

# Statin Mediated Vasodilation in the Vasculature

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*Dedicated to the memory of John McEachern*

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## **THESIS DECLARATION**

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Scott Copley and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Signed,

Scott Copley



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## THESIS ABSTRACT

Clinical trials have established the efficacy and safety of the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) in lowering cardiovascular morbidity and mortality in patients with and without coronary artery disease. Traditionally, the beneficial effects of statins have been ascribed entirely to their ability to lower serum cholesterol. Evidence indicates, however, that statins may exert cholesterol-independent or “pleiotropic” effects.

Statins may modulate vascular reactivity via the inhibition the RhoA/Rho-kinase pathway in both the vascular endothelium and the underlying vascular smooth muscle. To examine this hypothesis, we coupled the measurement of isometric force in isolated rat caudal artery segments with molecular analysis of the downstream targets of Rho-kinase in both the endothelium and smooth muscle.

We report that clinical concentrations of pravastatin inhibit  $\alpha_1$ -adrenoreceptor mediated vascular contraction through an endothelial-dependent mechanism. Our results suggest that this is mediated by an increase in P[Ser1177]eNOS phosphorylation, consistent with increased eNOS activation, increased nitric oxide production and the inhibition of the RhoA/Rho-kinase pathway in the vascular endothelium.

In the context of ThromboxaneA<sub>2</sub> (TxA<sub>2</sub>) receptor-mediated contraction we report that acute high dose simvastatin administration causes a robust reduction in contraction. We describe a concomitant increase eNOS Ser1177 phosphorylation, suggesting activation of eNOS and increased NO production, however, experiments in which

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eNOS was inhibited suggest that this mechanism does not account for the majority of relaxation. Perhaps more importantly, we report the increased activation of smooth muscle myosin phosphatase that may account for simvastatin-mediated relaxation in this preparation.

Extending these results to a chronic setting we examined the consequence of 7-day statin administration on rats. Using non-invasive tail cuff we demonstrate reductions in the systolic blood pressure of healthy rats treated with clinically relevant doses of simvastatin for 7 days. Using a perfused isolated heart model we report reduced TxA<sub>2</sub>-receptor mediated coronary perfusion pressure in hearts isolated from these animals and a reduction in TxA<sub>2</sub>-receptor mediated contraction in isolated blood vessels. Western blot analysis revealed an increase in the expression of endothelial nitric oxide synthase (eNOS) that was concomitant with these effects. Additional administration of high dose simvastatin further reduced TxA<sub>2</sub>-receptor mediated contraction via disinhibition of smooth muscle myosin phosphatase.

These results suggest that statins may be a viable treatment option to effect acute vasodilatation in patients with normal cholesterol levels but with abnormal vasomotor reactivity and/or endothelial dysfunction.

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## COMMON ABBREVIATIONS

[Ca <sup>2+</sup> ] <sub>cyt</sub>	cytosolic Ca <sup>2+</sup> concentration
5-HT	serotonin
AA	arachidonic acid
ACh	acetylcholine
BP	blood pressure
cAMP	cyclic guanosine monophosphate
cGMP	cyclic adenosine monophosphate
CAD	coronary artery disease
CHD	coronary heart disease
COX	cyclooxygenase
CPI-17	PKC-potentiated inhibitory protein of 17 kDa
CRP	C-reactive protein
CSFP	coronary slow flow phenomenon
DAG	diacylglycerol
EC	effective concentration
ECL	enhanced chemiluminescence
EDHR	endothelial derived hyperpolarizing
EET	epoxyeicosatrienoic acid
E <sub>max</sub>	maximal contraction factor
EDRF	endothelial derived relaxing factor
HMG-CoA	3-hydroxy-3-methyl-glutaryl-CoA reductase
eNOS	endothelial nitric oxide synthase
ET-1	endothelin-1
FH	familial hypercholesterolemia
FPP	farnesyl pyrophosphate
GGPP	geranylgeranyl pyrophosphate
GSNO	S-nitrosoglutathione
GTP	guanosine triphosphate
HDL	high density lipoprotein
HR	heart rate
HUVEC	human umbilical vein endothelial cell
IgG	immunoglobulin G

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ILK	integrin linked kinase
IP <sub>3</sub>	inositol trisphosphate
L-NAME	<i>N</i> <sub>ω</sub> -Nitro-L-arginine methyl ester
LC <sub>20</sub>	20 kDa light chains of myosin
LDL	low density lipoprotein
MAP	mean arterial pressure
MI	myocardial infarction
MLCK	myosin light chain kinase
MLCP	myosin light chain phosphatase
MS	multiple sclerosis
MYPT1	myosin phosphatase targeting subunit
NO	nitric oxide
NOS	nitric oxide synthase
OD	optical density
P	statistical probability
PCI	percutaneous coronary intervention
PGI <sub>2</sub>	prostacyclin
PI3K	phosphatidylinositol 3-kinase
PKA	protein kinase A
PKC	protein kinase C
PKG	protein kinase G
PLC	phospholipase C
PP1c	protein phosphatase type 1
RA	rheumatoid arthritis
ROK	Rho associated kinase (Rho-kinase)
ROS	reactive oxygen species
S.E.M	standard error of the mean
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
Ser	serine
SR	sarcoplasmic reticulum
TBS	tris buffered saline
TBS-T	tris buffered saline – Tween 20
TCA	trichloroacetic acid

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Thr	threonine
TPR	total peripheral resistance
TRPC	transient receptor potential channel
TxA <sub>2</sub>	thromboxane A <sub>2</sub>
VSM	vascular smooth muscle
ZIPK	zipper interacting protein kinase