

## RESEARCH

# Midnight salivary cortisol secretion associated with high systolic blood pressure in type 1 diabetes

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## Abstract

**Objective:** To explore associations between high midnight salivary cortisol (MSC) secretion and high blood pressure (BP) in type 1 diabetes (T1D).

**Methods:** Cross-sectional study of 196 adult patients with T1D (54% men). Associations between high MSC ( $\geq 9.3$  nmol/L) and high systolic BP ( $>130$  mmHg), and high diastolic BP ( $>80$  mmHg) were explored for all patients, users and non-users of antihypertensive drugs (AHD). Adjustments were performed for age, sex, diabetes-related variables, p-creatinine, smoking, physical inactivity, depression and medication.

**Results:** The prevalence of high MSC differed between patients with high and low systolic BP in all 196 patients: 39 vs 13% ( $P = 0.001$ ); in 60 users of AHD: 37 vs 12% ( $P = 0.039$ ), and in 136 non-users of AHD: 43 vs 13% ( $P = 0.012$ ). Significant associations with high systolic BP were for all patients: physical inactivity (adjusted odds ratio (AOR) 6.5), high MSC (AOR 3.9), abdominal obesity (AOR 3.7), AHD (AOR 2.9), age (per year) (AOR 1.07), and p-creatinine (per  $\mu\text{mol/L}$ ) (AOR 1.03); for 60 users of AHD: high MSC (AOR 4.1) and age (per year) (AOR 1.11); for 136 non-users of AHD: abdominal obesity (AOR 27.4), physical inactivity (AOR 14.7), male sex (AOR 9.0), smoking (AOR 7.9), and age (per year) (AOR 1.08). High MSC was not associated with high DBP.

**Conclusions:** In adult patients with T1D, high systolic BP was associated with physical inactivity, high MSC secretion, abdominal obesity, p-creatinine, age, and AHD, the latter indicating treatment failure.

## Key Words

- ▶ antihypertensive drugs
- ▶ cortisol
- ▶ hypertension
- ▶ sex differences
- ▶ obesity
- ▶ physical inactivity
- ▶ smoking
- ▶ treatment failure
- ▶ type 1 diabetes

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## Introduction

Type 1 diabetes (T1D) is an autoimmune disease, characterised by insulin deficiency due to pancreatic  $\beta$ -cell loss leading to hyperglycaemia (1). T1D is associated with increased risk for myocardial infarction, heart failure and ischemic stroke (2). Hypertension is a major risk factor for cardiovascular disease (CVD), and antihypertensive therapy reduces atherosclerotic CVD and heart failure (3). In a large Swedish population study the prevalence of hypertension in patients with T1D was 35%, almost twice as high as in the non-diabetic population (2).

Several factors might contribute to the development of hypertension, such as increased cortisol secretion (4, 5, 6, 7, 8, 9), obesity (8, 9), physical inactivity (10), smoking (11), and depression (12), and higher blood pressure (BP) have been observed in men (13). Increased cortisol secretion is one factor that might contribute to treatment-resistant hypertension (14).

Increased cortisol secretion is implicated in the development of atherosclerosis, CVD and CV mortality (15, 16, 17). Several disturbances of the

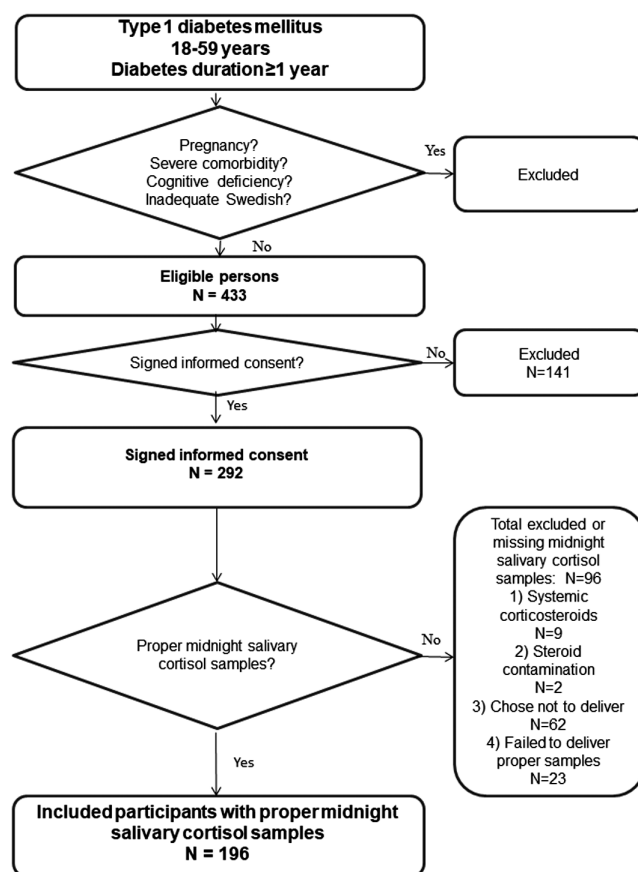
hypothalamus–pituitary–adrenal (HPA) axis in patients with T1D have been demonstrated. The disturbances include increased basal hyperactivity (18), exaggerated nocturnal rise in plasma cortisol (19), greater cortisol responses to corticotropin-releasing hormone (CRH) stimulation (20), and impaired glucocorticoid negative feedback (18, 21). Impaired glycaemic control, hypoinsulinaemia, hypoglycaemia episodes, impaired kidney function, depression, physical inactivity, smoking and other types of substance abuse, all affect the levels of cortisol secretion (21, 22, 23, 24, 25, 26, 27, 28).

In our previous research we showed that high systolic BP was associated with abdominal obesity (9), and with the macrophage-derived inflammatory marker soluble 163 (29). We also previously found that depression, smoking and physical inactivity were associated with high midnight salivary cortisol (MSC) secretion ( $\geq 9.3$  nmol/L) in these patients with T1D (22).

We hypothesise that high MSC secretion contributes to hypertension and to treatment failure of antihypertensive drugs (AHDs). The main aims were to explore whether high MSC ( $\geq 9.3$  nmol/L) was associated with systolic BP  $>130$  mmHg and/or diastolic BP  $>80$  mg. We controlled for potential covariates such as age, diabetes duration, sex, abdominal obesity, HbA1c, hypoglycaemia episodes, depression, physical inactivity, smoking, plasma-creatinine, AHD, and the use of antidepressants.

## Materials and methods

This study had a cross-sectional design. The patients were recruited by specialist diabetes physicians or diabetes nurses at regular follow-up visits at one outpatient’s diabetes clinic in southern Sweden during the period 03/25/2009 to 12/28/2009. The catchment population was 125,000. Inclusion criteria were T1D, age 18–59 years, diabetes duration  $\geq 1$  year, and performed measurements of MSC (Fig. 1). Exclusion criteria were systemic corticosteroid treatment; pregnancy; severe comorbidities or cognitive deficiency (cancer, hepatic failure, end-stage renal disease (ESRD), diagnosed Cushing’s syndrome/disease, stroke with cognitive deficiency, psychotic disorder, bipolar disorder, severe personality disorder, mental retardation, severe substance abuse); or inadequate knowledge of Swedish. BP and waist circumference (WC) measurements, saliva and blood samples were collected, supplemented by data from electronic medical records. Data were analysed for all, and separately for users and non-users of AHD. Depression was assessed by a self-report instrument. The study was



**Figure 1** Flow chart showing excluded and included patients with T1D.

approved by the Regional Ethical Review Board of Linköping University, Linköping (Registration no. M120-07, T89-08). All patients provided written informed consent.

## Midnight salivary cortisol

Each patient collected one MSC sample between 23.30 and 00.30 h, using the Salivette® sampling method (Sarstedt, Nümbrecht, Germany) (22, 30, 31).

Patients had a restriction period of 30 min prior to sampling when they were told not to eat, drink, smoke, use snuff, or perform physical exercise, and a period of 60 min prior to sampling when they should avoid brushing their teeth. MSC samples delivered within 1 week from BP measurements were included. The samples were centrifuged and frozen at  $-25^{\circ}\text{C}$  until assayed within a year. The Roche Cobas Cortisol assay®, a competitive electrochemiluminescence immunoassay (ECLIA) was used on an Elecsys 2010 immunoanalyser system (Roche Diagnostics) (32). The intra-coefficient of variation was  $<3\%$ .

High MSC was defined as  $\geq 9.3$  nmol/L (22, 30, 33), which corresponds to the 83rd percentile in these participants.

Out of 292 patients who provided informed consent to participate, nine subjects were excluded due to the use of systemic corticosteroids, two subjects using topical steroids had very high MSC values (82 and 72 nmol/L) and were therefore excluded as contamination was suspected, and 85 subjects chose not to deliver or failed to deliver proper MSC samples (Fig. 1) (22). Finally, there were proper MSC samples for 196 participants.

### Blood pressure and waist circumference

A nurse measured BP once on the patient's right arm, with the patient in the sitting position. High systolic BP was defined as  $>130$  mmHg, and high diastolic BP as  $>80$  mmHg. The treatment targets for AHD, recommended in the Swedish National Guidelines for Diabetes in 2009, were systolic BP  $\leq 130$  and diastolic BP  $\leq 80$  mmHg (34).

Waist circumference was measured according to standard procedures by a nurse. Abdominal obesity was defined as waist circumference (meters)  $\geq 0.88$  for women and as  $\geq 1.02$  for men (9, 22, 35, 36, 37).

### Episodes of hypoglycaemia

A severe episode of hypoglycaemia was defined as needing help from another person. Episodes during the last 6 months prior to recruitment were registered.

### HbA1c and p-creatinine

HbA1c (presented as mmol/mol and %) was analysed with an Olympus AU clinical chemistry. The intra-coefficient of variation was  $<3\%$ .

P-creatinine ( $\mu\text{mol/L}$ ) was assayed by an AU2700® instrument (Beckman Coulter). The intra-coefficient of variation was  $<3\%$ .

### Smoking and physical inactivity

Smokers were defined as having smoked any amount of tobacco during the last year (22). Levels of physical activity performed at work and during leisure time were assessed by interviews performed by skilled nurses and physicians at the regular follow-up visits. Physical inactivity was defined as moderate activities, such as 30 min of walking, less than once a week (22, 37).

### Self-reported depression

Depression was assessed by the Hospital Anxiety and Depression Scale – the depression subscale (HADS-D) which consists of seven statements rated from 0 to 3. Depression was defined as HADS-D  $\geq 8$  points as recommended by the constructors of the test (38), and as in our previous research (22, 30, 36, 39, 40). A major characteristic of HADS-D is that potential symptoms of somatic disease are not included (38).

### Medication

AHD were Ca antagonists (ATC codes C08CA01-02); angiotensin-converting enzyme (ACE) inhibitors (ATC codes C09AA-BA); angiotensin II antagonists (ATC codes C09CA-DA); diuretics (ATC codes C03AA03 and C03CA01); selective beta-adrenoreceptor antagonists (ATC code C07AB). The use of AHD was dichotomised into users and non-users of AHD.

Antidepressants were SSRIs (ATC codes N06AB04 and N06AB10); SNRIs (ATC code N06AX16); combined serotonin and norepinephrine reuptake inhibitors (ATC code N06AX21); tricyclic antidepressants (ATC code N06AA04); and/or tetracyclic antidepressants (ATC code N06AX11). The use of antidepressants was dichotomised into users and non-users of antidepressants.

### Statistical analysis

Analyses of data distribution using histograms revealed that MSC, systolic and diastolic BP, were not normally distributed. Data were presented as median values (quartile ( $q$ )<sub>1</sub>,  $q$ <sub>3</sub>; range), and analyses were performed with Mann–Whitney  $U$  test. Fisher's exact test were used to analyze categorical data. Crude odds ratios (CORs) were calculated. Variables with  $P \leq 0.10$  for the CORs, and sex and age (independent of  $P$  values), were entered in multiple logistic regression analyses (Backward: Wald) with systolic BP  $>130$  mmHg and diastolic BP  $>80$  mmHg as dependent variables for all, users of AHD and non-users of AHD. In non-users of AHD, multiple logistic regression analyses (Backward: Wald) were performed with high MSC as a dependent variable. The Hosmer and Lemeshow test for goodness-of-fit and Nagelkerke  $R^2$  were used to evaluate each of these multiple logistic regression analyses models. Confidence intervals (CIs) of 95% were used.  $P < 0.05$  was considered statistically significant. SPSS® version 23 (IBM) was used for the statistical analyses.

## Results

One hundred and ninety-six patients with T1D participated in this study (54% men, 18–59 years, diabetes duration 1–55 years). Sixty patients (31%) were users of AHD and 136 (69%) were non-users of AHD. The patients used either multiple daily insulin injections (MDII) (90%) or continuous s.c. insulin infusion (CSII) (10%). Seven patients (4%) had cardiovascular complications.

Baseline data, comparisons between users and non-users of AHD, and comparisons between patients with high and low MSC are presented in Table 1. The 60 users of AHD compared to the 136 non-users were older ( $P < 0.001$ ), had longer diabetes duration ( $P < 0.001$ ), and higher prevalence of high systolic BP (32 vs 10%,  $P = 0.001$ ). The 34 patients with high MSC compared to the 162 with low MSC were older ( $P = 0.002$ ), and had higher prevalence of high systolic BP ( $P = 0.001$ ), smoking ( $P = 0.002$ ), and depression ( $P = 0.002$ ).

In Table 2 comparisons are presented between patients with high and low systolic BP. In the users of AHD,

the patients with high systolic BP had higher prevalence of high MSC ( $P = 0.039$ ). In the non-users of AHD, patients with high systolic BP had higher prevalence of physical inactivity ( $P = 0.009$ ), high MSC ( $P = 0.012$ ), depression ( $P = 0.022$ ), and abdominal obesity ( $P = 0.025$ ).

In Table 3 associations with high systolic BP are presented for all patients. Physical inactivity (adjusted odds ratio (AOR) 6.5), high MSC (AOR 3.9), abdominal obesity (AOR 3.7), AHD (AOR 2.9), age (per year) (AOR 1.07), and p-creatinine (per  $\mu\text{mol/L}$ ) (AOR 1.03) were associated with high systolic BP.

In Table 4 associations with high systolic BP are presented separately for users and non-users of AHD. In the users of AHD, high MSC (AOR 4.1) and age (per year) (AOR 1.11) were associated with high systolic BP. In the non-users of AHD, abdominal obesity (AOR 27.4), physical inactivity (AOR 14.7), male sex (AOR 9.0), smoking (AOR 7.9), and age (per year) (AOR 1.08), were associated with high systolic BP.

There were no associations between high MSC and high diastolic BP, neither for all patients ( $P = 0.63$ ), users of AHD ( $P > 0.99$ ), nor non-users of AHD ( $P = 0.63$ ).

**Table 1** Baseline characteristics, comparisons between users and non-users of AHD, and between patients with high and low MSC.

|   | All patients             | Users of antihypertensive drugs |                |                    | High midnight salivary cortisol ( $\geq 9.3$ nmol/L) |                |           |
|---|--------------------------|---------------------------------|----------------|--------------------|--|----------------|-----------|
|   |                          | Yes                             | No             | $P^a$              | Yes  | No             | $P^a$     |
| <i>n</i>  | 196                      | 60                              | 136            | –                  | 34   | 162            |           |
| Sex   |                          |                                 |                |                    |  |                |           |
| Men   | 106 (54)                 | 38 (63)                         | 68 (50)        | 0.090              | 17 (50)  | 89 (55)        | 0.71      |
| Women   | 90 (46)                  | 22 (37)                         | 68 (50)        |                    | 17 (50)  | 73 (45)        |           |
| Age (years)                                     | 43 (32, 51; 18–59)       | 49 (40, 55)                     | 40 (29, 49)    | $< 0.001^b$        | 48 (42, 53)  | 42 (29, 50)    | 0.002     |
| Diabetes duration (years)                       | 20 (11, 29; 1–55)        | 29 (20, 35)                     | 16 (9, 26)     | $< 0.001^b$        | 22 (15, 33)  | 19 (10, 29)    | 0.28      |
| MSC (nmol/L)                                    | 5.0 (3.1, 7.5; 1.9–47.0) | 5.4 (3.0, 8.4)                  | 4.9 (3.2, 7.0) | 0.36 <sup>b</sup>  | 12.0 (10.8, 14.2)                                    | 4.5 (2.9, 6.1) | $< 0.001$ |
| High MSC ( $\geq 9.3$ nmol/L)                   | 34 (17)                  | 12 (20)                         | 22 (16)        | 0.54               | –  | –              |           |
| Systolic BP (mmHg)                              | 120 (110, 130; 90–160)   | 130 (121, 139)                  | 120 (110, 125) | $< 0.001^b$        | 125 (120, 135)                                       | 120 (110, 130) | 0.030     |
| High systolic BP ( $> 130$ mmHg)                | 33 (17)                  | 19 (32)                         | 14 (10)        | 0.001              | 13 (38)  | 20 (12)        | 0.001     |
| Diastolic BP (mmHg)                             | 70 (70, 75; 55–100)      | 70 (70, 78)                     | 70 (65, 75)    | 0.058 <sup>b</sup> | 70 (70, 78)  | 70 (69, 75)    | 0.34      |
| High diastolic BP ( $> 80$ mmHg)                | 9 (5)                    | 5 (8)                           | 4 (3)          | 0.14               | 1 (3)  | 8 (5)          | $> 0.99$  |
| Abdominal obesity <sup>c</sup>                  | 29 (15)                  | 11 (19)                         | 18 (14)        | 0.39               | 5 (15)   | 24 (15)        | $> 0.99$  |
| Hypoglycaemia (severe episodes)                 | 9 (5)                    | 1 (2)                           | 8 (6)          | 0.28               | 1 (3)  | 8 (5)          | $> 0.99$  |
| HbA1c   |                          |                                 |                |                    |  |                |           |
| mmol/mol  | 63 (54, 71; 25–110)      | 64 (53, 74)                     | 61 (54, 69)    | 0.18               | 62 (54, 71)  | 63 (53, 71)    | 0.88      |
| %   | 7.9 (7.1, 8.6; 4.4–12.2) | 8.0 (7.0, 8.9)                  | 7.8 (7.1, 8.4) |                    | 7.8 (7.1, 8.6)                                       | 7.9 (7.0, 8.6) |           |
| P-creatinine <sup>d</sup> ( $\mu\text{mol/L}$ ) | 70 (62, 77; 28–182)      | 70 (62, 80)                     | 68 (61, 76)    | 0.16 <sup>b</sup>  | 66 (58, 71)  | 70 (62, 78)    | 0.027     |
| Smoking <sup>e</sup>                            | 16 (9)                   | 6 (10)                          | 10 (8)         | 0.59               | 8 (24)   | 8 (5)          | 0.002     |
| Physical inactivity <sup>f</sup>                | 19 (10)                  | 5 (8)                           | 14 (11)        | 0.80               | 7 (21)   | 12 (8)         | 0.054     |
| Depression                                      | 20 (10)                  | 8 (13)                          | 12 (9)         | 0.44               | 9 (26)   | 11 (7)         | 0.002     |
| AHD   | 60 (31)                  | –                               | –              | –                  | 12 (35)  | 48 (30)        | 0.54      |
| Antidepressants                                 | 13 (7)                   | 6 (10)                          | 7 (5)          | 0.22               | 4 (12)   | 9 (6)          | 0.25      |
| Continuous s.c. insulin infusion                | 20 (10)                  | 5 (8)                           | 15 (11)        | 0.80               | 3 (9)  | 17 (10)        | $> 0.99$  |

Results are presented as median ( $q_1$ ,  $q_3$ ; min-max) or  $n$  (%).

<sup>a</sup>Fisher's exact test unless otherwise indicated. <sup>b</sup>Mann-Whitney  $U$  test. Missing values for all/users of AHD/non-users of AHD: <sup>c</sup>Abdominal obesity  $n = 6/1/5$ ; <sup>d</sup>creatinine  $n = 7/1/6$ ; <sup>e</sup>smoking  $n = 10/1/9$ ; <sup>f</sup>physical inactivity  $n = 12/1/11$ .

**Table 2** Comparisons between patients with high and low systolic BP for all, users and non-users of antihypertensive drugs.

|                                 | High systolic BP (>130 mmHg) |                |                       |                |                |                                 |                |                |                       |     |                                     |                       |  |  |  |
|---------------------------------|------------------------------|----------------|-----------------------|----------------|----------------|---------------------------------|----------------|----------------|-----------------------|-----|-------------------------------------|-----------------------|--|--|--|
|                                 | All patients                 |                |                       |                |                | Users of antihypertensive drugs |                |                |                       |     | Non-users of antihypertensive drugs |                       |  |  |  |
|                                 | Yes                          | No             | <i>P</i> <sup>a</sup> | Yes            | No             | <i>P</i> <sup>a</sup>           | Yes            | No             | <i>P</i> <sup>a</sup> | Yes | No                                  | <i>P</i> <sup>a</sup> |  |  |  |
| <i>n</i>                        | 33                           | 163            |                       | 19             | 41             |                                 | 14             | 122            |                       |     |                                     |                       |  |  |  |
| Sex                             |                              |                |                       |                |                |                                 |                |                |                       |     |                                     |                       |  |  |  |
| Men                             | 22 (67)                      | 84 (52)        | 0.13                  | 13 (68)        | 25 (61)        | 0.77                            | 9 (64)         | 59 (48)        | 0.40                  |     |                                     |                       |  |  |  |
| Women                           | 11 (33)                      | 79 (48)        |                       | 6 (34)         | 16 (39)        |                                 | 5 (36)         | 63 (52)        |                       |     |                                     |                       |  |  |  |
| Age (years)                     | 51 (44, 56)                  | 42 (29, 50)    | <0.001 <sup>b</sup>   | 54 (51, 58)    | 45 (34, 52)    | 0.003 <sup>b</sup>              | 45 (40, 49)    | 38 (28, 49)    | 0.12 <sup>b</sup>     |     |                                     |                       |  |  |  |
| Diabetes duration (years)       | 25 (14, 35)                  | 19 (10, 29)    | 0.082 <sup>b</sup>    | 29 (18, 44)    | 28 (21, 35)    | 0.99 <sup>b</sup>               | 22 (8, 29)     | 16 (9, 26)     | 0.39 <sup>b</sup>     |     |                                     |                       |  |  |  |
| High MSC (≥9.3 nmol/L)          | 13 (39)                      | 21 (13)        | 0.001                 | 7 (37)         | 5 (12)         | 0.039                           | 6 (43)         | 16 (13)        | 0.012                 |     |                                     |                       |  |  |  |
| Systolic BP (mmHg)              | 140 (135, 140)               | 120 (110, 125) | <0.001 <sup>b</sup>   | 140 (140, 145) | 125 (120, 130) | <0.001 <sup>b</sup>             | 135 (135, 140) | 120 (110, 125) | <0.001 <sup>b</sup>   |     |                                     |                       |  |  |  |
| High diastolic BP (>80 mmHg)    | 5 (15)                       | 4 (2)          | 0.008                 | 3 (16)         | 2 (5)          | 0.31                            | 2 (14)         | 2 (2)          | 0.053                 |     |                                     |                       |  |  |  |
| Abdominal obesity               | 9 (27)                       | 20 (13)        | 0.058                 | 4 (21)         | 7 (18)         | 0.73                            | 5 (36)         | 13 (11)        | 0.025                 |     |                                     |                       |  |  |  |
| Hypoglycaemia (severe episodes) | 2 (6)                        | 7 (4)          | 0.65                  | 1 (5)          | 0              | 0.32                            | 1 (7)          | 7 (6)          | 0.59                  |     |                                     |                       |  |  |  |
| HbA1c                           |                              |                |                       |                |                |                                 |                |                |                       |     |                                     |                       |  |  |  |
| mmol/mol                        | 60 (55, 71)                  | 63 (53, 70)    | 0.54 <sup>b</sup>     | 64 (53, 74)    | 65 (54, 73)    | 0.94 <sup>b</sup>               | 60 (55, 71)    | 61 (53, 69)    | 0.69 <sup>b</sup>     |     |                                     |                       |  |  |  |
| %                               | 7.7 (7.2, 8.7)               | 7.9 (7.0, 8.5) |                       | 8.0 (7.0, 8.9) | 8.1 (7.1, 8.8) |                                 | 7.7 (7.2, 8.6) | 7.8 (7.0, 8.4) |                       |     |                                     |                       |  |  |  |
| P-creatinine (µmol/L)           | 70 (57, 86)                  | 69 (62, 76)    | 0.62 <sup>b</sup>     | 71 (60, 88)    | 70 (63, 78)    | 0.86 <sup>b</sup>               | 70 (50, 83)    | 68 (61, 76)    | 0.67 <sup>b</sup>     |     |                                     |                       |  |  |  |
| Smoking                         | 5 (15)                       | 11 (7)         | 0.17                  | 4 (10)         | 2 (10)         | >0.99                           | 3 (21)         | 7 (6)          | 0.081                 |     |                                     |                       |  |  |  |
| Physical inactivity             | 7 (21)                       | 12 (8)         | 0.051                 | 2 (10)         | 3 (8)          | 0.65                            | 5 (36)         | 9 (8)          | 0.009                 |     |                                     |                       |  |  |  |
| Depression                      | 8 (24)                       | 12 (7)         | 0.008                 | 4 (21)         | 4 (9)          | 0.25                            | 4 (29)         | 8 (7)          | 0.022                 |     |                                     |                       |  |  |  |
| AHD                             | 19 (58)                      | 41 (25)        | 0.001                 | –              | –              | –                               | –              | –              | –                     |     |                                     |                       |  |  |  |
| Antidepressants                 | 2 (6)                        | 11 (7)         | >0.99                 | 0              | 6 (15)         | 0.16                            | 2 (14)         | 5 (4)          | 0.15                  |     |                                     |                       |  |  |  |

Results are presented as median (*q*<sub>1</sub>, *q*<sub>3</sub>) or *n* (%).

<sup>a</sup>Fisher's exact test unless otherwise indicated. <sup>b</sup>Mann-Whitney *U* test. For missing values, see Table 1.



**Table 3** Associations with high systolic BP in all patients.

|                                       | High systolic BP (>130 mmHg) |        |                  |                |
|---------------------------------------|------------------------------|--------|------------------|----------------|
|                                       | COR (95% CI)                 | P      | AOR (95% CI)     | P <sup>a</sup> |
| Sex (men)                             | 1.9 (0.9–4.1)                | 0.12   | 1.5 (0.5–4.2)    | 0.42           |
| Age (per year)                        | 1.08 (1.04–1.12)             | 0.001  | 1.07 (1.02–1.13) | 0.012          |
| Diabetes duration (per year)          | 1.03 (1.00–1.06)             | 0.052  | 0.99 (0.95–1.03) | 0.57           |
| High MSC ( $\geq 9.3$ nmol/L)         | 4.4 (1.9–10.1)               | 0.001  | 3.9 (1.4–10.6)   | 0.007          |
| Abdominal obesity                     | 2.6 (1.0–6.3)                | 0.040  | 3.7 (1.2–11.8)   | 0.026          |
| Hypoglycaemia (severe episodes)       | 1.4 (0.3–7.2)                | 0.67   | –                | –              |
| HbA1c (mmol/mol)                      | 1.02 (0.99–1.05)             | 0.20   | 1.02 (0.99–1.05) | 0.20           |
| P-creatinine (per $\mu\text{mol/L}$ ) | 1.02 (1.00–1.04)             | 0.039  | 1.03 (1.00–1.05) | 0.043          |
| Smoking                               | 2.3 (0.7–7.2)                | 0.15   | –                | –              |
| Physical inactivity                   | 3.1 (1.1–8.7)                | 0.029  | 6.5 (1.5–28.6)   | 0.013          |
| Depression                            | 4.0 (1.5–10.8)               | 0.006  | 1.9 (0.6–6.6)    | 0.29           |
| AHD                                   | 4.0 (1.9–8.8)                | <0.001 | 2.9 (1.1–7.3)    | 0.027          |
| Antidepressants                       | 0.9 (0.2–4.2)                | 0.88   | –                | –              |

<sup>a</sup>Multiple logistic regression analyses (Backward: Wald): Variables with *P* values  $\leq 0.10$  for the CORs, sex and age are included in the analyses; *n* = <sup>a</sup>180; Nagelkerke *R*<sup>2</sup>: <sup>a</sup>0.341; Hosmer and Lemeshow Test: <sup>a</sup>0.142.

## Discussion

The principal finding in this study of 196 adult patients with T1D was that patients with high systolic BP (>130 mmHg) compared to patients with low systolic BP, had higher prevalence of high MSC ( $\geq 9.3$  nmol/L). This was the case for both users and non-users of AHD. In all patients, physical inactivity, high MSC, abdominal obesity, AHD, p-creatinine, and age, were independently associated with high systolic BP. In the users of AHD, high MSC and age were associated with high systolic BP. In the non-users of AHD, abdominal obesity, physical inactivity, male sex, smoking, and age, were associated with high systolic BP. In the non-users of AHD, high MSC was not independently associated with systolic BP. No association between high diastolic BP ( $\geq 80$  mmHg) and high MSC was found in any group.

The first strength of this study was that the population of patients with T1D was well defined. Patients with severe somatic or psychiatric comorbidities and/or substance abuse were excluded. Of particular importance is that no patients with diagnosed Cushing's syndrome/disease (4, 5, 7), ESRD (4, 6) or severe substance abuse were included (25, 26). All patients using systemic corticosteroids, and two patients using topical steroids with extreme MSC values were excluded as contamination was suspected (22). We have previously controlled that the MSC levels did not differ between users and non-users of inhaled steroids, and we have performed non-response analyses (22). The non-response analyses showed no differences regarding age, diabetes duration, sex, metabolic variables, smoking, physical inactivity, or depression, between those who delivered and those who did not deliver

MSC samples (22). Second, salivary cortisol measurement has advantages compared to blood measurements as it is non-invasive. Blood sampling can be stressful leading to increased cortisol secretion. Beneficial is also that participants can collect samples in their normal environment (31). Third, the cut-off level we chose to indicate high MSC has clinical implications. In previous research this cut-off level for high MSC was highly predictive of Cushing's disease in patients with clinical features of hypercortisolism (33). Fourth, we presented our results for all patients, and separately for users and non-users of AHD. Fifth, we have adjusted for relevant variables such as age, sex, glycaemic control, abdominal obesity, severe hypoglycaemia episodes, depression, smoking, physical inactivity, and kidney function, which all have been associated with either hypertension or increased cortisol secretion, or both (4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 21, 22, 23, 24, 27, 28).

The main limitation was that only one MSC sample was collected from each patient. Due to the inconvenience of midnight sampling, we anticipated a lower participation rate if we had demanded repeated samplings. A second limitation was that we did not perform any dexamethasone suppression tests for the participants with high MSC values. A third limitation was that we did not have any matched controls without T1D.

There is clear evidence from previous research that increased cortisol secretion contributes to the development of hypertension (4, 5, 6, 7), which in turn has impact on the development of atherosclerosis, CV disease and mortality (3, 7, 15, 16, 17). We found a clear independent association between high MSC and high systolic BP in all patients which supports previous research (4, 5, 6, 7).

**Table 4** Associations with high systolic BP for users and non-users of AHD.

|                                 | High systolic BP (>130 mmHg)    |       |                  |                |                  |       |                                     |                |                  |       |                  |                |
|---------------------------------|---------------------------------|-------|------------------|----------------|------------------|-------|-------------------------------------|----------------|------------------|-------|------------------|----------------|
|                                 | Users of antihypertensive drugs |       |                  |                |                  |       | Non-users of antihypertensive drugs |                |                  |       |                  |                |
|                                 | COR (95% CI)                    | P     | AOR (95% CI)     | P <sup>a</sup> | COR (95% CI)     | P     | AOR (95% CI)                        | P <sup>b</sup> | COR (95% CI)     | P     | AOR (95% CI)     | P <sup>b</sup> |
| Sex (men)                       | 1.4 (0.4-4.4)                   | 0.58  | 0.8 (0.2-3.2)    | 0.78           | 1.9 (0.6-6.1)    | 0.26  | 9.0 (1.3-63.3)                      | 0.028          | 1.0 (0.4-4.4)    | 0.97  | 1.08 (1.01-1.16) | 0.027          |
| Age (per year)                  | 1.10 (1.02-1.19)                | 0.010 | 1.01 (1.02-1.21) | 0.014          | 1.04 (0.99-1.10) | 0.11  | 1.04 (1.01-1.16)                    | 0.027          | 1.04 (0.96-1.05) | 0.38  | -                | -              |
| Diabetes duration (per year)    | 1.00 (0.96-1.05)                | 0.97  | -                | -              | 1.02 (0.97-1.07) | 0.38  | -                                   | -              | 4.2 (1.1-15.7)   | 0.033 | 2.7 (0.6-12.9)   | 0.21           |
| High MSC (≥9.3 nmol/L)          | 4.2 (1.1-15.7)                  | 0.033 | 4.1 (1.0-16.6)   | 0.048          | 5.0 (1.5-16.2)   | 0.008 | 2.7 (0.6-12.9)                      | 0.21           | 4.4 (1.3-15.3)   | 0.018 | 27.4 (3.3-226)   | 0.002          |
| Abdominal obesity               | 1.3 (0.3-5.0)                   | 0.74  | -                | -              | 4.4 (1.3-15.3)   | 0.018 | 27.4 (3.3-226)                      | 0.002          | 1.3 (0.1-11.0)   | 0.84  | -                | -              |
| Hypoglycaemia (severe episodes) | -                               | >0.99 | -                | -              | 1.3 (0.1-11.0)   | 0.84  | -                                   | -              | -                | -     | -                | -              |
| HbA1c (mmol/mol)                | 1.01 (0.96-1.05)                | 0.81  | -                | -              | 1.02 (0.98-1.07) | 0.27  | -                                   | -              | -                | -     | -                | -              |
| P-creatinine (per µmol/L)       | 1.01 (0.99-1.04)                | 0.19  | -                | -              | 1.00 (0.95-1.05) | 0.96  | -                                   | -              | -                | -     | -                | -              |
| Smoking                         | 1.1 (0.2-6.4)                   | 0.95  | -                | -              | 4.1 (0.9-18.3)   | 0.062 | 7.9 (1.2-53.2)                      | 0.033          | 1.5 (0.2-9.5)    | 0.005 | 14.7 (2.7-81.7)  | 0.002          |
| Physical inactivity             | 1.5 (0.2-9.5)                   | 0.70  | -                | -              | 9.3 (1.7-22.8)   | 0.005 | 14.7 (2.7-81.7)                     | 0.002          | 2.5 (0.5-11.2)   | 0.24  | 3.2 (0.5-20.1)   | 0.21           |
| Depression                      | 2.5 (0.5-11.2)                  | 0.24  | -                | -              | 5.7 (1.5-22.3)   | 0.012 | 3.2 (0.5-20.1)                      | 0.21           | -                | -     | -                | -              |
| Antidepressants                 | -                               | >0.99 | -                | -              | 3.9 (0.7c22.3)   | 0.13  | -                                   | -              | -                | -     | -                | -              |

<sup>a,b</sup>Multiple logistic regression analyses (Backward: Wald); Variables with P values ≤0.10 for the CORs, sex and age are included in the analyses; n = 60/<sup>a</sup>123; Nagelkerke R<sup>2</sup>: 0.277/<sup>b</sup>0.381; Hosmer and Lemeshow Test: 0.154/<sup>b</sup>0.136.

In the users of AHD, the association between high MSC and high systolic BP was direct without any mediators. However, the number of patients using AHD was low, and the CIs for the CORs for the potential mediators (smoking, physical inactivity and depression) were wide, which indicate a profound statistical uncertainty of these estimates.

Despite the even higher prevalence of high MSC secretion in the non-users of AHD with high systolic BP, the association between MSC and high systolic BP was not sustained after adjustments for age, depression, physical inactivity and smoking. We have previously shown that these four variables were associated with high MSC in these patients (22). In the non-users of AHD, we found three targets for intervention in order to reduce high systolic BP – abdominal obesity, physical inactivity and smoking – which is in accordance with previous research (8, 10, 11). We did not find any association between depression and hypertension, which differs from previous research (12). As the CI for the AORs between depression and high systolic blood pressure was very wide, indicating a statistical uncertainty, we suggest exploration in a larger population sample. The association between male sex and high systolic BP in the sub group of non-AHD users, is in accordance with previous research (13).

In this study we clearly showed that patients with T1D with high systolic BP had a high prevalence of high MSC, both in users and non-users of AHD. Increased cortisol secretion is one important cause of treatment-resistant hypertension according to previous research (14). In clinical practice we suggest that MSC should be measured in T1D patients with high systolic BP. We also suggest in case of high MSC, that a dexamethasone test should be performed to explore whether the patients have impaired glucocorticoid-negative feedback (14, 18, 21). The cut-off value we chose (MSC ≥9.3 nmol/L) showed previously a high predictive value to distinguish Cushing's disease from pseudo-Cushing's syndrome (33). Several types of disturbances of the HPA axis functioning in T1D have been demonstrated (18, 19, 20, 21), but other factors might contribute to increased MSC secretion, which should be explored. We have previously shown that depression, physical inactivity and smoking were associated with high MSC in these patients with T1D (22). We suggest further research on causes and consequences of HPA axis dysfunction in patients with T1D. We are planning to explore the prevalence of high MSC in a normative Swedish population sample for comparison in further research.

In conclusion, physical inactivity, high MSC, abdominal obesity, AHD, p-creatinine, and age, were

independently associated with high systolic BP in adult patients with T1D. Our hypothesis that high MSC secretion might contribute to hypertension and to treatment failure of AHD was supported.

#### 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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