Increased blood pressure and hyperdynamic cardiovascular responses in carriers of a common hyperfunctional variant of adenylyl cyclase 6

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Running Title Page:

Running head: Effect of ADCY6 variant on BP and vascular function

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Abstract

Adenylyl cyclase is a critical regulator of metabolic and cardiovascular function. We have recently identified a genetic variant (A674S) in ADCY isoform 6 (ADCY6). Subsequent studies demonstrated that the expression of this ADCY6 variant paralleled an increase in adenylyl cyclase-mediated functions. However, the impact of this hyperfunctional variant on cardiovascular function is unknown. Therefore we evaluated the hemodynamic profile of carriers of ADCY6 A674S. The association of ADCY6 A674S with anthropometric and hemodynamic parameters was assessed in 364 healthy Caucasian subjects. The allele encoding this variant was present in 6.9% of subjects and these individuals had increased blood pressure. To determine the hemodynamic basis for increased blood pressure (BP) in carriers of ADCY6 A674S, we assessed forearm blood flow (FBF), and cardiac output (Q) at rest, during handgrip-exercise (to test vasodilator responsiveness), and with lower body negative pressure (to test forearm vasoconstrictor as well as heart rate [HR] responsiveness) in a sub sample of 21 subjects. At rest, Q and BP were higher in carriers of ADCY6 A674S. This was paralleled by an increase in plasma renin activity, but not in plasma norepinephrine. With handgrip-exercise, FBF and vasodilator responses were greater in carriers of ADCY6 A674S. Responses to reactive hyperemia were not different between genotypes. With lower body negative pressure, the HR response to this orthostatic stress was markedly higher in carriers of ADCY6 A674S.

These data indicate that the relatively common hyperfunctional ADCY6 A674S variant underlies a hyperdynamic cardiovascular response and increased blood pressure.
Introduction

The adenylyl cyclases (ADCY) are a ubiquitously expressed family of enzymes that catalyze the generation of cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP). They regulate a broad range of cellular functions (Patel et al., 2001) and are critical effectors for a number of G protein–coupled receptors (GPCRs). Adenylyl cyclase activation has been suggested to be the rate-limiting step in the GPCR signalling cascade (Ostrom et al, 2000). In vascular cells, activity of adenylyl cyclase regulates acute functional effects including vascular reactivity as well as cellular growth, hypertrophy, and apoptosis (Gros et al., 2006). Furthermore, alterations in the regulation of adenylyl cyclase activity have been implicated in the pathogenesis of hypertension, heart failure, and diabetes (Matsumoto et al., 2005; Roth et al., 1999; Tepe and Liggett, 1999; Roth et al., 2002; Moxham and Malbon, 1996).

Nine membrane-bound isoforms of adenylyl cyclase have been cloned—grouped into 3 major subfamilies comprising the following: Group 1: ADCY1, ADCY3, ADCY8, Group 2: ADCY2, ADCY4, ADCY7 and Group 3: ADCY5, ADCY6 (Patel, 2001). Additionally, ADCY9 has been characterized as a distinct (and atypical) isoform (Sunahara and Taussig, 2002) with restricted expression, and a soluble adenylyl cyclase has been characterized that is the predominant form in mammalian sperm (Wuttke et al., 2001). Each isoform has a specific pattern of tissue/organ distribution and a specific pattern of regulation by G proteins, calcium/calmodulin, and protein kinases (Sunahara and Taussig, 2002; Wuttke et al., 2001; Wang and Brown, 2004).

Variability in cyclic AMP synthesis was thought to be determined predominantly either by the extent of adenylyl cyclase expression, by the specific characteristics of the GPCRs linked to enzyme activation, or by variation in the concentration of the “regulatory factors” (i.e., G
proteins, protein kinases, ions) (Feldman and Gros, 1998). The importance of “genetic”
variability has been unappreciated; that is, the expression of genetic structural variants of the
enzyme that differ by function and/or activity. However, recent studies by us and others have
suggested that variability in the expression of ADCY genetic variants may be an important
regulator of adenylyl cyclase-mediated responses (Small et al., 2003; Ikoma et al., 2003;
Nordman et al., 2008).

Several genetic variants have been described for a range of G proteins and GPCRs linked
to adenylyl cyclase (Rana et al., 2001; Siffert, 2003). Expression of these variants leads to
alterations in receptor-mediated activation of adenylyl cyclase as well as alterations in
downstream effector pathways. The identification of dysfunctional genetic variants of ADCY is
presently limited to those in ADCY6, ADCY3 and ADCY9, of which the latter has a much more
restricted distribution than other isoforms (Small et al., 2003). A single nucleotide polymorphism
(SNP) in intron 17 of gene encoding the very widely expressed ADCY6 isoform (Wang and
Brown, 2004) was identified in a Japanese population (Nordman et al., 2008). However, the
impact of this variant on adenylyl cyclase function is currently unknown. In our initial studies,
we discovered a relatively common (~7% in Caucasians) missense SNP in ADCY6, with the
trivial name of ADCY6 A674S (Gros et al., 2005). In a mammalian vascular cell system,
specifically rat vascular smooth muscle cells transfected using adenoviral constructs, we have
shown that expression of the ADCY6 A674S variant isoform resulted in enhanced adenylyl
cyclase activity and function compared to expression of wild type ADCY6. Further in humans,
expression of the ADCY6 A674S variant correlates with i) increased adenylyl cyclase enzymatic
activity and ii) enhanced adenylyl cyclase-mediated vascular responses (Gros et al., 2007).
However, whether there are detectable alterations in cardiovascular or phenotypic characteristics associated with expression of this variant in humans was unknown.

Consequently, the present studies were performed to assess the association of the ADCY6 A674S variant with phenotypic characteristics within a large group of healthy, younger human subjects. Additionally, in a subset of these subjects we have examined the impact of expression of this genetic variant on hemodynamic responses both at rest and with exercise and orthostatic stress. Data to be presented demonstrate that carriers of the ADCY6 A674S genetic variant have increased blood pressure related to a hyperdynamic profile consistent with the effect of increased adenylyl cyclase function.
Methods

Participants. A total of 364 healthy Caucasian volunteers (age range was 18 to 50 years) were screened. Exclusion criteria included, but were not limited to: history of cardiovascular events, average alcohol intake >2 units per day, pregnancy, and use of antihypertensive/blood pressure altering drugs or anticoagulants. Recruitment was based on local advertising and email invitations for volunteers within the Robarts Research Institute and the University of Western Ontario.

Of the 25 individuals that we identified as expressing the ADCY6 genetic variant we were able to recruit seven individuals (3 male) all of whom were heterozygotic carriers of ADCY6 A674S (hereafter referred to as the ADCY6 variant group) for more detailed analysis of cardiovascular responsiveness. Fourteen individuals (7 male) who did not express the genetic variant were recruited as a Control group. The mean ±SEM ages of the control and ADCY6 variant groups were 26 ± 1 and 26 ± 1 years, respectively. By self-report, all participants were nonsmokers who were free of cardiovascular and neurological disease. Participants reported to the laboratory following at least a 3-h fast and having abstained from caffeine, alcohol, and exercise for a minimum of 12 hours. Preceding experimentation, participants were encouraged to maintain typical water consumption and sleeping behaviors. While the menstrual phase was not assessed, data between males and females displayed no perceptible difference and were, therefore, pooled. Informed consent was obtained for all analyses, with approval from the University of Western Ontario Research Ethics Review Board.

Measurements. During the initial screening, the five measurements of seated blood pressure and heart rate were averaged and recorded (BP Tru, VSM, Vancouver, British Columbia, Canada).
A 10 ml blood sample was taken for biochemical and genetic determinations. Data on sex, weight, height, waist circumference, and smoking status were also obtained. Waist circumferences were normalized by sex, based on the sex specific-ATP III-recommended upper limits (Third Report of the National Cholesterol Education Program [NCEP] Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults [Adult Treatment Panel III] final report. 2002).

In the follow-up study of cardiovascular responsiveness, all measurements were performed with the participants in the supine position in a quiet, darkened room kept at a relatively constant temperature (21–23°C). Heart rate was recorded via a 3 lead ECG (Pilot 9200, Colin Medical Instruments, San Antonio, TX), blood pressure was measured continuously by finger photoplethysmography (Finapres 2300, Ohmeda, Englewood, CO) and validated intermittently via sphygmomanometry. Brachial artery mean blood velocity (4.7 MHz) and diameter (10 MHz imaging transducer) were assessed by pulsed-wave Doppler ultrasound (Echo Ultrasound Imaging; GE Vingmed System Five). Artery images were recorded on a S-VHS video cassette recorder (SVO-9500 MDP, Sony; Tokyo, Japan). Cardiac output (Q) was derived on a beat-by-beat basis from the transfer function of the finger pulsatile pressure waveform, as validated for these maneuvers (Shoemaker et al., 2007; Frances et al., 2008). All analog data were sampled at 1000 Hz using a data-acquisition system (PowerLab, ADInstruments, Colorado Springs, CO). Cardiac output was normalized to body surface area (BSA) (Qc) using the equation of DuBois and DuBois (DuBois and DuBois, 1916): BSA(m²) = 0.007184 × weight (kg)0.425 × height (cm)0.725. Stroke volume (corrected for BSA) was determined from the quotient of Q and HR. Forearm blood flow (FBF) was calculated as FBF = blood velocity × r² ×
60 / V, where r is the brachial artery radius, 60 is to convert flow from s to min and V is volume of the forearm.

**Handgrip exercise:** Adenylyl cyclase activation by a range of metabolic factors is an important determinant of the vasodilator response to exercise. To assess whether those subjects expressing the ADCY6 genetic variant demonstrated an exaggerated vasodilatory response to exercise we performed handgrip protocols. Baseline measures were recorded for 60 seconds, followed by three repeated 5-sec handgrip contractions with 60 s of recovery following each trial. The handgrip contraction intensity was 40% of the maximal voluntary contraction force, determined before this protocol from 3 to 5 trials of voluntary maximal hand-gripping. The dilator response to this contraction was assessed over a 5-sec average immediately following each contraction and averaged to produce the mean FBF responses.

**Flow mediated dilation:** Beyond adenylyl cyclase activation, vasodilator responses to exercise may also be mediated by endothelial factors (e.g. nitric oxide) in response to enhanced endothelial shear stress (Clifford and Hellsten, 2004). This flow-mediated dilation (FMD) is predominantly mediated by endothelial responses. Thus, to determine whether any alterations seen in handgrip responses might be related to endothelial factors, a reactive hyperemia and FMD protocol was performed. While supine, a rapid inflation/deflation pneumatic cuff (Hokanson®, D.E. Hokanson Inc., Bellevue, WA) was placed around the right forearm immediately distal to the olecranon process to provide forearm ischemia and avoid excessive myogenic contributions to brachial artery dilation following ischemia (Betik et al., 2004; Corretti et al., 2002). Ultrasound parameters were set to optimize longitudinal, B-mode images.
of the lumen/arterial wall interface. Specifically, central and forearm hemodynamics were recorded during 5 min of baseline, forearm cuff inflation to >200 mmHg for 5 min and then for 3 min after cuff deflation.

Lower body negative pressure: To determine whether any alterations in vasodilator responses between groups might be related to a generalized vascular “hyper-reactivity” characterized by both exaggerated vasodilator and vasoconstrictor responses we assessed blood flow and cardiac hemodynamic responses following lower body negative pressure (LBNP). The legs and hips were sealed in an air-tight container connected to a vacuum source (Kimmerly and Shoemaker, 2002). Data collection for each test commenced following 5 min of undisturbed rest in the supine position. An initial 5 min baseline period was followed by 5 min of LBNP at –40 mm Hg and, and finally, 5 min of rest at atmospheric pressure.

Blood processing and analysis: Blood samples were obtained from an antecubital fossa vein at baseline and during the LBNP protocol. A 3 ml sample of whole blood for determination of catecholamines was mixed with 75 μl of EGTA-glutathione anticoagulant and was centrifuged at 4°C for 15 min. A 6 ml sample of whole blood for determination of renin activity was collected in two 3 ml samples in 4 ml BD vacutainers, each containing 7.2 mg of EDTA and centrifuged at 4°C for 15 min.

Catecholamines were extracted from the plasma by adding acid-washed alumina and mixing by inversion. The pellet was washed with distilled water and the catecholamines were released into 0.1 M perchloric acid and separated by centrifugation. The concentrations of norepinephrine were determined in duplicate by HPLC with electrochemical detection (2465
ElectroChemical Detector, Waters, Milford, CT) and quantified by determining the area under the peak. Working and internal standards were used to correct the measurements. Renin activity assays were performed commercially by Gamma-Dynacare Medical Laboratories, London, Ontario.

**DNA analysis.** Genomic DNA was extracted from whole blood as previously described (Gros et., 2005). Briefly, genotyping of the ADCY6 A674S variant was performed using exon-specific DNA amplification followed by purification using shrimp alkaline phosphatase (Roche, Mannheim, Germany) and exonuclease I (ExoI; New England Biolabs, Mississauga, Canada) and capillary pheresis in an automated DNA sequencer as recently described (Cao and Hegele, 2003).

**Data analysis.** For the initial population screening, the statistical significance of differences in quantitative variables between control and ADCY6 variant groups was determined by student’s t-test for unpaired data (Prism 4.0, GraphPad Software, San Diego, CA). In the sub-sample studied for cardiovascular responsiveness, the associations of genotype on baseline values and responsiveness (defined as the change in each variable from rest) were assessed using a mixed one-way repeated measures analysis of variance (ANOVA) (SAS® v.9.1, SAS Institute Inc., Cary, NC). Post hoc analysis of significant interactions was assessed using Bonferroni analysis. Data are presented as mean ± standard error and statistical significance was assumed when $p < 0.05$. 
Results

Genotype and allele frequencies of ADCY6 A674S variant: In the population of 364 healthy Caucasian volunteers we identified 25 individuals (24 heterozygotes and 1 homozygote) who had ADCY6 p.A674S (g.2714G>T) variant in their genomes. The genotype frequencies for 2714G/G, 2714G/T and 2714T/T were 0.931, 0.066 and 0.003 respectively. The 2714T allele frequency was 0.035 and there was no deviation of genotype frequencies from Hardy-Weinberg expectations. These findings were similar to our previously reported frequency of ADCY6 A674S in a Caucasian population (Gros et al., 2007).

ADCY6 variant associations: Participants were classified according to g.2714G>T genotype with the single 2714T/T homozygote added to the 2714G/T heterozygote group (S674 subjects), while the 2714G/G homozygotes formed the comparator group (A674 subjects). Seated systolic blood pressure was increased in ADCY6 S674 subjects compared to ADCY6 A674 subjects ($p < 0.05$; Table 1). Diastolic blood pressure also tended to be higher in the ADCY6 variant group, although this difference was of borderline significance ($p = 0.05$) (Table 1).

Waist circumference was reduced in the ADCY6 S674 variant group compared to the ADCY6 A674-expressing individuals (Controls) (Table 1). In contrast, mean body mass index did not differ according to genotype.

Baseline hemodynamic and biochemical assessments: Of the 25 individuals that we identified as expressing the ADCY6 genetic variant we were able to recruit seven individuals (3 male) who were heterozygotic carriers of ADCY6 A674S (hereafter referred to as the ADCY6 variant
group) for more detailed analysis of cardiovascular responsiveness. Fourteen individuals (7 male) who did not express the genetic variant were recruited as a Control group.

Baseline assessments were performed after approximately 20 minutes of quite supine rest. Systolic, mean arterial and diastolic blood pressures were all significantly increased in the ADCY6 variant group (Table 2). Also, compared to the control group, those expressing the ADCY6 variant had increased mean heart rate and cardiac output (both $p < 0.05$).

Under baseline conditions, plasma renin activity (PRA) was greater in the ADCY6 variant group ($0.63 \pm 0.11$ ng/L/s) ($p < 0.05$) compared with control subjects ($0.31 \pm 0.11$ ng/L/s). In contrast, baseline levels of plasma norepinephrine were similar between groups (Control = $1.28 \pm 0.25$ nmol/L, ADCY6 variant= $1.38 \pm 0.24$ nmol/L) ($p > 0.1$).

*Handgrip exercise responses:* Baseline measures of forearm blood flow (FBF) did not differ between genotypic groups ($p > 0.05$). With exercise, FBF increased in both ADCY6 variant and control groups. However, compared with control subjects, the exercise-induced increase in FBF was almost 4 times greater in those expressing the ADCY6 variant (Fig. 1, increased ~30% in the control subjects ($p < 0.05$) vs. ~120% in the ADCY6 variant group, $p < 0.001$).

*Reactive hyperemia and flow mediated dilation (FMD):* To determine whether the increase in FBF seen following handgrip exercise was due to a generalized enhancement of vasodilatory responses- not specific to adenylyl cyclase mediated effects, we next assessed the (endothelial-dependent) effect of reactive hyperemia on FBF. When compared to Control subjects, the ADCY6 variant group displayed no difference in baseline FBF ($p = 0.181$, Fig. 2) prior to the FMD procedure. Also, the initial hyperemic response to the deflation of the cuff was also
unaffected by genetic status ($p > 0.05$; Fig. 2). The extent of FMD in the brachial artery (between 45-90 sec following cuff deflation), did not differ between the two groups, with the increases in the diameters of the control group being $8.7 \pm 0.5 \%$ and of the ADCY6 variant group being $9.2 \pm 0.7 \%$ ($p > 0.05$). Thus, endothelial-mediated vasodilatory responses were not enhanced in individuals expressing the ADCY6 variant.

*Lower body negative pressure (LBNP):* During LBNP, both groups demonstrated reductions in FBF ($p < 0.05$; Fig. 3 A). However, the forearm vascular response to LBNP was not greater in the ADCY6 variant group. In fact, the increase in $\text{TPR}_c$ in response to LBNP was greater in the control than the ADCY6 variant group (Fig. 3 B; $p < 0.05$). In contrast to the unchanged or blunted vascular responses to LBNP, the cardiac chronotropic responses to LBNP - mediated indirectly via baroreceptor unloading - were exaggerated in the ADCY6 variant group. Compared to the insignificant increase in HR seen in controls following LBNP, in ADCY6 variants, HR was significantly increased ($p < 0.05$) (Fig. 4). The increase in HR was 14 beats·min$^{-1}$ greater in the ADCY6 variant group during LBNP vs. controls ($p < 0.05$) (Fig. 4). Notably, cardiac output in the ADCY6 variant group was significantly higher than controls both at rest and with LBNP (but not during the recovery phase, Fig. 5A). However, the magnitude of fall in cardiac output during LBNP was similar in both groups ($p > 0.05$) (Fig. 5B).

With LBNP, the increase in plasma norepinephrine with LBNP did not differ between groups (control $+0.86 \pm 0.10$; ADCY6 $+0.93 \pm 0.13$ nmol/L) ($p > 0.05$). Plasma renin activity was essentially unaltered with acute orthostatic stress and the extent of change was similar between the control ($-0.05 \pm 0.03$ ng/L/s) and ADCY6 variant ($-0.07 \pm 0.02$ ng/L/s) groups.
Discussion

Our recent studies had identified a relatively common amino acid variant of ADCY6, namely A674S (Gros et al., 2005; Gros et al., 2007). Further, we have shown previously that this variant is hyperfunctional \textit{in vitro} in the context of enzymatic activity as well as in the context of adenylyl cyclase-mediated vascular responses (Gros et al., 2007). However, the significance of its expression in regards to phenotypic characteristics, as well as \textit{in vivo} effects on responses to acute exercise, and vasoconstrictor stimuli were unknown. The present studies have confirmed that the ADCY6 A674S variant is present in \(~7\%\) of Caucasians, and further demonstrate that its expression is associated with increased blood pressure and hyperdynamic cardiovascular responses characterized by 1) increased cardiac output and heart rate 2) elevated plasma renin activity 3) a markedly greater vasodilator response to handgrip exercise and 4) augmented heart rate response to baroreceptor unloading (LBNP), without impact on reflex-mediated sympathetic forearm vasoconstriction. Together, the findings indicate that the expression of the ADCY6 A674S genetic variant leads to a hyperdynamic cardiovascular system both under baseline conditions and in response to stressors that activate adenylyl cyclase.

\textit{Determinants of increased blood pressure in subjects expressing the ADCY6 A674S genetic variant.} It could be reasonably argued that the major functional determinants of cAMP-mediated regulation of blood pressure relate to the effects of adenylyl cyclase activation on: i) cardiac contractility/heart rate, ii) the renin-angiotensin system (RAS) axis- via cAMP-mediated regulation of renin release iii) vascular reactivity- predominantly via vasodilator mechanisms. In regards to the impact of expression of the ADCY6 variant on cardiac function, under baseline
conditions cardiac output was higher in the ADCY6 variant group. This effect seems to have been primarily related to the increased heart rate, since stroke volume did not differ significantly between groups. The observation that cardiac output was elevated, without appreciable reductions in afterload (higher systolic pressure and normal vascular resistance) in this group suggests that cardiac filling pressures were also sustained or elevated.

In regards to the potential effect of expression of the ADCY6 variant on vascular reactivity, total peripheral resistance was not reduced (nor was baseline FBF increased). Acutely, increased adenylyl cyclase activation (as would be seen with infusion of the non-selective beta adrenergic agonist, isoproterenol) is associated with decreased peripheral resistance- related to both direct and baroreflex-mediated responses. Notably, in our initial studies, subjects carrying the A674S ADCY6 genetic variant demonstrated enhanced adenylyl cyclase-mediated vasodilator responses as determined by the extent of isoproterenol-mediated vasorelaxation assessed by LVDT (Gros et al., 2007). However, chronically, the direct effects of adenylyl cyclase activation on vascular reactivity would be expected to be modulated by its actions in regulating renin release. In the ADCY6 variant group, plasma renin activity was increased compared to the Control group. Thus, the failure to detect a reduced total peripheral resistance in subjects expressing the ADCY6 variant might reflect the chronic effects of increased renin activity, leading to enhanced aldosterone-mediated effects on intravascular filling and/or increased ambient angiotensin II-mediated effects. However, regardless of the countervailing balance of enhanced adenylyl cyclase-mediated vasodilation vs. enhanced adenylyl cyclase-mediated renin release on peripheral resistance, altogether, our findings support the hypothesis that the increased blood pressure in the ADCY6 variant group is primarily related to increased cardiac output and increased plasma renin activity.
Notably the hemodynamic responses to handgrip exercise support the hypothesis that subjects with the ADCY6 A674S variant have exaggerated adenylyl cyclase-mediated hemodynamic responses. Acute exercise causes rapid and large changes in muscle perfusion (Shoemaker et al., 1997a; Shoemaker et al., 1997b; Shoemaker et al., 1997c; Shoemaker et al., 1998). Among the multiple mechanisms mediating this effect is the role of metabolic factors released by working muscles. The mediators of exercise hyperemia that are released from contracting skeletal muscle include adenosine and prostaglandins that act through GPCRs to affect intracellular calcium levels in vascular smooth muscle (Betik et al., 2004; Marshal, 2007) via an adenylyl cyclase-regulated pathway. The pronounced difference in the FBF to handgrip exercise in the ADCY6 variant group is consistent with the suggestion of this genetic variant producing a “hyper-functional” phenotype. These alterations in vasodilator responses were not paralleled by significant differences in flow-mediated vasodilator processes as assessed by the effects of reactive hyperemia. This latter response is predominantly mediated by endothelial factors that are (largely) independent of adenylyl cyclase activation. Thus, these findings in aggregate would suggest that the alterations in hand grip responses in individuals expressing the ADCY6 variant are not related to a generalized enhancement of vasodilator responses.

Interestingly, the ADCY6 variant group had ~4 cm decreased waist circumference reflecting a 10% decrease in abdominal girth, without a significant difference in body mass index. Waist circumference has been increasingly appreciated as a surrogate for abdominal fat and, more importantly, as a highly predictive risk factor for the development of the metabolic syndrome and, ultimately, atherosclerotic complications (Pouliot et al., 1994). The observation that waist circumference, but not body mass index, was reduced in healthy, younger ADCY6
variant subjects suggests that the difference in waist circumference reflected a difference in abdominal fat, but not a difference in “whole-body” fat.

In summary, these data indicate that the expression of a novel, relatively common genetic variant of ADCY6 is associated with an increase in adenylyl cyclase function that parallels increased blood pressure and cardiac output and exaggerated adenylyl cyclase-mediated responses to exercise as well as altered distribution of body fat. Whether these findings have implications in regards to the development of cardiovascular disease and/or complications related to visceral obesity— the metabolic syndrome and diabetes, remain to be determined. However, these studies do indicate that genetic regulation of GPCR-mediated adenylyl cyclase activation is an important determinant of human cardiovascular function.
Acknowledgements

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References


Footnotes

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Legends for Figures

Figure 1. Forearm blood flow responses in the handgrip exercise study. The 3 trials of exercise were combined for the control, (■) and ADCY6 S674 (□) variant groups. * indicates $p < 0.05$ between groups. † indicates $p < 0.05$ from baseline within groups. Data represent the mean ± standard error.

Figure 2. The mean forearm blood flow (FBF) responses from the control (■) and ADCY6 variant (□) groups during baseline, reactive hyperemia, and flow mediated dilation. * indicates $p < 0.05$ from baseline. Data represent the mean ± standard error.

Figure 3. Effect of lower body negative pressure (LBNP) on forearm blood flow and on total peripheral resistance A. Changes in forearm blood flow during LBNP. There were no differences between the control (■) and ADCY6 variant (□) groups at baseline. Both groups show significant vasoconstriction and comparable FBF responses to LBNP and recovered to values not different than baseline. * indicates $p < 0.05$ from baseline. Data represent the mean ± standard error. B. Changes in total peripheral resistance ($TPR_c$) during LBNP. Note the large increase in $TPR_c$ in the control group with LBNP vs. the nonsignificant increase in the ADCY6 variant group. * indicates $p < 0.05$ between groups. † indicates $p < 0.05$ from baseline. Data represent the mean ± standard error.

Figure 4. Increases in heart rate during lower body negative pressure (LBNP) in the control (■) and ADCY6 variant (□) groups. Note the large HR response in the ADCY6 variant group.
compared to the modest response in the control group. * indicates \( p < 0.05 \) between groups. † indicates \( p < 0.05 \) from baseline. Data represent the mean ± standard error.

**Figure 5.** Cardiac output responses during the lower body negative pressure (LBNP) in the ADCY6 variant (□) and control (■) groups. Panel A shows the data during rest (baseline), LBNP, and recovery. Panel B shows the percentage change in \( Q_c \) during LBNP. * indicates \( p < 0.05 \) between groups. Data represent the mean ± standard error.
Table 1: Anthropometric and hemodynamic profiles of individuals in screening study.

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<th>Control</th>
<th>ADCY6 variant</th>
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<td>Number (m/f)</td>
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<td>Age (yr)</td>
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<td>WC (%, gender-corrected)</td>
<td>86±1</td>
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<td>110±1</td>
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<td>Heart rate (beats · min⁻¹)</td>
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Values are Mean ± Standard Error. (m/f), male/female ratio; WC, waist circumference -- gender corrected using the ATP III “upper limit of normal” standards of 88 cm for females and 102 cm for males; BMI, body mass index; BP, blood pressure. *p < 0.05 vs. Control (Wild-type).
Table 2: Baseline anthropometric and cardiovascular status in participants of hemodynamic studies

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<td>TPRc (mm Hg·L⁻¹·min⁻¹·m²)</td>
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<td>25.5±1.8</td>
</tr>
</tbody>
</table>

Values are Mean ± standard error. M/F, male/female ratio; Qc, cardiac output corrected for body surface area; SVc, stroke volume corrected for body surface area; HR, heart rate; MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; TPRc, TPR corrected for body surface area. *p < 0.05 vs. Control (wild-type).
Figure 1

**Forearm blood flow**

(ml · min⁻¹ · 100 ml tissue⁻¹)

- Control
- ADCY6 Variant

Baseline

Exercise

*
Figure 2

- Forearm blood flow (ml·min⁻¹·100 ml tissue⁻¹)
  - Baseline
  - Initial
  - 60–90 s

- Control
- ADCY6 Variant
Figure 4

Heart rate (beats · min⁻¹)

Baseline  LBNP  Recovery

Control  ADCY6 Variant

* Indicates statistical significance.
† Indicates a significant difference compared to the control group.