Augmented supraorbital skin sympathetic nerve activity responses to symptom trigger events in rosacea patients

*Kristen Metzler-Wilson1,2; *Kumika Toma2,5; Dawn L. Sammons3,6; Sarah Mann2; Andrew J. Jurovcik2; Olga Demidova2; & Thad E. Wilson1,2,3,4

1Marian University College of Osteopathic Medicine, Indianapolis, IN, USA; 2Ohio Musculoskeletal and Neurological Institute, 3Department of Specialty Medicine, & 4Department of Biomedical Sciences, Ohio University Heritage College of Osteopathic Medicine, Athens, OH, USA; 5School of Kinesiology, College of Health Professions, Marshall University, Huntington, WV, USA; & 6Oakview Dermatology, Athens, OH

* contributed equally to the project and equally share first authorship

Running head: Role of the SNS in rosacea

Word Count: 4499; Figures: 3; Tables: 1

Author Conflicts of Interest: None

Keywords: supraorbital nerve, rosacea triggers, mental stress, physical stress, heat stress, autonomic dysfunction

Address for correspondence:
Thad E. Wilson, Ph.D.
Marian University College of Osteopathic Medicine
3200 Cold Spring Rd
Indianapolis, IN 46222-1997
Phone/fax: 317-955-6256
Email: twilson@marian.edu
ABSTRACT

Facial flushing in rosacea is often induced by trigger events. However, trigger causation mechanisms are currently unclear. This study tested the central hypothesis that rosacea causes sympathetic and axon reflex mediated alterations resulting in trigger-induced symptomatology.

Twenty rosacea patients and age/sex-matched controls participated in one or a combination of symptom triggering stressors. Protocol 1: Forehead skin sympathetic nerve activity (SSNA; supraorbital microneurography) was measured during sympathoexcitatory mental (2 min serial subtraction of novel numbers) and physical (2 min isometric handgrip) stress. Protocol 2: Forehead skin blood flow (laser-Doppler flowmetry) and transepithelial water loss/sweat rate (capacitance hygrometry) were measured during sympathoexcitatory heat stress (whole-body heating via perfusing 50°C water through a tube-lined suit). Protocol 3: Cheek, forehead, forearm, and palm skin blood flow were measured during non-painful local heating to induce axon reflex vasodilation. Heart rate and mean arterial pressure (MAP) were recorded via finger photoplethysmography to calculate cutaneous vascular conductance (CVC; flux*100/MAP).

Higher patient transepithelial water loss was observed (rosacea=0.20±0.02 vs. control=0.10±0.01 mg/cm²/min, p<0.05). Heart rate and MAP changes were not different between groups during sympathoexcitatory stressors or local heating. SSNA during early mental (32±9 and 9±4% increase) and physical (25±4 and 5±1% increase, for rosacea and controls, respectively) stress was augmented in rosacea (both p<0.05). Heat stress induced more rapid sweating and cutaneous vasodilation onset in rosacea compared to controls. No axon reflex vasodilation differences were observed between groups. These data indicate that rosacea affects SSNA, and that hyper-responsiveness to trigger events appears to have a sympathetic component.
INTRODUCTION

Rosacea is a chronic inflammatory skin disease affecting 2.3 to 10% of workers in Germany, Ireland, and Sweden in well-controlled large cohort studies (Tan and Berg 2013) and a projected 16 million Americans (2013a). Rosacea causes both increased financial burden and reduced quality of life (Aksoy et al. 2010; Beikert et al. 2013). Rosacea’s etiology and mechanisms are unclear; however, trigger events such as physical, mental, and thermal stress cause symptomatology including facial flushing (Del Rosso 2012; Del Rosso et al. 2013). Neural reflexes (sympathetic and local axon) may contribute to rosacea symptomatology, as these reflexes are actively engaged in symptom-mediating stress responses induced by triggers.

Physical, mental, and thermal stress increase skin sympathetic outflow (Cui et al. 2006; Muller et al. 2013; Vissing and Hjortso 1996; Wilson et al. 2006), which can be quantified directly via post-ganglionic skin sympathetic nerve activity (SSNA) and indirectly via sympathetic end-organ responses (skin blood flow and sweat gland secretions). It is currently unknown if rosacea patients have altered skin sympathetic outflow during triggering stresses. One difficulty with addressing this question is that SSNA contains multiple signals and is not uniform across the body (Charkoudian and Wallin 2014; Iwase 2009), while rosacea symptoms are normally confined to the head and neck (Pelle 2012). Fortunately, Nordin and colleagues adapted microneurography to impale cutaneous nerves from the head (Nordin 1994; Nordin et al. 1986; Nordin and Thomander 1989); only the supraorbital nerve contained suitable and measurable efferent sympathetic outflow traffic (Nordin 1990). Thus, quantifying supraorbital skin sympathetic outflow during baseline and trigger stimuli may give insight into potential rosacea mechanisms.

Facial-related SSNA has only rarely been described, and never in a dermatology population with facial involvement. In contrast, skin blood flow has been partially described in
the literature (Drott et al. 2002; Guzman-Sanchez et al. 2007; Mark et al. 2003; Shanler and Ondo 2007; Sibenge and Gawrodger 1992). Many of these studies, however, are difficult to interpret because of lack of controls and inappropriate use of laser-Doppler methodology. For example, blood pressure and laser-Doppler flowmetry must be assessed simultaneously to interpret sympathetic-induced flux changes (e.g., increased arterial blood pressure can increase flux independent of cutaneous vasodilation) and blood flow standardization must be employed, as absolute values are meaningless unless blood vessel density and other parameters are known (but are unknown in all non-invasive studies). Although also under sympathetic control (Shibasaki et al. 2006), sweat gland function, to our knowledge, has not been evaluated in rosacea.

Axon reflexes involve local peripheral neural circuits where a cutaneous afferent stimulus causes a local sympathetic-like end-organ response, yet these sensory-motor responses are not mediated by brainstem autonomic control centers (Holzer 1997; Rubino and Burnstock 1996). Noxious axon reflexes associated with stinging induced by cholinergic iontophoresis are augmented in severe vs. milder rosacea (Drummond and Su 2012). It is possible that in some patients with rosacea, triggers are mediated via sympathetic-like axon reflexes involving warmth, which activate transient receptor potential vanilloid subtype (TRPV) ion channels in cutaneous afferents, inducing vasodilation (Wong and Fieger 2010). We recently quantified cheek and forehead non-noxious axon reflexes via laser-Doppler flowmetry, local heating, and topical afferent nerve block (Metzler-Wilson et al. 2012). It is possible that this non-noxious test can identify the sympathetic-like effects of axon reflexes in trigger events.

Our aim was to test whether rosacea triggers induce hyper-responsive sympathetic and local axon reflex responses via fundamental alterations in neural reflexes. These exaggerated sympathetic and sympathetic-like responses may trigger symptomatology and local
inflammation, and over time cause disease progression. Thus, to identify whether changes in sympathetic and axon reflex responses occur in rosacea, we tested the following hypotheses: 1) We hypothesized that augmented supraorbital SSNA and cutaneous end-organ responses to mental and physical stress would occur in rosacea vs. controls. Mental and physical stressors were used as sympathoexcitatory triggers during direct supraorbital sympathetic outflow quantification. 2) We hypothesized that augmented cutaneous end-organ responses to whole-body thermal stress would occur in rosacea vs. controls. Thermal stress was also used as a sympathoexcitatory trigger, but because supraorbital SSNA recordings cannot be maintained for the time necessary for a whole-body heating protocol in our hands, only indirect supraorbital sympathetic outflow was quantified. 3) We hypothesized that greater skin blood flow reflex changes would result from local heating in affected compared to unaffected skin and vs. controls. Non-noxious local heating can engage axon reflexes, producing sympathetic-like responses.

METHODS

Participants. Eleven otherwise healthy erythematotelangiectatic rosacea patients (3 women, 8 men) and 11 age/sex-matched healthy controls participated in Protocol 1. Two to 4 wks later, 16 of these participants (8 rosacea, 8 control) participated in Protocol 2. Nine otherwise healthy erythematotelangiectatic rosacea patients (5 women, 4 men) and 9 age/sex-matched healthy controls participated in Protocol 3. One Protocol 3 control participant’s data were not analyzed due to a technical issue. By design, there were no significant age differences (p=0.808), but patients were significantly heavier than controls (p=0.015); collapsed means±SD for all protocols were age=35±15 and 25±5 yr and body mass index=24±4 and 25±4 kg/m², for women and men, respectively.
Participant health was assessed by medical history and physical exam including 12-lead electrocardiogram, and rosacea classification was determined based on established guidelines (Wilkin et al. 2002). Exclusion criteria were: smoking; pregnancy; hypertension; obesity; neural, cardiovascular, gastrointestinal, hematological, musculoskeletal, or other dermatological diseases; or medications or supplements that could not be temporarily withheld. All experiments conformed to the Declaration of Helsinki and were approved by the local Institutional Review Board. Each participant gave both written and verbal informed consent to participate in this study.

Participants refrained from caffeine, alcohol, and exercise for 12-24 hr and prescription drugs for at least 4 half-lives prior to testing conducted in the late morning or early afternoon in a temperature- and humidity-controlled laboratory (23-25°C, relative humidity <50%). All participants rested awake and supine for at least 30 min prior to experimentation.

**Measurements.** The supraorbital nerve tract was first identified and mapped via external stimulation using a square-wave electrical current (Grass Telefactor, West Warwick, RI) delivered via wand electrode. Nerve stimulation of this type produces afferent sensations and small muscle contractions. Multiunit supraorbital SSNA was measured by inserting a custom sterile tungsten microneurography electrode (2 MΩ impedance, 0.2 mm shaft diameter; FHC Inc., Bowdoin, ME) through the skin without local anesthesia. A ground electrode was then inserted close to the recording electrode but outside the sensory innervation area. The recording electrode was then positioned within the nerve fascicle and adjusted until SSNA bursts were clearly identified using previously established criteria (Delius et al. 1972; Hagbarth et al. 1972). In brief, these included 3:1 signal to noise ratio; skin afferent recordings; nonpulse-synchronous SSNA induction by startle, inspiratory gasp, and handgrip; and no relation to chemoreflex or
baroreflex perturbations. Figure 1 depicts a representative tracing of baseline and during skin affenter stimulation. The nerve signal was amplified, passed through a band-pass filter (500–5,000 Hz), and integrated with a 0.1 sec time constant (Iowa Bioengineering, Iowa City, IA). Mean voltage neurograms were visually displayed and recorded on a data acquisition system (Biopac Systems, Goleta, CA). The nerve signal was also routed to a loudspeaker for monitoring throughout the study.

Skin blood flow was indexed via integrated laser-Doppler flow probes (Moor Instruments, Wilmington, DE) placed on skin sites devoid of visible superficial and telangiectatic veins. In Protocol 3, optical sending and receiving fibers were housed in an 8-mm disk containing a heating element (Moor Instruments). Transepidermal water loss (TEWL) and sweat rate were measured via capacitance hygrometry using a custom ventilated chamber (Protocols 1 & 2: 0.5 or 2.83 cm², Protocol 3: 0.6 cm² chamber window) perfused with N₂ (150 ml/min). Consistent with the literature, we define TEWL as water loss during non-stimulated baseline conditions and sweat rate as water loss during sweat gland engagement during sympathoexcitatory conditions. Chamber humidity was measured using a high-sensitivity humidity-temperature probe (Sable Systems International, Las Vegas, NV) connected in series with the chamber.

Beat-by-beat finger arterial blood pressure was measured via volume clamp photoplethysmography that accounted for the vertical distance between the finger and heart (Finapres Medical Systems, Amsterdam, Netherlands). Heart rate (HR) was derived in real-time from the arterial pressure waveform (Finapres Medical Systems), and cardiac output was derived by ModelFlow methodology (Bogert and van Lieshout 2005). Oral temperature was measured via thermocouple (Omega, Stamford, CN; Protocol 2) located in the sublingual sulcus, and local skin temperature was measured via thermocouple (Omega) or thermistor (YSI, Yellow Springs,
OH) secured with surgical tape. All temperature data were routed to a bioamplifier (Sable Systems International; Protocols 1 & 2; Biopac Systems; Protocol 3).

**Experimental Protocols.** Protocol 1. SSNA was measured in the supraorbital nerve (Figure 1). A laser-Doppler flow probe for skin blood flow, ventilated capsule for TEWL/sweat rate, and thermistor for skin temperature were placed within the typical area of supraorbital innervation on the contralateral forehead since ipsilateral sensory-related mechanoreceptive afferents could not be avoided. After SSNA verification, baseline measurements were obtained, followed by mental arithmetic, re-baseline, then handgrip protocols.

**Mental Arithmetic:** To induce mental stress, participants continuously performed serial subtractions of the number 7 or 11 from a multiple-digit number for 2 min, with a novel starting number provided every ~30 sec. Participants indicated answers via finger gesture of the last numerical digit to avoid jaw or arm movement, which could cause microneurography electrode artifact. Due to occasional baseline neurogram shift and/or if the participant did not give mental task maximal effort, in some subjects, the task was repeated after ~15 min re-baseline. This type of mental arithmetic increases fibular, tibial, and supraorbital SSNA (Iwase et al. 1997; Muller et al. 2013; Nordin 1990).

**Handgrip:** To induce physical stress, participants performed 2-min isometric handgrip. Participants performed maximal voluntary contractions while measuring force via compression dynamometry (Biopac Systems) to determine a target submaximal force of 30%. Handgrip force feedback was provided via verbal cues. This task increases fibular SSNA in an effort-dependent manner that is not related to the amount of muscle mass involved (Silber et al. 2000; Vissing and Hjortso 1996; Vissing et al. 1991; Wilson et al. 2006).
Protocol 2. Thermoregulatory effectors (skin blood flow and sweating) were engaged by whole-body heating. Each participant wore a high-density tube-lined suit (Med-Eng Systems, Ottawa, Canada) that covered the entire body except the hands, feet, and head and allowed for skin temperature control via changing the temperature of water perfusing the suit. Following a thermoneutral baseline (34°C water perfusion), whole-body heating was performed by 50°C water perfusion. This method generally increases skin temperature to ~38°C and core temperature by ~1.0°C above baseline after 30-60 min heating. Because of pronounced symptom triggering and heat intolerance in participants with rosacea, whole-body heating was often terminated before the ~1.0°C temperature increase (average heating ~30 min). At the completion of whole-body heating, 24°C water was perfused to cool the participant. Whole-body heating increases SSNA, cutaneous vascular conductance (CVC), and sweat rate (Cui et al. 2006; Normell and Wallin 1974; Wilson et al. 2001) and appears to follow a pattern of initial suppression of SSNA, then dramatically increased internal temperature (Iwase et al. 2002).

Protocol 3. Local cutaneous vasodilation was engaged via direct skin heating within focal forehead, cheek, forearm, and palm areas, while skin blood flows were indexed via laser-Doppler flowmetry; these sites included two facial sites that can flush with rosacea and both glabrous and hairy control sites. Local heating protocols can be completed and interpreted in these skin sites (Metzler-Wilson et al. 2012). Following 6 min baseline, local skin temperature was standardized to 32°C for 6 min. Skin temperature was then increased by 0.1°C/2-3 sec and was maintained at 42°C for 30 min. Increasing skin temperature at this rate ensured that pain fibers were not activated, as pain induction can alter the skin blood flow (Magerl and Treede 1996). After 30 min or when red blood cell flux plateaued, local skin temperature was increased to 43°C and was maintained for 6 min to verify achievement of maximal skin blood flow. Local heating induces...
axon reflex vasodilation followed by sustained nitric oxide-mediated vasodilation, previously identified via sensory afferent and nitric oxide synthase blockade (Johnson et al. 2014).

**Data Analysis.** Data were acquired at 1000 (Protocols 1 & 2) or 62.5 (Protocol 3) Hz using a data acquisition system and analyzed with commercially available software (Biopac Systems). Rosacea and control subject characteristics were evaluated by 2-tailed independent *t*-test. Critical p-value=0.05. Data are presented as means±SE unless otherwise noted.

**Protocol 1.** Supraorbital SSNA was calculated as the integral of the integrated SSNA neurogram (Young et al. 2009), averaged in 15-sec bins, normalized to baseline, and expressed as percent change from baseline. Normalization accounts for individual SSNA variation and electrode locations within the nerve. CVC was calculated by red blood cell flux times 100, then that quantity was divided by mean arterial pressure (MAP). TEWL and sweat rate were determined by the product of measured N₂ flow rate and humidity change across the skin surface divided by the skin area exposed to N₂. HR, cardiac output, MAP, CVC, and sweat rate were averaged in 1-min bins and expressed as absolute change from baseline. Group differences were tested via Wilcoxon signed rank test (SSNA) and independent *t*-test (HR, cardiac output, MAP, CVC, and TEWL/sweat rate).

**Protocol 2.** CVC and sweat rate were determined as in Protocol 1. HR, cardiac output, MAP, facial skin temperature, oral temperature, CVC, and sweat rate were averaged in 1-min bins throughout the thermal stress. Data from the final min of heat stress were compared via independent *t*-test. Thermoregulatory effectors’ onset thresholds were determined as the time when the variable exponentially increased from baseline. These thresholds can provide an indirect indication of central sympathetic outflow to sweat glands and cutaneous blood vessels (Shibasaki et al. 2006).
Protocol 3. CVC for each site was determined as in Protocol 1 and expressed as percent of maximal (derived from the highest plateau value). Averages of 60-sec bins were obtained during initial baseline, 32°C standardization, plateau at 42°C, and plateau at 43°C; additionally, 5-sec bins were obtained during the site’s initial peak. HR and MAP were also averaged in 60-sec bins. Two-way (skin site by time) repeated-measures ANOVA was utilized, with Student-Newman-Keuls post hoc analysis when significant main effects were observed.

RESULTS

Protocol 1. Augmented supraorbital SSNA responses occurred in rosacea compared to controls during both physical (Figures 2 and 3A) and mental (Figure 3B) stress. Because mental stress responses can vary and some nerve recordings were difficult to maintain, correlation coefficients were obtained on the percent increase in supraorbital SSNA within the initial portion of mental arithmetic between multiple bouts in some subjects. These correlations were 0.96 in rosacea (n=7) and 0.87 in controls (n=4), indicating high between-bout reproducibility. During the baseline period preceding mental stress, rosacea patients showed higher forehead TEWL than controls (0.20±0.02 vs. 0.10±0.01 mg/cm²/min; p=0.03), and baseline CVC was similar (36±5 vs. 28±3 flux/mmHg; p=0.18, for rosacea and controls, respectively). Thermoregulatory effectors did not significantly change during physical or mental stress in the normothermic laboratory environment (ΔCVC <1% of baseline and Δsweat rate <0.01 mg/cm²/min for all subjects and conditions). During the physical stress protocol, the groups displayed similar forehead temperatures during baseline (33.18±0.35 and 33.29±0.27°C) and during the handgrip stressor (33.16±0.36 and 33.39±0.28°C, for rosacea and controls, respectively; p=0.60). Similarly, during the mental stress protocol, the groups displayed similar forehead temperatures during baseline (33.23±0.29 and 33.38±0.28°C) and during the stressor (33.20±0.29 and 33.±0.48°C, for rosacea
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and controls, respectively; \( p=0.55 \). HR and MAP changes from baseline were not different between rosacea and controls during either stressor. Change in HR (handgrip: 4±2 bpm and 7±1 bpm, \( p=0.28 \); mental arithmetic: 11±2 bpm for both groups, \( p=0.92 \)) and \( \Delta \)MAP (handgrip: 6±1 and 9±2 mmHg, \( p=0.21 \); mental arithmetic: 9±2 and 11±2 mmHg, \( p=0.37 \); for rosacea and controls, respectively) were not different between groups during either stress, while \( \Delta \)cardiac output was higher during mental arithmetic (1.2±0.3 vs. 0.4±0.2 L/min, \( p=0.04 \)) but not handgrip (0.3±0.1 vs. 0.2±0.1 L/min, \( p=0.59 \)) in rosacea.

**Protocol 2.** Whole-body heating occurred until internal temperature increased 1°C from baseline or subjects self-terminated the protocol (duration: 16-47 min for rosacea and 27-53 min for controls). During the thermoneutral baseline period, oral (36.1±0.5 and 36.0±0.2°C, \( p=0.84 \)) and forehead skin (33.7±0.3 and 33.8±0.4°C, \( p=0.39 \); for rosacea and controls, respectively) temperatures were not different between groups. Whole-body heating increased oral (0.94±0.37 and 0.88±0.12°C; \( p=0.88 \)) and forehead skin (1.51±0.26 and 1.17±0.48°C, for rosacea and controls, respectively; \( p=0.21 \)) temperature equally in both groups. Whole-body heating increased HR (27±1 and 28±4 bpm; \( p=0.90 \)) and cardiac output (1.92±0.31 and 1.64±0.34 L/min; \( p=0.55 \)) similarly in both groups but had a variable effect on MAP (-6±3 and 4±6 mmHg; \( p=0.19 \), for rosacea and controls, respectively). During whole-body heating, the sweating onset threshold in rosacea occurred at 10:53±1:44 compared to 18:04±2:34 (min:sec) in controls (\( p=0.02 \)). However, sweating only trended higher during thermal stress in rosacea compared to controls (0.72±0.19 and 0.41±0.14 mg/cm²/min; \( p=0.10 \)). The cutaneous vasodilation onset threshold during whole-body heating occurred at 12:19±1:57 in rosacea compared to 19:59±1:52 (min:sec) in controls (\( p<0.01 \)). Whole-body heating increased CVC to a greater extent in rosacea compared to controls (451±40 and 352±33% increase from baseline; \( p=0.04 \)).
Protocol 3. CVC was not different between rosacea and controls within a given skin site during initial baseline or 32°C standardization. Similarly, initial peak response to local heating was not different between rosacea and controls within a given skin site (Table 1). Plateau values for each skin site, expressed as percent increases from baseline, were not different between groups (forehead $p=0.66$, cheek $p=0.23$, forearm $p=0.77$, and palm $p=0.67$). HR did not change across the local heating protocol (rosacea: 62±4 to 62±5 bpm, $p=0.84$; control: 65±4 to 65±4 bpm, $p=0.90$; for baseline and end-heating, respectively), but MAP did increase slightly in rosacea (rosacea: 89±4 to 93±5 mmHg, $p=0.02$; control: 95±3 to 97±3 mmHg, $p=0.32$; for baseline and end-heating, respectively). No pain was reported during increasing skin temperature or with prolonged 42-43°C local heating.

DISCUSSION

This study aimed to determine if rosacea causes changes in sympathetic outflow and axon reflex-mediated vasodilation. We believe this is the first direct quantification of a facial nerve’s sympathetic outflow in rosacea. Our primary new findings are: 1) trigger-induced supraorbital sympathetic outflow hyper-responsiveness in rosacea during physical and mental stress; 2) increased sympathetic drive in rosacea demonstrated by earlier onset of sweating and cutaneous vasodilation during whole-body heat stress; and 3) similar axon reflex mediated vasodilation in rosacea during non-painful local heating in both affected and non-affected skin. Thus, it appears that sympathetic outflow is affected by rosacea, while local axon reflexes remain intact.

Rosacea presentation often suggests a neurological component in addition to the traditionally defined inflammatory disease process, as sympathoexcitatory triggers induce flushing (2013b), and associated sympathectomies decrease symptoms (Drott et al. 2002; Schram and James 2012). Providing further indication of a neural link, Schwab and colleagues (Schwab
et al. 2011) identified increased myelinated neuron number in erythematotelangiectatic rosacea compared to healthy skin. However, providing direct evidence of sympathetic outflow differences in the face is difficult. To our knowledge, only Nordin (Nordin 1990) has identified supraorbital SSNA. SSNA contains sympathetic signals to sweat glands, cutaneous blood vessels, and piloerector muscles and is often used as a central sympathetic outflow index because it is not governed by reflexes (e.g., baroreflexes and chemoreflexes) that influence sympathetic outflow via muscle or renal sympathetic nerve activity (Menon et al. 2007; Vissing 2000; Vongpatanasin et al. 1999). Less is known about supraorbital SSNA, as facial skin may or may not resemble peripheral hairy or glabrous skin as indexed via fibular and tibial nerves. Because this study provides the first sympathetic outflow quantification in rosacea, it has the potential to directly evaluate rosacea’s neurological component.

Augmented supraorbital SSNA increases were observed during isometric handgrip exercise in rosacea vs. controls, which widened further as handgrip duration increased. To our knowledge, supraorbital SSNA has never been assessed during physical stress. Handgrip has been used extensively to induce fibular SSNA increases (Vissing 2000; Wilson et al. 2006); it may be more reproducible and tolerable for patients than mental or thermal stress. Arousal level likely dictates the increased supraorbital SSNA, but whether supraorbital SSNA tracks previous fibular SSNA observations (Seals 1993; Vissing and Hjortso 1996) is unclear. Physical exercise including “carrying and lifting” is a trigger event for many rosacea patients (2013b), and this higher outflow may be one reason.

We also observed augmented supraorbital SSNA increases during mental stress in rosacea compared to controls. Previously, increased supraorbital (Nordin 1990) and fibular (Muller et al. 2013) SSNA have been identified in healthy subjects during mental stress. Iwase and colleagues (Iwase et al. 1997) also identified increased tibial SSNA in plantar hyperhidrosis.
Because of likely proximity differences in needle placement within a nerve and the current recommended SSNA analysis procedures which evaluate the integral rather than burst frequency, it is difficult to determine if rosacea subjects had increased baseline supraorbital SSNA. As a general qualitative observation, supraorbital baseline activity was fairly low in both groups (Figure 2B). However, in response to mental arithmetic, SSNA increased in both groups, with dramatically increased responses in rosacea that persisted throughout the task. This higher outflow may be a reason that mental stress is often a rosacea symptom trigger.

We employed a number of improvements in skin blood flow assessment compared to previous studies in this area: 1) CVC calculation rather than obtaining only flux measures, which are significantly affected by MAP; and 2) skin blood flow standardization to either baseline or maximal CVC to more accurately compare both persons and skin sites with varying blood vessel densities. Cutaneous vasodilation occurred sooner and CVC increased to a greater extent in rosacea compared to controls during whole-body heating. Subjects with rosacea increased oral temperature to the same degree as age-sex matched controls despite being heated for less time. It is possible that this higher skin blood flow observed in rosacea aided in the observed heat gain during the thermal stress. However, we cannot confirm this because we do not have an index of skin blood flow in non-facial skin. Baseline CVC was not significantly different in rosacea compared to controls in either protocol. This is not unusual, as visible surface veins and telangiectases were avoided and no patients actively flushed during normothermic baseline.

Whole-body heating induced symptoms such as flushing in most rosacea participants, and many self-terminated the heating protocol. In contrast to thermal stress, neither of the Protocol 1 stressors appeared to cause flushing or change CVC in patients or controls despite increased SSNA. Nordin (Nordin 1990) observed weak vasoconstriction and vasodilation during stressful perturbations when linking supraorbital SSNA to end-organ responses. Nordin’s skin blood flow
data, however, are more difficult to interpret because of lack of accounting for blood pressure changes and because of use of an atypical skin blood flow standardization procedure. In normothermic conditions, it is not unusual to observe a lack of change in CVC within the area of innervation of the common fibular nerve during isometric exercise (Wilson, et al. 2006). While an increase in SSNA with no end-organ change seems paradoxical, local factors can have dramatic effects on skin perfusion. Local skin temperature dramatically affects peripheral skin blood flow and alters the sympathetic drive to CVC relationship (Wenger et al. 1985). Room temperature was ~23°C in all conditions, which is in the range to suppress CVC, but during whole-body heating, forehead skin temperature increased indirectly because of warm blood perfusing the skin. Local temperature’s precise role in modulating facial skin blood flow responses remains unknown.

TEWL was increased in rosacea compared to controls. TEWL changes are often interpreted to indicate an impaired skin barrier. Jappe and colleagues (Jappe et al. 2005) suggested that rosacea results in a disrupted and inflamed facial skin barrier which could increase susceptibility to contact dermatitis and result in a more vigorous response to cutaneous irritation. Skin barrier issues in rosacea could prove clinically useful and warrant additional testing. To our knowledge, alterations in sweat rate responses in rosacea have not previously been identified during triggers. This may be because the more sophisticated methodology (i.e., capacitance hygrometry) used in this study can detect subtle water vapor content changes, and the dry N\textsubscript{2} provides a high gradient for evaporation without saturation. Other methodologies such as skin conductance (or its reciprocal: skin resistance) can be significantly affected by ionic fluid changes such as blood beneath the skin surface. During Protocol 1, sweat rates were not significantly different between groups. Sweating increased dramatically during whole-body heating and trended higher in rosacea. Additionally, the sweating threshold decreased (i.e.,
sweating was initiated earlier) in rosacea during whole-body heating, providing evidence of increased central sympathetic outflow to the region during heat stress. We are unaware of reports of sudomotor dysfunction in rosacea, however since we identified changes in SSNA in rosacea, and SSNA includes sudomotor activity, future investigations into possible alterations of sudomotor activity in rosacea may be warranted.

Contrary to our hypothesis, non-noxious local heating did not induce greater axon reflex changes in skin blood flow in affected compared to unaffected skin and compared to controls. The local heating response has been well-documented in dorsal and ventral hairy forearm skin (Johnson et al. 2014), and more recently in the cheek and forehead (Metzler-Wilson et al. 2012). The vasodilatory response to local warming is biphasic; topical afferent anesthetics can block the initial peak - thereby implicating a local axon reflex mechanism. Local heat stimulates TRPV receptors on warm sensory afferents. TRPV subtypes 1-4 are upregulated in affected skin in erythematotelangiectatic rosacea (Sulk et al. 2012). Despite this, we did not observe a difference in initial peaks, indicating intact axon reflexes in rosacea. The second peak during local heating is primarily nitric oxide-mediated. Currently, it is unknown if nitric oxide-mediated vasodilation is altered in rosacea. Local heating responses are central nervous system-independent as demonstrated by preservation despite nerve blocks (Johnson et al. 2014). Previous studies indicate that noxious heat increases skin blood flow in affected vs. unaffected skin in patients with papulopustular, but not erythematotelangiectatic, rosacea (Guzman-Sanchez et al. 2007). Additionally, noxious stinging induced by cholinergic iontophoresis augments axon reflexes in severe vs. milder rosacea (Drummond and Su 2012). Thus, axon reflex responses may be altered differently depending on rosacea classification and the presence of pain.

This was the first study to directly quantify sympathetic outflow \textit{in vivo} in rosacea. During both handgrip and mental arithmetic, individuals with rosacea had augmented
supraorbital SSNA compared to controls. This suggests hyperresponsive sympathetic outflow in rosacea. Indirect evidence of hyperresponsiveness was also collected during whole-body heating, where sweating and cutaneous vasodilation occurred earlier in rosacea compared to controls. Non-noxious local-heating induced axon reflexes were not different. Taken together, these results imply that sympathetic nerve activity is affected by rosacea, while local-heating induced skin axon reflexes remain intact. Since sympathectomy decreases flushing, sympathoexcitatory trigger events induce flushing, and individuals with rosacea have hyper-responsive sympathetic outflow to triggers, the neural influence in rosacea may need to be revisited in addition to the previously demonstrated changes in inflammatory mediators.
ACKNOWLEDGEMENTS

This project was supported by a grant from the National Rosacea Society (T.E.W.). The authors express appreciation to Michael Tomc, DO, Andrew LePorte, and Aiwane Iboaya for their technical assistance and to the subjects for their willing participation.

AUTHOR CONTRIBUTIONS

KM-W, DLS, KT, and TEW conceived and designed experiments.
OD, AJJ, SM, KM-W, KT, and TEW performed data collection.
OD, AJJ, SM, KM-W, KT, and TEW analyzed and interpreted data.
KM-W and TEW prepared figures.
KM-W, KT, and TEW drafted the manuscript.
All authors reviewed, edited, and approved the final version of the manuscript.

DISCLOSURES

The authors have declared no conflicts of interest.
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National Rosacea Society, Patient home page

National Rosacea Society, Rosacea triggers survey


Del Rosso JQ. Advances in understanding and managing rosacea: part 1: connecting the dots between pathophysiological mechanisms and common clinical features of rosacea with emphasis on vascular changes and facial erythema. The Journal of Clinical and Aesthetic Dermatology 5: 16-25, 2012.


Role of the SNS in rosacea


Table 1: Cutaneous vascular conductance (CVC) responses to non-noxious local heating in rosacea and age/sex-matched controls.

<table>
<thead>
<tr>
<th>CVC(^1) (% max)</th>
<th>Forehead</th>
<th>Cheek</th>
<th>Forearm</th>
<th>Palm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rosacea</td>
<td>Control</td>
<td>Rosacea</td>
<td>Control</td>
</tr>
<tr>
<td>Baseline(^2)</td>
<td>25 ± 10</td>
<td>17 ± 5</td>
<td>11 ± 4</td>
<td>19 ± 4</td>
</tr>
<tr>
<td>32° C Standardization(^3)</td>
<td>23 ± 8</td>
<td>17 ± 4</td>
<td>16 ± 4</td>
<td>25 ± 4</td>
</tr>
<tr>
<td>Initial Peak(^4)</td>
<td>68 ± 9</td>
<td>75 ± 5</td>
<td>77 ± 8</td>
<td>78 ± 5</td>
</tr>
</tbody>
</table>

\(^1\)CVC (\% max): the expression of CVC to maximal blood flow for the particular site. Values are expressed as mean±SE. No group comparisons were statistically significant (P>0.05).

\(^2\)Baseline: CVC responses at individual non-standardized skin temperature (rosacea = 30.7±0.4, 29.1±0.5, 29.1±0.5, 29.4±0.9; control 30.2±0.3, 29.5±0.3, 28.8±0.2, 28.8±0.6°C, for forehead, cheek, forearm, and palm, respectively)

\(^3\)32°C standardization: when local heaters were engaged to standardize blood flow between subjects and sites

\(^4\)Initial Peak: the first peak in the local heating response
FIGURE LEGENDS

**Figure 1.** Representative neurogram (first row) and ECG tracing (second row) during baseline and skin afferent stimulation. Arrow denotes a spontaneous baseline burst and squares represent light (open squares), moderate (gray squares), and firm (black squares) rhythmical brush strokes with a calligraphy brush within the area of innervation of the supraorbital nerve. Note that bursts are not related to ECG r-waves, and cutaneous mechanoreceptor activation is related to amplitude.

**Figure 2.** Representative neurograms during baseline and physical stress for (A) rosacea and (B) age/sex-matched controls. The first row depicts the representative skin sympathetic nerve activity (SSNA) tracing during baseline and during a sympathoexcitatory response to physical stress involving 2 min of 30% maximal isometric handgrip in one rosacea and one control subject. The second row depicts the arterial blood pressure (ABP) waveform. The third and fourth rows depict the corresponding skin blood flow (SkBF) and transepidermal water loss/sweat rate (TEWL/SR) from cutaneous end-organs on the contralateral side of the forehead.

**Figure 3.** Changes in skin sympathetic nerve activity (SSNA) during (A) physical stress involving 2 min of 30% maximal isometric handgrip and (B) mental stress involving 2 min of mental arithmetic for rosacea and age/sex-matched controls. Values are means±SE. **A:** Significant differences noted between group (rosacea and control, p<0.01), across time (p=0.03), and in the interactive term (p=0.03). **B:** Significant differences noted between group (rosacea and control, p=0.04), but no significant difference across time (p=0.37) or in the interactive term (p=0.35).
Figure 2A.
Figure 2B.
Figure 3.