Sexual Dimorphism and Sex Steroids Influence Cardiovascular Autonomic Neuropathy in Patients With Type 1 Diabetes

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The male predominance of cardiovascular disease is overridden by the presence of diabetes, as the sex-related burden of cardiovascular disease increases in postmenopausal women with diabetes (1). Sexual dimorphism in cardiovascular autonomic neuropathy (CAN) might contribute to these findings (1).

We conducted a cross-sectional study hypothesizing that *1*) the prevalence of CAN is different among women and men with type 1 diabetes, *2*) such a difference is dependent on the pre- or postmenopausal status of women, and *3*) sex steroids show opposite associations with CAN depending on sex.

In this study, we recruited 279 consecutive patients with type 1 diabetes attending our clinic (ClinicalTrials.gov identifier NCT02910271) (2,3). Inclusion and exclusion criteria are detailed elsewhere (2,3). Assuming a global prevalence of CAN of ~30% in our population of patients with type 1 diabetes (2) and setting α at 0.05 and β at 0.20 for a two-sided test, the inclusion of at least 160 men and 120 women was enough to identify a difference in prevalence among them above 15% (www.imim.cat/ofertadeserveis/software-public/granmo/).

The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the local ethics committee of Hospital Universitario Ramón y Cajal (approval date 22 January 2016, protocol ID 464/15). Informed consent was obtained from all participants included in the study. Patients did not receive any economic compensation for their participation.

For all study subjects, we reviewed medical history and clinical parameters of the patients at recruitment. Then, participants were submitted to a physical examination and biochemical assessments. Serum for sex steroid measurements was frozen at −80°C until thawed for analysis. Technical characteristics of the assays used for biochemical and hormone measurements have been described in detail elsewhere (2,4). We performed hormone assays at the same time using kits from the same production lots. Cardiovascular autonomic function was assessed using Ewing tests (Monitor OneDx System device; Qmed, Inc., Eatontown, NJ) and by the changes in blood pressure and heart rate after active standing (3).

Data are shown as means ± SD or counts (percentage) with 95% CI (lower limit; upper limit). Patients were classified by age using a cutoff of 45 years. Analysis of discrete variables used logistic binary regressions, and continuous variables were submitted to two-way ANOVA tests. We assessed the association between sex steroids and Ewing tests by Spearman correlations. Statistical significance was set at a P value <0.05.

The features of participants are detailed in Table 1. In women, CAN was early/mild in 39 cases (32%) and definitive in 4 (3%). In men, CAN was early/mild in 40 cases (25%) and definitive in 2 (1%). On the whole, the prevalence of CAN was not significantly different between men and women (Table 1). However, it was much more frequent in women >45 years of age than in younger women or in men regardless of age. The odds ratio of CAN was 4.5 (95% CI 1.4; 14.1) in older women compared with older men, 5.4 (95% CI 2.0; 14.7) compared with younger women, and 6.4 (95% CI 2.4; 17.2) compared with younger men.

Men with CAN showed lower total testosterone (T) and T/estradiol (E_2) ratios than those without CAN. T and T/E_2 ratios were directly correlated with Ewing scores. Women with CAN had higher T/E_2 ratios than those without CAN. A

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Table 1—Demographic characteristics, clinical features, and sex steroid profile of the whole group of study participants and as a function of sex and age

		Women			Men		
Variable	All patients (n = 279)	All (n = 121)	≤45 years (n = 99)	>45 years (n = 22)	All (n = 158)	≤45 years (n = 124)	>45 years (n = 34)
Age (years)[b]	36 ± 12 [35; 37]	36 ± 13 [34; 38]	32 ± 9 [30; 34]	56 ± 7 [53; 59]	36 ± 11 [34; 38]	32 ± 8 [31; 33]	51 ± 5 [49; 53]
DKA at diagnosis	121 (43) [38; 49]	52 (43) [35; 52]	42 (42) [33; 52]	10 (46) [27; 65]	69 (44) [36; 52]	56 (45) [27; 54]	13 (38) [24; 55]
CSII[a]	55 (20) [16; 25]	31 (26) [19; 34]	25 (25) [18; 35]	6 (27) [13; 48]	24 (15) [10; 22]	17 (14) [9; 21]	7 (21) [10; 37]
Duration of diabetes (years)[bc]	20 ± 11 [19; 21]	20 ± 11 [19; 21]	16 ± 8 [14; 18]	35 ± 8 [31; 39]	20 ± 11 [18; 22]	17 ± 10 [15; 19]	29 ± 6 [27; 31]
Never smokers	167 (60) [54; 65]	72 (41) [51; 68]	64 (65) [55; 73]	8 (36) [20; 57]	95 (60) [52; 67]	75 (61) [52; 69]	20 (59) [42; 74]
Antiaggregant therapy[bc]	21 (8) [5; 11]	10 (8) [5; 15]	2 (2) [1; 7]	8 (36) [20; 57]	11 (7) [4; 12]	2 (2) [0; 6]	9 (27) [15; 43]
Statin therapy[abc]	78 (28) [23; 34]	26 (22) [16; 31]	11 (11) [6; 19]	15 (68) [47; 84]	52 (33) [26; 41]	28 (23) [16; 31]	24 (71) [54; 83]
Antihypertensive therapy[bc]	42 (15) [11; 20]	16 (14) [9; 21]	4 (4) [2; 10]	12 (55) [35; 73]	26 (17) [12; 23]	13 (11) [6; 17]	13 (38) [24; 55]
Oral contraception treatment	11 (4) [2; 7]	11 (10) [6; 17]	11 (11) [6; 19]	— —	— —	— —	— —
Microangiopathy[b]	71 (25) [21; 31]	36 (30) [22; 38]	20 (20) [14; 29]	16 (73) [52; 87]	35 (22) [16; 29]	23 (19) [13; 26]	12 (35) [22; 52]
Coronary heart disease[b]	6 (2) [1; 5]	4 (3) [1; 8]	1 (1) [1; 6]	3 (14) [5; 33]	2 (1) [0; 5]	1 (1) [0; 4]	1 (3) [1; 2]
Peripheral artery disease	35 (15) [9; 17]	13 (11) [6; 18]	6 (6) [3; 13]	7 (32) [16; 53]	22 (14) [9; 20]	17 (14) [9; 21]	6 (18) [8; 34]
BMI (kg/m^2)[b]	25 ± 4 [25; 26]	24 ± 4 [23; 25]	24 ± 4 [23; 25]	26 ± 3 [25; 27]	25 ± 4 [24; 26]	25 ± 4 [24; 26]	26 ± 3 [25; 27]
Obesity	30 (11) [8; 15]	11 (9) [5; 16]	7 (7) [4; 14]	4 (18) [7; 39]	19 (12) [8; 18]	15 (12) [8; 19]	4 (12) [5; 27]
Waist circumference (cm)[abc]	84 ± 12 [83; 85]	78 ± 11 [76; 80]	76 ± 10 [74; 78]	85 ± 12 [81; 90]	89 ± 11 [87; 91]	88 ± 11 [86; 90]	93 ± 9 [89; 96]
Office systolic BP (mmHg)[abc]	120 ± 16 [118; 122]	114 ± 16 [111; 117]	110 ± 12 [108; 112]	133 ± 21 [124; 142]	124 ± 14 [122; 126]	122 ± 13 [120; 124]	130 ± 16 [124; 136]
Office diastolic BP (mmHg)[a]	72 ± 10 [71; 73]	69 ± 9 [67; 71]	69 ± 8 [67; 71]	70 ± 13 [64; 76]	73 ± 11 [71; 75]	72 ± 11 [70; 74]	77 ± 8 [74; 79]
Heart rate (bpm)[c]	74 ± 12 [73; 75]	77 ± 12 [75; 79]	78 ± 12 [76; 80]	72 ± 11 [67; 77]	72 ± 12 [70; 74]	71 ± 12 [69; 73]	76 ± 11 [72; 80]
eGFR (mL/min/1.73 m^2)[ab]	95 ± 18 [93; 97]	89 ± 18 [86; 92]	91 ± 18 [87; 95]	79 ± 13 [73; 85]	97 ± 17 [94; 100]	99 ± 17 [96; 102]	88 ± 17 [82; 94]
HbA_{1c} (%)	7.6 ± 1.3 [7.5; 7.8]	7.7 ± 1.4 [7.5; 8.0]	7.6 ± 1.4 [7.3; 7.9]	7.8 ± 1.0 [7.4; 8.2]	7.5 ± 1.3 [7.3; 7.7]	7.5 ± 1.2 [7.3; 7.7]	7.3 ± 1.4 [7.1; 8.1]
HbA_{1c} (mmol/mol)	60 ± 14 [58; 62]	61 ± 15 [58; 64]	60 ± 15 [57; 63]	62 ± 11 [57; 67]	58 ± 15 [56; 60]	58 ± 14 [56; 61]	56 ± 16 [50; 62]
Total cholesterol (mmol/L)	4.4 ± 0.7 [4.3; 4.5]	4.6 ± 0.7 [4.5; 4.7]	4.6 ± 0.7 [4.5; 4.7]	4.5 ± 0.8 [4.1; 4.9]	4.3 ± 0.7 [4.2; 4.4]	4.3 ± 0.7 [4.2; 4.4]	4.5 ± 0.6 [4.3; 4.7]
HDL cholesterol (mmol/L)[a]	1.5 ± 0.4 [1.5; 1.6]	1.6 ± 0.3 [1.6; 1.7]	1.6 ± 0.3 [1.5; 1.7]	1.6 ± 0.3 [1.5; 1.7]	1.4 ± 0.3 [1.4; 1.5]	1.3 ± 0.3 [1.3; 1.4]	1.4 ± 0.4 [1.3; 1.5]
LDL cholesterol (mmol/L)	2.6 ± 0.6 [2.5; 2.7]	2.6 ± 0.5 [2.5; 2.7]	2.6 ± 0.5 [2.5; 2.7]	2.5 ± 0.6 [2.4; 2.7]	2.6 ± 0.6 [2.5; 2.7]	2.5 ± 0.7 [2.4; 2.6]	2.7 ± 0.5 [2.5; 2.9]
Triglycerides (mmol/L)	0.9 ± 0.4 [0.9; 1.0]	0.8 ± 0.4 [0.7; 0.9]	0.8 ± 0.4 [0.7; 0.9]	0.8 ± 0.2 [0.7; 0.9]	0.9 ± 0.4 [0.8; 1.0]	0.9 ± 0.4 [0.8; 1.0]	0.9 ± 0.3 [0.8; 1.0]
FSH (IU/L)[abc*]	9 ± 19 [7; 11]	17 ± 27 [12; 22]	6 ± 7 [4.4; 7.4]	59 ± 36 [43; 75]	4 ± 2 [4; 4]	3 ± 2 [3; 3]	4 ± 2 [3; 5]
LH (IU/L)[abc*]	7 ± 8 [6; 8]	11 ± 11 [9; 13]	7 ± 8 [6; 9]	25 ± 10 [21; 29]	4 ± 2 [4; 4]	4 ± 2 [4; 4]	4 ± 2 [3; 5]

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Table 1—Continued

Variable	All patients (n = 279)	Women			Men		
		All (n = 121)	≤45 years (n = 99)	>45 years (n = 22)	All (n = 158)	≤45 years (n = 124)	>45 years (n = 34)
Total T (nmol/L)[abc*]	14.0 ± 11.7 [12.6; 15.4]	1.6 ± 0.9 [1.4; 1.8]	1.6 ± 0.8 [1.4; 1.8]	1.2 ± 0.7 [0.9; 1.5]	22.6 ± 7.0 [21.5; 23.7]	23.3 ± 7.2 [22.0; 24.6]	20.0 ± 5.4 [18.0; 21.0]
Total E_2 (pmol/L)[abc*]	231 ± 294 [196; 266]	416 ± 392 [345; 487]	461 ± 373 [382; 540]	234 ± 410 [52; 416]	103 ± 40 [97; 109]	28 ± 11 [26; 30]	27 ± 9 [24; 30]
Total T/E_2 molar ratio[abc*]	146 ± 138 [129; 163]	10 ± 13 [8; 12]	7 ± 8 [5; 8]	22 ± 21 [13; 31]	242 ± 99 [226; 258]	247 ± 104 [229; 266]	224 ± 80 [196; 252]
SHBG (nmol/L)[abc*]	69 ± 41 [64; 74]	94 ± 50 [85; 103]	91 ± 51 [80; 102]	107 ± 42 [88; 126]	51 ± 21 [48; 54]	49 ± 20 [45; 53]	60 ± 22 [52; 68]
Calculated free T (pmol/L)[abc*]	233 ± 205 [208; 258]	16 ± 14 [14; 19]	17 ± 15 [14; 20]	11 ± 7 [8; 14]	384 ± 124 [365; 404]	409 ± 124 [387; 431]	292 ± 71 [267; 317]
Calculated free E_2 (pmol/L)[bc*]	3.7 ± 4.1 [3.2; 4.2]	5.8 ± 5.7 [4.8; 6.8]	6.5 ± 5.3 [5.4; 7.6]	3.1 ± 6.1 [0.4; 5.8]	2.2 ± 1.0 [2.0; 2.4]	0.6 ± 0.3 [0.6; 0.7]	1.9 ± 0.7 [1.7; 2.2]
Calculated free T/E_2 molar ratio[abc*]	116 ± 110 [103; 129]	7 ± 8 [6; 8]	5 ± 5 [4; 6]	14 ± 13 [8; 20]	193 ± 79 [181; 205]	200 ± 83 [185; 215]	169 ± 58 [135; 183]
Prevalence of CAN[bc]	85 (30.5) [25.4; 36.1]	43 (35.5) [27.6; 44.4]	28 (28.3) [20.4; 37.8]	15 (68.2) [47.3; 83.6]	42 (26.6) [20.3; 34.0]	31 (25.0) [18.2; 33.3]	11 (32.4) [19.1; 49.2]

Continuous and discrete variables are shown as mean ± SD and *N* (%), respectively. Numbers below those statistics denote 95% CI [lower limit; upper limit]. BP, blood pressure; bpm, beats per minute; CSII, continuous subcutaneous insulin infusion; DKA, diabetes ketoacidosis; eGFR, estimated glomerular filtration rate; FSH, follicle-stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone binding globulin. Comparisons among groups were performed by two-way ANOVA or binary logistic regression analyses. [a]Significant differences between men and women. [b]Significant differences among older and younger patients independently of sex. [c]Statistically significant interaction between sex and age-group. *11 women taking hormonal contraceptives were excluded.

significant correlation was observed between free T and orthostatism test values.

According to our findings, women with type 1 diabetes >45 years of age show a marked increase in the risk of suffering from CAN with respect to other subgroups of patients. In agreement with our previous hypothesis (5), we also observed that circulating T and its relation to E_2 may have opposite associations with CAN in men and women. The higher concentrations of T in our women with CAN suggest that androgen excess might be associated with autonomic dysfunction, although the T/E_2 ratio increase largely relied on the profound decrease of E_2 found in older women. Conversely, T was positively associated with heart rate variability in men, and those men with CAN had lower T levels.

Nonetheless, our older women also had more cardiovascular risk factors than the other study subgroups. Whether this fact was related only to the estrogen decline of menopause is unclear (1). We have to bear in mind that these women also had a longer duration of diabetes, which is one of the most important predictors for cardiovascular outcomes. However, older men also had a longer duration of diabetes but did not show an increased prevalence of CAN.

If confirmed in other populations, the very large prevalence of CAN among postmenopausal women with type 1 diabetes in our study, despite the obvious limitations of its relatively small sample size and observational design, would make CAN screening mandatory for the accurate stratification of their cardiovascular risk. The consequences of the management of male hypogonadism and female hyperandrogenism in patients with type 1 diabetes merit further research.

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Author Contributions. L.N.-C. recruited subjects, researched and analyzed data, and wrote the manuscript. H.F.E.-M. and M.L.-R. designed the study, researched and analyzed data, contributed to the discussion, and reviewed and edited the final version. S.A.D. recruited patients, researched data, and contributed to the discussion. L.J.-M. and A.G.-C. processed and assayed samples and contributed to the discussion. E.F.-D. and B.D.A. recruited patients, processed samples, and contributed to the discussion. All the authors approved the final version of the manuscript. L.N.-C. and M.L.-R. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
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