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An updated normative data-set from the Autonomic Reflex Screen representative of Southwestern Ontario

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ABSTRACT

BACKGROUND: In evaluating autonomic dysfunction, the autonomic reflex screen (ARS) is an established set of standardized tests to evaluate the presence and severity of autonomic dysfunction. Our laboratory previously reported normative data on 121 healthy individuals; however, the sample size in older individuals was reduced compared to other age groups. Therefore, the objective of the current study was to provide updated normative values representative of young, middle aged and older individuals from Southwestern Ontario.

METHODS: 252 healthy individuals completed quantitative sudomotor axon reflex testing, heart rate responses to deep breathing (HRDB), and Valsalva maneuver using standard protocols of the ARS.

RESULTS: All four sweat sites demonstrated a significant effect of sex ($p < 0.001$). In addition, the proximal leg, distal leg and foot were all significantly affected by age ($p < 0.001$). Cardiovagal parameters, measured via HRDB and Valsalva ratio revealed a significant regression with age ($p < 0.001$). These results show similar trends with previously reported normative datasets. All normative data as a function of age and sex, where appropriate, are expressed as percentiles (2.5th, 5th, 95th, 97.5th).

CONCLUSION: The current study provides updated normative data describing autonomic functioning in healthy individuals obtained from the sudomotor and cardiovagal components of the Autonomic Reflex Screen.

KEY WORDS: autonomic reflex screen; normative data; aging; QSWEAT; cardiovagal functioning; autonomic nervous system

INTRODUCTION

In evaluating autonomic dysfunction, the autonomic reflex screen (ARS) has been established as a set of four standardized tests to evaluate the presence and severity of autonomic dysfunction (Low 2003). The ARS provides an assessment of sudomotor, cardiovascular and adrenergic functioning. The ARS is also an important clinical tool in the evaluation and diagnosis of autonomic disorders. While a considerable amount of data has been collected to provide normative data from healthy controls subjects for each test of the ARS, the sample from which the normative data was collected comes from a single laboratory in the United States. In addition, the normative sudomotor data was collected using different methodology with equipment that is not commercially available in Canada (Mayo-built sudorometer). In the present study, normative data for sweat responses were obtained using a gel preparation of Acetylcholine (ACh) and QSWEAT device (WR Medical Electronics Co., Stillwater, MN). In contrast, the normative data previously reported used a solution-based vehicle to deliver ACh (10% weight:volume ratio mixture) and a Mayo-built sudorometer. Even though the two measurement devices have been previously compared (Low and Benarroch 2008), it is important to generate a normative dataset using a standard piece of equipment that is commercially available in Canada and thus relevant to the majority of practice in Canada.

Furthermore, the need for normative data that is representative of the population is crucial in clinical practice. To accurately assess the presence or absence of disease, patient characteristics must be weighed against representative datasets to judge whether the measurements fall within 'normal' limits. Thus, clinicians require the most up-to-date and representative sample that can be acquired. However, given the differences in normative data between different geographical regions and laboratories it is important for individual clinics to generate their own normative dataset (Low and Benarroch 2008). Our laboratory previously reported normative data on 121 healthy individual (Ives, Berger, and Kimpinski

2013); however, the sample size in older individuals was reduced compared to other age groups. Moreover, due to the smaller sample size, individuals in their 5th decade of life (40-49 years) were grouped with individuals in their 8th decade of life (70-79 years). Given the inherent variability of the autonomic nervous system, the significant effect of age on various autonomic parameters, and the prevalence of autonomic dysfunction in older populations we aimed to increase our sample size across all age groups and to report normative data on young, middle aged and older age groups.

Therefore, the objective of the current study was to provide normative values with respect to sweat volumes obtained using the QSWEAT device, and two standard tests of cardiovagal functioning that is representative of young, middle aged and older individuals from Southwestern Ontario.

MATERIAL and METHODS

Participants

All healthy participants were recruited from the general population and were examined to confirm the absence of any neurological conditions including any autonomic dysfunction. Additionally, due to the potential influence on the autonomic nervous system, healthy participants were excluded if they fell under any one of the following categories: i) pregnant or lactating females, ii) clinically significant coronary artery disease, iii) concomitant therapy with anticholinergic, alpha- and beta-adrenergic antagonists or other medications which could interfere with autonomic functioning, and iv) failure of other organ systems or systemic illness that could affect autonomic function or participants' ability to cooperate. All research involving humans was reviewed prior to beginning the research. Ethical approval for this study was obtained from the Health Sciences Research Ethics Board at Western University and written informed consent was obtained from all study participants prior to participating in the study.

Autonomic Testing

All study participants underwent standard tests of autonomic function as previously described (Low 2003). In brief, quantitative sudomotor axon reflex testing was performed to provide an assessment of post-ganglionic sympathetic fiber integrity using a QSWEAT device (WR Medical Electronics Co., Stillwater, MN). A gel preparation of 0.55M Ach in a 2.4%wt./v agarose gel was used in the current study. Ach was transdermally iontophoresed with a constant current generator (2mA) for 5 minutes at four standard sites (forearm, proximal leg, distal leg and foot), followed by an additional 5 minutes of post-stimulus recording. Total sweat volumes were calculated as the area under the curve across 10-minutes of testing. Cardiovagal function was assessed using heart rate responses to deep breathing (HRDB) at a pace of 6 cycles/min for 8 consecutive breaths and Valsalva ratio (VR) calculated from a Valsalva maneuver. Average HR responses to DB were calculated as the five highest consecutive peak-to-trough heart rate differences which were then averaged to provide an average heart rate response. To calculate a VR, participants were instructed to maintain an expiratory pressure of 40mmHg, held for 15 seconds. The VR was calculated as the highest heart rate generated from the maneuver divided by the lowest heart rate achieved following maneuver release. Heart rate was continuously recorded using an electrocardiography (ECG) device (Model 3000 Cardiac Trigger Monitor, IVY Biomedical Systems, Inc., Branford, CT). All data were recorded and analyzed using WR Testworks™ software.

Statistical Analysis

Descriptive statistics are presented as mean $\pm$ standard deviation and percentile (2.5th, 5th, 95th, 97.5th). To evaluate the effect of age and sex on autonomic parameters, a multiple stepwise regression analysis was performed. An alpha level of 0.05 was used to denote significance. The procedures for setting normative values were completed according to the methods outlined by O'Brien and Dyck (1995) (O'Brien and Dyck 1995). All statistical analyses were performed using SPSS® statistical software version 21 for Windows (SPSS, Inc., Chicago, IL).

RESULTS

A total of 252 participants were including in the current study (Table 1). Sudomotor function (QSWEAT):

The effect of sex (X_2) was significant at all four sites ($p < 0.001$), and age (X) was a significant factor at the proximal leg (PL) ($p = 0.001$), distal Leg (DL) ($p < 0.001$) and foot ($p = 0.002$). The regression for the forearm (FA) was $Y = 0.85X_2 + 0.81$, where Y = total sweat volumes (μL) and X_2 = sex (0, male; 1, female) ($p < 0.001$).

For the PL, DL and foot the relationships for the affect of Age (X) expressed in years and Sex (X_2) (0, male; 1, female) on total sweat volumes (Y ; μL) were as follows: PL: $Y = -0.01X + 0.71X_2 + 1.44$; DL: $Y = -0.02X + 0.92X_2 + 1.70$; Foot: $Y = -0.01X + 0.43X_2 + 1.24$. Subsequent separation of male and female sweat data revealed that females maintained a significant affect of age on sweat volumes at the PL, DL and foot (Figure 1: B-D). In contrast, the effect of age on sweat volumes in males was only significant at the DL (Figure 1G). Normal sweat volumes at the forearm, proximal leg, distal and foot for both male and female controls for the 5th percentile, mean and 95th percentile for young (18-29 years), middle aged (30-59 years) and old (60-94 years) are presented in Table 2 (males) and Table 3 (females).

Cardiovaagal parameters: Heart rate responses to deep breathing and Valsalva ratios varied significantly by age ($p < 0.001$), but not sex. Therefore, the results for males and females were combined to produce the following expressions: HRDB: $Y = -0.25X + 29.9$, where Y = average heart rate response to deep breathing (bpm) and X = age (years) (Figure 2A). VR: $Y = -0.008X + 2.2$, respectively, where Y = Valsalva ratio and X = age (years) (Figure 2B). Normal values for the 2.5th and 5th percentiles, mean and 95th and 97.5th percentiles for HRDB and VR in young (18-29 years), middle aged (30-59 years) and old (60-94 years) are presented in Table 4 and 5, respectively.

DISCUSSION

Autonomic Reflex Screen Data

Sudomotor function (QSWEAT): In the present study, normative data for sweat responses were obtained using a gel preparation of Ach and QSWEAT device. In contrast, the normative data previously reported used a solution-based vehicle to deliver Ach and a Mayo-built sudorometer. The main advantage of using solution is that it is quick and easy to mix, whereas gel must be prepared in advance of testing. However, there are technical difficulties in working with solution which can cause the solution to leak into the recording compartment of the multi-compartmental sweat cell. In contrast, the gel solution prevents leaking and ensures that a constant connection between sweat capsule and skin is maintained throughout the test. In previous validations of the QSWEAT volumes versus QSART, a positive linear regression was reported to indicate the QSWEAT accurately estimates mayo- QSART, but that QSWEAT volumes were approximately double that of QSART (Low and Benarroch 2008). In the current study, male and female QSWEAT volumes were smaller compared to normative values obtained from mayo-built QSART. However, our updated data with a larger sample size produced values much more similar to that of QSART. For example, Low et al. (2003) reported mean foot sweat volumes of young, middle aged and older females of 1.27 μL , 1.05 μL and 0.84 μL , respectively. In the current study, our female group produced mean foot sweat volumes of 1.08 μL , 1.00 μL and 0.65 μL , respectively. Furthermore, the normative data obtained using the commercially available QSWEAT device with gel preparation compared favorably with other normative datasets. For QSWEAT measurements, a consistent effect of sex was found at all four sites with females demonstrating lower mean sweat volumes than their male counterparts. Furthermore, there was a significant effect of age at the proximal leg, distal leg and foot. These QSWEAT results demonstrate reduced sweat volumes with age in females, which is consistent with the results of Low et al. (1997), whom also reported a significant effect of sex (all sites) and age (PL, DL and foot) in 357 healthy controls (Low et al. 1997).

Cardiovaagal parameters: Cardiovaagal parameters as measured through heart rate responses to deep breathing and Valsalva ratios varied significantly by age, but not sex. Both cardiovaagal parameters

showed a progressive decline with age, which remains consistent with previous reports(Ives et al. 2013; Low et al. 1997). The lack of a significant effect of sex remains consistent with the work of Ives et al. (2013), but differs from the normative dataset produced by Low et al. (1997). In comparing the two tests of cardiovagal function, the effects of age on VR was significant, but not as strong as HRBD. Controversial effects of age on VR have been reported. However, in the current study there was a significant slope of 0.008 per year, which is in line with the work of both Ingall et al. (1990) and Low et al. (1997) who reported slopes of 0.01 and 0.0002 per year, respectively(Ingall, McLeod and O'Brien 1990; Low et al. 1997). Furthermore, the smaller effect of age on VR as compared to HRDB is likely due to the greater complexity of the maneuver and the smaller degree of variability between individuals. Furthermore, HRDB provides a more reliable cardiovagal measure as both the efferent and afferent limbs of the reflex arc are mediated by the vagus nerves. In contrast, the Valsalva maneuver involves complex physiology with a combination of parasympathetic and sympathetic mechanisms.

CONCLUSION

The current study provides updated normative data describing autonomic functioning in healthy individuals obtained from the sudomotor and cardiovagal components of the Autonomic Reflex Screen. In previously reported normative datasets, specific inquiries into autonomic function in older healthy individuals were limited by sample size. In the current article we aimed to increase our sample size to better address characteristics of autonomic functioning across young, middle aged and older populations. Importantly, our study addresses regional variations using commercially available equipment that may be of potential interest to laboratories and clinics across Canada.

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Table 1. Subject Characteristics

<i>Variable</i>	<i>n = 252</i>
Age (years)	42 ± 21
Range (years)	18-94
Sex	114 M, 138 F
Age group:	
18-29 years	n = 123 (55 m, 68 f)
30-59 years	n = 59 (29 m, 30 f)
60-94 years	n = 70 (40 m, 30 f)
Values expressed as mean ± SD. M, male; F, female.	

Table 2. Male QSWEAT Responses (total sweat volume [μ L]): 5th, Mean and 95th Percentile Values

<i>Sites</i>	<i>18-29 Years</i>			<i>30-59 Years</i>			<i>60-94 Years</i>		
	5 th	Mean	95 th	5 th	Mean	95 th	5 th	Mean	95 th
Forearm	0.10	1.67	5.12	0.13	1.49	3.17	0.07	1.89	4.74
Proximal leg	0.42	1.92	4.60	0.14	1.57	3.62	0.03	1.68	4.84
Distal leg	0.48	1.97	4.44	0.07	1.87	4.52	0.02	1.33	3.61
Foot	0.33	1.49	3.76	0.16	1.26	2.52	0.03	1.25	3.35

QSWEAT, quantitative sudomotor axon reflex test; μ L, microliters.

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Table 3. Female QSWEAT Responses (total sweat volume [μL]): 5th, Mean and 95th Percentile Values

Sites	18-29 Years			30-59 Years			60-94 Years		
	5 th	Mean	95 th	5 th	Mean	95 th	5 th	Mean	95 th
Forearm	0.09	0.91	2.57	0.11	0.68	1.59	0.07	0.77	2.14
Proximal leg	0.34	1.31	3.26	0.27	0.94	1.99	0.08	0.70	2.58
Distal leg	0.12	1.25	2.64	0.11	0.98	2.36	0.06	0.52	1.23
Foot	0.27	1.08	2.64	0.04	1.00	2.70	0.08	0.65	1.60

QSWEAT, quantitative sudomotor axon reflex test; μL , microliters.

Table 4. Heart rate response to Deep Breathing: 2.5, 5, Mean, 95, and 97.5 Percentile Values

<i>Age</i>	<i>Percentile (bpm)</i>				
	<u>2.5</u>	<u>5</u>	<u>Mean</u>	<u>95</u>	<u>97.5</u>
<i>18-29 Years</i>	10	10.5	23.7	38.8	43.4
<i>30-59 Years</i>	6.6	8.5	20.1	34.0	35.9
<i>60-94 Years</i>	3.9	4.7	11.8	21.0	35.9

bpm, beats per minute

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Table 5. Valsalva Ratio: 2.5, 5, Mean, 95, and 97.5 Percentile Values.

<i>Age</i>	<i>Percentile</i>				
	<u><i>2.5</i></u>	<u><i>5</i></u>	<u><i>Mean</i></u>	<u><i>95</i></u>	<u><i>97.5</i></u>
<i>18-29 Years</i>	1.3	1.5	2	2.8	2.9
<i>30-59 Years</i>	1.4	1.5	1.9	2.6	2.7
<i>60-94 Years</i>	1.2	1.2	1.7	2.6	2.8

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FIGURE LEGEND

Figure 1. Linear regression for females (A-D) and males (E-H) at the forearm (FA), proximal leg (PL) distal leg (DL) and foot as a function of age. Females maintained a significant effect of age at the PL ($p<0.001$), DL ($p<0.001$) and foot ($p=0.007$). Males maintained a significant effect of age at the DL ($p=0.001$). Dashed lines represent the 5th and 95th percentiles, solid line represents the linear regression.

Figure 2. Linear regression for heart rate response to deep breathing (A) and Valsalva ratio (B) as a function of age. HRDB and VR show a significant decline with age ($p<0.001$). Dashed lines represent the 5th and 95th percentiles, solid line represents the linear regression.

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Figure 1.

Figure 2.