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Potentiation of the NO-cGMP pathway and blood flow responses during dynamic exercise in healthy humans

Jacqueline K. Limberg[1], **Katherine R. Malterer**[1], **J. Mikhail Kellawan**[2], **William G. Schrage**[1,2], **Brad W. Wilkins**[1], **Wayne T. Nicholson**[1], **John H. Eisenach**[1], **Michael J. Joyner**[1], and **Timothy B. Curry**[1]

[1]Department of Anesthesiology, Mayo Clinic College of Medicine, Rochester, Minnesota

[2]Department of Kinesiology, University of Wisconsin, Madison, Wisconsin, USA

Abstract

Purpose—Previous work has shown nitric oxide (NO) contributes to ~15% of the hyperemic response to dynamic exercise in healthy humans. This NO-mediated vasodilation occurs, in part, via increases in intracellular cyclic guanosine monophosphate (cGMP), which is catabolized by phosphodiesterase. We sought to examine the effect of phosphodiesterase-5 (PDE-5) inhibition on forearm blood flow (FBF responses to dynamic handgrip exercise in healthy humans and the role of NO. We hypothesized exercise hyperemia would be augmented by sildenafil citrate (SDF, PDE-5 inhibitor). We further hypothesized any effect of SDF on exercise hyperemia would be abolished with intra-arterial infusion of the NO synthase (NOS) inhibitor L-N^G-monomethyl arginine (L-NMMA).

Methods—FBF (Doppler ultrasound) was assessed at rest and during 5 minutes of dynamic forearm handgrip exercise at 15% of maximal voluntary contraction under control (saline) conditions and during 3 experimental protocols: 1) oral SDF (n=10), 2) intra-arterial L-NMMA (n=20), 3) SDF and L-NMMA (n=10). FBF responses to intra-arterial sodium nitroprusside (NTP, NO donor) were also assessed.

Results—FBF increased with exercise ($p<0.01$). Intra-arterial infusion of L-NMMA resulted in a reduction in exercise hyperemia (17±1 to 15±1 mL/dL/min, $p<0.01$). Although the hyperemic response to NTP was augmented by SDF (Area under the curve: 41±7 vs 61±11 AU, $p<0.01$), there was no effect of SDF on exercise hyperemia ($p=0.33$).

Conclusions—Despite improving NTP-mediated vasodilation, oral SDF failed to augment exercise hyperemia in young, healthy adults. These observations reflect a minor contribution of NO and the cGMP pathway during exercise hyperemia in healthy young humans.

Contact information for correspondence: Timothy B. Curry, MD, PhD, 200 First St. SW, Rochester, MN 55905, (507) 255-6051 (phone), (507) 255-4267 (fax), curry.timothy@mayo.edu.

ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments.

CONFLICT OF INTEREST

The authors declare no relevant conflicts of interest.

Keywords

blood flow; regional; sildenafil; exercise; nitric oxide; cyclic GMP

INTRODUCTION

Blood flow to exercising skeletal muscle increases proportionally to match oxygen delivery with metabolic demand (Shepherd 1983). Extensive work from our group and others has shown that nitric oxide (NO), a potent vasodilator, contributes to ~15% of the hyperemic response to dynamic exercise in healthy young humans [Reviewed in (Joyner and Casey 2015)]. The majority of our mechanistic understanding has come from the use of NO synthase (NOS) inhibitors; however, the effects of these drugs have been shown to be dependent on the type of exercise (Green et al. 2005), the interpretation of the altered baseline flow (Bradley et al. 1999; Duffy et al. 1999; Dyke et al. 1995; Katz et al. 1996; Maiorana et al. 2003; Radegran and Saltin 1999), and the timing of measurements [e.g. steady-state exercise versus recovery from exercise (Dyke et al. 1995; Saltin et al. 1998; Shoemaker et al. 1997)]. Consistent with this, NOS has been shown to play a greater (Duffy et al. 1999; Dyke et al. 1995; Gilligan et al. 1994; Maxwell et al. 1998; Schrage et al. 2004) or lessor (Endo et al. 1994; Frandsenn et al. 2001; Heinonen et al. 2011; Martin et al. 2006; Radegran and Saltin 1999; Wilson and Kapoor 1993) role in the blood flow response to dynamic exercise.

NO activates the intracellular enzyme soluble guanylate cyclase in vascular smooth muscle, thereby increasing the formation of cyclic guanosine monophosphate (cGMP), leading to smooth muscle relaxation and vasodilation. The effect of NO is terminated by phosphodiesterase enzymes, including the cGMP-selective phosphodiesterase-5 (PDE-5). PDE-5 is the isozyme responsible for the majority of cGMP degradation in smooth muscle cells and PDE-5 inhibition has been shown previously to effectively increase resting blood flow to select vascular beds [e.g. corpus cavernosa, lungs (Ghofrani et al. 2006; Moreland et al. 1998)], including the skeletal muscle vasculature (Attina et al. 2008). PDE-5 inhibition with the pharmacological agent sildenafil citrate has also been shown to improve exercise tolerance in persons with pulmonary hypertension (Singh et al. 2006) and in healthy adults during hypoxia (Hsu et al. 2006). Furthermore, data from Attina and colleagues suggest that sildenafil citrate can improve post-exercise blood flow in hypertensive patients (Attina et al. 2008); however, the effect of PDE-5 inhibition on skeletal muscle blood flow responses during dynamic exercise has not been examined directly. With this, it is possible NO plays only a minor role (~15%) in exercise hyperemia because PDE-5 limits complete activation of the NO-cGMP pathway and there is evidence to suggest that the NO pathway can be potentiated in young healthy individuals (Ferguson et al. 2013; Lee et al. 2015). Understanding the role of PDE-5 in regulating peak NO-mediated vasodilation during exercise will provide important information that could be translated clinically to improve exercise tolerance in conditions with limited NO bioavailability. There are also intriguing new data that suggest blood flow during exercise may be uncoupled from metabolic demand (Shepherd et al. 2016); thus, studying the effect of PDE-5 inhibition on exercise hyperemia

may also provide further insight into changes in blood flow in response to increased oxygen consumption.

Taken together, we proposed that inhibition of PDE-5 would increase skeletal muscle blood flow responses to dynamic exercise via potentiation of the NO-cGMP pathway. We examined the effect of the PDE-5 inhibitor sildenafil citrate on forearm blood flow responses to dynamic handgrip exercise in healthy humans. We hypothesized forearm blood flow responses to exercise would be increased with administration of sildenafil citrate. We further hypothesized any increase in forearm blood flow responses with sildenafil citrate would be absent with intra-arterial infusion of the NO synthase inhibitor L-N^G-monomethyl arginine (L-NMMA) – demonstrating the specificity of sildenafil citrate for the NO pathway.

METHODS

Subjects

This study was approved by the Institutional Review Boards at the Mayo Clinic and University of Wisconsin – Madison and was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments. Thirty subjects (24 females/16 males, age = 25±1 yrs, BMI = 22.4±0.3 kg/m^2) gave informed, written consent prior to participation. Subjects were non-smokers, were free from pulmonary, cardiovascular, endocrine, or neurological diseases, and were not taking any medications with known cardiovascular effects. Subjects refrained from exercise, alcohol, and caffeine for 24 hours prior to the study.

Instrumentation

Subjects were positioned supine and heart rate was recorded continuously from a 3-lead electrocardiogram. A 20-gauge, 5-cm catheter was placed in the brachial artery of the non-dominant arm under aseptic conditions after local anesthesia. A three-port connector was placed in series with a pressure transducer to allow simultaneous administration of study drugs and continuous arterial blood pressure monitoring. Brachial artery blood velocity was measured using Doppler ultrasound (Vivid 7 Ultrasound, GE Healthcare, Chalfont St. Giles, UK) (Joyner and Dietz 1997). In Protocols 1 and 3, a commercial interface unit (Multigon Industries, Yonkers, NY) processed the angle-corrected, intensity-weighted Doppler audio information into a flow velocity signal that was sampled in real time. In Protocol 2, the Doppler audio information was converted into a real-time digital flow velocity signal using Fast Fourier Transform (Herr et al. 2010). This method of signal processing has been validated by both in vitro and in vivo methods (Herr et al. 2010). All data were recorded continuously on a computer and analyzed offline (PowerLab Chart5, ADInstruments, Colorado Springs, CO). Brachial artery diameter was measured digitally off-line from two-dimensional, longitudinal images. Forearm blood flow (hyperemia) was calculated as the product of blood velocity and vessel cross sectional area and is expressed relative to forearm size ($mL \cdot dL^{-1} \cdot min^{-1}$). To account for changes in blood pressure and to assess vasodilation, forearm vascular conductance (FVC) was calculated ($mL \cdot dL^{-1} \cdot min^{-1} \cdot 100\ mmHg^{-1}$).

For each protocol, measurements were made at baseline (rest), during forearm exercise, and during incremental infusion of sodium nitroprusside (NTP, Protocols 1 and 3). A minimum of 10 minutes was allowed between exercise and NTP trials to ensure blood flow returned to baseline levels. Subjects were enrolled in 3 separate protocols which included a control condition, followed by an experimental condition with addition of: 1) oral sildenafil citrate (n=10), 2) intra-arterial L-NMMA (n=20), 3) oral sildenafil citrate and intra-arterial L-NMMA (n=10). Our rationale for including multiple research cohorts was based on the half-life of sildenafil and L-NMMA. Both sildenafil and L-NMMA have half-lives >1 hour (Mayer et al. 1999; Nichols et al. 2002); which prohibit our ability to adequately study each independent condition in the same individuals on a single study day. Data from subjects in Protocol 2 were published previously (Harrell et al. 2015; Kellawan et al. 2015).

Forearm exercise

Forearm exercise consisted of a quiet resting period, followed by 5 minutes of submaximal, dynamic handgrip exercise as previously described (Tschakovsky et al. 2002). Briefly, subjects rhythmically squeezed a handgrip ergometer (20 contractions/min) using a load that was 15% of their maximal voluntary contraction (MVC). Blood velocity data were averaged across 30 seconds at the end of rest and steady-state (5-min) exercise. Two-dimensional images of the brachial artery were obtained at baseline and after 5 minutes of exercise and were saved for off-line measurements of brachial artery diameter and calculation of forearm blood flow.

Sodium nitroprusside (NTP)

Sodium nitroprusside is an exogenous, endothelial-independent NO donor which was administered to the forearm via the brachial artery catheter to activate the NO-cGMP pathway independent of endothelial NOS. Four incremental doses (0.25, 0.5, 1, and 2 μg·dL forearm vol^{-1}·min^{-1}) were infused at rates between 2–3 mL·min^{-1} for a minimum of 2-min to allow blood flow to reach steady state. The last 30-sec of blood velocity data for each infusion of sodium nitroprusside was averaged to obtain the velocity for that dose. Brachial artery diameter was measured immediately after the blood velocity measurements were completed.

Sildenafil citrate (SDF; Protocols 1 and 3)

After completion of control measurements, sildenafil citrate (100 mg) was administered orally with a sip of water. This dose is the maximum recommended oral dose for the treatment of erectile dysfunction and was similar to other studies examining the effect of sildenafil citrate on hemodynamics (Dishy et al. 2001; Kimura et al. 2003). One hour was allowed for sildenafil citrate to reach peak concentrations and have maximal effects on forearm blood flow (Schalcher et al. 2002).

L-N^G-monomethyl arginine (L-NMMA; Protocols 2 and 3)

A loading dose (50 mg) of L-NMMA was administered intra-arterially in the instrumented forearm over 5–10 minutes to locally inhibit NO synthase. In Protocol 2, the loading dose began immediately after 5 minutes of handgrip exercise. In Protocol 3, the loading dose was

administered one hour after sildenafil citrate administration. In both protocols, after the completion of the loading dose, a maintenance infusion of L-NMMA began (1 $mg \cdot min^{-1}$) and continued for the remainder of the study (Dinenno and Joyner 2003, 2004).

Statistical analysis

Subject demographics were compared between protocols using a one-way analysis of variance (ANOVA). Hemodynamic data were compared using two-way repeated measures ANOVA to examine the main effect of condition (SDF, L-NMMA, SDF + L-NMMA) and time (exercise) or dose (NTP), as well as the interaction between condition and time/dose. To account for potential changes in baseline hemodynamics, the hyperemic response to exercise was reported as a change from baseline (Δ, $FBF_{Condition} - FBF_{Baseline}$). Forearm blood flow responses to sodium nitroprusside infusions were also assessed using an area under the curve (AUC) analysis. Data are presented as mean ± standard error of the mean. $P<0.05$ was considered significant.

Sample size was determined by power test equations with $\alpha=0.05$ and power=0.80 using differences reported from previously published data, which suggested that 6 subjects per group would be necessary to detect a 2.6 ± 1.7 $mL \cdot dL^{-1} \cdot min^{-1}$ change in forearm blood flow with sildenafil citrate (Attina et al. 2008). Because little is known about the effect of sildenafil citrate on exercise hyperemia, we exceeded n=6 in each cohort.

RESULTS

Subject demographics did not differ significantly between protocols (See Table 1).

Exercise

See Table 2 and Figure 1. Dynamic handgrip exercise resulted in an increase in heart rate (Main effect of exercise, $p<0.05$) and brachial artery diameter (Main effect of exercise, $p\leq0.05$). No exercise-mediated changes in blood pressure were observed in Protocols 1 and 3 (Main effect of exercise, Protocol 1, p=0.15; Protocol 3, p=0.49), but blood pressure increased in Protocol 2 (Main effect of exercise, $p<0.01$). Heart rate was significantly increased following oral SDF when compared to control conditions (Main effect of SDF, $p<0.01$), but no changes in heart rate were observed when SDF was combined with L-NMMA (Main effect of SDF, p=0.18). There was no effect of SDF on blood pressure (Main effect of SDF, p=0.76; Main effect of SDF+L-NMMA, p=0.54), or brachial artery diameter (Main effect of SDF, p=0.83; Main effect of SDF+L-NMMA, p=0.66). There was no effect of L-NMMA on brachial artery diameter (Main effect of L-NMMA, p=0.06) or heart rate (Main effect of L-NMMA, p=0.44), although an increase in blood pressure was observed (Main effect of L-NMMA, $p<0.01$).

Forearm blood flow and forearm vascular conductance increased with exercise (Main effect of exercise, FBF: $p<0.01$; FVC: $p<0.01$). Blood flow and vascular conductance were not altered by sildenafil citrate (Main effect of SDF, FBF: p=0.81; FVC: p=0.59). L-NMMA resulted in a reduction in blood flow and vascular conductance alone (Main effect of L-NMMA, FBF: $p<0.01$; FVC: p=0.02) and when combined with sildenafil citrate (Main effect of SDF+L-NMMA, FBF: p=0.04; FVC: p=0.03). When responses were assessed as a

change from baseline [Absolute (Δ)], any differences between forearm blood flow during control and experimental conditions (SDF, L-NMMA, SDF+L-NMMA) were no longer observed (Δ FBF p-value range 0.07–0.16). In regards to forearm vascular conductance, although there was no effect of SDF (p=0.33) or SDF+L-NMMA (p=0.053), there was a significant effect of L-NMMA (p=0.04) on the change in forearm vascular conductance (Δ FVC).

Sodium nitroprusside

Reductions in blood pressure (Main effect of NTP, p<0.01) and increases in brachial artery diameter (Main effect of NTP, p<0.01) and heart rate (Main effect of NTP, p<0.01) were observed with intra-arterial infusion of sodium nitroprusside. Blood pressure and brachial artery diameter were not altered by oral SDF (Main effect of SDF: Blood pressure, p=0.98; Diameter, p=0.48; Main effect of SDF+L-NMMA: Blood pressure, p=0.53; Diameter, p=0.87). Although heart rate increased following oral SDF when compared to control conditions (Main effect of SDF, p=0.02), there was no change in heart rate following SDF plus L-NMMA (Main effect of SDF+L-NMMA, p=0.65).

Forearm blood flow and vascular conductance increased with infusion of sodium nitroprusside (Main effect of NTP, FBF: p<0.01; FVC: p<0.01; Table 3). The increases in blood flow and conductance with sodium nitroprusside infusion were greater following oral sildenafil citrate when compared to control (Main effect of SDF, ΔFBF: p<0.01; ΔFVC: p<0.01; Figure 2). Blood flow and vascular conductance with infusion of sodium nitroprusside did not appear to be altered following sildenafil citrate plus L-NMMA (Main effect of SDF+L-NMMA, FBF: p=0.51; FVC: p=0.48; Table 3). However, when responses were assessed as a change from baseline (Δ), differences between control and sildenafil citrate plus L-NMMA were observed (Main effect of SDF+L-NMMA, ΔFBF: p=0.04; ΔFVC: p=0.049; Figure 2); thus L-NMMA did not alter the vasodilatory effect of sildenafil citrate on sodium nitroprusside-mediated increases in blood flow and vascular conductance.

DISCUSSION

Results from the present study show that sildenafil citrate has no effect on the steady-state blood flow response to dynamic exercise in healthy humans. We also found that the NOS inhibitor, L-NMMA, had only a minor effect on exercise hyperemia. The lack of an effect of sildenafil citrate and a limited effect of L-NMMA on exercise hyperemia together support the idea of physiological redundancy such that activation of the NO-cGMP pathway in healthy humans plays a relatively minor role in normal exercise vasodilation (Endo et al. 1994; Gordon et al. 2002; Martin et al. 2006; Shoemaker et al. 1997). Our results further suggest that the role for NO in exercise hyperemia in healthy adults is not limited by PDE-5 activity. Consistent with this, other vasodilator pathways besides the NO-cGMP pathway have been shown contribute to exercise hyperemia, including: 1) prostacyclin, 2) adenosine triphosphate (ATP), and 3) K^+ channels [Reviewed in (Joyner and Casey 2015)]. It is also likely that in healthy humans the NO-cGMP pathway is maximally activated in a way that further augmentation of its effects does not alter exercise responses (i.e. “ceiling effect”). Consistent with this, we did observe a trend for an increase in *resting* blood flow with

sildenafil citrate in the healthy adults studied. These data suggest PDE-5 may be capable of keeping up with cGMP metabolism at rest; however, if the NO-cGMP pathway was maximally activated during exercise, further increases in cGMP levels might not appreciably affect forearm blood flow.

Despite no observable effect of sildenafil citrate on exercise hyperemia in healthy adults (Table 2, Figure 1), its administration may be effective in improving exercise hyperemia in older adults and/or clinical conditions. Consistent with this, other approaches to potentiate the NO pathway (e.g. dietary nitrate) have been effective at improving exercise hyperemia in such populations (Casey et al. 2015; Ferguson et al. 2013). In addition, sildenafil citrate has been shown previously to increase resting forearm blood flow in conditions with impaired endothelial function such as smoking (Kimura et al. 2003; Vlachopoulos et al. 2004) and cardiovascular disease (Halcox et al. 2002; Hryniewicz et al. 2005; Schofield et al. 2003). Additionally, Attina and colleagues have shown sildenafil citrate to improve post-exercise blood flow in hypertensive patients, despite little-to-no effect in normotensive controls (Attina et al. 2008). Thus, in the presence of impaired endothelial NOS (e.g. conditions exhibiting endothelial dysfunction) and/or upregulated PDE-5, sildenafil citrate may be a viable therapeutic option to restore blood flow via increases in cGMP (Dishy et al. 2004; Kimura et al. 2003; Robinson et al. 2006). Future work in this area is necessary.

As proof-of-principle, we also infused sodium nitroprusside to examine the effect of sildenafil citrate on exogenous NO-cGMP activation. Our results show that sildenafil citrate increases the hemodynamic response to sodium nitroprusside infusion approximately two-fold [Table 3; (Blaise et al. 2010; Dishy et al. 2001)]. After taking into consideration a baseline effect, the increase in sodium nitroprusside-mediated vasodilation with sildenafil citrate was not altered with co-infusion of L-NMMA (Figure 2). The lack of an effect of L-NMMA is to be expected given L-NMMA inhibits the activity of endothelial NOS and sodium nitroprusside-mediated vasodilation is achieved independent of the endothelium. These data thus highlight the ability of sildenafil citrate to increase forearm blood flow independent of endothelial NOS (eNOS), and also confirm effective dosing. With this, it is important to note that although we hypothesized any effect of sildenafil citrate on exercise hyperemia would be specific to the NO-pathway (with a primary focus on eNOS), there are other NOS isoforms, as well as non-NO sources of cGMP (e.g. natriuretic peptides), that should be considered in future work.

Experimental Considerations

First, sildenafil citrate was administered orally, resulting in systemic distribution of the drug. While sildenafil citrate is not associated with clinically significant changes in systemic hemodynamic parameters (Jackson et al. 1999), it may cause increases in muscle sympathetic nerve activity (MSNA) and plasma norepinephrine (Dopp et al. 2013; Phillips et al. 2000). Enhanced vasoconstriction due to an increase in MSNA could partially mask any increase in exercise hyperemia as a result of sildenafil citrate. However, an increase in sodium nitroprusside-mediated vasodilation with combined sildenafil citrate suggests this is unlikely to have a major effect on present findings. Second, our results are specific to the low (15% MVC) exercise workload studied. The contribution of NO-mediated vasodilation

has been shown to be workload-specific and may be altered when combined with environmental stressors [e.g. hypoxia (Casey et al. 2011; Wilkins et al. 2008)]. Therefore we are unable to comment on whether conclusions would be altered with the use of sildenafil citrate under more physiological stressful conditions. Third, data were collected across three separate research cohorts in both men and women, thus limiting our ability to control for inter-subject variability. However, we have shown previously that the role of NO in exercise hyperemia is unlikely to be sex-dependent (Kellawan et al. 2015). In addition, the method of signal processing differed between protocols – resulting in lower absolute blood flow values in Protocol 2. Importantly, both methods of signal processing are commonly used, validated, and are consistent with flow velocity signals produced by the Doppler signal converter (Herr et al. 2010). Thus, differences in methodological approaches between protocols are unlikely to alter main conclusions.

CONCLUSIONS

Despite improvements in sodium nitroprusside-mediated vasodilation, oral sildenafil citrate failed to augment exercise hyperemia in young, healthy subjects. These observations most likely reflect a minor contribution of NO and the cGMP pathway during exercise hyperemia in healthy young humans. With this, we propose the importance of the NO-cGMP pathway may be greater in persons with underlying endothelial dysfunction where even modest increases in cGMP through sildenafil citrate may result in significant improvements in vasodilator responses and highlight this as an important area for follow-up work.

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ABBREVIATIONS

ACH	Acetylcholine
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
AU	Arbitrary units
AUC	Area under the curve
BMI	Body mass index
cGMP	Cyclic guanosine monophosphate

eNOS	Endothelial nitric oxide synthase
FBF	Forearm blood flow
FVC	Forearm vascular conductance
L-NMMA	L-N^G-monomethyl arginine
MSNA	Muscle sympathetic nerve activity
MVC	Maximal voluntary contraction
NO	Nitric oxide
NOS	Nitric oxide synthase
NTP	Sodium nitroprusside
PDE-5	Phosphodiesterase-5
SDF	Sildenafil citrate.

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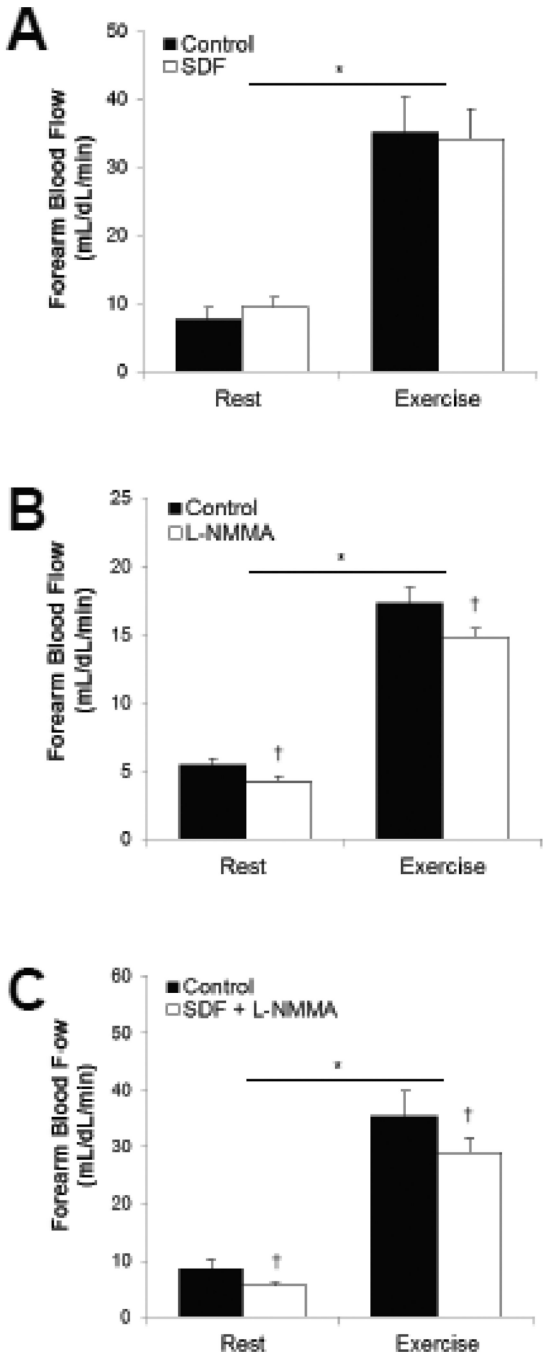

Fig. 1. Hyperemic response to dynamic handgrip exercise and role of the NO-cGMP pathway

Forearm blood flow increased with exercise (Main effect of exercise, $p<0.01$). Blood flow was not altered by sildenafil citrate (Main effect of SDF, $p=0.81$). L-NMMA resulted in a reduction in blood flow alone (Main effect of L-NMMA, $p<0.01$) and when combined with sildenafil citrate (Main effect of SDF+L-NMMA, $p=0.04$). $^{*}p<0.05$ vs Rest, $^{\dagger}p<0.05$ vs Control.

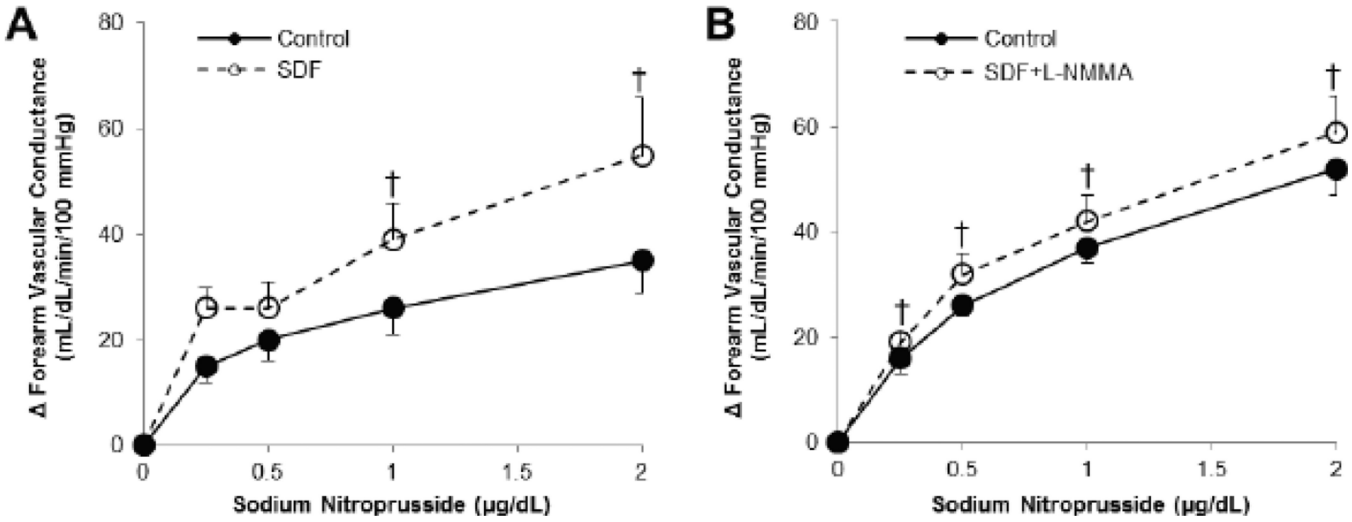

Fig. 2. Vasodilatory responses to intra-arterial infusion of sodium nitroprusside

The rise in forearm vascular conductance in response to intra-arterial sodium nitroprusside infusion was calculated ($FVC_{infusion} - FVC_{baseline}$). Sodium nitroprusside-mediated vasodilation was increased in oral SDF (**A:** Main effect of drug versus control, $p<0.01$) and remained high when sildenafil citrate was combined with L-NMMA (**B:** Main effect of drug versus control, $p=0.049$). $^{\dagger}p<0.05$ vs Control.

Table 1

Subject demographics

	Protocol 1 (Sildenafil)	Protocol 2 (L-NMMA)	Protocol 3 (Sildenafil+L-NMMA)
Sex (M/F)	4/6	11/9	1/9
Age (years)	26 ± 1	26 ± 1	24 ± 2
Height (cm)	169 ± 3	172 ± 2	171 ± 3
Weight (kg)	67 ± 3	68 ± 2	62 ± 4
Body mass index (kg/m^2)	23 ± 1	23 ± 1	20 ± 1
Forearm volume (mL)	900 ± 75	895 ± 47	787 ± 71
Maximal voluntary contraction (kg)	35 ± 4	36 ± 2	33 ± 3

Data are from: Protocol 1 (n=10), Protocol 2 (n= 20), Protocol 3 (n=10), unless otherwise noted. Maximal Voluntary Contraction (Protocol 3, n=8). No significant differences between protocols ($p>0.05$ for all).

Table 2

Hemodynamic responses to dynamic forearm exercise.

	Protocol 1		Protocol 2		Protocol 3	
	Control	**SDF**	**Control**	**L-NMMA**	**Control**	**SDF+L-NMMA**
Heart Rate (beat/min)						
Baseline	60 ± 4	65 ± 4†	61 ± 2	61 ± 2	62 ± 2	58 ± 3
5 minutes	64 ± 3*	70 ± 5*†	64 ± 2*	63 ± 2*	65 ± 2*	63 ± 3*
Δ	4 ± 1	5 ± 1	3 ± 2	2 ± 1	3 ± 1	4 ± 1
Mean Arterial Blood Pressure (mmHg)						
Baseline	91 ± 3	91 ± 3	88 ± 2	90 ± 1†	92 ± 3	91 ± 3
5 minutes	94 ± 2	93 ± 3	90 ± 2*	92 ± 2*†	91 ± 3	94 ± 4
Δ	3 ± 2	2 ± 1	2 ± 1	2 ± 1	−1 ± 1	3 ± 1
Brachial Artery Diameter (cm)						
Baseline	0.36 ± 0.02	0.36 ± 0.02	0.38 ± 0.01	0.38 ± 0.01	0.32 ± 0.01	0.32 ± 0.02
5 minutes	0.38 ± 0.02*	0.38 ± 0.02*	0.40 ± 0.01*	0.40 ± 0.01*	0.34 ± 0.01*	0.33 ± 0.02*
Δ	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.01
Forearm Blood Flow (mL/dL forearm volume/min)						
Baseline	8±2	10±1	5±1	4±0†	9±1	6±1†
5 minutes	35±5*	34±4*	17±1*	15±1*†	35±5*	29±2*†
Δ	27±4	25±4	12±1	11±1	27±3	23±2
Forearm Vascular Conductance (mL/dL forearm volume/min/100 mmHg)						
Baseline	8±2	11±1	6±1	5±0†	9±2	6±1†
5 minutes	37±5*	37±5*	19±1*	16±1*†	38±5*	30±2*†
Δ	29±4	26±4	13±1	12±1†	29±4	24±2

*p<0.05 vs Baseline;

†p<0.05 vs Control

Table 3

Hemodynamic responses to intra-arterial infusion of sodium nitroprusside.

	Protocol 1		Protocol 3	
	Control	SDF	Control	SDF+LNMMA
Heart Rate (beat/min)				
Baseline	61 ± 3	63 ± 4	59 ± 2	59 ± 3
0.25 μg/dL/min	61 ± 4	63 ± 5	59 ± 2	56 ± 2
0.5 μg/dL/min	59 ± 4	62 ± 5	60 ± 2	56 ± 2
1.0 μg/dL/min	61 ± 4	64 ± 5	60 ± 3	60 ± 3
2.0 μg/dL/min	63 ± 5^{abcd}	67 ± 5^{abcd}	62 ± 3^{abc}	62 ± 2^{abc}
Mean Arterial Blood Pressure (mmHg)				
Baseline	90 ± 3	88 ± 2	89 ± 3	89 ± 3
0.25 μg/dL/min	89 ± 2	86 ± 2	86 ± 3^{a}	87 ± 3^{a}
0.5 μg/dL/min	87 ± 3^{a}	86 ± 2^{a}	84 ± 3^{ab}	85 ± 3^{ab}
1.0 μg/dL/min	86 ± 2^{a}	84 ± 2^{a}	83 ± 3^{ab}	83 ± 3^{ab}
2.0 μg/dL/min	85 ± 3^{abc}	84 ± 2^{abc}	80 ± 3^{abcd}	81 ± 3^{abcd}
Brachial Artery Diameter (cm)				
Baseline	0.36 ± 0.02	0.36 ± 0.02	0.32 ± 0.02	0.32 ± 0.02
0.25 /dL/min μg	0.37 ± 0.02	0.37 ± 0.02	0.33 ± 0.01^{a}	0.33 ± 0.02^{a}
0.5 μg/dL/min	0.37 ± 0.02^{a}	0.37 ± 0.02^{a}	0.34 ± 0.02^{ab}	0.33 ± 0.02^{ab}
1.0 μg/dL/min	0.37 ± 0.02^{ab}	0.39 ± 0.02^{ab}	0.35 ± 0.02^{abc}	0.35 ± 0.02^{abc}
2.0 μg/dL/min	0.38 ± 0.02^{abc}	0.39 ± 0.02^{abc}	0.35 ± 0.02^{abcd}	0.36 ± 0.02^{abcd}
Forearm Blood Flow (mL/dL forearm volume/min)				
Baseline	5±1	9±2	10±3	5±1
0.25 μg/dL/min	19±3^{a}	31±4^{a†}	24±3^{a}	20±2^{a}
0.5 μg/dL/min	22±4^{a}	31±6^{a}	31±4^{ab}	31±4^{ab}
1.0 μg/dL/min	28±5^{a}	41±7^{a†}	39±5^{abc}	39±4^{abc}
2.0 μg/dL/min	35±6^{abc}	54±10^{abcd†}	49±6^{abcd}	52±6^{abcd}
AUC	41±7	61±11†	53±5	62±7†
Forearm Vascular Conductance (mL/dL forearm volume/min/100 mmHg)				
Baseline	6±1	10±2	12±3	6±1
0.25 μg/dL/min	21±4^{a}	36±5^{a†}	28±4^{a}	24±2^{a}
0.5 /dL/min μg	26±5^{a}	36±7^{a}	38±4^{ab}	38±4^{ab}
1.0 μg/dL/min	32±6^{a}	49±9^{a†}	49±5^{abc}	48±5^{abc}
2.0 μg/dL/min	41±6^{abc}	65±13^{abcd†}	64±6^{abcd}	65±7^{abcd}
AUC	48±8	73±13†	67±6	78±8†

^{a}p<0.05 vs Baseline,

^{b}p<0.05 vs 0.25 μg/dL/min,

[c] p<0.05 vs 0.5 μg/dL/min,

[d] p<0.05 vs 1.0 μg/dL/min;

[†] p<0.05 vs Control.

AUC = Area Under the Curve (AU).