Published in final edited form as:

Artery Res. 2014 September 1; 8(3): 115–118. doi:10.1016/j.artres.2014.03.002.

ENDOTHELIN-1 SYTEM ACTIVITY IN ADULTS WITH BORDERLINE HIGH LDL-CHOLESTEROL

Kyle J. Diehl¹, Brian L. Stauffer^{1,2,3}, Jared J. Greiner¹, and Christopher A. DeSouza^{1,2}

¹Integrative Vascular Biology Laboratory, Department of Integrative Physiology, University of Colorado, Boulder, CO 80309

²Integrative Vascular Biology Laboratory, Department of Medicine, University of Colorado Denver and the Health Sciences Center, Aurora, CO 80045

³Integrative Vascular Biology Laboratory, Denver Health Medical Center, Denver, CO 80204

Abstract

Background—Modest elevations in plasma low-density lipoprotein (LDL)-cholesterol have been shown to confer a significant increase in cardiovascular risk. Endothelin (ET)-1 is a vasoconstrictor peptide with proatherogenic properties. The experimental aim of this study was to determine whether ET-1 system activity is elevated in adults with borderline high LDL-cholesterol, independent of other cardiometabolic abnormalities.

Methods—Forearm blood flow (FBF; plethysmography) responses to intra-arterial infusion of ET-1, selective ET_A receptor blockade (BQ-123), and non-selective ET_{A/B} blockade (BQ-123 + BQ-788) were determined in 40 middle-aged and older adults (45–70 years): 20 with optimal/near optimal LDL-cholesterol (<3.4 mmol/L) and 20 with borderline-high LDL-cholesterol (3.4–4.1 mmol/L).

Results—Both groups demonstrated a similar, non-significant (~10%) reduction in FBF to ET-1. BQ-123 and BQ-123+788 elicited a modest, but significant, increase in FBF (~15–20%) in each group. However, there were no group differences in the FBF responses to either selective ET_A or non-selective ET_{A/B} receptor antagonism.

Conclusion—Borderline-high LDL-C is not associated with increased ET-1 mediated vasoconstrictor tone. Disrupted ET-1 system activity may not contribute to the increased cardiovascular risk burden with borderline-high LDL-cholesterol.

© 2014 Elsevier B.V. All rights reserved.

Correspondence: Christopher DeSouza, Ph.D., Integrative Vascular Biology Laboratory, Department of Integrative Physiology, University of Colorado, 354 UCB, Boulder, CO 80309, TEL: (303) 492-2988, FAX: (303) 492-6778.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

INTRODUCTION

Low-density lipoprotein (LDL) cholesterol is a major atherogenic fraction of total cholesterol [1, 2] and an important predictor of myocardial infarction in middle-aged adults [3]. In fact, the risk of cardiovascular disease is estimated to increase by ~30% for every 30 mg/dL rise in plasma LDL-cholesterol concentration, suggesting increased cardiovascular risk burden at levels below the clinically recognized elevated range of >130 mg/dL [1]. Indeed, the relative risk of heart disease is nearly twofold higher in adults with borderline high LDL-cholesterol (3.4–4.1 mmol/L [30–159 mg/mL]) compared with adults with optimal/near optimal LDL-cholesterol levels (<3.4 mmol/L [130 mg/dL]) [1]. Moreover, nearly 40% of the adults in the United States have LDL-cholesterol levels in the borderline high range. Thus, understanding the mechanisms involved with the increased vascular risk with borderline high LDL-cholesterol has great public health importance.

Endothelin (ET-1)-1 is a powerful vasoconstrictor peptide that in addition to contributing to the regulation of vascular tone is also involved in the development and progression of atherosclerotic vascular disease [4, 5]. Indeed, enhanced ET-1 system activity elicits a proinflammatory vascular phenotype and ET-1 immunoreactivity is prominent in the walls of atherosclerotic human vessels [6, 7]. Moreover, ET-1 promotes fibrous tissue formation and inhibits endothelial nitric oxide synthesis [8, 9]. Increased ET-1 vasoconstrictor tone is common characteristic of numerous cardiovascular conditions and risk factors [10–12], including clinical hypercholesterolemia [13]. Whether this vascular impairment is already apparent in the borderline-high cholesterolemic state with its corresponding increase in vascular risk is unknown.

Accordingly, the experimental aim of this study was to determine whether ET-1 system activity is elevated in adults with borderline high LDL-cholesterol, independent of other cardiometabolic abnormalities. If so, this may contribute mechanistically to endovascular dysfunction and increased cardiovascular risk in this population.

METHODS

Subjects

Forty, sedentary middle-aged adults were studied: 20 with optimal/near optimal LDL-C (<130 mg/dL) and 20 with borderline-high LDL-C (130–159 mg/dL) [14]. All subjects were sedentary, non-obese, non-smokers, normotensive, non-medicated (including vitamins) and free of overt cardiovascular disease as assessed by physical examination, fasting blood chemistries and electrocardiogram and blood pressure at rest and during incremental exercise performed to volitional exhaustion. All women were at least 1 year postmenopausal and had never taken or had discontinued use of hormone replacement therapy at least 1 year before the start of the study. Written informed consent was obtained according to the guidelines of the University of Colorado at Boulder.

Intra-Arterial Infusion Protocol

All of the studies were performed between 7 AM and 10 AM after a 12-hour overnight fast in a temperature-controlled room as previously described [15]. Briefly, a 5-cm, 20-gauge

catheter was inserted into the brachial artery of the nondominant arm under local anesthesia (1% lidocaine). Forearm blood flow (FBF) at rest and in response to each pharmacological agent was measured by strain-gauge venous occlusion plethysmography (D.E. Hokanson, Bellevue, Washington). To rule out the possibility of nonspecific group differences to vasoconstrictor agents, vascular responses to norepinephrine were determined. Norepinephrine was infused at a rate of 260 pmol/min for 5 minutes, and FBF was measured during the last 3 minutes. After a 20-minute rest period to allow FBF to return to baseline levels, ET-1 (Clinalfa, AG) was infused at a rate of 5 pmol/min for 20 minutes, and FBF was measured during the last 3 minutes. After a 30-minute rest period to allow resting FBF to return to baseline, BQ-123 (Clinalfa, AG), a selective ET_A receptor antagonist, was infused at a rate of 100 nmol/min for 60 minutes. FBF was measured every 10 minutes throughout the infusion period. After 60 minutes of BQ-123 infusion, FBF was measured every 10 minutes during the combined infusion of BQ-123 and BQ-788. BQ-788, a selective ET_B receptor antagonist, was infused at a rate 50 nmol/min for 60 mins.

Statistical Analysis

Differences in subject baseline characteristics and the magnitude of change in FBF to norepinephrine and ET-1 were determined by between-groups analysis of variance (ANOVA). Group differences in FBF responses to BQ-123 and BQ-123+BQ-788 were determined by repeated-measures ANOVA. There were no significant gender interactions, therefore the data were pooled and presented together. Data expressed as means $\pm$ SEM. Statistical significance was set at $P < 0.05$.

RESULTS

Selected subject characteristics are presented in the Table. By design, total cholesterol and LDL-C levels were the only variables significantly different ($p < 0.05$) between the groups. The vasoconstrictor response to norepinephrine was not significantly different between the groups optimal/near optimal (from 4.0 ± 0.3 to 3.0 ± 0.3 mL/100 mL tissue/min) and borderline high (from 4.1 ± 0.3 to 3.0 ± 0.2 mL/100 mL tissue/min) LDL-C groups. Resting FBF was reduced by $\sim 10\%$ in response to ET-1 in both the optimal/near optimal (from 4.0 ± 0.2 to 3.6 ± 0.2 mL/100 mL tissue/min) and borderline high (from 3.9 ± 0.2 to 3.6 ± 0.3 mL/100 mL tissue/min) LDL-C groups (Figure). In response to BQ-123, there was a significant increase in FBF ($\sim 15\%$) from baseline in both groups. The addition of BQ-788 did not significantly alter FBF in either group (Figure). Importantly, there were no group differences in FBF responses to either BQ-123 ($P = 0.77$) or BQ-123 + BQ-788 ($P = 0.51$).

DISCUSSION

The ET-1 system not only plays a central role in the regulation of vascular tone, but is also involved in the etiology of atherosclerotic vascular disease [4, 15]. The primary finding of the present study is that borderline-high LDL-cholesterol is not associated with enhanced ET-1 system activity. Indeed, forearm vascular responses to exogenous ET-1, selective ET_A receptor antagonism and non-selective ET_{A/B} receptor blockade were almost identical between adults with LDL-cholesterol in the optimal/near optimal and borderline-high levels.

Cardillo and colleagues [13] were the first to demonstrate a relation between hypercholesterolemia and ET-1 system activity. In a seminal study, employing the same isolated forearm model as used herein, Cardillo et al. [13] reported that ET_A receptor-mediated ET-1 vasoconstrictor tone was significantly elevated in middle-aged adults with high LDL-cholesterol concentrations (5.8 ± 0.2 mmol/L [223 ± 9 mg/dL]). The results of the present study compliment and extend these findings by demonstrating that ET-1 vasoconstrictor is not elevated at subclinical levels of LDL-cholesterol. This finding is in contrast to conditions such as prehypertension [12] and impaired fasting glucose [16] where ET-1-mediated vasoconstrictor tone is already elevated to comparable levels seen with clinical hypertension [17] and overt diabetes [18]. Interestingly, borderline high LDL-cholesterol is associated with diminished nitric oxide-mediated endothelial vasodilation [19], thus modest elevations in LDL-cholesterol appear to have differential effects on two key elements of endothelial vasomotor regulation. Moreover, given the inhibitory interaction between ET-1 and nitric oxide [20], lack of enhanced ET-1 system activity argues against an ET-1-related mechanism underlying endothelial vasodilator dysfunction with borderline high LDL-cholesterol.

It should be noted that we did not measure circulating plasma concentrations of ET-1. Since the vast majority of ET-1 produced by the endothelium (>80%) is released towards the vascular smooth [21], circulating levels of ET-1 do not appropriately reflect the vascular effects of the peptide [22]. Rather, we employed a pharmacological approach involving the intra-arterial infusion of exogenous ET-1 and ET receptor antagonists providing a more direct and specific biological assessment of ET-1 system activity *in vivo* [13, 17].

In conclusion, the results of the present study demonstrate that borderline high LDL-cholesterol is not associated with increased ET-1 vasoconstrictor activity. Although elevations in LDL-cholesterol in the borderline high range is linked with both diminished endothelial vasodilator function [19, 23] and increased cardiovascular risk burden [1], disrupted ET-1 system activity does not appear to be an contributing factor.

Acknowledgments

We would like to thank all the subjects who participated in this study. This study was supported by National Institutes of Health awards HL077450, HL076434, and UL1 TR000154, American Heart Association awards 0840167N and 09PRE2230382.

References

1. Grundy SM, Cleeman JJ, Merz CN, Brewer HB Jr, Clark LT, Hunninghake DB, Pasternak RC, Smith SC Jr, Stone NJ. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III Guidelines. *J Am Coll Cardiol*. 2004; 44:720–32. [PubMed: 15358046]
2. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. *Circulation*. 1998; 97:1837–47. [PubMed: 9603539]
3. Cremer P, Nagel D, Labrot B, Mann H, Muehle R, Elster H, Seidel D. Lipoprotein Lp(a) as predictor of myocardial infarction in comparison to fibrinogen, LDL cholesterol and other risk factors: results from the prospective Gottingen Risk Incidence and Prevalence Study (GRIPS). *Eur J Clin Invest*. 1994; 24:444–53. [PubMed: 7957500]

4. Miyauchi T, Masaki T. Pathophysiology of endothelin in the cardiovascular system. *Annual Reviews of Physiology*. 1999; 61:391–415.
5. Luscher T, Barton M. Endothelins and endothelin receptor antagonists: therapeutic considerations for a novel class of cardiovascular drugs. *Circulation*. 2000; 102:2434–2440. [PubMed: 11067800]
6. Ihling C, Szombathy T, Bohrmann B, Brockhaus M, Schaefer HE, Loeffler BM. Coexpression of endothelin-converting enzyme-1 and endothelin-1 in different stages of human atherosclerosis. *Circulation*. 2001; 104:864–9. [PubMed: 11514370]
7. Ravalli S, Szabolcs M, Albala A, Michler RE, Cannon PJ. Increased immunoreactive endothelin-1 in human transplant coronary artery disease. *Circulation*. 1996; 94:2096–102. [PubMed: 8901657]
8. Guarda E, Katwa L, Myers P, Tyagi S, Weber K. Effects of endothelins on collagen turnover in cardiac fibroblasts. *Cardiovascular Research*. 1993; 27:2130–2134. [PubMed: 8313419]
9. Lavalley M, Takamura M, parent P, Thorin E. Crosstalk between endothelin and nitric oxide in the control of vascular tone. *Heart Failure Reviews*. 2001; 6:265–276. [PubMed: 11447301]
10. Dashwood M, Tsui J. Endothelin-1 and atherosclerosis: potential complications associated with endothelin-receptor blockade. *Atherosclerosis*. 2002; 160:297–304. [PubMed: 11849651]
11. Kelly J, Whitworth J. Endothelin-1 as a mediator in cardiovascular disease. *Clinical and Experimental Pharmacology and Physiology*. 1999; 26:158–161. [PubMed: 10065339]
12. Weil BR, Westby CM, Greiner JJ, Stauffer BL, DeSouza CA. Elevated endothelin-1 vasoconstrictor tone in prehypertensive adults. *Can J Cardiol*. 2012; 28:347–53. [PubMed: 22244775]
13. Cardillo C, Kilcoyne C, Cannon R, Panza J. Increased activity of endogenous endothelin in patients with hypercholesterolemia. *Journal of American College of Cardiology*. 2000; 36:1483–1488.
14. 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. *Circulation*. 2002; 106:3143–421. Third. [PubMed: 12485966]
15. Weil BR, Westby CM, Van Guilder GP, Greiner JJ, Stauffer BL, DeSouza CA. Enhanced endothelin-1 system activity with overweight and obesity. *Am J Physiol Heart Circ Physiol*. 2011; 301:H689–95. [PubMed: 21666117]
16. Diehl KJ, Templeton DL, Ma J, Weil BR, Greiner JJ, Stauffer BL, Desouza CA. Impaired fasting blood glucose is associated with increased endothelin-1 vasoconstrictor tone. *Atherosclerosis*. 2013; 229:130–3. [PubMed: 23643051]
17. Cardillo C, Kilcoyne C, Wacławski M, Cannon R, Panza J. Role of endothelin in the increased vascular tone of patients with essential hypertension. *Hypertension*. 1999; 33:753–758. [PubMed: 10024340]
18. Cardillo C, Campia U, Bryant MB, Panza JA. Increased activity of endogenous endothelin in patients with type II diabetes mellitus. *Circulation*. 2002; 106:1783–7. [PubMed: 12356630]
19. Diehl KJ, Stauffer BL, Greiner JJ, Weil BR, DeSouza CA. Nitric oxide-mediated endothelium-dependent vasodilation is impaired with borderline high-LDL cholesterol. *Clin Transl Sci*. 2012; 5:21–6. [PubMed: 22376252]
20. Iglarz M, Clozel M. Mechanisms of ET-1-induced endothelial dysfunction. *J Cardiovasc Pharmacol*. 2007; 50:621–8. [PubMed: 18091577]
21. Wagner OF, Christ G, Wojta J, Vierhapper H, Parzer S, Nowotny PJ, Schneider B, Waldhausl W, Binder BR. Polar secretion of endothelin-1 by cultured endothelial cells. *J Biol Chem*. 1992; 267:16066–8. [PubMed: 1644793]
22. Schiffrin EL. Vascular endothelin in hypertension. *Vascul Pharmacol*. 2005; 43:19–29. S1537–1891. [PubMed: 15955745]
23. Walker AE, Eskurza I, Pierce GL, Gates PE, Seals DR. Modulation of vascular endothelial function by low-density lipoprotein cholesterol with aging: influence of habitual exercise. *Am J Hypertens*. 2009; 22:250–6. [PubMed: 19114985]

Figure.

Forearm blood flow response to ET-1 (top panel), BQ-123, selective ETA receptor antagonism (middle panel) and BQ-123 + BQ-788, ETA and ETB receptor blockade (bottom panel) in the optimal/near optimal LDL-C and borderline high LDL-C groups.

Table

Selected Subject Characteristics.

Variable	Optimal/Near Optimal LDL-C (n=20)	Borderline-High LDL-C (n=20)
Age, yr	59±2	59±2
Sex, M/F	11/9	11/9
Body mass, kg	80.5±3.5	76.9±3.6
BMI, kg/m ²	27.2±0.8	26.3±0.9
Body fat, %	34.1±2.3	34.1±1.8
Waist circumference, cm	91.8±2.7	88.0±3.0
Systolic BP, mmHg	126±2	122±2
Diastolic BP, mmHg	78±1	78±2
Total cholesterol, mmol/L	4.7±0.1	5.6±0.1 *
LDL-cholesterol, mmol/L	2.7±0.1	3.7±0.1 *
HDL-cholesterol, mmol/L	1.4±0.1	1.3±0.1
Triglycerides, mmol/L	1.2±0.1	1.4±0.1
Glucose, mmol/L	5.2±0.1	5.1±0.1
Insulin, pmol/L	38.1±4.9	40.2±4.8

BMI indicates body mass index; BP, blood pressure; LDL, low-density lipoprotein; HDL, high-density lipoprotein; Values are mean±SEM.

* P < 0.05 vs. optimal/near optimal LDL-C.