JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
COMMENTARY

Involvement of advanced glycation end-products in 'hyperglycemic memory'

The aim of diabetes treatment is to prevent diabetic complications. However, many patients still suffer from diabetic complications. Therefore, it is important to elucidate the mechanism that connects hyperglycemia to the development of diabetic complications. Several hyperglycemia-induced metabolic abnormalities, such as activation of the polyol pathway, formation of advanced glycation end-products (AGEs), activation of protein kinase C isoforms and oxidative stress, have been reported to involve in the development of diabetic complications[1]. In addition, we previously showed that reactive oxygen species (ROS) from mitochondria was the major cause of diabetes-induced oxidative stress, and this mitochondrial ROS could cause the activation of three other metabolic abnormalities[2]. However, there remain many unanswered questions in the mechanism of diabetic complications.

The 'hyperglycemic memory' reported by Engerman and Kern[3] in 1987 is one of such questions. In their study, 35 normal dogs were divided into one non-diabetic group and three alloxan-induced diabetic groups, which were prospectively identified according to glycemic control: poor control for 5 years, good control for 5 years and poor control for 2.5 years followed by good control for 2.5 years. To achieve good control, insulin was injected to maintain glycemic control comparable with that of the normal dogs. Diabetic retinopathy developed in the poor control for 5 years group, but not in the good control for 5 years group. In the poor control for 2.5 years followed by good control for 2.5 years group, retinopathy was absent or indeterminate at 2.5 years of poor control, whereas, surprisingly, it was found to have subsequently developed despite good glycemic control after a further 2.5 years. Thus, episodes of poor glycemic control during the earlier stages of diabetes could promote diabetic complications later in the course of the disease, even after good blood glucose control is therapeutically achieved.

In the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC)[4], the 'hyperglycemic memory' was referred to again. In 1993, the DCCT reported that in type 1 diabetes, intensive insulin therapy dramatically reduced the occurrence and severity of diabetic microvascular complications compared with conventional insulin therapy. After the publication of the DCCT results, most of the participants in the conventional therapy group adopted intensive therapy. Therefore, glycated hemoglobin (HbA1c) values for the intensive and conventional therapy groups were reported to be comparable after the approximately 14-year EDIC follow-up study with more than 90% of the DCCT participants. Henceforth, the participants in the conventional therapy group continued to have a higher incidence of complications, whereas the intensive therapy group continued to have a lower incidence of complications. The DCCT/EDIC group called this phenomenon 'metabolic memory.' A similar phenomenon was also reported in type 2 diabetes in the United Kingdom Prospective Diabetes Study 80, which was given the name 'legacy effect.' These large trials clinically proved that the phenomenon of 'hyperglycemic memory' existed both in type 1 and type 2 diabetes.

The DCCT group also investigated cross-sectional associations between skin collagen AGEs and the presence of diabetic microvascular complications in DCCT subjects (the DCCT Skin Collagen Ancillary Study). They obtained a skin biopsy sample from the subjects before the DCCT closeout and have since published a series of articles. In 2015, new interesting clinical data about AGEs and metabolic memory was published in the *Journal of Diabetes*[5].

In 1999, three AGEs (furosine, pentosidine and carboxymethyl-lysine [CML]), two solubility abnormalities in collagen (acid and pepsin soluble collagen) and protein-bound relative fluorescence from a skin biopsy sample were measured, and the relationships between 5 years of intensive control of glycemia and indicators of skin collagen AGEs were examined[6]. Furosine is glycated collagen, an early Amadori product, and pentosidine and CML are products of glycoxidation and AGE formation. The biopsy results showed that long-term intensive treatment caused lower levels of these AGEs parameters in parallel with a reduction in HbA1c. In addition, the severity of microvascular complications was significantly associated with these AGEs parameters, and interestingly, the associations generally remained significant even after adjustment for HbA1c. In particular, furosine was the parameter most consistently associated with diabetic complications. These results suggested that accumulation of AGEs was associated with the progression of diabetic complications, and skin collagen AGEs might be a marker for diabetic complications independent of HbA1c. In 2005, Genuth *et al.*[7] investigated whether AGEs in skin collagen, which were obtained from the subjects of DCCT before DCCT closeout, could predict the risk of progression of

*Corresponding author. Takeshi Nishikawa
Tel.: +81-96-373-5169
Fax: +81-96-366-8397
E-mail address: takeshi@kumamoto-u.ac.jp
Received 28 July 2015; revised 29 July 2015; accepted 29 July 2015

microvascular disease. Their results showed that these skin collagen AGEs, especially furosine and CML, were highly correlated with the progression of retinopathy and nephropathy over 10 years in the EDIC study. Interestingly, the combination of furosine and CML also predicted the progression of retinopathy and nephropathy even after adjustment for the mean DCCT HbA1c. However, the predictive effect of mean DCCT HbA1c vanished after adjustment for furosine and CML. Therefore, the authors claimed that AGEs formation associated with prior HbA1c could explain the future risk of diabetic microvascular complications, and might contribute to the 'metabolic memory.'

In the present article, the DCCT Skin Collagen Ancillary Study investigated whether the addition of four AGEs (glucosepane, hydroimidazolones of methylglyoxal and glyoxal, and carboxyethyl-lysine) could improve the associations with the incident of diabetic microvascular events 13–17 years after DCCT[5]. Though the four AGEs, glucosepane, hydroimidazolones of methylglyoxal, glyoxal and carboxyethyl-lysine, were not available in 1999, it was reported that glucosepane was the most abundant in skin. As a result, the 10-AGEs panel was associated with future progression of diabetic microvascular complications independent of either mean DCCT or EDIC HbA1c levels. In addition, the four added AGEs enhanced the association of the original six AGEs with the risk of retinopathy and neuropathy development. Furthermore, the effect of mean DCCT HbA1c on the progression of diabetic microvascular complications during the EDIC study vanished after adjustment using the whole panel of AGEs similar to their reports of 2005. Therefore, the present study reconfirmed that AGEs formation was involved in 'metabolic memory.'

To show that some sort of metabolic abnormality, such as AGEs accumulation, contributes to the 'hyperglycemic memory,' it will probably be necessary to clarify that this metabolic abnormality is involved in three processes: (i) the metabolic abnormality is induced by hyperglycemia; (ii) the metabolic abnormality continues for a while or remains after hyperglycemia is improved; and (iii) cellular damage progresses without hyperglycemia because of the metabolic abnormality. Therefore, hyperglycemic memory-related metabolic abnormality might exist as a branch of the path from hyperglycemia to diabetic complications (Figure 1).

In consideration of the series of reports from the DCCT Skin Collagen Ancillary Study Group, the AGEs hypothesis might satisfy the three processes. It suggests that the accumulation of AGEs is a key factor in the pathogenesis of diabetic complications, which can explain the mechanism of 'hyperglycemic memory.' However, it is important to note that in these reports, AGEs were measured in skin collagen and not in tissues related to diabetic complications. As it is inconceivable that skin collagen AGEs affect the pathogenesis of diabetic retinopathy, nephropathy and neuropathy, skin collagen AGEs are likely markers of diabetic vascular complications.

It has been reported that AGEs elicit oxidative stress through their binding to the receptor of AGEs (RAGE)[8]. Also, hyperglycemia has been reported to increase mitochondrial ROS, and this increased mitochondrial ROS increases intracellular formation of AGEs[2], and the expression of the RAGE and its activating ligands[1]. Taken together, a close association is known to exist between AGEs and oxidative stress. Therefore, accumulation of AGEs in the skin collagen of subjects from the DCCT before DCCT closeout might also suggest increased oxidative stress at that time, and oxidative stress can predict future risk of

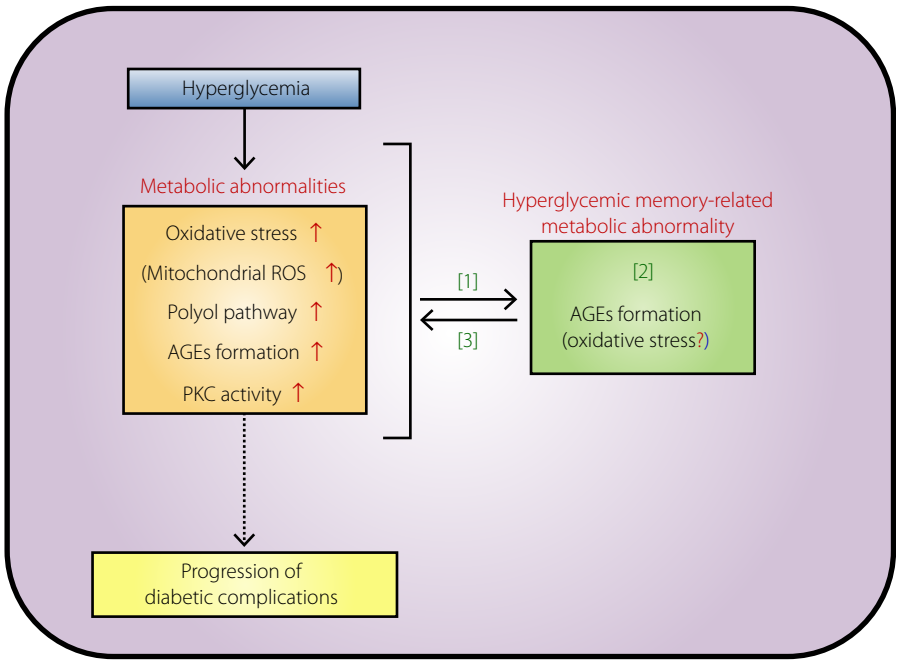

Figure 1 | Model of the pathogenesis of diabetic complications in regard to 'hyperglycemic memory.' Oxidative stress, activation of the polyol pathway, formation of advanced glycation end-products (AGEs) and activation of protein kinase C (PKC) isoforms have been reported as hyperglycemic pathways to developing diabetic complications. In addition, we previously showed that mitochondrial reactive oxygen species (ROS) was the major cause of diabetes-induced oxidative stress. Hyperglycemic memory-related abnormality is involved in three processes: (i) the metabolic abnormality is induced by hyperglycemia; (ii) the metabolic abnormality continues for a while or remains after hyperglycemia is improved; and (iii) the cellular damage progresses without hyperglycemia because of the metabolic abnormality. AGEs formation might be one of candidates for the mechanism of the 'hyperglycemic memory.'

developing diabetic complications. Indeed, Paneni *et al.*[9] reported that ROS generation was elevated when endothelial cells were cultured in high glucose for 3 days followed by normal glucose for 3 days, and ROS generation was not inhibited by 3 weeks of insulin treatment in streptozotocin-induced diabetic mice. In addition, they claim that persistent mitochondrial adaptor $p66^{Shc}$ upregulation and mitochondrial translocation were associated with continued ROS generation, and this finding could explain a part of the mechanisms of 'hyperglycemic memory.'

The series of reports by the DCCT Skin Collagen Ancillary Study Group are very interesting and informative. The hypothesis from these reports clearly indicates that AGEs are a strong candidate for the mechanism of the 'hyperglycemic memory.' However, the exact mechanism of the phenomenon remains uncertain. It has been reported that treatment with pyridoxamine dihydrochloride, which inhibits formation of AGEs, is associated with a lower average change in serum creatinine concentration at 52 weeks among patients with lower creatinine ranges (serum creatinine <1.85 mg/dL)[10]. Therefore, it could be interesting to investigate the effect of pyridoxamine dihydrochloride on 'hyperglycemic memory.'

ACKNOWLEDGMENT

This work was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science, Japan (No. 26461340 to TN).

DISCLOSURE

The authors declare no conflict of interest.

Takeshi Nishikawa[1,2]*, Eiichi Araki[2]
Departments of [1]Molecular Diabetology and [2]Metabolic Medicine, Faculty of Life Sciences, Kumamoto University, Chuo, Kumamoto, Japan

REFERENCES

1. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001; 414: 813–820.
2. Nishikawa T, Edelstein D, Du XL, *et al.* Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 2000; 404: 787–790.
3. Engerman RL, Kern TS. Progression of incipient diabetic retinopathy during good glycemic control. *Diabetes* 1987; 36: 808–812.
4. Nathan DM, Cleary PA, Backlund JY, *et al.* Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J Med* 2005; 353: 2643–2653.
5. Genuth S, Sun W, Cleary P, *et al.* Skin advanced glycation end products glucosepane and methylglyoxal hydroimidazolone are independently associated with long-term microvascular complication progression of type 1 diabetes. *Diabetes* 2015; 64: 266–278.
6. Monnier VM, Bautista O, Kenny D, *et al.* Skin collagen glycation, glycoxidation, and crosslinking are lower in subjects with long-term intensive versus conventional therapy of type 1 diabetes: relevance of glycated collagen products versus HbA1c as markers of diabetic complications. *Diabetes* 1999; 48: 870–880.
7. Genuth S, Sun W, Cleary P, *et al.* Glycation and carboxymethyllysine levels in skin collagen predict the risk of future 10-year progression of diabetic retinopathy and nephropathy in the diabetes control and complications trial and epidemiology of diabetes interventions and complications participants with type 1 diabetes. *Diabetes* 2005; 54: 3103–3111.
8. Yamagishi S, Maeda S, Matsui T, *et al.* Role of advanced glycation end products (AGEs) and oxidative stress in vascular complications in diabetes. *Biochim Biophys Acta* 2012; 1820: 663–671.
9. Paneni F, Mocharla P, Akhmedov A, *et al.* Gene silencing of the mitochondrial adaptor $p66^{Shc}$ suppresses vascular hyperglycemic memory in diabetes. *Cir Res* 2012; 111: 278–289.
10. Lewis EJ, Greene T, Spitalewiz S, *et al.* Pyridorin in type 2 diabetic nephropathy. *J Am Soc Nephrol* 2012; 23: 131–136.

Doi: 10.1111/jdi.12405