatment (11).

Atenolol is used to manage tachycardia and tremor, antianxiety drugs to alleviate nervousness.

Beta-blockers non-cardio selective should be avoided, because they inhibit peripheral T4 to T3 conversion, thus worsening of the hypothyroidism present in certain tissues.

Among agents with the potential to decrease TH through suppression of TSH, TRIAC, has been successful to reduce goiter size, and alleviate some symptoms, without a thyromimetic effect on peripheral tissues (compare to L-T3, TRIAC is eliminated faster and has high affinity for the β but not α TR) (11).

Goal of management is to maintain a normal serum TSH level and a eumetabolic state and offer appropriate genetic counseling and prenatal diagnosis. Inappropriate treatment of eumetabolic patients results in hypothyroidism and need for TH replacement (Figure 2).

Although, RTH has been associated to other kind of neoplasia that requires vigilance (thyroid neoplasia, hepatocellular carcinoma, renal cell carcinoma…), there is no increased incidence of thyrotropic adenoma. Our patient hasn't present any other neoplasia to the moment.

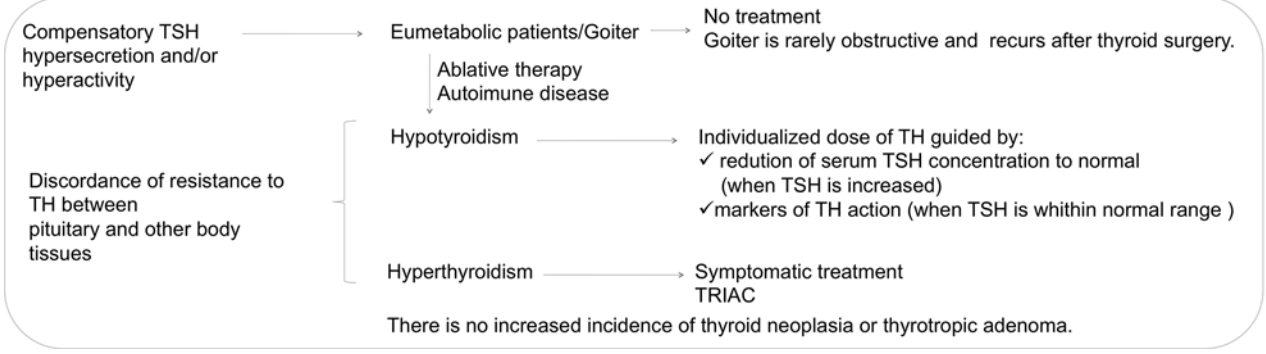

Figure 2. Current management strategies of resistance to thyroid hormone.

CONCLUSION

Reduced sensitivity to thyroid hormone (RSTH) is more common then formerly suspected (3).

Cell-specific thyroid hormone (TH) deprivation, sufficiency and excess can coexist in syndromes of RSTH.

Goiter and tachycardia are the most common reasons leading to testing and – ultimately – the diagnosis of RSTH (3).

Laboratory findings are very characteristic, almost pathognomonic, of RSTH (3,4,6).

Genetic analysis of the suspected individuals is a short cut to diagnosis, but absence of a mutation does not rule out the suspected defect, particularly when dealing with mosaicism and non-TR RTH (3,6). In such instances, a biochemical diagnosis should be secured by measuring the response to incremental doses of L-T4 and/or LT-3.

Inappropriate treatment in RSTH complicates the follow up and outcome.

Genetic factors, variability of tissue expression of iodothyronine cell membrane transports and intracellular enzymes could explain the lack of phenotype/genotype correlation (6).

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Metástase gigante de carcinoma papilífero

Giant metastasis of thyroid papillar carcinoma

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SUMÁRIO

O carcinoma papilífero da tireoide, o mais comum deste órgão, geralmente se apresenta como lesões parenquimatosas pequenas e, eventualmente, com metástases cervicais numerosas, raramente volumosas. É descrito um caso raro de uma paciente do gênero feminino, 44 anos, com um tumor cervical anterior, nodular e volumoso há nove anos. Após o tratamento cirúrgico, o anatomopatológico mostrou tratar-se de metástases linfonodais de carcinoma papilífero. O objetivo deste estudo é relatar um caso clínico de apresentação incomum de carcinoma papilífero da tireoide, de diagnóstico inicial difícil e apresentando-se com metástases linfonodais volumosas. Arq Bras Endocrinol Metab. 2014;58(9):967-9

SUMMARY

Papillary thyroid carcinoma, the most common type of thyroid cancer is usually presented as small parenchymatous lesions and, eventually, with cervical lymph node metastasis, rarely voluminous. Here we describe a rare case of a 44-year-old woman presenting a visible anterior cervical tumor, nodullary and voluminous, for nine years. After surgical treatment, the anatomical pathology sample revealed that the mass was composed of several cervical lymph node metastatic lesions of a papillary thyroid carcinoma. We report the discovery of an uncommon papillary thyroid carcinoma manifestation, with a difficult initial diagnosis and presenting voluminous lymph node metastases. Arq Bras Endocrinol Metab. 2014;58(9):967-9

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INTRODUÇÃO

O carcinoma papilífero da tireoide, a neoplasia mais comum deste órgão, abrange 75% (1) a 85% (2) dos 60.220 novos casos estimados de câncer de tireoide nos Estados Unidos em 2013. Desses tumores da tireoide, 75,24% ocorrem em mulheres (3), normalmente em jovens adultos. A idade média de diagnóstico do câncer de tireoide é de 54 em homens e 48 em mulheres (4). Em pacientes com câncer papilífero da tireoide, há alta incidência de metástases cervicais ao primeiro diagnóstico, dependendo não só do estadiamento como também de qual método é utilizado na pesquisa por potenciais metástases (5). Metástases para linfonodos cervicais ocorrem em 20% a 50% dos carcinomas papilíferos de tireoide no exame anatomopatológico de peças cirúrgicas (6) e raramente são volumosas. O objetivo do presente trabalho é relatar um caso de apresentação incomum de carcinoma papilífero da tireoide, de diagnóstico inicial difícil e apresentando-se com metástases linfonodais volumosas, atendido no Serviço de Cirurgia de Cabeça e Pescoço da Santa Casa de São Paulo.

RELATO DE CASO

Mulher, 44 anos, branca, solteira, natural e procedente de Santo André/SP, foi admitida no Pronto-Socorro municipal de Santo André devido à presença de tumor cervical que apresentou ulceração, sangramento e extravasamento de conteúdo amarelo (Figuras 1 e 2). Após cuidados iniciais, foi encaminhada ao Serviço de Cirurgia de Cabeça e Pescoço da Santa Casa de São Paulo. Queixava-se de nódulo cervical anterior há nove anos, inicialmente de crescimento rápido, permanecendo com volume estável nos últimos dois anos. Referia

ser o nódulo doloroso e incômodo no que diz respeito à mobilidade e estética. Há cinco anos procurou serviço médico, sendo sugerida a não intervenção cirúrgica. Paciente negava sintomas compressivos e apresentava hirsutismo desde os 20 anos de idade e sinais de virilizacão, comprovados ao exame clínico, frequência e volume menstrual irregulares e hipertensão arterial sistêmica controlada com uso de captopril 25 mg/dia.

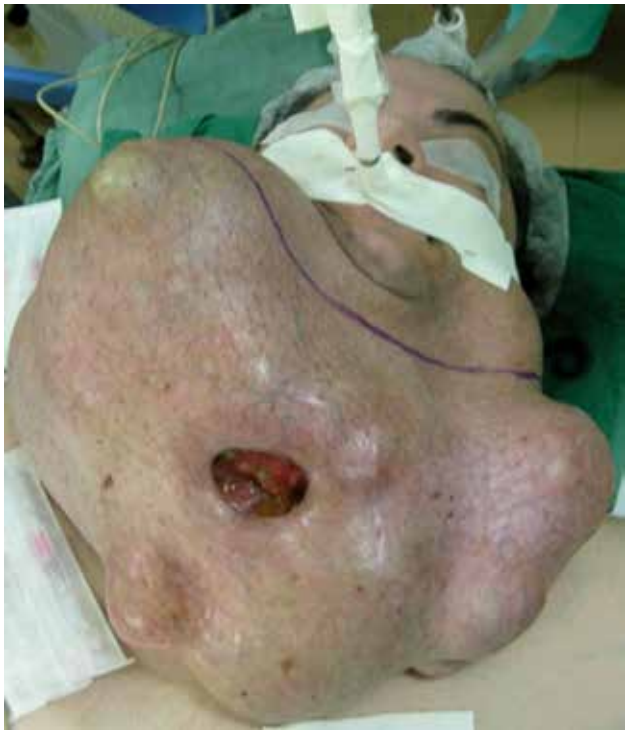

Figura 1. Apresentação do tumor ulcerado em visão frontal.

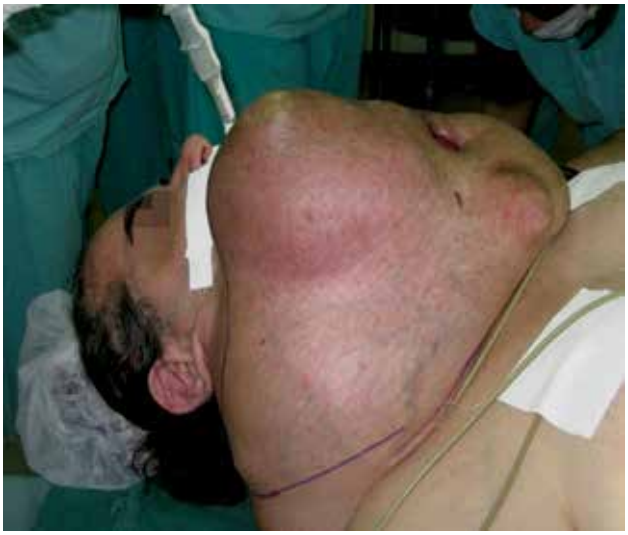

Figura 2. Apresentação do tumor em visão de perfil.

A orofaringolaringoscopia e a rinoscopia foram normais. No exame físico, a paciente era brevilínea, obesa, normotensa, eufônica, apresentava exame neurológico normal e não possuía alterações respiratórias. A dosagem de hormônios tireoidianos foi normal e a radiografia de tórax não apresentava alargamento mediastinal.

O diagnóstico inicial foi de bócio gigante, sendo indicado tratamento cirúrgico. A paciente foi submetida à cervicotomia exploradora com ressecção de grande área de pele que recobria a lesão. No intraoperatório, notou-se que a totalidade da lesão encontrava-se fora da loja tireoidiana, anteriormente aos músculos pré-tireoidianos, sendo ressecada sem intercorrências, exceto pela secção do músculo esternocleidomastóideo, com posterior ressutura, e ligadura das veias jugulares anteriores e externas. A peça cirúrgica pesou 7,2 kg e mediu 37 cm x 30 cm (Figura 3). Durante a cirurgia não foi localizado nenhum nódulo tireoidiano palpável, sendo encerrada sem a ressecção da glândula. O exame anatomopatológico de congelação não foi conclusivo, apesar de sugerir presença de tecido tireoidiano.

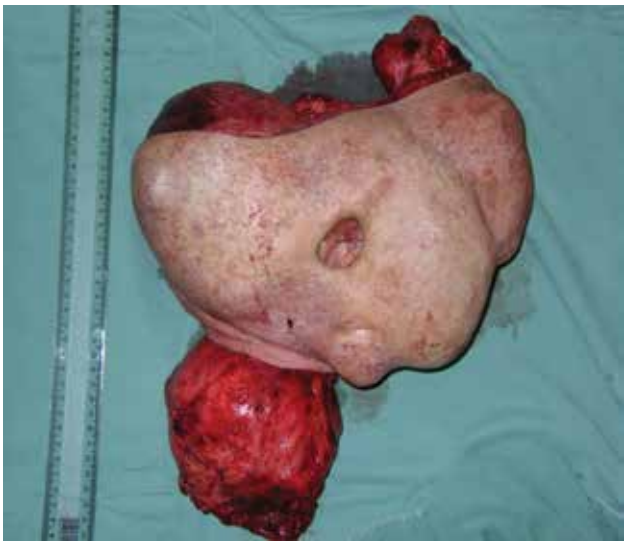

Figura 3. Peça cirúrgica de 7,2 kg, medindo 37 cm x 30 cm.

Paciente evoluiu sem complicações. O anatomopatológico mostrou tratar-se de metástases linfonodais de carcinoma papilífero. Indicada tireoidectomia total onde foram encontrados dois focos de carcinoma papilífero menores que 2 cm. No pós-operatório de três meses, observou-se a presença de linfonodos palpáveis na região cervical, sendo realizado novo esvaziamento à esquerda. Paciente evoluiu com depressão, necessitando de medicação específica, mas com bom controle hormonal e programação de dose de iodo 131. Após

sete meses da última cirurgia, paciente faleceu em casa, sem causas definidas.

DISCUSSÃO

O carcinoma papilífero costuma evoluir de forma benigna, apresentando bom prognóstico quando submetido a tratamento adequado e intervenção precoce. Mostramos um caso de apresentação atípica, primeiro pelo volume descomunal das metástases cervicais desproporcional à pequena agressividade local e a distância. Apesar de volumosos, os linfonodos metastáticos não invadiam as estruturas adjacentes e também não causavam sintomas compressivos esperados, exceto incômodo relativo pelo próprio volume e peso. Sem dúvida houve falha na interpretação diagnóstica inicial, quando acreditamos tratar-se de uma lesão própria da glândula. Ao rever os exames tomográficos (Figura 4), notamos a musculatura tão delgada que não podíamos individualizá-la, dificultando a percepção de uma glândula tireoide pouco alterada e a presença dos nódulos fora da loja tireoidiana. A necessidade de três cirurgias subsequentes para obter o controle macroscópico da neoplasia não parece ter trazido maiores prejuízos à paciente, assim como não estabelecemos uma relação direta do óbito da paciente, seja com a neoplasia ou com o processo terapêutico.

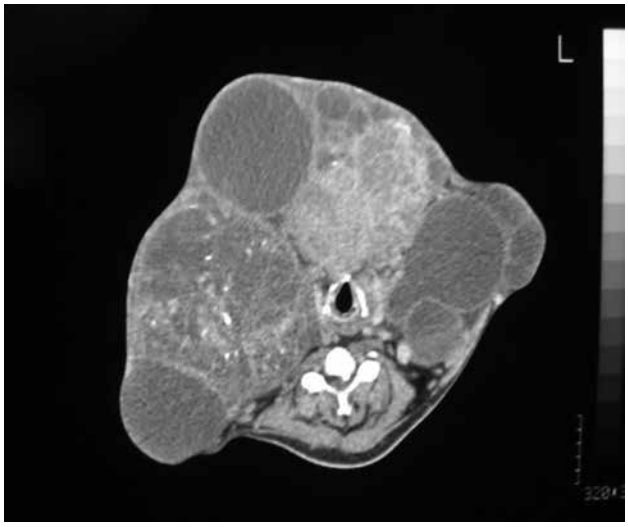

Figura 4. Imagem de tomografia computadorizada evidenciando nódulos fora da loja tireoidiana.

CONCLUSÃO

O caso apresentado representa uma evolução atípica do carcinoma papilífero da tireoide, tanto no que diz respeito à sua apresentação clínica quanto ao seu desfecho.

Declaração: os autores declaram não haver conflitos de interesse científico neste estudo.

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Angiotensin-II induced insulin resistance

Resistência à insulina induzida por angiotensina-II

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We read with great interest the article by Lima-Martínez and cols. (1). They studied the relationship between epicardial adipose tissue (EAT) thickness and plasma levels of adiponectin in Venezuelan patients. And they found a significant association between EAT thickness and both metabolic syndrome components and adiponectin concentration. The authors also reported a strong correlation between left ventricular mass and EAT thickness. The article has important messages. But there are some items to be clarified.

Angiotensin II (AII), the major hormone of the renin-angiotensin system, plays an important role in the pathogenesis of hypertension and atherosclerosis. Evidence has suggested that AII impairs insulin sensitivity (2). Hypertensive subjects and animal models have shown improvements in insulin resistance in response to treatment with angiotensin I converting enzyme (ACE) inhibitors or AII type 1 receptor (AT1R) blocker (3). The exact mechanisms for the AII-induced insulin resistance remain largely unknown. But Ran and cols. previously showed that long-term AII infusion decreased the circulating adiponectin concentration without affecting the gene expression in rats, and this may facilitate the development of insulin resistance. And AT1R blocker ameliorated the AII-induced hypoadiponectinemia (4).

Left ventricular hypertrophy (LVH) is well known to be associated with increased cardiac risk. Regression of LVH over a period of a few months has been reported with ACE inhibitors and angiotensin receptor blockers (ARBs) (5). Regression of LVH continues gradually over time (three years or more) and may be associated with complete reversal of LVH and other abnormalities induced by hypertension such as left atrial enlargement and diastolic dysfunction (5).

Lima-Martínez and cols. mentioned that 16 out of 27 patients in their series were on ACE inhibitor or ARB therapy (1). Considering the above mentioned data, antihypertensive therapy may have influenced left ventricular measurements and plasma levels of adiponectin. For these reasons, the authors have better mentioned this point as a limitation of the study.

Disclosure: no potential conflict of interest relevant to this article was reported.

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Response to the letter: Angiotensin-II induced insulin resistance

Resistência à insulina induzida por angiotensina-II

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We have examined with attention the comments of the letter to the Editor in regards to our recently published article (1), and want to thank its authors for their interest in our work.

Indeed, it has been demonstrated that, upon acting on the AT1 receptor, angiotensin II activates matrix metalloproteases that release the epidermal growth factor (EGF), binding to its receptor promotes the activation of mammalian target of rapamycin (mTOR) and ribosomal S6 kinase-1, both of which inhibit phosphatidylinositol 3-kinase insulin signaling, thus favoring insulin resistance (2-4). Interestingly, some clinical studies have demonstrated that treatment with either angiotensin I-converter enzyme inhibitors (ACEI) or angiotensin II receptor antagonist (ARA) reduces the incidence of *diabetes mellitus* in high risk patients (5,6).

Our study compared a group of metabolic syndrome (MS) patients (31 subjects) with a control group (27 subjects), and, as pointed out in the Results section, 13 out of 31 MS patients presented high blood pressure (41.9%), whereas none of the subjects in the control group were hypertensive (0%), not receiving therefore hypertension medication. As mentioned in the study, 10 of the 13 patients with high blood pressure and MS were treated with monotherapy (6 with ACEI and 4 with ARA), and the other three were on a combined therapy of ARA with calcium antagonist (2), and ACEI with a diuretic (1). Our study found that patients with MS revealed a greater thickness of epicardial adipose tissue (EAT) (5.69 ± 1.12 *vs* 3.52 ± 0.80 mm; p = 0.0001) in comparison with the subjects in the control group (1). As far as we know, no study has demonstrated that ARAs or ACEIs decrease EAT thickness, and although it is described that both antihypertensives drugs reduce insulin resistance, we observed that the study group had greater basal plasma insulin concentrations than those of the control group (6.08 ± 5.22 *vs.* 2.10 ± 0.54; p = 0.0001), and even greater triglyceryde/HDL-C ratio (4.52 ± 2.37 *vs.* 2.10 ± 1.15; p = 0.0001), which is considered a surrogate marker of insulin resistance (7).

Furthermore, a larger left ventricular mass (60.28 ± 14.00 *vs.* 53.01 ± 8.40; p = 0,019) and lower adiponectin plasma levels (11.20 ± 2.65 *vs.* 14.95 ± 3.87; p = 0,0001) were found in subjects with MS as compared to the control group. In agreement with the comments of the letter to the Editor, both ACEI and ARA have been shown to reduce left ventricular mass; however, it is worth noting that only subjects belonging to the MS group were receiving such therapy, whereby it can be asserted that this aspect had little incidence on the results. Furthering into the beneficial effects of ACEI and ARA, new analyses were carried out, and upon comparing the patients receiving these drugs (n = 13) with those MS patients receiving different medication or not receiving antihypertensives (n = 18), the levels of insulin (5.37 ± 4.03

vs. 6.58 ± 6.0; p = 0.507), left ventricular mass (63.72 ± 57.78; p = 0.252) and epicardial fat (5.37 *vs.* 5.92; p = 0.169) were not different; however, the levels of adiponectin were significantly higher in those MS patients being treated with ACEI and ARA (12.53 ± 2.93 *vs.* 10.23 ± 1.98; p = 0,02), though they continued to be lower than those subjects in the control group (12.53 ± 2.93 *vs.* 14.95 ± 3.87; p = 0.037). It is important to investigate the beneficial metabolic effects of this type of antihypertensive, using appropriate study designs. Since there were no high blood pressure subjects in our control group using hypertension medication, we consider that this possible effect of ACEI and ARA was not a limitation to achieve our objective.

Disclosure: no potential conflict of interest relevant to this article was reported.

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Red cell distribution width in subclinical hypothyroidism

Distribuição dos glóbulos vermelhos ampliada no hipotiroidismo subclínico

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We have read the article "The value of red blood cell distribution width (RDW) in subclinical hypothyroidism" by Hea Min Yu and cols. (1). They aimed to investigate the relationship between the subclinical hypothyroidism and RDW levels in a healthy population. They concluded that RDW levels were correlated with euthyroid and subclinical thyroid status.

This study gives important information on this clinically relevant condition. Thanks to the authors for their contribution. We think that some points should be discussed. Some markers have been found to be associated with early and late complications in many conditions. Inflammatory cytokines, high-sensitivity C-reactive protein (CRP), natriuretic peptides, neurohormones have recently established to be useful markers for diagnosis and prognosis in many diseases. However, these markers are very expensive and are not easily used in clinical practice. Elevated RDW is a measure of the variability in size of circulating erythrocytes and is expressed as the coefficient of variation of the erythrocyte volume. As several routine haematology instruments can analyse erythrocyte volume, RDW is available in most clinical settings. The ready availability of this parameter without additional cost may encourage its wider use in clinical practice.

Several studies have reported that elevated RDW levels are associated with poor prognosis in the setting of atherosclerosis, heart failure, stroke, peripheral arterial disease, older age (2). However, RDW may also reflect ethnicity, neurohumoral activation, renal dysfunction, hepatic dysfunction, nutritional deficiencies (i.e. iron, vitamin B_{12}, and folic acid), bone marrow dysfunction, inflammatory diseases, chronic or acute systemic inflammation (3) and use of some medications like antihypertensive therapy (4).

In addition, the authors used the formula developed and validated in the Modification of Diet in Renal Disease (MDRD) to estimate glomerular filtration rate (GFR). However, MDRD formula might measure higher GFR in younger age groups and lower GFR in older individuals compared to the Cockcroft-Gault equation (5). Although the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) recently published an equation for GFR estimate using the same variables (serum creatinine level, age, sex, and race) as the MDRD formula, the CKD-EPI equation more accurately categorized the individuals with respect to long-term clinical risk compared with the MDRD formula (6).

As a conclusion, we strongly believe that the findings obtained from the current study will lead to further studies examining the relationship between inflammation and subclinical hypothyroidism (7). Not only RDW but also mean platelet volume,

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neutrophil lymphocyte ratio, CRP and uric acid are easy methods to evaluate the inflammation in patients with subclinical hypothyroidism (8). These markers might be useful in clinical practice (9). Finally, it would be better if the authors might define how much time between blood collection and arrival to the laboratory they specified on measuring RDW levels, because of the delaying blood sampling can cause abnormal results in RDW measurements.

Disclosure: no potential conflict of interest relevant to this article was reported.

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Response to the letter: Red cell distribution width in subclinical hypothyroidism

Distribuição dos glóbulos vermelhos ampliada no hipotiroidismo subclínico

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We read the paper by Sevket BALTA and cols. and thanks for your interest in our study entitled "Red Cell Distribution Width in Subclinical Hypothyroidism" (1). As we mentioned in the discussion part, the RDW can be affected by some disease conditions such as recent blood transfusion, renal dysfunction, hepatic dysfunction, anemia related nutritional deficiencies (i.e. iron, vitamin B_{12}, and folic acid), bone marrow dysfunction, inflammatory diseases, chronic or acute systemic inflammation (2-5) and some medications (6) is already well known. And this point could be one of the major limitations of our paper. So, we tried to gather other multiple potential confounding factors as much as possible and to adjust confounding factors. And the reason what the participants comprised healthy subjects with no known systemic diseases and who were not taking any medication that may affect thyroid function, and were not pregnant or within the first year of the postpartum period is to rule out the confounding factors. Because of this aspect your paper pointed out, in fact, more investigations and prospective studies are needed to clarify the relations between RDW and subclinical hypothyroidism before application in the clinical field.

And we, authors used the formula developed and validated in the Modification of Diet in Renal Disease (MDRD) to estimate glomerular filtration rate (GFR). As your paper mentioned, according to the current trends, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation replace the Modification of Diet in Renal Disease (MDRD) Study equation (7-9). We all agree with your this opinion and can afford to accept your advice. Although we used the MDRD study equation in our paper as usual, in the further study we would plan to use the CKD-EPI equation instead.

When it comes to the additional final question, considering laboratory system of our hospital, we can ensure that analysis of blood sample is not delayed enough to cause abnormal results in RDW measurements.

In addition, because we also have confidence that relationship between inflammatory factors and subclinical hypothyroidism is exist (4,10,11), we guess that the studies deal with inflammatory factors such as RDW, mean platelet volume, neutrophil lymphocyte ratio, CRP, and so on, would have value and should be keep on investigating.

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PTPN2, a potential therapeutic target for type 1 diabetes?

PTPN2 é um alvo terapêutico potencial no diabetes tipo 1?

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We read with great interest the article by Rheinheimer and cols. (1), showing that four hundred and eighty six patients with type 1 diabetes (T1D) and 484 non-diabetic subjects were conducted to discuss the rs1893217 (T/C) polymorphism in protein tyrosine phosphatase, non-receptor type 2 gene (PTPN2) gene for T1D from Southern Brazil, by which the C allele was observed in 14.5% of the T1D patients and 12.2% of the non-diabetic subjects (P = 0.152). Moreover, the frequencies of this variant did not differ markedly between T1D patients and non-diabetic subjects when assuming recessive (T/C + T/T *versus* C/C), dominant (T/T *versus* T/C + C/C), or additive (C/C *versus* T/T) model. The clinical and laboratory characteristics of T1D patients did not differ markedly among the three genotypes of the rs1893217 polymorphism. These findings suggest that PTPN2 gene polymorphism may not correlate with T1D. However, Espino-Paisan and cols. (2) genotyped 439 T1D Spanish subjects and 861 controls for PTPN2 rs2542151 and rs478582, showing that the frequency of rs2542151 G carriers was significantly higher in the early-onset patients compared with late-onset patients (P = 0.023) and with controls (P = 0.005), while the analysis of rs478582 did not reach statistical significance.

Type 1 diabetes is an inflammatory disease of the pancreatic islet, where insulin producing β-cells are preferentially destroyed to varying degrees by the concerted action of autoreactive T-cells and monocytic cells. Single nucleotide polymorphism in PTPN2 encodes T cell protein tyrosine phosphatase (TCPTP). TCPTP can attenuate T cell activation and proliferation *in vitro* and blunt antigen-induced responses *in vivo*, where T cell-specific TCPTP-deficient mice lowered the *in vivo* threshold for TCR-dependent CD8(+) T cell proliferation (3). Consistently, TCPTP-deficient mice developed widespread inflammation and autoimmunity that was transferable to wild-type recipient mice by CD8(+) T cells alone. This autoimmunity was related to increased serum levels of pro-inflammatory cytokines and anti-nuclear antibodies, T cell infiltrates in non-lymphoid tissues, and liver disease (3). PTPN2 mRNA and protein are expressed in human islets and rat beta-cells and increased by cytokines (4). Transfection with PTPN2 siRNAs inhibited basal- and cytokine-induced PTPN2 expression in rat beta-cells and dispersed human islets cells. Decreased PTPN2 expression exacerbated interleukin (IL)-1beta + interferon (IFN)-gamma-induced beta-cell apoptosis and turned IFN-gamma alone into a proapoptotic signal (4). Similarly, PTPN2 knockdown exacerbated type I IFN-induced apoptosis in INS-1E, primary rat, and human beta cells. PTPN2 silencing and exposure to type I and II IFNs induced BAX translocation to the mitochondria, cytochrome c release, and caspase 3 activation (5).

Collectively, available evidence suggests that PTPN2 may play a potential role in the pathogenesis of T1D, and may give therapeutic potential for T1D. However, further studies are still needed to clarify the role of PTPN2 in T1D, either the genetic susceptibility of PTPN2 in different populations, or the immunologic mechanisms played in T1D. Therefore, with more studies about PTPN2 in T1D, the clear mechanisms of PTPN2 played in T1D may be addressed in the future.

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PTPN2 gene polymorphisms are associated with type 1 *diabetes mellitus* in Brazilian subjects?

Polimorfismos no gene PTPN2 *estão associados com diabetes melito em brasileiros?*

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We appreciated the correspondence by Liu and cols. that was sent to this journal regarding our manuscript "The rs1893217 (T/C) polymorphism in PTPN2 gene is not associated with type 1 *diabetes mellitus* in subjects from Southern Brazil" (1).

We agree with Liu and cols. regarding the potential role of PTPN2 in the pathogenesis of type 1 *diabetes mellitus* (T1DM), which has been reinforced by several experimental studies (2). However, our study found no evidence of a significant association between the *PTPN2* rs1893217 (T/C) polymorphism and T1DM risk (1), while Espino-Paisan and cols. (3) reported that in subjects from Spain the minor allele of the *PTPN2* rs2542151 polymorphism was more prevalent in early-onset T1DM patients (age of onset < 16 years) as compared to late-onset patients and non-diabetic controls. No significant difference was found between control and T1DM late-onset groups. Of note, the rs2542151 polymorphism is in strong linkage disequilibrium with the rs1893217 polymorphism in subjects of European descent (4). One possible explanation for these discordant results is that Espino-Paisan and cols. (3) only observed an association between the rs2542151 polymorphism and early-onset T1DM. Thus, our results are in agreement with their data regarding an absence of association with late-onset T1DM. It is worth noting that in our sample the mean age of T1DM onset was 17.3 ± 10.1 years (1), more similar to that of their late-onset group. Another possible explanation is different genetic backgrounds and environmental risk factors between Brazilian (1) and Spanish populations (3). It is well known that genetically different individuals exposed to varied environmental factors will have different pathways leading to β-cell loss and, consequently, disease onset and evolution (2).

Moreover, *Steck and cols.* (5) reported that the *PTPN2* rs1893217 polymorphism seems to predict islet autoimmunity (hazard ratio = 1.42, 95% CI 1.02-1.99) but not T1DM development, after controlling for family history of T1DM and HLA high-risk genotypes. The absence of an association with T1DM is in agreement with our reported data (1). Unfortunately, we did not analyze HLA-high risk genotypes in our population to know if this genetic background would modify the association between the rs1893217 polymorphism and T1DM in different subgroups. Interactions with non-HLA genes also might influence the effects of the rs1893217 polymorphism on T1DM susceptibility.

Therefore, we agree that available evidence suggests that PTPN2 may play a potential role in the pathogenesis of T1DM, and may have a therapeutic potential for this disease. However, different therapeutic strategies might be required depending on the genetic background of the affected individuals. Furthermore, multicenter studies with larger sample numbers, and controlling for HLA-high risk genotypes and

presence of autoantibodies are necessary to define the role of *PTPN2* polymorphisms in the Brazilian population as well as in other populations. Prospective studies following children since the development of islet autoimmunity to progression to T1DM are also needed as they may offer further insights regarding the association of the *PTPN2* rs1893217 polymorphism and T1DM.

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3. Nome completo, endereço postal, e-mail, telefone e fax do autor para correspondência.
4. Título abreviado de, no máximo, 40 caracteres para títulos de página.
5. Palavras-chave (recomenda-se usar *MeSH terms* e até 5).
6. Número de palavras – excluindo a página de rosto, resumo, referências, figuras e tabelas.
7. Tipo do manuscrito.

Resumos

Todos os artigos originais, comunicados rápidos e relatos de casos deverão ser apresentados com resumos de, no máximo, 250 palavras. O resumo deve conter informações claras e objetivas sobre o estudo de modo que possa ser compreendido, sem consulta ao texto. O resumo deve incluir quatro seções que refletem os títulos das seções do texto principal. Todas as informações relatadas no resumo devem possuir origem no MS. Recomenda-se o uso de frases completas para todas as seções do resumo.

Introdução

O propósito da introdução é estimular o interesse do leitor para o trabalho em questão com uma perspectiva histórica e justificando seus objetivos.

Materiais e Métodos

Deve ser descrito em detalhe como o estudo foi conduzido de forma que outros investigadores possam avaliar e reproduzir o trabalho. A origem dos hormônios, produtos químicos incomuns, reagentes e aparelhos deve ser indicada. Para os métodos modificados, apenas as novas modificações devem ser descritas.

Resultados e Discussão

A seção Resultados deve apresentar brevemente os dados experimentais tanto no texto quanto por tabelas e/ou figuras. Deve-se evitar a repetição no texto dos resultados apresentados nas tabelas. Para mais detalhes sobre a preparação de tabelas e figuras, veja a seguir. A Discussão deve focar na interpretação e no significado dos resultados, com comentários objetivos, concisos, que descrevam sua relação com outras pesquisas nessa área.

Na Discussão, deve-se evitar a repetição dos dados apresentados em Resultados, pode-se conter sugestões para explicá-los e deve-se terminar com as conclusões.

Autoria
Os **ABE&M** adotam as diretrizes de autoria e de contribuição definidas pelo Comitê Internacional de Editores de Periódicos Médicos (www.ICMJE.org). A coautoria irrestrita é permitida. O crédito de autoria deve ser baseado apenas em contribuições substanciais para:

1. concepção e desenho, análise ou interpretação de dados;
2. redação do artigo ou revisão crítica do conteúdo intelectual;
3. aprovação final da versão a ser publicada.

Todas essas condições devem ser respeitadas. O primeiro autor é responsável por garantir que todos os autores contribuíram para a realização do MS e concordaram com seu conteúdo e sua submissão aos **ABE&M**.

Conflito de Interesses
Uma declaração de conflito de interesse para todos os autores deve ser incluída no documento principal, seguindo o texto, na seção Agradecimentos. Mesmo que os autores não tenham conflito de interesse relevante a divulgar, devem relatar na seção Agradecimentos.

Agradecimentos
A seção Agradecimentos deve incluir os nomes das pessoas que contribuíram para o estudo, mas não atendem aos requisitos de autoria. Os autores são responsáveis por informar, a cada pessoa listada na seção de agradecimentos, sua inclusão e qual sua contribuição. Cada pessoa listada nos agradecimentos deve dar permissão – por escrito, se possível – para o uso de seu nome. É da responsabilidade dos autores coletar essas informações.

Referências
As referências da literatura devem estar em ordem numérica (entre parênteses), de acordo com a citação no texto, e listadas na mesma ordem numérica no final do manuscrito, em uma página separada. Os autores são responsáveis pela exatidão das referências. O número de referências citadas deve ser limitado, como indicado anteriormente, para cada categoria de apresentação.

Tabelas
As tabelas devem ser apresentadas no mesmo formato que o artigo (Word). Atenção: não serão aceitas tabelas com arquivos em Excel. As tabelas devem ser autoexplicativas e os dados não devem ser repetidos no texto ou em figuras e conter as análises estatísticas. As tabelas devem ser construídas de forma simples e serem compreensíveis sem necessidade de referência ao texto. Cada tabela deve ter um título conciso. Uma descrição das condições experimentais pode aparecer em conjunto como nota de rodapé.

Gráficos e Figuras
Todos os gráficos ou figuras devem ser numerados. Os autores são responsáveis pela formatação digital, fornecendo material adequadamente dimensionado. Todas as figuras coloridas serão reproduzidas igualmente em cores na edição *online* da revista, sem nenhum custo para os autores. Os autores serão convidados a pagar o custo da reprodução de figuras em cores na revista impressa. Após a aceitação do manuscrito, a editora fornecerá o valor dos custos de impressão.

Fotografias
Os **ABE&M** preferem publicar fotos de pacientes sem máscara. Encorajamos os autores a obter com os pacientes ou seus familiares, antes da submissão do MS, permissão para eventual publicação de imagens. Se o MS contiver imagens identificáveis do paciente ou informações de saúde protegidas, os autores devem enviar autorização documentada do próprio paciente, ou pais, tutor ou representante legal, antes de o material ser distribuído entre os editores, revisores e outros funcionários dos **ABE&M**. Para identificar indivíduos, utilizar uma designação numérica (por exemplo, Paciente 1); não utilizar as iniciais do nome.

Unidades de Medida
Os resultados devem ser expressos utilizando o Sistema Métrico. A temperatura deve ser expressa em graus Celsius e tempo do dia, usando o relógio de 24 horas (por exemplo, 0800 h, 1500 h).

Abreviaturas Padrão
Todas as abreviaturas no texto devem ser definidas imediatamente após a primeira utilização da abreviatura.

Pacientes
Para que o MS seja aceito para submissão, todos os procedimentos descritos no estudo devem ter sido realizados em conformidade com as diretrizes da Declaração de Helsinque e devem ter sido formalmente aprovados pelos comitês de revisão institucionais apropriados, ou seu equivalente.

As características das populações envolvidas no estudo devem ser detalhadamente descritas. Os indivíduos participantes devem ser identificados apenas por números ou letras, nunca por iniciais ou nomes. Fotografias de rostos de pacientes só devem ser incluídas se forem cientificamente relevantes. Os autores devem obter o termo de consentimento por escrito do paciente para o uso de tais fotografias. Para mais detalhes, consulte as Diretrizes Éticas.

Os pesquisadores devem divulgar aos participantes do estudo potenciais conflitos de interesse e devem indicar que houve essa comunicação no MS.

Animais de Experimentação
Deve ser incluída uma declaração confirmando que toda a experimentação descrita no MS foi realizada de acordo com padrões aceitos de cuidado animal, como descrito nas Diretrizes Éticas.

Descrição Genética Molecular
Usar terminologia padrão para as variantes polimórficas, fornecendo os números de rs para todas as variantes relatadas. Detalhes do ensaio, como, por exemplo, as sequências de iniciadores de PCR, devem ser descritos resumidamente com os números rs. Os heredogramas devem ser elaborados de acordo com as normas publicadas em Bennett et al. J Genet Counsel. 2008.17:424-33. DOI 10.1007/s10897-008-9169-9.

Nomenclaturas
Para genes, use a notação genética e símbolos aprovados pelo Comitê de Nomenclatura HUGO Gene (HGNC) – (http://www.genenames.org/~~V).

Para mutações, siga as diretrizes de nomenclatura sugeridas pela Sociedade Human Genome Variation (http://www.hgvs.org/mutnomen/).

- Fornecer e discutir os dados do equilíbrio Hardy-Weinberg dos polimorfismos analisados na população estudada. O cálculo do equilíbrio de Hardy-Weinberg pode ajudar na descoberta de erros de genotipagem e do seu impacto nos métodos analíticos.
- Fornecer as frequências originais dos genótipos, dos alelos e dos haplótipos.
- Sempre que possível, o nome genérico das drogas deve ser referido. Quando um nome comercial de propriedade é usado, ele deve começar com letra maiúscula.
- Siglas devem ser usadas com moderação e totalmente explicadas quando usadas pela primeira vez.

TRABALHOS APRESENTADOS EM INGLÊS

O MS deve ser escrito em inglês claro e conciso. Evite jargões e neologismos. A revista não está preparada para realizar grandes correções de linguagem, o que é de responsabilidade do autor. Se o inglês não é a primeira língua dos autores, o MS deve ser revisado por um especialista em língua inglesa ou um nativo. Para os não nativos da língua inglesa e autores internacionais que gostariam de assistência com sua escrita antes da apresentação, sugerimos o serviço de edição científica do *American Journal Experts* (http://www.journalexperts.com/index.php) ou o *PaperCheck* (http://www.papercheck.com/).

Instructions for authors

General Information

We emphasize the importance of following these instructions carefully. Failure to do so will delay the processing of your manuscript.

Manuscripts should be submitted solely to **ABE&M** and may not have been published, or be under consideration for publication, in any substantial form in another periodical – rofessional or lay.

Manuscripts must be written in idiomatic English or Portuguese and conform to the specifications described below. If authors are not fluent in written medical and scientific English, they are strongly encouraged to have their manuscripts reviewed and edited by an expert English writer prior to submission. This will increase the chances that the paper will be accepted and will speed the publication of those manuscripts that are accepted.

Papers that do not meet these requirements will be returned to the author for necessary revision before formal review.

All submissions are initially evaluated in depth by the scientific editors. Papers that do not conform to the general criteria for publication will be returned to the authors without detailed review, typically within three to five days. Otherwise, manuscripts will be sent to reviewers (most commonly two).

Manuscript Categories

Reports of original research may be submitted to **ABE&M** as an Original Article or Brief Report. Other special categories of manuscripts are described below. All manuscripts must adhere to the word count limitations, as specified below, for text only; the word count does not include the abstract, references, or figure/table legends. The word count must be noted on the title page, along with the number of figures and tables. The format is similar for all manuscript categories and it is described in detail in "Manuscript Preparation" section.

Original Articles

Original Article is a scientific report of the results of original research that has not been published or submitted for publication elsewhere (either in print or electronically). Represent a substantial body of laboratory or clinical work. In general, original paper should not exceed 3,600 words in the main text, include no more than six figures and tables and 35 references.

Review Articles

The **ABE&M** publishes review articles which present a balanced perspective on timely issues within the field of clinical endocrinology. All reviews are submitted upon invitation and are subject to peer review. Articles in this category are requested by the Editors to authors with proven experience in the field. Authors considering the submission of uninvited reviews should contact the editors in advance to determine whether the topic that they propose is of current potential interest to the Journal. These manuscripts should be no longer than 4,000 words in the main text, include no more than four figures and tables and 60 references. The author should mention the source and/or request authorization for use of previously published figures or tables.

Consensus Statements

Consensus Statements related to the endocrine and metabolic health standards and healthcare practices may be **submitted by professional societies, task forces, and other consortia.** All such submissions will be subjected to peer review, must be modifiable in response to criticisms, and will be published only if they meet the Journal's usual editorial standards. These manuscripts should typically be no longer than 3,600 words in the main text, include no more than six figures and tables and 60 references.

Brief Report

Brief report should consist of new data of sufficient importance to warrant immediate publication. It is a succinct description of focused study with important, but very straightforward, negative or confirmatory results. Brevity and clarity are always likely to enhance the chance of a manuscript being accepted for publication. A maximum of 1,500 words in the main text plus up to 20 references and normally no more than two illustrations (tables or figures or one of each).

Case Report

A brief communication presenting collected case reports, or single case reports of clinical or scientific significance. These reports should be concise and focused. They should address observations of patients or families **that add substantially to the knowledge of the etiology, pathogenesis and delineation of the natural history or management of the condition described**. Brevity and clarity are always likely to enhance the chance of a manuscript being accepted for publication. These manuscripts should be 2,000 words or less, with no more than four figures and tables and 30 references.

Letter

Letters to the Editor may be submitted in response to work that has been published in the Journal. Letters should be short commentaries related to specific points of agreement or disagreement with the published work. Letters are not intended for presentation of original data unrelated to a published article. Letters should be no longer than 500 words with no more than five complete references, and may not include any figures or tables.

Manuscript Preparation

General Format

The Journal requires that all manuscripts be submitted in a single-column format that follows these guidelines:

- The manuscript must be submitted in MS-Word format.
- All text should be double-spaced with 2 cm margins on both sides using 11-point type in Times Roman or Arial font.
- All lines should be numbered throughout the entire manuscript and the entire document should be paginated.
- All tables and figures must be placed after the text and must be labeled. Submitted papers must be complete, including the title page, abstract, figures, and tables. Papers submitted without all of these components will be placed on hold until the manuscript is complete.

All submissions must include:

- A cover letter requesting that the manuscript be evaluated for publication in **ABE&M** and any information relevant to your manuscript. Elsewhere on the submission form authors may suggest up to three specific reviewers and/or request the exclusion of up to three others.

The manuscript must be presented in the following order:

1. Title page.
2. Structured Abstract (or summary for case reports).
3. Main text.
4. Tables and Figures. They must be cited in the main text in numerical order.
5. Acknowledgments.
6. Funding statement, competing interests and any grants or fellowships supporting the writing of the paper.
7. Reference list.

Title Page

The title page must contain the following information:

1. Title of the article (a concise statement of the article's major contents).
2. Full names, departments, institutions, city and country of all co-authors.
3. Full name, postal address, e-mail, telephone and fax numbers of the corresponding author.
4. Abbreviated title of not more than 40 characters for page headings.
5. Up to five keywords or phrases suitable for use in an index (it is recommended to use MeSH terms).
6. Word count – excluding title page, abstract, references, figures and tables.
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All Original Articles, Brief Reports, Reviews, Case Reports should be submitted with structured abstracts of no more than 250 words. The abstract must be self-contained and clear without reference to the text and should be written for a general journal readership. The abstract format should include four sections that reflect the section headings in the main text. All information reported in the abstract must appear in themanuscript. Please use complete sentences for all sections of the abstract.

Introduction

The article should begin with a brief introductory statement that places the work to follow in historical perspective and explains its intent and significance.

Materials and Methods

These should be described and referenced in sufficient detail for other investigators to repeat the work. The source of hormones, unusual chemicals and reagents, and special pieces of apparatus should be stated. For modified methods, only the modifications need be described.

Results and Discussion

The Results section should briefly present the experimental data in text, tables, and/or figures. For details on preparation of tables and figures, see below. The Discussion should focus on the interpretation and significance of the findings with concise objective comments that describe their relation to other work in that area. The Discussion should not reiterate the Results.

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1. conception and design, or analysis and interpretation of data.
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All these conditions must all be met. The corresponding author is responsible for ensuring that all appropriate contributors are listed as authors and that all authors have agreed to the manuscript's content and its submission to **ABE&M**.

Conflict of interest
A conflict of interest statement for all authors must be included in the main document, following the text, in the Acknowledgments section. If authors have no relevant conflict of interest to disclose, it should be indicated in the Acknowledgments section.

Acknowledgments
The Acknowledgments section should include the names of those people who contributed to a study but did not meet the requirements for authorship. The corresponding author is responsible for informing each person listed in the acknowledgment section that they have been included and providing them with a description of their contribution so they know the activity for which they are considered responsible. Each person listed in the acknowledgments must give permission – in writing, if possible – for the use of his or her name. It is the responsibility of the corresponding author to collect this information.

References
References to the literature should be cited in numerical order (in parentheses) in the text and listed in the same numerical order at the end of the manuscript on a separate page or pages. The author is responsible for the accuracy of references. The number of references cited should be limited, as indicated above for each category of submission.

Tables
Tables should be submitted in the same format as your article (Word) and not another format. Please note: we cannot accept tables as Excel files within the manuscript. Tables should be self-explanatory and the data they contain must not be duplicated in the text or figures. Tables must be constructed as simply as possible and be intelligible without reference to the text. Each table must have a concise heading. A description of experimental conditions may appear together with footnotes at the foot of the table. Tables must not simply duplicate the text or figures.

Figures and Legends
All figures must display the figure number. Sizing the figure: The author is responsible for providing digital art that has been properly sized, cropped, and has adequate space between images. All color figures will be reproduced in full color in the online edition of the journal at no cost to the authors. Authors are requested to pay the cost of reproducing color figures in print (upon acceptance of the manuscript, the publisher will provide price quotes).

Photographs
The **ABE&M** strongly prefers to publish unmasked patient photos. We encourage all prospective authors to work with families prior to submission to address the issue of permission for review and possible publication of patient images. If your submission contains ANY identifiable patient images or other protected health information, you MUST provide documented permission from the patient (or the patient's parent, guardian, or legal representative) before the specific material will be circulated among editors, reviewers and staff for the purpose of possible publication in **ABE&M**. If it is necessary to identify an individual, use a numerical designation (e.g. Patient 1) rather than using any other identifying notations such as initials.

Units of Measure
Results should be expressed in metric units. Temperature should be expressed in degrees Celsius and time of day using the 24-hour clock (e.g., 0800 h, 1500 h).

Standard Abbreviations
All abbreviations in the text must be defined immediately after the first use of the abbreviation.

Experimental Subjects
To be considered, all clinical investigations described in submitted manuscripts must have been conducted in accordance with the guidelines in The Declaration of Helsinki and must have been formally approved by the appropriate institutional review committees or its equivalent.

The study populations should be described in detail. Subjects must be identified only by number or letter, not by initials or names. Photographs of patients' faces should be included only if scientifically relevant. Authors must obtain written consent from the patient for use of such photographs. For further details, see the Ethical Guidelines.

Investigators must disclose potential conflict of interest to study participants and should indicate in the manuscript that they have done so.

Experimental Animals
A statement confirming that all animal experimentation described in the submitted manuscript was conducted in accord with accepted standards of humane animal care, as outlined in the Ethical Guidelines, should be included in the manuscript.

Molecular Genetic Description
- Use standard terminology for variants, providing rs numbers for all variants reported. These can be easily derived for novel variants uncovered by the study. Where rs numbers are provided, the details of the assay (primer sequences, PCR conditions, etc.) should be described very concisely.
- Pedigrees should be drawn according to published standards (See Bennett *et al.* J Genet Counsel. 2008;17:424-33. DOI 10.1007/s10897-008-9169-9).

Nomenclatures
- For genes, use genetic notation and symbols approved by the HUGO Gene Nomenclature Committee (HGNC) – (http://www.genenames.org/).
- For mutation nomeclature please use the nomenclature guidelines suggested by the Human Genome Variation Society (http://www.hgvs.org/mutnomen/).
- Provide information and a discussion of departures from Hardy-Weinberg equilibrium (HWE). The calculation of HWE may help uncover genotyping errors and impact on downstream analytical methods that assume HWE.
- Provide raw genotype frequencies in addition to allele frequencies. It is also desirable to provide haplotype frequencies.
- Whenever possible, drugs should be given their approved generic name. Where a proprietary (brand) name is used, it should begin with a capital letter.
- Acronyms should be used sparingly and fully explained when first used.

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Papers must be written in clear, concise English. Avoid jargon and neologisms. The journal is not prepared to undertake major correction of language, which is the responsibility of the author. Where English is not the first language of the authors, the paper must be checked by a native Engligh speaker. For non-native English speakers and international authors who would like assistance with their writing before submission, we suggest American Journal Experts for their scientific editing service (http://www.journalexperts.com/index.php) or PaperCheck (http://www.papercheck.com/).

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Eficácia no controle glicêmico com benefício adicional de perda de peso[1]

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CONTRAINDICAÇÕES: Pacientes com conhecida hipersensibilidade a dapagliflozina ou aos outros componentes da fórmula. **INTERAÇÕES MEDICAMENTOSAS:** A farmacocinética da dapagliflozina não foi alterada pela metformina, pioglitazona, sitagliptina, glimepirida, hidroclorotiazida, valsartana ou sinvastatina.

Referências bibliográficas: **1.** Forxiga® (dapagliflozina) comprimidos [bula do medicamento]. São Paulo, SP. Bristol-Myers Squibb Farmacêutica S.A.; 2014. **2.** Wright EM. Renal Na(+)-glucose cotransporters. Am J Physiol Renal Physiol. 2001;280(1):F10-F18. **3.** Lee YJ, Lee YJ, Han HJ. Regulatory mechanisms of Na(+)/glucose cotransporters in renal proximal tubule cells. Kidney Int Suppl. 2007;(106):S27-S35. **4.** Hummel CS, Lu C, Loo DD, Hirayama BA, Voss AA, Wright EM. Glucose transport by human renal Na+/D-glucose cotransporters SGLT1 and SGLT2. Am J Physiol Cell Physiol. 2011;300(1):C14-21. **5.** Resolução - RE No 2. 234, de 28 de Junho de 2013. Dapagliflozina: registro de medicamento novo. Diário Oficial da União 2013;124 (Supl):pp 18. **6.** Abdul-Ghani MA, DeFronzo RA. Dapagliflozin for the treatment of type 2 diabetes. Expert Opin Pharmacother. 2013;14(12):1695-1703.

Material destinado ao profissional de saúde.

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