European Journal of Ophthalmology / Vol. 15 no. 1, 2005 / pp. 56-61

Myogenic response reduction by high blood glucose levels in human retinal arterioles

M. BLUM[1], C. BRÄNDEL[1], U.A. MÜLLER[2]

[1]Department of Ophthalmology, Helios Klinikum Erfurt
[2]Department of Internal Medicine II, Friedrich-Schiller University, Jena - Germany

PURPOSE. *The effect of elevated blood glucose level on the myogenic response of human retinal arterioles to acute increases in blood pressure is investigated.*
METHODS. *The vascular response to raised blood pressure (Bayliss effect) was measured in 12 healthy volunteers by use of the retinal vessel analyzer (RVA). For a 9-minute period an on-line measurement of the diameter of a retinal branch arteriole was performed. After the first 3 minutes (baseline measurement) a second phase with 3 minutes of isometric exercise caused an acute rise in blood pressure, followed by 3 minutes of recovery (phase III). After the first session 100 g glucose were administered per os. After 30 minutes blood glucose was measured again and an identical second session was performed with higher blood glucose levels. The Wilcoxon test was used for statistical analysis.*
RESULTS. *During the first session a rise in mean arterial pressure of 22.8 (±8.4) mmHg was followed by an arterial vasoconstriction of –6.6 (±1.7) %. The administration of 100 g glucose resulted in a significant rise in blood glucose levels within 30 minutes between the two sessions (4.35 mmol/L vs 7.46 mmol/L) (p=0.002). The blood pressure rise of 25.7 (±7.3) mm Hg in the second session was associated with a significant loss in arterial vasoconstriction of –2.3 (±1.4) % (session I vs session II p=0.002).*
CONCLUSIONS. *The myogenic response of the arterial wall in human retinal arterioles was significantly reduced during acute rise of blood glucose levels. (Eur J Ophthalmol 2005; 15: 56-61)*

KEY WORDS. *Myogenic response, Retinal vessel analyzer, Retinal arterioles, Bayliss effect, Blood glucose*

Accepted: August 26, 2004

INTRODUCTION

In 1904, W.M. Bayliss was the first to describe the Bayliss effect, which is the myogenic constriction of arterioles when transmural pressure is elevated (1). The retina provides an opportunity for the *in vivo* investigation of a part of human circulation. The retinal vessel analyzer (RVA, IMEDOS Jena, Germany) has a high reproducibility and sensitivity for online measurement of retinal vessel diameters (2) and offers the unique chance to measure Bayliss effect on a noninvasive basis in human retinal arterioles (3). It was described in the literature two decades ago that hyperglycemia impairs retinal autoregulation in an animal model (4). Today, the concept of hyperglycemia-induced endothelial dysfunction is supported by a larger number of studies (5-7). The purpose of the present study was to define the influence of a sudden change in blood glucose on the myogenic response in human retinal arterioles.

METHODS

Subjects

One eye was studied in each of 12 healthy volunteers. Data on age and sex are shown in Table I. Pri-

1120-6721/056-06$15.00/0

or to the measurements all volunteers had a full ophthalmologic examination including best-corrected visual acuity, intraocular pressure measurement, slit-lamp biomicroscopy, and direct and indirect ophthalmoscopy. Exclusion criteria were hypertension, cardiovascular disease, suspected or diagnosed glaucoma, and diabetes mellitus. Subjects were taking no medication at the time of the study. The study was approved by the local research ethics committee, and written consent was obtained from each subject.

Experimental protocol

For details on the experimental protocol, see Figure 1. Each subject took part in two sessions of a continuous measurement of a retinal arterial vessel over a 9-minute period by the use of the RVA. After pupil dilation with one drop of tropicamide intraocular pressure (IOP) was measured (noncontact tonometer, Topcon CT 20D). Right before the first session started under fasting conditions blood glucose was measured (Sys-

TABLE I - DATA FROM ALL 12 SUBJECTS AND RESULTS OF SESSION 1

No.	Sex	Age, yr	Blood glucose, mmol/L	MAP 1-4, mmHg	MAPmax, mmHg	ΔMAP, mmHg	Vasoconstriction, %
1	M	39	4.9	78	100	22	-4.9
2	M	25	4.2	88	118	30	-10.1
3	M	26	4.1	75	103	28	-6.9
4	M	23	5.1	110	124	14	-6.9
5	F	21	4.3	89	121	32	-6.7
6	F	24	3.7	94	117	23	-7.4
7	M	25	4.4	81	106	25	-5.2
8	M	25	5.1	87	116	29	-9.0
9	F	19	4.2	79	114	35	-5.9
10	F	20	4.4	73	85	12	-6.3
11	M	22	3.7	86	99	13	-5.8
12	F	19	4.1	76	87	11	-3.8
Mean ± SD		24.0 ± 5.3	4.35 ± 0.47	84.7 ± 10.3	107.5 ± 12.9	22.8 ± 8.4	-6.6 ± 1.7

TABLE II - RESULTS OF SESSION 2 VS SESSION 1

No.	Blood glucose, mmol/L	Δ Blood glucose mmol/L	MAP 1-4 mmHg	MAP_{max} mmHg	ΔMAP mmHg	Vasoconstriction % vs	ΔVasoconstriction session 1, %
1	7.3	+2.4	85	116	31	-0.49	4.4
2	8.7	+4.5	92	122	30	-2.4	7.7
3	8.9	+4.8	84	108	24	-1.2	5.7
4	6.7	+1.6	113	135	22	-0.1	6.8
5	9.8	+5.5	85	124	39	-2.9	3.8
6	6.3	+2.6	94	122	28	-1.6	5.8
7	7.2	+2.8	85	107	22	-1.8	3.4
8	7.3	+2.2	86	116	30	-4.7	4.3
9	8.2	+4.0	82	114	32	-2.6	3.3
10	5.9	+1.5	76	93	17	-4.7	1.6
11	6.5	+2.8	90	112	22	-2.9	2.9
12	6.7	+2.6	72	84	12	-2.0	1.8
	7.46 ±1.2	+3.1 ±1.2	87.0 ±10.2	112.7 ±13.7	25.7 ±7.3	-2.3 ±1.4	4.3 ±1.9

tem One Touch II, Johnson & Johnson). IOP measurements were repeated after the session.

A 3-minute baseline measurement (phase I) of vessel diameter, mean arterial blood pressure (MAP), and ECG was performed first. MAP was calculated as

$$MAP = BP_{dia} + 1/3\ (BP_{sys} - BP_{dia})\ mmHg$$

where BP_{sys} = systolic blood pressure and BP_{dia} = diastolic blood pressure.

This was followed by a 3-minute period of isometric exercise (phase II) causing an acute raise in blood pressure. This was achieved by holding a 1.5 kg weight in the right hand. The rise in MAP was calculated as

$$MAP_{rise} = MAP_{max} - MAP_{bas}$$

with MAP_{max} = maximal MAP under isometric exercise and MAP_{bas} = mean MAP during phase I.

After adjusting the fundus camera to the subject's mydriatic eye the measurement area is defined by dragging a region of interest over a larger arterial vessel close to the papilla. Measurements can be started by mouse click and the diameter of the column of red blood cells is measured by the way of contrast from wall to wall which is defined as vessel width. Vessel diameter measurements during phase II were separated into six 30-second segments. The mean vessel diameter during phase I was used as baseline to calculate the resulting percentage change in vessel caliber due to MAPrise. During the last 3 minutes (phase III) recovery was documented with further continuous measurement of vessel diameter and MAP.

Induction of blood glucose change

After the first session all volunteers were given a 100 g oral glucose dose in warm fluid. Thirty minutes later blood glucose was measured again and a second identical 9 minute session was performed with elevated glucose levels.

Controls

Six subjects of the study group underwent the same protocol with two sessions for a second time, but without glucose intake to serve as a control group. The control group had three male and three female volunteers (Tab. I, Subjects 2, 3, 4, 6, 10, 12). For the retesting they met the same inclusion and exclusion criteria as for the exposition to glucose.

Statistical methods

The Wilcoxon test was used for statistical analysis of the data. Within one session the MAP values of the three phases as well as the vessel diameters were tested for significant changes. The measurements of both sessions including the blood glucose levels were compared for statistical significance.

RESULTS

Session 1

On average, session 1 (Tab. I) started with blood glucose of 4.35 (±0.47) mmol/L. Vessel diameters were 128.1 (±24.7) µm. MAP during phase I was 84.7 (±10.3) mmHg. During phase II MAP showed a significant increase to 107.5 (±12.9) mmHg (p=0.002). On average, MAP rise was 22.8 (±8.4) mmHg. MAP returned to 84.0 (±8.4) mmHg during phase III (not significant vs phase I; p=0.445). Heart rate increased from 71.5 (±9.1) to 88.8 (±12.2) under isometric exercise. The rise in MAP during phase II was associated with an arterial vasoconstriction of –6.6 (±1.7) %. No changes in IOP were measured before and after the session.

Session II

On average, session II (Tab. II) started with blood glucose of 7.46 (±1.2) mmol/L. Vessel diameters were 127.7 (±26.4) µm. Average MAP during phase I was 87.0 (±10.2) mmHg. As in session I, average MAP increased significantly to 112.7 (±13.7) mmHg (p=0.002) during isometric exercise. On average, MAP rise was 25.7 (±7.3) mmHg. MAP returned to 87.1 (±11.6) during phase III (not significant vs phase I; p=0.789). Heart rate increased from 73.4 (±9.8) to 90.2 (±12.9) under isometric exercise. The rise in MAP during phase II resulted in an arterial vasoconstriction of –2.3 (±1.4)%.

Again no changes in IOP were measured before and after the session.

Session I vs session II

The administration of 100 g glucose resulted in a significant change in blood glucose levels within 30 minutes between the two sessions (p=0.002).

The average baseline MAP during phase I in both sessions did not show a significant difference (p=0.105). The MAP rise during phase II was higher during session II. This difference, however, was not statistically significant (p=0.061). No difference was found in the IOP measurements between session I and session II. Furthermore, no difference was found in the vessel diameter at the start of session I and II.

The myogenic response of the arterial wall in retinal arteries was significantly reduced after acute rise of blood glucose levels (p=0.002) (Fig. 2).

Controls

The six subjects of the control group started session I with MAP of 92.3 (±10.6) mmHg vs 91.0 (±12.9) mmHg in session II (p=0.42). During phase II significant MAP rises of 15.5 (±5.0) mmHg and 16.8 (±7.8) mmHg were measured. Again the MAP rise in session II was higher but no statistical significance was shown (p=0.4).

Furthermore, no significant change in blood glucose levels within 30 minutes between the two sessions was found in the control group ((4.4 (±0.5) vs 4.3 (±0.5); p=0.16)). With a vasoconstriction of –8.0 (±4.2)% vs –7.6 (±4.1)% the myogenic response was not significantly changed during the second session (p=0.83) (Fig. 3).

DISCUSSION

The retina provides an opportunity for the *in vivo* investigation of a part of human circulation. Therefore a number of techniques for evaluating the vascular status of the retina have been evolved (8). The RVA is a commercially available system for the measurement of vessel diameters *in vivo* on a noninvasive basis which has shown a high reproducibility and sensitivity (2, 9). It offers the unique chance to measure the myogenic response of human retinal arterioles on a noninvasive basis (3). Clinically this test was used in a pilot study to differentiate a group of diabetics from healthy sub-

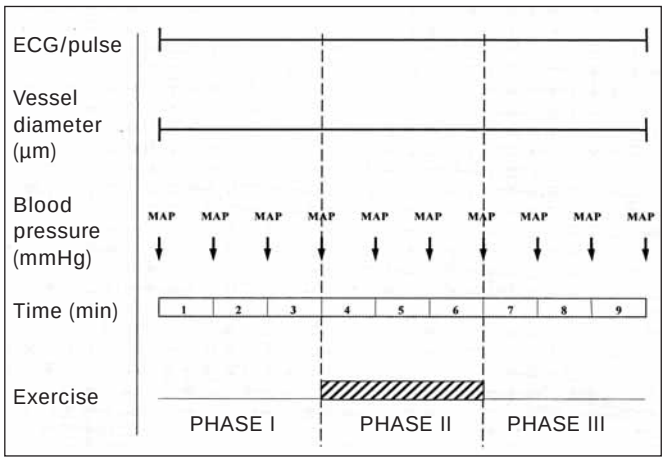

Fig. 1 - *The standard protocol. Two identical sessions were performed in every subject.*

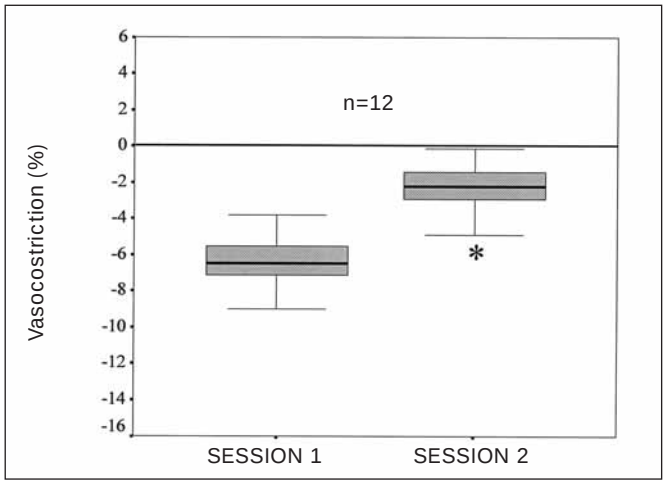

Fig. 2 - *Myogenic response of all 12 subjects in normoglycemia and hyperglycemia (*p=0.002).*

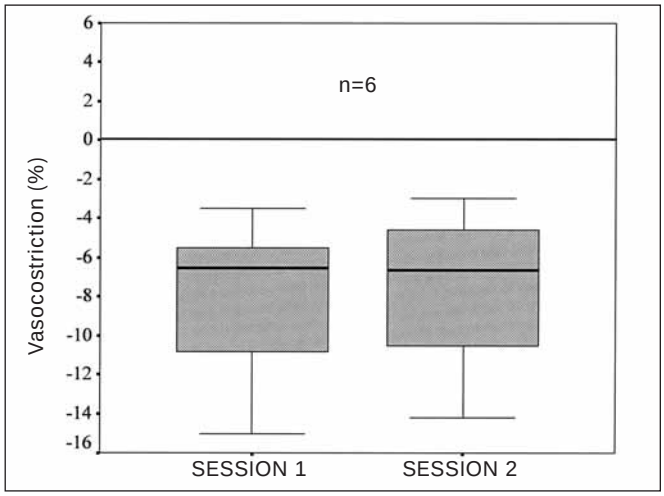

Fig. 3 - *Myogenic response of the six controls within normoglycemia in both sessions.*

jects (10). However, before implementation of a new test a validation and standardization of the testing method is mandatory. Acute hyperglycemia has been shown to increase the retinal blood flow in normal cats and dogs as early as 1983 (4). It is known that elevated blood glucose affects vascular endothelial cell function and the contractility of retinal pericytes (11, 12). Therefore it is necessary to investigate the influence of raised blood glucose on the Bayliss effect before this test setup is used on a larger number of patients.

In our study a setup similar to the oral glucose tolerance test (OGTT) as described and advocated by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus was used (13). The test was used in agreement with the ethics committee on healthy volunteers with 100 g of glucose load *per os*. All subjects reacted with a significant rise in blood glucose after 30 minutes. With elevated blood glucose levels the same rise in MAP was answered by a significantly reduced myogenic response.

As this reduced myogenic response under high blood glucose levels could also be caused by a lack of reproducibility of the test setup, the control group of six subjects was tested again without glucose intake. However, the data of the control group demonstrated that one can expect the same myogenic response to MAP rise under normoglycemia. As IOP did not show any changes before and after the sessions it can be excluded that IOP changes influence the vessel diameter.

The mechanism by which high blood glucose levels might influence the vessel wall is not fully understood. Endothelial dysfunction is believed to play a key role in regulation of vessel tone (14). *In vitro* studies have shown the effect of high glucose on the permeability of the capillary endothelium (15) and in an animal model reduced vessel tone of cerebral vessels under hyperglycemia has been reported to be caused by an endothelial mechanism (16). Further support for this concept was provided by studies showing a reduction in nitric oxide (NO) bioavailability in cultured aortic endothelial cells as well as retinal endothelial cells exposed to high glucose levels (17, 18). In animal model the role of reactive oxygen and nitrogen species was supported and recent studies have focused on the formation of nitrotyrosine (19). The *in vivo* measurements on human arterioles presented in our study are well in line with the literature.

However, there are limitations that must be taken into account while interpreting our results. The increase in blood glucose provoked in our setup will induce an increase in insulin plasma levels due to endogenous release of insulin in our healthy subjects within 30 minutes. Insulin itself has hemodynamic effects that we cannot distinguish from the glucose effect at present time. In isolated retinal arterioles from pig eyes a direct vasodilatory effect of insulin was reported (20). In our study both glucose and insulin levels are changing and to the best of our knowledge no non-invasive technique is clinically available to separate these two factors in human volunteers. We were unable to measure a significant change in the arterial vessel diameter at the start of our second session. Clinical data from Grunwald et al report that normoglycemia achieved with insulin injection results in a reduction of retinal flow (8, 21). This can only be achieved by a stronger vasoconstriction. However, we measured a significantly reduced vasoconstriction starting from the same vessel diameter as before. It is therefore not very likely that we have measured the effect of insulin, although we cannot exclude this entirely.

It should be stressed that the purpose of our study was to define the influence of a sudden change in blood glucose on the myogenic response. Even when results obtained with our methodology are cautiously interpreted it is very likely that changes in blood glucose levels will influence the measurement of the myogenic response. Further clinical evidence is given by studies where high blood glucose levels in non-diabetic subjects were linked with poor outcome in stroke patients and oxygen consumption in the retina was tested (22, 23). Future studies on the dynamics in vessel tone not only in subjects with diabetes but also in healthy subjects should therefore be carried out under control of blood glucose levels.

ACKNOWLEDGEMENTS

Supported by OphthalmoInnovation Thüringen (FKZ:13N8003).

Reprint requests to:
Marcus Blum, MD
Augenklinik, Helios Klinikum Erfurt
Nordhäusser Str. 74
99089 Erfurt, Germany
mblum@erfurt.helios-kliniken.de

REFERENCES

1. Bayliss WM. On the local reactions of the arterial wall to changes in internal pressure. J Physiol 1902; 28: 220-31.
2. Polak K, Dorner GT, Kiss B, et al. Evaluation of the new Zeiss retinal vessel analyzer. Br J Ophthalmol 2000; 84: 1285-90.
3. Blum M, Bachmann K, Wintzer D, Riemer T, Vilser W, Strobel J. Noninvasive measurement of the Bayliss effect in retinal autoregulation. Graefes Arch Clin Exp Ophthalmol 1999; 237: 296-300.
4. Ernest JT, Goldstick TK, Engerman RL. Hyperglycemia impairs retinal oxygen autoregulation in normal and diabetic dogs. Invest Ophthalmol Vis Sci 1983; 24: 985-9.
5. Chakravarthy U, Hayes RG, Stitt AW, McAuley E, Archer DB. Constitutive nitric oxide synthase expression in retinal vascular endothelial cells is suppressed by high glucose and advanced glycation end products. Diabetes 1998; 47: 945-52.
6. Enderle MD, Benda N, Schmuelling RM, Haering HU, Pfohl M. Preserved endothelial function in IDDM patients, but not in NIDDM patients, compared with healthy subjects. Diabetes Care 1998; 21: 271-7.
7. Haselton FR, Dworska EJ, Hoffman LH. Glucose-induced increase in paracellular permeability and disruption of ß-receptor signaling in retinal endothelium. Invest Ophthalmol Vis Sci 1998; 39: 1676-84.
8. Cioffi GA, Alm A. Ocular circulation. In: Kaufmann PL, Alm A, eds. Adler's Physiology of the Eye. Clinical Application. St. Louis: Mosby; 2003: 747-84.
9. Barcsay G, Seres A, Németh J. The diameters of the human retinal branch vessels do not change in darkness. Invest Ophthalmol Vis Sci 2003; 44: 3115-8.
10. Blum M, Kubetschka U, Hunger-Dathe W, Bachmann K, Müller UA, Strobel J. Autoregulation retinaler Arteriolen bei Patienten mit Diabetes mellitus und Normalprobanden. Klin Monatsbl Augenheilkd 2000; 216: 40-4.
11. McGinty A, Scholfield CN, Liu WH, Anderson P, Hoey DE, Timble ER. Effect of glucose on endothelin-1-induced calcium transients in cultured bovine retinal pericytes. J Biol Chem 1999; 274: 25250-3.
12. Williams SB, Goldfine AB, Timimi FK, et al. Acute hyperglycemia attenuates endothelium-dependent vasodilation in humans *in vivo*. Circulation 1998; 97: 1695-701.
13. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (1998). Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1998; 21(Suppl): S5-19.
14. Brown SM, Jampol LM. New concepts of regulation of retinal vessel tone. Arch Ophthalmol 1996; 114: 199-204.
15. Gillies MC, Su T, Stayt J, Simpson JM, Naidoo D, Salonikas C. Effect of high glucose on permeability of retinal capillary endothelium *in vitro*. Invest Ophthalmol Vis Sci 1997; 38: 635-42.
16. Cipolla MJ, Porter JM, Osol G. High glucose concentrations dilate cerebral arteries and diminish myogenic tone through an endothelial mechanism. Stroke 1997; 28: 405-11.
17. Du Y, Smith MA, Miller CM, Kern TS. Diabetes-induced nitrative stress in the retina, and correction by aminoguanidine. J Neurochem 2002; 80: 771-9.
18. Grainer WF, Posch K, Fleischhacker E, Wascher TC, Kostner GM. Increased superoxide anion formation in endothelial cells during hyperglycemia: an adaptive response or initial step of vascular dysfunction? Diabetes Res Clin Prac 1999; 45: 153-60.
19. El-Remessy AB, Abou-Mohamed G, Caldwell RW, Caldwell RB. High glucose-induced tyrosine nitration in endothelial cells: role of eNOS uncoupling and Aldose reductase activation. Invest Ophthalmol Vis Sci 2003; 44: 3135-43.
20. Su EN, Yu DY, Alder VA, Cringle SJ, Yu PK. Direct vasodilatory effect of insulin on isolated retinal arterioles. Invest Ophthalmol Vis Sci 1996; 37: 2634-44.
21. Grunwald JE, Riva CE, Baine J, Brucker AJ. Total retinal volumetric blood flow rate in diabetic patients with poor glycemic control. Invest Ophthalmol Vis Sci 1992; 33: 356-63.
22. Tiedeman JS, Kirk SE, Srinivas S, Beach JM. Retinal oxygen consumption during hyperglycemia in patients with diabetes without retinopathy. Ophthalmology 1998; 105: 31-6.
23. Weir CJ, Murray GD, Dyker AG, Lees KR. Is hyperglycemia an independent predictor of poor outcome after stroke? Results of a long term follow up study. BMJ 1997; 314: 1303-6.