Journal of Diabetes Science and Technology
2014, Vol. 8(5) 1042–1047

DOI: 10.1177/1932296814537039
dst.sagepub.com
SAGE

Glycemic Variability in Nondiabetic Morbidly Obese Persons: Results of an Observational Study and Review of the Literature

Sara J. Salkind, MS[1], Robert Huizenga, MD[2], Stephanie J. Fonda, PhD[1], M. Susan Walker, PhD[1], and Robert A. Vigersky, MD[1]

Abstract

Glycemic variability (GV) is correlated with oxidative stress which may lead to increased cardiovascular risk and poor clinical outcomes in people with prediabetes and diabetes. We sought to understand whether morbidly obese persons without diabetes by standard criteria have dysglycemia as measured by GV. We performed an observational study of GV metrics and carotid intima media thickness (CIMT) in 21 morbidly obese normoglycemic and 15 morbidly obese prediabetic applicants to *The Biggest Loser* television show. The results were compared to previously published studies in normoglycemic nonobese and obese individuals. Glucose was measured with a masked continuous glucose monitor (CGM) over 3 to 8 days and carotid intima media thickness (CIMT) was determined by ultrasound. CGM-derived GV metrics for GV were coefficient of variation (CV), standard deviation (SD), mean amplitude of glycemic excursions (MAGE), continuous overall net glycemic action–1 hour (CONGA1), and mean of daily differences (MODD). We found that morbidly obese subjects (n = 21) who were normoglycemic by standard criteria had higher GV (CV = 22%, SD = 24.2 mg/dl and MAGE = 48.6 mg/dl) than previous reports of normoglycemic, nonobese individuals (CV = 12-18%, SD = 11.5-15.0 mg/dl, and MAGE = 26.3-28.3 mg/dl). Morbidly obese prediabetic subjects (n = 15) had GV metrics indistinguishable from those morbidly obese subjects who were normoglycemic. CIMT was higher in both morbidly obese groups compared with historical age- and sex-matched controls. Normoglycemic and prediabetic morbidly obese individuals have higher GV compared with normal weight, nondiabetic individuals. We speculate that this may increase the risk for macrovascular disease through excessive oxidative stress.

Keywords

cardiovascular risk, continuous glucose monitoring, glycemic variability, morbid obesity, oxidative stress, prediabetes

It is hypothesized that glycemic variability (GV) may play a role in diabetic microvascular complications in people with type 2 diabetes through stimulation of oxidative stress (OS).[1,2] In vitro data suggest that GV is more deleterious than consistently high glucose concentrations alone,[3-5] but it is unclear what contribution GV makes to micro- and macrovascular complications in humans.[6] If GV causes OS and is an independent risk factor for complications for people with type 2 diabetes, it is important to understand when GV starts to appear. Obesity is a major risk factor for type 2 diabetes and cardiovascular disease,[7] which are both epidemic in the United States.[8,9] People with morbid obesity provide a unique opportunity to study the relationship of obesity and GV.

Herein, we present data on CGM-derived GV in normoglycemic and prediabetic morbidly obese individuals. The GV of a pure population of morbidly obese individuals has not previously published. We then summarize these new findings about GV in the context of the extant literature on CGM-derived GV in normal weight and obese people without diabetes.[10-18]

Research Design and Methods

We performed a nonrandom, uncontrolled, observational study of 40 morbidly obese (BMI ≥ 40) applicants to the tenth season of *The Biggest Loser*, a television show where participants compete to lose weight over a 6- to 7-month period (http://www.nbc.com/the-biggest-loser/). This was a

[1]Walter Reed National Military Medical Center, Bethesda, MD, USA
[2]Cedars-Sinai Medical Center, Los Angeles, CA, USA

Corresponding Author:
Robert A. Vigersky, MD, Diabetes Institute, Endocrinology Service, Walter Reed National Military Medical Center, 8901 Wisconsin Ave, Bethesda, MD 20889, USA.
Email: robert.a.vigersky.mil@health.mil

self-selected convenience sample who had applied for participation in *The Biggest Loser* and were those individuals who progressed through the preclinical screening process to reach the clinical evaluation. The measures obtained were part of a clinical evaluation of the applicants approved by the Institutional Review Board of Cedars-Sinai Medical Center. All applicants underwent metabolic screening prior to the selection of contestants for the television show. The metabolic screening included a hemoglobin A1C, fasting plasma glucose and insulin, blood glucose at 1 and 2 hours following a 75 g oral glucose load, and a fasting lipid profile. In addition, the percentage body fat by DXA scan was obtained using a GE Healthcare Lunar Densitometer, and carotid intima medial thickness (CIMT) was determined via high-resolution B-mode ultrasound images of the right common-carotid artery using a 7.5-MHz linear array transducer attached to Philips iE-33 (Philips Medical System, Andover, MA). CIMT was measured 5-10 mm below the common-carotid bifurcation during mid-diastole in the M-mode tracings by automated software and clinically blinded expert readers as previously described.[19] The subjects also wore a masked Dexcom Seven Plus® Real-Time CGM for 3 to 8 days. The participants calibrated twice daily and wore the CGM as directed. The mean absolute relative difference (MARD) between the calibration values and the corresponding CGM values was 7% and 17% in the prediabetes and normal glycemic groups, respectively.

Of the 40 applicants, 21 were normoglycemic by standard criteria (A1C <5.7% or 39 mmol/mol; fasting glucose < 100 mg/dL; 2-hour postprandial glucose load < 140 mg/dL). Fifteen subjects met the criteria for prediabetes (A1C 5.8-6.4%; 40 mmol/mol to 46 mmol/mol; 100 mg/dL < fasting glucose < 125 mg/dL; or 140mg/dL < postglucose load < 199 mg/dL), and 4 had type 2 diabetes. The data from the 4 subjects with type 2 diabetes were excluded and the data from the 36 normal and prediabetic subjects were analyzed. Ten of the subjects with normal glucose tolerance and 11 of the subjects with prediabetes were taking at least 1 prescription medication. In most cases these are not known to have effects on glucose metabolism or GV. One patient in the normoglycemic group was on metformin. In the prediabetes group 1 patient was on metformin/sitagliptin. The GV metrics of these 2 patients were not significantly different from their respective groups' so they were not excluded from the data analysis.

Measures

We used the glucose data from the CGM to characterize GV. Since applicants wore the CGM for different numbers of days, we selected 3 days of use for each applicant. In those who wore the sensor for more than 4 days, the middle 3 days of data were selected. If the participant had only 4 days of usable data, the first day was excluded. This process allowed us to standardize the amount of glucose data that we examined for each applicant. GV measures were calculated using EasyGV.[10] The metrics from EasyGV reported in this analysis are mean of daily differences (MODD), coefficient of variation (CV), continuous overall net glycemic action–1 hour (CONGA1), mean amplitude of glycemic excursion (MAGE), and the standard deviation of the glucose values (SD).

Carotid intima-medial thickness data were obtained on 15 of the 21 normoglycemic participants and 13 of the 15 participants with prediabetes.

Analyses

The analyses summarized the baseline characteristics of the 2 contestant groups with means ± standard deviations or frequencies, as appropriate, and then compared the 2 groups with *t* tests of the means (age, BMI, and all metabolic data), a chi-square test (for gender), and a Fisher exact probability test (for race/ethnicity). Next, because this cohort has not been characterized to date, the analyses examined the raw glucose data from the CGM for the contestants, also by group. Spearman correlations were then performed to examine the unpartial and partial association (net of metabolic syndrome, yes/no) between BMI and SD.

The analyses then compared the GV in *The Biggest Loser* applicants with that reported in publications of GV data in nondiabetic adult populations.[10-18] To do this comparison, we reviewed the English literature for studies that included data on nonpregnant adults where CGM, BMI, and parameters of GV were reported. We defined normal, overweight, obesity, and morbid obesity as BMIs of <25, 25-29.9, 30-39.0, and ≥40 kg/m^2, respectively, except in Asian populations, where a BMI ≥25 kg/m^2 is considered to be obese.[20] Eight studies met the search criteria.[10-18] Note that 2 studies were excluded because there were no data about weight or BMI[10] or because the study population studied was Asian and had mean BMI of 25 kg/m^2, which qualifies them as obese (see below).[15]

Spearman correlations were performed to examine the unpartial and partial association (net of metabolic syndrome, yes/no) between BMI and SD—the only measure of GV common to all studies—in *The Biggest Loser* contestants and the subjects from the published reports.

Results

Morbidly Obese People Without Diabetes—Biggest Loser Applicants

The baseline data for the morbidly obese *Biggest Loser* applicants in the nondiabetic and prediabetic groups were similar with respect to age, gender, degree of obesity (BMI and percentage body fat measured by DEXA scan), and lipid profiles (Table 1). The fasting plasma glucose, insulin, and A1C levels were significantly higher in those with prediabetes.

There was no significant difference in the GV metrics between the 2 nondiabetic (normoglycemic and prediabetic)

Table 1. Characteristics of *The Biggest Loser* Applicants.

	Normoglycemic, n = 21		Prediabetic, n = 15		
	Mean	SD	Mean	SD	*P* value
Age	32.0	10.4	33.7	10.7	.66
Gender					.61
Male	8		7		
Female	13		8		
Race/ethnicity					.68
Caucasian	14		10		
African American	5		3		
Hispanic	2		2		
Other	3		0		
BMI	49.6	10.1	51.4	6.9	.65
DXA-derived body fat (%)	51.86	3.96	50	4	.27
A1C (%) nmol/mmol	5.4/37	0.2/2	5.8/40	0.3/3	.03*
Diastolic blood pressure	132	17	135	12	
Systolic blood pressure	83	9	83	9	
Fasting plasma glucose	89	12	97	31	.1
2-hour postglucose load (mg/dL)	107	18	145	36	.0003*
Fasting serum insulin (μu/ml)	16.9	13.2	26.2	9.5	.03*
Hypertension (%)	76		93		.25
LDL (mg/dL)	119	29	111	22	.71
Triglycerides (mg/dL)	102	50	107	43	.63

P values are from *t* tests for the continuous variables (all denoted by means with standard deviations), a chi-square test for gender, and a Fisher exact probability test for race/ethnicity.
*Significant difference between the 2 groups.

morbidly obese groups (2 right columns in Table 2). The morbidly obese *Biggest Loser* applicants who were normoglycemic were hypoglycemic 11.2% of the time and hyperglycemic 11.8% of the time; the morbidly obese prediabetic subjects were hypoglycemic 4.6% of the time and hyperglycemic 17.7% of the time. The normoglycemic group spent significantly more time with blood sugars under 80 mg/dL ($P < .0001$), while the prediabetic group spent significantly more time with levels above 140 mg/dl ($P < .001$).There was no correlation between GV metrics and BMI in either morbidly obese group or when the groups were combined. In addition, there are no correlations between the percentage lean body mass and percentage fat mass with the metrics of GV. The CIMT did not differ between those morbidly obese subjects with normoglycemia (0.6 ± 0.1 mm) and those with prediabetes (0.6 ± 0.1 mm).

Comparison With Extant Literature

The GV data from 6 studies that met our search criteria are shown in Table 2 arranged in increasing order of SD. The range of GV in nonobese individuals (including those who are overweight) is a CV of 12-18%, an SD of 11.5-18 mg/dL and a MAGE of 26.3-28.3 mg/dL. There were very few glucose values in the hyper- or hypoglycemic range. These GV metrics are substantially lower than those in morbidly obese nondiabetic and prediabetic subjects. In 2 studies of obese normoglycemic individuals (with or without metabolic syndrome) the GV metrics were higher than normal: the CV is 24-28%, SD is 29.4-32.4 mg/dl, and MAGE is 25.2-37.6 mg/dl.[17,18] When the GV data from *The Biggest Loser* study were combined with those in the extant literature, there was a significant correlation between SD and BMI in subjects without diabetes ($r = .76$, $P = .01$). The trend persisted after those with metabolic syndrome ($r = .64$, $P = .06$) were excluded.

The CIMT results in the morbidly obese subjects (0.6 ± 0.1 mm) are similar to those reported in obese nonmetabolic syndrome subjects (0.71 ± 0.04 mm)[21] but exceed those reported in studies of nonobese (BMI = 24.7), similarly aged (28.4), normoglycemic (fasting glucose ~90 mg/dL) subjects (0.49 ± 0.05 for men and 0.48 ± 0.05 for women) in.[21]

Discussion

There are few studies exploring GV in truly normoglycemic individuals. Based on our review of the literature, we believe that we can now define a normal range of GV (CV of 12-18%; SD of 11.5-18 mg/dL; MAGE of 26.3-28.3 mg/dL) in those who are not obese (but may include those who are overweight) and who are normoglycemic by standard measures. It is in this context that we can better understand the results of *The Biggest Loser* study, which finds significant

Table 2. Glucometrics From CGM Data in Normoglycemic Nonobese Subjects Compared With *The Biggest Loser* Applicants.

	Nondiabetic adults[13]	Nondiabetic adults[11†]	Nondiabetic adults[16]	Nondiabetic adults[12]	Overweight without MS[18]	Nondiabetic adults[14]	*Biggest Loser* (Prediabetic)	*Biggest Loser* (Normal)
n	23	37	434	10	6	32	15	21
BMI	23.2 ± 2.2	24.9*	21.8	21.7 ± 1.4	29.0 ± 2.3	25.0 ± 2.0	51.4 ± 6.9	49.6 ± 10.1
A1C %	N/A	5.3 ± 0.3	N/A	N/A	N/A	5.3	5.8 ± 0.3	5.4 ± 0.2
Mean mg/dL	99.0 ± 15.0	96 ± 8	104	99.0 ± 8.0	74.3 ± 1.7	102.0 ± 7.0	117.0 ± 11.0	110.0 ± 14.0
SD mg/dL	11.5 ± 4.3	12.5 ± NA	14.2 ± NA	15.0 ± 4.0	15.6 ± NA	18.0 ± 4.0	23.7 ± 5.5	24.2 ± 10.0
CV %	12	13	13.6	15	21	18	20	22
MAGE mg/dL	28.3 ± 9.4	26.3 ± NA	N/A	N/A	N/A	N/A	50.0 ± 17.0	48.0 ± 26.0
CONGA1 mg/dL	N/A	N/A	N/A	N/A	N/A	N/A	103 ± 9.0	98.0 ± 14.0
MODD mg/dL	N/A	N/A	N/A	N/A	N/A	N/A	28.0 ± 6.0	28.0 ± 12.0
% of values <80 mg/dL	N/A	2.9††	2.4**	N/A	N/A	3.0**	4.6	11.2
% of values >140 mg/dL	N/A	0.3††	4.1	N/A	N/A	4.0	17.6	11.8
% of values 81-139 mg/dL	N/A	93.7††	93.5	N/A	N/A	93.0#	78.0	77.0

All values are reported as mean ± standard deviation. If a value was not reported in the original source, it is denoted here as N/A (not available).
*Median. **<70 mg/dL. #<70 mg/dL to 140 mg/dL. †Data shown are combined for ages 25 to ≥45 years as reported in JDRF study. ††Ranges in JDRF study are <70 mg/dl; >140 mg/dl, and 71-120 mg/dl.

dysglycemia as manifested by increased GV metrics in these morbidly obese individuals who are otherwise normoglycemic (as determined by traditional metrics of assessing glycemia). While it is not clear to us why there is no difference in GV metrics between the morbidly obese groups, we speculate that while there is a continuum of GV with increasing weight (as evidenced by the correlation or CV and BMI when all comparative groups are included), there is maximum effect on GV above a certain degree of obesity.

There are important implications of these observations if a linkage between GV and micro- and/or macrovascular disease can be established since there is an epidemic of obesity and morbid obesity in the United States[9] and the prevalence of morbid obesity is rising.[22]

That GV potentially plays a role in the development of microvascular disease is suggested by i*n vitro* data in human umbilical vein endothelial cells[3] and human retinal epithelial cells.[23] The umbilical vein study demonstrated that nitrotyrosine and 8-hydroxydeoxyguanosine (markers of OS) and apoptosis are greater when exposed to intermittently high glucose levels compared with consistently high glucose levels.[3] Human retinal epithelial cells produce more vascular endothelial growth factor in variable glucose levels in the culture media compared with consistently high glucose.[23] In human studies, there is an increased nitrotyrosine and 8-isoprostaglandin F2α (8-IsoPGF2α) in both normal and patients with type 2 diabetes when plasma glucose levels oscillate between 80 and 240 mg/dL during a euinsulinemic hyperglycemic clamp study.[24] In addition, a correlation was found between MAGE and urinary 8-IsoPGF2α in patients with poorly controlled type 2 diabetes,[25,26] although not in those with Type 1 diabetes.[27] Bragd et al found a correlation between the SD of fingerstick blood glucose values and peripheral neuropathy complications in patients with type 1 diabetes and suggested that the nervous system may be particularly susceptible to GV.[28] On the other hand, no relationship was found between GV (measured by fingerstick) and microvascular complications in the Diabetes Control and Complications Trial.[29]

Recent studies show that there is an association of GV with cardiovascular disease.[30-33] More recently, a positive correlation between 8-IsoPGF2 and left ventricular mass index has been found.[34] Buscemi et al demonstrated that those with high GV (as measured by the CV) was an independent predictor of poor vascular function as measured by flow mediated dilation.[17] Although our study did not assess vascular function, we did find a higher than normal CIMT in our morbidly obese normoglycemic subjects compared to data reported by others in similar age and nonobese individuals. This is consistent with Buscemi et al's observations that there was a correlation between CIMT and GV in a univariate analysis.[17] These data suggest that morbidly obese individuals with increased GV may have a greater risk of cardiovascular disease due to dysglycemia-induced OS even if they are normoglycemic by traditional criteria. Whether this is due to GV, hypertension (present in 76% of our normoglycemic and 93% of our prediabetic subjects, respectively), dyslipidemia, or some combination cannot be determined from our data. It is important to note that while our data and those of others suggest that GV may be a marker of vascular dysfunction, there is, as yet, no evidence of causality.

Our study has several strengths. Chief among them is that it is the first to investigate GV in a population of morbidly obese yet nondiabetic individuals. Another is that it includes a large enough sample size of nondiabetic and prediabetic morbidly obese individuals to analyze GV metrics using CGM to capture the data for the calculation of those metrics. Finally, it is one of the first studies to methodically examine the relationship between BMI and GV for individuals with normoglycemia or prediabetes.

The main limitation of our study is that the data are from an uncontrolled, cross-sectional, observational study that did not include contemporaneously studied normal weight and/or obese control groups. We recognize that it will be important to have age-, sex-, and glycemic-status-matched populations that vary only by BMI in future studies. In addition, our study included a relatively small number of subjects. However, the collective data from previous studies in normal subjects without obesity places this data in unique comparative context. We also have no data on the food consumption and physical activity of the subjects while the CGM data were being obtained. Additional research would also allow a discussion about which of these may influence GV in those without diabetes or prediabetes. In future research it would also be important to overtly measure OS to discuss the relationship between OS and GV more completely.

In summary, we found that morbidly obese individuals who are either normoglycemic or prediabetic by traditional measures have dysglycemia as demonstrated by their increased GV when compared to normal weight and obese individuals as described in the extant literature. We speculate that the elevated GV may be an important risk factor in the increased cardiovascular disease seen in obese and morbidly obese persons who are not diabetic.

Abbreviations

A1C, hemoglobin A1C; BG, blood glucose; BMI, body mass index; CGM, continuous glucose monitoring; CONGA, continuous overall net glycemic action; CV, coefficient of variation; DXA, dual X-ray absorptiometry; FPG, fasting plasma glucose; GV, glycemic variability; MAGE, mean amplitude of glycemic excursion; MODD, mean of daily differences; OS, oxidative stress; SD, standard deviation

Authors' Note

The opinions expressed in this article reflect the personal views of the authors and not the official views of the U.S. Army or the Department of Defense.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: RAV has received investigator-initiated research grants from DexCom. He has served on medical advisory boards of Bayer, Medtronic, and Sanofi.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

References

1. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes*. 2005;54:1615-1625.
2. Brownlee M, Hirsch IB. Glycemic variability—a hemoglobin A1C-independent risk factor for diabetic complications. *JAMA*. 2006;295:1707-1708.
3. Quagliaro L, Piconi L, Assaloni R, Martinelli L, Motz E, Ceriello A. Intermittent high glucose enhances apoptosis related to oxidative stress in human umbilical vein epithelial cells. *Diabetes*. 2003;42:2795-2804.
4. Piconi L, Quagliaro L, Assaloni R, et al. Constant and intermittent high glucose enhances endothelial cell apoptosis through mitochondrial superoxide overproduction. *Diabetes Metab Res Rev*. 2006;22:198-203.
5. Jones SC, Saunders HJ, Qi W, Pollock CA. Intermittent high glucose enhances cell growth and collagen synthesis in cultured human tubulointerstitial cells. *Diabetologia*. 1999;42:1113-1119.
6. Siegelaar SE, Holleman F, Hoekstra JBL, DeVries JH. Glucose variability: does it matter? *Endocrine Rev*. 2010;3:171-182.
7. Grundy SM. Pre-diabetes, metabolic syndrome, and cardiovascular risk. *J Am Coll Cardiol*. 2012;49:635-643.
8. Center for Disease Control Fact Sheet. 2011. Available at: http://www.cdc.gov/diabetes/pubs/pdf/ndfs_2011.pdf. Accessed March 7, 2013.
9. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of obesity in the United States, 2009-2010. National Center for Health Statistics Data Brief Number 82. January 2012.
10. Hill NR, Oliver NS, Choudhary P, Levy JC, Hindmarsh P, Matthews DR. Normal reference range for mean tissue glucose and glycemic variability derived from continuous glucose monitoring for subjects without diabetes in different ethnic groups. *Diabetes Technol Ther*. 2011;13:921-928.
11. Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study Group. Variation of interstitial glucose measurements assessed by continuous glucose monitors in healthy, nondiabetic individuals. *Diabetes Care*. 2010;33:1297-1299.
12. Hanaire H, Bertrand M, Guerci B, Anduze Y, Guillaume E, Ritz P. High glycemic variability assessed by continuous glucose monitoring after surgical treatment of obesity by gastric bypass. *Diabetes Technol Ther*. 2011;13:625-630.
13. Ma CM, Yin FZ, Wang R, et al. Glycemic variability in abdominally obese men with normal glucose tolerance as assessed by continuous glucose monitoring system. *Obesity*. 2011;19:1616-1622.
14. Mazze RS, Strock E, Wesley D, et al. Characterizing glucose exposure for individuals with normal glucose tolerance using continuous glucose monitoring and ambulatory glucose profile analysis. *Diabetes Technol Ther*. 2008;10:149-159.
15. Nomura K, Saitoh T, Kim GU, Yamanouchi T. Glycemic profiles of healthy profiles of healthy individuals with low fasting plasma glucose and HbA1c. *ISRN Endocrinol*. 2011;2011:1 -6.
16. Zhou J, Hong L, Ran X, et al. Establishment of normal reference ranges for glycemic variability in Chinese subjects using continuous glucose monitoring. *Med Sci Monit*. 2011;17:CR9-13.
17. Buscemi S, Re A, Batsis JA, et al. Glycaemic variability using continuous glucose monitoring and endothelial function in the metabolic syndrome and in Type 2 diabetes. *Diabetes Med*. 2010;27:872-878.
18. Buscemi S, Verga S, Cottone S, et al. Glycaemic variability and inflammation in subjects with metabolic syndrome. *Acta Diabetol*. 2009;46:55-61.

19. Ahmadi N, Eshaghian S, Huizenga R, Sosnin K, Ebrahimi R, Siegel R. Effects of intense exercise and moderate caloric restriction on cardiovascular risk factors and inflammation. *Am J Med*. 2011;124:978-982.
20. Examination Committee of Criteria for "Obesity Disease" in Japan, Japan Society for the Study of Obesity. New criteria for 'obesity disease' in Japan. *Circ J*. 2002;66:987-992.
21. Oren A, Vos LE, Uiterwall CSPM, Grobbee DE, Bots ML. Cardiovascular risk factors and increased carotid intima-media thickness in healthy young adults: The Atherosclerosis Risk in Young Adults (ARYA) Study. *Arch Intern Med*. 2003;163:1787-1792.
22. Sturm R, Hattori A. Morbid obesity rates continue to rise rapidly in the United States. *Int J Obes (Lond)*. 2013;37:889-891.
23. Sun J, Xu Y, Sun S, Sun Y, Wang X. Intermittent high glucose enhances cell proliferation and VEGF expression in retinal endothelial cells: the role of mitochondrial reactive oxygen species. *Mol Cell Biochem*. 2010;343:27-35.
24. Ceriello A, Mercuri F, Quagliaro L, et al. Detection of nitrotyrosine in the diabetic plasma: evidence of oxidative stress. *Diabetologia*. 2001;44:834-848.
25. Monnier L, Mas E, Ginet C, et al. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *JAMA*. 2006;295:1681-1687.
26. Monnier L, Colette C, Mas E, et al. Regulation of oxidative stress by glycaemic control: evidence for an independent inhibitory effect of insulin therapy. *Diabetologia*. 2010;53:562-571.
27. Wenholt IM, Kulik W, Michels RPJ, Hoekstra JBL, DeVries JH. Glucose fluctuations and activation of oxidative stress in patients with type 1 diabetes. *Diabetolgia*. 2008;51:183-190.
28. Bragd J, Adamson U, Backlund LB, Lins PE, Moberg E, Oskarsson P. Can glycaemic variability, as calculated blood glucose self-monitoring, predict the development of complications in type 1 diabetes over a decade? *Diabetes Metab*. 2008;34:612-616.
29. Kilpatrick ES, Rigby AS, Atkin SL. Effect of glucose variability on long-term risk of microvascular complications in type 1 diabetes. *Diabetes Care*. 2009;32:1901-1903.
30. Hermanides J, Vriesendorp TM, Bosman RJ, Zandstra DF, Hoekstra JB, DeVries JH. Glucose variability is associated with intensive care unit mortality *Crit Care Med*. 2010;38:838-842.
31. Temelkova-Kurktschiev TS, Koehler C, Henkel E, Leonhardt W, Fuecker K, Hanefeld M. Postchallenge plasma glucose and glycemic spikes are more strongly associated with atherosclerosis than fasting glucose or HbA1c level. *Diabetes Care*. 2000;23:1830-1834.
32. Su G, Mi S, Tao H, et al. Association of GV and the presence and severity of coronary artery disease in patients with type 2 diabetes. *Cardiovasc Diabetes*. 2011;10:19-27.
33. Hu Y, Liu W, Huang R, Zhang X. Postchallenge plasma glucose excursions, carotid intima-media thickness, and risk factors for atherosclerosis in Chinese population with type 2 diabetes. *Atherosclerosis*. 2010;210:302-306.
34. Di Flaviani A, Picconi F, Di Stefano P, et al. Impact of glycemic and blood pressure variability on surrogate measures of cardiovascular outcomes. *Diabetes Care*. 2011;34:1605-1609.