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Prevalence of subclinical hypercortisolism in type 2 diabetic patients from the Rio de Janeiro Type 2 Diabetes Cohort Study

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ABSTRACT

Aims: Subclinical hypercortisolism was reported to be more prevalent among diabetic, obese and hypertensive patients. Our primary aim was to investigate the prevalence of subclinical hypercortisolism in patients from the Rio de Janeiro Type 2 Diabetes (RIO-T2D) Cohort; and secondarily to assess its associated factors.

Methods: From May 2013 to August 2014, 393 diabetic outpatients underwent overnight 1 mg dexamethasone suppression test (DST). Patients with non-suppressive morning cortisol (≥1.8 μg/dl) were further evaluated with nocturnal salivary cortisol, two readings >0.35 μg/dl were considered confirmatory for subclinical hypercortisolism.

Results: One-hundred twenty-eight patients (32.6%) failed to suppress morning cortisol, and in 33 patients (8.6%) subclinical hypercortisolism was confirmed. Independent correlates of a positive DST were older age (OR: 1.04; 95% CI: 1.01–1.07; $p = 0.007$), number of anti-hypertensive drugs in use (OR: 1.26; 95% CI: 1.05–1.50; $p = 0.012$), longer diabetes duration (OR: 1.03; 95% CI: 1.004–1.06; $p = 0.023$), and presence of diabetic nephropathy (OR: 1.70; 95% CI: 1.01–2.87; $p = 0.047$). Independent correlates of confirmed subclinical hypercortisolism were a greater number of anti-hypertensive medications (OR: 1.54; 95% CI: 1.14–2.06; $p = 0.004$), shorter diabetes duration (OR: 0.92; 95% CI: 0.87–0.98; $p = 0.006$), and increased aortic stiffness (OR: 2.81; 95% CI: 1.20–6.57; $p = 0.017$); metformin use was protective (OR: 0.27; 95% CI: 0.10–0.73; $p = 0.010$).

Conclusion: Patients with type 2 diabetes had a high prevalence of subclinical hypercortisolism, and its presence was associated with more severe hypertension and increased aortic stiffness.

1. Introduction

Subclinical Cushing's syndrome (or subclinical hypercortisolism) is a condition defined by excessive production of cortisol without the classical signs or symptoms associated with the syndrome. Obesity, hypertension, glucose intolerance and diabetes mellitus are common features in these cases, which are also present in metabolic syndrome. Prevalence of this condition among general population ranges approximately between 0.2 and 2%, taking into account previous studies performed in patients with adrenal incidentaloma (Chiodini, 2011).

Routine testing for hypercortisolism in diabetes management is not worthwhile or cost-effective, so currently it is not recommended (Mullan et al., 2010; Nieman et al., 2008). Thus, some patients affected by hypercortisolism may be misdiagnosed during clinical management of diabetes due to its subtle and unspecific manifestations. However, overlooking hypercortisolism diagnosis may have serious consequences. Although clinically unapparent, hypercortisolism makes metabolic control more difficult to achieve and may increase the risk of future cardiovascular events (Terzolo et al., 2012). Moreover, some studies suggested that the prevalence of hypercortisolism in diabetic patients might be higher than what it was initially thought (Catargi et al., 2003; Chiodini et al., 2005; Leibowitz et al., 1996; Murakami et al., 2010; Taniguchi, Hamasaki, & Okamoto, 2008). Otherwise, a large-scale screening of diabetic patients for subclinical hypercortisolism may overestimate the prevalence of this condition due to false-positive results (Mullan et al., 2010). Currently, the Endocrine Society recommends that testing should be limited to specific clinical settings, such as in patients with uncommon characteristics for age or multiple and progressive features, especially those that are more strongly associated with Cushing's syndrome (Nieman et al., 2008).

Therefore, the primary aim of this study was to investigate the prevalence of subclinical hypercortisolism in a well-characterized cohort of type 2 diabetic patients with high cardiovascular risk

Conflict of interest: There is no conflict of interest.

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attending the outpatient clinic of a tertiary-care university hospital; and, as a secondary aim, to determine the factors associated with the presence of subclinical hypercortisolism, particularly the presence of macro- and microvascular degenerative complications, and some preclinical cardiovascular risk markers, such as left ventricular mass, carotid intima-media thickness and aortic stiffness.

2. Material and methods

In a cross-sectional study nested within a cohort of type 2 diabetic patients, the Rio de Janeiro Type 2 Diabetes (RIO-T2D) cohort, a total of 483 patients attending to the diabetes outclinic of a tertiary-care university hospital were screened. The study was performed between May 2013 and August 2014. All participants gave written informed consent, and the local Ethics Committee had previously approved the study protocol. The characteristics of this cohort, the baseline procedures and the diagnostic definitions have been detailed elsewhere (Cardoso et al., 2008; Cardoso, Ferreira, Leite, & Salles, 2013; Cardoso, Leite, Ferreira, & Salles, 2015; Salles, Leite, Pereira, Nascimento, & Cardoso, 2013). In brief, inclusion criteria were all adult type 2 diabetic individual up to 80 years old with either any microvascular (retinopathy, nephropathy or neuropathy) or macrovascular (coronary, cerebrovascular or peripheral artery disease) complication, or with at least two other modifiable cardiovascular risk factors. Exclusion criteria to enter the cohort were a body mass index >40 kg/m^2, serum creatinine ≥180 mmol/L, and the presence of any serious concomitant disease limiting life expectancy (Cardoso et al., 2008, 2013, 2015; Salles et al., 2013). For this study, 90 patients were further excluded because used corticosteroids by any route of administration during the previous year, were using oral contraceptives or anti-epileptic medications within the last 6 weeks, had depression or excessive alcohol intake, or had worsening liver or renal function, totaling 393 patients evaluated. The patients were clinically examined by a single endocrinologist to exclude discriminatory features of Cushing's syndrome. All patients were submitted to a thorough physical examination, laboratory evaluation, 2D echocardiogram, ultrasonographic carotid intima-media thickness (IMT) measurement, 24-hour ambulatory blood pressure monitoring (ABPM) and carotid-femoral (aortic) pulse wave velocity (PWV) measurement. Diagnostic criteria for macrovascular and microvascular degenerative diabetic complications were detailed previously (Cardoso et al., 2008, 2013, 2015; Salles et al., 2013). Laboratory evaluation included fasting glycemia, glycated hemoglobin (HbA_{1c}), lipid profile and serum creatinine. Glycemic control was assessed by the first HbA_{1c} obtained at cohort entry, by mean HbA_{1c} during the first year of follow-up (when the greatest HbA_{1c} reduction occurred) and by mean HbA_{1c} collected during the year before subclinical hypercortisolism investigation. Physically active patients were those who exercised at least 150 min per week. Diagnosis of dyslipidemia was defined by medical history, use of hypolipidemic drugs or according to NCEP-ATPIII (National Cholesterol Education Panel-Adult Treatment Panel III) criteria (NCEP, 2001). Office blood pressure was measured three times using a digital oscillometric blood pressure monitor (HEM-907 XL, Omron) with a suitable sized cuff. The first measure was discarded and BP considered was the mean between the last two readings. Arterial hypertension was diagnosed if mean SBP ≥140 mmHg or DBP ≥90 mmHg or if anti-hypertensive drugs had been prescribed (Mancia et al., 2013). Echocardiographic left ventricular mass was calculated by using Devereux's formula (Devereux & Reichek, 1977) and indexed to height$^{2.7}$. ABPM was recorded using Mobil O Graph (version 12) equipment, as previously reported (Salles et al., 2013). All patients used their prescribed anti-hypertensive medications during ABPM. Parameters evaluated were 24-hour systolic BP and diastolic BP. Measurement of aortic PWV was performed by the Complior device (Artec-Medical, Paris, France), as previously described (Cardoso et al., 2013). The mean value of three consecutive readings was used for analysis. The cut-off value for considering increased aortic stiffness was 10 m/s (Van Bortel et al., 2012). Bilateral common carotid artery IMT was measured by high-resolution ultrasound, as previously described (Cardoso et al., 2015).

2.1. Evaluation of subclinical hypercortisolism

The participants underwent 1 mg overnight dexamethasone suppression test (DST) on an outpatient basis. The patients were advised to take two tablets of 0.5 mg of dexamethasone at 2300 hours and the sample for cortisol was collected on the next morning between 0800 and 0900 hours. Serum cortisol of ≥1.8 μg/dl (50 nmol/l) was used to achieve 95% sensitivity rate (Nieman et al., 2008). Serum cortisol was measured by chemiluminescence immunoassay method (UniCel; Beckman Coulter, Inc., Fullerton, California, USA) with a lower detection limit of 0.3 μg/dl. The intra-assay and interassay coefficient of variation were 4.4–6.7% and 6.0–7.9%, respectively. All those who failed to suppress morning serum cortisol on DST to less than 1.8 μg/dl were referred for a second stage of the study, which consisted of late-night salivary cortisol measurements. Salivary cortisol test was performed at 2300 hours on two consecutive nights. Patients were instructed to refrain from eating, drinking, smoking or brushing their teeth for at least one hour before sampling. Saliva sampling was collected by inserting a swab (Salivette®, North Carolina, USA) into the patient's mouth until it became saturated and then stored in a provided kit in the refrigerator, until the following morning. Salivary cortisol levels were measured by electrochemiluminescence assay (ELECSYS®, Roche Diagnostics; intra-assay and interassay coefficient of variation: 2.8–6.1% and 4.1–33.4%, with a lower detection limit of 0.018 μg/dl). Two measurements of nighttime salivary cortisol >0.35 μg/dl were considered as confirmatory criterion of hypercortisolism (Beko et al., 2010; Tateishi et al., 2012). In case of discordant results, a third sample was collected. Twelve patients with discordant nocturnal salivary cortisol did not supply a third sample (4 of them died before a third sample was collected), totaling 381 patients with completed investigation. Fig. 1 illustrates the flow chart of subclinical hypercortisolism investigation.

2.2. Statistical analysis

Continuous variables with normal distribution were expressed as means and standard deviations (SD) or described as medians and interquartile ranges when they presented asymmetrical distribution. Prevalence rates and their 95% confidence intervals (CI) were estimated for both positive screened patients and for those with confirmed hypercortisolism. DST-negative patients served as controls and were compared with DST-positive patients and with those with confirmed hypercortisolism. Continuous variables were compared using unpaired *t*-test or Mann–Whitney U test, as appropriate. Categorical variables were compared by chi-squared test. Independent covariates associated with positive screening test and with confirmed hypercortisolism were determined by multiple logistic regressions. Candidate variables for predicting DST positive screening test were age, gender, diabetes duration, presence of degenerative diabetic complications, arterial hypertension, metformin and insulin use, serum triglycerides, left ventricular mass index, and increased aortic PWV. Candidate variables for predicting confirmed subclinical hypercortisolism were age, gender, diabetes duration, presence of diabetic nephropathy and cardiovascular disease, metformin and insulin use, 24-hour systolic blood pressure, left ventricular mass index, and increased aortic PWV. A stepwise backward selection process with *p*-value <0.10 was used as criterion to remain into the logistic models. Results were expressed as odds ratios (OR) and their 95% CI. Model fitness was examined by the Hosmer–Lemeshow goodness-of-fit test and by the areas under the receiver-operating

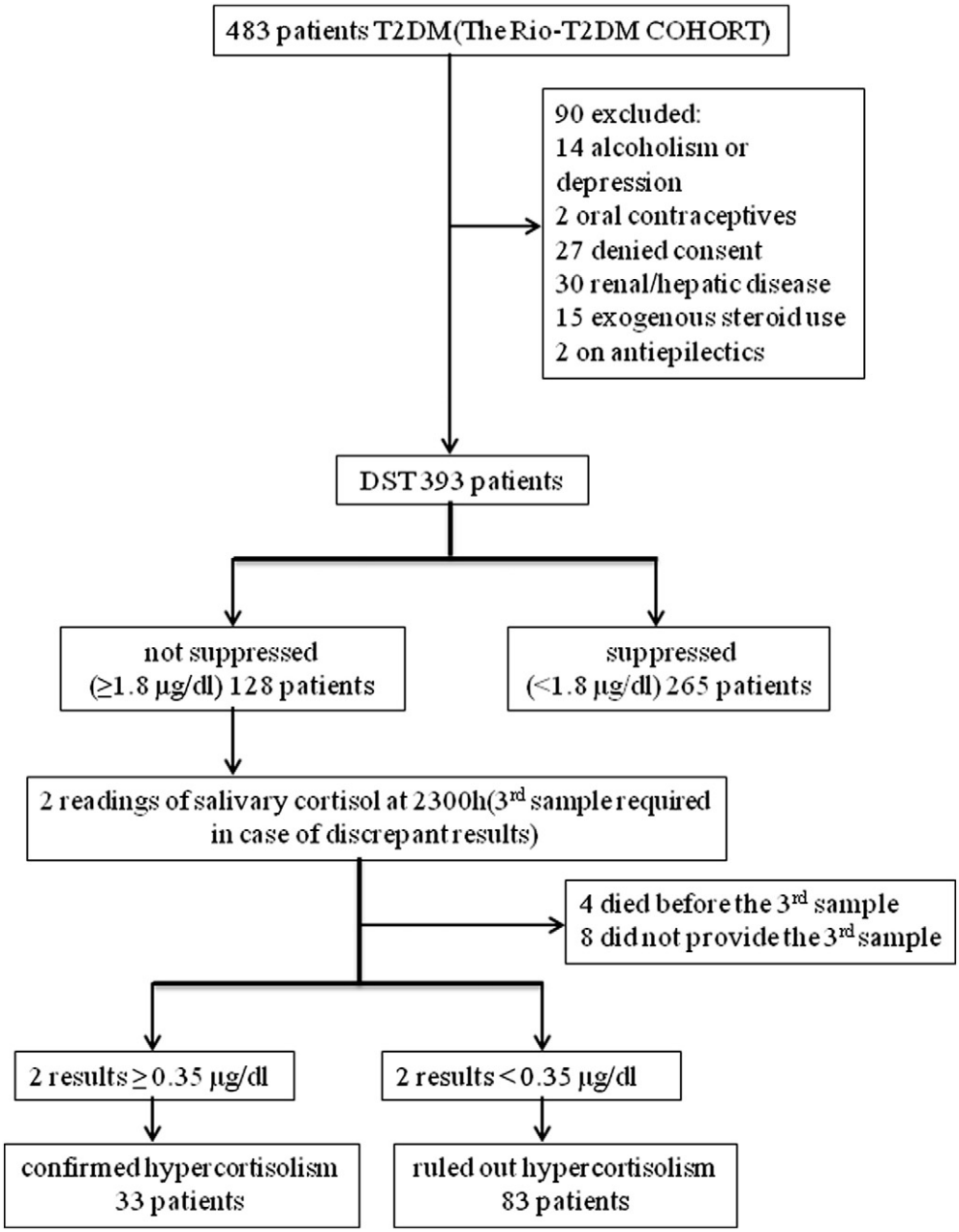

Fig. 1. Flow chart of the study.

characteristics (ROC) curves of the predicted probabilities. Statistical analysis was performed using SPSS 20.0 software (Statistical Process for the Social Sciences; SPSS, Inc., Illinois, USA) and two-tailed *p*-values <0.05 were considered statistically significant.

3. Results

Three-hundred and ninety-three patients, without features of Cushing's syndrome, underwent overnight 1 mg DST. One hundred and twenty-eight patients had non-suppressive morning serum cortisol (prevalence of 32.8%, 95% CI: 27.4–38.7%). Most of these patients, a total of 105, presented cortisol levels between 1.8 and 5 µg/dl. Diagnosis of hypercortisolism was confirmed in 33 cases by two nocturnal salivary cortisol measurements >0.35 µg/dl (prevalence of 8.6%, 95% CI: 6.2–12.2%).

Table 1 outlines the characteristics of patients with positive and negative screening DST and of those with confirmed hypercortisolism. The subgroup of patients who did not suppress morning serum cortisol after 1 mg dexamethasone screening test was older, had longer diabetes duration, had higher prevalence of degenerative complications, used less metformin and more anti-hypertensive drugs and had increased left ventricular mass index and increased aortic stiffness than those patients who suppressed morning cortisol. Similarly, patients with confirmed hypercortisolism had the same features of those patients considered false positive, except for a shorter diabetes duration and a lower glomerular filtration rate. Results of multiple logistic regression analysis for independent covariates associated with positive screening test and confirmed hypercortisolism are shown in Table 2. Older age (OR: 1.04, $p = 0.007$), greater number of anti-hypertensive drugs in use (OR: 1.26, $p = 0.012$), longer diabetes duration (OR: 1.03, $p = 0.023$), and presence of diabetic nephropathy (OR: 1.70, $p = 0.047$) were the variables independently associated a positive DST. A shorter diabetes duration (OR: 0.92, $p = 0.006$), the use of a greater number of

Table 1
Characteristics of diabetic patients with positive and negative screening test for hypercortisolism (DST) and with confirmed subclinical hypercortisolism (2 elevated values of night salivary cortisol).

Characteristics	Patients with negative screening ($n = 265$)	Patients with positive screening ($n = 128$)	Patients with confirmed hypercortisolism ($n = 33$)
Age, years	58 (8.9)	61 (8.6)*	62 (9.0)
Gender, male (%)	35.5	36.7	36.4
BMI kg/m^2	29.8 (4.5)	29.6 (4.8)	29.1 (3.8)
Waist circumference, cm	102 (11)	102 (11)	101 (10)
Smoking, current, ex (%)	41.1	34.4	33.3
Physical activity (%)	25.7	26.6	24.2
Diabetes duration (years)	7 (3–15)	10 (5–19)*	6 (3–10)
Dyslipidemia (%)	86.8	88.3	81.8
Use of statins (%)	74	79.7	63.6
Diabetes treatment (%)			
Metformin	91.7	85.2‡	78.8‡
Sulfonylurea	43	45.3	42.4
Insulin	44.9	54.7	54.5
Macrovascular complications (%)	25.7	34.4	45.1‡
Coronary artery disease	14.3	17.2	24.2
Cerebrovascular disease	7.9	9.4	15.2
Peripheral artery disease	12.1	20.3‡	21.2
Microvascular complications (%)			
Retinopathy	25.7	39.1†	24.2
Nephropathy	24	35.9‡	30.3
Peripheral neuropathy	20.8	33.1†	21.2
Hypertension (%)	84.2	92.2‡	97
Anti-hypertensive therapy (%)			
Number of medications	2 (1–3)	3 (2–4)*	3 (2–4)†
ACE/ARB Inhibitors	71.3	78.1	84.8
Beta-blockers	40.8	52.3‡	66.7†
Calcium channel blockers	26	32.8	39.4
Diuretics	56.6	72.7†	84.8†
Systolic blood pressure (mmHg)			
Clinic	145 (22)	149 (26)	150 (30)
24 h	126 (14)	129 (15)	133 (19)
Diastolic blood pressure (mmHg)			
Clinic	84 (12)	84 (14)	84 (15)
24 h	74 (9)	73 (10)	76 (13)
Right carotid artery intima-media thickness (mm)	1.01 (0.16)	1.02 (0.15)	1.03 (0.15)
Left carotid artery intima-media thickness (mm)	1.05 (0.17)	1.06 (0.15)	1.05 (0.15)
Aortic PWV >10 m/s (%)	19.6	32.8†	36.4
LVMI (g/height$^{2.7}$)	50 (15)	54 (18)‡	58 (24)†
Laboratory variables			
Fasting glycemia (mg/dL)	161 (72)	158 (68)	150 (68)
Entry HbA1c (%)	7.9 (1.8)	8.1 (2.0)	7.6 (1.5)
(mmol/mol)	63 (19.7)	65 (21.9)	60 (16.4)
Mean first-year HbA_{1c} (%)	7.6 (1.4)	7.7 (1.6)	7.5 (1.4)
(mmol/mol)	60 (15.3)	61 (17.5)	58 (15.3)
Mean previous-year HbA_{1c} (%)	7.9 (1.5)	7.9 (1.6)	8.2 (1.8)
(mmol/mol)	63 (16.4)	63 (17.5)	66 (19.7)
Cholesterol (mg/dl)	195 (46)	198 (46)	197 (45)
Triglycerides (mg/dl)	132 (97–200)	153 (99–232)	156 (110–243)
HDL-cholesterol (mg/dl)	43 (11)	43 (12)	41 (13)
LDL-cholesterol (mg/dl)	117 (38)	115 (40)	114 (44)
Glomerular filtration rate (ml/min/1.73 m^2)	99 (32)	86 (32)	84 (33)‡
GFR ≤ 60 ml/min/1.73 m^2 (%)	8.3	20.3*	27.3†
Albuminuria (mg/24 h)	12 (7–29)	14 (7–60)	10 (6–46)

Values expressed in means (SD), medians (IQR) or proportions.
Abbreviations: BMI, body mass index; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; HDL, high density lipoprotein; LDL, low density lipoprotein; HBA_{1C}, glycated hemoglobin; PWV pulse wave velocity; LVMI, left ventricular mass indexed to height$^{2.7}$; GFR, glomerular filtration rate.
* $P < 0.001$.
† $P < 0.01$.
‡ $P < 0.05$; for bivariate comparisons with the reference group with negative screening test for subclinical hypercortisolism.

anti-hypertensive medications (OR: 1.54, $p = 0.004$) and an increased aortic stiffness (OR: 2.81, $p = 0.017$) were the variables associated with an increased chance of having confirmed hypercortisolism. Metformin use was associated with a reduced chance of having confirmed hypercortisolism (OR: 0.27, $p = 0.010$).

4. Discussion

This study has two main findings: first, it demonstrated that subclinical hypercortisolism was a prevalent condition in type 2 diabetic patients with high cardiovascular risk. Second, patients with confirmed hypercortisolism had a worse cardiovascular profile: they used a greater number of anti-hypertensive medications and had an increased aortic stiffness; known factors associated with a worse prognosis in type 2 diabetes (Cardoso et al., 2013; Salles et al., 2013), despite having a shorter diabetes duration. These findings suggest that subclinical hypercortisolism may contribute to increase the cardiovascular risk in type 2 diabetes.

Previous studies in diabetes reported a prevalence of subclinical hypercortisolism varying from 0 to 9.4% (Budyal et al., 2015; Caetano, Silva Rdo, & Kater, 2007; Catargi et al., 2003; Chiodini et al., 2005;

Table 2
Results of multivariate logistic regression for variables independently associated with positive screening test and confirmed subclinical hypercortisolism.

Positive screening ($n = 128$)*	B (SE)	odds ratio	95% CI	p-value
Age (years)	0.39 (0.014)	1.04	1.010–1.069	0.007
Number of antihypertensive drugs in use	0.23 (0.091)	1.26	1.053–1.502	0.012
Diabetes duration (years)	0.31 (0.014)	1.03	1.004–1.059	0.023
Presence of diabetic nephropathy	0.53 (0.267)	1.70	1.006–2.868	0.047
Serum triglycerides (50 mg/dl increase)	0.072 (0.041)	1.08	0.992–1.164	0.077
Metformin use	−0.62 (0.362)	0.54	0.265–1.096	0.088
Hosmer–Lemeshow goodness-of-fit test $p = 0.898$				
Area under ROC curve of estimated probabilities: 0.682, 95% confidence interval: 0.626–0.738, $p < 0.001$				
Confirmed hypercortisolism ($n = 33$)**	B (SE)	odds ratio	95% CI	p-value
Diabetes duration (years)	−0.82 (0.30)	0.92	0.869–0.977	0.006
Number of antihypertensive drugs in use	0.43 (0.151)	1.54	1.144–2.064	0.004
Metformin use	−1.31 (0.507)	0.27	0.10–0.726	0.010
Increased aortic PWV (>10 m/s)	1.03 (0.434)	2.81	1.201–6.568	0.017
Hosmer–Lemeshow goodness-of-fit test $p = 0.649$				
Area under ROC curve of estimated probabilities: 0.737, 95% confidence interval: 0.646–0.827, $p < 0.001$				

Abbreviations: PWV, pulse wave velocity; CI, confidence interval; B, B coefficient; SE, standard error.
*Candidates variables were age, gender, duration of diabetes, presence of diabetic nephropathy and cardiovascular disease, arterial hypertension, metformin and use of insulin, serum triglycerides, left ventricular mass indexed to height$^{2.7}$, increased aortic PWV.
**Candidates variables were age, gender, duration of diabetes, presence of diabetic nephropathy and cardiovascular disease, metformin and use of insulin, 24-hour systolic blood pressure and increased aortic PWV.

Leibowitz et al., 1996; Mullan et al., 2010; Murakami et al., 2010; Newsome et al., 2008; Reimondo et al., 2007; Taniguchi et al., 2008; Terzolo et al., 2012). The marked differences in subclinical hypercortisolism prevalence between diabetic populations are mostly due to differences in methods in terms of sample size, inclusion criteria and screening tests used with various cut-offs (Beko et al., 2010; Terzolo et al., 2012). Two large recent studies in type 2 diabetes, performed with unselected outpatients, reported very low prevalences of subclinical hypercortisolism of 0.2% and 0.7% (Budyal et al., 2015; Terzolo et al., 2012). Different from our study, Terzolo et al. used 5 μg/dl as the cut-off value for DST (Terzolo et al., 2012). So, they couldn't exclude the possibility of having missed subclinical hypercortisolism. If they had used the most sensitive cut-off of 1.8 μg/dl, as many as 22.6% of their patients should have been submitted to further investigation. On the other hand, it is notable that most of our positively screened patients (approximately 82%) presented cortisol levels between 1.8 and 5 μg/dl. If the cut-off point of our study was elevated to 5 μg/dl, the overall prevalence of subclinical hypercortisolism would be only 1.7%. Nevertheless, in the other large study including 993 patients, they used for screening the same cut-off of DST we used, and observed a prevalence of only 3.7% (Budyal et al., 2015). Different from our study, both used a second step evaluation by undergoing a standard 2-day 2 mg DST, instead of nocturnal salivary cortisol. The present study was performed in a tertiary specialist hospital center for endocrinology and diabetes that manages patients with diabetes referred from secondary centers. Similar to another study, patients enrolled in the present study had at least two of the three characteristics: arterial hypertension, obesity and glycated hemoglobin >7%; but different from them that reported a prevalence of 0%, we observed a significant prevalence of subclinical hypercortisolism (Mullan et al., 2010). Also, in contrast to our findings, other studies with smaller number of outpatients with some selected characteristics, as obesity, or hypertension or glycated hemoglobin >7%, also reported a prevalence of 0% (Caetano et al., 2007; Mullan et al., 2010). Our prevalence was only comparable to two studies performed in hospitalized selected type 2 diabetes, which were 8.9 and 9.4 % (Chiodini et al., 2005; Murakami et al., 2010). The present study included patients with multiple high-risk features that are known to be associated with hypercortisolism in the absence of Cushing syndrome and a false positive DST (Nieman et al., 2008). So, the present data cannot be applicable to general diabetic population, but possibly only to type 2 diabetic patients with high cardiovascular risk.

Like Budyal et al., no difference between BMI, glycemic and blood pressure control was found between the subgroups of suppressors and non-suppressors in the present study (Budyal et al., 2015). Additionally, in the present study, non-suppressors had longer diabetes duration, greater prevalence of arterial hypertension and of degenerative complications than suppressors. Dissimilar to Terzolo et al., non-suppressors had a poor glycemic control and higher systolic blood pressures than suppressors (Terzolo et al., 2012). Whether the profile of non-suppressors could at least in part account for the high rate of positive screening remains uncertain. The increased level of cortisol found may not be the cause of patients' morbidity, but alternatively a marker of a more serious cardiovascular disease, once hypothalamo-hypophysis-adrenal (HHA) axis dysfunction and stimulation of proinflamatory cytokines production can contribute to type 2 diabetes, consequently increasing cortisol levels (Hackett, Steptoe, & Kumari, 2014; Androulakis et al., 2014). On the other hand, the association between subclinical hypercortisolism and increased cardiovascular risk has been consistently demonstrated. In a selected population of resistant hypertensive patients, subclinical hypercortisolism was associated with markers of worse cardiovascular prognosis (Martins, Conceicao, Muxfeldt, & Salles, 2012). Moreover, studies in adrenal incidentaloma have recently demonstrated that even low-grade cortisol hypersecretion may increase mortality rate (Di Dalmazi et al., 2014; Morelli et al., 2014). Elevated cardiovascular risk associated with hypercortisolism is possibly mediated by the mechanism of increasing vascular smooth muscle contractility and enhancing sensitivity to noradrenalin promoted by cortisol (Debono et al., 2014). A recent study has shown an extensive calcification in coronary arteries and noncalcified atherosclerotic plaques in patients with hypercortisolism (Neary et al., 2013). Another investigation (Androulakis et al., 2014) observed a greater number of biochemical markers for cardiovascular risk and increased values of carotid IMT, even when analyzing patients without overt risk factors, like normotensive or euglycemic individuals. These vascular disorders associated with hypercortisolism could contribute to aggravate arterial hypertension and consequently increase aortic stiffness.

Patients with positive DST and with confirmed hypercortisolism used less frequently metformin in comparison to those with negative screening, and in multivariate analysis the use of metformin was protective for the presence of subclinical hypercortisolism. This could in part be explained by the presence of diabetic nephropathy with moderate/severe reduction of renal function, a contraindication to metformin use. Moreover, metformin may induce less cortisol production by inhibiting the 11beta-hydroxysteroid dehydrogenase type 1 (11β-HSD1) enzyme; hence decreasing serum cortisol concentrations (Anagnostis, Athyros, Tziomalos, Karagiannis, &

Mikhailidis, 2009), which can justify less cortisol production related to the use of metformin. Consistent with such hypothesis, a recent study has shown reduction of proinflammatory cytokines production and reduced expression of the 11β-HSD1 enzyme in adipocytes treated with metformin (Esteves et al., 2014).

Diabetic patients who failed to suppress on 1 mg dexamethasone test (DST) were older than suppressors. Studies have previously demonstrated association between age and subclinical hypercortisolism (Di Dalmazi et al., 2014, Morelli et al., 2014). Increased concentration of cortisol was also identified in older patients with resistant hypertension (Martins et al., 2012). A possible explanation is that age is related to HHA axis activity, particularly increasing late-night cortisol nadir and subsequently releasing cortisol in the morning peak, therefore decreasing the sensitivity to glucocorticoid negative feedback (Pecori Giraldi et al., 2007; Veldhuis, Asharma, & Roelfsema, 2013).

However, despite growing evidence of increased cardiovascular risk in patients affected by subclinical hypercortisolism, except for patients with adrenal incidentaloma (Chiodini et al., 2010; Toniato et al., 2009), indication of adrenalectomy in the absence hypercortisolism stigmas remains controversial (Nieman et al., 2008). To date, no study has investigated the beneficial effects of adrenalectomy in patients with diabetes and subclinical hypercortisolism. Therefore, currently, investigation for hypercortisolism should only be applied in type 2 diabetes patients with a cushingoid appearance and hypertension or truncal obesity or dyslipidaemia (Nieman et al., 2008).

There are limitations in the study that deserve comment. First, cross-sectional studies do not support inferences of causality related to findings, but only associations. Second, the etiology of hypercortisolism of these patients was not investigated, if they were ACTH dependent or not. Most of the studies on subclinical hypercortisolism have shown that the etiology is ACTH-independent, usually caused by adrenal adenomas (Terzolo et al., 2007). Otherwise, the patients who presented high salivary cortisol levels are being followed-up and, up to now, no one presented features of Cushing syndrome. Third, it was not investigated in this population the prevalence obstructive sleep apnea, which should be considerable given the demographic characteristics of the studied population. We can speculate that there is a reasonable chance that the link between the increased cardiac risk profile and raised late night cortisol might be in part mediated by the presence of sleep apnea. Finally, our data were generated in a tertiary hospital and we cannot exclude the possibility of a selection bias toward greater risk of subclinical hypercortisolism than the general diabetic population. Otherwise, strengths of the study include its relative large number of well-characterized outpatients from one center and the fact that patients with potential confounding factors for false positive DST were excluded at baseline.

5. Conclusions

In a large sample of type 2 diabetic outpatients with high cardiovascular risk, 8.6% had subclinical hypercortisolism. Albeit these patients had shorter diabetes duration, they had greater prevalence of degenerative complications, more severe arterial hypertension and increased aortic stiffness, confirming a worse cardiovascular profile associated with the presence of hypercortisolism. If it will be cost-effective to investigate subclinical hypercortisolism in patients with shorter diabetes duration but with degenerative complications, more severe hypertension or with increased aortic stiffness, only future prospective studies confirming the beneficial effects of its treatment can answer this question.

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