Measuring Physical Stigmata of Niacin-Associated Skin Toxicity by Colorimetry, White-Light Spectroscopy, Laser Doppler Flowmetry, and Thermometry in Combination with Symptom Perception Scoring: Methods to Aid Development of Niacin Mimetics.

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Supplemental Tables

Study	Niacin dose/population	Objective Stigmata Assessment(s)	Subjective Symptom Perception	Findings	Stigmata/Symptom Correlation
***Rubor* by Colorimetry**					
Dunbar et al., 2016*	500 mg IRN, dosed once. (n=68, NHV)	Colorimetry (*rubor*) + LDF (plethora) + WLS (*rubor*) + Thermometry (*calor*)	FAST	All objective modalities correlated strongly with symptoms perception	Rapidity of *rubor* onset correlated most strongly with symptom perception.
Plethora by Laser Doppler Flowmetry					
Wilkin, 1988.[1]	5.71 mg/kg IRN, dosed once (n=11, NHV)	LDF Investigator observed visible *rubor*	N.A.	IRN-induced plethora occurs preferentially in facial vs forearm skin.	No objective vs subjective statistical correlation tested. Subjective *rubor* more common and more intense in face than forearm. Plethora by LDF in face > forearm.
Stern et al, 1991.[2]	500mg IRN tid x 5 days (n=4, NHV)	LDF + Thermometry (ΔMTCI)	Semi-quantitative symptom assessment (exact methodology not presented)	↓ΔMTCI over 5 days ↓LDF CPI (peak flow/basal flow) over 5 days ↓ Subjective flushing over 5 days	No objective vs subjective statistical correlation tested. *Calor* by thermometry and plethora by LDF followed the same trend as subjective symptoms.
Parson et al. 2013.[3]	1000 mg IRN+325mg ASA/PBO, dosed once. (n=30, NHV)	LDF	FAST	ASA ↓ IRN-induced hyperemia only in hairy vs glabrous skin.	Peak plethora by LDF correlated strongly with all symptoms on FAST and with overall flushing
Dunbar et al., 2016*	500 mg IRN, dosed once. (n=68, NHV)	LDF + Colorimetry + WLS + Thermometry	FAST	All objective modalities correlated strongly with symptoms perception	Time to onset of *rubor*, plethora (flux index ≥ 1.8), *calor*, and *frigor* significantly correlated with overall flushing severity as well as individual NASTy symptoms
***Calor* by Thermometry**					
Aberg and Svedmyr, 1971.[4]	250mg, 500mg, 1g IRN, and 500mg and 1 g Perycit), dosed once, crossover design. (n=10, NHV)	Thermometry (↑ in skin temperature). Investigator assessed erythema on 4-point scale (no, slight, moderate, intense redness)	4-point, patient reported warmth scale (no, slight, moderate, intense warmth).	IRN caused more intense symptoms and greater ↑ in skin temperature than Perycit.	No statistical correlation tested. Good subjective correspondence between warmth perception and thermometry.
Svedmyr et al., 1977.[5]	Individually titrated oral IRN + Indomethacin/PBO. (n=10, NHV)	Thermometry (↑ in skin temperature)	N.A	Indomethacin significantly reduced niacin induced ↑ in skin temperature	N.A

Study	Niacin dose/population	Objective Stigmata Assessment(s)	Subjective Symptom Perception	Findings	Stigmata/Symptom Correlation
Phillips and Lightman, 1981.[6]	25 mg niacin IV once preceded by 2 days of indomethacin/benoryl ate/PBO. (n=10, NHV)	Thermometry (↑ in skin temperature)	Subject reported symptoms, non-quantitative	Indomethacin and benorylate attenuated niacin induced ↑ in skin temperature and subjective symptoms severity.	Statistical correlation not tested. Both symptoms and *calor* ↓ with indomethacin & benorylate pretreatment.
Wilkin, 1982[7]	100 mg IRN, dosed once (n=9 for IRN, n=162 for all agents tested, NHV)	Thermometry (ΔMTCI) +/- facial reddening	N.A.	ΔMTCI ≥1.5 had high sensitivity, specificity and PPV for flushing. ΔMTCI strongly predicted binary flushing (investigator observed skin reddening).	N.A.
Wilkin et al, 1982.[8]	0.71, 1.43, 2.86, and 5.71 mg/kg IRN + 325 mg ASA or PBO, dosed once. (n=29, NHV)	Thermometry (ΔMTCI)	N.A.	Dose-dependent ↑ in ΔMTCI with IRN. Attenuated ↑ in ΔMTCI with ASA	N.A.
Fiedler et al., 1986.[9]	25 mg niacin IV. (n=30, 13 alcoholics+9 SCZ+9 NHV)	Thermometry (Δ MTCI)	N.A.	Alcoholics had ↓ hyperemic response to niacin versus NHV and SCZ. No difference between SCZ and NHV.	N.A.
Pontiroli et al., 1991.[10]	ACX 31.25, 62.5, 125, 250 mg on 5 different days (n=25. NHV)	Thermometry (↑in skin temperature)	Patient reported 10cm visual analog scale	ΔT, ΔT-AUC, rate of malar skin temp. rise (ΔA(a)), and subjective semi-quantitative flushing were dose dependent.	ΔT, ΔT-AUC , and ΔA(a) all correlated significantly with subjective flushing score. ΔA(a) had strongest correlation.
Rybakowski and Weterle, 1991.[11]	200 mg IRN, dosed once. (n=51, 33 SCZ+18 depressive patients)	Thermometry (↑ in skin temperature) Investigator assessed occurrence of erythema (binary)	N.A.	Clinical flushing not consistently reproduced by increases in skin temperature	N.A.
Hudson et al., 1997.[12]	200mg IRN, dosed once. (n=74, 28 SCZ+18 BPD+28 NHV)	Thermometry (ΔMTCI)	N.A.	SCZ patients had significantly impaired hyperemic response to niacin c.w BPD and NHV.	N.A.
Bouwer and Stein, 1997.[13]	100mg IRN, dosed once. (n=12, 6 social phobia patients+6 NHV)	Thermometry (↑ in skin temperature) Investigator assessed visible flushing (binary)	N.A.	6/6 social phobia pts had visible flushing vs 1/6 NHV. Skin temp. increased significantly more in social phobia vs NHV	N.A
Hudson et al., 1999.[14]	200mg IRN, dosed once. (n=53, 30 NHV+23 SCZ)	Thermometry (ΔMTCI)	N.A.	Significant differences in phospholipase A2 acticity between SCZ and NHV	N.A

Study	Niacin dose/population	Objective Stigmata Assessment(s)	Subjective Symptom Perception	Findings	Stigmata/Symptom Correlation
Nilsson et al., 2006.[15]	200 mg IRN, dosed once (n=47, 30 SCZ+17 NHV)	Thermometry (↑ in skin temperature)	N.A.	Patients with SCZ had longer time to peak skin temperature than NHV.	N.A
Nilsson et al, 2009.[16]	200 mg IRN, dosed once. (n=23 SCZ)	Thermometry (↑ in skin temperature) Electrodermal activity	N.A.	Low retest stability for thermometry using increases in skin temperature as measure of flushing	N.A.
Dunbar et al., 2016*	500 mg IRN, dosed once. (n=68, NHV)	Thermometry + Colorimetry + LDF + WLS	FAST	All objective modalities correlated strongly with symptoms perception	Rapidity of core hypothermia correlated most strongly with symptom perception.

Supplemental Table S1: Summary of studies using objective hyperemia assessment tools after systemically administered niacin. Only LDF and thermometry have been studied in this context. Colorimetry has not been used to study NASTy severity after systemic niacin, and neither has WLS. Only one study has studied correlation between objective hyperemia assessment (using LDF) and a semi-quantitative, detailed symptoms severity scale: the FAST©, (Parsons et al., 2013) showing strong correlations between individual symptoms of NASTy, as well as overall flushing, and peak blood flow by laser Doppler flowmetry (LDF). ASA-aspirin; CPI- Cutaneous Perfusion Index; FAST- Flushing assessment tool; IRN-immediate release niacin; NHV-normal healthy volunteers; N.A.- not assessed; PBO-placebo; Perycit- pentaerythrityltetranicotinate; SCZ-schizophrenics; BPD-bipolar disease. * Current investigation.

Supplemental Table S1 references

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Stigmata:	***Rubor***		**Plethora**	***Calor*/*Frigor***
Physical Property Measured:	Skin color	Color of hemoglobin in skin	Motion of red corpuscles (red-shift)	Temperature
Modality:	Clinical colorimetry	WLS	LDF	Thermometry
Individual-related factors				
Age	↓ basal skin color in >66 yrs old	No	No	No
Sex	Uncertain, probably not	No	No	No
Race	Yes	No	No	No
Anatomical site	Yes	Yes	Yes	Yes
Physical activity	Yes	Yes	Yes	Minor
Eating	No	No	No	No
Drugs/Medications	Yes	Yes	Yes	Yes
Lab values	Yes	Yes	No	No
Motion	Somewhat	Somewhat	Yes	No
Skin condition	Yes	Yes	Yes	Yes
Menstrual cycle	Likely	Likely	Yes	Likely
Posture	Yes	Likely	Yes	No
Device-related factors				
Probe configuration	n/a	Yes	Yes	n/a
Probe pressure	Yes	Yes	Yes	No
Environmental factors				
Ambient temperature	Yes	Yes	Yes	Yes
Convection	Yes	Yes	Yes	Yes
Ambient light	Yes	No	No	No
Mental stress	Yes	Uncertain	Yes	Uncertain
Temporal variations	Yes	Uncertain	Possibly	Uncertain

Supplemental Table S2: Variables influencing objective measurement of NASTy stigmata by colorimetry, WLS, LDF, and thermometry. An awareness of these is crucial in proper selection of any one or more of these techniques for any particular experiment to assess NASTy. Controlling these variables is also optimal to ensure valid and reproducible measurements.

SUPPLEMENTARY APPENDIX

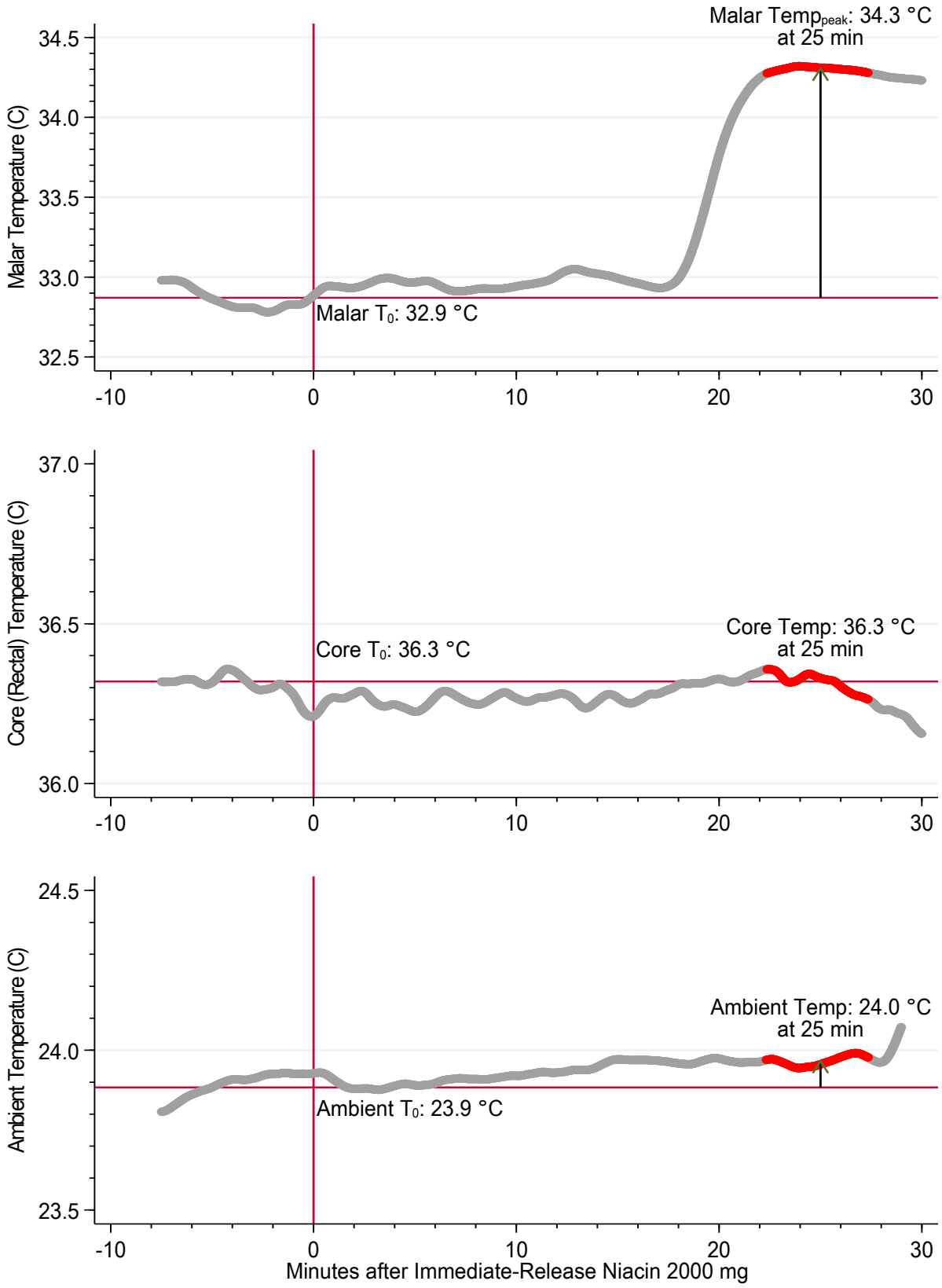
1.1 Wilkin's Malar Thermal Circulation Index (ΔMTCI)

The ΔMTCI is calculated as the ratio of peak to baseline skin thermal conductivity using the equation:

$$\Delta MTCI = \left(\frac{Tmp-Tap}{Tcp-Tmp}\right) \Big/ \left(\frac{Tmb-Tab}{Tcb-Tmb}\right)$$

Where Tmp, Tap and Tcp are *peak* malar, ambient and core temperatures, and Tab, Tcb and Tmb are *basal* ambient, core and malar temperatures, respectively.

1.2 Example Calculation for Wilkin's Delta Malar Circulation Index



Supplemental Figure S1: Calculation of delta-Malar Thermal Circulation Index (dMTCI). Each graph depicts one of the three smoothed temperatures contributing to the dMTCI: malar, core (rectal), and ambient. In each case, the baseline level was determined from -10 to 0 minutes, labeled T_0 on each graph. To determine the post-niacin peak malar temperature, we assessed the maximum temperature and defined a 5-minute window based on the time of the peak (red region on the line graph). The average temperature during that 5 minutes was taken as the peak malar temperature. Taking a window conservatively guards against over-estimating the peak, for example, if there were a spike in temperature that may or not be representative which influenced the maximum despite curve smoothing. Once the interval was defined for the malar peak, that same interval was used to define the identical time interval for the core and ambient temperatures. Note that at the time of the malar peak, there was little change in either the core temperature or ambient temperature, which is typical. Core temperature usually drops just after the malar temperature peaks. Ambient temperature sometimes rises slightly and slowly throughout the day, reflecting environmental conditions.

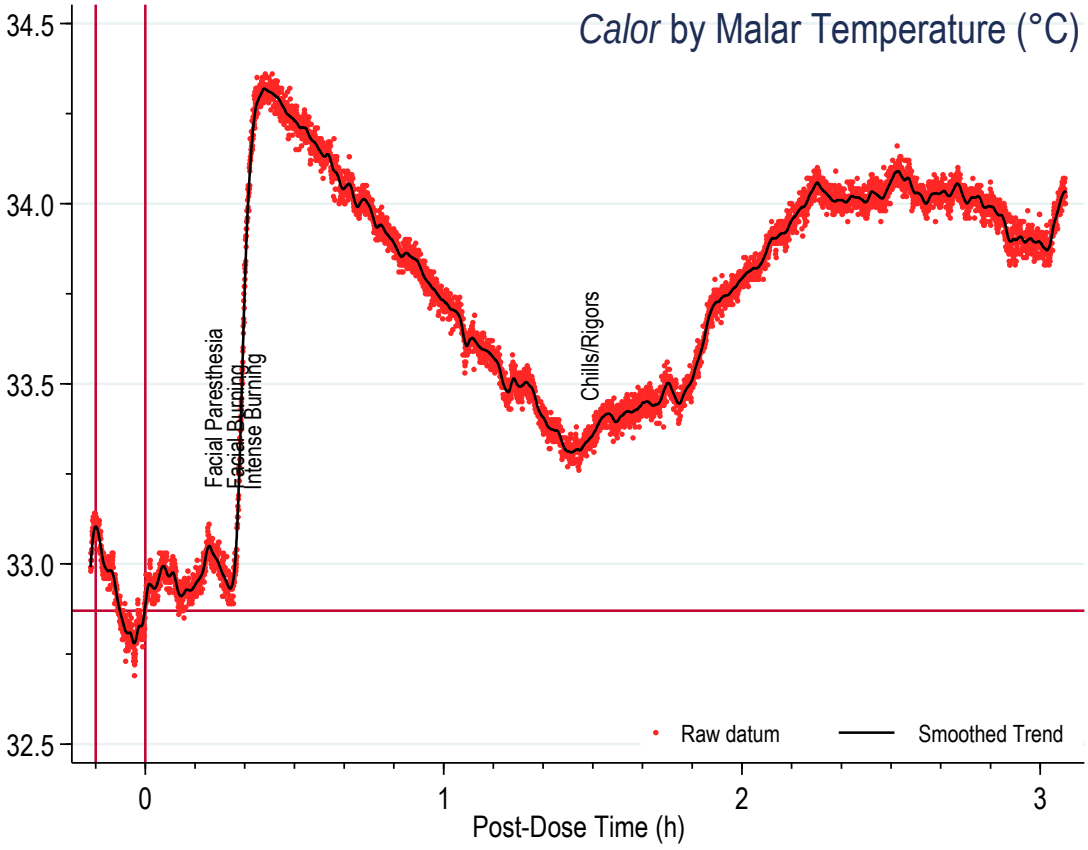
This process yields the 6 parameters used to calculate the dMTCI: malar0, malarP, core0, coreP, ambient0, and ambientP. The equations below show the formula for dMTCI as the variables, and again after substitution, and the solution for this subject after receiving 2g IR niacin. (For the equation, Tmb = malar0, Tmp = malarP, Tab = ambient0, Tap = ambientP, Tcb = core0, and Tcp = coreP.)

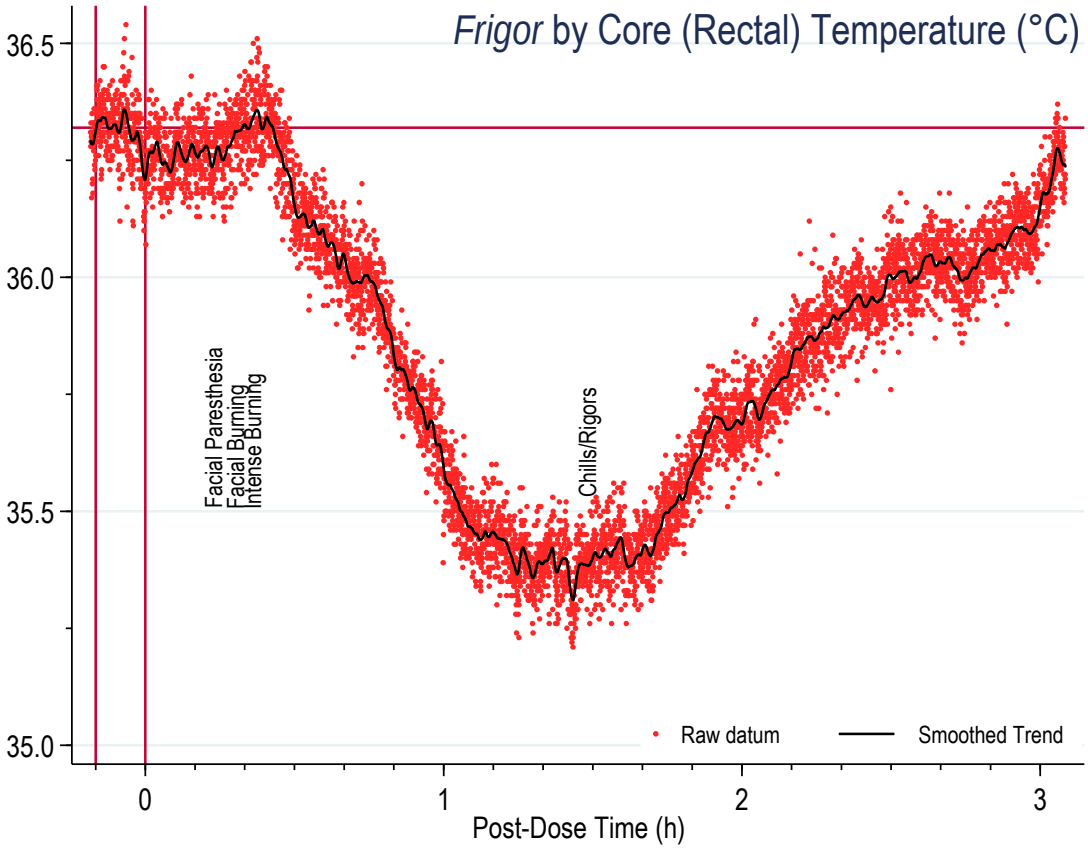
$$\Delta MTCI = \left(\frac{Tmp-Tap}{Tcp-Tmp}\right) \Big/ \left(\frac{Tmb-Tab}{Tcb-Tmb}\right) \quad \text{or} \quad \Delta MTCI = \left(\frac{malarP-ambientP}{coreP-malarP}\right) \Big/ \left(\frac{malar0-ambient0}{core0-malar0}\right)$$

Hence,

$$\Delta MTCI = \left(\frac{34.3-24.0}{36.3-34.3}\right) \Big/ \left(\frac{32.9-23.9}{36.3-32.9}\right)$$

$$\Delta MTCI = 1.95$$


Calor by Malar Temperature (°C)
34.5
34.0
33.5
33.0
32.5
Facial Paresthesia
Facial Burning
Intense Burning
Chills/Rigors
Raw datum
Smoothed Trend
0
1
2
3
Post-Dose Time (h)


Frigor by Core (Rectal) Temperature (°C)
36.5
36.0
35.5
35.0
Facial Paresthesia
Facial Burning
Intense Burning
Chills/Rigors
Raw datum
Smoothed Trend
0
1
2
3
Post-Dose Time (h)

Supplemental Figure S2: Reciprocal changes in malar *calor* (upper panel) and core body *frigor* (lower panel), by temperatures over 3 hours following 2000 mg immediate-release niacin. The striking fall in core temperature (*frigor*) follows massive heat loss from intense dermal vasodilatation approaches decrements seen in hypothermia, coinciding with the rigors and chills that frequently accompany NASTy effects.

2. DETAILED METHODS FOR EACH DEVICE

Stepwise application of colorimetry, white light spectroscopy (WLS), laser Doppler flowmetry (LDF), and thermometry to measure NASTy physical stigmata.

2.1 Pre-procedure preparation and precautions:

1) Ensure the study room is at a comfortable temperature, ideally 20-24°C, as described in later sections. Variations in ambient temperature should be minimized and ideally kept within this range.

2) Subjects should be allowed to acclimatize to room temperature for at least 20-30min before application of either colorimetry, WLS, LDF, or temperature probes. All heavy clothing should be removed early to allow adaptive vasoconstriction to subside by the time measurement begins.

3) The area of skin to be studied, in this case the face, should be clean. Unbearded men should shave and women should not wear makeup.

4) Ensure the subject is afebrile and does not have any inflammatory skin lesions where the laser and thermometry probes are to be applied. Inflammatory lesions cause increased blood flow measurable by all four methods.

5) In studies of several hours' duration, restrooms should be close to the study room. The ambient temperature of restrooms and any intervening hallways should be as close as possible to the study room temperature.

6) Drinks or food for subject ingestion should be served at room temperature.

7) Encourage subjects to empty their bowel and bladder before inserting the rectal thermistor for core body temperature measurement.

8) Prepare the malar temperature probe by covering the probe by transparent dressing like Tegaderm™ on the skin contact surface and moleskin bandage on the outside surface (for insulation from ambient temperature).

9) Ensure the subject is not near heating or cooling vents, areas of direct sunlight etc to minimize their effect on skin temperature. Perspiration, by causing heat loss can lower temperature readings and should be avoided by keeping temperatures comfortable.

2.2.1 Clinical Colorimetry

1) Attach the clinical colorimetry device to a PC. The device comes with a pre-programmed Excel sheet using Visual Basic for Applications (VBA).

2) Turn on the device. The Excel sheet should automatically start showing growing rows of numbers in the columns R, G, B, and EI. The EI is calculated using the equation in section 1.4.

3) Place the probe on the colors red, green, blue, black, and white on a standard color card (X-Rite Color Checker Classic, X-Rite, Grand Rapids, MI) for 30 seconds each. This establishes a reference standard.

4) Place the probe on the subject's malar eminence. Ensure that the skin does not have any rashes, plaques, excessive hair etc.

5) The Excel-sheet should now start recording different numbers in the RGB columns along with the EI.

Supplemental Figure S3: cyberDERM clinical tristimulus colorimeter.

6) Do not pause the device if measurement has to be interrupted for any reason. Instead, keep the probe on a black or white color on the reference color card. The marked change in recordings helps mark the times when recording was interrupted. Time synchronization is encouraged at these times.

7) Pause the recording at the end of measurement and detach the probe from the patient. Save the Excel-sheet.

2.2.2 Calculation of Erythema Index (EI)

The Erythema Index (EI) is calculated by the clinical colorimetry software using the following formula:

$$EI = 100\,X\,\log\left({I_{red}}/{I_{green}}\right)$$

where I_{red} and I_{green} are intensity of reflected red and green lights, respectively.

2.3 White light spectroscopy

1) Follow pre-procedure preparations as in section 2 above.

2) Attach the WLS device to a PC using the USB cord and open the software window. Next, power on the device and attach the probes to the slots provided for the probe cable on the device.

3) In the software window, open "New File" and configure device settings including frequency, display settings etc. Press the start button on the device or the "Record" button in the software window.

4) The WLS has time on the x-axis in 4 sec intervals and hemoglobin concentration in AUs on the y-axis. Before application of the probes, the tracing should be near or at 0 on the y-axis and the graph should start moving to the right reflecting passage of time on the x-axis.

5) Clean the skin gently with an alcohol swab. Do not rub very hard as it may increase skin perfusion. Affix the plastic laser probe-holder to the non-dominant cheek (left cheek in a right-handed person) using a pre-cut transparent double-stick tape.

Supplemental Figure S4: Upper panel: moorVMS-OXY^{TM} WLS device. **Lower panel:** Moor instruments OP1-1000 WLS probe. White light emitting and receiving fibres are separated by 1mm. The probe has the same dimensions as the LDF probe i.e 12.5 mm high and 8 mm outside diameter and uses the same probe holder as the LDF probe.

6) Fit the probe into the probe-holder. This allows quick and easy removal and reapplication of the probe, while maintaining the exact measurement site. The probe-holder also keeps the probe perpendicular to the skin, ensuring uniform sampling depth, though repeated removal/reinsertion may affect sampling depth by changing the angle, or the distance of the probe from the skin.

7) The tracing in the software window will be noted to leave the baseline on the x-axis, and appears somewhat irregular which confirms that data is recording.

8) Excessive motion at the probe-skin interface and in the probe cable should be minimized during the study period. It is probably best that the subjects not eat during the study period.

9) Ensure that the WLS probe is not pressed too hard against the skin as that can squeeze blood out of the tissues and falsely underestimate hemoglobin measurements.

10) Throughout the study, perfusion data should be saved at regular intervals and the device and study clock synchronized, as detailed below in "Time synchronization" section. Since various versions of Windows can crash unexpectedly, it is critical to save frequently. Some versions of the software save the data in the background but this is feature does not predictably pass down to new versions. Since quality control on this feature is lacking, we strongly advise saving independently

11) The probe can be removed from the probe-holder whenever the subject needs to move away from the monitor, for example to go to the bathroom. Allow the tracing to continue, lest elapsed times diverge from time of day. The probe can simply be inserted into the probe holder once the subject returns. The probe-

holder should not be removed under any circumstances throughout the study. Alternatively, the devices and the computer can be housed on a rolling cart that subjects can take to the bathroom

12) At the end of the study interval, stop the recording and follow on-screen prompts to save the data file. Probe and probe-holders may now be removed.

2.4.1 Laser Doppler Flowmetry:

1) Ensure the subject is relaxed and acclimatized to room temperature, for 20-30 minutes. Allow the LDF device to warm up for at least 15 minutes before commencing measurement.

2) Attach the laser Doppler to a PC using the USB cord and open the software window. Next, power on the laser Doppler and attach the probes to the slots provided for the probe cable on the device.

3) In the software window, open 'New File' and configure device settings including frequency, display settings etc. Press the start button on the device or the 'Record' button in the software window.

4) The Doppler graph has time on the x-axis in 4 sec intervals and skin perfusion in PUs on the y-axis, similar to WLS. Before application of the probes, the tracing should be near or at 0 on the y-axis and the graph should start moving to the right reflecting passage of time on the x-axis.

5) Clean the skin gently with an alcohol swab. Do not rub very hard as it may increase skin perfusion. Affix the plastic laser probe-holder as above with WLS.

6) Fit the probe into the probe-holder. This allows quick and easy removal and reapplication of the probe, same as WLS.

7) The tracing in the software window will be noted to leave the baseline on the x-axis and appears somewhat irregular which confirms that data is recording.

8) Excessive motion at the probe-skin interface and in the probe cable will lead to artifact and should be minimized during the study period. It is probably best that the subjects not eat during the study period.

9) Ensure the LDF probe is not pressed too hard against the skin, as that can provoke capillary vasodilatation.

10) Throughout the study, perfusion data should be saved at

Supplemental Figure S5: **Upper Panel:** moorVMS-LDF™ monitor. The device comes with one or two-channel options. Multiple modules can be combined for a multi-channel system. **Middle Panel:** VP1T probe with emitting and receiving fibres incorporated into a single probe to minimze motion artifact. Each fiber is 0.5 mm in diameter and separated by 0.5mm. The probe has a height of 12.5mm (at the probe tip) and 8mm outside diameter. The groove around the tip of the probe allows the probe to clip into the probe holder. L**ower panel:** PH1-V2F probe holder.

regular interval due to the reasons mentioned above for WLS, and detailed below.

11) The probe can be removed from the probe-holder whenever the subject needs to move away from the monitor, for example to go to the bathroom, similar to WLS above. Allow the tracing to continue, as for WLS. The probe can simply be re- inserted into the probe holder. The probe-holder should not be removed throughout the study. Alternatively, the devices and the computer can be housed on a rolling cart that subjects can take to the bathroom.

12) At the end of the study interval, stop the recording and follow on-screen prompts to save the data file. Probe and probe-holders may now be removed.

2.4.2 Graph Smoothing Methodology and Curve Parameterization

For WLS and LDF, to improve computational time, the sampling rates were compressed to 1 Hz for analysis by taking the median of the 40 readings for each second of the 8-hour observation. All points accompanied by zero voltage were excluded, because these represent times when the instrument's lamp was turned off and not recording valid readings, as when subjects went to the toilet. LDF tracings typically have non-physiological spikes in the positive direction. Because a key parameter is height of the peak, failure to remove these spikes might lead to severe overestimations of peak and bias the assessment of plethora away from null. It is therefore advisable to conservatively remove such artifacts. We did so by first fitting a Lowess curve to the raw data, and then identifying outliers defined as points more than 5 times the interquartile range above or below the median for a rolling window of observations. Each curve was visually inspected to confirm that such points properly identified artifacts. If not, such points were re-included; in practice, this method only identified artifacts. Next, we re-fit the Lowess curve after excluding confirmed outliers. We visually inspected the resulting smooth to assure good curve fitting. Curve parameters were then derived from the smooth, again to avoid overestimation of peak. To further guard against overestimating the peak, we first determined the peak, and then averaged the points within plus or minus 5 minutes of the peak so that a 10-minute window was used to determine the peak. This is again conservative, to avoid biasing away from null.

For all modalities, curve smoothing and parameterization were conducted in Stata v14.1 (College Park, TX). Pharmacodynamic curve parameters include c-max, incremental c-max/peak, time of max/peak, c-min, incremental c-min/nadir, time of min/nadir, and area under the curve (AUC) or area over the curve (AOC). Areas were calculated by the trapezoidal rule. Incremental areas were separately calculated for supra-basal area (i.e. AUC) and infra-basal area (i.e. AOC), and net incremental area (iAUC) as the difference.

2.5 Thermometry:

It is best to set up and start the temperature monitoring first to allow the probes to equilibrate to body temperature while any other device is being set up.

1) Connect the OctTemp logger to a PC using the accompanying cable. Test the logger link and logger battery using the MadgeTech software before starting recording.

2) Set the current PC time as the logger time then specify the start and stop times for temperature recording. Allow for a liberal margin past the anticipated study stop time to allow for any unforeseeable delays.

3) The logger may now be disconnected from the PC until data extraction. We prefer to continuously record on the PC.

Supplemental Figure S6: Madgetech OctTemp 8 channel thermocouple based temperature logger. Thermocouple probes were custom made and are detailed in Figure S6.

4) Mark a spot 4-6 inches from the tip on the rectal thermistor to ensure adequate depth of insertion. Dip the thermistor in KY jelly and insert into the rectum up to the mark. Again ensure that the urinary bladder is evacuated prior to placement of the thermistor.

5) Secure the thermistor with tape posterior and superior to the anus and course the cable through the intergluteal fold. This allows the subject to sit comfortably without being hindered by the thermistor wire, and helps it stay in place. Secure with a second tape at the top of the gluteal fold.

6) Lightly wipe the skin of the dominant face cheek with alcohol swab and place a 6cm x 7cm clear Tegaderm on the dominant cheek immediately below the cheek bone (contralateral to the LDF probe).

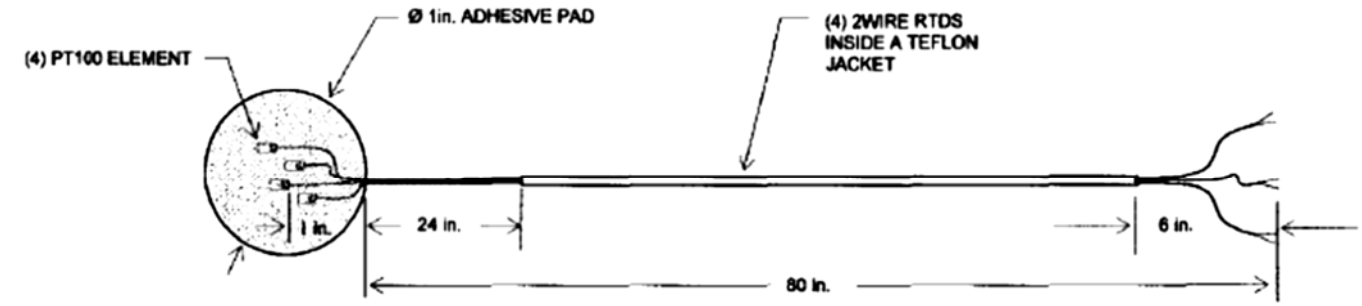
7) Place the moleskin-covered thermistor over the Tegaderm and cover with duoderm for additional insulation and apply medical tape around the edges. Ensure thermistor orientation so that the wire can be run over the ear without excessive twisting or kinking, while allowing free head movement.

8) Anchor the malar probe wire by tape immediately in front of the ear and a second tape over the mastoid process.

9) Place the ambient temperature probe in a plastic fenestrated ball (Wiffle ball) or tape to a secure spot away from any heating/cooling sources. This will guard against the ambient thermistor getting enmeshed in the bedding.

12) At the end of the study, remove all thermistors (rectal and malar) from the subject.

A



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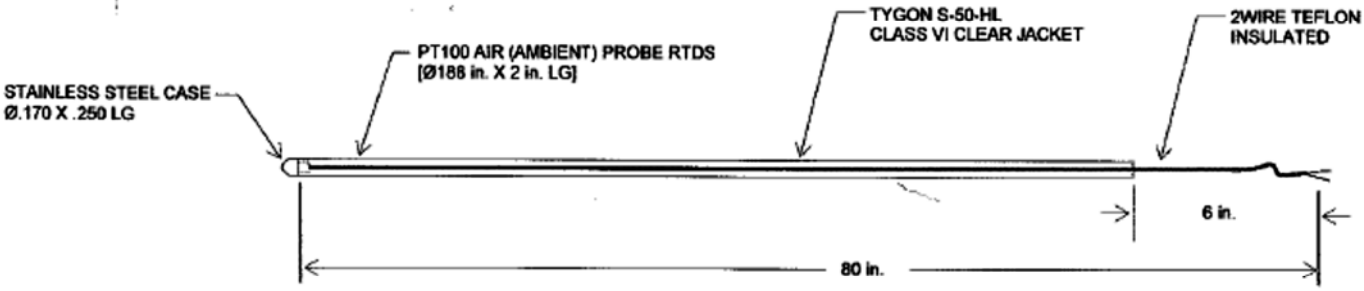

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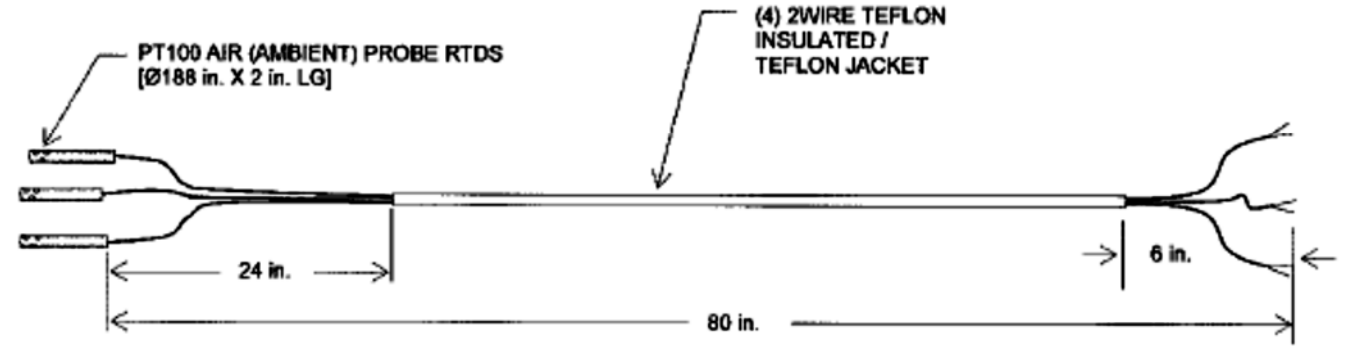


Supplemental Figure S7: Diagram depicting the custom made RTD (resistance temperature detectors) temperature probes used for dermal thermometry. The probe exploits the predictable change in resistance, in certain materials, produced by a change in temperature of the material. A) depicts the malar temperature probe consisting of 4 two-wire RTDs in an insulated Teflon jacket with the sensor element embedded in an adhesive pad for easy application. B) depicts the core temperature probe consisting of a single two-wire RTD with the wires in a semi-rigid clear jacket and the element encased in a steel case to enable rectal insertion.C) depicts the ambient temperature probe consisting of three two-wire RTD in an insulated Teflon jacket.

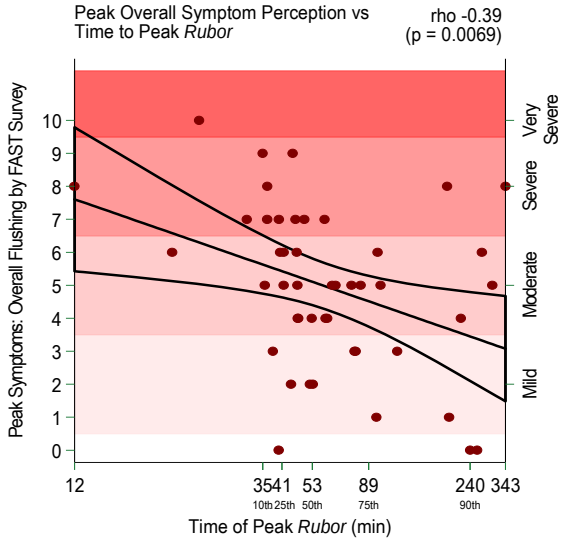
2.6 Time Synchronization

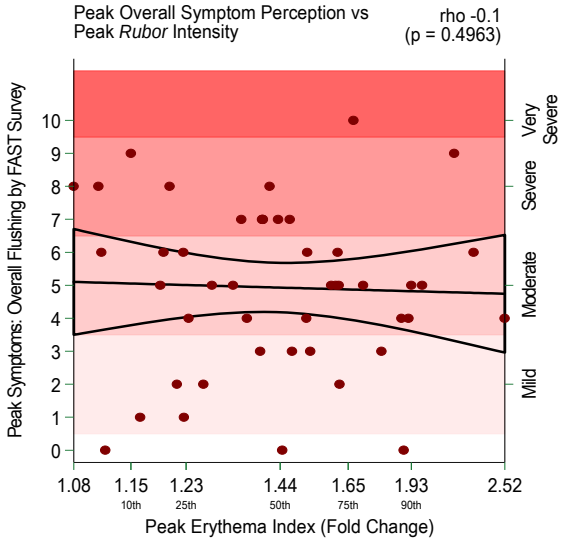
Because each device has a different internal clock, it is important to synchronize all devices to a standard digital clock for all study encounters. This allows accurate timing of colorimetry, WLS, LDF, and thermometry changes relative to each other at baseline and post-niacin ingestion. Synchronization also allows marking of significant events. For example time of niacin-ingestion or time during which the LDF probe might have been removed to allow subject movement.

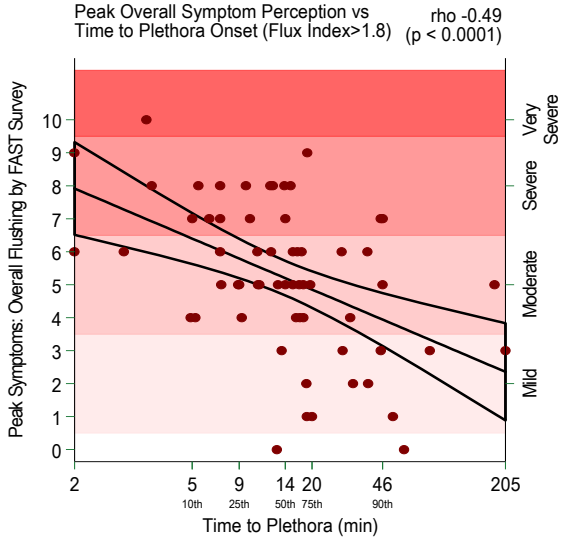
1) A standard digital clock should be placed in the study area, clearly visible with large numerals. Ideally, the same clock or model should be used for all subjects and study visits.

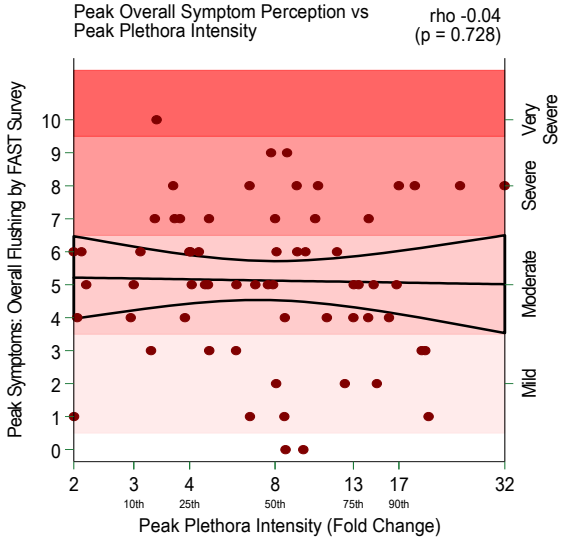
3) The laser Doppler and WLS elapsed times should be recorded at the same tim

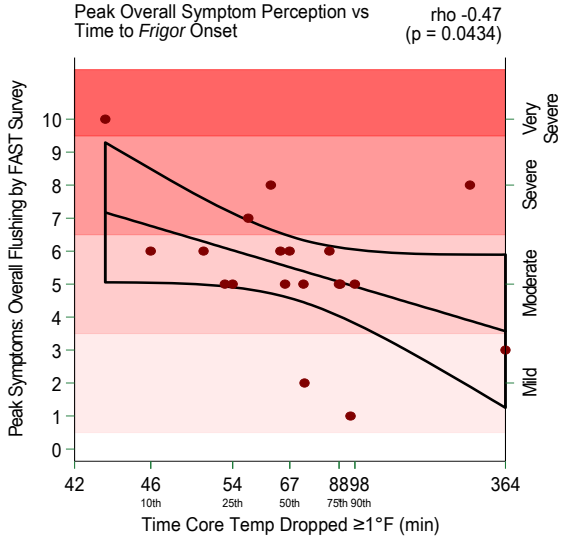
3. SUPPLEMENTAL DATA

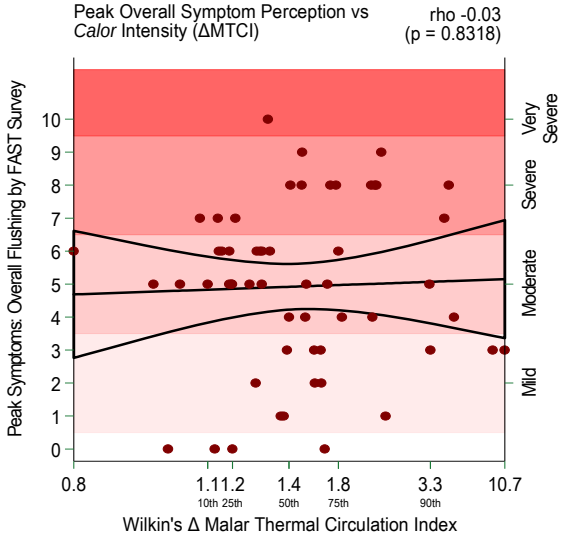












Supplemental Figure S8: Scatter plots corresponding to each bar in Figure 5 in main body of the manuscript, depicting the relationship between physical measurements of objective stigmata with overall subjective flushing perception. The y-axis in each panel represents peak overall flushing perception on the FAST survey from 1-10. The x-axis depicts $10^{th}/25^{th}/50^{th}/75^{th}$/and 90^{th} percentiles of the spread of physical measurements. The strength of the relationship between subjective flushing perception and physical stigmata measurements is assessed by Spearman's rho, given atop each panel. Top panels: representing the relationship of the colorimetric measurements of time to peak *rubor* (left), and peak *rubor* intensity (right), measured as erythema index, with peak overall flushing. Middle panels: relationship of plethora onset time by laser Doppler flowmetry (left), and peak plethora (right) with peak overall flushing perception. Lower panels: relationship of the time taken for *frigor,* i.e. core temperature to fall by 1degree Fahrenheit (left), and ΔMTCI (right) with peak overall flushing perception.