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editorials

original articles

case reports

FAMÍLIA NESINA® TAKEDA

Completa no cuidado do DM2[1*]

Nesina®
alogliptina

APRESENTAÇÕES:
12,5 mg; 25 mg[10]
POSOLOGIA:
1 comprimido 1x/dia[10]

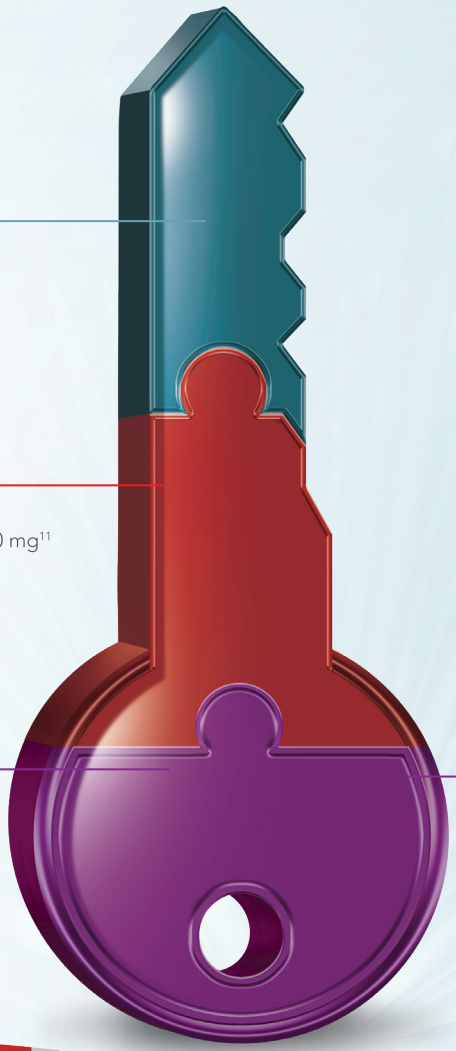
Nesina Met*
alogliptina+cloridrato de metformina

APRESENTAÇÕES:
12,5 mg + 850 mg; 12,5 mg + 1.000 mg[11]
POSOLOGIA:
1 comprimido 2x/dia[11]

Nesina Pio*
alogliptina+pioglitazona

LANÇAMENTO

APRESENTAÇÕES:
25 mg + 15 mg; 25 mg + 30 mg[12]
POSOLOGIA:
1 comprimido 1x/dia[12]



- **Maior SELETIVIDADE** vs. outros iDPP-4[2]
- **EFICÁCIA: o único inibidor da DPP-4** que demonstrou superioridade do controle **glicêmico vs. sulfonilureia** em 2 anos**[3-7]
- **SEGURANÇA: o único inibidor da DPP-4** com segurança cardiovascular comprovada em pacientes diabéticos Tipo 2 que apresentaram Síndrome Coronariana Aguda RECENTE[8,9]

- **Mecanismo de ação complementar:**
  MELHORA a função das células beta[13]
  MELHORA a resistência insulínica[13]
  RETARDA o uso da terapia insulínica[13]
- **REDUÇÃO rápida e potente** da HbA1c[14***]
- **EFICÁCIA em terapia de combinação** inicial ou em adição a metformina[12-16****]

*Considerando que o portfólio Takeda Diabetes oferece 3 medicamentos para ao tratamento do DM2: Nesina, Nesina Met e Nesina Pio, que atendem o tratamento com monoterapia, terapia dupla ou tripla de acordo com as diretrizes de tratamento do DM2 AACE/ACE. ** Glipizida. ***Comparada à terapia de pioglitazona + metformina após 52 semanas. **** Terapia combinada como 2ª ou 3ª linha de tratamento.

Referências bibliográficas: 1. Garber AJ, et al. Consensus statement by the american association of clinical endocrinologists and american college of endocrinology on the comprehensive type 2 diabetes management algorithm – 2017 executive summary. Endocrine Practice.2017. 23(2):207-238. **2.** Capuano A, et al. Dipeptidyl peptiades-4 inhibitors in type 2 diabetes therapy – Focus on alogliptin. Drugs Des Devel Ther 2013; 989- 1001. **3.** Del Prato S, et al. Durability of the efficacy and safety of alogliptin compared with glipizide in type 2 diabetes mellitus: a 2-year study. Diabetes Obes Metab. 2014;16(12):1239-46. **4.** Seck T, et al; Sitagliptin Study 024 Grou. Safety and efficacy of treatment with sitagliptin or glipizide in patients with type 2 diabetes inadequately controlled on metformin: a 2-year study. Int J Clin Pract. 2010;64(5):562-76. **5.** Goke B, Galwitz B, Eriksson JG, et al. Saxagliptin vs. glipizide as add-on therapy in patients with type 2 diabetes mellitus inadequately controlled controlled on metformin alone: long-term (52-week) extension of a 52- week randomised controlled trial. Int J clin Pract. 2013 Apr;67(4): 307-16. **6.** Galwitz B, et-al. 2-years efficacy and safety of linagliptin compared with glimepired in patients with type 2 diabetes inadequately controlled on metformin: a randomised, double-blind, non- inferiority trial. Lancet. 2012 Aug 4;380(9840):475-83.
7. Matthews DR, Dejager S, Ahren B, et al. Vildagliptin add-on to metformin produces similar efficacy and reduced hypoglycaemic risk compared with glimepiride, with no weight gain: results from a 2-year study. Diabetes Obes Metab. 2010;12(9): 780-9. **8.** White WB, et al. Alogliptin after acute coronary syndrome in patients with type 2 diabetes. N Engl J Med. 2013;369(14):1327-35. **9.** Zannad F, et al. Heart failure and mortality outcomes in patients with type 2 diabetes taking alogliptin versus placebo in EXAMINE: a multicentre, randomised, double-blind trial. Lancet.2015;385(9982):2067-76. **10.** Nesina® [Bula]. São Paulo: Takeda Pharma Ltda. **11.** Nesina Met®[Bula]. São Paulo: Takeda Pharma Ltda. **12.** Nesina Pio®[Bula]. São Paulo: Takeda Pharma Ltda. **13.** Van Raalte DH, et al. The effect of alogliptin and pioglitazone combination therapy on various aspects of beta-cell function in patients with recent-onset type 2 diabetes. Eur J Endocrinol. 2014;170(4):565-74. **14.** Bosi E, et al. Alogliptin as a third oral antidiabetic drug in patients with type 2 diabetes and inadequate glycaemic control on metformin and pioglitazone: a 52-week, randomized, double-blind, active-controlled, parallel-group study. Diabetes Obes Metab. 2011;13(12):1088-96. **15.** Triplitt C, et al. Pioglitazone and alogliptin combination therapy in type 2 diabetes: a pathophysiologically sound treatment. Vasc Health Risk Manag. 2010;6:671-90.
16. DeFronzo RA, et al. Efficacy and tolerability of the DPP-4 inhibitor alogliptin combined with pioglitazone, in metformintreated patients with type 2 diabetes. J Clin Endocrinol Metab. 2012;97(5):1615-22.

NESINA® PIO* - Alogliptina + cloridrato de pioglitazona. Indicações: para pacientes com 18 anos ou mais com diabetes mellitus tipo 2 como adjuvante à dieta e exercícios para melhorar o controle glicêmico em pacientes inadequadamente controlados com pioglitazona isoladamente, e para os quais a metformina é inapropriada devido a contraindicações ou intolerância; e em combinação com metformina (terapia de combinação tripla). **Contraindicações:** hipersensibilidade a alogliptina ou pioglitazona ou a qualquer um de seus excipientes; em pacientes com insuficiência cardíaca Classes I a IV de (NYHA). **Advertências e Precauções:** não deve ser utilizado para o tratamento de pacientes com diabetes mellitus tipo 1 ou para o tratamento da cetoacidose diabética. Existem relatos pós-comercialização de pancreatite aguda. Se houver suspeita de pancreatite, Nesina Pio deve ser descontinuado. Cautela ao associar Nesina Pio com agentes que sabidamente causam hipoglicemia como insulina e sulfonilureias. Dose menor de insulina ou sulfonilureia pode ser necessária. Pode ocorrer retenção de fluidos e Insuficiência Cardíaca Congestiva (ICC). Pacientes com ICC devem ser monitorados e o tratamento com Nesina Pio deve ser interrompido no caso de piora dos sintomas; estudos observacionais de longa duração não encontram aumentos significativos do risco de câncer de bexiga em pacientes diabéticos utilizando pioglitazona. No entanto, dados de estudo de curta duração sugerem a possibilidade de um pequeno aumento no risco de câncer de bexiga. Nesina Pio não deve ser iniciada em pacientes com câncer de bexiga; o tratamento com Nesina Pio deve ser iniciado com cautela em pacientes com aumento dos níveis das enzimas hepáticas ou evidência de doença hepática; foi observado aumento de peso relacionado à dose de pioglitazona isoladamente ou em combinação com outros antidiabéticos orais; pioglitazona pode causar reduções nos níveis de hemoglobina e hematócrito. Pacientes com síndrome do ovário policístico podem retomar a ovulação após o tratamento com pioglitazona. As pacientes devem, portanto, estar conscientes do risco de gravidez; Nesina Pio é categorizado como risco C e não deve ser usado durante a gravidez. Nesina Pio não deve ser administrado em mulheres em fase de amamentação. **Interações medicamentosas:** Um inibidor de CYP2C8 (como genfibrozila) pode elevar a ASC de pioglitazona e um indutor de CYP2C8 (como rifampicina) pode reduzir a ASC de pioglitazona. A pioglitazona não afeta a farmacocinética ou farmacodinâmica da digoxina, varfarina, femprocumona ou metformina, e em coadministração com sulfonilureias não parece afetar a farmacocinética da sulfonilureia. Hipoglicemia pode ocorrer quando pioglitazona é administrada com sulfonilureia ou insulina. **Reações adversas**: edema, aumento de peso corporal, redução dos níveis de hemoglobina e hematócrito, aumento da creatina quinase (creatinafosfoquinase), insuficiência cardíaca, disfunção hepatocelular, edema macular, fraturas ósseas em mulheres, infecção do trato respiratório superior, sinusite, insônia, distúrbios visuais, câncer de bexiga, cefaleia, dor abdominal, doença de refluxo gastroesofágico, prurido, erupção cutânea. **Posologia e modo de usar:** Um comprimido (25mg + 15mg ou 25mg + 30mg) uma vez ao dia. Pode ser administrado com ou sem alimentos. Em pacientes com insuficiência renal moderada e grave ajuste de dose é necessário por causa da alogliptina. Este medicamento não deve ser partido ou mastigado. MS – 1.0639.0274. **SE PERSISTIREM OS SINTOMAS, O MÉDICO DEVERÁ SER CONSULTADO. MEDICAMENTO SOB PRESCRIÇÃO MÉDICA.** NP_1115_0716_VPS.

Contraindicação: insuficiência cardíaca Classes I a IV de (NYHA). **Interação medicamentosa:** Hipoglicemia pode ocorrer quando administrado com sulfonilureia ou insulina.

NESINA® - alogliptina. Indicações: adjuvante à dieta e à prática de exercícios para melhorar o controle glicêmico em pacientes com diabetes mellitus tipo 2 em vários cenários clínicos. **Contraindicações:** indivíduos que apresentem histórico de hipersensibilidade à alogliptina ou aos demais componentes da fórmula. **Advertências e Precauções:** NESINA® não deve ser utilizado para o tratamento de pacientes com diabetes mellitus tipo 1 ou para o tratamento da cetoacidose diabética. Existem relatos pós-comercialização de pancreatite aguda. Se houver suspeita de pancreatite, NESINA deve ser descontinuado. Cauleta ao associar NESINA® com agentes que sabidamente causam hipoglicemia como insulina e sulfonilureias. Dose menor de insulina ou sulfonilureia pode ser necessária. Categoria "B" de risco para a gravidez. NESINA® não deve ser utilizado por mulheres grávidas sem orientação médica ou do cirurgião-dentista. **Interações medicamentosas:** Não foram observadas interações medicamentosas com substratos ou inibidores da CYP testados ou com medicamentos excretados pela via renal. **Reações Adversas:** Monoterapia com alogliptina nos ensaios clínicos pivotais combinados e controlados de fase 3 - Frequentes (≥ 1/100 a < 1/10): infeções no trato respiratório, nasofaringite, cefaleia, dor abdominal, doença de refluxo gastroesofágico, prurido, erupção cutânea. Pós-comercialização – Frequência desconhecida: hipersensibilidade, pancreatite aguda, disfunção hepática (incluindo insuficiência), doenças esfoliativas de pele, incluindo Síndrome de Stevens- Angioedema, urticária. **Posologia e modo de usar:** 25 mg uma vez ao dia. NESINA® pode ser administrado com ou sem alimentos. Em pacientes com insuficiência renal moderada e grave ajuste de dose é necessário. MS – 1.0639.0266. **SE PERSISTIREM OS SINTOMAS, O MÉDICO DEVERÁ SER CONSULTADO. MEDICAMENTO SOB PRESCRIÇÃO.** NS_0414_0115_VPS

Contraindicação: NESINA não deve ser usado por indivíduos que apresentem histórico de alergia (hipersensibilidade) à NESINA ou aos demais componentes da fórmula. **Interações medicamentosa:** Não foram observadas interações medicamentosas com substratos ou inibidores da CYP testados ou com medicamentos excretados pela via renal.

NESINA® MET*- Alogliptina + cloridrato de metformina. Indicações: adjuvante à dieta e à prática de exercício, para melhorar o controle glicêmico em pacientes adultos, a partir dos 18 anos, com *diabetes mellitus* tipo 2: que não conseguem o controle adequado com a dose máxima tolerada de metformina isolada; em combinação com a pioglitazona, em pacientes que não conseguem o controle adequado com a dose máxima tolerada de metformina e pioglitazona; em combinação com a insulina, quando a insulina numa dose estável e a metformina isolada não assegurarem o controle glicêmico. **Contraindicações:** hipersensibilidade à alogliptina, ou outro inibidor de DPP4, à metformina ou aos demais componentes da fórmula; cetoacidose diabética, pré-coma diabético; comprometimento renal moderado e grave e doença renal em fase terminal (depuração da creatinina <60 ml/min); doenças agudas ou crônicas com potencial para alterar a função renal ou causar hipóxia tecidual; comprometimento hepático; intoxicação alcoólica aguda, alcoolismo; **Advertências e Precauções:** não deve ser utilizado em pacientes com diabetes mellitus tipo 1. Caso suspeite de acidose metabólica, a administração de NESINA® MET* deve ser suspensa e o paciente deve ser imediatamente hospitalizado. NESINA® MET* não é recomendada em pacientes com comprometimento renal moderado e grave e doença renal em fase terminal (depuração da creatinina <60 ml/min). Alogliptina não foi estudada em pacientes com comprometimento hepático grave (> 9 na escala de Child--Pugh), portanto, a sua utilização não é recomendada nestes pacientes. Como NESINA® MET* contém metformina, o tratamento deve ser suspenso 48 horas antes de uma cirurgia eletiva com anestesia geral, raquianestesia ou epidural ou realização de exames contrastados. Devido ao risco acrescido de hipoglicemia em associação com a pioglitazona ou insulina, pode ser considerada uma dose mais baixa de pioglitazona ou insulina quando em associação com NESINA® MET*. Se houver suspeita de pancreatite, deve-se interromper o tratamento. NESINA® MET* é Categoria "B" de risco para a gravidez e, portanto, não deve ser utilizado por mulheres grávidas sem orientação médica ou do cirurgião-dentista. Recomenda-se cautela ao se administrar NESINA® MET* a lactantes. **Interações medicamentosas:** Não foram observadas interações medicamentosas com substratos ou inibidores da CYP testados ou com medicamentos excretados pela via renal para alogliptina. A acidose láctica pode ocorrer quando administrado metformina com agentes de contraste iodado ou álcool. A metformina pode diminuir as concentrações de vitamina B12. **Reações Adversas:** Monoterapia com alogliptina nos ensaios clínicos pivotais combinados e controlados de fase 3 - Frequentes (≥ 1/100 a < 1/10): infeções no trato respiratório, nasofaringite, cefaleia, dor abdominal, doença de refluxo gastroesofágico, prurido, erupção cutânea. Sintomas gastrointestinais ocorrem mais frequentemente durante o início do tratamento e se resolvem espontaneamente na maioria dos casos. Foram relatados casos isolados de hepatite ou anormalidade dos testes de função hepática que se resolvem com a descontinuação da metformina. **Posologia e modo de usar:** duas vezes ao dia com uma refeição. A dose pode ser ajustada com base na eficácia e tolerabilidade, sem exceder a dose máxima diária recomendada de 25 mg de alogliptina e 2000 mg de cloridrato de metformina. Este medicamento não deve ser partido, aberto ou mastigado. MS – 1.0639.0272. **SE PERSISTIREM OS SINTOMAS, O MÉDICO DEVERÁ SER CONSULTADO. MEDICAMENTO SOB PRESCRIÇÃO.** NM_0614_1115_VPS

Contraindicação: Hipersensibilidade às substâncias ativas ou a qualquer um dos excipientes mencionados na composição. **Interações medicamentosas:** pode ocorrer perda do controle glicêmico quando administrado com diuréticos, corticosteróides ou simpaticomiméticos.

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Radioguided localization of recurrent lymph nodes in differentiated thyroid cancer – Where are we now?

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Controlling loco-regional recurrence is an important goal for patients with differentiated thyroid cancer (DTC). A precise localization of suspected lesions is critical for patients with recurrence following a therapeutic neck dissection, as reoperations present increased morbidity. A focused excision may be justified, and marking of non-palpable lesions can help optimize surgical approach and increase tumor detection and removal.

Radioguided occult lesion localization (ROLL) was initially described for breast tumors, and is based on the intra-operatory guidance by a hand-held gamma-probe that detects radiation emitted from ^{99m}Tc-macroaggregated albumin (^{99m}Tc-MAA), previously injected into the lesion during an ultrasound or mammography. The excellent results of ROLL for non-palpable breast lesions, as evidenced by systematic reviews and meta-analysis (1), lead to its use in other clinical situations.

Radioguided procedures are also performed with radiopharmaceuticals smaller than ^{99m}Tc-MAA, capable of following the physiological lymphatic drainage from the injection site, and to identify and guide sentinel lymph node (SLN) biopsy. For patients with DTC, a recent systematic review proposes that lymphoscintigraphy could be used to guide compartment-oriented lymphadenectomy but not to restrict biopsy of SLNs only (2). Evidently, SLN biopsy is a procedure for initial staging and not an option for post-surgical restaging or recurrent disease localization.

In recent years, the interest in ROLL procedures to guide metastatic lymph node (LN) excision in DTC has increased. In this issue of *Archives of Endocrinology and Metabolism* (AE&M), Cerit and cols. present their experience using intralesional injection of ^{99m}Tc-MAA under ultrasound guidance for radioguided resection of lymph nodes in 11 patients with recurrent or persistent DTC (3). The authors used the term Guided intra-Operative Scintigraphic Tumor Targeting (GOSTT), originally proposed to include the use of different tracers, multimodality equipments, and 3D navigational systems (4).

Although several radiopharmaceuticals are described to localize recurrent/persistent metastatic LN in DTC, including tumor-avid tracers such as ^{99m}Tc-sestamibi (5), ^{18}F-FDG, and iodine-131 (6), Cerit and cols. use the classic concept of ROLL. Injection of ^{99m}Tc-MAA into the suspicious lymph nodes was performed under ultrasound (US) monitoring, 1 to 2 h prior to surgery. A diagnosis of DTC metastasis had been previously established via US-guided fine needle aspiration biopsy (FNAB) and FNAB-Tg washout level. So, it is not surprising that all of the 26 marked LNs revealed DTC metastasis on histopathologic examination.

It is also of note that metastasis were present in 14 from the total of 85 additional non-marked LNs removed (16%). In the study of Gulcelik, including the use of ROLL

for locoregional metastasis in 20 DTC patients, 36 out of 56 (64%) suspicious non-marked lesions were metastatic (7). Detection of metastasis in non-marked adjacent LNs points to the fact that disease may also be present in other LNs that were not removed. Even if the initial purpose of the surgery is not to eliminate all micrometastasis, it is not clear the impact of residual disease in future recurrences and what will be the role of radioiodine therapy or other systemic therapies in this scenario.

Regarding recurrence after ROLL, the authors state that even subcentimetric lesions were excised and local control of the disease was achieved in all patients after a median follow-up of 30 months (a 2nd ROLL was necessary in one case and 3 patients presented with lung or mediastinal metastasis during follow-up). Borsò and cols. (8) also found 33% to 40% recurrence rate after a follow-up of 29 months in 32 patients with DTC submitted to metastatic LN ROLL.

These findings do not invalidate the use of ROLL to improve identification of suspicious lesions, and possibly to increase the completeness of resection in a difficult surgical field, with the intention of improving prognosis of patients with recurrent DTC. However, despite the encouraging results, at this moment the limited number of patients and follow-up interval do not allow a clear definition of ROLL impact in long-term outcomes and clinical recurrence of DTC patients.

Available data suggests that radioguided dissection is safe and feasible in patients with loco-regional recurrence from DTC, and may be an alternative for controlling DTC LN recurrence with low morbidity. The procedure requires an intensive coordination among different specialties: surgeon, radiologist, nuclear medicine physician, and pathologist; and careful planning is required by the institutions that intend to adopt it.

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Metabolomics of thyroid nodules and the future

Regina Lúcia Elia Gomes[1], Eloisa M. M. Santiago Gebrim[1]

In this issue of the *Archives of Endocrinology and Metabolism* (AE&M), the use of 3T Magnetic Resonance Spectroscopy (MRS) for the evaluation of patients with thyroid nodules (TNs) is addressed in a very interesting prospective study by Aghaghazvini and cols (1).

The new field in biological science, Metabolomics, makes use of analytical and standard recognition approaches and bioinformatics (2,3). The metabolome is the final downstream product of gene expression reflecting changes in transcriptome and proteome (4). Metabolic, histological and cytological changes occur during the development and progression of carcinoma (5), and it is paramount to understand its biochemistry to allow the development of powerful diagnostic tools and to identify new biomarkers (2). Several studies have demonstrated that the metabolomics makes it possible to characterize different types of tumors in other organs than thyroid gland (2).

Magnetic resonance spectroscopy (MRS) is a noninvasive method that analyzes tissue metabolism, allowing the measurement of the concentration of metabolites in tissues and organs and making it possible to characterize the metabolic changes associated with cancer (6).

As is known, standard magnetic resonance imaging (MRI) plays a limited role in the evaluation of thyroid nodules as well as does not assess its functional status (7,8). The main difference between the standard MRI and the MRS is that in the MRI the spatial distribution of the water proton signals is used to generate an anatomical image of the analyzed tissue and in the clinical MRS methods there is suppression of the water signal to provide chemical information about the metabolites in which the data are shown as line spectra (6,9), or maps of metabolic images if the MRS data are acquired using a method called chemical shift imaging. These spectra can be analyzed quantitatively by the presence, absence or alteration of metabolites and semi-quantitatively by the calculation of the amplitude or integrals of the metabolite or metabolite ratios relative to the control (6).

Although MRS is used to assess cancer in various regions of the body, the neck region may create technical difficulties such as shimming and subject motion for in vivo spectroscopy, and it is the only noninvasive technique capable of measuring chemicals/metabolites within the body (7,8). Thyroid cancer was the first field in which the molecular diagnosis was performed using ex-vivo MRS (10,11) in Fine-Needle Aspiration Biopsy (FNAB) specimen or tissue obtained at the time of surgery. Few in vivo studies were performed, most of them with 1.5 T MRS, even less with 3T MRS. There are technical difficulties in performing in vivo thyroid spectroscopy, such as tumor movement as a result of swallowing and breathing, shimming difficulties due to large differences in magnetic susceptibility between the neck and air in the trachea and contamination of spectra by adjacent fat (7).

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In this study by Aghaghazvini and cols (1), the Choline (Chol) to creatine (Cr) ratio was assessed on each nodule, and the sensitivity (75%) is less than two main prior studies (King and cols. (12), 87%; Gupta and cols. (13), 100%) while the specificity is comparable to two studies. Further studies with larger number of patients are needed.

A review article by Minuto and cols. shows that there are other MRS studies in the literature demonstrating that benign and malignant thyroid lesions have a higher level of several amino acids (methionine, glycine, alanine, cysteine, glutamine, glutamate, isoleucine, leucine, lysine, phenylalanine, serine, tyrosine, valine), lactate and taurine, and a lower content of fatty acids than normal tissues. Papillary and follicular neoplasms have a higher concentration of taurine, lactate, phenylalanine and tyrosine, and lower concentrations of mi and scyllo-inositol, choline, phosphocholine and/or glycerophosphorus-line and unknown compounds in relation to follicular adenomas; increased levels of hypoxanthine and decreased levels of acetone in the thyroid lesions were also described, as well as new markers such as 3-hydroxybutyrate and -glucose and phosphocholine and formate. Four metabolites (creatine, scyllo-inositol, myo-inositol and uracil) were considerate candidates for selective biomarkers for thyroid cancer. The follicular thyroid adenomas have some metabolic characteristics of normal tissue and other features associated with thyroid cancer, which may denote an intermediate nature of these benign tumors, so that thyroid cancer may arise from preexisting follicular adenoma, or it may be a preneoplastic lesion (10).

Some metabolomic MRS studies have concluded that specific markers of benign and malignant tissues have been correlated with cell proliferation observed during tumor development (10).

The advancement in the MRI techniques as 3T and technology of software programs as MRS can improve the metabolomics of thyroid nodules in the future, perhaps even being a powerful complementary tool to other diagnostic methods.

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Guided intraoperative scintigraphic tumor targeting of metastatic cervical lymph nodes in patients with differentiated thyroid cancer: a single-center report

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ABSTRACT

Objective: Our aim was to present our experiences related to performing neck surgery using the guided intraoperative scintigraphic tumor targeting (GOSTT) procedure for patients who had locally recurrent or persistent differentiated thyroid cancer (DTC) and who had undergone previous thyroid surgery. **Subjects and methods:** We retrospectively evaluated 11 patients who had locally recurrent or persistent DTC, who had undergone previous surgery, and for whom reoperation was planned for metastatic cervical lymph nodes (LNs). We performed the neck surgery using the GOSTT procedure on all patients and at a single academic institution. **Results:** The 11 patients had a total of 26 LNs, as marked with a radiotracer, and those LNs' mean size was 14.7 ± 8.2 mm (range: 5–34 mm). Histopathological examinations revealed DTC metastasis in all 26 of the preoperatively marked LNs. Of the 11 patients, only one needed a reoperation in the neck; she had another successful surgery (also using the GOSTT procedure). In the evaluation of the patients' final status, all were disease-free in their necks. There also were no GOSTT-associated postoperative complications. **Conclusion:** The GOSTT procedure is a useful, successful, inexpensive, and comfortable procedure for marking and mapping metastatic LNs, especially in DTC patients who have undergone previous surgery. Arch Endocrinol Metab. 2018;62(5):495-500

Keywords

Differentiated thyroid cancer; guided intraoperative scintigraphic tumor targeting; radio-guided occult lesion localization; recurrent thyroid carcinoma

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INTRODUCTION

Globally, thyroid cancer is the most common endocrine neoplasia (1). Differentiated thyroid cancer (DTC) is the most frequent thyroid cancer subtype (2). Although DTC has an excellent outcome, with the use of standard pathological techniques, cervical lymph node (LN) metastases have been detected in 20 to 50% of patients in most series. LN metastases may be present even when the primary tumor is small and/or has an intrathyroidal location (3,4). During follow-ups, using neck ultrasonography (US) and monitoring thyroglobulin (Tg) levels can lead to greater identification of nonpalpable LN metastases in the central and lateral neck, particularly in patients who have been newly diagnosed or who have undergone previous thyroid surgery (5).

Various surgical approaches exist for lateral cervical LN dissection (6). Selective lateral LN dissection is the preferred surgical approach, as it does not lead to higher morbidity and minimizes local recurrence rates (6-9). In the "berry picking" procedure, which has been recommended for the local recurrence of LN metastases in areas that have been previously dissected, only suspicious and/or enlarged lymph nodes are removed (6). Biopsy-proven metastatic LNs in a compartment that previously underwent surgery should be considered for excision, but reoperations are associated with increased morbidity compared with primary tumor surgery (10,11). Reoperative surgery in the neck for recurrent DTC is a burdensome and demanding procedure for surgeons because of the presence of scarred and fibrotic tissues in that area (11).

Radio-guided surgery (RGS) is a popular modality in surgical practice. RGS allows a surgeon to identify lesions or tissues that have been preoperatively marked with radioactive substances (12). RGS is performed using either systemic or local injection of radioisotopes. Radio-guided occult lesion localization (ROLL), a type of GOSTT, was originally described for occult lesions in breast cancer (13). Recently, GOSTT has also been performed on patients who have locally recurrent or persistent DTC and who are undergoing a repeat neck surgery (5,12,14-24). ROLL is based on the intralesional injection of a radioactive substance that (because of its large size) does not migrate from the site of the interstitial injection. In the ROLL technique, the radiation that other tissues absorb is negligible. Therefore, this technique is presumably more advantageous than systemic radionuclide administration (15,16). In ROLL, a surgeon confirms complete removal of a lesion by repeatedly counting the surgical bed with the gamma probe. All of the published results indicate that GOSTT-associated radiation exposure to both health care professionals and patients is minimal. The radiation exposure doses for patients, GOSTT administrators, surgeons, nurses, and pathologists are all within the safety limits recommended by the International Commission on Radiological Protection (14). Routine monitoring of radiation exposure after the GOSTT procedure is not recommended for any nonnuclear medical professionals (14).

Our aim in this study was to present our experiences and results related to performing the GOSTT procedure on patients who had locally recurrent or persistent DTC and who had undergone previous thyroid surgery.

SUBJECTS AND METHODS

We retrospectively evaluated 11 patients who underwent total thyroidectomy with or without central and/or lateral LN dissection; these patients all had locally recurrent or persistent DTC and all had previously undergone thyroid surgery. Reoperation was planned for all 11 patients' metastatic cervical LNs to address 1 or more suspicious LNs that had been detected via neck US. We confirmed the diagnosis of LN metastasis due to DTC via US-guided fine needle aspiration biopsy (FNAB) and monitoring of the FNAB-Tg washout level (10). We then performed the repeat neck surgery using the GOSTT procedure for all patients.

GOSTT procedure

We percutaneously injected the radioactive particles directly into the lesion 1 to 2 h prior to surgery. Specifically, we used a 22-gauge needle and a syringe to inject 0.2 mCi of technetium-99m human albumin macroaggregate aliquot (Makro-Albumon, Medi-Radiopharma, Budapest, Hungary) in a volume of 0.1 ml suspension directly into each suspicious lymph node using 7.5 MHz linear transducers (GE Logiq 5 Pro system equipped with an 8–12 MHz linear probe, GE Medical Systems, Milwaukee, WI) under continuous US monitoring. We withdrew the needle under slight aspiration in order to minimize the release of residual radioactivity along the needle track. We marked the skin directly overlying each suspicious LN with a surgical skin marker under US guidance. A nuclear medicine specialist had previously prepared the radioactive material. The nuclear medicine department enforced radiation safety protocols, and we performed the procedure in the nuclear medicine department. The marked cutaneous projections of the lesions on the neck skin provided a useful guide for deciding upon the appropriate incision site at the beginning of surgery. During surgery, we used a handheld gamma-detection probe (Crystal CXS-SGV2 model, Crystal Photonics, Berlin, Germany) that was covered by a sterile probe cover to locate the suspicious lesions and decide upon the most appropriate excision of the radio-labeled lesions. We excised the premarked LNs along with any adjacent suspicious LNs or soft tissues in order to accomplish the selective LN dissection. We used the berry-picking procedure for patients who had undergone previous compartment-oriented dissections in the current suspicious region. After excision of all premarked and suspicious LNs, we monitored the suspicious area for residual radioactivity in each patient.

We performed the statistical analyses (in terms of mean, range, ratio, and percent) using SPSS version 16.0 (Chicago, IL) software.

We obtained informed consent from all patients before performing the FNAB and GOSTT procedures. The local ethics board approved this retrospective study.

RESULTS

Eleven patients who had recurrent or persistent DTC and who had undergone previous thyroid surgery were included to this study. The mean age of the patients

was 41.6 ± 18.3 years (range: 20–71 years). The female-to-male ratio was 5:6. The patients' tumor, node, and metastasis classifications, cancer stages, and histopathological characteristics are shown in Table 1. Each of the patients had undergone at least 1 surgery (range: 1–3) prior to the GOSTT procedure. The types of prior operations are also shown in Table 1. Eight patients had undergone radioactive iodine ablation before the GOSTT procedure.

A total of 26 LNs were marked for the GOSTT procedure across the 11 patients. The mean size of the marked LNs was 14.7 ± 8.2 mm (range: 5–34 mm). The sites of the lesions are shown in Table 2. No complications were encountered during the procedure. All the patients underwent successful excisions, even for lesions of less than 1 cm. We performed berry-picking in the 5 patients who had undergone previous compartment-oriented dissections in the current suspicious region (Patients 1, 2, 3, 10, and 11). Histopathological examinations revealed DTC metastasis in all of the 26 preoperatively marked LNs. There were no postoperative complications (Table 2).

All of the patients received postoperative radioactive iodine ablation treatment. The mean follow-up time after GOSTT was 30.0 ± 13.3 months (range: 10–50 months). Of the 11 patients, only 1 had recurrent disease in the neck, and she underwent another successful RGS to remove these metastatic lymph nodes (Patient 9). During the follow-up, 3 patients developed distant metastasis (in the lungs and the mediastinal LNs); two underwent mediastinal LN dissection as a result (Patients 4 and 10). At the final patient-status evaluation, all of the patients were disease-free in their necks, but 2 patients (Patients 9 and 10) were still living with disease because of distant metastasis to the lungs (Table 2).

DISCUSSION

Although thyroid cancer mostly follows an indolent course, the patients in our study had late-stage disease or required recurrent operations. All patients in this study had preoperative skin markings done via the GOSTT procedure and underwent successful excisions, even for lesions less than 1 cm. Serum Tg levels decreased in 8 of 11 patients during the early postoperative period. At the final patient-status evaluation, all of the patients were disease-free in their necks.

Compared to primary thyroid surgery, reoperation for locally recurrent or persistent DTC carries increased risks for damage to both the recurrent laryngeal nerve and the parathyroid glands. For reoperative procedures, the incidence of permanent vocal cord paralysis is 1–12% and the incidence of hypoparathyroidism is 1–4% (11,15,25). Even though recurrent neck surgery seems more difficult than primary surgery, the patients in our study experienced no GOSTT-associated postoperative complications. The operative time was also shorter than expected (operative time data is not available). The GOSTT procedure could be comfortable for surgeons during the incision and excision processes. Using a gamma probe also helps surgeons to confirm that all suspicious LNs have been excised.

Table 1. Patient and tumour characteristics

Patient no.	Sex	Age at Diagnosis (yr)	TNM classification	Stage	Histology of carcinoma	Operation(s) before RGS	No. of neck operation before RGS	Total I-131 dose before RGS
1	M	31	T1bN1aM0	Stage 1	Classical type PTC	TT, CLND	1	150
2	F	36	T1bN1bM0	Stage 1	Classical type PTC	TT, CLND, LLLND	2	118
3	F	23	T2N1aM0	Stage 1	Classical type PTC	TT, CLND	1	125
4	M	35	T1bN1bM1	Stage 2	Classical type PTC	TT, CLND, RLLND, LLLND	3	150
5	F	55	T3N1bM0	Stage 4a	Follicular variant PTC	TT	1	50
6	F	20	T3N1bM0	Stage 1	Diffuse sclerosing variant PTC	TT, CLND, RLLND	2	100
7	M	71	T3N1bM0	Stage 4a	Classical type PTC	TT	1	0
8	M	60	T1aN1bM0	Stage 4a	Follicular variant PTC	TT, CLND	1	0
9	F	27	T3N1bM1	Stage 2	Classical variant PTC	TT, LLLND	2	100
10	M	68	T2N1bM1	Stage 4c	Widely invasive FTC	TT, LLLND, RLLND	2	150
11	M	32	T1bN1aM0	Stage 1	Classical variant PTC	TT, CLND	1	0

M: male; F: female; No: number; PTC: papillary thyroid cancer; FTC: follicular thyroid cancer; LN: lymph node; TNM: tumour, node, metastasis; RGS: radioguided surgery; TT: total thyroidectomy; CLND: cantral LN dissection; RLND: right lateral LN dissection; LLND: left lateral LN dissection; RAI: radioactive iodine treatment.

Table 2. Characteristics of guided intraoperative scintigraphic tumour targeting procedure and follow up of patients after radioguided surgery

Patient no.	Site of LN in GOSST procedure (mm)	Size of LN in GOSST Procedure (mm)	Type of operation with GOSST procedure	No. of marked LN with GOSST procedure	No. of metastatic LN found	No. of total excised LN	Pre RGS (TSH,Tg, Anti-T)	Post RGS (early period) (TSH, Tg, Anti-T)	Compl	Distant metastasis	Tx after RGS	Follow up time after RGS (mo)	Final status in neck	Total follow up time (mo)	Final disease status
1	Left level VI	11	BP in left level VI	2	4	5	TSH: 1.5 Tg: 0.8 Anti-T: N	TSH: 0.2 Tg: 0.2 Anti-T: N	None		150 mCi I 131	48	Neck US negative TSH:0.05 Tg: < 0.2 Anti-T: N	72	LDF
2	Left level VA	14	BP in left level VA	1	1	3	TSH: 156 Tg: 65 Anti-T: N	TSH: 0.3 Tg: 0.2 Anti-T:N	None		150 mCi I-131	36	Neck US negative TSH: 0.01 Tg: 0.2 Anti-T: N	54	LDF
3	Left level VI	8	BP in left level VI	2	4	4	TSH: 11 Tg: 3.9 Anti-T: N	TSH: 147 Tg: 1.6 Anti-T:N	None		150 mCi I-131	28	Neck US negative TSH: 0.05 Tg: 0.2 Anti-T: N	82	LDF
4	Right level IV	34	RLLND	4	10	10	TSH: 100 Tg: 266 Anti-T: N	TSH:110 Tg: 168 Anti-T:N	None	Lung, mediastinal LN	400 mCi I-131, mediastinal LND	36	Neck US negative TSH: 0.01 Tg: 0.2 Anti-T: N	48	LDF
5	Left level VI and left level III	9	CLND, LLLND	2	2	18	TSH: 0.8 Tg: 0.4 Anti-T: P	TSH: 116 Tg: < 0.2 Anti-T:N	None		150 mCi I-131	24	Neck US negative TSH: 0.01 Tg: < 0.2 Anti-T: N	40	LDF
6	Right level VI and right level III	25	CLND, RLLND	3	6	12	TSH: 324 Tg: 8.4 Anti-T: N	TSH: 74 Tg: 1.4 Anti-T: N	None		150 mCi I-131	50	Neck US negative TSH: 0.01 Tg: 0.2 Anti-T: N	69	LDF
7	Right level VI and right level IV	9	CLND, RLLND	2	2	22	TSH: 74 Tg: 33 Anti-T:N	TSH: 121 Tg: 5.3 Anti-T: N	None		150 mCi I-131	15	Neck US negative TSH:0.01 Tg: 0.2 Anti-T: N	18	LDF
8	Right level III-IV-V	11	RLLND	3	4	20	TSH: 0.5 Tg: 3.3 Anti-T: P	TSH:0.01 Tg: 0.2 Anti-T: N	None		150 mCi I-131	24	Neck US negative TSH: 0.02 Tg: < 0.2 Anti-T: N	27	LDF
9	Left level VI and left level III-IV	9	CLND, LLLND	3	3	11	TSH: 227 Tg: 230 Anti-T: P	TSH: 149 Tg: 56 Anti-T: P	None	Lung	400 mCi I-131, LLLND with ROLL	18	Neck US negative TSH: 0.01 Tg: 11 Anti-T: N	30	LWD
10	Right level VI and right level IV	20	BP in right level VI and level IV	2	2	4	TSH: 116 Tg: 208 Anti-T: N	TSH:100 Tg: 500 Anti-T: N	None	Lung, mediastinal LN	600 mCi I-131 Mediastinal LND + TCİ?	42	Neck US negative TSH: 0.05 Tg: 145 Anti-T: N	85	LWD
11	Left level VI and right level VI	L – 12mm R – 5mm	BP in left and right level VI	2	2	2	TSH:0.14 Tg:<0.2 Anti-T: P	TSH:104 Tg: 6.7 Anti-T: P	None		150 mCi I-131	10	Neck US negative TSH:0.02 Tg < 0.2 Anti-T: N	12	LDF

LN: lymph node; BP: berry picking; GOSST: guided intraoperative scintigraphic tumour targeting; CLND: cantral LN dissection; RLND: right lateral; LN dissection; LLND: left lateral LN dissection; LDF: living disease free; LWD: living with disease; TCI: tyrosine kinase inhibitor; Tg: thyroglobulin (ng/mL); TSH: thyroid stimulating hormone (μIU/mL); Tx: treatment; RGS: radioguided surgery; RAI: radioactive iodine treatment; Compl: complication; Anti-T: anti-thyroglobulin antibody; P: positive (high); N: negative (in normal range).

Tükenmez and cols., using the ROLL technique, reported the first successful resection of nonpalpable LN metastases in 2 DTC patients (17). Three prospective studies were published in 2010, all of which indicated that the ROLL technique was safe and effective for the removal of nonpalpable DTC metastases (18-21). Terzioğlu and cols. reported the excision of 30 lesions from 21 patients undergoing reoperative thyroid and parathyroid surgery, all without any nerve injury or transient hypoparathyroidism. Tg levels decreased to less than 2 ng/ml in 86% of the patients with preoperatively elevated Tg levels (16). Gulcelik and cols. reported on 20 patients with metastatic DTC who underwent the ROLL technique with a 100% success rate and with no postoperative complications other than a single seroma (22). Borsò and cols., in a study of 32 patients, found no cervical DTC recurrences (all tests were radioiodine-negative) after performing surgery with the ROLL technique (26). In our cases, all of the 26 suspicious LNs revealed DTC metastasis on histopathologic examination. Of the 11 patients, only one needed a reoperation in the neck; she underwent another successful surgery (also using the GOSTT procedure).

The injection of a radioactive substance into a suspicious LN is technically similar to LN FNAB. Clinicians who can perform LN FNAB should be able to easily inject a radioactive substance into suspicious LNs.

In conclusion, preoperative mapping with US-guided intralesional radiotracer injection and intraoperative gamma-probe use, as compared to conventional methods, allows for faster intraoperative detection of metastatic lesions, could facilitate the surgical approach, and could improve surgical outcomes for patients who have locally recurrent or persistent DTC and who have previously undergone neck surgery. Thus, GOSTT is a convenient, comfortable, and inexpensive procedure for selected patients with locally recurrent or persistent DTC.

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3T magnetic resonance spectroscopy as a powerful diagnostic modality for assessment of thyroid nodules

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ABSTRACT

Objective: Magnetic resonance spectroscopy (MRS) is a powerful tool for structural studies of chemical compounds and biomolecules and also documented promising findings as a potential imaging technology in thyroid oncology. This prospective study was to ascertain the clinical significance of 3 Tesla MRS in the evaluation of patients with thyroid nodules (TNs) as an ancillary diagnostic technique for thyroid carcinoma. **Materials and methods:** Magnetic resonance spectroscopy at 3T at echo-times (TEs) 136 and 270 ms was carried out on 15 patients with total number of 32 TNs larger than 1 cm^3, which all were surgically resected. Choline (Chol) to creatine (Cr) ratio was assessed at 136 and 270 TEs on each nodule and a receiver operating characteristic (ROC) curve was used to determine optimal cut-off point. The findings were compared with histopathology of thyroid specimens. **Results:** There were 23 benign and 9 malignant lesions (7 papillary and 2 follicular thyroid carcinomas). The mean values of Chol/Cr at 136 and 270 TEs was 2.28 ± 3.65 and 1.52 ± 1.67 respectively and the difference between benign and malignant nodules was only significant at 136 TEs. The study revealed that Chol/Cr ratio cut-off point of 2.5 best correlates with histopathology results (sensitivity = 75%; specificity = 100%; PPV = 100%; NPV= 92%). **Conclusion:** This preliminary study showed that 3T magnetic resonance spectroscopy might be a specific modality for the evaluation of thyroid nodules in differentiation of benign from malignant thyroid tissue. However, a larger series would give much greater confidence that this state-of-the-art technology will worth pursuing in clinical practice. Arch Endocrinol Metab. 2018;62(5):501-5

Keywords
Magnetic resonance spectroscopy; thyroid nodules; thyroid carcinoma; choline

INTRODUCTION

Thyroid nodules are quite common and estimated as being clinically presented in 4-7% of the population (1). Early and precise differentiation of malignant and benign nodules stays a remarkable diagnostic problem, but is critical to the patient's management (2). Although there is not a consensus in the method of choice for initial evaluation, it is generally accepted that, adjunct with thyroid function tests, fine-needle aspiration cytology (FNAC), should be the first procedure to be done (3). Nevertheless, with a reported sensitivity of 68-98% (mean 83%) and a specificity of 72-100% (mean 92%); FNAC does not seem to be a perfect diagnostic method for the assessment of thyroid nodules (4-6).

The success of ultrasound as an inexpensive and noninvasive modality in discrimination benign from malignant nodules is presented in several reports (7,8). Although none of the sonographic properties have a reliable and sufficient sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) in ascertaining malignancy of the nodules, the sonographic features of the nodules can assists in selecting the nodules for needle aspiration (7,9). Computed tomography of neck is often restricted by different artifacts (10). Magnetic resonance spectroscopy (MRS) is the only non-invasive modality capable of measuring chemicals/metabolites within the body (11) as result of different magnetic frequency or chemical shifts (11). The concept that malignant cells develop a large number of proton nuclear magnetic resonance (1H NMR) visible molecules leads to potential role of MRS as a diagnostic modality in various cancers (12). It is a powerful tool that has been used the nuclear magnetic resonance in NMR spectroscopy based upon the hydrogen-1 nuclei within the molecules of a matter, for the purpose of ascertaining the structure of its molecules (13).

Literature about MRS application especially using 3T for thyroid lesions is very limited. Most of the studies done initially were ex vivo, either MRS was performed on FNAC specimen or on tissue obtained at surgery (14-16). Most few in vivo studies have been carried out with 1.5 T (12,15,17).

The present study was carried out to assess the findings of MRS of thyroid nodules using a 3T MRI and its correlation with histopathology obtained at surgery.

MATERIALS AND METHODS

The present study was conducted in a university-affiliated tertiary referral Hospital from September 2010 to February 2013. A total number of 15 patients presenting with thyroid nodule larger than 1 cm^3 were included in the study. Patients with previous thyroid surgery, known malignant disease or history of radiation were excluded. Moreover, patient's with contraindication for MRI such as claustrophobia and also being pace maker were excluded.

All MRI examinations were performed on a 3 Tesla MR unit (Magnetom Avanto; Siemens, Erlangen, Germany) with gradient strength of 33 mTs. Patients were positioned in supine position and were instructed not to swallow or move during the examination. Circularly polarized surface coil was placed over the neck. Fast scout scan in sagittal, axial and coronal planes was obtained. After localizing the lesion, the voxel was placed on the lesion and position checked in all three planes. The scan technique used was PRESS, single voxel technique. The sequence parameters employed with sampling numbers 512, averaging 16 and average scan time of 4.55 min. It was followed by water suppression pulses to be followed by data acquisition. Choline (Chol) to creatine (Cr) ratio was assessed at 136 and 270 TEs on each nodule. Image analyses were assessed by two radiologists and discrepant results were resolved by consensus and these findings were compared with the results of histopathological studies as gold standard test.

All patients had previously undergone ultrasound examination, FNAC and thyroid hormone measurements and also had previously been selected for surgery on the basis of their clinical and paraclinical results.

The study complies with the declaration of Helsinki and was approved by the institutional ethics committee of Tehran University of Medical Sciences, and all patients gave written informed consent.

Statistical analysis

The distribution of parameters was evaluated using probability plots and the Shapiro-Wilk test. Continuous variables are presented as mean ± SD, and categorical values as the absolute values and percentages. Fisher exact and chi-squared tests were applied to compare the qualitative variables and also T-test and *Mann–Whitney U test* between the quantitative variables in groups. The sensitivity, specificity, positive and negative predictive and also efficiency of values of each setting was acquired based on the definitive diagnosis established through histopathology. A receiver operating characteristic (ROC) curve analysis was done to determine optimal cut-off point for the discrimination of malignant from benign nodules. Cohen's kappa coefficient (κ) was applied inter-rater agreement of variables. Statistical analysis was performed using an IBM computer and PASW software, version 22.0 (SPSS, Inc., Chicago, USA).

RESULTS

A total of 15 patients (11 women, 4 men; mean age 39.5 ± 12.3 years, range 20–50) were included in this study. The total number of thyroid nodules was 32; 15 nodules were in the right lobe; 13 nodules in the left lobe and 4 nodules in the isthmus. Histological findings were malignant in 9 cases (seven cases of papillary carcinoma and two cases follicular carcinoma) and benign in 23 cases (Table 1). The volume of thyroid nodules was 11.67 cc; there was no significant difference in volume between malignant and benign nodules (p value > 0.05).

In term of metabolite profile semi quantitatively, significant choline peak was seen only in 7 cases (malignant = 7, benign = 0). The mean values of

Table 1. Histopathology of thyroid nodules of the included patients

	Frequency	Percent
Coloid cyst	4	12.5
Coloid nodule	7	21.9
Hashimoto	2	6.3
MNG	6	18.8
MNG with lymphocytic thyroiditis	2	6.3
Nodular hyperplasia with cystic degeneration	2	6.3
Follicular thyroid carcinoma	2	6.2
Papillary thyroid carcinoma	7	21.9

MNG: multinodular goiter.

Chol/Cr at 136 and 270 TEs was 2.28 ± 3.65 and 1.52 ± 1.67 respectively and the difference between benign and malignant nodules was only significant at 136 TEs (p value < 0.05) (Table 2). Seven malignant cases showed significant choline peak at 3.22 ppm whereas 2 out of 9 malignant cases failed to show it. Among 23 benign lesions, none showed choline peak at 3.22 ppm.

Correlation of spectroscopic findings with pathologic diagnosis was presented in Table 3.

The area under the ROC curve (AUC) of Chol/Cr was significant at 136 TEs (0.86, p value = 0.00) while was not statistically at 270 TEs (0.78, p value = 0.68). Roc curve study revealed that Chol/Cr ratio at 136 TEs with a cut-off point of 2.5 best correlates with histopathology results (sensitivity = 75%; specificity = 100%; PPV = 100%; NPV= 92%) (Figures 1 and 2, Table 4).

Table 2. Correlation between mean of different variables and pathology

	Pathology	N	Mean ± SD	*P* value
Chol/cr 136 ms	Benign	23	0.80 ± 0.60	0.018
	Malignant	9	6.60 ± 5.30	
Chol/cr 270 ms	Benign	10	0.80 ± 0.63	0.088
	Malignant	6	2.71 ± 2.20	

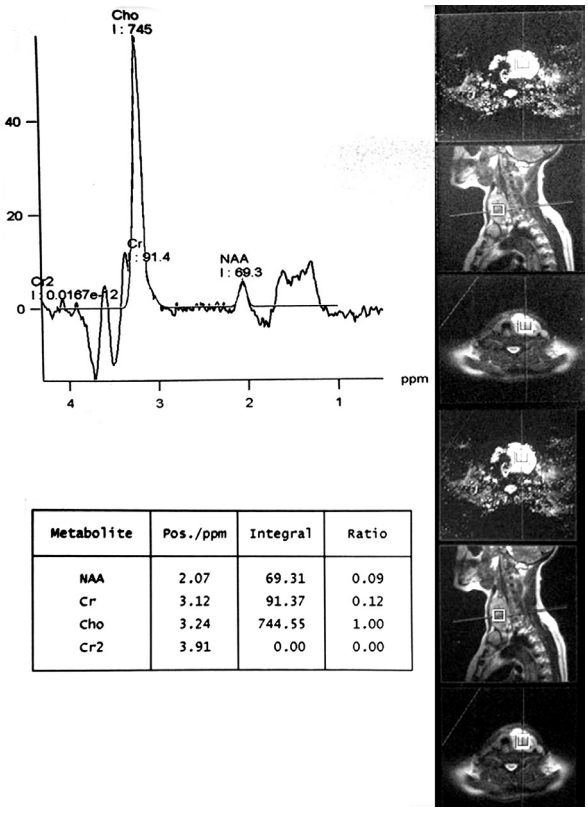

Figure 1. Graph of a malignant thyroid nodule with a very high choline (Chol) to creatine (Cr) ratio on MRS.

Table 3. Correlation of spectroscopic findings with pathologic diagnosis

Cho/cr	Number	Cut off 1.5		Cut off 2.5	
		TE 136	TE 270	TE 136	TE 270
Colloid cyst	4	0	0	0	0
Colloid nodule	7	1 of 7 (2.32)	0	0	0
Hashimoto	2	0	0	0	0
MNG	8	1 of 8 (1.67)	0	0	0
Nodular hyperplasia	2	1 of 2 (2.22)	1of 2 (2.32)	0	0
FCC	2	0	0	0	0
PTC	7	7 of 7	4 of 4	7 of 7	3 of 4 (2.39)

Table 4. Diagnostic values for Chol/Cr in cut off points at 136 TEs

Cut off	0.35	0.85	1.5	2.5
Sensitivity	1 (95% CI: 0.63 – 1)	0.88 (95% CI: 0.47 – 0.99)	0.75 (95% CI: 0.35 – 0.97)	0.75 (95% CI: 0.35 – 0.97)
Specificity	0.26 (95% CI: 0.10 – 0.48)	0.61 (95% CI: 0.39 – 0.80)	0.87 (95% CI: 0.66 – 0.97)	1 (95% CI: 0.85 – 1)
Efficiency	0.45 (95% CI: 0.27 – 0.64)	0.68 (95% CI: 0.49 – 0.83)	0.68 (95% CI: 0.49 – 0.83)	0.94 (95% CI: 0.79 – 0.99)
Positive Predictive value	0.32(95% CI: 0.15 – 0.56)	0.44 (95% CI: 0.20 – 0.70)	0.44 (95% CI: 0.19 – 0.70)	1.00 (95% CI: 0.45 – 1.00)
Negative Predictive value	1 (95% CI: 0.54 – 1)	0.93 (95% CI: 0.68 – 0.99)	0.93 (95% CI: 0.68 – 0.99)	0.92 (95% CI: 0.74 – 0.99)
Likelihood ratio of positive test	1.35 (95% CI: 1.06 – 1.72)	2.24 (95% CI: 1.26 – 3.97)	2.27 (95% CI: 1.26 – 3.97)	-
Likelihood ratio of negative test	-	4.87 (95% CI: 0.76 – 31.36)	4.87 (95% CI: 0.76 – 31.36)	4.00 (95% CI: 1.20 – 13.28)
Cohen's kappa coefficient (κ)	0.15 (95% CI: 0.01 – 0.30)	0.36(95% CI: 0.086 – 0.64)	0.36 (95% CI: 0.06 – 0.64)	0.82 (95% CI: 0.58 – 1.06)

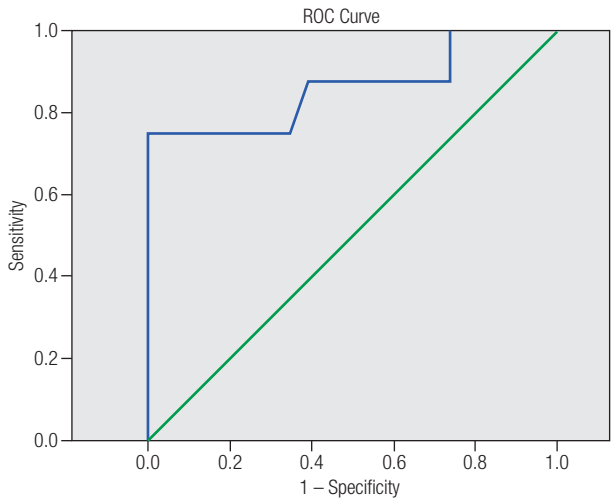

Figure 2. Receiver operating characteristic curve analysis for the prediction of malignant thyroid nodules based on choline (Chol) to creatine (Cr) ratio at 136 TE.

DISCUSSION

The study depicted that the findings of 3T MRS of thyroid nodules correlated with histopathology obtained surgically.

In term of imaging, ultrasonography, as a main modality in the management of patients with thyroid nodules, has some very useful discriminatory values in distinguishing benign from malignant lesion (4,7,18).

Though computed tomography and MR imaging studies allow a rapid and accurate assessment of the size of a goiter, its extension into the mediastinum, and its relationship to and impingement upon major structures with in the chest and neck, their accuracy is not high enough to different benign from malignant lesions (19).

Parallel in anatomical and functional changes, in addition to histologic and cytologic alterations in malignancies, metabolic processes at the cellular level are also identified to trace changes. These metabolic changes can be discovered and quantified using a state of the art technique acknowledged as magnetic resonance spectroscopy (MRS) which has recently gained interest as a potential cancer imaging technology e.g. in thyroid cancer. Literature about MRS of in vivo evaluation of thyroid lesions is very limited (17,20-22).

In a study, 1HMRS was carried out over tissue obtained at the time of surgery from 53 patients undergoing partial or total thyroidectomy for thyroid nodule (20). When compared with histological diagnosis, 1HMRS distinguished normal thyroid tissue from invasive papillary, anaplastic and medullary carcinoma with P values of < 0.001 with negative predictive value 100% and specificity of 52% (20).

Gupta and cols. studied 1.5T MRS in 25 patients with solitary thyroid nodule and they depicted a sensitivity and specificity of 100% and 94.11% respectively (21). They also had worked previously on 26 patients using 1.5T MRS and demonstrated sensitivity of 100% and specificity of 88.88% (22). King and cols. also worked 1HMRS in 13 patients of thyroid nodules with larger than 1 cm and demonstrated sensitivity of 87% and specificity of 100% (17).

In the current study, 3T MRS was carried out on 32 thyroid nodules in which the Chol/Cr ratio cut-off point of 2.5 best correlates with histopathology results (sensitivity = 75%; specificity = 100%; PPV = 100%; NPV = 92%).

In this study, 2 out of 9 malignant cases failed to delineate the choline peak at 3.22 ppm which most probably because of small size of follicular cell carcinoma lesions.

In addition, 3 benign thyroid nodules showed Chol/Cr more than 1.5 but less than 2.5 which could be due to hyperplastic foci as result of increased hypercelluraity as shown in 2 hyperplastic nodules in prior study (22).

In the current study, the sensitivity (75%) is less than two main prior studies (King and cols., 87%; Gupta and cols., 100%) while the specificity is comparable to two studies. It could be explained by two small follicular cancer lesions in our study.

In term of metabolite profile assessment, choline peak at 3.22 ppm is predominantly due to glycerophosphocholine and glycerophosphoethanolamine that form phospholipids of the cell membranes (23). The choline content rises in malignancy because of rapid multiplication and proliferation of cells (23). Height of choline peak depends on amount and nature of tissue under voxel. The creatinine peak indicates energy state of the cell (14,24).

In total, the numbers of cases especially cancers in this series is low and further studies with a larger series would give much greater confidence that the technique was worth pursuing in clinical practice.

In conclusion, magnetic resonance spectroscopy is a feasible option with promising results and it provided 100% specificity and 100% PPV in discrimination of benign from malignant thyroid nodules. Therefore, it can be complementary to other diagnostic techniques in patients with thyroid nodules; however, further

exploration especially considering large number of patients with different thyroid nodules sizes is needed to validate its clinical role.

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Is FGF-23 an early indicator of atherosclerosis and cardiac dysfunction in patients with gestational diabetes?

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ABSTRACT

Objective: Fibroblast growth factor 23 (FGF-23) is a phosphorus-regulating hormone and plays a role in the pathogenesis of myocardial hypertrophy. The aim of this study was to evaluate the association of FGF-23 levels with echocardiographic parameters and insulin resistance (IR) in patients with gestational diabetes. **Subjects and methods:** Fifty-four pregnant patients with gestational diabetes mellitus (GDM) (age, 31.12 ± 5.72 years) and 33 healthy pregnant women (age, 29.51 ± 4.92 years) were involved in the study. Fasting insulin, fasting plasma glucose (FPG), lipid profile, oral glucose tolerance test (OGTT), FGF23, echocardiographic parameters, and carotid artery intima-media thickness (CIMT) were evaluated in the two groups. **Results:** The two groups were not significantly different in age, sex, body mass index, lipid profile, or blood pressure. Insulin, homeostatic model assessment-insulin resistance (HOMA-IR), FGF-23 levels, CIMT, left ventricular (LV) mass, LV mass index and myocardial performance index (MPI) were significantly higher in the GDM group. HOMA-IR was positively correlated with FGF-23, and insulin was positively correlated with FGF-23. Additionally, FGF-23 was positively correlated with CIMT, LV mass index, and MPI. **Conclusion:** Our findings suggest that monitoring serum FGF-23 may be useful as a non-invasive indicator of subclinical atherosclerosis in patients with GDM. Arch Endocrinol Metab. 2018;62(5):506-13

Keywords
FGF-23; myocardial performance index; gestational diabetes mellitus

INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as a glucose intolerance with onset or first recognition occurring during pregnancy (1). It leads to maternal hyperglycemia, endothelial dysfunction, and abnormal regulation of vascular tone (2). GDM has serious adverse perinatal outcomes and increases long-term risk for the development of type 2 diabetes, obesity, and cardiovascular disease in mother and fetus (3).

Tissue Doppler imaging (TDI) was reported as appropriate for evaluating early changes in systolic and diastolic left ventricle (LV) function (4). The myocardial performance index (MPI) is a sensitive parameter widely used to quantitatively assess global ventricular function in a noninvasive way and combines systolic and diastolic intervals. It is related to morbidity and mortality in many cardiovascular diseases (5). MPI has been studied in several cardiac disorders including heart failure, hypertension, and diabetes, and it has been found to predict worsened morbidity and mortality (6-8). Left ventricular hypertrophy (LVH) is a marker of subclinical cardiovascular disease (CVD) and is associated with cardiovascular morbidity and mortality independent of established risk factors such as age, sex, and diabetes (9). The association between the left ventricular mass index (LVMI) and coronary flow reserve in patients with DM has been investigated in previous studies (10).

Fibroblast growth factor-23 (FGF-23) is a hormone involved in calcium–phosphate homoeostasis, primarily

produced and secreted by osteocytes (11). In the renal tubules, FGF-23 binds to the FGF receptor 1c and the klotho coreceptor to promote phosphaturia and lower circulating levels of 1,25-dihydroxyvitamin D (9). FGF-23 may also play a role in some metabolic processes, such as insulin resistance (IR), and may also be a marker of diabetes progression or may increase with diabetes-related complications (12). Moreover, serum FGF-23 was shown to be independently associated with LVH in chronic kidney disease (CKD) (13). It has been investigated in several diseases; however, to the best of our knowledge, the relationship between FGF-23 and myocardial function has not been studied yet in patients with GDM.

Carotid artery intima-media thickness (CIMT) assessment and carotid artery plaque identification using ultrasound are well-recognized tools for identification and monitoring of atherosclerosis. Elevated serum FGF-23 levels have been found to be associated with vascular diseases such as CIMT, arterial stiffness, and coronary atherosclerosis in patients with advanced CKD (14,15).

Therefore, we conducted a cross-sectional study to test the hypothesis that elevated FGF-23 concentrations are independently associated with LVMI, MPI, and CIMT in patients with gestational diabetes and to investigate the correlation between FGF-23 levels and IR in gestational diabetic patients compared to pregnant women with normal glucose tolerance.

SUBJECTS AND METHODS

This prospective study was conducted between 2014 May and 2015 May in the Department of Endocrinology and Metabolism of Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey. It was approved by the local ethical committee (Date: 01.08.2013, Decision Number: 2013712-10), and written informed consent was obtained from all subjects.

Sixty-four pregnant patients seen and diagnosed with GDM (the patient group) in endocrinology and metabolism outpatient clinics were included in the study. From the patient group, 10 patients were ruled out due to follow-up failure, and the study continued with 54 patients. Thirty-three consecutive healthy pregnant patients seen in an antenatal obstetrics and gynecology outpatient clinic were chosen as the control group.

Exclusion criteria

Patients were excluded if they had moderate to severe valvular heart disease, any rhythm other than the normal sinus rhythm, more than a mild degree of pericardial effusion, abnormal left ventricular systolic function (ejection fraction ≤ 50%), known coronary artery disease, clinical suspicion of coronary artery disease, uncontrolled hypertension, or acute illnesses. The patients who had previous impaired fasting glucose, impaired glucose tolerance or diabetes mellitus, family history of diabetes mellitus, chronic and acute renal failure, or a smoking habit and those who used calcium supplements or a vitamin D treatment were also ruled out from the study.

Study protocol

Fasting plasma glucose (FPG), LDL-cholesterol, HDL-cholesterol, triglycerides (TG), alanine aminotransferase (ALT), calcium, phosphate, insulin, 25 hydroxy vitamin D, parathyroid hormone (PTH), and fibroblast growth factor 23 (FGF-23) were measured at the 24th-28th gestational weeks in all subjects. Blood pressure and anthropometric measurements were performed, and body mass index was calculated. Additionally, an oral glucose tolerance test (OGTT) with 75 g glucose was performed at the 24th–28th gestational weeks in all subjects. Serum glucose levels were evaluated according to American Diabetes Association 2013 criteria (16).

Laboratory parameters

Blood samples for hormones and biochemical parameters were taken after an overnight fast from an antecubital vein between 08:00 a.m. and 09:00 a.m. Glucose was analyzed by the glucose hexokinase method. LDL, HDL, and TG were analyzed by the turbidymetric method (Siemens Dimension, Clinical Chemistry System, Newark, DE, USA), using appropriate commercial kits. Insulin was determined by a two-site chemiluminescent immunometric assay with solid-phase mouse monoclonal anti-insulin antibodies conjugated with calf intestine alkaline phosphatase in buffer solution (Immulite Insulin, Diagnostic Products Corporations, Los Angeles, CA) with 2 IU/mL sensitivity. The estimate of insulin resistance by the homeostasis model assessment (HOMA) score was calculated with the formula fasting serum insulin (lU/mL) x fasting plasma glucose (mmol/L)/ 22.5, as described by Matthews and coworkers (17). A HOMA index ≥ 2.5 indicated IR.

FGF-23 level measurement

Serum FGF-23 levels were determined, and venous blood samples were collected in tubes from the antecubital vein, followed by an overnight fast. The tubes were centrifuged at 2000g (10 min) to remove the serum. Aliquots of serum samples were stored at –80°C until FGF-23 assaying. Serum FGF-23 levels were determined using Human FGF-23 ELISA Kit (cat. number EZHFGF-23-32K) purchased from Millipore (USA) according to the manufacturer's instructions. The Millipore Human FGF-23 ELISA Kit employs the quantitative sandwich enzyme immunoassay technique.

Echocardiographic assessment

The echocardiographic examinations, including M-Mode, two-dimensional, SDE, and tissue Doppler echocardiography (TDI) recordings, were performed with Vivid 7 Pro (GE, Horten, Norway, 2–4 MHz phased array transducer). Measurements were made according to the American Society of Echocardiography guidelines by a single cardiologist unaware of the clinical data and averaged from five cardiac cycles.

For all participants, two-dimensional, M-Mode, and TDI echocardiography examinations were performed by the same physician. LV mass was calculated with the Devereux Formula (18) and indexed to body surface area.

In all TDI images, the systolic velocity duration was measured as ejection time (ET, ms), the time between the end of the systolic velocity and the beginning of early diastolic velocity was measured as isovolumetric relaxation time (IRT, ms), and the time between the end of the late diastolic velocity and the beginning of systolic velocity as isovolumetric contraction time (ICT, ms) MPI, which is calculated from systolic and diastolic time intervals, reflects the global left ventricular function with good reproducibility, and is independent from the left ventricle's geometry and heart rate.

The myocardial performance index was calculated using the formula (ICT + IRT)/ET.

Measurement of carotid intima-media thickness

Measurements of all participants' CIMT were performed by the same endocrinologist with the same ultrasound device (Logic P5 System, General Electric Medical Systems, Milwaukee, WI, USA) using a linear transducer of 12 MHz width. The carotid arteries were scanned in longitudinal projection. Intima media is defined as the distance between the beginning of the tunica intima and the beginning of the tunica adventitia. Intima media thickness measurements on the common carotid artery's far wall were bilaterally performed. For reproducible measurements, a high-quality image acquisition was used along a minimum length of 10 mm of an arterial segment. The mean CIMT was calculated from three consecutive examinations.

Statistical analysis

Statistical analysis was conducted with Statistical Package for Social Sciences 15.0 packet program (SPSS Inc., Chicago, IL). Descriptive statistics were shown as mean±standard deviation. The student t test was used to compare continuous variables between two groups. The difference between the groups regarding the qualitative variables was investigated with a Chi-square test. A p value less than 0.05 was considered statistically significant. Similarly, for bivariate correlation coefficients, parametric data was analyzed using Pearson's rho.

RESULTS

Fifty-four pregnant women (age, 31.12 ± 5.72 years) who had GDM and 33 healthy pregnant women (age, 29.51 ± 4.92 years) were involved in the study. The GDM patients' and controls' demographic and biochemical characteristics are presented in Table 1. There was no significant difference in age, sex, lipid parameters, body mass index (BMI), or systolic and diastolic blood pressure. FPG, oral glucose tolerance test first hour glucose ($OGTTglucose_{1)}$, oral glucose tolerance test second hour glucose ($OGTTglucose_{2)}$), insulin, HOMA-IR, FGF-23 levels, and mean CIMT were significantly higher in the GDM group.

Changes in the patient group's and control group's conventional and Doppler echocardiographic measurements are presented in Table 2. LV mass and LV mass index were significantly higher in the patient group. No significant difference existed between the groups' left ventricular end-diastolic dimensions (LV EDDs), left ventricular end-systolic dimensions (LV ESDs), interventricular septum thickness (IVS), posterior wall thickness (PW), left atrial dimension, left ventricular ejection fraction (LV EF), mitral E and A velocity, E/A, DT, or LA volume index.

Tissue Doppler echocardiographic parameters of the gestational diabetes and control groups are

presented in Table 3. MPI was significantly higher in the patient group. HOMA-IR did not show a positive correlation with FGF-23, PW, or IVS (r = 0.309, p = 0.008; r = 0.336, p = 0.004; and r = 0.356, p = 0.002, respectively), but it showed a negative correlation with HOMA-IR and LV EDD (r = -0.242, p = 0.039).

Table 1. Characteristics of patient and control groups

Parameters	Patient group n = 54	Control group n = 33	p
Age (years)	31.12 ± 5.72	29.51 ± 4.92	0.105
BMI (kg/m^2)	32.05 ± 5.84	30.12 ± 4.42	0.183
BSA (m^2)	1.81 ± 0.17	1.78 ± 0.15	0.437
Systolic BP (mmHg)	110.48 ± 10.63	106.36 ± 13.09	0.113
Diastolic BP (mmHg)	69.57 ± 9.06	66.45 ± 8.76	0.055
FPG (mg/dL)	89.70 ± 12.28	75.27 ± 6.25	< 0.001
OGTTglucose$_1$ (mg/dL)	189.53 ± 30.97	126.48 ± 26.22	< 0.001
OGTTglucose$_2$ (mg/dL)	151.68 ± 25.78	103.33 ± 22.36	< 0.001
LDL (mg/dL)	108.98 ± 33.99	117.05 ± 33.11	0.281
HDL (mg/dL)	65.86 ± 21.60	66.21 ± 10.46	0.930
TG (mg/dL)	200.73 ± 61.05	183.21 ± 50.27	0.169
ALT (U/L)	18.64 ± 7.27	18.15 ± 6.13	0.744
Insulin (uIU/L)	12.84 ± 9.10	9.07 ± 4.99	0.032
HOMA-IR	2.71 ± 1.98	1.75 ± 1.04	0.021
Calcium (mg/dL)	8.82 ± 0.30	8.75 ± 0.27	0.284
Phosphate (mg/dL)	3.58 ± 0.41	3.75 ± 0.36	0.069
25 OH D (ng/mL)	34.31 ± 4.25	33.93 ± 3.42	0.669
PTH (pg/mL)	39.06 ± 3.38	38.67 ± 3.39	0.600
CRP	3.13 ± 0.25	3.17 ± 0.23	0.446
FGF 23 (pg/mL)	162.24 ± 22.41	68.36 ± 10.96	< 0.001
CIMT (cm)	0.064 ± 0.009	0.057 ± 0.007	< 0.001

BMI: body mass index; BSA: body surface area; BP: blood pressure; FPG: fasting plasma glucose; OGTTglucose$_1$: oral glucose tolerance test first hour glucose; OGTTglucose$_2$: oral glucose tolerance test second hour glucose; LDL: low density lipoprotein; HDL: high density lipoprotein; TG: triglycerides; ALT: alanine aminotransferase; HOMA-IR: homeostatic model assessment index-insulin resistance; 25 OH D: 25 hydroxy vitamin D; PTH: parathyroid hormone; FGF-23: fibroblast growth factor; CIMT: carotid intima-media thickness.

Table 2. Conventional and Doppler echocardigraphic measurements of the gestational diabetes and control groups

Parameters	Patient group n = 54	Control group n = 33	p
LV EDD (mm)	46.00 ± 3.12	44.88 ± 5.25	0.274
LV ESD (mm)	28.12 ± 6.51	28.21 ± 3.01	0.946
IVS (mm)	9.92 ± 3.83	8.60 ± 1.63	0.063
PW (mm)	13.16 ± 3.60	8.17 ± 1.47	0.384
LV mass (g)	164.75 ± 42.04	139.30 ± 29.37	0.003
LV mass index (g/m^2)	90.47 ± 20.16	77.85 ± 15.87	0.003
Left atrial dimension (mm)	50.16 ± 11.79	48.81 ± 11.02	0.597
LV EF (%)	68.72 ± 4.74	68.63 ± 4.16	0.932
Mitral E velocity (cm/s)	83.11 ± 13.26	87.93 ± 14.59	0.117
Mitral A velocity (cm/s)	68.38 ± 11.13	71.36 ± 13.76	0.273
E/A	1.23 ± 0.24	1.25 ± 0.23	0.704
DT (ms)	170.48 ± 34.59	167.90 ± 35.23	0.739
LA volume index (mL/m^2)	27.81 ± 6.29	27.37 ± 5.67	0.741

LV: left ventricular; LV EDD: LV end-diastolic dimension; LV ESD: LV end-systolic dimension; IVS: interventricular septum thickness; PW: posterior wall thickness; EF: ejection fraction; E: early diastolic velocity; A: late diastolic velocity; DT: mitral E-wave deceleration time; LA: left atrium.

Insulin had a positive correlation with FGF-23, PW, and IVS (r = 0.271, p = 0.011; r = 0.264, p = 0.014; r = 0.282, and p = 0.008, respectively). Additionally, FPG had a positive correlation with BMI, CIMT, FGF-23, LV mass index, PW, IVS, RVL-MPI, IVS-MPI, and LVL-MPI (r = 0.274, p = 0.010; r = 0.209, p = 0.052; r = 0.519, p < 0.001; r = 0.291, p = 0.006; r = 0.312, p = 0.003; r = 0.406, p < 0.001; r = 0.240, p = 0.025; and r = 0.285, p = 0.007, respectively). FGF-23 had a positive correlation with CIMT, LV mass index, RVL-MPI, IVS-MPI, and LVL-MPI (r = 0.400, p < 0.001; r = 0.282, p = 0.008; r = 0.544, p < 0.001; r = 0.310, p = 0.004; and r = 0.268, p = 0.012, respectively) (Table 4).

DISCUSSION

Several previous studies demonstrated that FGF-23 is independently associated with several cardiovascular risk factors, such as endothelial dysfunction, increased arterial stiffness, LVH, left ventricular dilatation impaired vasoreactivity, cardiovascular mortality, and atherosclerosis in hemodialysis patients and healthy subjects (14,15). CIMT assessment and common carotid artery plaque identification by ultrasound are well-recognized tools for identification and monitoring of atherosclerosis. Elevated serum FGF-23 levels have been found to be associated with vascular diseases such as CIMT, arterial stiffness, and coronary atherosclerosis in patients with advanced CKD (16,17). Although Balci and cols. (19) reported a positive correlation between CIMT and FGF-23 in hemodialysis patients, Gungor and cols. (20) did not find any correlation. Several studies have indicated that a pregnancy complicated by GDM has a significant impact on endothelial function during pregnancy (21,22). However, the impact of FGF-23 on the early stages of atherosclerosis in patients with gestational diabetes is not defined. In this study, mean CIMT was significantly higher in patients with GDM, and a positive correlation between FGF-23 and CIMT was found. Association of FGF-23 with CIMT may be another possible mechanism underlying the development and progression of atherosclerosis in patients with GDM.

The myocardial performance index reflects LV systolic and diastolic function. MPI is also abnormal in individuals without overt cardiac disease who have risk factors such as diabetes mellitus and treated and untreated hypertension (17,23,24). In a recent study, MPI was measured by the conventional method and with tissue Doppler echocardiography. It was significantly higher in the diabetic group than in the control group (25). In patients with CKD, FGF-23 supraphysiologic levels were strongly associated with LVMI, left ventricular hypertrophy (LVH), cardiovascular events, and mortality (14,26). High prevalence of preclinical diastolic dysfunction is a well-established fact among diabetic patients. The evidence indicates that myocardial damage in diabetic subjects affects diastolic function before the systolic function (27). Using tissue Doppler and strain/strain rate echocardiography, a recent study showed that LV longitudinal systolic and diastolic functions were impaired in diabetic patients (28). However, limited studies have been conducted on GDM's effect on diastolic functions (29). Caliskan and

Table 3. Tissue Doppler echocardiographic parameters of the gestational diabetes and control groups

Parameters	Patient group n = 54	Control group n = 33	p
Left ventricular lateral wall			
Sm (cm/s)	10.75 ± 2.61	10.42 ±1.71	0.514
Em/Am	1.49 ± 0.42	1.34 ± 0.68	0.211
MPI	0.50 ± 0.94	0.44 ± 0.08	0.004
Interventricular septum			
Sm (cm/s)	8.22 ± 1.65	9.15 ± 2.07	0.024
Em/Am	1.28 ± 0.41	1.13 ± 0.55	0.154
MPI	0.52 ± 0.07	0.42 ± 0.09	0.002
Right ventricular lateral wall			
Sm (cm/s)	16.48 ± 3.73	18.87 ± 5.12	0.014
Em/Am	0.97 ± 0.35	0.84 ± 0.43	0.128
MPI	0.55 ± 0.09	0.41 ± 0.07	< 0.001

Sm: systolic myocardial velocity; Em: myocardial early diastolic velocity; Am: myocardial late diastolic velocity; MPI: myocardial performance index.

cols. (29) found that although LV systolic function and LVMI were similar between the two groups (GDM and control groups), the diastolic function parameters were impaired in the GDM group. In our study, although LVMI was significantly higher in the GDM group than in the control group, no significant difference existed in diastolic function parameters (mitral E/A ratio) between groups. Sm determined by TDI is another measure of LV systolic functions independent from the LV's shape (31). In our study, Sm had fewer patients with GDM, indicating the presence of possible subclinical systolic dysfunction. MPI was significantly higher in patients with GDM than in the control group. Additionally, FGF-23 showed a significant positive correlation with MPI. Therefore, we think MPI, which reflects diastolic and systolic functions, is a more important marker than mitral inflow velocity (E/A) in the evaluation of cardiac functions.

The data concerning the relationship between FGF-23 levels and IR are contradictory. Wahl and cols. (13) have observed that FGF-23 levels are greater in coronary artery disease patients who have diabetes. Garland and cols. (30) reported that increasing HOMA-IR was positively associated with FGF-23 in stage 3-5 chronic kidney disease patients. Holecki and cols. (31) showed increased levels of both circulating FGF-23 forms in elderly subjects are not associated with obesity or IR. Hanks and cols. (32) found that the surrogate marker of IR, HOMA-IR were positively associated with FGF-23. The relationship between

Table 4. Correlation between metabolic parameters, FGF-23, convensional and tissue doppler echocardiographic parameters of the all participants

Parameters		HOMA-IR	Insulin	FPG	OGTTglucose$_1$	OGTTglucose$_2$	FGF-23
BMI (kg/m^2)							
	r	0.048	0.025	0.274	0.075	0.234	0.181
	p	0.686	0.817	0.010	0.489	0.029	0.094
CIMT (cm)							
	r	0.136	0.126	0.209	0.339	0.156	0.400
	p	0.251	0.244	0.052	0.001	0.150	< 0.001
FGF-23							
	r	0.309	0.271	0.519	0.631	0.679	-
	p	0.008	0.011	< 0.001	< 0.001	< 0.001	-
LVMI (g/m^2)							
	r	0.097	0.076	0.291	0.180	0.262	0.282
	p	0.414	0.482	0.006	0.095	0.014	0.008
E/A							
	r	0.045	0.052	-0.086	-0.090	-0.070	-0.030
	p	0.707	0.632	0.430	0.407	0.517	0.783
LV mass (g)							
	r	0.159	0.109	0.312	0.178	0.259	0.298
	p	0.178	0.316	0.003	0.099	0.016	0.005
RVL-MPI							
	r	0.196	0.143	0.406	0.455	0.464	0.544
	p	0.096	0.187	< 0.001	< 0.001	< 0.001	< 0.001
IVS-MPI							
	r	0.087	0.153	0.240	0.230	0.172	0.310
	p	0.462	0.158	0.025	0.032	0.110	0.004
LVL-MPI							
	r	0.032	0.031	0.285	0.244	0.248	0.268
	p	0.788	0.774	0.007	0.023	0.021	0.012

BMI: body mass index; CIMT: carotid intima-media thickness; FGF-23: fibroblast growth factor 23; LVMI: left ventricular mass index; E: mitral early diastolic velocity; A: mitral late diastolic velocity; RVL: right ventricular lateral wall; LVL: left ventricular lateral wall; MPI: myocardial performance index; HOMA-IR: homeostatic model assessment index-insulin resistance; FPG: fasting plasma glucose; OGTTglucose$_1$: oral glucose tolerance test first hour glucose; OGTT glucose$_2$: oral glucose tolerance test second hour glucose

IR and FGF-23 has not been investigated in patients with GDM. This study showed a positive correlation between HOMA-IR and FGF-23. Association of FGF-23 with IR may be another possible mechanism underlying the development and progression of atherosclerosis in DM patients.

In conclusion, the present prospective study is the first in the literature that evaluated the relationship between FGF-23 and MPI in patients with gestational diabetes in the absence of CVD. Plasma FGF-23 levels were shown to be associated with increased LVMI, MPI, and CIMT in patients with GDM. The significant and positive association of FGF-23 plasma level with LVMI and MPI raises the possibility of a specific pathophysiologic effect of FGF-23 on left ventricular mass and function distinct from its effects on serum calcium, phosphorus, and intact parathormone. Additionally, this study showed that FGF-23 may play a role in the development of GDM with IR. Therefore, we believe that FGF-23 plays a role in the pathogenesis and development of preclinical atherosclerosis and cardiac dysfunction in patients with GDM.

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Impact of an inpatient multidisciplinary glucose control management program

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ABSTRACT

Objective: Glycemic control has been increasingly recognized as a critical element in inpatient care, but optimal management of blood glucose in the hospital setting remains challenging. The aims of this study were to describe and evaluate the impact of the implementation of an inpatient multidisciplinary glucose control management program on glucose control in hospitalized patients. **Materials and methods:** Retrospective analysis of medical records and glucose monitoring data obtained by point-of-care testing (POCT) in hospitalized patients before (May 2014) and after (June 2015 and May 2017) the implementation of the program. **Results:** We analyzed 6888, 7290, and 7669 POCTs from 389, 545, and 475 patients in May 2014, June 2015, and May 2017, respectively. Hyperglycemia (≥ 180 mg/dL) occurred in 23.5%, 19.6%, and 19.3% POCTs in May 2014, June 2015, and May/2017, respectively ($p < 0.001$), while severe hyperglycemia (≥ 300 mg/dL) was observed in 2.5%, 2.2%, and 1.8% of them, respectively ($p = 0.003$). Hyperglycemia (≥ 180 mg/dL) reduced significantly from May 2014 to June 2015 (16.3%, $p < 0.001$) and from May 2014 to May 2017 (17.8%, $p < 0.001$). No significant changes occurred in hypoglycemic parameters. **Conclusions:** The implementation of an inpatient multidisciplinary glucose control management program led to significant reductions in hyperglycemic events. The key elements for this achievement were the development of institutional inpatient glycemic control protocols, establishment of a multidisciplinary team, and continuing educational programs for hospital personnel. Altogether, these actions resulted in improvements in care processes, patient safety, and clinical outcomes of hospitalized patients. Arch Endocrinol Metab. 2018;62(5):514-22

Keywords
Hyperglycemia; hypoglycemia; inpatient glucose control; diabetes mellitus; systems, point-of-care

INTRODUCTION

Glycemic control has been increasingly recognized as a critical element in inpatient care (1-7). Several lines of evidence corroborate the concept that both hyperglycemia and hypoglycemia are associated with adverse outcomes to the patient. Hyperglycemia may occur in hospitalized patients with known diabetes or acutely ill individuals with previously normal glucose tolerance ("stress hyperglycemia") due to increased circulating counterregulatory hormones in response to stress. Irrespective of the cause, hyperglycemia on an inpatient setting is an independent marker of increased morbidity and mortality. During treatment of hyperglycemia, a major concern is the occurrence of hypoglycemia, which is also an independent risk factor for poor clinical outcomes (8-15). Therefore, great emphasis has been placed on optimizing the treatment of hospitalized patients with diabetes and hyperglycemia.

Based on data from multiples studies and clinical trials, the management of hyperglycemia in a hospital setting has recently evolved (16-23). Current consensus statements from the American Diabetes Association, American Association of Clinical Endocrinologists, Endocrine Society, and Brazilian Diabetes Association have recommended therapy of critically ill patients with persistent hyperglycemia, starting at a blood glucose level of 180 mg/dL; once insulin is started, the therapeutic glucose target should be within the 140-180 mg/dL range (1-6). In noncritical care patients, the recommended values are < 140 mg/dL for fasting glucose and < 180 mg/dL for random glucose, and

the preferable regimen is the use of a basal insulin along with premeal and supplemental insulin, instead of sliding scale insulin. Optimal glycemic control also includes the prevention of hypoglycemia (1-6,24-26). Reducing the variability in glucose levels may also be important in improving outcomes (27,28).

Despite existing evidence, the optimal glucose management in a hospital setting remains challenging, as the achievement of improved glycemic control in a hospital setting meets numerous obstacles. In this scenario, the American College of Endocrinology, the American Diabetes Association, and the Brazilian Diabetes Society have released calls to action outlining strategies for a successful implementation of inpatient glucose control management programs (2,5,7). System-based key issues outlined included the need for the development and evaluation of: 1) clinical protocols to guide management, clinical decisions, and prescriptions; 2) multidisciplinary glucose control management teams; and 3) provider-delivered educational programs to improve knowledge and address barriers to achieving glycemic control (2,5,7,29,30).

The aims of this study were to describe and evaluate the impact of the implementation of a multidisciplinary glucose control management program for hospitalized patients. The study was conducted to determine if such program would improve inpatient safety by reducing the number of hyperglycemic events.

MATERIALS AND METHODS

The study was conducted at the *Pró-Cardíaco Hospital*, a 99-bed, tertiary-care, medical and surgical center located in Rio de Janeiro, Brazil. The medical center comprises an emergency room, two adult intensive care units (ICU), one coronary care unit, one surgical intensive care unit, one surgical semi-intensive care unit, three clinical semi-intensive units, one oncology care unit, and one day clinic. The hospital has an extensive referral network and includes outpatient specialty treatment and imaging centers, clinics, and rehabilitation centers.

Since 2001, the ICUs of the hospital have developed a protocol of insulin treatment for critically ill patients. In 2012, we introduced a hospital-wide inpatient multidisciplinary glucose control management program (MGCP) to facilitate the development of uniform glucose management policies and staff education based on current clinical practice guidelines. The main hallmarks of this program were the development of an Institutional inpatient glycemic control protocols in January 2012, and the establishment of a multidisciplinary glycemic control team in June 2014.

Institutional inpatient glycemic control protocols

In 2012, institutional glycemic control protocols were developed in an effort to improve and standardize the glycemic control of hospitalized patients. These protocols are aligned with international and local recommendations (2-6) and are frequently revised and updated based on these recommendations and local assistance requirements.

According to the institutional protocol, blood glucose levels of all critical and noncritical care patients admitted to the hospital are monitored using point-of-care testing (POCT). Glucose monitoring may be suspended 72 hours after admission of noncritically ill patients and in those without diabetes or current illness, or not using medications associated with hyperglycemia or hypoglycemia. Monitoring may also be suspended in patients whose blood glucose measurements have been within the normal range for 72 hours. All results obtained by POCT are downloaded directly from the glucometer (Precision®, Abbott Diabetes Care Inc., Alameda, CA, USA) to the patient's electronic medical records. In case of glucose measurements ≤ 40 mg/dL or ≥ 300 mg/dL, a direct notification is sent via e-mail from the medical record to the endocrinologist in charge of the patient.

Insulin therapy is the method of choice for glycemic control in hospitalized patients with hyperglycemia. The institutional protocol recommends the discontinuation of antidiabetic drugs for most patients upon hospital admission for acute illness. Patients with type 1 or 2 diabetes receiving insulin as multiple daily injections require treatment with basal-bolus insulin regimens, and their insulin doses are modified according to the patient's clinical status.

In noncritical care patients, the glycemic targets set by the institutional protocol are < 140 mg/dL before meals and < 180 mg/dL for random glucose measurements. In patients with terminal illness and/or limited life expectancy, the glycemic target is < 180 mg/dL. In noncritical care patients, POCTs are performed based on the timing of the meals: before meals in patients receiving an oral diet, every 6 hours in those with continuous enteral or parenteral nutrition, and every 4 hours in patients not receiving diet. Patients

with hyperglycemia (glucose level ≥ 200 mg/dL) undergo more frequent glucose measurements for detection and treatment of hyperglycemia, prevention of hypoglycemia following supplemental insulin administration, and prevention of glycemic variability. The protocol recommends a basal-bolus insulin regimen, including a basal component with a long-acting insulin analogue (glargine or detemir) or intermediate-acting insulin (NPH) once or twice daily, and a bolus component with ultra-rapid-acting insulin (lispro) administered according to meals and supplemental doses according to glycemic levels. Ultra-rapid insulin analogues are the insulin of choice for the bolus component of the regimen at our institution, based on evidence in the literature showing better glycemic control in hospitalized patients with this type of insulin when compared with regular insulin, with a lower number of hypoglycemic episodes (24). The total dose of insulin administered is individualized and based on the patient's previous insulin regimen, glycemic levels, total body weight, clinical status, and nutritional therapy. Basal insulin is administered to all patients with previous insulin regimens and in those with sustained hyperglycemia. In insulin-naïve patients, the recommended initial total daily insulin dose is 0.2-0.5 U/kg/day, with approximately 50% of the dose administered as basal insulin (preferably glargine) and the remainder as bolus insulin. Bolus insulin contemplates the patient's diet and carbohydrate intake, as follows: bolus insulin before meals in patients on an oral diet, every 6 hours in those on continuous enteral or parenteral nutrition therapy, and 3 to 4 times a day before meals in patients receiving cyclic enteral nutrition. The supplemental component of bolus insulin is administered according to the patient's glucose level. Our institution has five different supplemental insulin regimens: (i) usual insulin dose; (ii) reduced insulin dose, recommended for patients at risk for hypoglycemia; (iii) increased insulin dose, recommended for patients with insulin resistance; (iv) a regimen for patients with terminal illness and/or limited life expectancy; and (v) a regimen for patients with no oral or enteral nutritional therapy (fasting).

For critical care patients, blood glucose levels are measured at one-hour intervals. Insulin therapy is started in critically ill patients with sustained hyperglycemia, defined as at least two glucose measurements ≥ 180 mg/dL, using continuous intravenous regular insulin infusion. In patients with continuous intravenous insulin infusion, the glycemic targets are 140-180 mg/dL, and glucose levels < 100 mg/dL should be avoided. Insulin infusion is adjusted every hour according to glucose levels. The continuous intravenous insulin infusion protocol used at the institution was adapted from the Yale Insulin Infusion Protocol for critically ill patients, as previously described (23).

Multidisciplinary glycemic control team

A multidisciplinary glycemic control team was created at our institution in June 2014. The main goal of its implementation was to develop a centralized multidisciplinary team to address barriers to achieving glycemic control in the hospital setting. The team is chaired by an endocrinologist and includes physicians (endocrinologist, intensivists, hospitalists, and house staff), nurse practitioners, pharmacists, dietitians, and POCT/laboratory medicine specialists. The team aims at promoting the correct implementation of protocols for management of hyperglycemia and hypoglycemia, educating physicians and nurses on the proper use of these protocols, performing continuous education of health care professionals, promoting clinical decision aids, and surveilling performance measures for quality improvement. The members of the team deliberate on regular monthly meetings and on a daily basis during continuous patient care.

Education programs for hospital personnel

The educational programs for hospital personnel are delivered on a regular basis and include the participation of nurses, house staff, physicians, pharmacists, and dietitians. Educational sessions are often offered in the hospital at different time periods to ensure delivery to as many staff members as possible. All staff members (physicians, nurses, pharmacists, and dietitians) are exposed to the educational program upon joining the hospital staff. Regular educational sessions are delivered at different hospital units (a total of 10 units) at a maximum interval of 6 months. Once weekly, the endocrinologist in charge delivers educational orientation to staff members during patient care (on-site training).

The main aspects outlined in these educational programs include the impact of inpatient glycemic control on patient care, introduction and reinforcement of protocols for management of hyperglycemia and hypoglycemia, information about patients at risk for

hypoglycemia and hyperglycemia, identification of signs of hypoglycemia, characteristics of the different types of insulin and administration routes (intravenous or subcutaneous for basal insulin administration, prandial or correction doses), a review of insulin requirements during health and illness, inpatient use of antihyperglycemic agents, the influence of diet, the importance of respecting the appropriate time of glucose measurement, and proper documentation of patient treatment.

Glucose monitoring by point-of-care testing

Glucose monitoring by POCT was performed with the glucometers Precision Xceed Pro (PXP)® and FreeStyle Precision Pro (FSPP)® (Abbott Diabetes Care Inc., Alameda, CA, USA). In noncritical patients, glycemic measurements with POCT used capillary blood samples obtained by fingertip puncture after local hygiene, while in critical patients, venous or arterial blood samples were used instead. The same type of blood source was used in each patient according to his or her clinical status. Areas with edema, lesions, hypoperfusion, and/or venous infusion routes with continuous infusion of solutions were avoided during blood drawing.

The glucometers underwent continuous quality control. All devices were calibrated every 24 hours with high and low glucose control samples; when calibration was not performed in 24 hours, the glucometer was automatically blocked from use or for POCT. All glucometers underwent a harmonization process every 6 months, consisting on a comparison of the results obtained by POCT with those obtained by the laboratory (Dimension®, Siemens Healthcare Diagnostics, Deerfield, IL, USA). During the study, the variation coefficient between the results obtained by the POCT and those by the laboratory was < 10.78% in all glucometers, which is aligned with standards of care (31).

Glycemic control quality indicators

Since the MGCP implementation, quality indicators of glycemic control, hypoglycemia, and hyperglycemia were monthly assessed using data from the POCTs. All results obtained by POCT were electronically downloaded directly from Abbott's Precision® glucometer to the software using the Abbott Precision Web System (Abbott Diabetes Care Inc., Alameda, CA, USA) and to the patient's electronic medical records, providing accurate data for the indicators. Calculation of the rates of hyperglycemia and hypoglycemia were as follows: (i) hyperglycemia (≥ 180 mg/dL) as the number of glucose measurements by POCT ≥ 180 mg/dL (numerator) divided by the total number of glucose measurements by POCT performed in that given period (denominator), (ii) severe hyperglycemia (≥ 300 mg/dL) as the number of glucose measurements by POCT ≥ 300 mg/dL (numerator) divided by the total number of glucose measurements by POCT performed in the period (denominator), (iii) hypoglycemia (≤ 70 mg/dL) as the number of glycemic measurement by POCT ≤ 70 mg/dL (numerator) divided by the total number of glycemic measurement by POCT performed in the period (denominator), (iv) severe hypoglycemia (≤ 40 mg/dL) as the number of glycemic measurement by POCT ≤ 40 mg/dL (numerator) divided by the total number of glycemic measurement by POCT performed in the period (denominator).

Adherence to the institutional inpatient glycemic protocols was measured regularly by a revision of the patient's prescriptions and medical records.

Data collection

We performed a retrospective analysis of the medical records of the patients admitted to the hospital. We analyzed the data obtained in May 2014, before the MGCP implementation, and in June 2015 and May 2017, after the MGCP implementation.

The inclusion criteria were all critical and noncritical patients admitted to the hospital, aged ≥ 18 years, who had blood glucose measured by POCT by Abbott's Precision® glucometer according to the institutional protocol, and a length of stay ≥ 2 days. The exclusion criteria were age < 18 years, length of stay shorter than 2 days, and admissions limited to the emergency room or to the day-clinic unit. We also excluded the results of POCT glucose monitoring obtained during surgical procedures.

We analyzed the quality indicators of glycemic control and the results of glucose monitoring obtained by POCT before the implementation of the MGCP in May 2014 and after the implementation of the MGCP in June 2015 and May 2017. Based on blood glucose levels, the patients were characterized as having hyperglycemia (≥ 180 mg/dL), severe hyperglycemia (≥ 300 mg/dL), hypoglycemia (≤ 70 mg/dL), or severe hypoglycemia (≤ 40 mg/dL). The rates of hyperglycemia (≥ 180 mg/dL), severe hyperglycemia

(≥ 300 mg/dL), hypoglycemia (≤ 70 mg/dL), and severe hypoglycemia (≤ 40 mg/dL) were calculated as described above.

Adherence to the institutional inpatient glycemic protocols was also analyzed by a revision of the patients' prescriptions and medical records in May 2014, June 2015, and May 2017. A prescription was considered to be not compliant to the institutional protocols if inadequate to the diet or clinical scenario. Inadequacy with the diet was present when the prescription was not conformed with the type of diet (*i.e.*, oral, enteral, and parenteral diets, or fasting), not coordinated with the POCT, or when insulin was not administered around mealtime. Inadequacy with the clinical scenario occurred when the insulin prescribed was not suitable for the patient's clinical status according to the institutional protocol (*i.e.*, intravenous insulin for critical patients, subcutaneous insulin for noncritical patients, basal-bolus insulin regimen for noncritical patients with sustained hyperglycemia and/or previous use of a basal-bolus insulin regimen, or supplemental subcutaneous insulin for noncritical patients in accordance to patients characteristics (usual insulin dose, risk of hypoglycemia, insulin resistance, terminal illness, and/or limited life expectancy).

The local institutional ethics committee approved the study.

Statistical analysis

Continuous data are presented as mean and standard deviation values or median values and range. Comparisons of categorical variables were performed with the Fisher's exact or chi-square test, while continuous variables were compared using Student's t test.

The statistical analyses were performed with the software programs SPSS, version 20 (SPSS Inc., Chicago, IL, USA), Minitab 16, and Excel Office 2010. Statistical significance was set at $p < 0.05$.

RESULTS

The clinical characteristics of the hospitalized patients undergoing glucose monitoring in May 2014, June 2015, and May 2017 are described in Table 1. The groups had comparable baseline clinical characteristics except for age, which differed among the groups.

We analyzed 6888, 7290, and 7669 POCTs from 389, 545, and 475 patients in May 2014, June 2015, and May 2017, respectively. The mean number of glucose measurements per patient was 2.39 ± 1.96, 2.39 ± 2.26, and 3.64 ± 2.76 in May 2014, June 2015, and May 2017, respectively. There was a significant increase in glucose monitoring from May 2014 to May 2017 ($p = 0.007$), but no differences between May 2014 and June 2015 ($p = 0.99$).

Table 2 describes the quality indicators of inpatient glycemic control. In May 2014, June 2015, and May 2017, the rates were 23.5%, 19.6%, and 19.3%, respectively, for hyperglycemia (≥ 180 mg/dL; $p < 0.001$), and 2.5%, 2.2%, and 1.8%, respectively, for severe hyperglycemia (≥ 300 mg/dL; $p = 0.003$).

Table 1. Baseline clinical characteristics of hospitalized patients with glucose measured by point-of-care testing (POCT)

	May 2014	June 2015	May 2017	p value
Number of patients undergoing POCT	389	545	475	
Age (years)	72.7 ± 16.6	73.0 ± 16.2	75.6 ± 14.6	0.04*
Admission				
Clinical patients	80.8%	83.9%	78.5%	0.086
Surgical patients	19.2%	16.1%	21.5%	
Patients' characteristics at admission				
Critical care	10.4%	13.4%	9.7%	0.141
Noncritical care	89.6%	86.6%	90.3%	
Diabetes mellitus	33.7%	30.4%	35.1%	0.176
Rate of hyperglycemia (≥ 180 mg/dL) on admission	22.1%	18.9%	19.6%	0.465
Length of stay (days)	5 (2-919)	5 (2-919)	5 (2-967)	0.98
Intravenous insulin protocol	7.3%	8.8%	6.5%	0.371

Data are expressed as mean ± standard deviation, mean (range), or percentage. * Statistically significant ($p < 0.05$).

The rates of hyperglycemia (≥ 180 mg/dL) reduced significantly from May 2014 to June 2015 (16.3%, p < 0.001) and from May 2014 to May 2017 (17.8%, p < 0.001). Similarly, the rates of severe hyperglycemia (≥ 300 mg/dL) reduced statistically significantly from May 2014 to May 2017 (28.5%, p = 0.003), but were non-statistically significant between May 2014 and June 2015 (13.6%, p = 0.175) (Figures 1 and 2).

There was no statistically significant change in hypoglycemic parameters over time (Table 2). Rates of hypoglycemia (≤ 70 mg/dL) were 0.9%, 1.8%, and 1.0% in May 2014, June 2015, and May 2017, respectively (p = 0.710), while the rates of severe hypoglycemia (≤ 40 mg/dL) showed a non-statistically significant decrease of 34.3% from May 2014 to May 2017 (p = 0.336).

Table 3 describes the rates of adherence to the institutional inpatient glycemic protocols. Adherence to the protocol improved over time. The proportion of prescriptions not compliant with the institutional protocols decreased from 34.2% in May 2014 to 10.1% in June 2015, and 7.5% in May 2017 (p < 0.001). We found significant decreases in diet and clinical scenario inadequacies over time from May 2014 to May 2017.

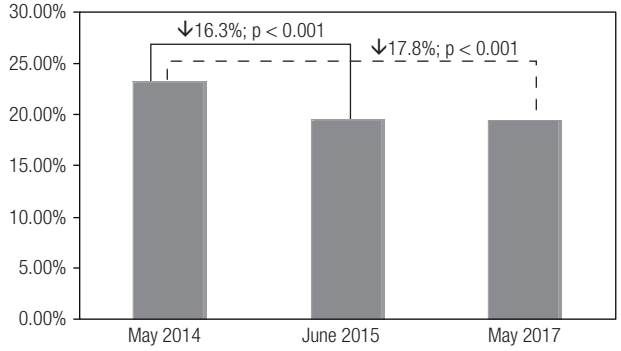

Figure 1. Rates of hyperglycemia (≥ 180 mg/dL) in hospitalized patients in May 2014, June 2015, and May 2017.

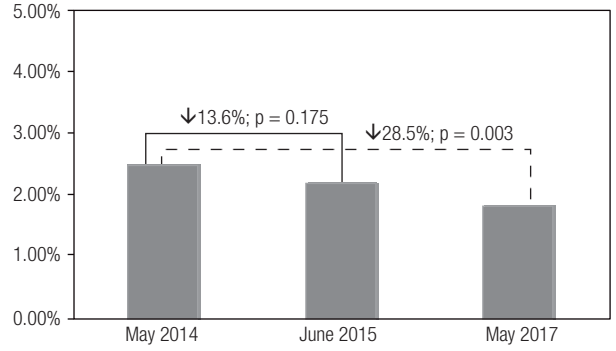

Figure 2. Rates of severe hyperglycemia (≥ 300 mg/dL) among hospitalized patients in May 2014, June 2015, and May 2017.

DISCUSSION

The implementation of an inpatient multidisciplinary glucose control management program (MGCP) had a positive impact on glycemic control in hospitalized patients at our center. We observed that the key elements for this achievement were the implementation

Table 3. Rates of adherence to the institutional inpatient glycemic protocols

	May 2014	June 2015	May 2017	p value
Prescriptions not compliant with the institutional protocols	34.2%	10.1%	7.5%	< 0.001*
Noncompliance with clinical scenario	20.0%	6.3%	5.2%	0.026*
Noncompliance with diet	17.4%	3.8%	2.5%	0.006*

Data are expressed as percentage. * Statistically significant (p < 0.05).

Table 2. Quality indicators of inpatient glycemic control

	May 2014	June 2015	May 2017	p value
Number of patients undergoing POCT	389	545	475	-
Number of glucose readings with POCT	6888	7290	7669	-
Glucose level – mean (SD) (mg/dL)	158.9 ± 60.7	150.5 ± 59.1	150.3 ± 57.8	< 0.001*
Glucose level – median (IQR) (mg/dL)	147.0 (118-188)	138.0 (1120-177)	137.0 (109-178)	< 0.001*
Rate of severe hypoglycemia (≤ 40 mg/dL)	0.2% (n = 12)	0.1% (n = 7)	0.1% (n = 9)	0.336
Rate of hypoglycemia (≤ 70 mg/dL)	0.9% (n = 65)	1.8% (n = 129)	1.0% (n = 79)	0.710
Rate of hyperglycemia (≥ 180 mg/dL)	23.5% (n = 1620)	19.6% (n = 1428)	19.3% (n = 1521)	< 0.001*
Rate of severe hyperglycemia (≥ 300 mg/dL)	2.5% (n = 175)	2.2% (n = 160)	1.8% (n = 143)	0.003*

SD: standard deviation; IQR: interquartile range; POCT: point-of-care testing. * Statistically significant (p < 0.05).

of institutional inpatient glycemic control protocols, establishment of a multidisciplinary glycemic control team, and continuous educational programs for hospital personnel. Altogether, these actions resulted in a significant reduction in hyperglycemic events and improved safety among inpatients.

The development of institutional inpatient glycemic control protocols was important in guiding initial management, clinical decisions, and prescriptions at our center. With the protocols, we were able to standardize our policies and the patients' glycemic control. Since the implementation of the institutional protocols, different educational programs were delivered for hospital staff training. These protocols need to be constantly reevaluated and updated based on newly available evidence in the literature and on local demands of patient care. Other centers have described similar improvements in clinical outcomes with the adoption of insulin protocols for glucose management in critical and noncritical patients (17,18,23,29,30,31-33).

Considered alone, the implementation of the institutional inpatient glycemic control was probably not enough to improve the process of care. The protocol was implemented in January 2012, and in May 2014, we observed that a great proportion of the prescriptions were still not compliant to the protocol. We then hypothesized that the staff education programs should be optimized and further actions should be taken, including the implementation of a multidisciplinary glycemic control team and the dissemination of quality indicators of glucose control. Indeed, we found that the implementation of hospital-wide glucose policies was best facilitated by targeted educational programs and clinical decision support infrastructure to facilitate acceptance by the hospital personnel. We then observed a significantly increased adherence to the institutional inpatient glycemic control protocols over time, accompanied by improved quality indicators of glycemic control in June 2015 and May 2017.

The establishment of a centralized multidisciplinary glycemic control team was a core and critical element in the development of our inpatient glucose management program. Through regularly scheduled monthly meetings and a culture of collaboration and teamwork, the members of the team promoted the implementation of protocols, education interventions, clinical decision aids, performance measures, and quality indicators of glycemic control across continuous inpatient care. In fact, we observed significant reductions of 17.8% in the rate of hyperglycemia (≥ 180 mg/dL) and 28.5% in the rate of severe hyperglycemia (≥ 300 mg/dL) from May 2014 to May 2017, before and after the implementation of the multidisciplinary glycemic control team, respectively. These reductions in hyperglycemic events were already observed one year before (in June 2015), and improved even further in May 2017, suggesting a continuous improvement in patient care and quality outcomes.

Our educational programs focused on the major challenges to optimal glucose management. Similar to other centers, the main obstacles we encountered included unanticipated nutritional changes, poor coordination of the POCT with the administration of insulin around mealtime, unanticipated changes in clinical status or medications, use of medications associated with increased insulin resistance (such as glucocorticoids, often in variable and changing doses), failure by clinicians of making adjustments in glycemic therapy based on daily blood glucose patterns, prolonged use of sliding scale insulin as monotherapy, multiple system/organizational barriers such as lack of communication and/or deficient knowledge of diabetes management among providers and caregivers (7,29,30,32,33). Notably, we demonstrated that a collaborative work of the nurses, dietitians, and physicians reduced the inadequacy of the prescription with the type of diet and improved the coordination of POCT and administration of insulin around mealtime.

The impact of the MGCP on hypoglycemic events in inpatients was less established. We observed a nonsignificant 34.3% reduction in the rate of severe hypoglycemia (≤ 40 mg/dL), which might be due to the low rate of such event at our center. The rates of hypoglycemia (≤ 70 mg/dL) were similar over time. Hypoglycemia is a possible unwanted consequence of improved control of hyperglycemia and may be associated with increased morbidity (3-5,21). Therefore, our results of reduced hyperglycemia without increased hypoglycemic events demonstrate that our institutional protocols were safe. Indeed, it has been demonstrated that the implementation of standardized insulin order sets with less strict glycemic targets and frequent glucose monitorization are associated with better glycemic control and produce expected benefits in terms of patient safety across different hospitals (18,23,25,29,30,32,33).

Our study has limitations inherent to its retrospective, nonrandomized design and the absence of a concurrent control group. This study was intended to evaluate

intermediary outcomes as a quality improvement for hospitalized patients, and we did not evaluate morbidity, mortality, or other important clinical outcome data other than the rates of hyperglycemia and hypoglycemia. Another limitation regarding the analysis of the glycemic data was the potential for an increased type I error (*i.e.*, a false-positive result) due to clustering of POCT values within patients and increased monitoring frequency upon observation of a hyperglycemic event. Indeed, the number of glucose monitoring tests among patients with normal glucose values may affect the proportion of abnormal values. Nevertheless, according to our institutional protocol, patients with hyperglycemia have more frequent POCT than those with normal glucose values.

Finally, despite the decrease in hyperglycemia rates, they still require further reduction, and efforts will be made for this purpose. Despite the fact that our quality indicators of glycemic control seem to be aligned with those of other hospitals, hyperglycemia in hospitalized patients is still frequently observed (34). The creation of a national benchmarking process would be important for the development of best practices and improved management of inpatient hyperglycemia (35).

In conclusion, the implementation of an inpatient multidisciplinary glucose control management program at our center was associated with improved care process and clinical outcomes, demonstrated by continued reductions in rates of hyperglycemic events. The key elements for these achievements were the development of institutional inpatient glycemic control protocols, establishment of a multidisciplinary team, and continuing educational programs for hospital personnel. Therefore, our results suggest that an inpatient multidisciplinary glucose control management program increased the awareness of the value of treating hyperglycemia in hospitalized patients, representing an important feature for inpatient safety and quality improvement.

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ABSI is a poor predictor of insulin resistance in Chinese adults and elderly without diabetes

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ABSTRACT

Objective: Recently, a new obesity index (A Body Shape Index, ABSI) based on waist circumference (WC) was developed, and high ABSI corresponds to a more central concentration of body volume. It is well known that central obesity is closely linked with insulin resistance (IR). Therefore, our study aimed to examine the discriminatory power of ABSI for IR in Chinese adults and elderly without diabetes. **Subjects and methods:** In 2007, a cross-sectional study was made. In this study, 570 individuals without diabetes were available for analysis (male: 56.1%, mean age: 62.3 ± 6.5 years). Insulin resistance was assessed by homeostasis model assessment (HOMA-IR). Areas under the receiver operating characteristic (ROC) curves were determined to identify variables/models that could predict insulin resistance. **Results:** ABSI was associated with IR, the cut-off points was 0.0785 m11/6kg-2/3 to identifying IR and the area under the ROC (AUC) curve was 0.618 (95%CI: 0.561-0.675), which was not better than body mass index BMI (AUC = 0.753; 95%CI: 0.706-0.801), WC (AUC = 0.749; 95%CI: 0.700-0.797), and fasting plasma glucose (FPG, AUC = 0.752; 95%CI: 0.705-0.799). Furthermore, combination with ABSI could improve the discriminatory power of other variables for IR. The AUC curve increased from 0.753 to 0.771for BMI, 0.749 to 0.754 for WC, 0.752 to 0.769 for FPG, respectively. **Conclusions:** ABSI is associated with IR in the general Chinese adults and elderly without diabetes, but the discriminatory power for IR is poor. It is recommended that ABSI be used in combination with other variables. Arch Endocrinol Metab. 2018;62(5):523-9

Keywords

Body shape index; body mass index; waist circumstance; insulin resistance

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INTRODUCTION

Insulin resistance is characterized by a decrease in the ability of insulin to stimulate the use of glucose by muscles and adipose tissues and to suppress hepatic glucose production and output (1). Insulin resistance plays a patho-physiological role in type 2 diabetes and metabolic disorders, and is frequently present in hypertension, coronary artery disease, cancer, endothelial dysfunction and depression (2-6). Early identification of insulin resistant individuals is important for managing the health problems associated with insulin resistance.

It is well known that obesity is closely linked with insulin resistance (7), and many studies have shown that obesity indexes, such as body mass index (BMI) and waist circumstance (WC), could estimate insulin resistance (8-11). Recently, Krakauer and Krakauer (12) developed a new obesity index, namely a body shape index (ABSI), based on WC that is approximately independent of height, weight, and BMI. High ABSI indicates that WC is higher than expected for a given height and weight and corresponds to a more central concentration of body volume (12). Krakauer and cols. (12,13) have shown that ABSI could predict mortality in the general American and British population, even better than BMI and WC. In consideration of the aforementioned characteristics of ABSI and the present findings (12,13), we conclude that ABSI might potentially be a good marker of insulin resistance. Since ABSI was developed, it has led to substantial international interests (14-22). However, to the best of our knowledge, the specific relationship between ABSI and insulin resistance about Chinese adults and elderly was not studied previously, only some foreign studies were reported (23-25). Therefore, the aims of our study were to examine the discriminatory power of ABSI for insulin resistance in Chinese adults and elderly without diabetes.

SUBJECTS AND METHODS

Study population

In 2007, a cross-sectional study was conducted among 711 adults and elderly in an urban community located in Chengdu, Sichuan province, China. The study was supported by mega-projects of science research for the 11th five-year plan, China (Trends in the incidence of metabolic syndrome and integrated control in China). Among the 711 individuals, 141 of them had no data about insulin or were diagnosed with diabetes. Therefore, the remaining 570 individuals were available for analysis (male: 56.1%, mean age: 62.3 ± 6.5 years). The study was approved by Ministry of Health of China, as well as by the Ethics Committee of West China Hospital of Sichuan University. All participants gave informed consent.

Data collection

Anthropometric measurements, such as height, weight and WC, were conducted at the time of interview. Height was measured using a digital stadiometer with a fixed vertical backboard and an adjustable head piece. Weight was measured on a digital scale. At the end of a normal exhalation, WC was measured to the midpoint between the lower border of the rib cage and the iliac crest. Blood samples were drawn from the antecubital vein in the morning after a 12-h fasting. Laboratory tests included fasting plasma glucose (FPG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG) and insulin. FPG, LDL-C, HDL-C and TG were measured enzymatically using a MODULAR P800 Analyzer (Roche Diagnostics). Fasting serum insulin was measured by radioimmunoassay (XH-6010, Xi'an, China). These chemistries were measured at the laboratory of West China Hospital (Chengdu, China).

Related definitions

Insulin resistance was assessed by homeostasis model assessment (HOMA-IR), which was calculated according to the following formula: fasting insulin (mU/mL) × fasting glucose (mmol/L)/22.5 (8). Insulin resistance was defined as being in the highest quintile of HOMA score (≥ 1.66), according to the previous studies (26,27). BMI was calculated as weight in kg/height in m^2. ABSI was defined as $WC/(BMI^{2/3}height^{1/2})$, expressing WC and height in m (12). Those with hypertension were defined as having systolic blood pressure of at least 140 mmHg and/or diastolic blood pressure of at least 90 mmHg and/or currently taking antihypertensive medications. Diabetes mellitus was defined as one of the following at follow-up assessment: (1) fasting plasma glucose ≥ 7.0 mmol/L; (2) a positive response to the question, "Has a doctor ever told you that you have diabetes?"or (3) current use of insulin or oral hypoglycemic agents (14).

Statistical analysis

Descriptive statistics (mean ± standard deviation, median + inter-quartile, percentages, etc.) were used to summarize demographic and metabolic characteristics. Correlations for normally distributed and skewed variables were assessed, respectively, by Pearson and Spearman correlation analysis. Krakauer and cols. (12) thought that ABSI expressed the excess mortality risk from high WC that was complementary to BMI and to other known risk factors. We also estimated whether combination with ABSI could improve the discriminatory power of other variables for insulin resistance. To estimate whether combination with ABSI could improve the discriminatory power of other variables for insulin resistance, different logistic regression models were developed. Areas under the receiver operating characteristic (ROC) curves were used to estimate the discriminatory power of each variable/model for insulin resistance. We use Hosmer and Lemeshow test to estimated whether combination with ABSI could improve the discriminatory power of other variables for insulin resistance. The statistic follows the chi-square distribution and a larger p value indicates the model fit better. In view of the influence of age and sex as variables, we run the analyses with the adjustment of age and sex.

We also set the optimal cut-off point, which represents the optimal combination of sensitivity and specificity for the study sample. For statistical analysis, SPSS software was used (version 17.0; SPSS, Chicago, IL), and statistical significance was defined as $p < 0.05$.

RESULTS

Demographic and metabolic characteristics

The demographic and metabolic characteristics of the 570 subjects (male: 56.1%, mean total age: 62.3 ± 6.5 years, mean male age: 63.3 ± 6.2 years, mean female age:61.0 ± 6.7) are provided in Table 1 according to gender. The mean total values were 23.5 ± 3.2 kg/

m^2, 82.0 ± 9.6 cm, 0.0786 ± 0.0047 m11/6kg-2/3 and 4.6 ± 0.7 mmol/L for BMI, WC, ABSI and FPG respectively, the mean male values were 23.4 ± 3.0 kg/m^2, 83.6 ± 9.3 cm, 0.0792 ± 0.0046$m^{11/6}kg^{-2/3}$ and 4.6 ± 0.8 mmol/L, and the mean female values were 23.6 ± 3.4 kg/m^2, 80.0 ± 9.7 cm, 0.0778 ± 0.0048$m^{11/6}kg^{-2/3}$ and 4.6 ± 0.7 mmol/L. Other variables are shown in Table 1. For age, SBP, DBP, HDL-C, insulin, HOMA, height, weight, and ABSI, there is statistically significant difference between male and female, and all the P values were less than 0.001. But for the other variables, there is no significant difference between male and female. Logistic regression analysis showed that ABSI, BMI, WC and FPG were independently associated with insulin resistance, with adjusted OR values 2.395 (95%CI: 1.358-4.225, p < 0.003), 1.357 (95%CI: 1.232-1.496, p < 0.001), 1.117 (95%CI: 1.081-1.154, p < 0.001) and 3.853 (95%CI: 2.557-5.807, p < 0.001) respectively. Correlation coefficients of ABSI with WC, BMI, height and weight were 0.621 (p < 0.001), 0.121 (p = 0.004), 0.168 (p < 0.001) and 0.180 (p < 0.001), respectively.

Areas under receiver operating characteristic curves for potential variables/models identifying insulin resistance

Table 2 presents the areas under the ROC curves identifying insulin resistance from potential variables. Among the anthropometric variables (BMI, WC, ABSI), the ROC curve analyses showed that ABSI had the poorest discriminatory power for insulin resistance,

Table 1. Demographic and metabolic characteristics

Variable	Total (570)	Male (320)	Female (250)
Age (years)	62.3 ± 6.5	63.3 ± 6.2	61.0 ± 6.7*
SBP (mmHg)	134.7 ± 19.1	136.7 ± 18.2	132.2 ± 19.9*
DBP (mmHg)	79.4 ± 10.1	80.6 ± 10.0	77.9 ± 10.0*
FPG (mmol/L)	4.6 ± 0.7	4.6 ± 0.8	4.6 ± 0.7
TG (mmol/L)	1.5 (1.1, 2.2)	1.7 (1.1, 2.0)	1.7 (1.2, 2.2)
HDL-C (mmol/L)	1.4 (1.3, 1.7)	1.4 (1.2, 1.6)	1.6 (1.3, 1.8)*
LDL-C (mmol/L)	3.0 ± 0.7	3.0 ± 0.8	3.0 ± 0.7
Insulin (mU/L)	5.2 (3.6, 7.3)	4.8 (3.4, 6.6)	5.6 (4.1, 8.1)*
HOMA	1.05 (0.72, 1.53)	0.98 (0.67, 0.98)	1.16 (0.84, 1.67)*
Height (cm)	162.2 ± 7.7	166.7 ± 5.9	156.6 ± 6.0*
Weight (kg)	62.0 ± 10.1	65.1 ± 9.4	58.0 ± 9.6*
BMI (kg/m^2)	23.5 ± 3.2	23.4 ± 3.0	23.6 ± 3.4
WC (cm)	82.0 ± 9.6	83.6 ± 9.3	80.0 ± 9.7
ABSI ($m^{11/6}kg^{-2/3}$)	0.0786 ± 0.0047	0.0792 ± 0.0046	0.0778 ± 0.0048*
Hypertension (%)	50%	51.2	48.4

Data are presented as means ± SD, median (interquartile range), or percentage. "*" means p < 0.05.

ABSI: a body shape index; BMI: body mass index; WC: waist circumference; SBP: systolic blood.

Table 2. Comparison of areas under receiver operating characteristic curves for potential variables identifying insulin resistance

Variable	Area under ROC Curve	95% CI
Biological variable		
FPG	0.752	0.705 – 0.799
TG	0.642	0.585 – 0.699
HDL-C	0.351	0.296 – 0.405
LDL-C	0.526	0.467 – 0.585
Anthropometric variable		
BMI	0.753	0.706 – 0.801
WC	0.749	0.700 – 0.797
ABSI	0.618	0.561 – 0.675

ROC: receiver operating characteristic; CI: confidence interval. Other abbreviations as in Table 1.

with an area under the ROC curve of 0.618 (95%CI: 0.561-0.675) (Table 2, Figure 1). Compared with ABSI, BMI and WC had better discriminatory power, as they had areas under the ROC curves of 0.753 (95%CI: 0.706-0.801) and 0.749 (95%CI: 0.700-0.797), respectively (Table 2, Figure 1). The areas under the ROC curves of other variables were less than 0.700, except FPG (Table 2).

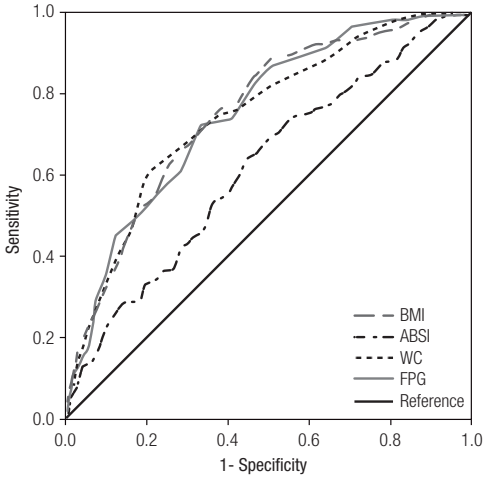

Figure 1. Receiver operating characteristic curves of BMI, WC, ABSI and FPG identifying insulin resistance.

Abbreviations as in Table 1.

We also estimated whether combination with ABSI could improve the discriminatory power of other variables for insulin resistance (Table 3). In combination with ABSI, BMI could improve the discriminatory power, and the area under the ROC curve increased from 0.753 to 0.771 (Tables 2 and 3). In combination with ABSI, WC and FPG could also improve the discriminatory power, and the areas under the ROC curves increased from 0.749 for WC to 0.754 in model 2, from 0.752 for FPG to 0.769 in model 3, respectively (Tables 2 and 3).

When considering age and sex as variables of adjustment in the analyses, we found that both age and sex affect the results as to BMI and WC, but as to ABSI and FPG, only sex alone affect the result. As to the ROC of different models, ABSI ROC curve analysis according to the sex, the male area under the curve is 0.689, female is 0.599, the difference is very big still.

The optimal cut-off points to identifying insulin resistance in our population were the following values: 24.2 kg/m^2 for BMI, 87.5 cm for WC, 0.0785 m11/6kg-2/3 for ABSI, 4.75 mmol/L for FPG, –1.26 for model 1, –1.31 for model 2 and –1.31 for model 3, respectively; and other parameters for these optimal cut-off points are shown in Table 4.

Table 3. Comparison of areas under receiver operating characteristic curves for different models identifying insulin resistance

Variable	Model 1	Model 2	Model 3
ABSI	√	√	√
BMI	√		
WC		√	
FPG			√
Model formula	-16.029+89.151*ABSI+0.312*BMI		-14.626+85.819*ABSI+1.343*FPG
Hosmer and Lemeshow test (p value)	0.133	0.302	0.091
AROC (95%CI)	0.771 (0.725 – 0.818)	0.754 (0.706 – 0.802)	0.769 (0.723 – 0.816)

For each model, ABSI in $m^{11/6}kg^{-2/3}$, TG in kg/m^2, WC in cm and FPG in mmol/L. The symbol "√" meant that the specific variable was included in the specific model. Abbreviations as in Tables 1 and 2.

Table 4. Sensitivity and specificity of different variables/models for identifying insulin resistance

Variable/Model	Optimal cut-off point	Sensitivity (%)	Specificity (%)	+ LR	–LR
BMI	24.2	72.2	66.8	2.17	0.42
WC	87.5	60.0	80.2	3.03	0.50
ABSI	0.0785	65.2	54.3	1.43	0.64
FPG	4.75	72.2	66.8	2.17	0.42
Model 1	-1.26	73.0	74.1	2.82	0.36
Model 2	-1.31	71.3	71.4	2.49	0.40
Model 3	-1.31	71.3	71.4	2.49	0.40

+LR: positive likelihood ratio; –LR: negative likelihood ratio. Other abbreviations as in Table 1.

DISCUSSION

Recently, ABSI was studied extensively, some studies declared it can predicted mortality of general people or affected incidence of obesity or metabolic disorder (3,7,12,23). The aims of our study were to examine the discriminatory power of ABSI for insulin resistance in Chinese adults and elderly without diabetes. Our findings showed that ABSI is associated with insulin resistance in the general Chinese adults and elderly without diabetes, but the discriminatory power for insulin resistance is poor. Furthermore, combination with ABSI could improve the discriminatory power of other variables for IR.

Insulin resistance is associated with many health problems, such as type 2 diabetes, metabolic disorders, hypertension, coronary artery disease, cancer, endothelial dysfunction, depression and pulmonary arterial hypertension (2-6,28). As to Asian, McKeigue and cols. (29) found that insulin resistance syndrome is prevalent in South Asian populations and associated with a pronounced tendency to central obesity. It can lead to a higher prevalence of diabetes (19% vs 4%), higher blood pressures, higher fasting and post-glucose serum insulin concentrations, higher plasma triglyceride, and lower HDL cholesterol concentrations. Lee and cols. (30) also found HOMA-IR could identify dysglycemia and type 2 diabetes mellitus. The direct relation between insulin resistance and fatness is well known (3,7). Banerji and cols. (31) also found that increased visceral fat was related to dyslipidemia and increased frequency of insulin resistance and may account for the increased prevalence of diabetes mellitus and cardiovascular disease in Asian Indians. The underlying mechanisms of obesity inducing insulin resistance include inflammation, mitochondrial dysfunction, hyperinsulinemia and lipotoxicity, oxidative stress, genetic background, aging, fatty liver, hypoxia and lipodystrophy (7,32). Many studies have shown that obesity indices, such as BMI and WC, could estimate insulin resistance (8-11), our findings confirmed these studies.

The new obesity indice, namely ABSI, is based on WC that is approximately independent of height, weight, and BMI (12). The developers of ABSI have claimed that the index is more related to visceral than peripheral fat, which indicates that ABSI might potentially be a good marker of insulin resistance. However, the present findings are opposite to the hypothesis. Currently, there is not sufficient information available for us to understand why, however, some speculations could be made. ABSI is based on an American population from NHANES 1999–2004 (mainly including Mexicans, blacks and whites) (12). The American study population of Krakauer and cols. (12) has a higher height, BMI and WC than our study population. On the other hand, Asian populations are more prone to abdominal obesity and low muscle mass compared with their Western counterparts (33-35). Therefore, the coefficients of ABSI based on American population might not be suitable for other populations, especially for Asian populations. For example, Haghighatdoost and cols. (15) concluded that ABSI was a weak predictor for CVD risks and metabolic syndrome among Iranian adults, and Maessen and cols. (16) suggested that ABSI was not capable to determine cardiovascular diseases presence in the Netherlandish population (16). The authors thought that body height might confound the values of ABSI for health problems (15,16). Cheung (18) showed that ABSI was less associated with incident hypertension than BMI and WC in the Indonesian population, which might be caused by the lower mean BMI and WC (18). Furthermore, the coefficients of ABSI might be influenced by the age, even if Krakauer and cols. (12) showed that ABSI correlation with mortality hazard held across the range of age. In an published article by us (14), the subjects were with a mean age of 48.1 ± 6.2 years, which were younger than the present population, and the correlation coefficients of ABSI with BMI, height and weight were 0.611 ($p < 0.001$), -0.040 ($p = 0.292$), 0.283 ($p < 0.001$) and 0.155 ($p < 0.001$), respectively. The correlation coefficients did not hold across the range of age. Those might imply the coefficients of ABSI might not be suitable for other populations. Although ABSI had poor discriminatory power for insulin resistance, it could improve the discriminatory power of other variables (Table 3).

The present study has some limitations that should be considered. Firstly, insulin resistance is traditionally determined by euglycaemic-hyperinsulinaemic clamp technique (36), but in general population, it is more convenient and cost effective to estimate it using HOMA IR, which is an established test in epidemiological studies (37,38). Secondly, insulin resistance based on HOMA IR has been defined differently in different studies. Values based on 50th percentile, 75th percentile, 90th percentile, lower boundary of the top quintile or tertile

of HOMA score have been used previously (39). We defined insulin resistance arbitrarily as HOMA score greater than the 80th percentile (≥ 1.66), but this is commonly practiced (26,27). Thirdly, for the absence of an oral glucose tolerance test, some individuals with diabetes might be included in the analysis, which could confound the results to some extent. Fourthly, no comparisons between different races might be another limitation. In addition, some other study considered that refined estimations of HOMA-IR levels could not exclude the indices such as age and sex (40). As to nondiabetic Spanish, there are gender-specific differences in HOMA-IR, with increased levels in women over fifty years of age that may be related with changes in body fat distribution after menopause. So, it meant that age and sex might influence the result of HOMA-IR, but our mode design did not regard sex and age as influencing factors.

Of course, the choice of statistical method is very important, because different statistical method may result in discriminating results. In our research, logistic regression analysis revealed that these variables associated with IR, and ROC suggested the identified ability. They are the common methods of clinical research.

In conclusion, our findings showed that ABSI is associated with insulin resistance in the general Chinese adults and elderly without diabetes, but the discriminatory power for insulin resistance is poor. It is recommended that ABSI be used in combination with other variables. Further studies about ethnic specificities of ABSI are needed and warranted, as well as the complementary values of ABSI to other known risk factors.

Authorship: Xiaoping Chen: guarantor of integrity of the entire study, manuscript editing, manuscript review; Kai Wu, Sen He and Yi Zheng: study concepts; Kai Wu: manuscript preparation; Sen He: study design, statistical analysis; Yi Zheng: data analysis.

All the Authors have read the manuscript and have agreed to submit it in its current form for consideration for publication in the AE&M.

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Exercise training improves quality of life in women with subclinical hypothyroidism: a randomized clinical trial

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ABSTRACT

Objective: The aim was to evaluate the quality of life (HRQoL) in women with subclinical hypothyroidism (sHT) after 16 weeks of endurance training. **Subjects and methods:** In the first phase, a cross-sectional study was conducted in which 22 women with sHT (median age: 41.5 (interquartile range: 17.5) years, body mass index: 26.2 (8.7) kg/m^2, thyroid stimulating hormone > 4.94 mIU/L and free thyroxine between 0.8 and 1.3 ng/dL were compared to a group of 33 euthyroid women concerned to HRQoL. In the second phase, a randomized clinical trial was conducted where only women with sHT were randomly divided into two groups: sHT-Tr (n = 10) – participants that performed an exercise program – and sHT-Sed (n = 10) – controls. Exercise training consisted of 60 minutes of aerobic activities (bike and treadmill), three times a week, for 16 weeks. The HRQoL was assessed by the SF-36 questionnaire in the early and at the end of four months. **Results:** Women with sHT had lower scores on functional capacity domain in relation to the euthyroid ones (77.0 ± 23.0 *vs.* 88.8 ± 14.6; p = 0.020). The sHT-Tr group improved functional capacity, general health, emotional aspects, mental and physical component of HRQoL after training period, while the sHT-Sed group showed no significant changes. **Conclusion:** After 16 weeks of aerobic exercise training, there were remarkable improvements in HRQoL in women with sHT. Arch Endocrinol Metab. 2018;62(5):530-6

Keywords
Hypothyroidism; quality of life; exercise training; functional capacity

INTRODUCTION

Thyroid hormones (T_3 and T_4) act on most body cells, and changes in serum concentrations of these hormones cause impact on health of people (1). When thyroid hormones are normal and thyroid stimulating hormone (TSH) is above the reference values, subclinical hypothyroidism (sHT) is characterized (2). It is estimated that the prevalence of sHT in the general population is around 4% to 10%, which is higher among women (3).

The sHT patients may present signs and characteristic symptoms of hypothyroidism such as: fatigue, weight gain, dyslipidemia, psychological disorders, cardiovascular disorders, and increased risk of coronary artery disease and mortality (3,4). The effects of hypothyroidism on health related quality of life (HRQoL) are well established (5-7), but the results are controversial in sHT (3,4).

There is evidence that sHT is associated to worse scores in HRQoL domains such as: functional capacity, health perception, vitality and emotional aspects (6-8). In contrast, some studies do not support these findings (9-11). In part, the divergence of results can be explained by the degree of change in TSH levels. It seems that worse HRQoL are observed in patients with TSH higher than 10 mIU/L (2,6), although recent cross-sectional study has not confirmed this hypothesis (11).

Conventional treatment of sHT is done through hormone replacement with levothyroxine (**L**-T_4) that is associated with decreased clinical manifestations in various organs and systems (4,12). It is emphasized, however, that it has produced conflicting results in the improvement of HRQoL and at the symptoms of patients in different studies (8,13).

Exercise is associated to a better HRQoL in different populations (14,15). However, few studies have investigated the effects of exercise on HRQoL of patients with sHT (16). Thus, this study aimed at evaluating if exercise improves HRQoL in women with sHT. According to previous studies with subclinical disorders (16,17), exercise is taken as hypothesis that also improve HRQoL in sHT.

SUBJECTS AND METHODS

Subjects

A total of 55 female participants were included, aged 20-60 years old, which composed two study groups: sHT group is consisted of 22 women recruited in the Endocrinology Service of Hospital and Maternity Terezinha de Jesus of *Faculdade de Ciências Médicas e da Saúde de Juiz de Fora*, Brazil. The inclusion criteria for the sHT group were: show two doses, with a minimum interval of four weeks of serum TSH above the adopted reference upper limit (4.94 mIU/L) and level of T_{4L} within the reference range (0.70 to 1.48 ng/dL). The control group consisted of 33 euthyroid women with normal values of serum TSH, anti-TPO (anti-thyroid peroxidase antibody) and free T4 within the reference ranges for the used kits and absence of thyroid disease history. Others criteria adopted for both groups were: absence of comorbidities and no physical exercise program for at least three months. Patients with sHT or euthyroid participants with any chronic and/or cardiovascular disease, smokers, those who were using drugs or substances that could interfere in thyroid function, in heart rate or blood pressure, as well as those with musculoskeletal inability toperform physical exercises were excluded from the study. The study was approved by the local ethics committee (no. 0164/10), and all participating groups signed free informed term of consent prior to participation in the study.

Study design

Initially a cross sectional study comparing patients with sHT and euthyroid women concerned to signs and symptoms of hypothyroidism and HRQoL was performed. Subsequently, only sHT patients were randomized to participate or not in the exercise program. The patients were followed in both prospective study groups without masking. A resting echocardiography and exercise cardiopulmonary test were performed for clinical assessment of cardiac structure and cardiorespiratory function before the prospective phase. Two patients with severe changes in blood pressure and heart rate during the exercise test were excluded from randomization. Patients who participated in exercise training (n = 10) were denominated sHT-Tr, while those who did not participate in exercise training (n = 10) were denominated sHT-Sed. Exercise training consisted of aerobic activities, three times a week, for 16 weeks, supervised by the authors. The sHT-Sed patients were instructed for maintaining their usual daily life activities. After four months of intervention or observation, the tests conducted at the beginning of the study were repeated.

Hormonal measures, level of physical activity and anthropometric measures

The TSH, T_4L and anti-TPO levels were measured by third generation chemiluminescenceimmunometric assay (BeckmanCoulter®, Access2®). The reference values for TSH and free T_4 were 0.35 to 4.94 mUI/mL and 0.70 to 1.48 ng/dL, respectively. The anti-TPO levels > 35 UI/mL were considered positive. The level of physical activity was assessed using the Baecke's Habitual Physical Activity Questionnaire, in its translated version and validated for Portuguese (18). In anthropometric assessment, body mass and height (Filizola scale with 0.1 kg and 10 mm precision, respectively), in order to calculate the Body Mass Index (BMI; kg/m^2) were measured.

Signs and symptoms of hypothyroidism – Clinical score

The specific signals and symptoms of hypothyroidism were evaluated by Billewicz scale modified (19). The scale consists of 12 clinical signals and symptoms of hypothyroidism: dry skin, rough skin, decreased sweating, weight gain, paresthesia, hoarseness, decreased hearing, constipation, periorbital edema, slow movements, cold skin, and slow Achilles reflex. A point (1) was assigned when the presence of signal or symptoms or zero (0) in his absence was found.

The maximum scale score is 12 points; scores lower than 3 are expected in euthyroid women; among 3 and 5, in sHT; and higher than 5 in hypothyroidism.

Measure of health related quality of life

The SF-36 (Medical Outcomes Study 36 – Item Short-Form Health Survey) was used to assess the HRQoL of participants in their translated version and validated for Portuguese (20). The SF-36 is consisted of 36 items distributed in eight dimensions: functional capacity, physical aspects, pain, general health, vitality, social aspects, emotional aspects and mental health. The answers are presented in likert scale. The score of each domain ranges from 0 to 100 points and higher the score, higher HRQoL. The physical component was calculated by the average of the following scales: functional capacity, physical aspects, pain and general health; and the mental component: vitality, social aspects, emotional aspects and mental health.

Exercise program

The exercise program consisted of aerobic activities with supervision of a physical education professional, involved in the study. The frequency of training was three times a week for 16 weeks. Each aerobic exercise session consisted of 60 minutes, divided into four phases: heating (5 minutes), ergometric bicycle (25 minutes), treadmill (25 minutes) and resting (5 minutes). The training was individually prescribed based on exercise cardiopulmonary test and maximum heart rate estimated by age (HR_{max} = 220 - age). The training intensity was controlled by HR between 65 and 75% of HR_{max}. The training was continuous and participants could walk, walk with inclination or run in treadmill. The HR was monitored during training sessions by HR monitor (Polar®). Blood pressure and rate of perceived exertion were measured every 10 minutes. After exercise session, stretching exercises were performed by participants and also stimulated to drink fluids.

Statistical analysis

Descriptive analysis was presented as mean ± standard deviation or median (1st quartile, 3rd quartile). In the cross sectional study, comparisons between patients and euthyroid women were measured using Student's t tests or Mann-Whitney test. A two way repeated measures analysis of variance (ANOVA) (2X2) was performed to determine if significance differences between groups and time. If significant main effects or interactions were present a Bonferroni post hoc analysis was conducted. For the analysis of qualitative variables, Fisher's Exact Test was used. Relationship among quantitative variables was performed using Pearson's correlation test. As clinically relevant differences was considered the differences of at least 10 points on the scale of 0 to 100 (21). To assess the internal consistency of SF-36, the alpha Cronbach was used. All analyzes were carried out by using the statistical package SPSS 24.0 (IBM Corp., Armonk, NY). The significance level was set at $P < 0.05$.

RESULTS

Cross sectional study

The general characteristics and LQRH scores of women with sHT and euthyroid are shown in Table 1. In the sHT group, a total of 41% showed positive anti-TPO, while all euthyroid presented anti-TPO negative. There were no significant differences among groups regarding

Table 1. General characteristics and quality life scores of women with subclinical hypothyroidism (sHT) and euthyroid

	sHT (n = 22)	Euthyroid (n = 33)
Age (years)	39.4 ± 10.6	38.8 ± 8.7
TSH (mU/L)	5.58 (5.16–7.53)	2.10 (1.94–2.53)*
T_4 (ng/dL)	0.95 (0.85–1.03)	0.98 (0.94–1.00)
Body mass (kg)	69.3 ± 17.0	69.8 ± 11.8
BMI (kg/m²)	26.4 ± 5.5	26.3 ± 5.0
Level of physical activity	7.1 ± 0.9	8.3 ± 1.3*
Signals and symptoms	3.5 ± 1.6	2.5 ± 1.0*
Domains and scores of SF-36		
Functional capacity	77.0 ± 23.0	88.8 ± 14.6*
Limitation by physical aspects	67.0 ± 40.4	81.1 ± 33.1
Pain	68.0 ± 26.6	68.4 ± 23.7
General health	71.1 ± 16.3	72.5 ± 19.2
Vitality	53.6 ± 23.0	58.0 ± 24.5
Social aspects	73.3 ± 29.2	70.1 ± 27.0
Limitation by emotional aspects	56.1 ± 45.3	57.6 ± 44.3
Mental health	66.4 ± 20.9	65.3 ± 24.5
Physical component	70.8 ± 20.3	77.7 ± 18.0
Mental component	62.3 ± 26.3	62.8 ± 25.4

Values: Mean ± SD and Median (Interquartile Range).
TSH: thyrotropin; T_4: free thyroxine; BMI: body mass index; Level of physical activity measured by Baecke test; Signals and Symptoms of hypothyroidism measured by Zulewski test.
* Patients with sHT versus controls, significant difference $p < 0.05$.

the variable potentials of confounding age (p = 0.85), body mass (p = 0.91), BMI (p = 0.94) and menopause status (p = 0.28). The sHT patients showed lower levels of physical activity (p < 0.001) and higher number of signals and symptoms (p = 0.02). Regarding LQRH, sHT patients showed lower scores on "functional capacity" domain (p = 0.02) compared to euthyroid. In other domains there were no significant differences observed between groups, however women with sHT showed consistently lower scores, except in "social aspects". No relationship was found between TSH, signals and symptoms and life quality (p > 0.05). The internal consistency of 36 questions of SF-36 was satisfactory (Cronbach's alpha = 0.92). All domains also showed satisfactory coefficients (0.70 to 0.90). This means that the investigated sample reported high level of consistency in the answers to the survey questions.

Randomized clinical trial

After randomization, there were no significant differences between the sHT-Tr and sHT-Sed groups related to age (p = 0.25), TSH (p = 0.85), T4 (p = 0.74), body mass (p = 0.35), BMI (p = 0.35), level of physical activity (p = 0.11), signals and symptoms (p = 0.53), menopause and all domains of life quality life (p > 0.05).

Analyses revealed a significant (group x time) interaction effect concerned functional capacity, general health, emotional aspects, psychological component, and physical component (Table 2). The sHT-Tr group showed improvement in these domains. On the other hand, the sHT-Sed group after four months of observation, showed no significant changes in all domains assessed by SF-36. Both groups showed no significant changes in signals and symptoms number (p > 0.05).

DISCUSSION

This study compared the HRQoL of women with sHT and the euthyroid and assessed the impact of physical exercise on this outcome. The main findings were: 1) sHT is associated to a worse perception of HRQoL; 2) Women with sHT showed improvements in multiple assessed domains of HRQoL after aerobic exercise training during four months.

In the cross sectional study, it was found that patients with sHT showed more signals and symptoms and lower functional capacity compared to control euthyroid group, what has been mentioned previously in other studies (6-8). Besides that, in our study, there was a greater presence of signs and symptoms in sHT patients compared to the control group. Similar results were beforehand observed (13). Previously, a study reported that the presence of signals and symptoms of thyroid dysfunction may be related to decreased quality

Table 2. General characteristics and quality life scores of patients with subclinical hypothyroidism before and after 4 months of exercise training (sHT-Tr) or observation (sHT-Sed)

	sHT-Tr (n = 10)		sHT-Sed (n = 10)		Group effect	Time effect	Interaction
	Baseline	4 months	Baseline	4 months			
Signals and symptoms	3.8 ± 2.0	3.3 ± 1.6	3.1 ± 1.4	3.1 ± 2.0	0.53	0.50	0.50
Domains and components of SF-36							
Functional capacity	73.0 ± 26.4	86.5 ± 9.4	85.0 ± 11.5	82.0 ± 17.7	0.58	0.21	0.049*
Limitation by physical aspects	65.0 ± 42.8	92.5 ± 12.1	75.0 ± 37.3	67.5 ± 44.2	0.55	0.37	0.12
Pain	67.7 ± 29.3	71.7 ± 17.6	72.7 ± 24.7	68.8 ± 24.3	0.92	0.99	0.36
General health	68.1 ± 17.4	83.0 ± 13.9	75.0 ± 16.2	69.5 ± 18.0	0.61	0.22	0.01*
Vitality	53.0 ± 30.7	70.0 ± 19.4	56.0 ± 16.3	56.5 ± 22.0	0.57	0.07	0.08
Social aspects	75.0 ± 27.0	87.5 ± 14.4	80.0 ± 28.4	77.5 ± 25.5	0.80	0.30	0.13
Limitation by emotional aspects	43.3 ± 49.8	90.0 ± 22.5	76.7 ± 35.3	63.3 ± 39.9	0.81	0.12	0.01*
Mental health	64.4 ± 26.5	76.4 ± 17.4	68.8 ± 17.1	68.0 ± 22.4	0.82	0.13	0.08
Physical component	68.5 ± 25.7	83.4 ± 6.6	76.9 ± 12.9	72.0 ± 20.2	0.82	0.29	0.04*
Mental component	59.0 ± 30.6	81.0 ± 14.4	70.4 ± 20.9	66.4 ± 24.3	0.86	0.09	0.02*

Values: Mean ± SD.

TSH: thyrotropin; T_4: free thyroxine; BMI: body mass index; Level of physical activity measured by Baecke test; Signals and symptoms of hypothyroidism measured by Zulewski test.

* Significant difference p < 0.05, ANOVA 2x2.

of life (11). But it is difficult to distinguish sHT from the euthyroid only by signals and symptoms (22). This could explain the lack of difference in quality of life comparing these two groups in some studies (11).

Regarding the quality of life, there is controversy concerned to the results of studies involving patients with sHT (3). In our study, a worse quality of life in women with sHT was observed, mainly through the reduction of functional capacity compared to the euthyroid ones. Corroborating our results, there are studies that observed lower scores of functional capacity in women with sHT, associated with the worst perceptions of health, vitality and emotional aspects (6-8).

An earlier research found that patients with sHT showed lower scores in physical and psychological aspects and greater complaints related to fatigue, which cause damage to their daily activities (5). According to these authors, the decreased quality of life negatively influences mood and enhance anxiety and depression rates in sHT. In addition, studies showed lower functional capacity, general health and physical aspect (6) and worst health perception by patients with sHT, especially in the vitality factor, related to the psychological aspect (8). However, studies on Italy (9), Australia (10) and Netherlands (11) populations prove no worsening in quality of life in patients with sHT.

Studies have earlier identified worst quality of life in sHT (9,11). The results could be related not for disease diagnosis itself, but by the fact that patients are labeled as "sick" or their conscience are sick. Quality of life is a multidimensional and subjective construct, difficult for defining and systematization, which makes complex its operationalization (23). This, in part, could explain the divergent results reported in previous studies. Furthermore, another explanation would be no standardization of the criteria for assessing quality of life (24).

In the follow-up study, exercise training improves HRQoL after four months of intervention. There were increased domain scores for functional capacity, general aspects of health, emotional aspects and psychological and physical component. After 4 months of training, the patients had functional capacity values, general health, emotional aspects and higher mental and physical component of the normative values of Brazilian healthy women of the same age (25). Similar benefits were observed by other researchers (16), who conducted a study which evaluates the influence of a medium impact exercise program in relation to quality of life and cardiorespiratory fitness of women with sHT. Participants were subjected to a program that consisted of activity for 3 weekly sessions of 60 minutes during 12 weeks. After 12 weeks of intervention, women showed improvement in quality of life, through a higher score in most domains of SF-36, especially in relation to vitality, general health, social aspects of health and mental health. Furthermore, participants who were subjected to exercise program increased their cardiovascular fitness. In patients with subclinical hyperthyroidism it was found improvement in relation to the disease symptoms, especially in the perception of fatigue after 12 weeks of aerobic training (17).

It is unknown the actual mechanism responsible for the psychological effects of physical exercise, although it is recognized that it is an interaction of psychophysiological factors. In patients with coronary artery disease, for example, poor quality of life is associated to reduced exercise ability (14,15) and more tendency to fatigue (15). It is known that physical exercise is the main intervention used for physical fitness improvement and that it is positively related to quality of life (14,15,23,26). A systematic review to assess the association between physical activity and quality of life found that, in cross-sectional and longitudinal studies, the highest level of physical activity was related to a better perception of quality of life in apparently healthy adults or in different conditions of disease, regardless of sex (23). Moreover, a meta-analysis concluded that individuals with chronic diseases who received intervention to increase the level of physical activity improved their quality of life and that those who received supervised interventions showed the best result (24).

In clinical practice, the decision for whether or not treating the patient with sHT is connected to observed signals and symptoms, including patient complaints (3). Treatment with levothyroxine is generally associated with reduced pain, improvements in overall health and physical aspects (13), but not necessarily with better quality of life (27,28). In the present study, from the clinical point of view, the improvement observed in the quality of life of patients after physical training was moderate to high magnitude (> 10 points in SF-36 scales) (21). Thus, it is plausible to speculate that the improvement in the quality life of patients with sHT may be due to increased level of physical activity, physical fitness improvement and reduced signals and symptoms. Therefore, physical activity can be used as a

strategy to improve health perception of these patients and should be encouraged by doctors and stimulate as a key element for the adoption of a healthy lifestyle for patients with sHT. Furthermore, the assessment of quality of life should be used in the diagnostic of these patients.

It is known that most patients with sHT tend to normalize spontaneously the TSH, especially those with TSH < 10 mIU/l. This normalization has no a well – defined pattern, although most patients may normalize their TSH levels between 6 and 18 months (29). In this same study, more than half of the patients had negative anti-TPO, and the changes in TSH were not correlated to the presence of anti-TPO (27). In this study, all patients practically showed TSH < 10 mIU/l. The effects of physical training on thyroid hormones are still a matter of debate in the literature and should be investigated in further studies. As limitations of the study, include the small sample size and the performed research only with women, not allowing, thus, expanding the results for male individuals.

In conclusion, the results suggest that women with subclinical hypothyroidism tend to have consistently lower scores on domains of quality of life compared to euthyroid women. However, physical exercise has been able to adjust these losses and therefore, should be encouraged in this group of patients. Further studies are necessary to better understand the optimal dose and the type of exercise would be more efficient to improve quality of life in women with sHT.

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Thyroid cancer burden and economic impact on the Brazilian public health system

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ABSTRACT

Objective: Recent data indicates an increasing incidence of thyroid cancer not accompanied by a proportional increase in mortality, suggesting *overdiagnosis*, which may represent a big public health problem, particularly where resources are scarce. This article aims to describe and evaluate the procedures related to investigation of thyroid nodules and treatment and follow-up of thyroid cancer and the costs for the Brazilian public health system between 2008 and 2015. **Materials and methods:** Data on procedures related to investigation of thyroid nodules and treatment/follow-up of thyroid cancer between 2008 and 2015 in Brazil were collected from the Department of Informatics of the Brazilian Unified Health System (Datasus) website. **Results:** A statistically significant increase in the use of procedures related to thyroid nodules investigation and thyroid cancer treatment and follow-up was observed in Brazil, though a reduction was noted for procedures related to the treatment of more aggressive thyroid cancer, such as total thyroidectomy with neck dissection and higher radioiodine activities such as 200 and 250 milicuries (mCi). The procedures related to thyroid nodules investigation costs increased by 91% for thyroid ultrasound ($p = 0.0003$) and 128% in thyroid nodule biopsy ($p < 0.001$). Costs related to treatment and follow-up related-procedures increased by 120%. **Conclusion:** The increase in the incidence of thyroid cancer in Brazil is directly associated with an increased use of diagnostic tools for thyroid nodules, which leads to an upsurge in thyroid cancer treatment and follow-up-related procedures. These data suggest that substantial resources are being used for diagnosis, treatment and follow-up of a potentially indolent condition. Arch Endocrinol Metab. 2018;62(5):537-44

Keywords
Thyroid cancer; economic impact; Brazilian public health system; costs; overdiagnosis

INTRODUCTION

Thyroid nodules are a very common condition, found by palpation in 4-7% of the adult population and in more than 50% if an image exam is used (1-4). Despite the general knowledge that thyroid nodules are seldom malignant, about 5%, some studies with necropsies have shown that thyroid cancer may be present in up to 36% of individuals who died from other causes not thyroid-related (5-9).

Indeed, thyroid cancer is the most common endocrine cancer, with an incidence rate of 7.57 per 100,000 women and 1.49 per 100,000 men in Brazil (10), though recent data indicate an increase in incidence worldwide (2,11-14). Interestingly, this increase is not accompanied by a proportional increase in mortality, suggesting the potential diagnosis of early-stage cancer associated with a lower risk of recurrence or the potential occurrence of *overdiagnosis*, which means diagnosing a disease that would never cause symptoms or death during a patient's expected life span (2,15-18).

This phenomenon has been documented in a recent South Korean study, which reported a dramatic rise in the diagnosis of thyroid cancer, reaching epidemic levels, due to the incorporation of a neck ultrasound as part of a routine screening check-up (11). In addition, data from the United States suggest that despite an overall increase in the incidence of thyroid cancers, this phenomenon was more prominent in regions with widely available health care access (15,19,20). Interestingly, such findings seem to be occurring worldwide, leading to increased concern over its public health impact (20).

Recent Brazilian data from the population-based cancer registry (RCBP) has demonstrated a significant increase in the incidence of thyroid cancer in the city of São Paulo from 2008 to 2012 (21). Although this

seems to occur throughout the country, the results are more impressive in the southern, southeastern and northeastern regions, where diagnostic tools are more widely available (10,15,22-25).

Since *overdiagnosis* may represent an outsize problem for public health services, it is fundamental to evaluate this phenomenon especially in developing countries, where resources are scarce (22). However, no data on the quantity of procedures performed to investigate a thyroid nodule, treat and follow thyroid cancer patients, as well as its costs for the Brazilian public health system (SUS) are currently available. Therefore, the aim of this study is to describe and discuss the procedures related to investigation of thyroid nodules, treatment and follow-up of thyroid cancer and the direct costs for the Brazilian public health system between 2008 and 2015.

MATERIALS AND METHODS

A retrospective study was performed using the Department of Informatics of the Unified Health System (Datasus) database (datasus.saude.gov.br) as the main source of information, accessed during the month of December 2016.

We considered all the procedures present in the algorithm proposed by the national endocrine society for the investigation and management of thyroid nodules (Figure 1 – supplementary material) (26). As thyroid-stimulating hormone TSH measurements are used to investigate thyroid disfunctions and there is not a specific Datasus code for TSH dosage requested for thyroid nodule evaluation, data about TSH measurements were not included.

The quantity and tariffs of the procedures between 2008 and 2015 were accessed through the TABNET link on the Datasus homepage. The data were organized according to the place where the procedure was performed, not by the patient's birthplace. We analyzed data from the entire country, stratified by the five Brazilian regions (south, southeast, northeast, north, and central-west). It was considered only the treatment related-procedures restricted to the 10th edition International Classification of disease code C-73 ('Thyroid Cancer") (27).

The tariffs were described in the Brazilian local currency, i.e. *reais*. Considering that the tariff (direct cost) of each procedure for our Public Health System, collected from the SIGTAP (sigtap.datasus.saude.gov.br), hasn't changed since 2008, there was no need for adjusting to the inflation rate.

The thyroid nodule investigation and treatment/follow-up-related procedures analyzed, as well as each tariff are described in Tables 1 and 2.

Brazilian population estimates were obtained from the Brazilian Institute of Geography and Statistics (IBGE) website to adjust the quantity of procedures per 100,000 people in each region. The incidence and mortality rate of thyroid cancer was obtained from the latest version of the National Cancer Institute/Population-based Cancer Registry (INCA/RCBP) database, using the tenth edition of the International Classification of Disease (ICD-10) code "C73" (27).

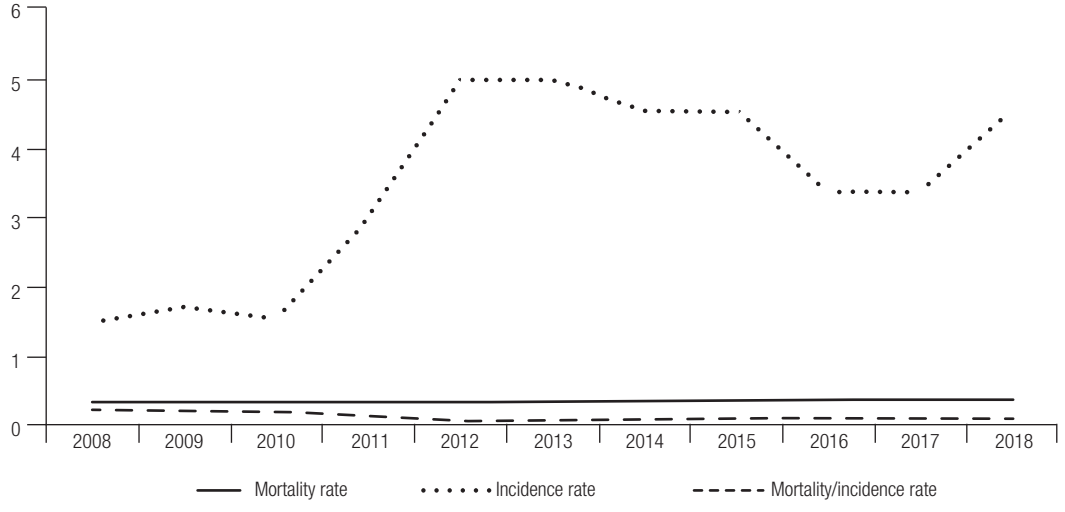

Figure 1. Incidence versus Mortality of Thyroid Cancer (ICD-10: C73) between 2008 and 2018 in Brazil (rate per 100,000 people). Source: Data obtained from the National Cancer Institute (INCA).

The statistical analysis was performed with Stata 13.0 (28). To verify the trend of each variable during the period of the study, a Spearman correlation was used. The significance level adopted was 5%.

Table 1. Thyroid Cancer Diagnosis-related Procedures Available at Datasus in December 2016 (sigtap.datasus.saude.gov.br)

Procedure name	Datasus code	Tariff (R$)
Thyroid ultrasound	02.05.02.012-7	24.20
Thyroid FNAB	02.01.01.047-0	23.73
Thyroid scintigraphy	02.08.03.002-6	77.28
Thyroid scintigraphy with suppression and/or stimulus*	02.08.03.003-4	107.30

* Suppression with T3 or T4/stimulus with recombinant human TSH (thyroid-stimulating hormone).
FNAB: fine-needle aspiration biopsy.

Table 2. Thyroid Cancer Treatment-related Procedures Available at Datasus in December 2016 (sigtap.datasus.saude.gov.br)

Procedure name	Datasus code	Tariff (R$)
Oncologic total thyroidectomy	04.16.03.027-0	2836.30
Total thyroidectomy with neck dissection	04.02.01.005-1	767.77
Total thyroidectomy with neck dissection in oncology	04.16.03.012-2#	1606.86
Trans sternal resection of thyroid cancer	04.16.03.036-0	4186.64
Trans sternal resection of goiter in oncology	04.16.03.005-0*	2618.25
RAI 30 mCi	03.04.09.005-0	443.70
RAI 50 mCi	03.04.09.006-9	614.70
RAI 100 mCi	03.04.09.002-6	1071.90
RAI 150 mCi	03.04.09.001-8	1289.90
RAI 200 mCi	03.04.03.003-4	1471.32
RAI 250 mCi	03.04.09.004-2 03.03.12.001-0	1810.32
WBS	02.08.03.004-2	338.70
Serum thyroglobulin	02.02.06.036-5	15.35

Code 04.16.03.012-2 was revoked in January 2013. Afterwards, codes 04.16.02.018-6 (unilateral neck dissection in oncology) and 04.16.03.027-0 (total thyroidectomy in oncology) were used to describe this procedure.
* Code 04.16.03.005-0 was revoked in January 2013. Afterwards, code 04.16.03.036-0 (trans sternal resection of thyroid cancer) was used to describe this procedure.
RAI: radioiodine treatment; WBS: whole body scan.

RESULTS

In 2008, thyroid cancer incidence rate was 1.51 per 100,000 individuals, rising progressively to 4.57 per 100,000 individuals in 2018 ($p = 0.06$). The mortality rate rose from 0.30 in 2008 to 0.36 in 2018 ($p = 0.004$). However, comparing the mortality rate to the incidence rate (mortality rate/incidence rate) there was a negative trend ($p = 0.07$, rho -0.0674, Figure 1).

Contributing to this incidence's upsurge, it was observed a statistically significant increase in the number of thyroid nodule investigation tools (thyroid ultrasound and fine-needle aspiration biopsy – FNAB), and treatment/follow-up-related procedures (oncologic total thyroidectomy and radioiodine treatment 100 mCi and 150 mCi) between 2008 and 2015 in Brazil and in all geographic regions (Tables 3, 4 and Figure 2). Data on the increase in treatment-related procedures per geographic region are described in the supplementary material.

The use of thyroid scintigraphy with or without stimulus and/or suppression has reduced during the analyzed period (Table 3).

However, the procedures related to more aggressive thyroid cancer treatment reduced significantly during the same period. For example, total thyroidectomy with neck dissection decreased from 0.21 per 100,000 people in 2008 to 0.12 per 100,000 people in 2015 ($p = 0.03$), while the number of higher radioiodine activities, such as 200 mCi and 250 mCi, both decreased from 0.33 and 0.32 per 100,000 people in 2008, to 0.26 and 0.19 per 100,000 people in 2015, respectively ($p = 0.0991$ and $p < 0.0001$). The comparison among levels of radioiodine used for thyroid cancer ablation is shown in Figure 3.

Considering treatment for low-risk patients, an increase in the use of lower doses of RAI (30-50 mCi) for thyroid cancer treatment was noted, although this data is only available since 2014.

Table 3. Number of Thyroid Cancer Diagnosis-related Procedures per 100,000 People, between 2008 and 2015 in the Brazilian Public Health System (SUS)

	2008	2009	2010	2011	2012	2013	2014	2015	p
Thyroid ultrasound	154.5	173.2	196.7	173.5	196.1	207.7	232.8	229.6	< 0.001
Thyroid FNAB	10.3	10.1	10.4	11.2	13.5	14.6	15.6	16.9	< 0.001
Thyroid scintigraphy	6.2	5.5	5.6	5.6	5.4	5.5	5.3	4.8	0.001
Thyroid scintigraphy with suppression/stimulus*	0.09	0.06	0.06	0.04	0.04	0.05	0.07	0.08	0.795

* Suppression with T3 or T4/stimulus with recombinant human TSH (thyroid-stimulating hormone).
FNAB: fine-needle aspiration biopsy.
Source: datasus.saude.gov.br.

Table 4. Number of Thyroid Cancer Treatment and Follow-up-related Procedures per 100,000 People, between 2008 and 2015 in the Brazilian Public Health System (SUS)

	2008	2009	2010	2011	2012	2013	2014	2015	p
Oncologic TT	0.8	0.9	1.0	1.1	1.2	1.7	1.8	1.9	< 0.001
TT with neck dissection	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.027
TT with neck dissection in oncology	0.48	0.56	0.65	0.73	0.84	-	-	-	< 0.001
Trans sternal resection of thyroid cancer*	0.03	0.04	0.03	0.04	0.02	0.02	0.02	0.03	0.046
RAI 30 mCi**	-	-	-	-	-	-	0.06	0.07	< 0.001
RAI 50 mCi**	-	-	-	-	-	-	0.04	0.07	< 0.001
RAI 100 mCi	0.4	0.5	0.7	0.7	0.8	0.9	0.9	0.9	0.027
RAI 150 mCi	0.4	0.5	0.7	0.7	0.8	0.9	0.8	0.8	0.055
RAI 200 mCi	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.127
RAI 250 mCi	0.3	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.007
WBS	3.8	3.7	4.2	4.6	4.4	5.1	4.9	4.9	0.008
Serum thyroglobulin	23.3	27.3	37.3	39.4	38.7	39.8	44.9	48.5	< 0.001

* Data includes codes 04.16.03.005-0 (from 2008 to 2013) and 04.16.03.036-0 (from 2013 to 2016).
** Data available since 2014.
RAI: radioiodine treatment; TT: total thyroidectomy; WBS: whole body scan.
Only the procedures restricted to the IDC: C73 were considered.
Source: Data obtained from datasus.saude.gov.br

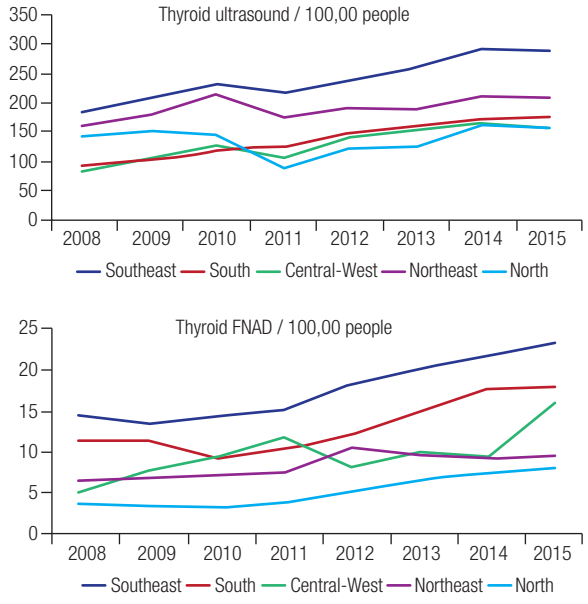

Figure 2. Increase in Numbers of Procedures Related to Thyroid Nodule Investigation between 2008 and 2015 in the Brazilian Public Health System (SUS) by Regions.
FNAB: fine-needle aspiration biopsy.

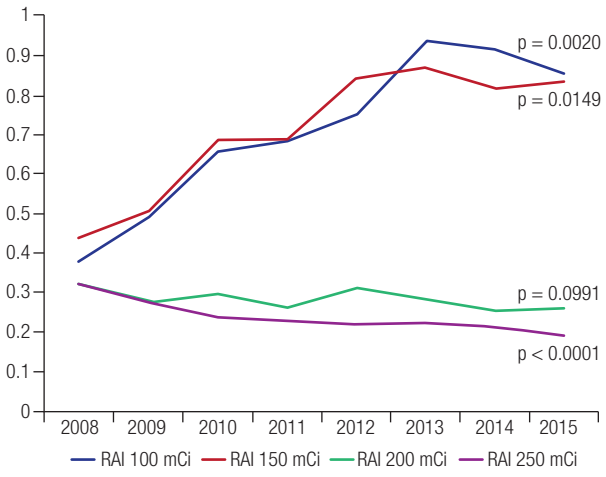

Figure 3. Radioactive Iodine Use per 100,000 People During 2008–2015 in the Brazilian Public Health System (SUS).
RAI: radioiodine treatment.

Regarding direct costs to the Brazilian public health system, a 84% increase in procedures related to thyroid nodule investigation costs was noted in this period. Thyroid ultrasound costs increased by 91% ($p = 0.0003$), and thyroid nodule biopsy (fine-needle aspiration biopsy) costs increased by 128% ($p < 0.001$) from 2008 to 2015 (Figure 4A). Similarly, there was a 120% increase in the total costs of treatment-related procedures performed during the same period, mainly due to the increase in the use of oncologic total thyroidectomy, radioiodine activities of 100 and 150 mCi RAI and follow-up procedures (Figure 4B). These procedures (diagnostic and therapeutic) altogether represented an expense of almost 230 million *reais* for the unified health system (SUS) in this 8-year period.

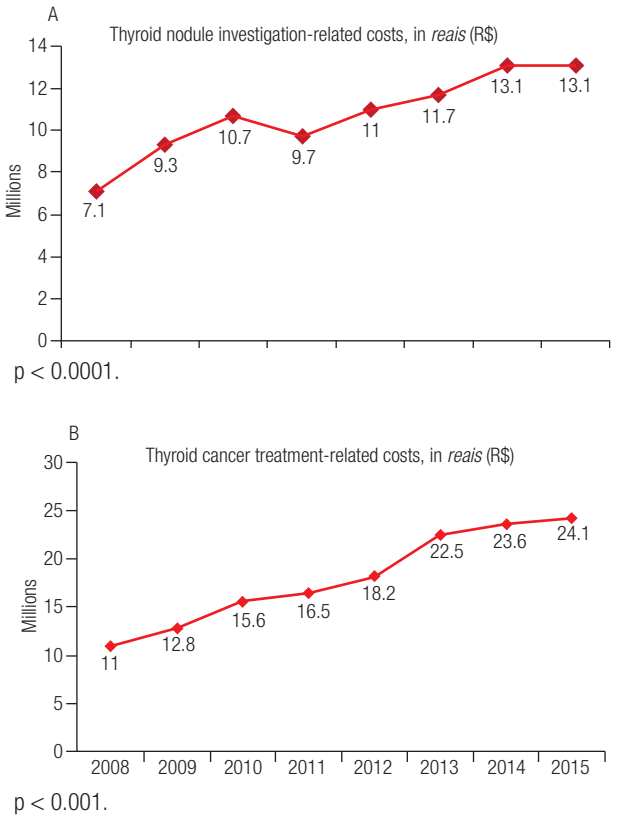

Figure 4. Increase in Costs Related to Thyroid Cancer Diagnosis (**A**) and Treatment (**B**) between 2008 and 2015 in the Brazilian Public Health System.

DISCUSSION

Our study demonstrates an important upsurge in the use of procedures related to thyroid nodule investigation and thyroid cancer treatment and follow-up in Brazil from 2008 to 2015. This overuse of resources has increased the costs of the disease for the Brazilian public health system.

Interestingly, this increase seems to be mostly driven by procedures related to early-stage cancer, as the use of more aggressive surgery and higher-dose radiation therapy has decreased over time. Although this trend might be interpreted as earlier diagnosis due to more intensive use of screening strategies, one would expect a reduction in mortality if this were true. Collectively, this evidence can be interpreted as a potential *overdiagnosis* of cases, which are unlikely to progress to overt or aggressive forms of cancer.

The clinical relevance of a nodule incidentally found by ultrasound is unclear because it probably will not prompt symptoms or neoplastic dissemination (29-31). Indeed, studies have shown that 19% to 68% of the population presents with a thyroid nodule on a neck ultrasound (32,33). In these cases, an early thyroid cancer diagnosis would not improve prognosis, but it may increase the risks related to unnecessary aggressive treatment (31).

To avoid *overdiagnosis* and overuse of resources, health systems in different countries are reconsidering the usefulness of neck ultrasound to screen for thyroid cancer in asymptomatic individuals. In the United Kingdom, only a thyroid specialist is allowed to order neck ultrasounds for patients with thyroid nodules (34). Along the same lines, the American Preventive Service Task Force (USPSTF) recently released its guidelines, in which it strongly recommends against using neck ultrasounds for thyroid cancer screening in asymptomatic people (35). Restricting the use of neck ultrasounds to cases in which palpable nodules are detected by a specialist could be an option for reducing healthcare resource utilization in the Brazilian public health system.

Despite the increase in total thyroidectomies performed during the period of this study, a significant reduction in the use of more complex surgeries, such as total thyroidectomies with neck dissection, was noted. This finding suggests that smaller tumours are being resected, without lymph node metastasis and probably with no important clinical repercussion, which corroborates the hypothesis of *overdiagnosis* (36-38). This concept is reinforced by recent data showing a similar effect with *watchful waiting* compared with surgery when a thyroid nodule is diagnosed as cancer (39,40).

Our results have also documented a significant increase in radioiodine treatment with 100 and 150 mCi, the most common activities prescribed for thyroid cancer patients with intermediate or high risk of recurrence. Lately, national and international guidelines on thyroid cancer management have recommended against the use of radioiodine in low risk of recurrence cases (26,41). Even so, Roman and cols. (42) observed that 30% of the patients with tumours < 1 cm still receive radioiodine activities despite recent guidelines against it (42). In our study, it is not clear if the rise observed in the use of RAI is associated with diagnosis of higher-risk tumours or if clinicians continue to prescribe RAI due to a lack of knowledge or for thyroid remnant ablation. In these cases, the use of RAI could facilitate the use of serum thyroglobulin measurements in the thyroid cancer follow-up (43). Nevertheless, the reduction in prescribing higher RAI activities (200 and

250 mCi) implies that less aggressive cases are being diagnosed.

There was a numerical increase in the use of low radioiodine activities (30-50 mCi), despite the small absolute number of those procedures, as they were only included in the list of authorized procedures by the Brazilian Public Health System in 2014. Recent studies have shown that the benefits of lower RAI doses equal higher doses, such as 100 mCi for low- to intermediate-risk patients, with fewer side effects and reduced costs (44,45). This may be a tendency as low-risk cases are being dignosed. However, the low request for these procedures may indicate that low-risk patients have not received any RAI treatment, which is the most recent standard of care expected for low-risk cases (46,47), or they have received 100 mCi despite the recent literature recommendations (46-49).

The southeastern region of Brazil had the highest increase in number of procedures as well as expenditures related to thyroid cancer diagnosis and treatment. This may have occurred due to the choice of using the Datasus search filter of patients' treatment place and not their birthplace. It is known that the southeastern region is the richest region and has the largest cancer centres in Brazil, which are referred centres for people from other regions. So the expectation on overuse of resources is higher. Nonetheless, this important upsurge in diagnostic and treatment-related procedures seems to be spread throughout the country, irrespective of region.

From 2010 to 2015, there was a 66% increase in costs related to cancer in Brazil, from 2.1 billion *reais* to 3.5 billion *reais*, according to the National Cancer Institute (INCA). During the 8-year study period, there was also a significant increase in the cost of thyroid nodule investigation and thyroid cancer treatment and follow-up in all Brazilian regions, proportionally higher than what was observed for other types of cancer (106% for thyroid cancer vs 66% for all types of cancer). This excessive expenditure for a potentially indolent disease adds on to the hypothesis of *overdiagnosis*.

Our study, however, must be read within the context of its design. The completeness of the INCA/RCBP database is questionable in some regions. Therefore, the inputs on thyroid cancer incidence may be underestimated. Also, the Datasus database depends on the registry of the procedures performed in each hospital or healthcare service across the country, thus it does not warrant a completely reliable source of information. Additionally, there are different codes to describe the same procedure, for example, "total thyroidectomy" *versus* "total thyroidectomy in oncology". This study considered only the code for total thyroidectomy in oncology, which excludes thyroid surgeries for benign diseases. However, the figures may be underestimated, as it is possible that the code for total thyroidectomy (not oncologic) also might have been used to describe surgeries for cancer. Another coding problem is that the code used to describe thyroid FNAB because it is used to describe thyroid as well as parathyroid FNAB. It is known that thyroid tumors are 16 times more common than parathyroid tumors (50). We therefore assumed all FNAB were related to thyroid cancer. Although this may result in a small overestimation of its use, there has been no change in the incidence of parathyroid tumors, and the documented increase over the last eight years is unlikely to have changed if the cases used for parathyroid disease were excluded. Finally, the treatment related-procedure codes that referred to other diseases despite thyroid cancer were disregarded as they might have overestimated the results.

In conclusion, the increasing incidence of thyroid cancer in Brazil seems to be directly associated with the performance of diagnostic procedures, as well as an increase in treatment-related procedures. These data suggest that large resources are allocated for diagnosis and treatment of a potentially indolent condition, which could remain unnoticed throughout one's lifetime. Therefore, it is important that thyroid cancer care be reexamined with a cost-conscious view, providing the best outcome that matters to the patient, relative to the cost of delivering it, especially in developing countries where healthcare resources are scarce.

Ethics approval and consent to participate: the study was approved by the Ethics Committee on Research from Federal University of Sao Paulo (Unifesp) under the National Research Database (Plataforma Brasil). The committee's reference number (CAAE) is: 58914516.8.0000.5505.

Availability of data and material: all data is available publicly at www.datasus.gov.br. The raw data on the statistical analysis is available from the corresponding author upon reasonable request.

Authors' contributions: Carolina Castro Porto Silva Janovsky designed the study, collected the data, conducted the statistical analysis and wrote the manuscript. Marcio Sommer Bittencourt contributed to the statistical analysis, contributed to the discussion and reviewed the manuscript. Maykon Anderson Pires de Novais contributed to the study design and reviewed the manuscript. Rui Monteiro de Barros Maciel contributed to the discus-

sion and reviewed the manuscript. Rosa Paula de Mello Biscolla designed the study, contributed to the discussion and reviewed the manuscript. Paola Zucchi designed the study, contributed to the discussion and reviewed the manuscript. All authors read and approved the final version.

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Evaluation of redox profiles in exogenous subclinical hyperthyroidism at two different levels of TSH suppression

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ABSTRACT

Objective: Evaluate the relationship between exogenous subclinical hyperthyroidism and oxidative stress through the analysis of the redox profile of patients with subclinical hyperthyroidism exogenous (SCH) grade I (TSH = 0.1 to 0.4 IU/mL) and grade II (TSH < 0.1 IU/mL). **Subjects and methods:** We analyzed 46 patients with SCH due to the use of TSH suppressive therapy with LT4 after total thyroidectomy along with 6 control euthyroid individuals (3M and 3W). Patients were divided into two groups, G1 with TSH ≥ 0.1-0.4 IU/mL (n = 25; and 7M 14W) and G2 with TSH < 0.1 IU/mL (n = 25; and 4M 21W). Venous blood samples were collected to measure the levels of markers for oxidative damage (TBARS, FOX and protein carbonylation), muscle and liver damage (CK, AST, ALT, GGT) and antioxidants (GSH, GSSG and catalase). **Results:** Individuals in G2 showed a GSH/GSSG ratio ~ 30% greater than those in G1 (p = 0.004) and a catalase activity that was 4 times higher (p = 0.005). For lipid peroxidation, the levels measured in G2 were higher than both control and G1 (p = 0.05). No differences were observed for both protein carbonyl markers. G1 and G2 presented with greater indications of cell injury markers than the control group. **Conclusion:** TSH suppression therapy with LT4 that results in subclinical hyperthyroidism can cause a redox imbalance. The greater antioxidant capacity observed in the more suppressed group was not sufficient to avoid lipid peroxidation and cellular damage. Arch Endocrinol Metab. 2018;62(5):545-51

Keywords
Subclinical hyperthyroidism; thyroidectomy; oxidative stress; antioxidants

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INTRODUCTION

Thyroid cancer is the most common malignancy to affect the endocrine system. Its incidence has increased worldwide in recent years with differentiated thyroid carcinoma (DTC) accounting for close to 90% of all cases (1). Treatments for DTC currently involve a partial or total thyroidectomy followed by radioiodine ablation and hormone suppression with levothyroxine (LT4) (2). The suppression of thyroid stimulating hormone (TSH) using LT4 at above physiological doses is widely used to reduce tumor recurrence, which induces exogenous subclinical hyperthyroidism (SCH) (3).

SCH is characterized by normal levels of thyroid hormones associated with low or undetectable TSH values (4). Studies have demonstrated that SCH has similar systemic effects to clinical hyperthyroidism that includes an increased metabolism resulting in a greater production of free radicals and alterations in redox balance leading to tissue damage (5-7). An imbalance between levels in free radicals and antioxidants is associated with some diseases, like diabetes and arterial coronary disease (8). Coronary disease is also associated with subclinical hyperthyroidism, because it leads to impaired endothelial function, oxidative stress and decreased insulin sensitivity (8-10).

An understanding of the effects associated with diseases and medications are important to prevent and treat possible collateral damage (11). Previous studies on oxidative stress in endogenous SCH have shown that there is an increase in lipid peroxidation and in the activity of antioxidant enzymes, such as SOD (5,12,13). However, until now, the oxidative profile of patients with exogenous SCH due to TSH suppression with LT4 as a treatment for DTC is not well known (8), as

well as the effect of different levels of TSH suppression on the oxidative profile.

Therefore, the aim of this study was to evaluate the redox status of euthyroid patients with exogenous SCH, comparing them according to two categories of TSH suppression: ≥ 0.1 to 0.4 IU/mL (grade 1) and < 0.1 IU/mL (grade 2) to the control group (euthyroid). The knowledge that the level of TSH suppression may influence the oxidative profile of SCH patients can be useful for making clinical decisions, including adjustments in the TSH levels that could be associated with an improved general health status.

SUBJECTS AND METHODS

Study and participants

A cross-sectional study was conducted. First, 74 patients were recruited from the Endocrine Clinic of the *Hospital Clementino Fraga Filho* (HUCFF, Rio de Janeiro, Brazil) that consisted of 6 euthyroid control individuals and 68 patients with exogenous SCH due to TSH suppressive therapy with LT4 after total thyroidectomy for DTC. All patients were 18 years of age or older and exhibited a stable health profile over the last year of follow-up exams. After a clinical examination, 22 subjects were excluded due to characteristics that could affect the oxidative stress measurements including: detectable thyroglobulin, positive whole body scan (PCI), C-reactive protein greater than 3.0 ng/L, possible current malignancy or serious illnesses, smokers, alcoholics and those on medications that could influence the oxidative stress.

None of the 46 SCH (34 women and 12 men) patients included in the study had active thyroid tissue and all had at least six months of suppressive therapy with LT4. Each had at least two dosages with TSH below the reference value (< 0.4 IU/mL) and normal FT4 (0.8 to 1.9 ng/dL) with undetectable thyroglobulin, anti-thyroglobulin antibody negative and negative whole-body scans.

This study was approved by the local ethics committee (040/11-CEP) and all patients gave their written consent before the study entry.

Procedures

The participants were divided according to the TSH value into three groups, as proposed by Biondi and cols. (2015): Control: 0.4 to 4 IU/ml; G1: TSH ≥ 0.1 to 0.4 IU/mL (n = 21) and G2: TSH < 0.1 IU/mL (n = 25). Fasting venous blood samples were collected at rest. Liver and muscle enzymes were measured in plasma because lipid peroxidation changes the membrane lipids and can lead to leakage of the cytosolic enzymes to the plasma (14,15).

The samples were centrifuged (1500 x g ≅ 4°C, 15 min) for plasma separation. Aliquots of 1 mL plasma were separated and stored at -80°C until analysis. After the plasma was removed, the erythrocytes were resuspended 1:1 in 0.9% saline and centrifuged (1500 X g ≅ 4°C, 15 min). The supernatant was discarded and the pellet was resuspended again at the same proportion until the process was performed three times.

The following hormones and biomarkers were considered in the study:

Thyroid-stimulating hormone – TSH: TSH was measured with a chemiluminescent immunometric assay third generation commercial kit using DPC® (Diagnostic Products Corporation, USA), following the manufacturer's instructions. The reading was performed in the Immulite 2000 system. The reference value for TSH was 0.4 to 4.0 IU/mL with an intra-assay variation of 3.8%-12.5% and inter-assay of 4.6%-12.5%.

Free thyroxine – FT4: Free T4 was measured by a chemiluminescent immunoenzymatic assay using the DPC® commercial kit (Diagnostic Products Corporation, USA), according to the manufacturer's instructions. The reading was performed in automatic device (Immulite 2000®). The reference value for T4 was 0.8-1.9 ng/dL with an intra-assay variation of 4.4%-7.5% and inter-assay variation of 4.8%-9.0%.

Biomarkers of cellular injury to liver and muscle

The plasma levels of the enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT) and creatine kinase (CK) were measured using commercial kits (Bioclin®, Brazil) following the manufacturer's specifications. Controls included the use of two control sera along with a Biocontrol normal and pathological (Bioclin®, Brazil).

Thiobarbituric acid reactive substances – TBARs

To determine plasma levels of TBARs, 50 µL of plasma was diluted with 150 µL of 100 mM phosphate buffer.

Next, an equal volume (200 µL) of 10% Trichloroacetic acid (TCA) was added, mixed and allowed to incubate for 15 min at 4°C before centrifugation (2200 x g for 15 minutes). To the supernatant (300 µL), an equal volume of TBA (465 mM thiobarbituric acid in 0.1 M Dimethyl Sulfoxide – DMSO) was added and the mixture heated at 95°C for 2 hours. After 15 min of cooling to room temperature, the absorbance of samples (200 µL) were measured at 532 nm in an ELISA plate. The concentration was calculated by a standard curve of TMP and the values presented in nmol MDA (16).

Ferrous oxidation xylenol orange – FOX

To 25 µL plasma sample, 25 µL of 40 mM butylated hydroxytoluene (BHT; 45 mg in 5 mL of 100% methanol) was added. The samples were vortexed for 1 minute and then centrifuged (5000 x g, 5 min ≅ 4°C). To the supernatant (20 µL), 360 µL of the working reagent (100 mM orange xylenol, 25 mM sulfuric acid and 250 mM ferrous sulfate diluted in 90% methanol) was added. The mixture was incubated at room temperature protected from light. After 2h, samples were centrifuged (10,000 x g, 10 min ≅ 4°C). The absorbance of the supernatant (200 µL) was read in an Elisa plate 560 nm. The lipid hydroperoxide concentration was calculated using the molar extinction coefficient of 4.46 x 10^4 $M^{-1}cm^{-1}$. The values are presented as nmol/L (17-19).

Protein carbonylation

To measure protein carbonylation, plasma (200 µL) was treated with 500 µL of 10 mM 2,4-dinitrophenylhydrazine (DNPH). A control blank was prepared at the same time for each sample. Next, 200 µL of the sample was mixed with 500 µL of HCl (2.5 M) followed by an incubation for one hour in the dark with vortexing every 15 minutes. This was followed by the addition of 1 mL of 10% TCA (10%) and centrifugation (15,000 x g 5 min ≅ 4°C). The supernatant was discarded and the prellet was washed with 1 mL of a 1:1 (v/v) mixture of ethyl acetate and ethanol 1:1. This was repeated three times. After the last wash, the pellet was resuspended in 1 mL of urea (6 M) and incubated at 37°C with shaking for 15 minutes. The absorbance was measured at 375 nm, and the data were calculated by molar extinction coefficient and expressed as mmol/mg protein (20).

Reduced gluthatione/gluthatione oxidized – GSH/GSSG ratio

GSH and GSSG were evaluated to determine if there was an upregulation of the antioxidant system that could explain the absence of cell damage. Assays for reduced gluthatione (GSH) and gluthatione oxidized (GSSG) were performed as described by Rahman and cols. (21). Briefly, 70 µL of sulfosalicylic acid (0.23 M) was added to plasma (200 µL). The mixture was immediately centrifuged (15,000 x g for 10 min ≅ 4°C). The supernatant was removed, aliquoted and stored at -80°C until the time of analysis.

To perform GSH measurements, 1.4 mL of buffer (100 mM potassium phosphate and 5 mM EDTA) was added to 200 µL of plasma. Then, 120 µL of 1.7 mM 5,5-Dithiobis (2-nitrobenzoic acid) (DTNB) and 120 µL glutathione reductase (500 units) were added. The mixture was homogenized for thirty seconds before the addition of 120 µL of 0.8 mM β-nicotinamide adenine dinucleotide 2'-phosphate (β-NADPH). The absorbance at 420 nm was measured for 120 seconds every thirty seconds. The concentration was expressed in µM based on the change in absorbance generated by the formation of 2-nitro-5-thiobenzoic. The final concentration was obtained from a linear regression of a GSH standard curve.

Oxidized glutathione (GSSG) was measured by adding 2 µL of vinylpyridine to 200 µL of plasma followed by a 1h incubation in the dark. Next, 12 µL of triethanolamine was added to adjust the pH. The subsequent steps were identical to the GSH assay.

Catalase

Enzyme activity was measured in erythrocytes as described by Aebi (22). Briefly, 10 µL of erythrocytes were diluted 200-fold and analyzed in 1 mL of a solution containing 50 mM PBS (pH 7.0) in the absence or presence of 30 mM H_2O_2. Absorbance was measured at 240 nm for 90 seconds. The absorbance of the samples and blank were recorded every 15 seconds. The results were expressed as k/mg Hb.

Statistics

All data were expressed as mean ± standard deviation. The comparisons between the two groups of differing TSH suppression levels were performed with the Kruskal-Wallis test and a Dunn's test for multiple comparisons. All analyses were made using GraphPad Prism software, version 6. Statistical significance was defined by a $p < 0.05$.

RESULTS

Table 1 shows the general characteristics of the participants grouped according to their TSH suppression levels. There were no statistical differences between the groups in terms of age, height or weight. Figure 1A shows that control group have regular TSH levels that are higher than G1 or G2. However, G2 was more suppressed than G1 (TSH = 0.03 ± 0.22 IU/mL vs. TSH = 0.005 ± 0.022 IU/mL, $p < 0.001$, respectively). The FT4 concentration was similar in both groups (G1 and G2) with the FT4 levels in G2 being higher than the control group (Figure 1B).

Table 1. Sample characteristics

	Control	G1 (TSH ≥ 0.1 to 0.4 IU/mL)	G2 (TSH < 0.1 IU/mL)
Age (years)	52.2 ± 7.1	46 ± 16	55 ± 11
Weight (kg)	67 ± 9.5	78 ± 14.2	75.9 ± 16.4
Height (m)	1.7 ± 0.1	1.7 ± 0.1	1.6 ± 0.1
Gender	3 women; 3 men	14 women; 7 men	21 women 4 men

Control group – euthyroid (6); G1-TSH ≥ 0.1 to 0.4 UI/mL (n = 21); G2-TSH < 0.1 UI/mL (n = 25). Data are presented as mean ± SD.

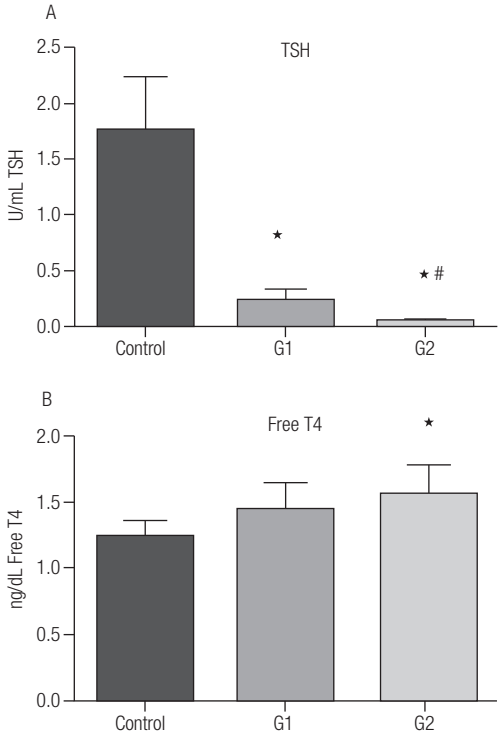

Figure 1. Hormonal concentration of (**A**) Thyroid stimulating hormone (TSH) and (**B**) Free thyroxine FT4. Control group – euthyroid (6); G1-TSH ≥ 0.1 to 0.4 UI/mL (n = 21); G2-TSH < 0.1 UI/mL (n = 25). Data are presented as mean ± SD. * $p < 0.05$ vs. control; # $p < 0.05$ vs. G1.

The level of lipid peroxidation as measured by TBARS was similar in all three groups (Figure 2A). In contrast, when the levels were measured by the FOX assay, G2 showed an increase in lipid peroxidation in comparison to the control and G1 (Figure 2B). While the lipid peroxidation levels in G2 suggested oxidative damage, the evaluation of the extent of protein carbonylation showed no significant differences between the three groups (Figure 2C).

Both of the SCH groups (G1 and G2) did present higher values than the control group in the plasma concentration of all cell damage markers (Table 2). In the group comparisons, G1 appeared to show greater

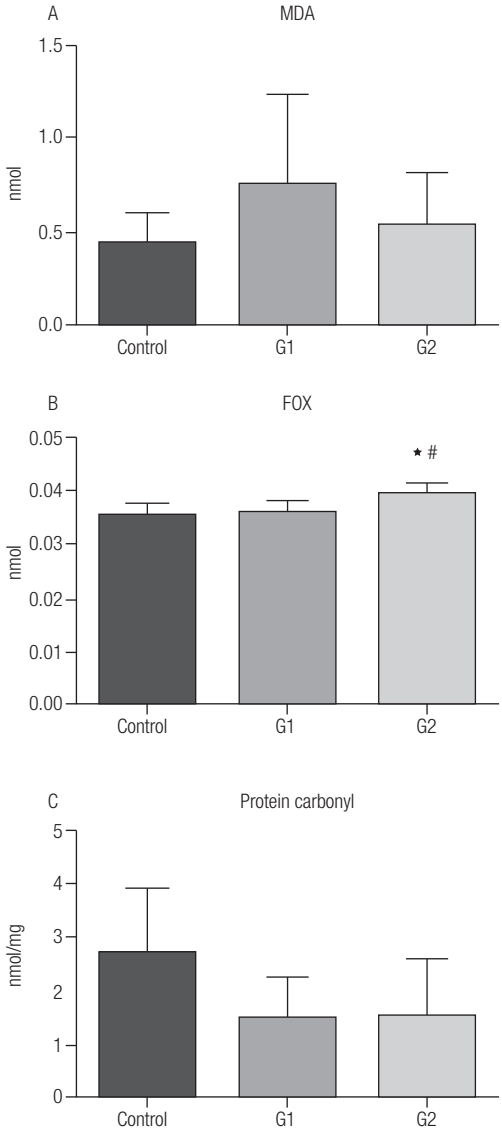

Figure 2. Lipid peroxidation (MDA – malondialdehyde) (**A**), FOX (**B**), protein carbonyl (**C**). Control group – euthyroid (6); G1-TSH ≥ 0.1 to 0.4 UI/mL (n = 21); G2-TSH < 0.1 UI/mL (n = 25). Data are presented as mean ± SD. *$p < 0.05$ vs. control; #$p < 0.05$ vs. G1.

Table 2. Plasma biomarkers of cellular damage

	Control	G1 (TSH ≥ 0.1 to 0.4 IU/mL)	G2 (TSH < 0.1 IU/mL)
AST (U/L)	10.51 ± 1.33	22.19 ± 0.99*	22.36 ± 0.94*
ALT (U/L)	27.03 ± 3.58	40.53 ± 2.47*	43.32 ± 2.41*
GGT (U/L)	17.83 ± 3.3	42.29 ± 5.08*	42.60 ± 4.52*
CK (U/L)	65.44 ± 30.48	135.9 ± 9.32*	143.1 ± 19.43*

AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma glutamine transferase; CK: creatine kinase. Control group – euthyroid (6); G1-TSH ≥ 0.1 to 0.4 UI/mL (n = 21); G2-TSH < 0.1 UI/mL (n = 25). Data are presented as mean ± SD. *p < 0.05 vs. control; p < 0.05 vs. G1.

significant differences than G2 to controls for AST (control vs G1, p = 0.0003; control vs G2, p = 0.001); ALT (control vs G1, p = 0.0008; control vs G2, p = 0.01), GGT (control vs G1, p = 0.02; control vs G2, p = 0.03), and CK (control vs G1, p = 0.01; control vs G2, p = 0.04).

Figure 3A shows that the GSH concentration was higher in both suppressed groups. For GSSG, its concentration was lower in G2 when compared with G1 and control group (Figure 3B). To estimate the redox balance of these patients, GSH/GSSG ratio was calculated (Figure 3C). The GSH/GSSG ratio was smaller in G1 than control (p = 0.0053) with the ratio of G2 being greater than G1. The catalase activity (Figure 3D) was lower in G1 than control and G2 (p = 0.0006 and 0.03, respectively).

DISCUSSION

There is a consensus in the literature that hyperthyroidism induces lipid peroxidation (5,12,13), which is hallmark of oxidative stress. Yavuz and cols. (8) showed that SCH promotes oxidative stress that was associated with endothelial impairment. However, the study did not relate the level of oxidative stress to different TSH levels. To date, no research has evaluated the level of lipid peroxidation among patients with regards to different levels of suppression. The aim of the study reported here was to compare the redox profiles of patients with exogenous SCH considering two categories of TSH suppression: ≥ 0.1 to 0.4 IU/mL (G1) and < 0.1 IU/L levels of TSH suppression in exogenous SCH (4).

Our measurements of lipid peroxidation using the FOX assay showed higher levels associated with the exogenous subclinical hyperthyroid state in the G2 group compared with the less suppressed (G1) and

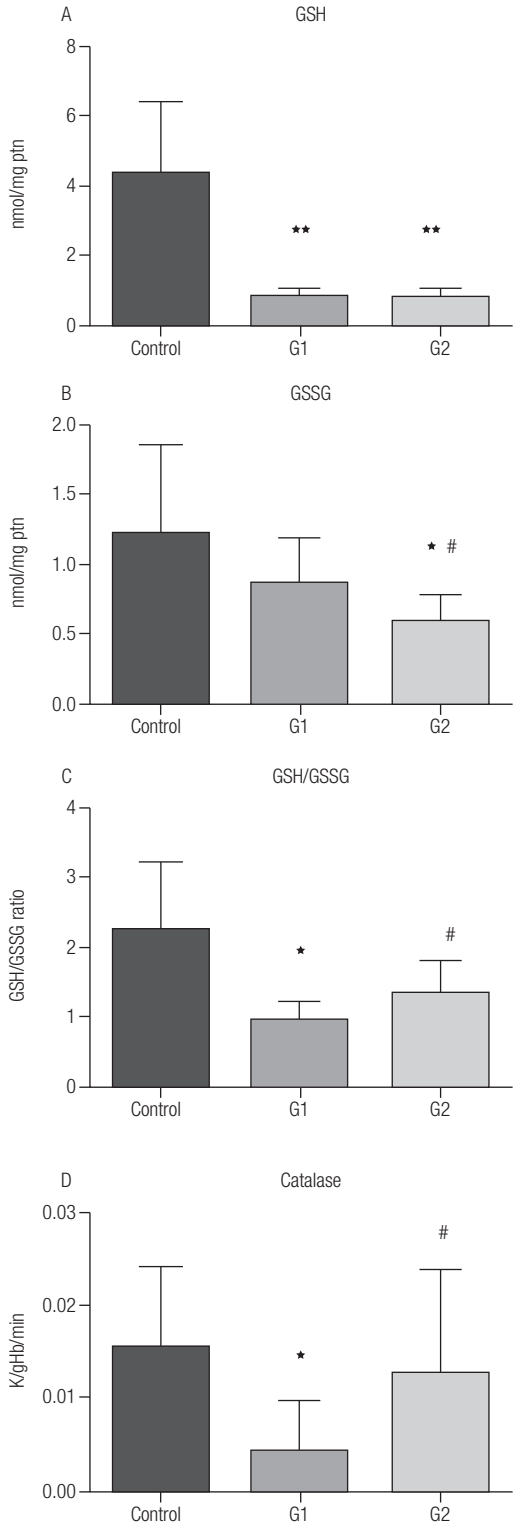

Figure 3. Antioxidant profile: (**A**) GSH; (**B**) GSSG; (**C**) GSH/GSSG and (**D**) Catalase. G1-TSH ≥ 0.1 to 0.4 UI/mL (n = 21); G2-TSH < 0.1 UI/mL (n = 25). GSH, reduced glutathione; GSSG, oxidized glutathione; GSH/GSSG, ratio reduced glutathione/oxidized glutathione. Control group – euthyroid (6); G1-TSH ≥ 0.1 to 0.4 UI/mL (n = 21); G2-TSH < 0.1 UI/mL (n = 25). Data are presented as mean ± SD. *p < 0.05 vs. control; #p < 0.05 vs. G1.

control group (Figure 2B). In contrast, measurements by TBARS did not show any differences between the groups. While both the FOX and TBARS assays measure lipid peroxidation, these methods have different affinities for the intermediaries of the lipid peroxidation cascade. As such, it is not uncommon for their results to show different behaviors (17), which suggests that G2 group did experience oxidative stress.

Another oxidative marker is carbonyl groups on proteins (23). Here, the SCH groups did not show any difference when compared with control (Figure 2C). This was unexpected since Mseddi and cols. (24) had observed that patients with Hashimoto's thyroiditis had increased plasma protein carbonyl. Considering that Hashimoto's thyroiditis is associated with an increased inflammatory profile and oxidative stress (25) while the SCH patients here had no active inflammatory process, the presence or absence of differences in protein carbonyl levels can be associated with the type of disease. Since our patients have exogenous subclinical hypertodism, the results suggest that exogenous hormonal variation alone could not significantly increase protein carbonyl levels.

Elevated T4 levels, together with free radical production, has been associated with hepatic cellular damage as observed by transaminases in the plasma (26). Another study in rats also showed also that an excess of T4 increased liver injury as measured by increases in AST, ALT, and GGT plasma concentration in rats (27). The results from the G2 group, which have higher T4 concentrations in comparison to the control group (Figure 1B), are consistent with these previous reports with increases in AST, ALT and GGT (Table 2). However, the G1 group, which has T4 levels within the normal range (Figure 1B), also presented with an increase in the levels of the biomarkers for cell lesion (Table 2). In this case, the increased in these biomarkers of hepatic lesion can be associated to the reduction of redox equilibrium observed in this group (Figure 3C), also in agreement with Massarah and Boumendjal (26). It is interesting to observe that both AST and GGT were higher than the normal reference values in G1 and G2 (normal values: AST < 32 U/L, GGT < 38 U/L), indicating hepatic damage induced by TSH suppression. As well as the hepatic biomarkers of cell damage, we also observed an increased plasma concentration of CK, which is characteristic for muscular damage (28). These measured increases in circulating enzyme levels in patients with TSH suppression suggest an affect not only on hepatic tissue, but also muscle.

Defensive measures to prevent cellular damage from oxidative processes include increased antioxidants either through enzymatic or non-enzymatic reactions. The major endogenous non-enzymatic antioxidant is glutathione, which can be in its reduced (GSH) or oxidized (GSSG) form and the ratio of GSH to GSSG provides indications of the tissue redox balance (29). In the G1 and G2 groups, the reduced glutathione (GSH) level, antioxidant was lower when compared with the control group (Figure 3A), which suggests a reduced antioxidant capacity in both TSH suppressed groups. For the oxidized form (GSSG), G2 had a lower concentration compared to G1 and control group (Figure 3B). The redox equilibrium calculated from the GSH/GSSG ratio was diminished in G1 compared to the control and G2 groups (Figure 3C). The reduced imbalance in redox equilibrium observed for the G2 group is most likely a result of the increase in catalase activities (Figure 3D). In a rat model of hyperthyroidism, catalase liver activity, GPx, and lipid peroxidation were all increased suggesting that increased oxidative damage, as observed in G2, can induce increases of antioxidants enzymes activity to control the imbalance (26). This is consistent with our results for G2, since it was the only group that presented with an increase in lipid peroxidation (Figure 2B) and it was the most TSH suppressed group (G2).

This study is the first attempt to understand redox status and possible cellular damage from oxidative stress in SCH patients presenting with TSH at different levels. The results suggest that TSH suppression therapy with LT4 that leads to subclinical hyperthyroidism can cause different oxidative responses when patients are segregated by their TSH suppression levels into two groups (TSH < 0.01 IU/mL; TSH between 0.1 to 0.4 IU/mL). Both groups showed hepatic damage, but the group with lower TSH (G2) displayed higher levels of lipid peroxidation as well as increased antioxidant capacity. In conclusion, we encourage subclinical hyperthyroidism researchers to categorize patients by their TSH levels as suggested by Biondi and cols. 2015, since different oxidative damage can aggravate or attenuate diseases associated with subclinical hyperthyroidism. Future studies will help understand how these different interfere on physiological functions.

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Lipid disorders among Black Africans non-users of lipid-lowering medication

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ABSTRACT

Objective: Angola is a sub-Saharan African country where the population has scarce access to lipid-lowering medication. We sought to determine the frequency of lipid disorders among Angolan non-users of lipid-lowering medication. **Material and methods:** A cross-sectional descriptive study was carried out in a sample of 604 workers from the public sector. Blood pressure and anthropometric data were measured along with biochemical parameters including total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C). LDL-C to HDL-C ratio (LDL-C/HDL-C) was obtained from LDL-C and HDL-C levels. **Results:** High frequencies of elevated blood pressure (44.8%), metabolic syndrome (20.2%), increased TC (39.2%) and increased LDL-C (19.3%) were found. Low HDL-C was more frequent in women (62.4% *vs.* 36.1%, $p < 0.001$). Isolated hypercholesterolemia was more frequent in men (9.6% *vs.* 2.5%, $p < 0.001$). Among men TC, TG, LDL-C and LDL-C/HDL-C ratio were higher and HDL-C was lower in obese than in low-weight and normal-weight participants. Among women TC, TG, LDL-C and LDL-C/HDL-C ratio were higher in obese than in normal-weight participants. Significant linear trend of increasing TC and LDL-C levels as age increased was detected for both genders (p for trend < 0.05). **Conclusion:** The results of our study showed a high frequency of lipid disorders in Angolan non-users of lipid-lowering medication. Arch Endocrinol Metab. 2018;62(5):552-9

Keywords
Lipid disorders; Black Africans; lipid-lowering medication

INTRODUCTION

Dyslipidemia is a multifactorial lipid disorder that is dependent on genetic, environmental and lifestyle factors and is described as one of the most relevant risk factors for cardiovascular diseases (1). Indeed, dyslipidemia has been identified as one of the major modifiable risk factors for ischemic heart disease among young adults (2).

The definition of dyslipidemia is the presence of abnormal blood lipid levels, encompassing increased plasma levels of total cholesterol (TC), low-density lipoprotein (LDL-C) and triglycerides (TG), as well as decreased levels of high-density lipoprotein (HDL-C) (3).

In sub-Saharan Africa, the majority of countries is experiencing a rapid demographic and epidemiological transition (4,5). Several studies have reported the prevalence of cardiovascular risk factors in sub-Saharan African populations (6-8), but these data are limited to a few countries (9,10).

Angola is a sub-Saharan African country where in the last few years, significant economic growth and increased urbanization has occurred (11). These changes may imply an increasing prevalence of diverse cardiovascular risk factors such as obesity, insufficient physical activity, high blood pressure and dyslipidemia.

The Angolan population has scarce access to lipid-lowering pharmacological therapy, which offers an ideal opportunity to analyze the lipid profile in a large sample with minimal interfering factors. Therefore, in this study, we sought to determine the frequency of lipid disorders in an Angolan population consisting of non-users of lipid-lowering medication.

MATERIAL AND METHODS

Study design

This cross-sectional descriptive study was carried out in a sample consisting of civil servants working at Agostinho Neto University (UAN) in Luanda, Angola. The survey was conducted in the Department of Physiology from the Faculty of Medicine. All data were collected from 2009 to 2010, and details of the study design were described elsewhere (12,13). The project was approved by the Independent Ethics Committee on Research of the Faculty of Medicine of Agostinho Neto University following the standard procedures in human research in accordance with the Declaration of Helsinki.

Subjects ≥ 20 years of age working at UAN were invited to participate in a survey of cardiovascular risk factors. From the eligible sample comprising 1,458 staff members, 614 (42%) responded to the invitation. Only nine subjects (1.4%) were excluded from the present analysis due to use of lipid-lowering medication.

Demographics including socioeconomic class, educational level and medical history were collected during an interview using a structured questionnaire, as previously reported (14). Participants were classified as non-smokers (never and former smokers) and current smokers (daily and occasional smokers).

Socioeconomic classes were categorized into quartiles according to average monthly household income: first quartile (low class), second quartile (middle class), third quartile (upper middle class) and fourth quartile (upper class). Education levels were classified into three categories based on the number of years of education: low (≤ four years of education), middle (five to 12 years of education) and high (≥ 13 years of education) (13).

Biochemical analyses

Participants reported to the Faculty of Medicine after 12h of fasting. They were asked to refrain from smoking, physical exercise and caffeinated beverages for at least 12 hours before the visit. Clinical exams were performed in a temperature-controlled room (22°C – 23°C) between 8 a.m. and noon. For determination of serum levels of TC, TG, HDL-C and glucose, venous blood was obtained by standard *forearm venipuncture* and processed immediately using commercially available kits (Biosystems S.A. Costa Brava 30, Barcelona, Spain).

All biochemical parameters were analyzed by enzymatic methods with a spectrophotometer (Biosystems BTS 310, Barcelona, Spain). LDL-C was calculated as previously described (15), and very low-density lipoprotein cholesterol (VLDL-C) was calculated as triglycerides/5 for those participants with TG < 400 mg/dL according to the Third Report of the National Cholesterol Education Program Expert Panel on detection, evaluation and treatment of high blood cholesterol in adults (NCEP-ATP III) (16). The LDL-C to HDL-C ratio (LDL-C/HDL-C) was obtained from LDL-C and HDL-C levels.

Diabetes mellitus was defined as fasting glucose ≥ 126 mg/dL or the use of antidiabetic drugs (17).

Dyslipidemia was classified into four phenotypes in accordance with V Brazilian Guidelines on Dyslipidemia and Atherosclerosis: isolated hypertriglyceridemia (TG ≥ 150 mg/dL), isolated hypercholesterolemia (LDL ≥ 160 mg/dL), mixed hyperlipidemia (TG > 150 and TC ≥ 200 mg/dL) and low HDL-C (isolated reduction of HDL-C, men < 40 mg/dL and women < 50 mg/dL or combined with either increased LDL-C or increased TG) (18).

Metabolic syndrome was defined based on the presence of three or more of the following conditions: waist circumference (WC) > 102 cm (men) or > 88 cm (women), systolic blood pressure (SBP) ≥ 130 mmHg and/or diastolic blood pressure (DBP) ≥ 85 mmHg and/or BP-lowering treatment, fasting triglyceride levels ≥ 150 mg/dL (1.70 mmol/l) or treatment for hypertriglyceridemia, HDL-C < 40 mg/dL (1.04 mmol/l) (men) or < 50 mg/dL (1.30 mmol/l) (women), treatment for dyslipidemia, fasting glucose level ≥ 110 mg/dL or use of anti-diabetic medication (16).

Anthropometric measurements

Anthropometric measures of weight, height, WC and hip circumference (HC) were obtained with participants barefoot and wearing only underwear. Weight was measured to the nearest 0.1 kg using a previously calibrated mechanical scale (SECA GmbH & Co, Germany). A stadiometer fixed to the scale was used to measure body height to the nearest 0.5 cm.

WC and HC were measured twice using an inextensible 1-cm-wide tape measure. WC was measured at the end of a normal expiration at the midpoint between the lower border of the rib cage and the top of the iliac crest (19). The waist-to-hip ratio (WHR) was calculated from the WC and HC.

BMI was calculated as the weight in kilograms divided by height in meters squared (kg/m²). According to the BMI values, the participants were classified as low weight (< 18.5 kg/m²), normal weight (18.5–24.9 kg/m²), overweight (25.0–29.9 kg/m²) and obese (≥ 30.0 kg/m²) (20).

Hemodynamic measurements

Blood pressure measurement was performed after a five-minute rest in triplicate in the non-dominant arm with the arm at the level of the heart. Systolic blood pressure (SBP), diastolic blood pressure (DBP) and HR were obtained using a validated, automated digital oscillometric sphygmomanometer (Omron 705CP, Tokyo, Japan). The readings were repeated at three-minute intervals. The mean of the last two readings was recorded. Elevated blood pressure was defined as SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg and/or the use of antihypertensive drugs.

Statistical analysis

Statistical analysis was performed using SPSS software, version 20.0 (SPSS Inc., Chicago, IL, USA). The data's normality was examined using the Kolmogorov-Smirnov test. Continuous variables were reported as mean ± standard deviation (SD) or as proportions.

Student's *t* test was used to compare the means of two groups. Linear association between lipid fractions and categories of nutritional status was tested with ANCOVA. The chi-squared test was used to compare proportions. A test for linear trends (Jonckheere-Terpstra test) was performed to test the linear association between the age range as the independent variable and lipid fractions as the dependent variable. Normal distribution curves of total cholesterol and triglycerides were drawn separately for gender and median (P^{50th}) with corresponding interquartile intervals ($P^{25th} – P^{75th}$) provided. The level of significance was set at $p < 0.05$.

RESULTS

Demographic data are summarized in Table 1. As expected, the sample comprised predominantly black individuals. Regarding educational level and socioeconomic status, approximately 60% had at least five years of formal education, and almost 68% were classified as members of the middle/upper socioeconomic class.

Table 1. Demographic characteristics of sample

	N	%
Gender		
Male	291	48
Female	314	52
Total	605	100
Age range (years)		
22-31	88	14.5
32-41	137	22.6
42-51	207	34.2
52-61	149	24.6
62-72	24	4.0
Race/ethnicity		
White	5	0.8
Black	577	95.4
Mulattos	23	3.8
Education level		
Low	243	40.2
Medium	150	24.8
High	212	35.0
Socioeconomic Class		
Low	191	31.6
Middle	203	33.6
Upper	209	34.5
MS	2	0.3

MS: missing data.

Table 2 shows the sample's general characteristics, emphasizing the comparison between genders. The age was similar between genders, but higher BMI and WC were observed in women than in men. A significant gender difference was not detected for SBP and DBP. Moreover, lipid profile was similar between genders, but HDL-C was higher in women than in men ($p < 0.001$).

Overall, a high frequency of elevated blood pressure (44.8%), metabolic syndrome (20.2%), increased TC (39.2%) and increased LDL-C (19.3%) was found. Moreover, gender interfered with the frequency of some risk factors. Obesity, metabolic syndrome and low HDL-C were more frequent in women than in men. On the other hand, smoking and isolated hypercholesterolemia were more frequent in men than in women (Table 3).

Table 4 shows the association between lipid fraction and nutritional status for both genders. Among men, TC, TG, LDL-C and the LDL-C/HDL-C ratio were higher, and HDL-C was lower in obese participants than in low-weight and normal-weight participants.

Table 2. Anthropometric and biochemical characteristics of sample stratified by gender

	Male	Female	*p value*
Age (years)	45.1 ± 11.1	43.8 ± 10.0	0.18
Weight (kg)	67.7 ± 4.7	69.1 ± 15.7	0.33
Height (cm)	167 ± 7.0	159.5 ± 6.6	< 0.001
BMI (kg/m^2)	24.0 ± 4.2	27.1 ± 5.8	< 0.001
WC (cm)	79.9 ± 12.8	83.7 ± 13.5	< 0.001
HC (cm)	91.4 ± 9.4	99.5 ± 1.5	< 0.001
WHR	0.87 ± 0.08	0.84 ± 0.09	< 0.001
SBP (mmHg)	137 ± 23	133 ± 27	0.09
DBP (mmHg)	83 ± 14	82 ± 14	0.86
Heart rate (bpm)	67 ± 10	69 ± 10	0.003
Glucose (mg/dL)	95 ± 20	92 ± 19	0.31
TC (mg/dL)	190 ± 42	192 ± 36	0.24
LDL-C (mg/dL)	125 ± 41	125 ± 38	0.79
HDL-C (mg/dL)	44 ± 10	48 ± 11	< 0.001
TG (mg/dL)	101 ± 42	99 ± 38	0.34
VLDL-C (mg/dL)	20± 8	20 ± 7	0.34

BMI: body mass index; WC: waist circumference; HC: hip circumference; WHR: waist-to hip ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; VLDL-C: very low-density lipoprotein cholesterol; TG: triglycerides.

Table 3. Risk factors in the sample and stratified by gender

	All	Male	Female	*p value*
Elevated blood pressure	44.8	46.0	43.6	0.30
Diabetes	5.1	5.2	5.1	0.56
Obesity	19.2	8.9	28.7	< 0.001
Metabolic Syndrome	20.2	16.2	23.9	0.01
Smoking	6.1	8.2	4.1	0.03
TC ≥ 200	39.2	38.8	39.5	0.47
Isolated hypercholesterolemia	6.0	9.6	2.5	< 0.001
LDL-C ≥ 160 mg/dL	19.3	20.6	18.2	0.25
Isolated hypertriglyceridemia	4.6	6.2	3.2	0.06
TG ≥ 150 mg/dL	10.7	12.4	9.2	0.13
Mixed hyperlipidemia	1.7	1.7	1.6	0.903
Low-HDL-C	49.8	36.1	62.4	< 0.001

Data are presented as proportions (%). Elevated blood pressure: SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg or normotensive on antihypertensive medication. Diabetes: fasting glucose ≥ 126 mg/dL or on medication. TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high density lipoprotein cholesterol; TG, triglycerides. Isolated hypercholesterolemia: isolated elevation of LDL-C (≥ 160 mg/dL); Isolated hypertriglyceridemia: isolated elevation of TGs (≥ 150 mg/dL); Mixed hyperlipidemia: Increased LDL-C (≥ 160 mg/dL) combined with increased TG (≥ 150 mg/dL). Low HDL-C: isolated reduction of HDL-C (Men < 40 mg/dL, Women < 50 mg/dL) or in association with elevated LDL-C and TG.

A similar difference was found in overweight participants compared with low-weight participants, but in TG levels, such a difference was not detected between overweight and normal-weight men. On the other hand, among women, TC and LDL-C were higher in obese and overweight participants than in normal-weight participants, and TG was higher in obese participants than in low-weight and normal-weight participants. The LDL-C/HDL-C ratio was higher in obese participants than in normal-weight participants, but HDL-C was not different across the categories of nutritional status.

Table 4. Association between lipid fraction and weight status

	Low weight	Normal weight	Overweight	Obese
Male				
n (%)	16 (5.6)	168 (58.3)	78 (27.1)	26 (9.0)
TC (mg/dL)	168 ± 26	184 ± 39	199 ± 46*†	214 ± 33*†
TG (mg/dL)	78 ± 34	98 ± 36	108 ± 49†	123 ± 46*†
LDL-C (mg/dL)	104 ± 25	119 ± 39	135 ± 46*†	149 ± 36*†
HDL-C (mg/dL)	48 ± 14	46 ± 10	42 ± 10*†	40 ± 8*†
LDL-C/HDL-C	2.4 ± 1.0	2.8 ± 1.3	3.4 ± 1.4*†	3.9 ± 1.5*†
Female				
n (%)	11(3.6)	111(36.4)	93 (30.5)	90 (29.5)
TC (mg/dL)	191± 29	186 ± 35	196 ± 33*	197 ± 39*
TG (mg/dL)	88 ± 37	94 ± 34	97 ± 40	109 ± 41#*
LDL-C (mg/dL)	125 ± 29	118 ± 35	129 ± 34*	129 ± 44*
HDL-C (mg/dL)	48 ± 7	49 ± 11	47 ± 12	46 ± 11
LDL-C/HDL-C	2.7 ± 0.7	2.6 ± 1.1	2.9 ± 1.2	3.1 ± 1.5*

Data are mean ± standard deviation. TC: total cholesterol; TG: triglycerides; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; LDL-C/HDL-C: LDL-C to HDL-C ratio. *$p < 0.05$ *vs.* normal weight, #$p < 0.05$ *vs.* overweight, †$p < 0.05$ *vs.* low weight. Comparisons adjusted for age.

A significant linear trend of increasing TC, LDL-C and TG levels as well as decreasing HDL-C levels with increasing age was observed among women (p for trend < 0.05) (Figure 1). On the other hand, a significant linear trend of increasing TC and LDL-C levels with increasing age was detected (p for trend < 0.01), but no significant linear trend was detected between age range and HDL-C and TG among men.

Figure 2 shows the distribution curves of TC and TG for women and men. The medians with interquartile intervals for TC were 191 mg/dL (170-215 mg/dL) and 189 mg/dL (159-217 mg/dL) in women and men, respectively. Likewise, the medians with corresponding interquartile intervals for TG were 91 mg/dL (75-118 mg/dL) and 96 mg/dL (74-120 mg/dL), respectively. The areas under the distribution curves provide the proportion of individuals with TC and TG above the 75th percentile.

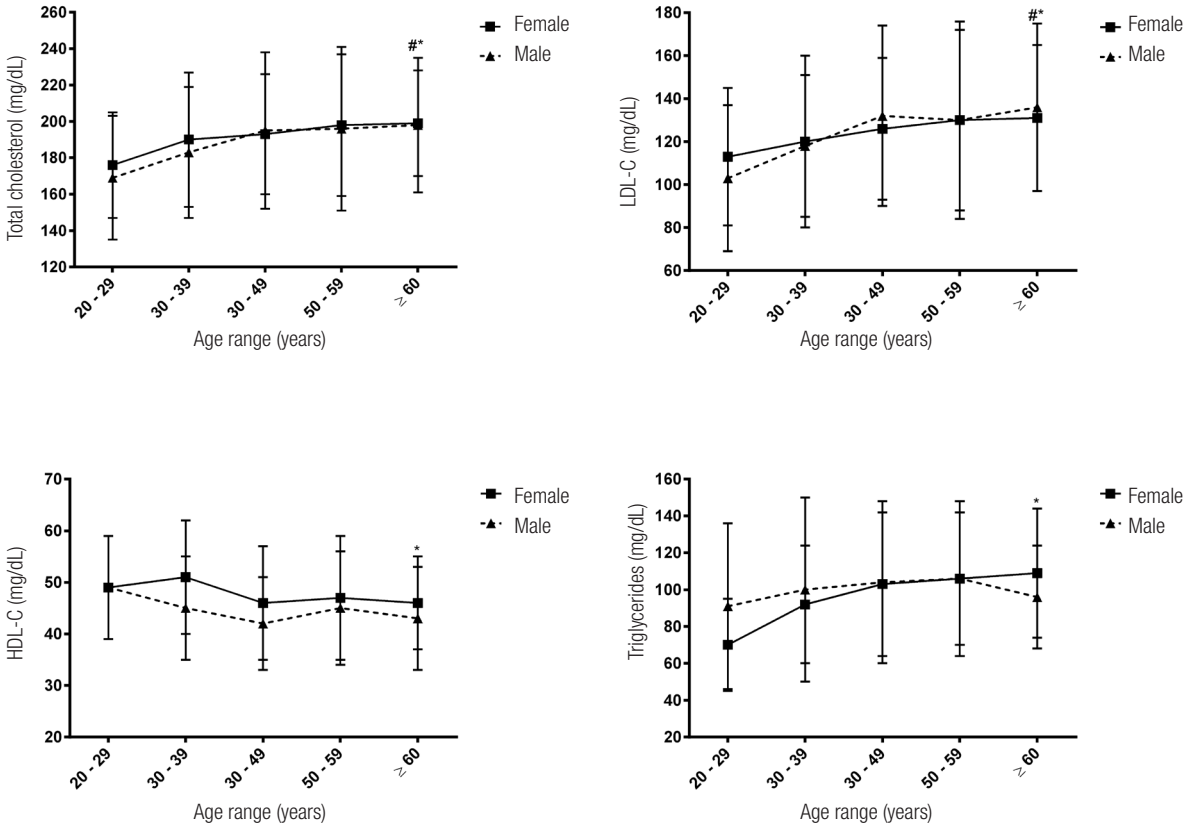

Figure 1. Test for significant linear trend between age range and total cholesterol, LDL-C, HDL-C and triglycerides. # men, *p* for trend < 0.05 ; *women, *p* for trend < 0.05.

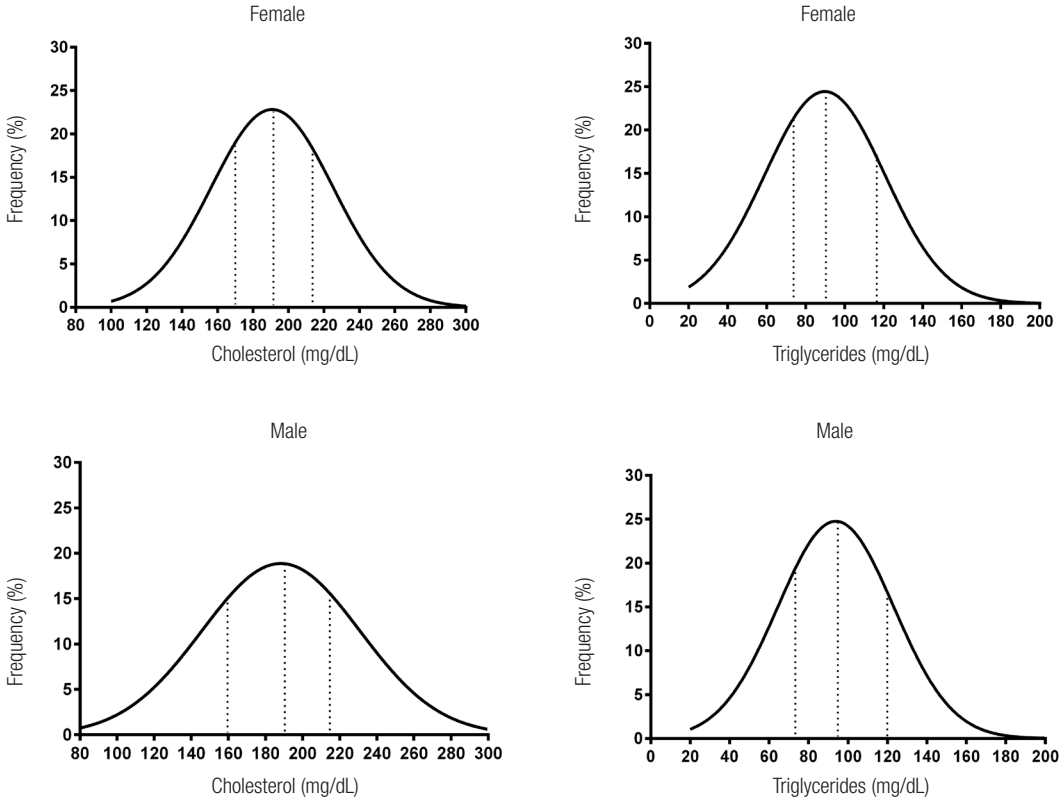

Figure 2. Nornal distribution curves of total cholesterol and triglycerides separately by gender. Dot lines indicate 25th, 50th and 75th percentiles from the left to the right.

DISCUSSION

The present study was carried out in Angolan civil servants, predominantly black and of middle/upper socioeconomic class. Higher frequencies of obesity, metabolic syndrome and low HDL-C were found in women than in men. On the other hand, obesity seems to cause the lipid profile to undergo greater changes in men than in women. Moreover, a significant linear trend of increasing TC and LDL-C levels as age increased was detected for both genders.

Epidemiologic studies have shown that elevated serum levels of cholesterol in the population increases the risk for coronary heart disease (21,22). Moreover, dyslipidemia, among other risk factors, has been implicated in the pathogenesis of stroke (23).

Data from the 2003 through 2006 NHANES survey were analyzed to determine the proportion of the US population with abnormalities in the lipid profile (24). Despite the existence of efficacious and cost-effective treatment guidelines, high prevalence of lipid abnormalities such as high LDL-C (27.0%), elevated TG levels (30.0%) and low HDL-C (23%) was reported. Although the study described here is not a population-based cohort, compared with the US population, Angolan civil servants have a lower proportion of high LDL-C (19.3%) and elevated TG (10.7%) and a much higher proportion of low HDL-C (49.8%), which is in great part affected by the huge proportion of low HDL-C observed in women (62.4%). Indeed, this finding is alarming given the strong association of low HDL-C with atherosclerosis (25).

An expert panel from the American College of Cardiology/American Heart Association recommended that all individuals over 20 years of age with LDL-C levels above 190 mg/dL should be encouraged to either start or continue statin use for prevention of coronary heart disease and stroke (26). However, statins are still widely underused in the clinical setting, and poor adherence to statin therapy has become a major concern for preventive cardiology (27).

Recently, a Finnish population-based study showed the cardiovascular burden of medication non-adherence (28). The rate of adherence to statins was 58% in men and 60% in women, which the authors classified as intermediate. In addition, compared with adherent patients, the adjusted odds ratio for stroke death in non-adherents was 2.04 (CI95% 1.72–2.43) at the year of death or at the end of follow-up.

Regarding African populations, most of the studies dealing with adherence to statin therapy were conducted among people of African descent living outside Africa. One of these studies reported that ethnicity (African American) was among the predictors of non-adherence to statin therapy (29). Moreover, among white and black Medicare beneficiaries discharged from the hospital following an ischemic stroke, the adherence to statin therapy was lower in African Americans than in white subjects (30).

Lack of access to medical care has been considered one of the plausible explanations for the differences in the adherence to statin therapy between black and white subjects. However, a previous study has shown that even without access barriers, African Americans were less adherent to medication therapies, including statins (31).

To our knowledge, the adherence rate to lipid-lowering pharmacological therapy has not been documented in black African countries. Recently, a retrospective longitudinal study among Ghanaian heart failure patients has shown that statin users had a higher survival rate than those non-users who underwent another therapeutic option, but therapy adherence was not within the study's scope (32).

In the current study, around 60% of the volunteers claimed to have a good, or high, education level, and 68.1% comprised the middle or upper socioeconomic class. However, the mean values for the metabolic parameters were similar to those previously reported in the lower socioeconomic strata from black African populations (33). This result reflects the epidemiological transition of the Angolan population because even with enhanced socioeconomic status, the frequency of metabolic syndrome and elevated cholesterol levels remain meaningfully high, and this scenario tends to get worse as age advances.

Because this study was cross-sectional, it is not appropriate to establish a causal relationship between non-adherence to lipid-lowering therapy and cardiovascular risk factors. Indeed, this study was not designed to address this particular issue. In addition, as the sample was composed of public sector workers, the results should not be extrapolated to the whole Angolan population. However, it is worth wondering whether a high frequency of lipid disorders without treatment was observed in a population comprising almost two-thirds of the middle/upper-income segment and what to

expect from a representative sample of the population comprising approximately 85% of the low-income class.

In summary, a high frequency of lipid disorders was observed among Angolan non-users of lipid-lowering medication. Longitudinal studies should be conducted to highlight the long-term consequences of non-treated lipid disorders in the Angolan population.

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Congenital hyperinsulinism in two siblings with *ABCC8* mutation: same genotype, different phenotypes

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SUMMARY

Congenital hyperinsulinism (CHI) is a heterogenous disease caused by insulin secretion regulatory defects, being *ABCC8/KCNJ11* the most commonly affected genes. Therapeutic options include diazoxide, somatostatin analogues and surgery, which is curative in focal CHI. We report the case of two siblings (born two years apart) that presented themselves with hypoketotic hyperinsulinemic persistent hypoglycemias during neonatal period. The diagnosis of diffuse CHI due to an *ABCC8* compound mutation (c.3576delG and c.742C>T) was concluded. They did not benefit from diazoxide therapy (or pancreatectomy performed in patient number 1) yet responded to somatostatin analogues. Patient number 1 developed various neurological deficits (including epilepsy), however patient number 2 experienced an entirely normal neurodevelopment. We believe this case shows how previous knowledge of the firstborn sibling's disease contributed to a better and timelier medical care in patient number 2, which could potentially explain her better neurological outcome despite their same genotype. Arch Endocrinol Metab. 2018;62(5):560-5

INTRODUCTION

Congenital hyperinsulinism (CHI) is the most common cause of persistent hypoglycemia in infancy and it can potentially lead to permanent neurological damage (1,2). It is the result of a group of genetic disorders which can lead to severe and persistent hypoglycemia (3). Estimated incidence is 1/50,000 live births (4).

The disease is caused by mutations in various genes related to insulin secretion regulation (5), such as: *ABCC8* (6), *KCNJ11* (7), *GCK* (8), *GLUD1* (9), *HADH* (10), *SLC16A1* (11), *HNF4A* (12), *HNF1A* (13), *UCP2* (14), *HK1* (15) and *PGM1* (16). The most common mutations affect *ABCC8* and *KCNJ11* genes and lead to inactivation of the ATP-dependent potassium channel (K_{ATP}) subunits (SUR1 and Kir6.2 respectively), resulting in unregulated insulin β-cell secretion (2,4,7,17).

Histologically the disease is characterized by Langerhans islet hyperplasia and can present as diffuse (18) or focal form (19), which is at least partially determined by the type of genetic defect (3). The two forms are clinically indistinguishable and the best way to differentiate is by performing a 18F-fluoro-L-DOPA positron emission tomography (PET) (20).

Pharmacotherapeutic options (3,21) include oral diazoxide as first line treatment and glucagon, somatostatin analogues and calcium channel blockers. In cases of focal hyperplasia, the treatment of choice is surgical removal of the affected tissue, which is usually curative (19). In diffuse forms that are unresponsive to medical therapy, near-total pancreatectomy can be an alternative, although risking complications such as diabetes (3).

We report the case of two siblings diagnosed in neonatal period with CHI who were found to

have the same genetic defect but different long-term disease course. The previous knowledge of the firstborn sibling's disease and how it responds to different therapeutic options led to a more appropriate therapeutic intervention in the second sibling, which we believe contributed decisively to a better neurological outcome, even with the same disease genotype. We believe this is a prime example of how a timely diagnosis and appropriate therapeutic strategy are determinant to a better clinical outcome.

CASE REPORT – PATIENT 1

An early term large-for-gestational age – birth weight: 4,200 g (SDS +2.84) and APGAR 9/10 – male patient born to nonconsanguineous parents presented with severe and persistent hypoglycemia 36 hours after birth, with impaired consciousness level, hypotonia and bradycardia. This was his 28-year-old mothers first gestation and she developed gestational diabetes at the 20th week (controlled with diet). There was no family history of hypoglycemic disorders. Starting on the fourth day of life, he required continuous infusion of concentration glucose (glucose infusion rate 8-10 mg/kg/minute) to maintain normal blood glucose.

Abdominal ultrasound and computed tomography did not identify any pancreatic abnormalities. Laboratory studies (Table 1) demonstrated very low levels of ketone bodies and free fatty acids in the presence of low glycemic values and inappropriately high insulin levels. Glucagon stimulation test yielded positive results – blood glucose increased 46.8 mg/dL 30 minutes after glucagon administration. Genetic analyses, performed at 5 years of age, identified biallelic *ABCC8* mutation – a frameshift mutation, c.3576delG, inherited from his mother and a nonsense mutation, c.742C>T, inherited from an unaffected father.

He was initially started on diazoxide (titrated to 25 mg/kg/day) and later added hydrochlorothiazide. When he was 3 months old, he was submitted to exploratory abdominal surgery and near-total pancreatectomy (> 90%) due to persistent severe hypoglycemic episodes associated with seizures. Histology revealed diffuse pancreatic islets hyperplasia. Postoperatively, there were no improvements, so, at the age of two, he began gastrostomy continuous enteral feeding using complex carbohydrates and was switched to subcutaneous octreotide (20 µg/kg/day).

Table 1. Laboratory findings at the time of hypoglycemia

Patient (Age)	Patient 1 (1 month)	Patient 2 (< 1 month)
Blood glucose (70-110 mg/dL)	28	12
Insulin (9.7-97.2 pmol/L)	64.6	110.4
C Peptide (0.30-1.42 nmol/L)	0.9	2.3
Hydroxybutyrate (< 300 µmol/L)	Undetectable	Undetectable
Free fatty acids (0.4-0.7 nmol/L)	0.33	0.43
Cortisol (137.9-689.7 nmol/L)	855.3	634.6
GH (< 20 µg/L)	5.4	11
Carnitines:		
Total (38-68 nmol/mL)	60	42
Free (27-49 nmol/mL)	46	
Acilcarnitines (7-19 nmol/mL)	14	
Lactate (0.6-2.3 mmol/L)	0.9	13.4
Pyruvate (80-160 µmol/L)	67	54.5
Glutamic Acid (0.2-2.8 mg/dL)	0.77	
Ammonia (10-65 µmol/L)	61	45
Urinary organic acids	Negative	Negative

Neurologic evaluation at 5 years of age demonstrated psychomotor development retardation, hemiparesis, dysesthesias and involuntary movements of the left arm. magnetic ressonance imaging (MRI) showed diffuse cerebral atrophy and a T2 hyperintensity of the medial temporal right lobe, suggestive of hypoglycemic sequelae (22). He was diagnosed with partial epilepsy and started on oxcarbazepine and, subsequently, valproic acid.

The result of a continuous glucose monitoring performed at 13 years of age, while on medical therapy with octreotide and nifedipine, is shown in Figure 1A. By the age of 16 the patient exhibited improvement of his glycemic profile. Octreotide was switched to lanreotide (60 mg each 30 days) when he was 18 years old.

At the time of writing, the patient is 20 years old and remains without any recent hypoglycemic episodes although occasionally postprandially hyperglycemic (diabetes criteria not met). His BMI (body mass index) is 22.8 kg/m^2, thus showing improvement since infancy (BMI > 97th percentile). He is 169 cm tall (SDS -1.15) (23) and target height was: 177.5 cm (SDS + 0.25). IGF-1 and IGFBP-3 remained normal during all follow-up and pubertal development was adequate. He remains with slight neuropsychological deficits, without recent clinical epileptic activity. He is currently medically managed with lanreotide 60 mg each 30 days, methylphenidate (started at 12 years of age due to attention deficit disorder) and carbamazepine.

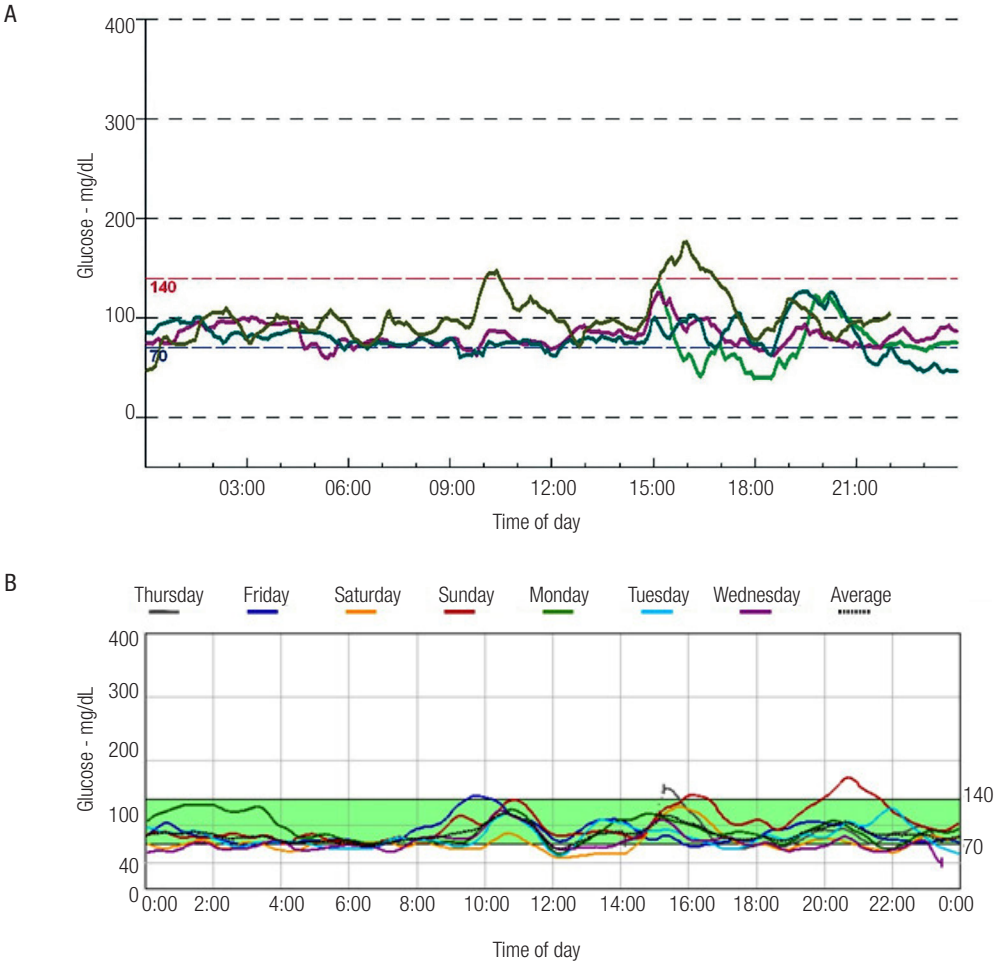

Figure 1. Results of both patients' subcutaneous glucose monitoring: (**A**) Patient number 1's 24-hour monitoring. Various periods of low blood glucose (40-60 mg/dL) were observed. (**B**) Patient number 2's 6-days monitoring. Various periods of low blood glucose (40-60 mg/dL) were also registered in this case.

CASE REPORT – PATIENT 2

An early term – birth weight: 3,660 g (SDS +1.64) and APGAR 9/10 – female patient, sister of patient number 1 (born 2 years after him), presented with severe hypoglycemia (20 mgl/dL) one hour after birth. This was her mother's second gestation and she, once more, developed gestational diabetes at the 20th week (managed with dietary measures). During her first days of life she required continuous infusion of glucose (glucose infusion rate 12 mg/kg/minute) to maintain euglycemia.

Abdominal imaging and laboratory studies (Table 1), including glucagon simulation test, revealed similar results as patient number 1. Genetic analyses found the same compound mutation.

At the age of one month, she was started on diazoxide (12 mg/kg/day) and hydrochlorothiazide but showed no benefit. One month later diazoxide was switched to octreotide (20 mg/kg/day) and she was started on continuous gastrostomy enteral feeding. During infancy and adolescence, her hypoglycemic episodes became less frequent and less severe and there were no recorded hyperglycemic episodes – results of continuous glucose monitoring performed when she was 13 are shown in Figure 1B. She was switched to lanreotide (60 mg each 30 days) when she was 16.

Currently the patient is 18 years old and remains with occasional non-severe hypoglycemic episodes but is otherwise euglycemic. Her psychomotor and intellectual development remains normal. BMI values (BMI > 97th percentile) consistent with obesity were observed until she was 6 years old, however her present BMI is 22.6 kg/m^2. Her final height is 160 cm (SDS -0.60) (23) and target height was: 165.4 cm (SDS+

0.23). No GH/IGF axis abnormalities were found throughout follow-up. She is currently medically managed with lanreotide 60 mg each 28 days and is on a fractionated diet.

DISCUSSION

We describe the case of two siblings diagnosed with diazoxide-unresponsive CHI due to the same compound heterozygous *ABCC8* mutation, yet different disease courses.

In the presence of CHI, molecular biology plays an important role in providing a precise diagnosis and potentially guiding the pharmacotherapy (24). *ABCC8* gene mutation are the most commonly found defects in patients with CHI (6). Monoallelic *ABCC8* mutations can cause both diazoxide-responsive and unresponsive CHI, however most biallelic mutations result in diazoxide-unresponsive form (25).

Our patients presented with biallelic *ABCC8* mutations – c.3576delG and c.742C>T (already referred to in a Spanish genetic mutation database (17)). The former mutation, has only been described in heterozygosity and associated with the focal form of the disease (26,27). The latter has been associated with CHI both in homozygosity and heterozygosity presenting as histologically diffuse disease (28,29). Both mutations result in premature termination codons. Therefore, here we describe two cases of a novel (to the best of our knowledge) compound mutation that retains the histological features (diffuse disease based on pancreatic histology of patient number 1) of the c.742C>T mutation. Functional studies could confirm the resultant protein grade of dysfunction, as other authors have done in different compound mutations (25).

The first therapeutic approach usually consists of diazoxide. It inhibits pancreatic insulin secretion through interaction with the SUR1 subunit of the K_{ATP} and therefore in case of abnormalities in these proteins it might not be effective (3,21,30). Such is the case in *ABCC8* mutations, making most of these patients diazoxide-unresponsive, as were these subjects.

In patients with diazoxide-unresponsive CHI, somatostatin analogues, usually administered as subcutaneous injections, have shown some promise (3,21). They act on somatostatin receptors (SSTR2 and SSTR5), inhibiting the secretion of various hormones, namely insulin. They have been used for over 20 years in the long-term control of diazoxide-unresponsive forms of the disease (31), however they have yet to be officially approved for this purpose. In addition to its side-effect profile (which includes gastrointestinal symptoms, biliary lithiasis and rare cases of necrotizing enterocolitis), there is a risk of worsening hypoglycemia (due to suppression of GH and glucagon production) and tachyphylaxis (due to downregulation of somatostatin receptors), which can limit its long-term use (3,21). Octreotide is the drug with the most clinical experience, however in the last years there have been reports of successful lanreotide (long-acting somatostatin analogue) use (32). The presented cases represent how both octreotide and lanreotide can be effective in attaining blood glucose control both post (patient number 1) and pre-operatively (patient number 2) in case of diffuse diazoxide-unresponsive CHI. These drugs seemed better tolerated than diazoxide.

Alternative pharmacotherapy options include nifedipine (a calcium channel blocker that has had mixed results (18)) and glucagon (used for short-term control of hypoglycemias (31)). Dietary treatment (21) can be sufficient in some cases and involves frequent glucose enriched oral feedings and continuous or frequent enteral feedings.

Surgical removal remains the best option in cases of an identifiable focal lesion associated with CHI – 94% can be rendered euglycemic at the time of discharge (33). In cases of medically refractory diffuse CHI, near-total pancreatectomy (95-98%) may be the only therapeutic option to cure or at least facilitate the medical management. The surgical outcome in case of diffuse CHI is highly variable: persistent hypoglycemia in about 50% of patients and diabetes in 20%, however most improve their glycemic control (3). This option must be carefully weighted also due to its potential complications (namely diabetes and exocrine pancreatic insufficiency (34)) and the fact that spontaneous remission can be observed in some cases (31). Regarding our cases, near-total pancreatectomy was performed in patient number 1 due to the severity and neurological repercussions of his hypoglycemic events and the lack of alternative pharmacotherapeutic options, even though by that time there was not a molecular diagnosis nor any other data to estimate the pancreatic extent of the disease (PET was not available and there were no macroscopic pancreatic lesions on surgical exploration). There was no clinical benefit

post-operatively and this contributed to the decision to withhold surgery in patient number 2 – also given the new-found knowledge of the extent of CHI (diffuse involvement based on patient number 1 pancreatic histology) associated with this mutation.

There have been reports of patients with CHI due to HNF4A (35) and HNF1A mutations (36) who present initially with hyperinsulinemic hypoglycemia and develop maturity-onset diabetes of the young type (MODY) later in life. ABCC8 mutations have been implied in cases of neonatal diabetes (frequently transient), MODY (37) and there are reports of hyperinsulinemic hypoglycemic disorder in early life progressing to insulinopenic diabetes later on (38). Regarding our cases, patient number 1 continues to record periods of post prandial hyperglycemia, although diabetes has been excluded using oral glucose tolerance test and glycated hemoglobin measurement. This phenomenon is most likely a complication of near-total pancreatectomy rather than another feature of her disease/genotype. Additional support to this hypothesis is presented by the fact that there were no hyperglycemic episodes recorded to date in the case of patient number 2, with the same disease genotype. Nevertheless, given these cases concern a new compound mutation, the risk of progression to diabetes in adulthood is largely unknown and should not be disregarded.

An interesting finding is the fact that the mother developed gestational diabetes in both pregnancies, although hyperglycemia did not persist after deliveries. It has already been established that maternal hyperglycemia can induce fetal hyperinsulinemia and be responsible for macrosomia and neonatal hypoglycemia (39). Nevertheless, no link has ever been implied between maternal hyperglycemia and the presence of insulin regulatory gene mutations (the mother was found to be carrier of the *ABCC8* c3576delG mutation).

Prolonged and severe hypoglycemias expose patients to a poor neurological outcome and, indeed, some series report neurodevelopment delay issues in as much as 30% of CHI patients (18). This risk may be higher in cases of neonatal onset and diazoxide-unresponsive CHI (3). Some MRI brain injury patterns have been identified (22), such as cerebral cortex T2 hyperintense lesions while sparing cerebellum, brainstem, and thalamus – this was the case of patient number 1. Taking this into account, the better neurodevelopment observed in patient number 2 can probably be linked to a more rapid and appropriate treatment approach due to the previous knowledge of her sibling's disease. In particular, octreotide was initiated when she was 2 months old while her brother only started at 2 years of age, which probably led to a lesser frequency and severity of hypoglycemic episodes during her infancy and could explain her better neurological outcome.

In conclusion, we report the case of two siblings with diazoxide-resistant CHI caused by the same compound *ABCC8* mutation. Their therapeutic management had some differences and this could offer a potential explanation for the distinct neurological outcome, despite the same genetic basis for the disease. This case highlights the importance of the first siblings index case clinical and molecular diagnosis and how it seemed to contribute to a better disease knowledge and medical care in patient number 2, ultimately resulting in a better outcome in spite of the same disease genotype.

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Insulin autoimmune syndrome in an occidental woman: a case report and literature review

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SUMMARY

Insulin autoimmune syndrome (IAS, Hirata's disease) is a rare hypoglycemic disorder characterized by spontaneous hypoglycemia associated with extremely high circulating insulin levels and positive anti-insulin antibody results. Thus far, most cases have been reported in Asian countries, notably Japan, with few cases reported in western countries. As a possible cause, it is associated with the use of drugs containing sulfhydryl radicals, such as captopril. This report refers to a 63-year-old female Brazilian patient with a history of postprandial hypoglycemia. After extensive investigation and exclusion of other causes, her hyperinsulinemic hypoglycemia was considered to have likely been induced by captopril. Most cases of IAS are self-limiting. However, dietary management, corticosteroids, plasmapheresis, and rituximab have already been used to treat patients with IAS. In our case, after discontinuation of captopril, an initial decrease in insulin autoantibody levels was observed followed by improvement in episodes of hypoglycemia. Although it is a rare disease, IAS should be considered in the differential diagnosis of endogenous hyperinsulinemic hypoglycemia. Patients with suspected IAS must be screened for autoimmunity-related drugs for insulin. Initial clinical suspicion of IAS can avoid unnecessary costs associated with imaging examinations and/or invasive surgical procedures. Arch Endocrinol Metab. 2018;62(5):566-70

INTRODUCTION

Insulin autoimmune syndrome (IAS), or Hirata's disease, is a rare hypoglycemic disorder first described in 1970 by Hirata and cols. (1). Most cases have been reported in Japanese and Koreans, and few cases in western countries have been described (2,3). It is characterized by spontaneous postprandial hypoglycemia, high levels of immunoreactive insulin, and anti-insulin antibodies. Anti-insulin antibodies triggered by viruses and drugs bind to insulin and proinsulin, resulting in initial hyperglycemia and further stimulation of insulin secretion. Eventually, antibody-binding capacity is exceeded, and unbound free insulin causes hypoglycemia. Dissociation of antibodies also contributes to hypoglycemia (4).

In a Japanese cohort of 197 patients with IAS, 43% were exposed to drugs with sulfhydryl radicals (5), including captopril, methimazole, and tiopronin (4,6). The mechanism by which these radicals lead to the development of IAS is unknown. This paper describes a case of IAS in a South American patient after use of captopril, the most prescribed antihypertensive medicine in our field.

CASE REPORT

A 63 year old female Brazilian patient complained of recurrent episodes of postprandial hypoglycemia (registered capillary blood glucose of 47 mg/dL), approximately three hours after breakfast, three times a week, with the following symptoms: palpitation, sweating, shakiness and nausea, dizziness, visual blurring, and sleepiness, which improved after sugar intake. These episodes began five years ago with increased frequency in the past three months. Comorbidities were systemic hypertension diagnosed five years ago, obesity and metabolic syndrome, and regular use of captopril 50 mg/day.

There was no personal or family history of diabetes mellitus or other endocrinopathies, such as insulinoma,

or previous use of oral hypoglycemic drugs such as sulfonylureas or exogenous insulin. Dietary recall revealed high consumption of foods rich in simple carbohydrates and fats, with low fiber intake. She had a sedentary lifestyle, BMI of 32.2 kg/m^2, abdominal circumference of 107 cm, and absence of acanthosis nigricans.

Laboratory results are presented in Table 1. Initial blood glucose (8-hour fast), serum insulin, and C-peptide levels were 91 mg/dL (reference range: 65 – 99 mg/dL), 620.9 µIU/mL (reference range: 2.6 – 24.9 µIU/mL), and 3.65 ng/mL (0.9 – 7.1 ng/mL), respectively. Our patient was hospitalized for a prolonged fasting test. After 62 hours of fasting, hyperinsulinemic hypoglycemia was observed with high levels of anti-insulin antibodies (294.8 U/mL; non-reactive < 5 U/mL) (Table 1). Endoscopic ultrasonography and abdominal magnetic resonance imaging showed no pancreatic or extra-pancreatic lesions. Anamnesis and detailed clinical investigation ruled out oral hypoglycemic use. Screening for anti-nuclear factor, serum protein electrophoresis, serology for viral hepatitis, and other autoimmune diseases were negative.

The patient underwent continuous glucose monitoring using the Continuous Glucose Monitoring System (CGMS) (Guardian Real Time®; Medtronic, Minneapolis, USA) to detect hypoglycemia within 72 hours. Episodes of hypoglycemia occurred in the late postprandial period (three hours after breakfast) and were concordant with neuroglycopenic symptoms: dizziness, visual blurring, and sleepiness. The lowest glucose value found was 48 mg/dL (Figure 1).

Given high levels of anti-insulin antibodies, hyperinsulinemia, late postprandial hypoglycemia, and clinical presentation coinciding with starting captopril use, the diagnostic hypothesis of IAS was proposed and captopril was discontinued. Six months later, the patient presented a decline in circulating levels of anti-insulin antibodies (100.0 U/mL) and reported reduced frequency of hypoglycemic symptoms (Table 1) to approximately once per month.

The patient underwent a new CGMS examination (Figure 2), and the lowest glucose value was 66 mg/dL three hours after breakfast without symptoms. The test also revealed sporadic high glucose levels (> 200 mg/dL). A 2-hour 75 gram oral glucose tolerance test (OGTT) was performed; fasting glycemia level was found to be 110 mg/dL, and 2-hour glucose overload was 276 mg/dL. Further workup showed an HbA1c level of 5.6% (Table 1).

Due to this development and presence of metabolic syndrome (7), it was decided to prescribe metformin at the dose of 1000 mg/day, as previously described in another case of IAS (8). At subsequent visits, the patient did not present an increase in episodes of hypoglycemia, nor did worsening of the clinical picture. The patient was also referred for professional nutritional evaluation and dietary plan of 1800 kcal/day, increased fiber intake, reduced lipids, and replacement of simple carbohydrates with complex ones.

In due course, we performed high-resolution HLA class II test and obtained positive results for DRB1*0701, DRB1*0901P, DQB1*0202, and DQB1*0303 haplotypes.

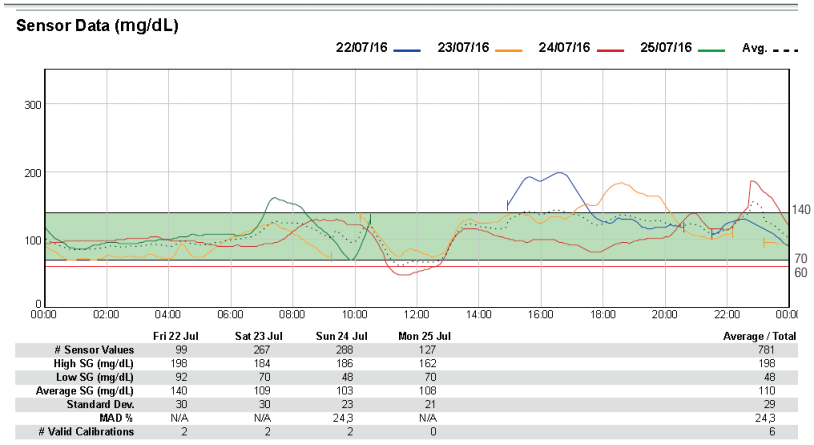
Table 1. Clinical and laboratory results during initial and subsequent visits

Parameter	September 25, 2015	November 6, 2015*	September 20, 2016**	Reference Range	Methods
Fasting blood glucose	91	58	97	65–99 mg/dL	Hexokinase
Serum insulin	620.9	246.5	> 300	2.6–24.9 µIU/mL	Electrochemiluminescence
Serum pro-insulin	-	28.2	-	0.5–3.5 pmol/L	ELISA
Serum C-peptide	3.65	2.6	-	0.9–7.1 ng/mL	Chemiluminescence
Hemoglobin A1c	-	-	5.6	4.1–5.7%	HPLC
Serum anti-insulin antibody	-	294.8	100	Non-reactive < 5 IU/mL	ELISA
Serum anti-GAD antibody	-	2.1	-	Non-reactive < 10 IU/mL	ELISA
Total cholesterol	211	-	230	< 200 mg/dL	Enzymatic
LDL	123.2	-	150	< 130 mg/dL	Friedewald Formula
HDL	51	-	47	< 50 mg/dL	Enzymatic Colorimetric
Triglycerides	184	-	164	< 150 mg/dL	GPO - Trinder

Anti-GAD antibody: anti-glutamic acid decarboxylase antibody; LDL: low-density lipoprotein; HDL: high-density lipoprotein; ELISA: enzyme-linked immunosorbent assay.

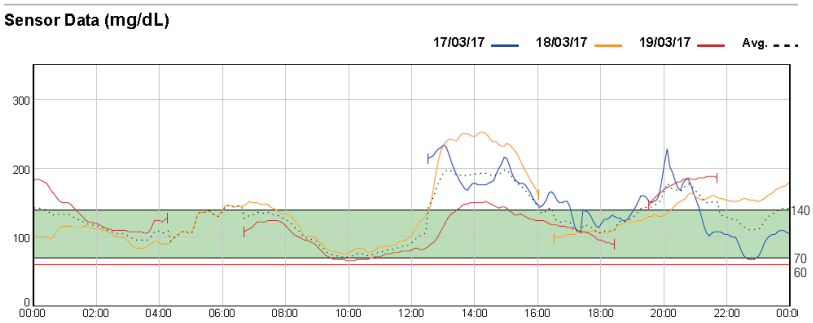
* Results obtained in the prolonged fasting test (62 hours) after episode of hypoglycemia accompanied by neuroglycopenic symptoms.

** Results obtained after suspension of captopril. It was discontinued in February 2016 with significant improvement in the symptoms of hypoglycemia.



	Fri 22 Jul	Sat 23 Jul	Sun 24 Jul	Mon 25 Jul	Average / Total
# Sensor Values	99	267	288	127	781
High SG (mg/dL)	198	184	186	162	198
Low SG (mg/dL)	92	70	48	70	48
Average SG (mg/dL)	140	109	103	108	110
Standard Dev.	30	30	23	21	29
MAD %	N/A	N/A	24,3	N/A	24,3
# Valid Calibrations	2	2	2	0	6

Figure 1. Initial continuous glucose monitoring using the Continuous Glucose Monitoring System: Data of glucose excursions on three consecutive days, 24 h per day. The dotted line represents the average glucose level for three days.



	Fri 17 Mar	Sat 18 Mar	Sun 19 Mar	Average / Total
# Sensor Values	138	283	221	642
High SG (mg/dL)	234	252	188	252
Low SG (mg/dL)	68	76	66	66
Average SG (mg/dL)	149	135	121	133
Standard Dev.	41	46	34	42
MAD %	39,8	11,3	2,8	21,0
# Valid Calibrations	3	2	2	7

Figure 2. Follow-up continuous glucose monitoring using the Continuous Glucose Monitoring System: Data obtained after 13 months of discontinuation of captopril, for three consecutive days, 24 hours per day. The dotted line represents the average glucose level for three days.

DISCUSSION

IAS is an uncommon etiology of spontaneous hypoglycemia and was first described by Hirata and cols. in 1970 (1). To our knowledge, this is the fourth case reported in Brazil (8-10). It affects men and women indiscriminately but is more frequent in patients aged > 40 years (11).

Susceptibility to IAS is related to specific HLA class II alleles, which are 10-30 times more prevalent in Japanese and Koreans than in Caucasians (12). Most cases are associated with HLA DR4, DRB1*0406, DQA1*0301, and DQB1*0302 (2,13). Our patient was positive for DRB1*0701, DRB1*0901P, DQB1*0202, and DQB1*0303. We have not found in the literature other cases with positivity for these haplotypes. In Brazil, Alves and cols. (9) described a case of a seven year old child with HLA DRB1*1104 haplotype, thus far unrelated to the syndrome. Such data suggest that the genetic spectrum of IAS seems to be broader and more heterogeneous than previously thought, leading to the need for further studies to understand the disease in patients from western countries.

IAS may occur with fasting or exacerbated exercise hypoglycemia; however, it is classically characterized by late postprandial hypoglycemia, high insulin levels, and positive results for anti-insulin antibodies.

The pathogenic mechanisms that lead to the development of IAS are not fully understood. They seem to be associated with the formation of insulin-antibody complexes, which hinder the physiological mechanism of insulin action. After a meal, anti-insulin antibodies bind to secreted insulin in response to increased glycemia. This binding reduces the availability of insulin to its receptors in the liver and peripheral

tissues causing hyperglycemia and additional secretion of insulin. The hyperglycemic effect is dose-dependent according to anti-insulin levels. Parallel to the decrease in blood glucose, the insulin bound to the antibodies is released, which results in free insulin concentrations disproportionately high for glycemia. Thus, late postprandial hypoglycemia occurs (4,12). Consistent with the kinetics of the insulin-antibody complex in IAS, we could infer that our patient presented with hyperglycemia at the beginning of the postprandial period, explained by the antibodies that bound to the endogenous insulin, with subsequent late occurrence of hypoglycemia when the antibodies dissociated from the insulin, increasing availability. Another possible mechanism behind IAS is the presence of a high-capacity, low-affinity paraprotein, capable of causing hypoglycemia associated with high plasma insulin levels and relatively low C-peptide levels. A plausible mechanism is delayed clearance of insulin, which is still available to bind its receptor due to the relatively weak affinity of the IgA anti-insulin for insulin (4,14).

Initially, we performed magnetic resonance imaging and endoscopic ultrasonography of the pancreas to rule out insulinoma or other extrapancreatic tumors, which would not justify the high levels of anti-insulin antibodies observed in our case. We did not measure plasma sulfonylureas because it was an unlikely diagnosis for our patient. Autoimmune insulin receptor disease (insulin resistance type B) is also part of the differential diagnosis. It is caused by the agonistic effect of antibodies against the insulin receptor resulting in significant insulin resistance and paradoxical hypoglycemia. In these cases, anti-receptor insulin antibodies are usually positive whereas anti-insulin antibodies are negative (4,15). Therefore, considering that our patient did not present acanthosis nigricans, and laboratory results showed high levels of insulin and anti-insulin antibodies, IAS seems to be the most likely diagnosis.

Insulin-immunoglobulin complexes (macro-complexes) may pose a significant challenge to the measurement of hormones by immunoassay and may also interfere with bioactivity of the hormones to cause clinical disorders. Church and cols. demonstrated that immunoprecipitation with polyethylene glycol (PEG) must be used with caution in screening for insulin-antibody complexes as gel filtration chromatography (GFC) with addition of exogenous insulin enhances sensitivity for the identification of insulin immunocomplexes (16). It is noteworthy that we did not consider the possibility of laboratory analytical interference, and we did not test heterophile antibodies.

IAS is frequently associated with other autoimmune diseases (mainly Graves' Disease), rheumatologic diseases, previous exposure to exogenous insulin, and use of medications (5,6,12,17). Among drugs, those containing sulfur/sulfhydryl radicals, for example methimazole, captopril, D-penicillamine, hydralazine, glutathione, methionine, mercaptans, imipenem, penicillin G, α-lipoic acid, and diltiazem, have been reported to potentially result in IAS (4,6,18). Our patient did not present any clinical or laboratory evidence of other diseases nor did she have a history of prior insulin use. The only positive finding was captopril use for treatment of hypertension.

Consistent with the pathophysiology of IAS, we have detected late postprandial hypoglycemia in our patient (Figure 1). Once we raised the diagnostic hypothesis of IAS, the medication containing sulfhydryl radical (captopril) was suspended. Six months later, the patient reported improvement in the clinical picture with a decrease in the frequency of hypoglycemic episodes, as shown in Figure 2. In the follow-up CGMS, we also observed hyperglycemia with values > 200 mg/dL throughout the day, which was not seen in the initial CGMS. These findings led us to perform OGTT, and results were consistent with the diagnosis of diabetes mellitus (fasting glucose level: 110 mg/dL; 2-hour glucose level: 276 mg/dL), raising the question of the concomitant possibility of insulin resistance, which could become more evident with the fall in the anti-insulin antibody levels. Our patient had metabolic-syndrome contributing to the clinical picture. Impaired glucose tolerance and overt diabetes do not rule out a diagnosis of IAS, and high HbA1c levels are common in these patients[4].

Regarding clinical manifestations, reported cases of IAS have varied from mild, transient, to very severe presentations (18). Most patients with drug-induced IAS can achieve remission of the disease soon after stopping use of the medication (6).

Nutritional management is recommended with small, frequent meals and reduction of rapidly-absorbed carbohydrates to avoid rapid elevation of blood glucose levels (18). Alpha-glucosidase inhibitors have also been used to reduce or prevent hypoglycemic episodes (11,12). In more severe or prolonged cases, glucocorticoids, immunosuppressants, and even

plasmapheresis may be useful as adjuvant therapy (3,15). Other reported alternatives include pancreatectomy, diazoxide, and octreotide (15). Immunoadsorption using a reusable adsorber system loaded with sheep antigens directed against human immunoglobulin followed by two doses of 1 g of rituximab has been reported effective in a patient with IAS refractory to prednisolone and azathioprine therapy (19). Finally, in the medical literature, meals containing reduced amounts of carbohydrates and corticosteroids were described as the most effective treatments for IAS (11,12).

Because our patient had a mild condition with infrequent hypoglycemia and without severe hemodynamic repercussions, we opted for lifestyle-changes with nutritional dietary management, and suspension of the medication captopril, a known trigger. As previously discussed, to reduce the possibility of concomitant insulin resistance, we initiated the use of metformin. Paiva and cols. reported the use of metformin in the follow-up of a 55 year old Brazilian male patient with suspected IAS with good results (8), which supported use in our patient.

In conclusion, IAS is part of the differential diagnosis of hyperinsulinemic hypoglycemia. It is a rare disease whose pathophysiology is still poorly understood. Initial clinical suspicion of IAS can avoid unnecessary expenses involving imaging examinations and/or invasive surgical procedures, and patients must be screened for autoimmunity-related drugs for insulin. Long-term longitudinal studies are needed to improve understanding of the disease and to improve treatment.

Patient consent: written informed consent has been obtained from the patient.

Acknowledgements and author contributions: MZR Reis wrote the article. ARP Quidute contributed to the writing and discussion of the article and was the named physician involved in care of the patient. ARP Quidute, EGP Fontenele and MZR Reis conducted literature review. ARP Quidute, EGP Fontenele and RMM Junior critically revised the manuscript for intellectual content. VO Fernandes and RMM Junior contributed to the diagnostic examinations. AP Abreu was involved in clinical management. All authors read and approved the final manuscript.

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