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# The $\alpha_{2C}$-adrenoceptor deletion322-325 variant and cold-induced vasoconstriction

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## Abstract

**Objectives—**Cold-induced vasoconstriction is mediated in part by selective enhancement of local $\alpha_{2C}$–adrenoceptor ($\alpha_{2C}$-AR) activity. A common insertion-deletion variant in the $\alpha_{2C}$-AR gene (*ADRA2C* del322-325) results in an approximately 85% reduction of agonist-mediated function *in vitro*. We tested the hypothesis that individuals with the *ADRA2C* del322-325 variant have attenuated vasoconstriction in response to cold.

**Methods—**Cutaneous digital blood flow (flux) was measured by laser Doppler flowmetry in a controlled environment at room temperature and during two cycles of graduated local heat and cold exposure in 31 subjects. Temperature-response curves were analyzed to estimate the following measures: $E_{min}$ (minimal flux during cooling), and $ET_{50}$ and $ET_{90}$ (the local temperature at which flux decreased by 50% and 90% respectively).

**Results—**We found no significant genotypic differences in $E_{min}$ (24.3±19.5, 30.0±20.5, and 21.5 ±25.9 AU for ins/ins, ins/del, and del/del genotypes, respectively; P=0.48), $ET_{50}$ (25.5±6.0, 25.1 ±6.7, and 25.1±7.1 °C; P=0.99), or $ET_{90}$ (20.5±4.7, 22.1±4.0, and 20.8±6.7 °C; P=0.77) in either the first or second heating and cooling cycle (cycle 1 values presented).

**Interpretation—**The *ADRA2C* del322-325 variant did not affect vascular sensitivity to local cold exposure.

### Keywords

adrenergic receptor; microcirculation; ADRA2C; cold; laser Doppler flowmetry

## Introduction

Cold-induced vasoconstriction of peripheral blood vessels serves as a physiologic reflex to conserve central body heat during cold exposure. This occurs via a reflex increase in sympathetic activity coupled with local alterations resulting in increased vascular sensitivity to norepinephrine [8,11]. Vasoconstriction in response to cold is mediated by the rapid selective enhancement of local $\alpha_2$–adrenergic receptor ($\alpha_2$-AR) activity [19,12] and is inhibited by an $\alpha_2$-AR antagonist [9]. Excessive cold-induced vasoconstriction is the hallmark of Raynaud's phenomenon, a clinical disorder that affects between 8-11% of the general population [34], and Raynaud's phenomenon has been linked to $\alpha_2$-AR dysfunction [14,15].

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There are three subtypes of $\alpha_2$-ARs, $\alpha_{2A}$, $\alpha_{2B}$, and $\alpha_{2C}$ [4,28]. At 37°C, $\alpha_2$-AR mediated vasoconstriction is conveyed primarily by the $\alpha_{2A}$ subtype [8], and the $\alpha_{2C}$-AR has minimal activity, as it is not expressed at the cell surface, but sequestered predominantly in the Golgi apparatus [19]. Hence, the $\alpha_{2C}$-AR was originally thought to be a "silent receptor [8]." However, cooling activates rho-kinase signaling [1] and evokes a redistribution of $\alpha_{2C}$-ARs to the cell surface, enhancing vasoconstrictor responses to norepinephrine [19] and mediating vasoconstriction in response to cold. Furthermore, selective inhibition of the $\alpha_{2C}$-AR attenuates the increased sensitivity to adrenergic agonists during cold exposure [8]. Thus, the $\alpha_{2C}$-AR is a key mediator of vasoconstriction in response to cold and, therefore, has become a drug target for the development of subtype selective antagonists. Indeed, pharmacological treatment with such an investigational selective $\alpha_{2C}$–AR antagonist has been explored as a potential therapy for Raynaud's phenomenon [37].

A common genetic variant in the human $\alpha_{2C}$-AR gene (*ADRA2C*) results in the deletion of four amino acids, del322-325 [30]. The frequency of the deletion allele is 42.5% and 6.3% in African-Americans and Caucasians, respectively [32], and it results in a reduction of approximately 85% of agonist-mediated function *in vitro* [30,31]. In view of the $\alpha_{2C}$-AR's key role in cold-induced vasoconstriction, the decreased function of the receptor encoded by the del322-325 variant may result in attenuation of cold-induced vasoconstriction. However, to date, the effects of this variant on vascular sensitivity to cold have not been defined.

We previously developed a method to measure changes in skin blood flow in response to controlled incremental local cold exposure [16], and we used it in the present study to examine the hypothesis that subjects with the *ADRA2C* del322-325 variant have decreased vascular sensitivity to cooling. Since exposure to cold is required for $\alpha_{2C}$-ARs to translocate to the cell surface and become active, we performed a second cold exposure test by repeating the heating/cooling cycle to examine whether previous cold exposure would change subsequent skin flux responses to cold.

## Methods

### Subjects

The study was approved by the Vanderbilt University Institutional Review Board, and all subjects provided written informed consent. To enrich the study population for the genotype of interest, we studied 31 African-American subjects, since the *ADRA2C* del322-325 variant is rare in Caucasians. Unrelated males and females were eligible to participate if they were African-American, between 18-40 years of age, nonsmokers, and had no clinically significant abnormality based on medical history and physical examination. No laboratory screening was performed for this study, though for 29 of the 31 subjects, red blood cell (RBC) count values were available from previous recent studies, and these are included in Table 1. Ethnicity was determined by self-report. Subjects were free of medications for at least 2 weeks. Since cutaneous blood flow varies with the menstrual cycle [6], all women were studied in the luteal phase of the menstrual cycle.

### Protocol

Subjects were admitted to the General Clinical Research Center (GCRC) of the Vanderbilt University Medical School at 8:00 am on the morning of the study day after an overnight fast. All studies were performed in a temperature-controlled room (24.0±1.0°C). After 30 minutes of acclimatization, a 20 G intravenous cannula was inserted into an antecubital arm vein for blood sampling. After an additional 30 minutes of supine comfortable rest, resting blood pressure and heart rate were obtained by a semi-automated device (Dinamap MPS; GE Medical

Systems, Waukesha, Wis, USA), and a venous blood sample (12 mL) was drawn from the uncuffed arm for determination of plasma norepinephrine concentration.

The subject's contralateral hand was then inserted to a fixed location inside a thermally inert plexi-glass box. Laser probes (DP1T-V2, Moor Instruments, Devon, UK) were attached to the pulp of the distal phalanges of the third and fourth digits. Skin blood flow was assessed by laser Doppler flowmetry (DRT4, Moor Instruments, Devon, UK), and skin temperature by a thermocouple. The laser Doppler flowmeter produces an output signal based on the reflection of light off moving red blood cells (RBCs). This signal is proportional to the microvascular blood flow (flux), a product of the number of RBCs in the sampling volume and the mean velocity [3]. Laser Doppler flux is proportional to blood flow, though the absolute value of the arbitrary units depends on many factors. There is considerable inter-individual variability in flux measurements; therefore the greatest utility of laser Doppler lies in assessing relative within-subject changes in flow, rather than absolute flux differences between subjects [3]. Skin flux and skin temperature were recorded continuously on an attached computer. The box temperature was controlled by a modified heating and air conditioning unit (Whirlpool ACE082XR). A metal baffle placed before the airflow inlet ensured even temperature distribution within the box, as confirmed in preliminary studies [16].

After an acclimatization period of at least 60 minutes, baseline skin flux and temperature measurements were recorded at room temperature for 10 minutes. The temperature in the box enclosing the hand was then increased gradually to 40.5° within 5 minutes and maintained at that temperature for 20 minutes (Figure 1) [16]. During the following 60 minutes, the temperature in the box was decreased stepwise at 2 minute intervals in approximately 2.5°C decrements to a minimum temperature of 8°C. We then performed a second cold exposure test by repeating the heating/cooling cycle to examine whether previous cold exposure would change subsequent skin flux responses to cold (Figure 1). During each cooling cycle, if skin flux did not change over three stepwise temperature decrements, this value was considered to represent maximum vasoconstriction in response to cold, and the cooling cycle was ended.

Blood samples (12 mL each) were drawn, and blood pressure was measured after 20 minutes at 40.5 °C during the 2 heating cycles, and at the minimum temperature during the 2 cooling cycles.

### Catecholamine Determination

Blood was collected into cooled heparinized tubes, immediately placed on ice, and centrifuged at 4°C for 10 minutes at 3,000 rpm. Plasma was harvested and stored in tubes containing 40 μL of reduced glutathione (6%) at -20°C until assayed. Norepinephrine concentrations were measured by high-performance liquid chromatography using electrochemical detection with dihydroxybenzylamine as the internal standard [17].

### Preliminary Studies

We performed a series of studies to optimize the heating/cooling protocol and to assess reproducibility. Preliminary studies on within-subject reproducibility of $ET_{50}$ (n=4) yielded a day-to-day difference of 1.6±2.9°C.

### Genotyping

After amplification of DNA fragments by polymerase chain reaction (PCR), *ADRA2C* del322-325 genotyping was performed by DNA fragment analysis as described previously [23]. In brief, a fluorescently labeled forward primer (5′-6-FAM-AGACGGACGAGAGCAGCGCA-3′) and a reverse primer (5′-AGGCCTCGCGGCAGATGCCGTACA-3′) were used to amplify DNA fragments by PCR.

Amplicons were denatured at 95°C for 5 min, and fragment analysis was performed on an ABI 3730 Genetic Analyzer and its Genotyper V.1.0.1 software. The success rate for genotyping was 100%. We use the term "*ADRA2C*" when referring to the gene and "$\alpha_{2C}$-AR" when referring to the receptor encoded by the gene. Individuals homozygous for the *ADRA2C* insertion (wild type) allele, homozygous for the deletion allele, and heterozygous are termed *ADRA2C* "ins/ins," "del/del," or "ins/del," respectively.

### Data Analysis and Statistics

Skin temperature and skin blood flow (flux) were calculated as the average of the readings in the two fingers. Baseline skin temperature and flux were calculated as the average of values obtained over 10 minutes after subjects had been acclimated in the room for at least 60 minutes. For the heating/cooling cycles, for each subject, average flux during every 2 minute temperature step was plotted against the box temperature, and nonlinear regression analysis was used to fit a sigmoidal curve (Prism v4.0, GraphPad Software Inc., San Diego, CA) (Figure 2). The software provides estimates for the steady-state blood flow during the heating stimulation ($E_{stim}$), the minimum blood flow achieved during cooling ($E_{min}$), the box temperature at which flux decreased by half the maximal effect ($ET_{50}$), and the box temperature at which flux decreased by 90% ($ET_{90}$) (Figure 2). We interpreted $E_{min}$ to reflect maximal cold-induced vasoconstriction, and $ET_{50}$ and $ET_{90}$ to reflect the sensitivity to cooling. The software also calculates the $R^2$ value as a measure of goodness of curve fit.

Skin temperature, which is determined by both ambient temperature and flux, was analyzed with the following parameters: $T_{stim}$ (the maximum skin temperature during heating stimulation), $T_{min}$ (the skin temperature at the time point of $ET_{90}$, at which flux had decreased to 90% of the maximum change ($E_{stim}$ - $E_{min}$)), and $T_{mid}$ (the skin temperature at the time when box temperature measured 24.5 °C, a midpoint, approximately 71 minutes into the study). Mean arterial blood pressure was calculated as the diastolic blood pressure plus one third of the systolic minus the diastolic pressure. All values are reported as mean ± standard deviation. A Kruskal-Wallis or chi square test was used to compare values in subjects with different *ADRA2C* genotypes. A repeated measures analysis of variance (ANOVA) was used to compare plasma norepinephrine concentrations and mean arterial pressure readings, using *ADRA2C* genotype as a covariate. All tests were two-tailed, and a P-value of $< 0.05$ was considered significant. Analyses were performed with the statistical software SPSS (SPSS v14.0, SPSS Inc., Chicago, IL).

## Results

### Subject Characteristics

Demographic characteristics arranged by *ADRA2C* genotype are shown in Table 1. There were no differences among subjects of different genotypes in any of the variables.

### Skin Flux and Skin Temperature Measures

The mean (± SD) goodness-of-fit for the temperature-flux response curves, expressed as $R^2$, was 0.89 ± 0.09. There were no significant differences in baseline skin blood flow (flux), $E_{min}$, $ET_{50}$, or $ET_{90}$ in either the first or second heating or cooling cycle between genotypes (Table 2). Similarly, genotype was not associated with the skin temperature responses measured by $T_{stim}$, $T_{min}$, and $T_{mid}$. The distributions of $ET_{50}$ and $ET_{90}$ for the first heating/cooling cycle are shown in Figure 3. The stimulated flux during the first heating cycle ($E_{stim}$) was lower in the *ADRA2C* del/del genotype group than the ins/del and ins/ins genotype groups (P=0.01), but there was no difference in $E_{stim}$ during the second cycle (P=0.64). There were no differences in the flux or skin temperature parameters between cycles 1 and 2.

### Cardiovascular Measures and Plasma Norepinephrine Concentrations

Among subjects with different *ADRA2C* genotypes, there were no significant differences in mean arterial blood pressure (MAP) at baseline (Table 1, P=0.69) or during the course of the study (all P>0.21), and MAP did not change over time (P=0.78). Similarly, plasma norepinephrine concentrations were not different at baseline (390.5±258.4, 347.9±165.3, and 327.0±146.7 pg/dL, for ins/ins, ins/del, and del/del genotypes respectively, P=0.99), and the values did not change significantly during the study (P=0.98).

## Discussion

This is the first study to examine the effects of a common, functionally significant deletion polymorphism in the $\alpha_{2C}$-adrenergic receptor on cold-induced vasoconstriction. Baseline skin blood flow and skin temperature were not associated with *ADRA2C* genotype, and neither the magnitude of vasoconstriction during cooling ($E_{min}$), nor the sensitivity of vasoconstriction to cold ($ET_{50}$) differed among *ADRA2C* genotypes.

Cold-induced vasoconstriction is an intricate physiologic defense mechanism that serves to reduce heat loss. Cooling induces vasoconstriction of arterioles via rapid augmentation of $\alpha_2$-AR activity. Both *in vitro* and *in vivo* studies have shown that inhibition of $\alpha_2$-ARs impairs the cold-induced vasoconstriction response [8,9]. $\alpha_{2C}$-ARs are located predominantly in small arteries, arterioles, and veins [10,13,24] and, although “silent” at 37°C, become the major effectors of vasoconstriction during cooling [11]. In response to cold, the $\alpha_{2C}$-ARs translocate from the Golgi apparatus, where they are sequestered at physiologic temperature, to the cell surface, amplifying vasoconstriction in response to norepinephrine [19]. The response is likely further magnified by increased local sympathetic activation, resulting in greater norepinephrine release from the nerve terminal [21]. *In vivo* studies suggest that $\alpha_2$–AR upregulation is quite rapid and that $\alpha_2$–AR mediated vasoconstriction can be seen as early as 1-5 minutes into cooling [7]. While the important role of $\alpha_{2C}$-ARs in cold-induced vasoconstriction is well established, other mechanisms are also involved. For example, the production of reactive oxygen species in the smooth muscle cell mitochondria appears to function as a temperature sensing mechanism initiating the cascade [2]. Subsequently, rho-kinase acts as a mediator whereby the $\alpha_{2C}$-AR translocates to the plasma membrane [1,35].

Recent studies have shown that both adrenergic and non-adrenergic mechanisms contribute to varying degrees, at different times, to the vascular response to cooling. The adrenergic mechanism, mediated by $\alpha_{2C}$-AR, is responsible primarily for the early response to cooling (within the first 10 minutes). After prolonged cooling (>30 minutes), non-adrenergic mechanisms become more prominent [21,38]. Adrenergic blockade does not prevent this late phase vasoconstriction. Inhibition of nitric oxide synthase appears to play a role in this late response, though the sympathetic cotransmitter neuropeptide Y [21] and endothelin [39] may potentially be involved.

The *ADRA2C* del322-325 variant results from the in-frame deletion of 12 nucleotides in the coding region. The variant is located in the third intracellular loop of the $\alpha_{2C}$-AR, in close proximity to the $G_i$ coupling domain. The deletion variant of the $\alpha_{2C}$-AR exhibited impaired agonist-receptor-G protein coupling in transfected cells, resulting in approximately 85% loss of function [30]. Studies to elucidate the significance of this variant in man are limited. One case-control study showed that African-American subjects who were homozygous for the *ADRA2C* del322-325 variant had an increased risk of heart failure, compared to heterozygotes and non-carriers [33]. Additionally, a small controlled study in healthy subjects suggested that individuals homozygous for the deletion variant had higher blood pressure and norepinephrine secretion [27]. However, more recent studies in larger cohorts did not show an association of the *ADRA2C* del322-325 variant with blood pressure and heart rate [23,25], more specific

markers of resting sympathetic activity [23], or early markers of congestive heart failure [5]. Despite the importance of the $\alpha_{2C}$-AR in the vascular response to cold, there have been no studies to assess the effect of *ADRA2C* genetic variants on cold-induced vasoconstriction.

Given the finding that *ADRA2C* del322-325 leads to a marked loss of function *in vitro*, our hypothesis was that the *ADRA2C* variant del322-325 would be associated with a decreased vasoconstrictor response to local cooling. Our main outcome was the cooling response, assessed by the variables $E_{min}$, $ET_{50}$, and $ET_{90}$, as well as $T_{min}$ and $T_{mid}$. There were no differences in these variables in either cycle among genotype groups. During the first cycle, there was a trend to a lower baseline flux and a statistically significant lower stimulated flux ($E_{stim}$) in individuals with the homozygous deletion, findings that were not observed during the second cycle. These findings were of marginal significance and are likely to reflect incomplete acclimatization of skin flux responses to the experimental conditions. Moreover, there was no difference in the maximum skin temperature during cycle 1. Additionally, as multiple factors lead to inter-individual variability in flux values, laser Doppler is most informative when assessing relative within-subject changes (such as $ET_{50}$) [3].

We expected carriers of the deletion variant to show an attenuated vasoconstrictor response to cold. The study was sufficiently powered to detect physiologically important differences; the sample size provided 80% power with $\alpha$=0.05 to detect a 6±5 °C difference in $ET_{50}$ between subjects homozygous for the insertion allele and subjects with at least one deletion allele. Though non-adrenergic mechanisms contribute to the late cold-induced vasoconstriction response, in our study, we believe that in using the temperature-response curves and $ET_{50}$ measurements, we selected for adrenergic-dependent responses. The $ET_{50}$ of our temperature-response curves corresponded to a skin temperature typically in the range of 29-31°C, measured after approximately 10-15 minutes of gradual cooling. The contribution of $\alpha_{2C}$-AR activity should have been near maximal during this time of our cooling protocol.

Possible explanations for the lack of effect of the *ADRA2C* del322-325 variant on cold-induced vasoconstriction could be functional redundancy in the vasoconstrictor and vasodilator systems [20], opposing effects of pre- and postsynaptic hypofunctional $\alpha_{2C}$-ARs on vasoconstriction, and that the $\alpha_{2C}$-AR may be sufficient, but not necessary for cold-induced vasoconstriction *in vivo*. Additionally, it is also possible that despite 85% reduced agonist-mediated function *in vitro*, residual activity of $\alpha_{2C}$-AR del/del may be sufficient to effect *in vivo* cooling responses.

Compensatory variation in factors such as $\alpha_{2A}$-ARs, rho-kinase [29,36], or nitric oxide [18], may mask any apparent loss-of-function associated with $\alpha_{2C}$-AR variants. For example, the vasodilation in response to warming is in part related to nitric oxide release [22]. In our study, there was a trend toward decreased $E_{stim}$ flux in individuals with the *ADRA2C* del/del genotype (Table 2). One could theorize that this lower plateau vasodilation reflects decreased nitric oxide production. If this decreased nitric oxide production carried forward into the cooling phase as well, this could mask effects of the *ADRA2C* deletion. In such a situation, the deletion variant $\alpha_{2C}$-AR could still be hypofunctional, trending toward decreased vasoconstrictor activity, but if coupled with an attenuated nitric oxide response, this could shift the balance and restore vasoconstriction to “normal” levels.

Our study was designed to apply local heat and cold stimuli while avoiding extreme temperatures. Cutaneous blood flow is often low at room temperature. Therefore, after obtaining baseline measures, we applied a standardized local heat exposure to achieve vasodilation, reflected by an elevation in flux to steady-state. For the heat stimulation, a box temperature of 40.5°C was selected, as this temperature has been shown to cause vasodilation, while higher temperatures may elicit nociceptive responses that increase skin blood flow via a mechanism independent of temperature itself [22,26]. After heating, we gradually decreased

the ambient temperature until flux reached a minimum. All temperatures in our testing sequence were within a range typically experienced in a temperate climate. The gradual cooling process allowed us to determine skin blood flow responses over the whole range of temperatures, such that we could accurately quantify the sensitivity of an individual's vasoconstrictor response to cold. We repeated the heating and cooling cycle to account for any residual baseline environmental effects present in the first cycle, as well as to assess whether upregulation of $\alpha_{2C}$-AR mediated signaling during cooling in the first cycle would affect responses during the second cooling cycle.

There are several limitations of our study. Our *in vivo* study design allowed us to examine local vascular responses and the effects of genetic variants within a physiologic system. A potential drawback to any *in vivo* study is that the effect of a genetic variant may be attenuated by local and systemic homeostatic and counterregulatory systems. Central sympathetic nervous system activity, partially regulated by $\alpha_{2C}$-ARs, could also affect peripheral vasoconstriction. Thus, we made an effort to avoid systemic responses triggered by heat or cold pain, and the lack of changes in mean arterial blood pressure and plasma norepinephrine concentrations during the study indicates that systemic effects, if present, were minimal.

We believe that our parameters extrapolated from the temperature-response curves reflect adrenergic, $\alpha_{2C}$-AR-mediated mechanisms, since the $ET_{50}$ was measured at a temperature and time point at which cold-induced vasoconstriction has been shown to be most adrenergically mediated. However, validating this using an $\alpha_2$-AR antagonist could have been very informative. Additionally, pharmacologic inhibition of nitric oxide production or rho kinase could have helped elucidate whether there were compensatory changes in these pathways masking effects of the *ADRA2C* del/del genotype.

In conclusion, in this study, the *ADRA2C* del322-325 variant did not affect vascular sensitivity to cold. Future exploration should involve *in vitro* studies to examine the effect of the deletion variant on cold-induced receptor translocation to the cell surface and *in vivo* studies using specific $\alpha_{2C}$-AR antagonists. Moreover, the effect of genetic variants in associated proteins, e.g. rho-kinase, on cold-induced vasoconstriction will be of interest.

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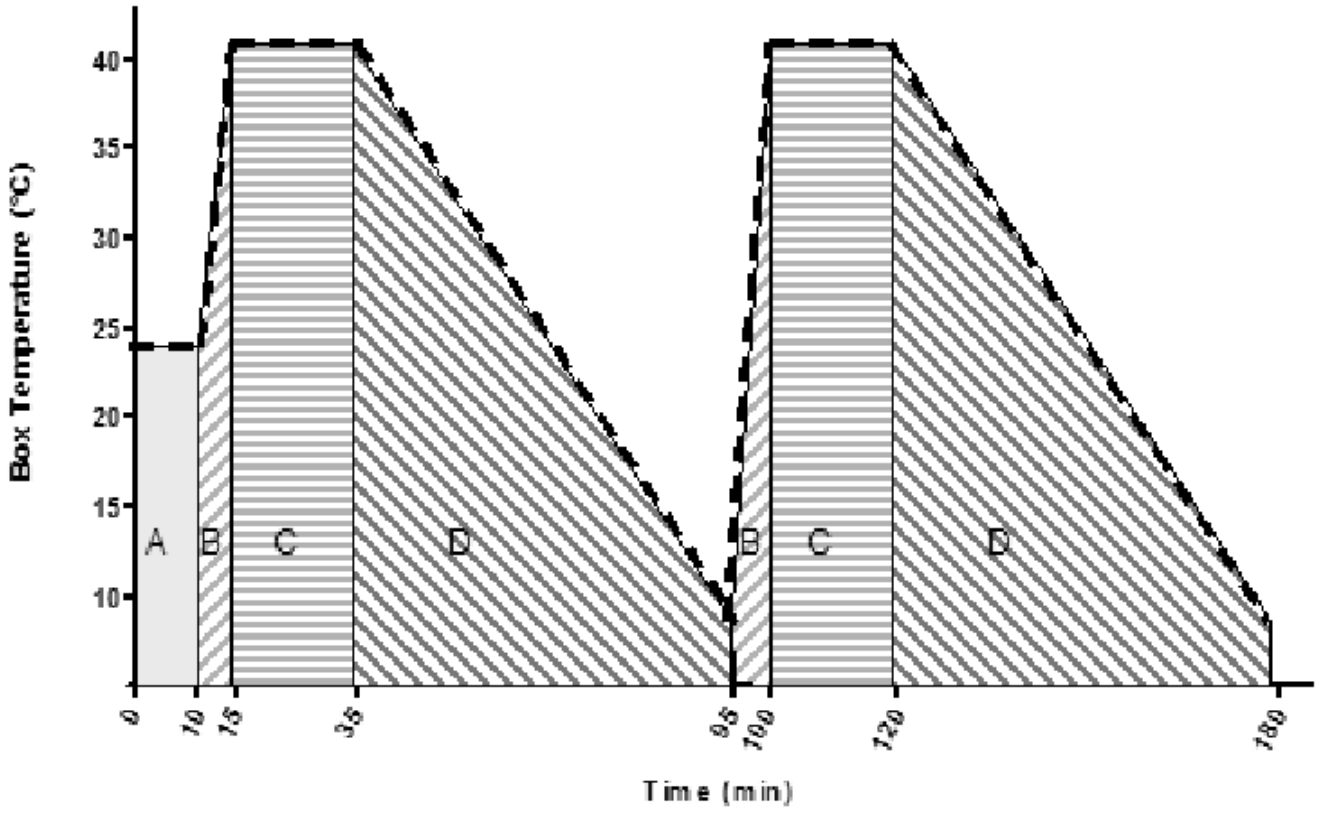


**Figure 1. Study Design**

After 60 minutes of acclimatization, baseline recordings were performed at room temperature for 10 minutes (“A”). Each heating/cooling cycle consisted of three phases: preheating (5 minutes, “B”), maximum temperature held at 40.5°C (20 minutes, “C”), and stepwise cooling (approximately 60 minutes, “D”).

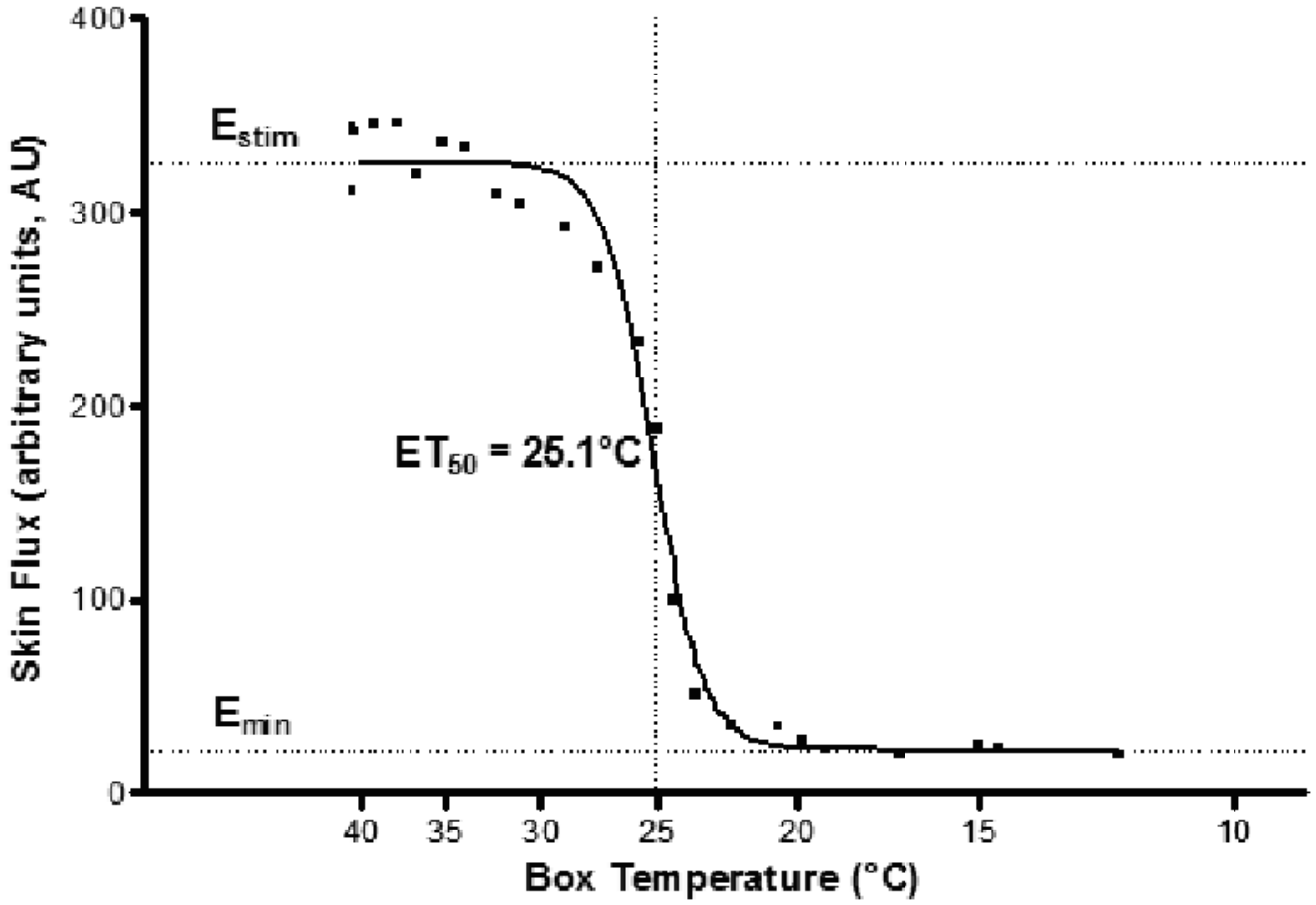


**Figure 2. A representative curve of the relationship between box temperature and skin blood flow**

The measurement parameters $E_{stim}$, (steady-state blood flow after heating), $E_{min}$ (minimal flux during cooling), and $ET_{50}$ (the box temperature at which flux decreased by 50% of the maximal change, $E_{stim}$ - $E_{min}$) are depicted.

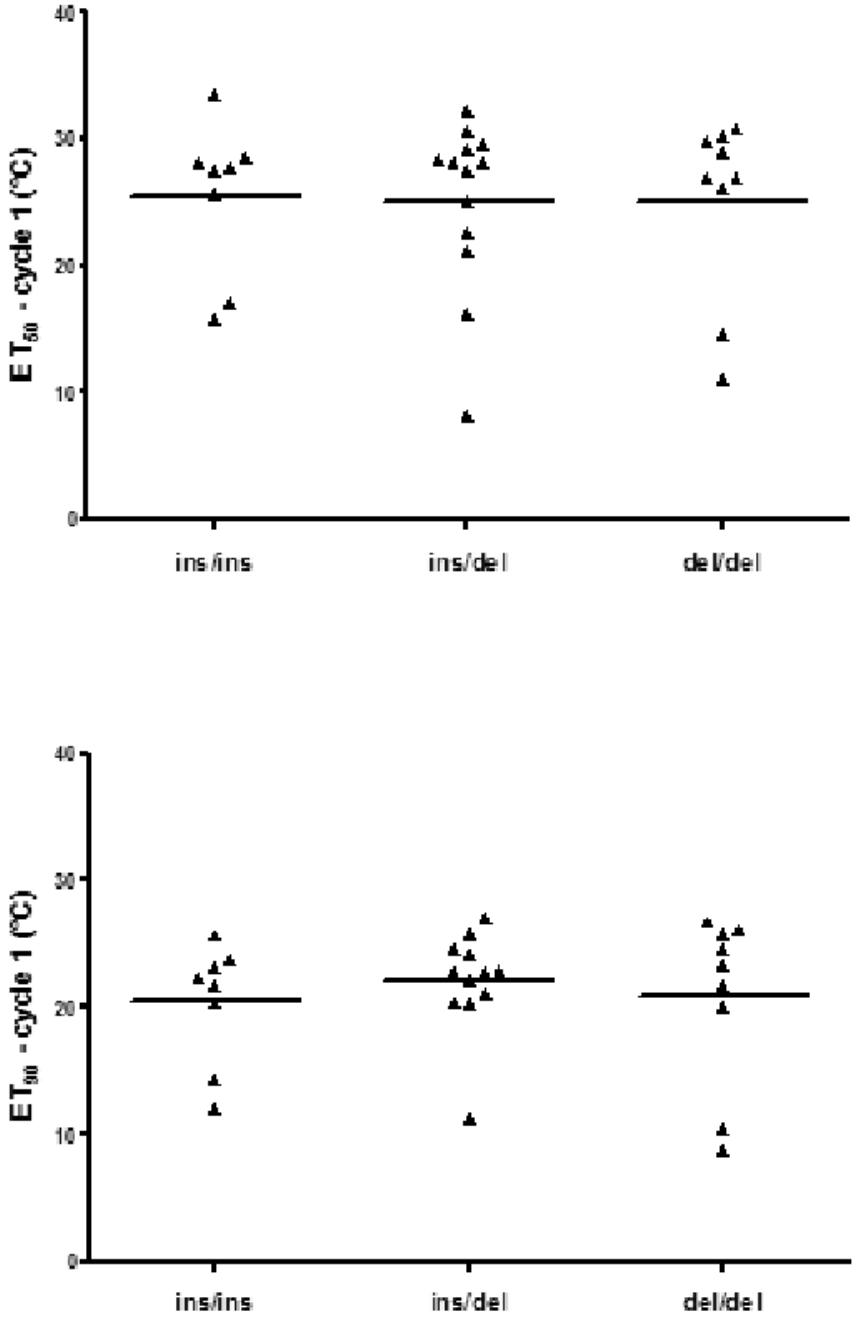


**Figure 3. $ET_{50}$ (upper panel) and $ET_{90}$ (lower panel) in 31 subjects during cycle 1, stratified by *ADRA2C* genotype**

$ET_{50}$ and $ET_{90}$ represent the box temperature at which flux decreased by 50% and 90% of the maximal change, respectively. The horizontal line represents the mean. There were no statistically significant differences among genotypes.

## Table 1

**Demographic characteristics**

Values represent mean ± standard deviation.

| | ADRA2C genotype | | | |
|---|---|---|---|---|
| | **ins/ins (N=8)** | **ins/del (N=14)** | **del/del (N=9)** | **P-value** |
| Age (years) | 27.9±5.9 | 29.1±6.7 | 25.2±3.6 | 0.44 |
| Height (m) | 1.69±0.11 | 1.72±0.14 | 1.70±0.12 | 0.91 |
| BMI ($kg/m^2$) | 28.5±6.4 | 30.9±9.6 | 25.9±3.9 | 0.52 |
| Female:Male Ratio (n:n) | 5:3 | 8:6 | 6:3 | 0.44 |
| Mean arterial pressure at baseline (mm Hg) | 89.3±9.5 | 89.2±9.0 | 87.0±6.6 | 0.69 |
| Red Blood Cell Count (mil/μl) | 4.62±0.31 | 4.60±0.46 | 4.57±0.41 | 0.42 |

## Table 2

**Skin blood flow and skin temperature parameters during the two heating/cooling cycles among ADRA2C del322-325 insertion/deletion genotypes**

$E_{stim}$ and $E_{min}$ represent the stimulated and minimum fluxes achieved during heating and cooling, respectively. $ET_{50}$ and $ET_{90}$ represent the box temperature at which flux decreased by 50% and 90% of the maximal change, respectively. $T_{stim}$ represents the maximum skin temperature achieved during heating; $T_{min}$ represents the skin temperature at the time flux decreased by 90%. $T_{mid}$ represents the skin temperature at the box temperature of 24.5°C. All values represent mean ± standard deviation. P-values are for comparisons among genotype groups.

| | ADRA2C genotype | | | |
|---|---|---|---|---|
| | ins/ins | ins/del | del/del | P-value |
| **CYCLE 1** | | | | |
| Baseline Flux | 240.5±133.3 | 159.6±123.0 | 99.0±122.4 | 0.10 |
| $E_{stim}$ | 291.2±83.0 | 184.6±92.1 | 170.5±73.9 | 0.01 |
| $E_{min}$ | 24.3±19.5 | 30.0±20.5 | 21.5±25.9 | 0.48 |
| $ET_{50}$ (°C) | 25.5±6.0 | 25.1±6.7 | 25.1±7.1 | 0.99 |
| $ET_{90}$ (°C) | 20.5±4.7 | 22.1±4.0 | 20.8±6.7 | 0.77 |
| Baseline Temp | 30.8±4.2 | 29.3±4.3 | 27.4±3.6 | 0.22 |
| $T_{stim}$ (°C) | 37.9±0.6 | 37.6±0.6 | 38.2±1.0 | 0.34 |
| $T_{min}$ (°C) | 25.1±3.1 | 26.3±3.6 | 24.1±4.6 | 0.58 |
| $T_{mid}$ (°C) | 30.1±2.9 | 31.5±2.9 | 29.4±3.9 | 0.37 |
| **CYCLE 2** | | | | |
| $E_{stim}$ | 330.0±145.6 | 281.3±94.5 | 253.6±134.9 | 0.64 |
| $E_{min}$ | 38.1±39.8 | 22.7±25.5 | 19.5±26.5 | 0.43 |
| $ET_{50}$ | 27.7±7.9 | 24.9±6.3 | 27.5±5.7 | 0.54 |
| $ET_{90}$ | 22.7±7.7 | 19.4±6.2 | 20.9±6.7 | 0.84 |
| $T_{stim}$ (°C) | 37.4±0.6 | 37.3±0.9 | 37.4±0.4 | 0.61 |
| $T_{min}$ (°C) | 26.5±6.3 | 23.4±4.3 | 25.0±3.9 | 0.77 |
| $T_{mid}$ (°C) | 31.2±4.7 | 30.8±2.1 | 31.4±2.9 | 0.78 |