Is diabetes in Cushing's syndrome only a consequence of hypercortisolism?

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Abstract

Objective: Diabetes mellitus (DM) is one of the most frequent complications of Cushing's syndrome (CS). The aim of this study was to define the changes in insulin sensitivity and/or secretion in relation to glucose tolerance categories in newly diagnosed CS patients.

Design: Cross-sectional study on 140 patients with CS.

Methods: A total of 113 women (80 with pituitary disease and 33 with adrenal disease, aged 41.7±15.7 years) and 27 men (19 with pituitary disease and eight with adrenal disease, aged 38.1±20.01 years) at diagnosis were divided according to glucose tolerance into normal glucose tolerance (CS/NGT), impaired fasting glucose and/or impaired glucose tolerance (CS/prediabetes), and diabetes (CS/DM) groups.

Results: Seventy-one patients had CS/NGT (49.3%), 26 (18.5%) had CS/prediabetes and 43 (30.8%) had CS/DM. Significant increasing trends in the prevalence of family history of diabetes ($P<0.001$), metabolic syndrome ($P<0.001$), age ($P<0.001$) and waist circumference ($P=0.043$) and decreasing trends in HOMA-β ($P<0.001$) and oral disposition index (DIo) ($P<0.002$) were observed among the groups. No significant trends in fasting insulin levels, area under the curve for insulin (AUC_{INS}), Matsuda index of insulin sensitivity (ISI-Matsuda) and visceral adiposity index were detected.

Conclusions: Impairment of glucose tolerance is characterized by the inability of β-cells to adequately compensate for insulin resistance through increased insulin secretion. Age, genetic predisposition and lifestyle, in combination with the duration and degree of hypercortisolism, strongly contribute to the impairment of glucose tolerance in patients with a natural history of CS. A careful phenotypic evaluation of glucose tolerance defects in patients with CS proves useful for the identification of those at a high risk of metabolic complications.

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Introduction

Cushing's syndrome (CS) results from chronic exposure to glucocorticoid (GC) excess and is characterized by high mortality, mainly due to cardiovascular complications, such as myocardial failure, cerebrovascular disease and thromboembolism (1, 2). In a recent cohort study, Dekkers *et al*. (3) have shown that CS patients with a long-term increased risk of myocardial infarction require adequate monitoring and single-risk factor management.

Diabetes mellitus (DM) is one of the most frequent complications of CS, and its prevalence is considered to range from 20 to 50%, although it may actually be underestimated (4). Indeed, the prevalence of glucose tolerance defects increases up to 70% when impaired fasting glycaemia (IFG) and impaired glucose tolerance (IGT) are included (5).

Nevertheless, the American Diabetes Association (ADA) continues to define DM in CS as a 'specific type of diabetes secondary to endocrinopathy', although some authors judge it as a classical form of type 2 diabetes (6, 7). Indeed, DM in CS occurs as a consequence of insulin

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resistance and impaired insulin secretion induced by GC excess (8). Insulin resistance is due to the effects of GCs on the liver, skeletal muscle and adipose tissue. In particular, in the skeletal muscle, GC excess has major effects on glucose metabolism via interference at several steps in the insulin signalling cascade, resulting in reduced glucose uptake and glycogen synthesis, and on protein metabolism, resulting in proteolysis (7, 8). GC excess also leads to increased lipolysis. The consequent elevation of amino acid and free fatty acid levels contributes to further impairment of insulin signalling and favours hepatic gluconeogenesis (4). Moreover, GC excess induces a significant redistribution of adipose tissue from the subcutaneous to the visceral region and also impairment of secretion of adipocytokines such as TNFα (TNF) and IL6 and a decrease in adiponectin levels with an increase in leptin levels (9, 10, 11, 12). Furthermore, it determines β-cell dysfunction because of reduced insulin sensitivity and direct β-cell function impairment, through genomic actions that lead to decreased insulin exocytosis and β-cell apoptosis, which may be further negatively influenced by the treatment undergone (8, 9, 10, 11, 12, 13, 14).

This study aimed to define the changes in insulin secretion and/or sensitivity in a large population of newly diagnosed CS patients according to glucose tolerance and to evaluate whether these changes are influenced by hormonal, metabolic and clinical parameters.

Subjects and methods

Patients

We retrospectively revised data collected from 140 consecutive patients with active CS (pituitary and adrenal dependent), newly diagnosed, and recruited at the Endocrinology Units of the Universities of Palermo and Naples between 2000 and 2012.

Data were collected for 113 women (80 with pituitary disease and 33 with adrenal disease, aged 41.67±15.7 years) and 27 men (19 with pituitary disease and eight with adrenal disease, aged 38.07±20.01 years).

The diagnosis of CS was based, according to international criteria, on high daily urinary free cortisol (UFC) levels (at least three samples), absent cortisol suppression after a low-dose dexamethasone test (>1.8 μg/dl) and lack of cortisol rhythm (midnight cortisol levels >7.5 μg/dl); in addition, the diagnosis of Cushing's disease was based on the presence of normal or high plasma adrenocorticotrophic hormone (ACTH) levels and adequate cortisol suppression after a high-dose dexamethasone test (>80%), evidence of pituitary adenoma at RMN and results of inferior petrosal sinus sampling, when required (15). Instrumental examinations were carried out for diagnosis definition.

Exclusion criteria were subclinical CS, adrenal or pituitary cancer, and recurrent CS. No women taking oestrogens and/or progesterone as contraceptives or postmenopausal hormones in replacement therapy were included.

Seven CS patients with IFG were treated with only diet and one with metformin; 13 patients with IGT were treated with diet and four with metformin; one patient with IGF+IGT was treated with metformin; and 12 diabetic patients were treated with diet, 18 with metformin, and one with sulphonylurea. To avoid an effect on insulin sensitivity and secretion indexes, all the diabetic patients treated with oral hypoglycaemic agents stopped taking them 2 days before the evaluation, while 12 patients treated with insulin stopped using bedtime insulin the day before the metabolic evaluation.

This study was approved by the Institutional Review Boards at the Faculty of Medicine of the University of Palermo and Naples. At the time of hospitalization, all the patients signed informed consent for the scientific use of their data. The participants remained anonymous during the database analysis.

Study design

This is a retrospective, cross-sectional study. All the patients underwent complete clinical and metabolic evaluation as good clinical practice during diagnosis. The patients were subdivided into three groups: normal glucose tolerance (NGT), prediabetes (with IFG, IGT or both) and diabetes (known or newly diagnosed by oral glucose tolerance test (OGTT)). BMI, systolic and diastolic blood pressure, measured according to international criteria (16), waist circumference (WC), measured at the midpoint between the lower rib and the iliac crest, and waist/hip ratio (WHR) were evaluated. After an overnight fast, lipid (total cholesterol, HDL-C and LDL-C, and triglycerides), HbA1c, glycaemia and serum insulin profiles were evaluated.

A complete evaluation of the pituitary–adrenal axis, 8-, 16- and 24-h ACTH and cortisol levels, UFC levels as a mean of three samples from 24-h urine collection, and serum cortisol levels after a low-dose dexamethasone suppression test (1 mg) was carried out. OGTT, performed by measuring plasma blood glucose and insulin levels every 30 min for 2 h after a 75-g oral glucose load, was carried out in 66 patients.

Insulin sensitivity was estimated indirectly using basal insulin and glucose values to calculate the homeostatic model of insulin resistance (HOMA2-IR) (glycaemia (mmol/l)×insulinaemia (μU/ml)/22.5) and using glucose and insulin values obtained during the OGTT to calculate the Matsuda index of insulin sensitivity (ISI-Matsuda) (10 000/glucose (mg/dl)×insulin (μU/ml)×glucose mean×insulin mean) (17, 18). A composite measure of β-cell function relative to insulin sensitivity, assessed by the oral disposition index (DIo), was calculated as ($\Delta Insulin_{0-30}/\Delta Glucose_{0-30}$)×(1/fasting insulin). The trapezoidal method was used for the calculation of the area under the curve for insulin ($AUC_{2\text{-h insulin}}$) and area under the curve for glucose ($AUC_{2\text{-h glucose}}$) (19). The HOMA-β was calculated as 360×(insulin)/((glucose)−63) % (glucose in mg/dl) (17).

The visceral adiposity index (VAI) was calculated according to gender, where TG is the triglyceride level expressed in mmol/l and HDL is the HDL-C level expressed in mmol/l:

i) VAI=(WC/39.68+(1.88×BMI))×(TG/1.03)×(1.31/HDL) for males;
ii) VAI=(WC/36.58+(1.89×BMI))×(TG/0.81)×(1.52/HDL) (20) for females.

The metabolic syndrome (MS) was diagnosed according to the NCEP ATP III criteria, whereas DM and prediabetes were diagnosed according to the ADA criteria (6, 21).

Hormone and biochemical assays

Serum insulin, glucose and lipid levels were measured by ELISA (DRG Instruments GmbH, Marburg, Germany). The normal insulin range was 5–19 UI/ml. LDL-C levels were measured using the Friedewald formula (total cholesterol−(HDL+(TG/5)). HbA1c levels were determined by HPLC with an ion-exchange resin (HA8121, Hi-AutoA1c, Menarini, Florence, Italy). UFC levels were measured by RIA (Diagnostic System Laboratories, Inc., Roche Diagnostics GmbH, Mannheim , Germany) with a normal range of 36–137 μg/24 h. ACTH levels were measured using ELISA (DRG Instruments GmBH) with a normal range of 7.6–66.1 pg/ml. Serum cortisol levels were measured using RIA (Diagnostic Systems Laboratories, Inc.) with a reference range of 22.8–217 ng/ml. The conversion factors for the International System (SI) were as follows: glucose mg/dl vs mmol/l: 0.0555; insulin mUI/ml vs pmol/l: 6.945; total cholesterol and HDL-C mg/dl vs mmol/l: 0.0259; triglycerides mg/dl vs mmol/l: 0.0113; and cortisol ng/ml vs nmol/l: 3.180.

Statistical analysis

The Statistical Packages for Social Science SPSS version 17 (SPSS, Inc.) was used for data analysis. The normality of quantitative variables was tested with the Shapiro–Wilk test. Baseline characteristics are presented as means±S.D. for continuous variables; rates and proportions were calculated for categorical data. Differences between two groups were detected using unpaired Student's *t*-test for continuous variables (after testing for equality of variance: Levene's test) and the χ^2 test and Fisher's exact test (when appropriate) for categorical variables. The ANOVA trend analysis for quantitative variables and χ^2 for trend for categorical variables were carried out for the three groups: CS/NGT, CS/prediabetes and CS/DM. A *P* value <0.05 was considered to be statistically significant.

Results

Metabolic and clinical parameters according to gender

The clinical, phenotypic and biochemical features of all the patients grouped according to gender are listed out in Tables 1, 2 and 3. No differences were found between men and women, except for BMI (F vs M: 31.35±6.03 vs 28.78±5.52 kg/m^2; $P=0.039$) and VAI (F vs M: 2.82±1.51 vs 1.46±0.75; $P<0.001$).

Metabolic and clinical parameters according to glucose tolerance

Of the 140 patients with CS, 71 were classified as having CS/NGT (49.3%), 26 (18.5%) CS/prediabetes and 43 CS/DM (30.8%) (Table 4). Significant increasing trends were observed in age (CS/NGT=35.05±16.34; CS/prediabetes =41.27±12.97; CS/DM=50.58±14.64 years; $P<0.001$) and WC (CS/NGT=100.85±14.77; CS/prediabetes=106.28±17.49; CS/DM=106.76±16.00 cm; $P=0.043$). A non-significant trend was observed in BMI (CS/NGT=30.00±5.07; CS/prediabetes=31.18 kg/m^2 ±6.08; CS/DM=32.07±7.19; $P=0.072$) (Table 4).

In addition, an increasing trend was observed in the familial history of DM (CS/NGT=29.6%; CS/prediabetes=46.2%; CS/DM=65.1%; $P<0.001$) and cardiovascular disease (CS/NGT=54.9%; CS/prediabetes=76.9%; CS/DM=81.4%; $P=0.002$), MS (CS/NGT=39.4%; CS/prediabetes=76.9%; CS/DM=93%; $P<0.001$) and hypertension (CS/NGT=67.6%; CS/prediabetes=76.9%; CS/DM=86%; $P<0.026$) (Table 4).

Table 1 Clinical and phenotypic features of all the 140 CS patients at diagnosis grouped according to gender. Data is presented as mean ± S.D. or as *n*(%).

	Women (*n* = 113)	**Men** (*n* = 27)	**Total** (*n* = 140)	***P***
Age (years)	41.67 ± 15.7	38.07 ± 20.01	40.98 ± 16.61	0.314
BMI (kg/m^2)	31.35 ± 6.03	28.78 ± 5.52	30.86 ± 6.01	0.039
WC	104.04 ± 15.53	102.16 ± 17.33	103.68 ± 15.83	0.609
WHR	1.01 ± 0.09	1.03 ± 0.05	1.01 ± 0.09	0.242
Family history of				
Diabetes	48 (42.5)	13 (48.1)	61 (43.6)	0.593
Cardiovascular disease	75 (66.4)	19 (70.4)	94 (74.1)	0.691
Dyslipidaemia	30 (26.5)	9 (33.3)	39 (27.9)	0.480
Smoking	40 (35.4)	14 (51.9)	54 (38.6)	0.115
Pituitary disease	80 (78.8)	19 (70.4)	99 (70.7)	0.965
Adrenal disease	33 (29.2)	8 (29.6)	41 (29.3)	0.965
Autoimmune thyroid disease	13 (11.5)	1 (0.4)	14 (10)	0.607
Multinodular goitre	14 (12.4)	2 (7.4)	16 (11.4)	0.737
Muscle hypotrophy	46 (40.7)	9 (33.3)	55 (39.3)	0.481
Moon face	58 (51.3)	12 (44.4)	70 (50)	0.520
Facial plethora	59 (52.2)	13 (48.1)	72 (51.4)	0.704
Buffalo hump	44 (38.9)	6 (22.2)	50 (35.7)	0.103
Purple striae	45 (39.8)	13 (48.1)	58 (41.4)	0.430
Ecchymosis	25 (22.1)	7 (25.9)	32 (22.9)	0.673
Coronary heart disease	19 (16.8)	4 (14.8)	23 (16.4)	1
Coagulopathy	7 (6.2)	3 (11.1)	10 (7.2)	0.406
Peripheral vascular disease	5 (4.4)	2 (7.4)	7 (5)	0.620
Cerebral vascular disease	10 (8.8)	2 (7.4)	12 (8.6)	1
Depression	27 (23.9)	5 (18.5)	32 (22.9)	0.550
Osteoporosis	25 (22.1)	7 (25.9)	32 (22.9)	0.673
Fractures	10 (8.8)	5 (18.5)	15 (10.7)	0.144
Vertebral collapse	11 (9.7)	2 (7.4)	13 (9.3)	1
Arthropathy	39 (34.5)	8 (29.6)	47 (33.6)	0.629
Hepatic steatosis	42 (57.5)	9 (69.2)	51 (59.3)	0.547

No differences were found in venous thrombosis, fasting insulin levels, total HDL-C and LDL-C levels, triglyceride levels, AUC_{INS}, ISI-Matsuda and VAI (Table 4). A decreasing trend was observed in HOMA-β (CS/NGT = 170.65 ± 43.07; CS/prediabetes = 163.46 ± 77.52; CS/DM = 87.11 ± 76.94; $P < 0.001$) and DIo (CS/NGT = 3.04 ± 3.68; CS/prediabetes = 0.87 ± 1.12; CS/DM = 0.70 ± 1.07; $P < 0.001$) (Table 4). As expected, a significant increasing trend was observed in fasting glucose levels (CS/NGT = 4.77 ± 0.64; CS/prediabetes = 5.43 ± 0.97; CS/DM = 7.79 ± 2.68 mmol/l; $P < 0.001$), $AUC_{2\text{-h glucose}}$ (CS/NGT = 14 756 ± 3038; CS/prediabetes = 17 314 ± 4890; CS/DM = 24 707 ± 8882; $P = 0.005$) and HbA1c levels (CS/NGT = 34.97 ± 3.4; CS/prediabetes = 39.89 ± 5.1; CS/DM = 54.20 ± 12.4 mmol/mol; $P < 0.001$) (Table 4).

Hormonal parameters according to glucose tolerance

Circadian rhythms for ACTH and cortisol, UFC and cortisol after the administration of 1 mg dexamethasone did not differ significantly among the three groups (Table 4).

Discussion

The results of this study indicate that, in agreement with the literature data, the prevalence of CS is high in women (80.71%). The sexual dimorphism of CS in women has already been documented (22). To date, it is estimated that Cushing's disease occurs five times more frequently in women than in men (23). Women with CS have a higher BMI than men, with similar values of WC, because in CS the gender differences in fat distribution are lost precisely because the adipose tissue is redistributed from the peripheral to the visceral region, leading to the well-known phenotype characterized by gynoid obesity, independently of sex (24, 25, 26, 27). In our study, CS women had a significantly higher VAI, consequent on the influence of GC excess on visceral adipose dysfunction. The VAI is a gender-specific index, which was modelled on healthy men and women (20, 28). The increase in the VAI, specifically observed in CS women, confirms the loss of cardiovascular protection in relation to gender, and CS women even developed worse visceral adipose dysfunction (indirectly expressed by the VAI) compared with men,

Table 2 Hormonal parameters of all the 140 CS patients at diagnosis grouped according to gender. Data is presented as mean ± S.D.

	Women ($n=113$)	**Men** ($n=27$)	**Total** ($n=140$)	***P***
8-h ACTH (pg/ml)	55.13 ± 44.27	58.79 ± 46.89	55.85 ± 44.64	0.715
16-h ACTH (pg/ml)	45.48 ± 36.2	44.33 ± 34.12	45.42 ± 35.69	0.969
24-h ACTH (pg/ml)	48.14 ± 27.42	41.76 ± 35.2	46.26 ± 29.79	0.475
8-h Cortisol (nmol/l)	817.26 ± 225.78	791.82 ± 279.8	814.08 ± 235.32	0.626
16-h Cortisol (nmol/l)	747.30 ± 232.14	610.56 ± 241.68	728.22 ± 238.5	0.142
24-h Cortisol (nmol/l)	629.64 ± 313.73	508.8 ± 225.78	594.66 ± 292.56	0.115
Urinary free cortisol (μ/24 h)	393.39 ± 348.45	518.92 ± 457.98	416.87 ± 372.76	0.122
Cortisol after an overnight dexamethasone suppression test (nmol/l)	295.74 ± 248.04	397.5 ± 384.78	314.82 ± 276.66	0.344

as also observed in other endocrine diseases, such as acromegaly, prolactinoma and diabetes (28, 29, 30, 31). However, despite this evidence, the VAI in CS has a strong application limit. The reason for this is that it is an indicator of early cardiometabolic risk and an important diagnostic tool in all borderline conditions in which overt MS is not present (32). Indeed, in our cohort of CS patients, 62.9% had overt MS, and so the VAI cannot be considered a specific index for these patients. This is explained by the fact that three of the variables making up the VAI (WC, TG and HDL) are dichotomically expressed in the criteria of MS. In a recent letter, the applicative limits of VAI in single patients have been pointed out and it has been recommended that for proper application in an individual patient or in small sample studies the application of the VAI is not suggested, above all in the

Table 3 Metabolic parameters of all the 140 CS patients at diagnosis grouped according to gender. Data is presented as *n*(%) or as mean ± S.D.

	Women ($n=113$)	**Men** ($n=27$)	**Total** ($n=140$)	***P***
Metabolic syndrome[a]	75 (66.4)	13 (48.1)	88 (62.9)	0.078
Diabetes or fasting glucose ≥5.6 mmol/l	42 (37)	10 (8)	52 (46)	0.017
High blood pressure	84 (74.3)	21 (67.8)	105 (75)	0.711
High triglycerides	38 (33.6)	9 (33.3)	47 (33.6)	0.977
Low HDL-C	72 (63.7)	5 (18.5)	77 (55)	<0.001
Increased WC	94 (83.2)	20 (74.1)	114 (81.4)	0.279
Hypercholesterolaemia	49 (43.4)	12 (44.4)	61 (43.6)	0.919
Impaired fasting glucose (IFG)	6 (5.3)	2 (7.4)	8 (5.7)	0.651
Impaired glucose tolerance (IGT)	14 (12.4)	3 (11.1)	17 (12.1)	1
IFG + IGT	0	1 (3.7)	1 (0.7)	0.193
Diabetes mellitus	36 (31.9)	7 (25.9)	43 (30.7)	0.548
Total cholesterol (mmol/l)	5.19 ± 0.93	4.79 ± 0.88	5.11 ± 0.93	0.049
HDL-C (mmol/l)	1.27 ± 0.33	1.35 ± 0.36	1.29 ± 0.34	0.243
LDL-C (mmol/l)	3.11 ± 0.87	2.74 ± 0.79	3.04 ± 0.86	0.037
Triglycerides (mmol/l)	1.58 ± 0.60	1.32 ± 0.57	1.53 ± 0.60	0.059
Fasting glucose (mmol/l)	5.79 ± 2.08	5.97 ± 2.12	5.82 ± 2.08	0.753
Fasting insulin (UI/ml)	13.16 ± 6.28	14.73 ± 7.53	13.46 ± 6.54	0.407
HOMA2-IR	2.00 ± 0.95	2.21 ± 1.10	2.04 ± 0.98	0.432
HOMA-β	142.99 ± 69.9	146.44 ± 88.13	143.66 ± 72.38	0.935
$AUC_{2\text{-h glucose}}$[b]	18 168 ± 7180	17 822 ± 5703	18 095 ± 6854	0.987
$AUC_{2\text{-h insulin}}$[b]	9627 ± 5721	9602 ± 6696	9622 ± 5891	0.974
ISI-Matsuda[b]	4.16 ± 3.48	3.53 ± 1.83	4.02 ± 3.19	0.615
Oral disposition index (DIo)[b]	1.88 ± 3.07	1.43 ± 1.27	1.78 ± 2.78	0.913
HbA1c (%)	6.24 ± 1.13	6.5 ± 1.13	6.29 ± 1.13	0.357
HbA1c (mmol/mol)	45.0 ± 12.4	48.0 ± 12.4	45.0 ± 12.4	0.357
VAI	2.76 ± 1.41	1.43 ± 1.27	2.51 ± 1.40	<0.001

[a]According to the Adult Treatment Panel (ATP) III criteria.
[b]In a subgroup of 66 patients without known diabetes.

Table 4 Clinical and biochemical features of all the 140 CS patients divided into three groups (normal glucose tolerance (CS/NGT), prediabetes (CS/prediabetes) and diabetes (CS/DM)). Data is presented as mean ± S.D. or as *n*(%).

	CS/NGT (*n* = 71)	**CS/prediabetes** (*n* = 26)	**CS/DM** (*n* = 43)	***P***
Age (years)	35.05 ± 16.34	41.27 ± 12.97	50.58 ± 14.64	< 0.001
BMI (kg/m^2)	30.00 ± 5.07	31.18 ± 6.08	32.07 ± 7.19	0.072
WC	100.85 ± 14.77	106.28 ± 17.49	106.76 ± 16.00	0.043
WHR	1.01 ± 0.09	1.02 ± 0.09	1.01 ± 0.08	0.900
Male	14 (19.7)	6 (23.1)	7 (16.3)	0.696
Female	57 (80.3)	20 (76.9)	36 (83.7)	0.696
Family history of				
Diabetes	21 (29.6)	12 (46.2)	28 (65.1)	< 0.001
Cardiovascular disease	39 (54.9)	20 (76.9)	35 (81.4)	0.002
Dyslipidaemia	18 (25.4)	10 (38.5)	11 (25.6)	0.863
Smoking	23 (32.4)	10 (38.5)	21 (48.8)	0.082
Pituitary disease	48 (67.6)	21 (80.8)	30 (69.8)	0.704
Adrenal disease	23 (32.4)	5 (19.2)	13 (30.2)	0.704
Metabolic syndrome[a]	28 (39.4)	20 (76.9)	40 (93)	< 0.001
Hypertension	48 (67.6)	20 (76.9)	37 (86)	0.026
Venous thrombosis	7 (5)	5 (4.4)	2 (7.4)	0.620
Hormonal parameters				
8-h ACTH (pg/ml)	51.11 ± 42.87	65.85 ± 34.47	57.86 ± 52.00	0.365
16-h ACTH (pg/ml)	40.90 ± 36.18	55.58 ± 35.24	45.58 ± 35.47	0.530
24-h ACTH (pg/ml)	41.27 ± 31.00	52.66 ± 20.32	51.00 ± 31.50	0.209
8-h Cortisol (nmol/l)	782.28 ± 209.88	817.26 ± 235.32	858.6 ± 270.3	0.102
16-h Cortisol (nmol/l)	734.58 ± 190.8	721.86 ± 228.96	718.68 ± 340.26	0.815
24-h Cortisol (nmol/l)	556.5 ± 238.5	607.38 ± 295.74	645.54 ± 365.7	0.249
Urinary free cortisol (μ/24 h)	436.25 ± 334.20	376.82 ± 322.03	409.54 ± 457.89	0.668
Cortisol after an overnight dexamethasone suppression test (nmol/l)	302.1 ± 273.48	333.9 ± 263.94	321.18 ± 292.56	0.783
Metabolic parameters				
Total cholesterol (mmol/l)	4.95 ± 0.87	5.60 ± 1.08	5.09 ± 0.86	0.258
HDL-C (mmol/l)	1.31 ± 0.35	1.32 ± 0.37	1.22 ± 0.29	0.237
LDL-C (mmol/l)	2.94 ± 0.83	3.36 ± 0.99	3.00 ± 0.80	0.586
Triglycerides (mmol/l)	1.47 ± 0.71	1.74 ± 0.97	1.64 ± 0.7	0.215
Fasting glucose (mmol/l)	4.77 ± 0.64	5.43 ± 0.97	7.79 ± 2.68	< 0.001
Fasting insulin (UI/ml)	13.54 ± 5.23	17.06 ± 8.05	10.97 ± 6.39	0.079
HOMA2-IR	1.97 ± 0.79	2.57 ± 1.19	1.83 ± 1.05	0.671
HOMA-β	170.65 ± 43.07	163.46 ± 77.52	87.11 ± 76.94	< 0.001
$AUC_{2\text{-h glucose}}$[b]	14 756 ± 3038	17 314 ± 4890	24 707 ± 8882	0.005
$AUC_{2\text{-h insulin}}$[b]	10 615 ± 6259	10 398 ± 5769	7104 ± 4854	0.066
ISI-Matsuda[b]	4.25 ± 1.93	3.35 ± 1.95	4.33 ± 5.38	0.947
Oral disposition index (DIo)[b]	3.04 ± 3.68	0.87 ± 1.12	0.70 ± 1.07	0.002
HbA1c (%)	5.35 ± 0.31	5.80 ± 0.47	7.11 ± 1.13	< 0.001
HbA1c (mmol/mol)	34.97 ± 3.4	39.89 ± 5.1	54.20 ± 12.4	< 0.001
VAI	2.36 ± 1.44	2.43 ± 1.11	2.82 ± 1.46	0.102

[a]According to the Adult Treatment Panel (ATP) III criteria.
[b]In a subgroup of 66 patients without known diabetes.

presence of morbid obesity, pendulous abdomen, severe hypertriglyceridaemia and/or fibrate use (33).

The results of our study indicate that DM patients are significantly older, have a higher prevalence of family history of DM and exhibit a marked deterioration of insulin secretion indexes.

The familial history of DM appears to predispose CS patients to worse metabolic derangement. It is worth noting that DM is a polygenic disease, and among all the genetic factors described, the presence of relatives affected by the disease remains one of the most important determinants for predicting the onset of disease (34). The role of age in the development of type 2 diabetes is unanimously recognized, and these findings were confirmed in our cohort, i.e. CS/DM patients are older than CS/NGT and CS/prediabetes patients.

A condition of insulin resistance has been reported in hypercortisolism (35). Surprisingly, neither ISI-Matsuda nor HOMA-IR was significantly affected in CS/DM patients in comparison with that in the other CS patients. By

contrast, a worsening of insulin secretion relative to insulin resistance (DIo), but not of absolute insulin secretion (AUC_{INS}), was observed in CS/DM patients in addition to the reduction of HOMA-β (3, 12). Indeed, the DIo, which expresses the ability of β-cells to adequately compensate for insulin resistance through increased insulin secretion, has been shown to predict the development of diabetes in adults (19). Therefore, the DIo could be used for evaluating diabetes risk linked to the CS condition.

These data show that patients with CS/DM have a strong insulin secretion defect without a worsening of insulin sensitivity in comparison with CS/NGT and CS/prediabetes patients. Therefore, diabetes in CS could probably be strongly influenced by β-cell defect that would occur after a long period of insulin resistance. This observation is not new, even if the data available are on mice or on patients with exogenous hypercortisolism (36). However, these data need to be investigated further.

Recently, many studies have been conducted on the usefulness of evaluating the hypothalamus–pituitary–adrenal axis in patients with DM. To date, this evaluation is not recommended (37, 38). Moreover, there are no data that can define any differences between patients with classic DM and those with CS and DM.

From the data obtained in our study, we can hypothesize that a time-dependent prodromal period, influencing both CS and DM, progresses together with other variables that can play a distinct role in the determination of diabetes in CS, such as the duration of disease, degree of hypercortisolism and genetic factors. The first consideration points out that the duration of disease is a parameter that is not always determinable in CS because the onset of CS is subtle and is usually diagnosed some time after the real onset of the disease. On the other hand, the degree of hypercortisolism may be variable: obviously marked hypercortisolism should exert an effect different from that of slight hypercortisolism. In this light, recently, it has been suggested that GC action may be influenced by GC receptor (*GR* (*NR3C1*)) polymorphisms (39). Some *GR* polymorphisms have been demonstrated to impair GC sensitivity and can be associated with altered metabolic profiles. In particular, *N363S, BCL1* and *ER22/EK* are associated with diabetes, hypertension and dyslipidaemia, while A3669G *GR* polymorphism seems to be protective in CS (39).

A further confirmation of the complex and heterogeneous variables implicated in the development of diabetes in CS is emerging from the different effects of pasireotide on glucose tolerance in patients with CS: some

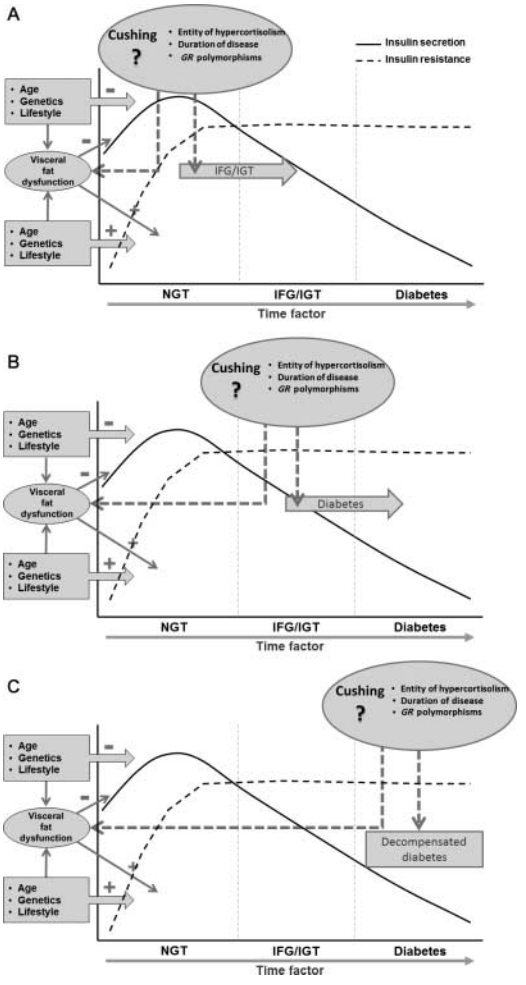

Figure 1

Diabetes in CS is due to complex and heterogeneous pathophysiological mechanisms linked only in part to insulin resistance.
(A) Patients with normal glucose tolerance. Age, genetic predisposition and lifestyle in association with visceral adipose dysfunction determine an early onset of insulin resistance and trigger reduction of insulin secretion. CS onset, through its specific factors, favours the impairment of glucose tolerance and the development of IFG/IGT (latent diabetes is triggered). (B) Patients with IFG/IGT. Age, genetic predisposition and lifestyle in association with visceral adipose dysfunction determine a worsening of insulin resistance and a significant reduction of insulin secretion. CS onset, through its specific factors, favours the impairment of glucose tolerance and the development of diabetes (latent diabetes is revealed). (C) Patients with diabetes. Age, genetic predisposition and lifestyle in association with visceral adipose dysfunction determine a significant progression of insulin resistance and a strong reduction of insulin secretion. CS onset, through its specific factors, together with the DM-specific ones, completely eliminates glucose tolerance, determining the decompensation of diabetes (diabetes mellitus is overt).

of them can develop overt diabetes, while others maintain NGT. This difference is most probably due to the interplay between pasireotide β-cell effects and genetic/environmental factors (40). Moreover, the complexity of the pathogenesis of diabetes in CS could also be supported by the evidence that in a large number of patients with CS, DM persists also after definitive treatment when total resolution of CS has been achieved (41).

Based on the above, we could suggest that in CS diabetes is due to the action of non-modifiable risk factors, such as age and genetic predisposition, and modifiable risk factors, such as lifestyle, which are independent of hypercortisolism. When CS becomes overt through some specific factors, such as the exact duration of hypercortisolism, *GR* polymorphisms and degree of hypercortisolism, it contributes to the impairment of glucose tolerance. Therefore, in the presence of the above-mentioned characteristics, if a patient has NGT, s/he will develop prediabetes or diabetes; if a patient has prediabetes, s/he will develop DM; and if a patient is diabetic, s/he will exhibit a worsening of glycaemic control with decompensated diabetes (Fig. 1).

Clearly, obesity and MS are additional factors that are associated with chronic inflammation contributing to the further development of insulin resistance and progression to diabetes. Local hyperactivity of GCs in abdominal adipose tissue contributes to the pathogenesis of MS and ongoing development of DM, steps that may vary in a CS patient in relation to different mechanisms arising in the history of the disease (27, 42). Therefore, careful phenotypic evaluation of glucose tolerance defects in patients with CS is necessary, using, if possible, accurate examinations to verify insulin secretion and sensitivity, before beginning medical treatment for CS, which can further impair glucose tolerance. In addition, this phenotypic evaluation could be essential above all for the identification of the best therapeutic management of diabetes in CS.

In conclusion, the results of this study suggest that DM is not a simple consequence of hypercortisolism and that both genetic predisposition for DM and hypercortisolism, working together, exert dangerous effects on glucose metabolism. However, increased insulin resistance and decreased insulin secretion entities may be extremely different in a single CS patient during the course of the disease.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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