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Review Article

Biological variation of cardiovascular risk factors in patients with diabetes

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Abstract

Biological variation refers to the natural fluctuations found when repeated measurements are made in a biological system. Generally, biological variation remains within narrow boundaries in health, but may differ in pathological states, with implications for the diagnosis and monitoring of disease processes. In disease, biological variation may alter such that any subsequent measurement may need to have a greater difference compared with a healthy control to be biologically relevant. Treatments such as insulin or anti-hypertensive therapy have been shown to reduce biological variability closer to normal levels and theoretically this may help prevent complication development or progression in conditions such as diabetes. This article reviews how biological variation can influence our identification and assessment of vascular risk factors in a person with diabetes. The role of biological variation in the diagnosis of diabetes (glucose and HbA_{1c}) is then examined. Finally, the influence that common treatments in diabetes have in modifying biological variation is described.

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Introduction

All measurements of components in biological fluids are subject to variability, comprising both analytical and biological variations. Analytical variability is a term used to describe the error introduced through measuring the component required. Causes of analytical fluctuations include pre-analytical variation attributable to differences in sample collection and handling, such as the fall in glucose concentrations when a specimen is not collected into a container that includes a preservative. Analytical error in the measuring instrument being used usually comprises of both intra-assay (within a batch of samples) imprecision and interassay (or between-batch) imprecision. Superimposed on this there can be a calibration bias, which does not in itself change the analytical variability, but will lead to consistently higher or lower test results compared with the true value. Lastly, post-analytical variation can occur when, for example, there is an inaccurate representation or transcription of results onto the final test report. While this analytical variation can be determined for most biological analytes, it cannot easily be calculated for many clinical variables such as blood pressure measurement.

Although steps can be taken to minimize analytical variability, biological variation, in contrast, is the inherent variability in the concentration of a component in blood or other fluid. This is caused by either intra- or inter-individual variation for any specific variable. Intra-individual variation can occur either in a cyclical or random pattern. The former occurs in a predictable fashion, such as in the diurnal variation in cortisol, the monthly changes during the female reproductive cycle or even in a seasonal cycle such as that for vitamin D concentrations. Additionally, there can also be the natural unpredictable random variation around a subject's homeostatic set point [1]. Inter-individual variability describes the biological differences found in a measurement between individuals (rather than within) amongst the population being studied. Figure 1 demonstrates how intra- and inter-individual variation can vary depending on the test being measured. For the test in Fig. 1a, intra-individual variability is large but inter-individual variability small, whereas for the test in Fig. 1b the opposite is the case. It means that, in Fig. 1a, the distribution of values when an individual is tested repeatedly is similar to that of the population as a whole, whereas in Fig. 1b individuals stay close to their 'set point', which can be substantially different from the set point of another subject.

Both the analytical and biological variations of laboratory samples have implications for the diagnosis and monitoring of disease. The coefficient of variation, a popular means of expressing the intra- and inter-individual variation of a test, is

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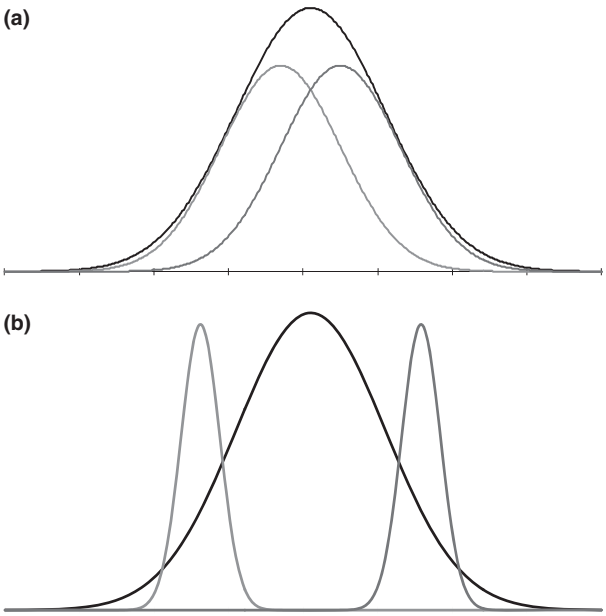

FIGURE 1 Schematic representation of two individuals (in grey) as part of the population distribution (in black) of two tests (a) and (b).

often calculated using data from a healthy population. Table 1 shows examples of the coefficient of variation for glycated haemoglobin, glucose and lipids [2]. There is evidence that estimates of biological variation are reassuringly constant in healthy individuals from population-based studies such as the Third National Health and Nutrition Examination Study (NHANES III) [3], a survey of a non-institutionalized civilian US population. The effect of any specific illness on the biological variation of an analyte can be variable, such that it may or may not remain unchanged. Thus, although the homeostatic point for a test may be changed by illness, it does not correspond that the within-individual variability of the test will necessarily change as well [1]. However, sometimes in disease the intra-individual variation is different to that of the healthy state. If there is indeed a rise in intra-individual variation, then a subsequent measurement may need to differ more from the previous one in order to be biologically significant.

Table 1 Coefficient of variation of intra- and inter-individual of analytes using in the management of cardiovascular risk and diabetes

Analyte	Intra-individual variation (CVw)	Inter-individual variation (CVg)
HbA_{1c}	1.9	5.7
Glucose (plasma)	4.5	5.8
Glucose (serum)	6.1	6.1
Cholesterol	5.4	15.2
HDL cholesterol	7.1	19.7
Triglycerides	20.9	37.2

CVw, coefficient of variation for intraindividual variation; CVg, coefficient of variation for interindividual variation.

In order to review current literature on biological variation, we performed a structured literature search using PubMed according to the patient, intervention, comparison, outcome (PICO) method, including relevant literature published online up to October 2012. We focused on the most salient papers concerning cardiovascular risk and biological variation in diabetes in this narrative review.

Relating biological variation to the development of diabetes complications

Glucose variability and microvascular complications

Glucose variability is often determined from data collected by the self-monitored measurement of seven-point daily glucose profiles (pre-/post-meal and bedtime), by laboratory-measured glucose profiles or by using continuous glucose monitoring. There remains considerable debate around which of the many statistical measures is the best assessment of this variability [4]. One of the simplest and most readily identifiable methods is by calculating the standard deviation of glucose measurements and/or the coefficient of variation, the latter taking account of the mean value. Another measure that is widely used is calculation of the mean amplitude of glycaemic excursions, which attempts to prioritize periods of marked glucose excursions.

Mean absolute glucose change is another method used to assess glucose variability and is the summated change in glucose per unit of time. This measure was first used in the intensive care setting and demonstrated that high glucose variability was associated with both intensive care and in-hospital death [5].

Alternative methods include calculating the mean absolute value of the differences between glucose values on two consecutive days at the same time and continuous overlapping net glycaemic action. Predictably, despite the different approaches taken, all measures are usually closely related to one another [6,7].

The measurement of glycated haemoglobin has become the cornerstone of glycaemic assessment in patients with diabetes. However, patients with similar glycated haemoglobin (HbA_{1c}) levels or mean blood glucose levels can have markedly different daily glucose excursions that will not be identified by the HbA_{1c} or mean blood glucose result alone. The role of this variability in the pathology of diabetes and the development of microvascular and macrovascular complications is subject to much debate and is still largely unresolved [4,8,9].

Hyperglycaemia is thought to cause vascular damage by the overproduction of superoxide mitochondrial electron-transport chain, as hypothesized in a unifying theory of microvascular complications developed by Brownlee [10]. Hyperglycaemia results in activation of production of reactive oxygen species, which in turn results in increased formation of advanced glycation end products and other

changes resulting in vascular damage. Fasting plasma glucose instability has been shown to be a predictor of cardiovascular mortality in the elderly patients with Type 2 diabetes [11] and this was examined further by Ceriello *et al*. [12]. They examined the effects of oscillating glucose levels in 27 patients with Type 2 diabetes and 22 control subjects by performing the euinsulinaemic hyperglycaemic clamp and showed that oscillating glucose between 5 and 15 mmol/l resulted in significantly increased endothelial dysfunction (using flow-mediated dilatation) and increased oxidative stress [measured using 24-h urine concentrations of free 8-iso prostaglandin $F_{2\alpha}$ (8-iso $PGF_{2\alpha}$) and plasma 3-nitrotyrosine] compared with continuous hyperglycaemia at levels of either 10 or 15 mmol/l glucose.

In an influential paper from 2006, Monnier *et al*. provided evidence that glucose variability was a major cause of increased reactive oxygen species production [13]. The authors studied 21 patients with Type 2 diabetes (two diet controlled and 19 on oral hypoglycaemic agents) compared with 21 age- and sex-matched control subjects and determined oxidative stress by measuring 24-h urine concentrations of the isoprostane free 8-iso prostaglandin $F_{2\alpha}$ (8-iso $PGF_{2\alpha}$) as well as determining glucose variability by collecting continuous glucose monitoring levels for three consecutive days. They showed the average urinary 8-iso $PGF_{2\alpha}$ was higher in the patients with diabetes and, crucially, that glucose fluctuations in these subjects, as assessed by mean amplitude of glycaemic excursion, was associated with raised isoprostane concentrations, independently of the mean glucose.

More recent studies have found difficulty in reproducing the findings of Monnier *et al*. De Vries' group sought to establish a similar relationship in 21 patients with Type 1 diabetes [14], but instead found there was no association between 8-iso $PGF_{2\alpha}$ and mean amplitude of glycaemic excursion ($r^2 = 0.08$), despite the range of glucose variability in their study patients being greater than in Monnier *et al*.'s and them measuring the 8-iso $PGF_{2\alpha}$. by a more specific method.

More recently, a study by Monnier *et al*. examined both Type 1 diabetes and Type 2 diabetes treated with insulin or oral agents [15] and again assessed the patients' oxidative stress by measuring 24-h urine collections for 8-iso $PGF_{2\alpha}$. The non-insulin-treated patients with Type 2 diabetes had the highest levels of 8-iso $PGF_{2\alpha}$, being independently associated with mean amplitude of glycaemic excursion and HbA_{1c}. However, in patients with Type 1 diabetes and patients with Type 2 diabetes treated with insulin, both associations disappeared, with 8-iso $PGF_{2\alpha}$ concentrations in fact being within the non-diabetic range. Indeed, when patients with Type 2 diabetes who were taking oral hypoglycaemic agents were started on insulin treatment, their subsequent excretion rate of 8-iso $PGF_{2\alpha}$ reduced by more than 50%, unlike patients whose treatment was intensified using oral hypoglycaemia agents, where their level of 8-iso $PGF_{2\alpha}$ remained unchanged. This study therefore suggested that insulin therapy itself may inhibit the production of reactive oxygen species, although how this might relate to the subsequent development of diabetes complications remains speculative.

Other authors have attempted to establish if glucose variability is associated with the development of complications by examining large epidemiological or trial data. In a retrospective study by Takao *et al*. [16] looking at 170 patients with Type 2 diabetes followed for a mean of 33 years, the standard deviation (SD) of their fasting plasma glucose measurements was used as a variability boundary. The patients were classified on the basis of their mean HbA_{1c} or fasting plasma glucose and also their fasting plasma glucose SD (low or high). This revealed the risk of retinopathy development and progression was similar in the group showing good control but high variability when compared with the group who had poor control but low variability. The authors concluded that fasting plasma glucose variability is a risk factor for developing retinopathy, independent of the mean fasting plasma glucose or HbA_{1c}. This is in contrast to other studies examining the seven-point profile based on the Diabetes Control and Complications Trial (DCCT) data set that examined 1441 individuals with Type 1 diabetes. Kilpatrick and co-workers determined the mean glucose area under the curve and glucose variability within 24 h and between study visits (both as standard deviation). They then related these to the risk of developing retinopathy, nephropathy and neuropathy, showing that within-day and between-day variability in blood glucose was not independently related to the development or progression of either complication [17,18]. This corroborated data from Service and O'Brien who examined glucose variability in the same DCCT individuals by calculating the SD, M-value and mean amplitude of glycaemic excursion and they too concluded that the meaurements of glucose variability did not provide any additional risk assessment information beyond that provided by mean capillary blood glucose [19]. One explanation for the lack of agreement between studies may be that Takao *et al*. were studying patients with Type 2 diabetes, while patients in the DCCT had Type 1 diabetes, this being consistent with Monnier's observation that insulin treatment seems to reduce reactive oxygen species. Alternatively, it could be that the fasting plasma glucose variability calculated by Takao *et al*. is a measure that is more akin to their HbA_{1c} variability (see below) than their within-day glucose variability.

Glycated haemoglobin (HbA_{1c}) variability

Measurement of glycated haemoglobin, predominately HbA_{1c}, is now fundamental to the management of people with diabetes. It is the most widely used measurement of prior glycaemic control and is used to monitor long-term glycaemic control, predict the risk of development of

complications and is employed as an assessment of the quality of diabetes care [20].

To examine HbA_{1c} variability on the risk of development of retinopathy and nephropathy, Kilpatrick *et al.* examined the DCCT data of 1441 patients with Type 1 diabetes. The HbA_{1c} variability was closely related to the mean HbA_{1c} for these patients. Unsurprisingly, mean HbA_{1c} was predictive of both retinopathy and nephropathy, but when the standard deviation of HbA_{1c} between the 3-monthly visits was included in the statistical modelling, the variability in HbA_{1c} either added to or explained the risk indicated by mean HbA_{1c} alone. This suggested that increased HbA_{1c} variability in patients with Type 1 diabetes could be a major contributor to the risk of microvascular disease over and above that of mean HbA_{1c} value [21]. A previous analysis of DCCT data showed little effect of HbA_{1c} variability on complication risk [22]; however, subsequent analyses from other study data have confirmed the association between HbA_{1c} variability and complication risk, especially with regard to the development of nephropathy [18,23].

The reason or reasons why HbA_{1c} variability seems to predict microvascular risk are speculative. It is possible that HbA_{1c} is simply more sensitive at detecting the effect of glucose changes than traditional daily glucose profiles, or that patients with variable HbA_{1c} are those in whom the rest of their diabetes management is suboptimal.

Mean glucose is the major determinant of the HbA_{1c} value in patients with diabetes and therefore is the main reason for between-individual variability in this group of patients. However, in some subjects their HbA_{1c} level tracks consistently higher or lower than their mean glucose would suggest. It is still not clear whether such high or low 'glycators' are at different risk of developing microvascular complications, even if their mean glucose levels are the same [24,25].

Insulin resistance variability

Insulin resistance is an integral factor in the development of Type 2 diabetes and has been shown to have an association with increased cardiovascular mortality. For example, in one study, 1521 men aged 42–60 years who had no history of cardiovascular disease or diabetes had their fasting serum insulin measured along with other cardiovascular risk factors. The study showed that fasting serum insulin level, as a continuous variable, was directly associated with the risk of cardiovascular death ($P = 0.006$) [26]. Population studies such as these give an indication of the effect that between-individual differences in insulin resistance can have on risk, but are not able to assess within-subject (intra-individual) variability.

In order to establish the intra-individual variability of insulin resistance, Jayagopal *et al.* examined 12 post-menopausal women with diabetes and 11 weight- and age-matched control subjects and determined the variability of the homeostasis model assessment of insulin resistance (HOMA-IR) in a 4-day interval on 10 consecutive occasions [27]. They showed that, not only was the mean insulin resistance increased in the women with Type 2 diabetes, but their intra-individual variation was substantially greater compared with the control subjects without diabetes. A similar finding was found in another insulin-resistant state of polycystic ovary syndrome [28]. This increase in variability has yet to be adequately explained as the main determinant of insulin resistance in a population, namely subject weight, did not alter significantly during either study. Likewise, whether this variability is contributory to the cardiovascular risk associated with insulin resistance is unknown.

Blood pressure variability

Untreated hypertension is associated with increased cardiovascular risk [29] and has been shown to be related to the development and progression of nephropathy in patients with diabetes [30]. Along with mean blood pressure, its variability and/or the maximum blood pressure reached may have a role in the aetiology. Rothwell *et al.* examined visit-to-visit blood pressure variability in patients without diabetes with treated hypertension and also patients with previous transient ischaemic attacks [31]. Systolic blood pressure was measured at 4-monthly intervals and the variability was defined as the standard deviation or the coefficient of variation. They showed that visit-to-visit variability in systolic blood pressure was a strong predictor of subsequent stroke in the group with previous transient ischaemic attack and the variability was also a strong predictor of stroke and coronary events in the group with treated hypertension.

Blood pressure variability has been examined in the DCCT cohort of 1441 patients with Type 1 diabetes [32] in respect of the development of microvascular complications. The participants had their blood pressure measured at least annually. This study showed increasing visit-to-visit variability in both systolic and diastolic blood pressure to be associated with the development of nephropathy. However, blood pressure variability was less of a feature for worsening diabetic retinopathy. The data suggested that a DCCT patient on the 95th centile of systolic blood pressure variability (SD 13.3 mmHg) could have 2.34 times the nephropathy risk of a patient on the 2.5th centile (SD 3.7 mmHg) for a given mean systolic blood pressure.

Lipid variability

Dyslipidaemia is another important contributory factor in the development of cardiovascular disease. Hyperglycaemia, particularly in Type 2 diabetes, can adversely affect the lipid profile, increasing triglycerides, lowering HDL cholesterol concentrations and increasing the formation of small dense LDL particles [33]. The intra-individual variability of total

cholesterol, triglycerides, LDL and HDL in patients with Type 1 and Type 2 diabetes who were not on any lipid-lowering medication has been examined [34]. Samples taken from 60 patients at 3- to 6-monthly intervals for at least 4 years showed there was significantly higher variability for total and HDL cholesterol compared with data in subjects without diabetes. The intra-individual biological variability in subjects with diabetes fell outside the 95% confidence intervals for variability without diabetes for both total cholesterol (coefficient of variation 8.4 vs. 5.5–6.5%) and HDL cholesterol (9.1 vs. 6.4–8.4%). The variability of triglycerides was at least double that of total cholesterol, HDL and LDL cholesterol and biological variation accounted for the greatest proportion of total variability for all lipid measurements. As is the case for insulin resistance, it is not currently known whether this increased variability in diabetes compounds any cardiovascular risk already present because of the atherogenic lipid profile.

Like variations in HbA_{1c}, it is difficult to determine if any association between lipid fluctuations and increased cardiovascular risk could at least be partly explained by confounding factors contributing to cardiovascular disease, such as poor or intermittent compliance to a healthy lifestyle [35].

Role of biological variation in diagnosing diabetes

Fasting blood glucose

The definition of diabetes is based on blood glucose concentrations that are associated with an increased risk of developing microvascular complications, particularly retinopathy [36]. Selvin *et al*. analysed data from the National Health and Nutrition Examination Survey III (NHANES III) second examination [37], where repeated examinations were conducted in 685 patients approximately 2 weeks after the original visit. The authors showed that the 2-h glucose levels had substantially more variability compared with either fasting glucose or HbA_{1c}. They found that a single fasting glucose reading of > 7.0 mmol/l was a fairly reliable indicator of diabetes with 70% repeatability, but a single 2-h glucose reading was much less so.

This variability of glucose readings supports a previous study examining 193 patients who were newly diagnosed with Type 2 diabetes who had a fasting glucose sample measured on two consecutive days [38]. The variability of fasting plasma glucose was assessed by comparison of percentage differences with averaged fasting plasma glucose and showed a 95% biological variability of 13.7%. The authors highlighted that this variability should be considered in patients who have a fasting blood glucose of 6.0–6.9 mmol/l as they may well have a subsequent fasting plasma glucose above the diagnostic criterion. The variance of these differences increased with increasing average fasting plasma glucose.

The timing of the sample is crucial as it also has an effect on fasting blood glucose; the dawn phenomenon may affect the glucose levels. It is not known how much the circadian rhythm and other hormones affect this biological variation.

Oral glucose tolerance test

The reproducibility of the oral glucose tolerance test, or lack of it, is well described [28–31]. One of the most comprehensive studies was a cohort of women in Sweden where all 64-year-old women in Gothenburg were invited to have a 75-g oral glucose tolerance test, which was repeated within 2 weeks if the result did not show normal glucose tolerance [39]. In total, 4856 women were invited to take part, of whom 82% responded and 2595 (53%) participated. More than 40% of women with an oral glucose tolerance test result indicating diabetes at the first test did not fulfil the criteria at the second test. Furthermore, of the patients who had impaired glucose tolerance diagnosed on the first test, more than 50% had a normal result on the second test. This poor reproducibility, mainly a consequence of variable 2-h glucose results, is supported by a study examining 31 patients without diabetes who had a repeat test 48 h after the initial test [40]. For the fasting sample, the mean fasting plasma glucose was identical on the two occasions, 71% varied by less than 10% and 97% varied by less than 20%. In comparison, the 2-h reading showed much higher variability; only 45% varied by less than 10% and 39% varied by greater than 20%. The NHANES III study [37] was able to compare oral glucose tolerance test glucose values with HbA_{1c} and showed the 2-h glucose levels had substantially more variability [coefficient of variation 16.7% (15.0–18.3)] compared with either fasting glucose [5.7% (5.3–6.1)] or HbA_{1c}, which had the lowest coefficient of variation of 3.6% (3.2–4.0). As a consequence, they also noted that the prevalence of undiagnosed diabetes using the 2-h glucose values was 9% (95% CI 5.6–12.2) using a single examination. If two abnormal results from two examinations were required, then the prevalence decreased to 6.7% (3.9–9.5%) a 2.3% (–3.5 to –1.1%) absolute difference. If a positive oral glucose tolerance test in either examination 1 or examination 2 was the criterion, then the prevalence was 11.2% (8.0–14.7%) a 2.2% (1.1–3.5%) absolute difference. This again further supported the high intra-individual variability in the 2-h oral glucose tolerance test.

Glycated haemoglobin (HbA_{1c})

The biological variability of glycated haemoglobin in 48 patients without diabetes was examined by Rohfling *et al*. [41] where fasting blood samples were collected weekly for 12 weeks for HbA_{1c} and fasting plasma glucose. They showed the intra-individual variation [calculated by SD was 0.08% (coefficient of variation 1.7%)]. Kilpatrick *et al*. [42] examined the biological variation of HbA_{1c}, whereby 12

patients without diabetes had fasting blood taken on 10 2-weekly occasions. They showed that, although the intra-individual variation was low, the inter-individual variation was high, suggesting that mean non-diabetic HbA_{1c} values varied markedly between (but not within) subjects. Therefore, within an HbA_{1c} reference interval of 20–42 mmol/mol (4–6%), an individual whose initial HbA_{1c} was 20 mmol/mol (4%) would have to increase their normal HbA_{1c} by 12 standard deviations to lie above the reference range, whereas for another patient starting at 42 mmol/mol (6%) it may only have to alter by 2 standard deviations. As these two subjects without diabetes may have initially had similar glucose tolerance, it means that the person at 20 mmol/mol (4%) could be markedly hyperglycaemic by the time they are identified as having an HbA_{1c} which is abnormal compared with the population without diabetes.

Braga *et al*. performed a review of biological variation of HbA_{1c} by examining nine studies [43]. Perhaps predictably they showed a substantial difference between the variability of HbA_{1c} in healthy individuals and persons with diabetes, with the intra-individual biological variability for HbA_{1c} being less than 2% in healthy individuals, whereas in people with diabetes the coefficient of variation ranged from 2 to 10%.

Subject age and ethnicity also affect HbA_{1c}. In individuals without diabetes < 40 years old, the 97.5th percentile for HbA_{1c} was 37 mmol/mol (5.6%) and rose to 44 mmol/mol (6.2%) for individuals without diabetes over the age of 70 years using the NHANES cohort [44,45]. This rise could not be explained by any differences in glycaemia between the age groups. Ethnicity consistently affects HbA_{1c}; for example, in individuals with impaired glucose tolerance in the Diabetes Prevention Programme, HbA_{1c} was compared in five racial and ethnic groups. After adjusting for cofounding factors, mean HbA_{1c} levels were 40 mmol/mol (5.78%) for white people, 41 mmol/mol (5.93%) for Hispanics, 42 mmol/mol (6.00%) for Asians, 43 mmol/mol (6.12%) for American Indians and 44 mmol/mol (6.18%) for black people ($P < 0.001$) [44].

The influence of treatments used in diabetes on biological variation

Treatments influencing glucose variability

The type of insulin used in treatment of diabetes can have a marked effect on glucose variability. Basal insulin preparations such as neutral protamine hagedorn (NPH) and the insulin analogue detemir (which is a derivate of human insulin soluble at neutral pH) have been compared [46]. After 26 weeks of treatment, there was no difference in mean fasting plasma glucose or in the nine-point self blood-glucose monitoring profiles. The biological variability of glucose (calculated by standard deviation and coefficient of variation percentage of the self blood-glucose monitoring profile) was lower for determir compared with NPH (1.3 vs. 1.4 mmol/l, $P = 0.021$). A potential advantage of continuous subcutaneous insulin infusion could be the reduction of glucose excursions, even compared with multiple daily injection therapy. This has been examined by Brutomesso *et al*., who examined 39 Type 1 patients with a mean HbA_{1c} 59 ± 10 mmol/mol (7.6 ± 0.8%) [47]. They were randomly assigned to a continuous subcutaneous insulin infusion with insulin lispro, or multiple daily injections with lispro and glargine. After 4 months they were switched to the alternative treatment. Blood glucose was measured using seven-point self-monitored readings. During the last month of each treatment, blood glucose variability was analysed using glucose standard deviation, mean amplitude of glycaemic excursion and average daily risk range showed that, during continuous subcutaneous insulin infusion, glucose variability was lower, blood glucose levels were lower and hyperglycaemic episodes were fewer. Continuous subcutaneous insulin infusion therapy compared with multiple daily injection therapy has also been examined using continuous glucose monitoring systems [48]. In this study, 36 patients with Type 1 diabetes were treated with continuous subcutaneous insulin infusion and 77 patients had multiple daily injection therapy. Interstitial glucose concentration was measured continuously over 72 h. HbA_{1c} was higher in this study: 69 ± 15 mmol/mol (8.5 ± 1.4%). Glucose variability was analysed using mean amplitude of glycaemic excursion, coefficient of variation, continuous overall net glycaemic action (CONGA)2 and CONGA4. They showed the glucose variability was lower in the insulin infusion therapy group only when HbA_{1c} was < 58 mmol/mol (7.5%), and glucose variability was actually worse in the patients treated with insulin infusion therapy when the HbA_{1c} was > 77 mmol/mol (9.2%), which could be attributable to more frequent use of correction boluses in patients in the insulin infusion therapy group compared with those in the multiple daily injection group.

Glucagon-like polypeptide 1 treatments such as exenatide, increase glucose-dependent insulin secretion and should thereby reduce postprandial glycaemia and, as a consequence, glucose variability. The effects of exenatide and the basal insulin, glargine, on glucose variability have been examined. Two hundred and eighty-two patients with Type 2 diabetes were randomized to receive exenatide and 267 matched patients received glargine insulin daily [49]. The study showed that, although the mean of the seven-point glucose values were similar between the two groups, the standard deviation of glucose values in the exenatide group was significantly lower than in those taking glargine.

Treatments influencing lipids

The effect of statins on the variability of lipid measurements in patients with Type 2 diabetes was examined by Sathyapalan *et al*. [50]. They performed a crossover study on 26

patients with Type 2 diabetes taking either simvastatin 40 mg daily or atorvastatin 10 mg daily for 3 months. They showed that there was no statistically significant difference in coefficient of variation of total cholesterol, HDL or LDL cholesterol or triglycerides between the two statins and that the coefficients of variation were comparable with patients not taking lipid-lowering treatment. Whilst lipid variability may not therefore vary between differing statin treatments, the authors showed that treating hyperlipidaemia in patients with diabetes to consistently (i.e. on > 95% of visits) achieve an LDL < 2.0 mmol/L meant maintaining a mean of LDL 1.5–1.6 mmol/l, and to maintain a total cholesterol of, say, less than 4.0 mmol/l meant attaining mean values of 3.3–3.4 mmol/l. These average values are likely to be more difficult to achieve than the thresholds for the targets would suggest and are actually much lower than the evidence from which the targets were originally derived.

Treatments influencing blood pressure

To examine the effect of anti-hypertensive medication on blood pressure in patients with diabetes, Fratolla *et al*. [51] gave patients with Type 2 diabetes the calcium antagonist lacidipine for 4 weeks in a double-blind placebo-controlled trial. Lacidipine reduced both the mean and standard deviation for 24 h, daytime and night-time blood pressure, and particularly for systolic blood pressure. Amlodipine, another long-acting dihydropyridine calcium antagonist, has been shown to prevent more stroke attacks compared with angiotensin-converting enzyme inhibitors, diuretics and β-blockers [52] in meta-analyses [53–57], which also may be a reflection on blood pressure variability reduction .

Further evidence of different classes of anti-hypertensive medications having different effects on blood pressure variability is from a systematic review and meta-analysis in which it was shown that angiotensin-converting enzyme inhibitors and angiotension 2 receptor antagonists led to a relatively increased systolic blood pressure variability, whilst calcium channel blockers reduced visit-to-visit systolic blood pressure variability compared with placebo [58]. This may explain the results from the renin–angiotensin system study looking at the effect of enalapril and losartan compared with placebo in patients with Type 1 diabetes, where there was a significant reduction in systolic and diastolic blood pressure in the two treatment groups, but there was no beneficial renal histological changes compared with placebo. As previously discussed [32], this may be explained by the benefits related to mean blood pressure reduction being lost by the lack of effect on blood pressure variability.

Conclusion

The possible role of risk factor variability in the development of micro- and macrovascular complications remains one of the last unanswered questions in diabetes. While there is some evidence that blood pressure variability may influence microvascular complications, the role of glucose variability is far from clear because of the conflicting data that exists. By comparison, there is little doubt that the biological variability in these measures and in lipids can influence whether a patient is treated with a lipid-lowering or anti-hypertensive agent, or indeed be diagnosed with diabetes at all. Once treated, variability in these measurements can then still affect the targets we aim for in individual patients. Taken together, healthcare staff need to be aware how biological fluctuations can fundamentally shape the way we manage our patients with diabetes.

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Competing interests

None declared.

References

1 Fraser CG. *Biological Variation: From Principles to Practice*. Washington DC: AACC Press, 2001.
2 Westgard QC. *Desirable Specifications for Total Error, Imprecision, and Bias, Derived from Intra- and Inter-Individual Biological Variation*. 2012. Available at www.westgard.com/biodatabase1.htm Last accessed 16 October 2012.
3 Lacher DA, Hughes JP, Carroll MD. Estimate of biological variation of laboratory analytes based on the third national health and nutrition examination survey. *Clin Chem* 2005; **51**: 450–452.
4 Siegelaar SE, Holleman F, Hoekstra JB, DeVries JH. Glucose variability; does it matter? *Endocr Rev* 2010; **31**: 171–182.
5 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.
6 Rodbard D. New and improved methods to characterize glycemic variability using continuous glucose monitoring. *Diabetes Technol Ther* 2009; **11**: 551–565.
7 Weber C, Schnell O. The assessment of glycemic variability and its impact on diabetes-related complications: an overview. *Diabetes Technol Ther* 2009; **11**: 623–633.
8 Kilpatrick ES, Rigby AS, Atkin SL. For debate. Glucose variability and diabetes complication risk: we need to know the answer. *Diabet Med* 2010; **27**: 868–871.
9 Ceriello A, Ihnat MA. 'Glycaemic variability': a new therapeutic challenge in diabetes and the critical care setting. *Diabet Med* 2010; **27**: 862–867.
10 Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* 2005; **54**: 1615–1625.
11 Muggeo M, Verlato G, Bonora E, Zoppini G, Corbellini M, de Marco R. Long-term instability of fasting plasma glucose, a novel predictor of cardiovascular mortality in elderly patients with non-insulin-dependent diabetes mellitus: the Verona Diabetes Study. *Circulation* 1997; **96**: 1750–1754.
12 Ceriello A, Esposito K, Piconi L, Ihnat MA, Thorpe JE, Testa R *et al*. Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients. *Diabetes* 2008; **57**: 1349–1354.

13 Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP et al. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *J Am Med Assoc* 2006; **295**: 1681–1687.

14 Wentholt IM, Kulik W, Michels RP, Hoekstra JB, DeVries JH. Glucose fluctuations and activation of oxidative stress in patients with type 1 diabetes. *Diabetologia* 2008; **51**: 183–190.

15 Monnier L, Colette C, Mas E, Michel F, Cristol JP, Boegner C et al. Regulation of oxidative stress by glycaemic control: evidence for an independent inhibitory effect of insulin therapy. *Diabetologia* 2010; **53**: 562–571.

16 Takao T, Ide T, Yanagisawa H, Kikuchi M, Kawazu S, Matsuyama Y. The effect of fasting plasma glucose variability on the risk of retinopathy in type 2 diabetic patients: retrospective long-term follow-up. *Diabetes Res Clin Pract* 2010; **89**: 296–302.

17 Kilpatrick ES, Rigby AS, Atkin SL. The effect of glucose variability on the risk of microvascular complications in type 1 diabetes. *Diabetes Care* 2006; **29**: 1486–1490.

18 Siegelaar SE, Kilpatrick ES, Rigby AS, Atkin SL, Hoekstra JB, Devries JH. Glucose variability does not contribute to the development of peripheral and autonomic neuropathy in type 1 diabetes: data from the DCCT. *Diabetologia* 2009; **52**: 2229–2232.

19 Service FJ, O'Brien PC. The relation of glycaemia to the risk of development and progression of retinopathy in the Diabetic Control and Complications Trial. *Diabetologia* 2001; **44**: 1215–1220.

20 Little RR, Sacks DB. HbA1c: how do we measure it and what does it mean? *Curr Opin Endocrinol Diabetes Obes* 2009; **16**: 113–118.

21 Kilpatrick ES, Rigby AS, Atkin SL. A1C variability and the risk of microvascular complications in type 1 diabetes: data from the Diabetes Control and Complications Trial. *Diabetes Care* 2008; **31**: 2198–2202.

22 The Diabetes Control and Complications Trial Research Group. The relationship of glycemic exposure (HbA_{1c}) to the risk of development and progression of retinopathy in the Diabetes Control and Complications Trial. *Diabetes* 1995; **44**: 968–983.

23 Sugawara A, Kawai K, Motohashi S, Saito K, Kodama S, Yachi Y *et al.* HbA_{1c} variability and the development of microalbuminuria in type 2 diabetes: Tsukuba Kawai Diabetes Registry 2. *Diabetologia* 2012; **55**: 2128–2131.

24 McCarter RJ, Hempe JM, Gomez R, Chalew SA. Biological variation in HbA1c predicts risk of retinopathy and nephropathy in type 1 diabetes. *Diabetes Care* 2004; **27**: 1259–1264.

25 Lachin JM, Genuth S, Nathan DM, Rutledge BN. The hemoglobin glycation index is not an independent predictor of the risk of microvascular complications in the Diabetes Control and Complications Trial. *Diabetes* 2007; **56**: 1913–1921.

26 Lakka HM, Lakka TA, Tuomilehto J, Sivenius J, Salonen JT. Hyperinsulinemia and the risk of cardiovascular death and acute coronary and cerebrovascular events in men: the Kuopio Ischaemic Heart Disease Risk Factor Study. *Arch Intern Med* 2000; **160**: 1160–1168.

27 Jayagopal V, Kilpatrick ES, Jennings PE, Hepburn DA, Atkin SL. Biological variation of homeostasis model assessment-derived insulin resistance in type 2 diabetes. *Diabetes Care* 2002; **25**: 2022–2025.

28 Jayagopal V, Kilpatrick ES, Holding S, Jennings PE, Atkin SL. The biological variation of insulin resistance in polycystic ovarian syndrome. *J Clin Endocrinol Metab* 2002; **87**: 1560–1562.

29 Warlow C, Sudlow C, Dennis M, Wardlaw J, Sandercock P. Stroke. *Lancet* 2003; **362**: 1211–1224.

30 Adler AI, Stratton IM, Neil HA, Yudkin JS, Matthews DR, Cull CA *et al.* Association of systolic blood pressure with macrovascular and microvascular complications of type 2 diabetes (UKPDS 36): prospective observational study. *Br Med J* 2000; **321**: 412–419.

31 Rothwell PM, Howard SC, Dolan E, O'Brien E, Dobson JE, Dahlof B *et al.* Prognostic significance of visit-to-visit variability, maximum systolic blood pressure, and episodic hypertension. *Lancet* 2010; **375**: 895–905.

32 Kilpatrick ES, Rigby AS, Atkin SL. The role of blood pressure variability in the development of nephropathy in type 1 diabetes. *Diabetes Care* 2010; **33**: 2442–2447.

33 Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. *J Am Med Assoc* 2002; **287**: 2570–2581.

34 Tsalamandris C, Panagiotopoulos S, Allen TJ, Waldrip L, Van Gaal B, Goodall I *et al.* Long-term intraindividual variability of serum lipids in patients with type I and type II diabetes. *J Diabetes Complications* 1998; **12**: 208–214.

35 Waden J, Forsblom C, Thorn LM, Gordin D, Saraheimo M, Groop PH. A1C variability predicts incident cardiovascular events, microalbuminuria, and overt diabetic nephropathy in patients with type 1 diabetes. *Diabetes* 2009; **58**: 2649–2655.

36 Engelgau MM, Thompson TJ, Herman WH, Boyle JP, Aubert RE, Kenny SJ *et al.* Comparison of fasting and 2-hour glucose and HbA1c levels for diagnosing diabetes. Diagnostic criteria and performance revisited. *Diabetes Care* 1997; **20**: 785–791.

37 Selvin E, Crainiceanu CM, Brancati FL, Coresh J. Short-term variability in measures of glycemia and implications for the classification of diabetes. *Arch Intern Med* 2007; **167**: 1545–1551.

38 Ollerton RL, Playle R, Ahmed K, Dunstan FD, Luzio SD, Owens DR. Day-to-day variability of fasting plasma glucose in newly diagnosed type 2 diabetic subjects. *Diabetes Care* 1999; **22**: 394–398.

39 Brohall G, Behre CJ, Hulthe J, Wikstrand J, Fagerberg B. Prevalence of diabetes and impaired glucose tolerance in 64-year-old Swedish women: experiences of using repeated oral glucose tolerance tests. *Diabetes Care* 2006; **29**: 363–367.

40 Olefsky JM, Reaven GM. Insulin and glucose responses to identical oral glucose tolerance tests performed forty-eight hours apart. *Diabetes* 1974; **23**: 449–453.

41 Rohlfing C, Wiedmeyer HM, Little R, Grotz VL, Tennill A, England J *et al.* Biological variation of glycohemoglobin. *Clin Chem* 2002; **48**: 1116–1118.

42 Kilpatrick ES, Maylor PW, Keevil BG. Biological variation of glycated hemoglobin. Implications for diabetes screening and monitoring. *Diabetes Care* 1998; **21**: 261–264.

43 Braga F, Dolci A, Mosca A, Panteghini M. Biological variability of glycated hemoglobin. *Clin Chim Acta* 2010; **411**: 1606–1610.

44 Herman WH, Ma Y, Uwaifo G, Haffner S, Kahn SE, Horton ES *et al.* Differences in A1C by race and ethnicity among patients with impaired glucose tolerance in the Diabetes Prevention Program. *Diabetes Care* 2007; **30**: 2453–2457.

45 Pani LN, Korenda L, Meigs JB, Driver C, Chamany S, Fox CS *et al.* Effect of aging on A1C levels in individuals without diabetes: evidence from the Framingham Offspring Study and the National Health and Nutrition Examination Survey 2001–2004. *Diabetes Care* 2008; **31**: 1991–1996.

46 Haak T, Tiengo A, Draeger E, Suntum M, Waldhausl W. Lower within-subject variability of fasting blood glucose and reduced weight gain with insulin detemir compared to NPH insulin in patients with type 2 diabetes. *Diabetes Obes Metab* 2005; **7**: 56–64.

47 Bruttomesso D, Crazzolara D, Maran A, Costa S, Dal Pos M, Girelli A *et al.* In Type 1 diabetic patients with good glycaemic control, blood glucose variability is lower during continuous subcutaneous insulin infusion than during multiple daily injections with insulin glargine. *Diabet Med* 2008; **25**: 326–332.

48 Lepore G, Corsi A, Dodesini AR, Nosari I, Trevisan R. Continuous subcutaneous insulin infusion is better than multiple daily insulin injections in reducing glucose variability only in type 1 diabetes with good metabolic control. *Diabetes Care* 2010; **33**: e81.

49 McCall AL, Cox DJ, Brodows R, Crean J, Johns D, Kovatchev B. Reduced daily risk of glycemic variability: comparison of exenatide with insulin glargine. *Diabetes Technol Ther* 2009; **11**: 339–344.
50 Sathyapalan T, Atkin SL, Kilpatrick ES. Variability of lipids in patients with Type 2 diabetes taking statin treatment: implications for target setting. *Diabet Med* 2008; **25**: 909–915.
51 Frattola A, Parati G, Castiglioni P, Paleari F, Ulian L, Rovaris G *et al*. Lacidipine and blood pressure variability in diabetic hypertensive patients. *Hypertension* 2000; **36**: 622–628.
52 Wang JG, Li Y, Franklin SS, Safar M. Prevention of stroke and myocardial infarction by amlodipine and angiotensin receptor blockers: a quantitative overview. *Hypertension* 2007; **50**: 181–188.
53 Turnbull F. Effects of different blood-pressure-lowering regimens on major cardiovascular events: results of prospectively-designed overviews of randomised trials. *Lancet* 2003; **362**: 1527–1535.
54 Neal B, MacMahon S, Chapman N. Effects of ACE inhibitors, calcium antagonists, and other blood-pressure-lowering drugs: results of prospectively designed overviews of randomised trials. Blood Pressure Lowering Treatment Trialists' Collaboration. *Lancet* 2000; **356**: 1955–1964.
55 Staessen JA, Wang JG, Thijs L. Cardiovascular prevention and blood pressure reduction: a quantitative overview updated until 1 March 2003. *J Hypertens* 2003; **21**: 1055–1076.
56 Staessen JA, Li Y, Thijs L, Wang JG. Blood pressure reduction and cardiovascular prevention: an update including the 2003–2004 secondary prevention trials. *Hypertens Res* 2005; **28**: 385–407.
57 Staessen JA, Wang J. Blood-pressure lowering for the secondary prevention of stroke. *Lancet* 2001; **358**: 1026–1027.
58 Webb AJ, Fischer U, Mehta Z, Rothwell PM. Effects of antihypertensive-drug class on interindividual variation in blood pressure and risk of stroke: a systematic review and meta-analysis. *Lancet* 2010; **375**: 906–915.