Published in final edited form as:

Pediatr Diabetes. 2012 December ; 13(8): 607–610. doi:10.1111/j.1399-5448.2012.00882.x.

Ascorbic Acid Blocks Hyperglycemic Impairment of Endothelial Function in Adolescents with Type 1 Diabetes

Robert P. Hoffman, MD^{1,2}, Amanda S. Dye, MD¹, and John A. Bauer, PhD²

¹Division of Pediatric Endocrinology, Metabolism and Diabetes Department of Pediatrics, The Clinical Research Center of The Ohio State University College of Medicine and Public Health

²Research Institute at Nationwide Children's Hospital, Columbus, Ohio

Abstract

Objective—To determine whether acute ascorbic acid infusions alters the effect of hyperglycemia on endothelial function in adolescents with type 1 diabetes.

Research Design and Methods—The forearm blood flow (FBF) reactive hyperemic response to 5 min of upper arm occlusion was studied in 8 adolescents with type 1 diabetes during euglycemic and hyperglycemic insulin clamp (40 mU/m²/min) with and without ascorbic acid infusion (3 mg/min).

Results—The ratio of post-occlusion to pre-occlusion FBF decreased during hyperglycemia without ascorbic acid ($p=0.013$) but did not change during hyperglycemia with ascorbic acid. The changes during hyperglycemia were different between the two studies ($p=0.038$). Similar results were found when the percent change in forearm vascular resistance following occlusion was assessed.

Conclusions—These results indicate that antioxidant treatment with ascorbic acid blocks acute hyperglycemic impairment of endothelial function in adolescents with type 1 diabetes.

Introduction

Adults and adolescents with type 1 diabetes have impaired endothelial function (1,2). Hyperglycemia-induced oxidative stress plays a significant pathophysiological role in this impairment (3,4). Specifically, hyperglycemia increases oxygen radicals which decreases the nitric oxide stress response (5). Quite interestingly, a direct adverse effect of glucose variability on endothelial cells has, also, been demonstrated. Protein kinase-c levels, apoptotic rates, and biochemical markers of endothelial cell dysfunction are higher in endothelial cells exposed to intermittent glycemic levels of 90 or 360 mg/dl than in cells exposed continuously to either 90 or 360 mg/dl (6). It is thought that the glucose fluctuations prevent the cells from adapting to the hyperglycemic condition. The adverse effect of oscillating glucose levels has been confirmed in adults with and without type 2 diabetes. Oscillating hyperglycemic and euglycemic clamp over 24 hours leads to greater impairment in endothelial function (measured using flow mediated vasodilation) and greater oxidative damage via reactive nitrogen species than does constant hyperglycemia (7).

Corresponding address; Robert P. Hoffman, M.D., Nationwide Children's Hospital ED422, 700 Children's Drive, Columbus, OH 43205, Telephone: 614-722-4425, Fax: 614-722-4440, Robert.Hoffman@nationwidechildrens.org.

Author Contribution

Robert P. Hoffman was the principal investigator of the funding grant. He developed the proposal, analyzed the data and wrote the manuscript. Amanda S. Dye helped with performing the studies and collecting the data. John A. Bauer assisted with protocol development and editing the manuscript.

Antioxidant treatment with acute intravenous ascorbic acid or 10 days of oral ascorbic acid blocks the dilatory effect of hyperglycemia on endothelial function during hyperglycemic clamp (8) or oral glucose tolerance testing (9) in healthy adults. A 12 hour ascorbic acid infusion restores endothelial function to normal levels in adults with recent onset or intermediate duration, well-controlled type 1 diabetes (10,11). In patients with poorly-controlled type 1 diabetes ascorbic acid infusion, alone, only partially restores endothelial function. It is unknown whether ascorbic acid prevents the acute effect of hyperglycemia in patients with type 1 diabetes, particularly adolescents. The aim of study was to test whether ascorbic acid blocks the acute deleterious effect of hyperglycemia on endothelial in adolescents with type 1 diabetes.

Methods

Subjects

Eight, Tanner Stage 2-4, adolescents (3 female, 5 male) with type 1 diabetes were recruited from the Pediatric Diabetes Clinic of Nationwide Children's Hospital. Their mean age was 14.2 ± 1.5 years (mean $\pm$ SD) and their mean body mass index was 22.0 ± 3.3 kg/m². Mean HgbA1c of $8.4 \pm 1.1\%$ and mean duration of diabetes was 5.1 ± 4.2 years. Type 1 diabetes was defined by American Diabetes Association Criteria, plus a fasting C-peptide of less than 0.4 ng/mL, insulin monotherapy since diagnosis, and an absence of a history of oral hypoglycemic agents and acanthosis nigricans on exam. All subjects were nonsmokers by report. Subjects with BP > 95%tile, smoking, pregnancy, uncorrected hypothyroidism, microalbuminuria (random urine microalbumin/creatinine > 0.02 mg albumin/mg creatinine), overt nephropathy, or early renal failure (serum creatinine > 1.0 mg/dl) were excluded. Subjects on any medications other insulin or levothyroxine were excluded. The study was approved by the Institutional Review Board and informed consent was obtained from parent or legal guardian. Proper assent was obtained from all subjects.

Protocol

Subjects were admitted to the Clinical Research Center at Ohio State University after an overnight fast. Only water intake was allowed after 10 PM. All studies were done at room temperature. Subjects continued home insulin regimen of multiple daily injections or continuous subcutaneous insulin infusion. Basal insulin was continued, while the morning bolus was withheld. Intravenous catheters were placed in the antecubital fossa of each arm. One was used for administration of glucose and insulin and one for blood sampling. After determination of the fasting plasma glucose level (YSI Model 2300; Yellow Springs Instruments, Yellow Springs, OH), insulin infusion was initiated at a rate of 40 mU/m²/min to bring the glucose level to a target of 90-95 mg/dl. Higher insulin rates were used in 4 studies (3 ascorbic acid, 1 control) because of prolonged hyperglycemia. Samples were taken at five minute intervals for the determination of plasma glucose. When euglycemia was achieved, dextrose was added to maintain this level for 30 minutes with a minimum total of 60 minutes of insulin infusion. In those subjects in whom the insulin level was increased it was reduced to and maintained at 40 mU/m²/min at the beginning of euglycemia. Endothelial function measurements were performed at the end of euglycemia. After completion of euglycemia, insulin infusion was continued and dextrose infusion rate was increased to raise plasma glucose level to 200 mg/dl for 60 minutes. Endothelial function was measured at the end of hyperglycemia.

All subjects returned for a second ascorbic acid study which was identical to the first except that ascorbic acid infusion 3 mg/min was begun at the same time as the insulin infusion (12).

Assessment of Endothelial Function

Endothelium dependent vasodilatory response was quantified as both the ratio of forearm blood flow (FBF, rFBF) from before to after upper arm occlusion and as the percent change in forearm vascular resistance (FVR, %FVR) (13,14). Forearm blood flow (FBF) was measured using strain gauge venous occlusion plethysmography using a Hokanson AI6 plethysmograph. Two minutes of baseline FBF was recorded after which the upper arm cuff was inflated to 200 mmHg pressure for 5 minutes to occlude flow. It was then released and FBF measured for one minute. FVR will be calculated by dividing mean arterial blood pressure by FBF. Arterial blood pressure will be measured using automated sphygmomanometer. Mean intra-observer coefficient of variation (CV) for FBF before upper arm occlusion is 5.1% and 7.4% after upper arm occlusion, the CV for percent fall in FVR following upper arm occlusion was 4.5%. This method of testing endothelial function assesses resistance vessel function. Results closely correlate with results from endothelial function assessed by intra-arterial acetylcholine infusion (14), and correlate well with the nitrite/nitrate ratio, an index of NO synthesis (15).

Statistical Analysis

Repeated measures analysis of variance was used to determine differences in rFBF, systolic blood pressure (SBP), diastolic blood pressure (DBP) and MAP pcFVR to changes in plasma glucose with and without ascorbic acid. Systat 11 (SAS, Systat Software Inc, Chicago, IL) was used to perform statistical analysis. Data are expressed as mean $\pm$ SE. Differences were considered significant at $p<0.05$ and tendencies are mentioned at $p<0.1$.

Results

Plasma glucose levels did not differ between the study sessions. Mean baseline glucose for the ascorbic acid study was 206.39 mg/dl and 169.23 for the control study. Mean levels at the end of euglycemia were 89.2 and 77.4 mg/dl and at the end of hyperglycemia 223.8 and 220.9 mg/dl, respectively.

Mean pre-occlusion and post occlusion FBF were 3.6 ± 0.4 and 20 ± 2 ml/dl min during euglycemia without ascorbic acid and 7.0 ± 1.1 and 29 ± 4 ml/dl min during hyperglycemia, respectively. FVR values without ascorbic acid were 24 ± 3 and 4.6 ± 0.7 mmHg dl min/ml pre and post occlusion during euglycemia and 14 ± 3 and 3.2 ± 0.5 during hyperglycemia. Mean pre-occlusion and post occlusion FBF were 4.0 ± 0.6 and 19 ± 2 ml/dl min during euglycemia with ascorbic acid and 5.1 ± 0.7 and 24 ± 3 ml/dl min during hyperglycemia, respectively. FVR values with ascorbic acid were 24 ± 4 and 5.3 ± 0.6 mmHg dl min/ml pre and post occlusion during euglycemia and 18 ± 3 and 4.1 ± 0.8 during hyperglycemia.

Table 1 shows the results for blood pressure, rFBF, and pcFVR for the studies. For blood pressure SBP tended to be slightly higher during ascorbic acid infusion ($p=0.067$). Repeated measures analysis of variance revealed a significant difference in effect of hyperglycemia on the rFBF for the two studies (ascorbic acid by glucose interaction, $p=0.038$). Specifically, rFBF decreased from euglycemia to hyperglycemia in the control study, $p=0.013$, but did not change during the ascorbic acid study. For pcFVR these same differences were found, but did not quite reach statistical significance (ascorbic acid by glucose interaction, $p=0.070$; control, euglycemia vs hyperglycemia, $p=0.090$).

Conclusions

This study demonstrates that acute ascorbic acid infusion minimizes acute, hyperglycemic impairment of endothelial function in adolescents with type 1 diabetes. Specifically, hyperglycemia caused a fall in the reactive hyperemic response to arterial occlusion, as

evidenced by a significant fall in rFBF and near significant fall in pcFVR during the control study. These decreases did not occur during hyperglycemia with ascorbic acid infusion. Importantly, the differences in responses to hyperglycemia were different between the two studies as indicated by the significant and near significant ascorbic acid by glucose interactions.

These results extend previous findings that ascorbic acid blocks the acute effect of hyperglycemia in normal adults (8,9) to adolescents with type 1 diabetes. In these previous studies with hyperglycemic clamp or oral glucose tolerance testing, insulin levels increased at the same time as the glucose levels. The current results, thus, demonstrate that the hyperglycemic impairment occurs without a change in plasma insulin since our patients were insulin deficient and treated with a continuous insulin infusion. The beneficial effects of ascorbic acid indicates that acute increases in oxidative free radicals are likely responsible for hyperglycemic-induced endothelial impairment.

The first limitation of our study is the failure to randomize the order of control and ascorbic acid infusion. The study was performed in this order to confirm that acute hyperglycemia impaired endothelial function before doing the ascorbic acid studies. The second limitation is that we cannot be sure that our results would translate to other methods of measuring endothelial function. The reactive hyperemic response as measured with venous occlusion plethysmography measures the endothelial response of the resistance vessels while the more traditional method measures conduit vessel function. The method used in this study correlates well with results from intra-arterial acetylcholine infusion (11). Resistance vessels changes are more likely to cause long term tissue injury. The studies cited above demonstrated the acute hyperglycemia impairs conduit vessel endothelial function in healthy adults (5,6). Lastly, because of the small number of subjects we cannot assess the effects of diabetes duration or glucose control on the potential benefits of ascorbic acid.

Functional abnormalities of the vascular endothelial lining precede overt cardiovascular disease in patients with and without diabetes (3,4,16,17). Furthermore, abundant short-term evidence indicates that endothelial dysfunction likely precedes microvascular, as well as macrovascular, complications in patients with type 1 diabetes (18,19). The fact that ascorbic acid infusion prevents, as well as restores, hyperglycemic-induced endothelial impairment indicates that its use may be helpful in preventing long-term diabetic complications.

Acknowledgments

This study was supported by the National Institutes of Health NIDDK grant R21DK083642-01 and the American Reinvestment and Recovery Act of 2009. The project described was supported by Award Number UL1RR025755 from the National Center for Research Resources. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health. The authors thank Karen Carter, Lauren Bird and Jesse Haines (Research Institute at Nationwide Children's Hospital) for their help with recruiting subjects and performing the research and the nurses of the CRC for their help with the insulin clamps.

References

1. Jarvisalo MJ, Raitakari M, Toikka JO, et al. Endothelial dysfunction and increased arterial intima-media thickness in children with type 1 diabetes. *Circulation*. 2004; 109:1750–5. [PubMed: 15023875]
2. Newkumet KM, Goble MM, Young RB, Kaplowitz PB, Schieken RM. Altered blood pressure reactivity in adolescent diabetics. *Pediatrics*. 1994; 93:616–21. [PubMed: 8134217]
3. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circulation research*. 2000; 87:840–4. [PubMed: 11073878]

4. Stehouwer CD, Lambert J, Donker AJ, van Hinsbergh VW. Endothelial dysfunction and pathogenesis of diabetic angiopathy. *Cardiovascular research*. 1997; 34:55–68. [PubMed: 9217873]
5. van Oostrom O, de Kleijn DP, Fledderus JO, et al. Folic acid supplementation normalizes the endothelial progenitor cell transcriptome of patients with type 1 diabetes: a case-control pilot study. *Cardiovasc Diabetol*. 2009; 8:47. [PubMed: 19706161]
6. Hirsch IB, Brownlee M. Should minimal blood glucose variability become the gold standard of glycemic control? *Journal of diabetes and its complications*. 2005; 19:178–81. [PubMed: 15866065]
7. Ceriello A, Esposito K, L P, et al. Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetes patients. *Diabetes*. 2008; 57:1349–54. [PubMed: 18299315]
8. Mullan BA, Ennis CN, Fee HJP, Young IS, McCance DR. Pretreatment with intravenous ascorbic acid preserves endothelial function during acute hyperglycaemia (R1). *Clinical and experimental pharmacology & physiology*. 2005; 32:340–5. [PubMed: 15854139]
9. De Marchi S, Prior M, Rigoni A, Zecchetto S, Rulfo F, Arosio E. Ascorbic acid prevents vascular dysfunction induced by oral glucose load in healthy subjects. *Eur J Intern Med*. 2012; 23:54–7. [PubMed: 22153532]
10. Ceriello A, Esposito K, Ihnat M, Thorpe J, Giugliano D. Long-term glycemic control influences the long-lasting effect of hyperglycemia on endothelial function in type 1 diabetes. *J Clin Endocrinol Metab*. 2009; 94:2751–6. [PubMed: 19491221]
11. Ceriello A, Piconi L, Esposito K, Giugliano D. Telmisartan shows an equivalent effect of vitamin C in further improving endothelial dysfunction after glycemia normalization in type 1 diabetes. *Diabetes Care*. 2007; 30:1694–8. [PubMed: 17456844]
12. Ceriello A, Kumar S, Piconi L, Esposito K, Giugliano D. Simultaneous control of hyperglycemia and oxidative stress normalizes endothelial function in type 1 diabetes. *Diabetes care*. 2007; 30:649–54. [PubMed: 17327335]
13. Duck MM, Hoffman RP. Impaired endothelial function in healthy African-American adolescents compared with Caucasians. *The Journal of pediatrics*. 2007; 150:400–6. [PubMed: 17382119]
14. Higashi Y, Yoshizumi M. New methods to evaluate endothelial function: method for assessing endothelial function in humans using a strain-gauge plethysmography: nitric oxide-dependent and -independent vasodilation. *Journal of Pharmacological Sciences*. 2003; 93:399–404. [PubMed: 14737008]
15. Sanada M, Higashi Y, Nakagawa K, et al. Hormone replacement effects on endothelial function measured in the forearm resistance artery in normocholesterolemic and hypercholesterolemic postmenopausal women. *The Journal of clinical endocrinology and metabolism*. 2002; 87:4634–41. [PubMed: 12364448]
16. McGill HC Jr, McMahan CA, Herderick EE, Malcom GT, Tracy RE, Strong JP. Origin of atherosclerosis in childhood and adolescence. *The American journal of clinical nutrition*. 2000; 72:1307S–15S. [PubMed: 11063473]
17. Landmesser U, Merten R, Spiekermann S, Buttner K, Drexler H, Hornig B. Vascular extracellular superoxide dismutase activity in patients with coronary artery disease: relation to endothelium-dependent vasodilation. *Circulation*. 2000; 101:2264–70. [PubMed: 10811593]
18. Jin SM, Noh CI, Yang SW, et al. Endothelial dysfunction and microvascular complications in type 1 diabetes mellitus. *Journal of Korean medical science*. 2008; 23:77–82. [PubMed: 18303203]
19. Pennathur S, Heinecke JW. Oxidative stress and endothelial dysfunction in vascular disease. *Current diabetes reports*. 2007; 7:257–64. [PubMed: 17686400]

Table 1

Blood pressure and reactive hyperemic responses during euglycemic and hyperglycemic clamp with and without ascorbic acid infusion

	Control		Ascorbic Acid		p-values		
	Euglycemia	Hyperglycemia	Euglycemia	Hyperglycemia	Glucose Effect	Ascorbic Acid Effect	Interaction
SBP (mmHg)	106±4	110±8	110±5	117±6	0.16	0.067	0.59
DBP (mmHg)	61±3	60±3	62±4	60±2	0.54	0.84	0.72
MAP (mmHg)	76±3	78±2	78±4	79±3	0.48	0.59	0.61
rFBF	5.9±0.6 ^a	4.6±0.5 ^a	5.1±0.6	4.9±0.5	0.10	0.69	0.038
pcFVR	-80.1±2.0 ^b	-75.3±3.5 ^b	-76.3±2.0	-76.6±2.9	0.23	0.66	0.070

SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; rFBF, ratio of post upper arm occlusion to pre-occlusion forearm blood flow; pcFVR, percent change in forearm vascular resistance from post-occlusion to pre-occlusion.

^ap=0.013

^bp=0.090