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Videocapillaroscopy in Diabetes

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Capillaroscopy is a method of recognized value in the study of morphological and functional abnormalities of the microcirculation. Its use in the clinical field dates from the early twentieth century, extending the clinical applications gradually over the years, to be considered a “routine investigation” in (nowadays) rheumatological practice.

The introduction of videocapillaroscopy with optical probes in contact resulted in a significant leap forward in the study of microcirculation.4,5 Videocapillaroscopy is indicated in all diseases whose pathogenesis recognizes anatomical and/or functional abnormalities in microcirculation.6

The advantages of capillaroscopy can be identified in the absence of invasiveness, in its remarkable sensitivity and specificity together with ease procedure and speed execution time. The relatively videocapillaroscopy low cost could be considered another big advantage of this procedure.

Videocapillaroscopy allows clinicians to acquire informations on morphological and hemodynamic parameters that could be useful like predictive value of extension, gravity and evolution of disease. In this sense videocapillaroscopy could be considered a double profile examination, with diagnostic and prognostic values in the same time.7

Among the many diseases related with abnormal capillaries there is diabetes mellitus. The interest of the microcirculation being altered glucose metabolism is well documented both in the course of diabetes type I and type II.

Chronic hyperglycemia is responsible for the metabolic alterations in endothelial cells, and also of the smooth muscle of the vessel walls, resulting in impairment of contractile properties together with permeability and hemodynamic modifications.

Parietal elasticity alteration transforms the vessels in rigid ducts by thickened walls, partly due to the non-enzymatic glycosylation of proteins and partly due to the altered metabolism of myo-inositol.8

Decreased of parietal compliance cause the appearance of ectasia, with microaneurysms related to pressor stress.

In the context of diabetic angiopathy we can distinguish nonspecific changes especially at the level of the arteries of large- and medium-caliber, and more specific, also if not exclusive, changes usually localized in the microvessel.

Microvessel videocapillaroscopy study seems to be very interesting in diabetes because microangiopathy is the most common and fearsome complication of diabetes and be-
cause it seems that the alterations of cutaneous microvessels and of the fundamental substance of connective constitute the earliest manifestations of the disease.

Modifications of cutaneous microvessel results in thickening of the capillary basement membrane, endothelial cells proliferation and vascular leakage.

Vascular leakage, detectable by fluorescence videocapillaroscopy, only in part seems to be related to an increase in the amplitude of the system to “small pores”, responsible for the trans-capillary diffusion. In fact, the accumulation of mucopolysaccharides, to which depends the thickening of the basal membrane, also change the selectivity of the process of filtration.

The capillary flow appears grainy and slowed by the presence of microaggregates which often interrupt the continuity of the blood stream. This dynamic alteration is the consequence of an increased friction, in part connected to the marked tortuosity of the capillaries, in part to the increase of blood viscosity and platelet adhesiveness.

The data so far in the literature about the capillaroscopic characteristics of diabetic microangiopathy are discordant. It was reported a provision of the capillaries to “shoal of fish” in diabetes mellitus type I and increased morphological variability of the loops with increasing diameter.

A further and interesting capillaroscopic modification consists in a progressive dilation of efferent portion of the loop, homogeneous and centripetal, such as to give the appearance to capillary classic “elephant nose,” (Figures 1 and 2) provided that the diameter exceeds at least 5 times the physiological value (7-18 microns according Grassi) and that it is repetitive.

Other capillaroscopic alterations consist of sacculare aneurysm apical and lateral of the loops. (Figures 3 and 4) Arborescent aspects, expression of neo-vascularization, and microhaemorrhages more or less pronounced, associated with slowing and grain flow, (Figures 5-7) are interpreted as an expression of greater severity of microangiopathy.11
Although not yet encoded a capillaroscopic pattern characteristic, in patients with diabetes mellitus can detect morphological expression of microangiopathy such as, for example, the homogeneous increase of the diameter of the capillaries (especially at the level of the stretch venular) and the presence of loops of look convoluted. (Figures 8-11).

These anomalies, although not of specific diagnostic value, can play a significant role in the characterization of diabetic microangiopathy.12

With the use of fluorescence capillaroscopy was detected a difference between the permeability alterations typical of diabetes mellitus and those features of systemic sclerosis.13 In the first case, in fact, the alteration of the permeability is homogeneously distributed throughout the course of the loop capillary (venular and arteriolar tract).

In scleroderma, however, the defect is asymmetric and is accompanied by loss of focal tracer.14 The analogy between two seemingly distant disease, such as diabetes and systemic sclerosis, supports the hypothesis already advanced from the Jordan (“diabetes as a disease of the connective deposit”) of a final common pathway of damage microcirculatory unit, regardless of the initial pathogenic noxa.15

CONFLICTS OF INTEREST: None.

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Additive and Antagonistic Effects among Combination of Agonists of Peroxisome Proliferator-Activated Receptor gamma (PPARg) on Transcriptional Activity

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ABSTRACT

Objective: The Angiotensin-II receptor blocker telmisartan and sulfonylurea glimepiride may have clinical usefulness as partial agonists of PPARg. We investigated additive and antagonistic effects among combinations of telmisartan, glimepiride, and the Thiazolidinedione (TZD) selective PPARg agonist pioglitazone on transcriptional activity of PPARg.

Materials/Methods: The receptors of pCMX-PPARg and pCMX-RXR, and PPRE-Luc reporter gene were transfected into CV1 cells, and treated with following agents, and luciferase assay was performed. Moreover, mammalian two-hybrid assay was done using GAL4 responsive reporter tk-MH100(UAS)×4-Luc and the chimeric receptor GAL4-PPARg.

Results: Telmisartan increased transactivation of PPARg dose dependently. Activation by telmisartan 10 µM was 58.8% of that by pioglitazone 10 µM. Glimepiride also increased transactivation of PPARg dose dependently. Activation by glimepiride 10 µM was 49.8% of that by pioglitazone 10 µM. Addition of telmisartan 5 µM significantly enhanced transactivation by glimepiride 10 and 50 µM. Moreover, addition of pioglitazone 0.5 µM significantly enhanced transactivation by glimepiride 10 and 50 µM. Mammalian two-hybrid assay showed additive effect between glimepiride and telmisartan on binding of SRC-1 to PPARg. On the other hand, addition of glimepiride 10 and 50 µM reduced transactivation by pioglitazone 5 µM to 74% and 70%, respectively.

Conclusion: Partial agonists of PPARg additively enhanced transactivation by other agonists, whereas high concentration of partial agonists reduced transactivation by full agonist antagonistically.

KEYWORDS: Glimepiride; Telmisartan; Pioglitazone; Nuclear receptor.

ABBREVIATIONS: ARB: Angiotensin–II receptor blocker; PPARg: Peroxisome proliferator-activated receptor gamma; PPRE-Luc: PPAR responsive element-luciferase; TZD: Thiazolidinedione.

INTRODUCTION

Peroxisome proliferator-activated receptor gamma (PPARg) is a transcriptional factor involved in adipocyte differentiation and insulin sensitization. It forms a heterodimer with retinoid X receptor and binds to peroxisome proliferator responsive elements in the promoter of target genes and regulates their expression. The thiazolidinedione derivative (TZD) pioglitazone is a high-affinity ligand of PPARg and clinically useful insulin sensitizer. However, pioglitazone therapy is associated with adverse effects such as weight gain, edema, and fluid retention; therefore, new agents that activate PPARg with less adverse effects are expected.
Recently, it was reported that the Angiotensin-II receptor blocker (ARB) telmisartan and sulfonylurea glimepiride act as ligands of PPARγ and their clinical usefulness in this context has received some attention, although characterization of these new PPARγ ligands has not been fully clarified. Crystal structure of PPARγ binding domains revealed a large binding pocket, which may explain the diversity of PPARγ ligands.20,21 Indeed, some studies showed that telmisartan bound to different sites of PPARγ from TZDs, and recruited coactivators/corepressors in different manner from TZDs.6-8,20 Although the characterization of glimepiride has not been clearly determined, glimepiride has been reported to have extra pancreatic effects or actions of improving insulin sensitivity as well as stimulatory effect on islet beta-cells.22,23 The extrapancreatic effects of glimepiride would partly originate from activation of PPARγ. Therefore, interactions of these ligands should be studied to determine their metabolic efficacy for the development of new therapeutic strategies against insulin resistance and type 2 diabetes. Namely, combination therapy of telmisartan with glimepiride might enhance PPARγ activity without adverse effects caused by TZDs.

We investigated additive and antagonistic effects among combinations of PPARγ ligands such as pioglitazone, telmisartan, and glimepiride on transcripational activity of PPARγ, and speculated the efficacy of combination therapy by these agents in clinical use.

MATERIALS AND METHODS

Materials

Pioglitazone was a kind gift from Takeda Chemical Industries (Osaka, Japan). Telmisartan and glimepiride were kind gifts from Boehringer-Ingelheim (Tokyo, Japan) and Sanofi-Aventis Pharma (Tokyo, Japan), respectively.

Plasmids

The human RXR expression plasmid pCMX-RXR, human PPARγ expression plasmid pCMX-PPARγ, and Luciferase (Luc) reporter gene containing the tk promoter fused to three copies of the PPRE derived from rat AOX, (tk-[PPRE]×3-Luc), were described elsewhere.24 GAL4 responsive reporter tk-(MH100)×4-Luc and the chimeric receptor GAL4-PPARγ were also previously described.24 The expression plasmid SRC-1 fused with GAL4 DNA binding domain GAL4-PPARγ was described elsewhere.24,25

Cell Culture and Transient Transfection

The monkey kidney cell line CV1 was grown in DMEM containing 10% fetal bovine serum, penicillin G (100 U/ml), and streptomycin (100 mg/ml) at 37 °C under 5% CO2. CV1 cells were trypsinized and seeded at a density of 2×105 cells/well in 6-well plates. Using the calcium phosphate technique, the cells were co-transfected with receptor of GAL4-PPARγ and tk-(MH100)×4-Luc reporter gene. In the other experiment, the CV1 cells were also co-transfected using the same method with receptors of pCMX-PPARγ and PPREE-Luc reporter gene. The total amount of transfected plasmid was adjusted to 2 µg/well by adding empty vector (pCMX). After transfection for 20 h, the medium was replaced with fresh DMEM containing 10% Dextran-coated charcoal-stripped (DCC) fetal bovine serum (10% DCC serum) in the presence or absence of ligands. After 24 h incubation, the cells were harvested and Luc activity was measured. Transfection efficiencies were normalized by ß-galactosidase activity.

Mammalian Two-Hybrid Assay

Using calcium phosphate technique CV1 cells in 6-well plates were co-transfected with 800 ng Luc reporter gene containing tk-(MH100)×4 promoter, 1 µg of ß-galactosidase expression plasmid, 900 ng of expression plasmid for hPPARγ fused with VP16 transactivating domain, and 360 ng of plasmid for SRC-1 fused with GAL4 DNA binding domain. pCMX was added to adjust the total DNA amount. After incubation for 24 h, the cells were harvested and Luc activities measured and normalized with ß-galactosidase activity.

Statistical Analysis

Results are expressed as mean ± SD from ≥4 transfections performed in duplicate. Data were analyzed by ANOVA post hoc tests versus control using Stat View 4.0 software (Abacus Concepts, Berkley, CA, USA). P<0.05 was considered significant.

RESULTS

Dose-Dependent Transactivation of PPARγ by Pioglitazone, Telmisartan, and Glimepiride

Telmisartan and glimepiride as well as pioglitazone increased transactivation of PPARγ dose dependently using the chimeric receptor GAL4-PPARγ and the GAL4-responsive reporter of tk-(MH100)×4-Luc (Figure 1A). Telmisartan ≥1 µM activated PPARγ significantly, as did glimepiride ≥5 µM. Transcriptional activity by 10 µM of telmisartan and 10 µM of glimepiride was 58.8% and 49.8% of that by 10 µM of pioglitazone. Telmisartan and glimepiride increased transactivation of PPARγ dose dependently on the PPRE using the receptor of PPARγ and PPARγ responsive reporter (PPREE)×3-Luc (Figure 1B). Transcriptional activity by 10 µM of telmisartan and 10 µM of glimepiride was 53.9% and 49.8% of that by 10 µM of pioglitazone. These results suggest that telmisartan and glimepiride bind to and transactivate PPARγ and thereby act as partial agonists of PPARγ.
Additive Effects of Telmisartan on Transactivation of PPARγ by Glimepiride

Telmisartan 5 µM potentiated transactivation of PPARγ additively by each concentration of glimepiride using the chimeric receptor GAL-4 PPARγ and the reporter of tk-(MH100)×4-Luc. Especially, significantly increased activations by 10 and 50 µM of glimepiride were potentiated by addition of 5 µM of telmisartan (Figure 2A). Furthermore, 5 µM of telmisartan potentiated transactivation of PPARγ by each concentration of glimepiride on PPRE using the receptor of PPARγ and reporter of (PPRE)×3-Luc. In particular, significantly increased activations by 10 and 50 µM of glimepiride were potentiated by addition of 5 µM of telmisartan (Figure 2B).

Additive Effects of Pioglitazone on Transactivation of PPARγ by Glimepiride

Pioglitazone 0.5 µM potentiated transactivation of PPARγ additively by each concentration of glimepiride using the chimeric receptor GAL4-PPARγ and the reporter of tk-(MH100)×4-Luc. Especially, significantly increased activations by 10 and 50 µM of glimepiride were potentiated by addition of 0.5 µM of pioglitazone (Figure 3A). Furthermore, 0.5 µM of pioglitazone potentiated transactivation of PPARγ by each concentration of glimepiride on PPRE using the receptor of PPARγ and reporter of (PPRE)×3-Luc. In particular, significantly increased activations by 10 and 50 µM of glimepiride were potentiated by addition of 0.5 µM of pioglitazone (Figure 3B).

Antagonistic Effects Glimepiride on Transactivation of PPARγ by Pioglitazone

High concentrations (10 and 50 µM) of glimepiride reduced transactivation of PPARγ by 5 µM of pioglitazone using the chimeric receptor GAL4-PPARγ and the reporter of tk-(MH100)×4-Luc (Figure 4A). Moreover, 50 µM of glimepiride reduced transactivation of PPARγ by 5 µM of pioglitazone using the receptor of PPARγ and reporter of (PPRE)×3-Luc (Figure 4B).

Additive Binding Activity of PPARγ to SRC-1 by Glimepiride and Telmisartan

To examine the additive effects of glimepiride and telmisartan on the interaction of PPARγ with co-activator, mam-

Figure 1: Dose-dependent transactivation of PPARγ by pioglitazone, telmisartan, and glimepiride. (A) Chimeric receptor of GAL4-PPARγ and GAL4 responsive reporter of tk-(MH100(UAS))×4-Luc were transfected into CV1 cells in the absence or presence of various ligands. Increasing amounts of ligand were added. Luciferase activities induced by pioglitazone (Piog; closed bar), telmisartan (Telm; grey bar), and glimepiride (Glim, open bar) are represented. (B) Receptors of PPARγ and R×R expression plasmids were transfected into CV1 cells together with PPRE-tk-Luc in the absence or presence of various ligands. Increasing amounts of ligand were added. Luciferase activities induced by Piog (closed bar), Telm (grey bar), and Glim (open bar) are represented. Results are mean ± SD from indicated numbers of transfections in duplicate. *P<0.05; §P<0.05 vs. respective control: (no ligand).

Figure 2: Additive effects of telmisartan on transactivation of PPARγ by glimepiride. (A) Chimeric receptor of GAL4-PPARγ and GAL4 responsive reporter of tk-(MH100(UAS))×4-Luc were transfected into CV1 cells in the absence or presence of increasing concentrations of glimepiride together with (closed bar) or without 5 µM of telmisartan (open bar). (B) Receptor of PPARγ and R×R expression plasmids were transfected into CV1 cells together with PPRE-tk-LUC in the absence or presence of increasing concentrations of glimepiride together with (closed bar) or without 5 µM of telmisartan (open bar). Results are mean ± SD from indicated numbers of transfections in duplicate. *P<0.05; §P<0.05 vs. respective control: (no ligand).
malian two-hybrid assay using VP16-PPARg, GAL4-SRC-1, and the reporter of GAL-Luc was performed. Both glimepiride and telmisartan enhanced Luc activity by the binding between VP16-PPARg and GAL4-SRC-1. The interaction between VP16-PPARg and GAL4-SRC-1 was more enhanced by addition of both glimepiride and telmisartan than by that of glimepiride or telmisartan alone (Figure 5).

**DISCUSSION**

Telmisartan directly activated PPARg dose dependently; telmisartan 10 µM activated transcriptional system using GAL4-PPARg chimera receptor and GAL4 responsive reporter gene and that using receptor of PPARg and reporter gene including PPRE to 58.8% and 53.8% of the level achieved by 10 µM of pioglitazone, respectively. These data suggest that telmisartan binds and activates PPARg because the structure of telmisartan is similar to that of TZD. Glimepiride 10 µM also activated PPARg to 49.8% of the level by 10 µM of pioglitazone in similar condi-
TZD and other partial agonists of PPARγ. Difference of binding affinity (to H12) might bring different degrees of activation of PPARγ. The carboxy terminal activation domain (AF-2) in H12 of LBD is proposed to undergo induced conformational changes after binding to ligand. The present experiment showed that two ligands, glimepiride and telmisartan, had additive activations of PPARγ, suggesting that each ligand did not change binding capacity to the receptor for each ligand. Recent study showed that some combinations of ligands, fatty acid and indole acetate or indomethacin, induce additive or synergistic activation of PPARγ. This study confirmed the data about the combination effect of telmisartan and glimepiride. We do not have definitive data about the binding site of glimepiride to PPARγ. However, it was reported that glimepiride competitively binds PPARγ with rosiglitazone, suggesting that glimepiride has partly similar binding site of PPARγ to TZD.

Because different ligands of PPARγ might recruit coactivator in different manner, interactions about the recruitment of coactivator by each ligand should be investigated. The present results suggest that telmisartan, glimepiride, and pioglitazone induced interactions between PPARγ and SRC-1 depending on the transcriptional activity of PPARγ (data not shown). Glimepiride also induced additive interaction between PPARγ and SRC-1. As for SRC-1, these ligands recruited the coactivator to PPARγ depending on the activity, although different ligands were reported to recruit coactivators such as DRIP205, TIF-2, and PGC-1 in different manners. A previous article reported that DRIP205 was recruited by rosiglitazone and telmisartan in similar degree, and PGC-1 by rosiglitazone and telmisartan depending on the transcriptional activity of PPARγ, whereas TIF-2 was recruited not by telmisartan but by rosiglitazone. These findings suggest that SRC-1 and PGC-1 were recruited by ligands depending on the transcriptional activity of PPARγ, but not TIF-2. Different manners of cofactor recruitment would result in different induction of genes. Schuup, et al. suggested the genes of prostacyclin receptor and glycerol kinase induced by TZDs were less stimulated by ARBs, and that these ligands had some transcriptional activity of PPARγ with less side effects including edema or body weight gain.

A number of clinical trials reported that telmisartan improved insulin sensitivity and lowered plasma triacylglycerides in type 2 diabetic patients. These effects were remarkably prominent by administration of telmisartan among all ARBs, suggesting that the observed effects might originate from activation of PPARγ. Glimepiride has been reported to stimulate insulin secretion with less increasing of body weight. These effects might originate from stimulating activity of PPARγ with less effect inducing edema or retention of body fluid. Clinically, combination therapy of telmisartan with glimepiride is suggested to induce additive effect on activity of PPARγ. Although clinically approved doses of telmisartan and glimepiride individually have only slight effect on activation of PPARγ, the combination therapy of both drugs might bring additive effects on activation of PPARγ. Although we have no definite data about combination therapy of glimepiride and telmisartan improving insulin sensitivity, these drugs would potentially induce additively transcriptional activity of PPARγ without side effects. This combination therapy might provide a new therapeutic option for better cardiovascular management in metabolic diseases and might initiate the development of new classes of combination therapy. Furthermore, activity of PPARγ by natural ligand might be potentiated by these agents.

On the other hand, our data showed that high concentrations of glimepiride and telmisartan inhibited activity of PPARγ by pioglitazone. Previous in vitro experiments showed that partial agonists inhibited adipocyte differentiation stimulated by TZDs. However, it seems improbable that glimepiride and telmisartan would clinically inhibit activity of pioglitazone on PPRE, because clinical doses of glimepiride and telmisartan bring only low concentration (1 μM) in blood stream. Contrarily, there are some reports that telmisartan had some beneficial metabolic effects in type 2 diabetic patients and animal models treated with rosiglitazone. Derosa, et al. reported that administration of telmisartan improved glucose tolerance in type 2 diabetic patients treated with rosiglitazone without body weight gain. Zanchi, et al. reported that telmisartan prevents weight gain and obesity through activation of PPAR delta-dependent pathways. Clinically, combined administration of telmisartan and low-dose pioglitazone would be an attractive therapy with respect to weight control because it might improve insulin sensitivity with less increase of edema than pioglitazone monotherapy.

In conclusion, we observed additive effects of PPARγ partial agonists such as telmisartan and pioglitazone on transcriptional activities of PPARγ, suggesting that combination therapies with these agents might improve insulin sensitivity in type 2 diabetic patients with few side effects.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.
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Insulin is a Gift of Life for people with Diabetes

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ABSTRACT

Although Diabetes Mellitus (DM) or sugar diabetes was identified centuries ago, it was not known why this metabolic disorder develops until JR Macleod and Frederick Banting made the discovery. DM is due to deficiency of insulin produced by beta cells of the pancreas. Prior to this discovery and its application around 1921, many people with this disorder died from diabetic coma. Thus insulin made a milestone in the treatment of DM, in early part of the 20th century and it is the same today. However, commercialism prevails in the care of DM with too much twist in the therapy. It is now the norm to prescribe oral anti-diabetic agents in all adults with diabetes called Type 2 DM. Oral anti-diabetic drugs lower fasting blood glucose and HbA1c but not 2h postprandial blood glucose. The latter is related to diabetic complications. Thus, although patients are not dying from diabetic coma because of insulin but they are developing whole gamut of complications which have increased the morbidity and mortality in patients with DM. Thus the emphasis of this article is to resurge the use of insulin as a gift of life for patients with DM and a therapy which permits a healthy and active life for them.

KEYWORDS: Diabetes; Diabetes Mellitus; Insulin; Pancreatic beta cells; Postprandial blood glucose; Fasting blood glucose; Glycosylated haemoglobin; Gift of life; Diabetic complications.

HISTORICAL PERSPECTIVE OF USE OF INSULIN

During May 1921, the Canadian researcher Frederick Banting under the supervision of John Macleod and helped by graduate student Charles Best attempted to extract the anti-diabetic secretion from the pancreas of a dog. By 1922, the team was successful in obtaining a useful extract which they named insulin. They published their work in 1922 under the authorship of Banting and Best in which they reported on the successful use of a pancreatic extract for normalizing blood sugar levels in diabetic dogs. On January 11, 1922 they were presented with an opportunity to try pancreatic extract on a 14 year old boy named Leonard Thompson. This young Toronto resident had diabetes since 1919. He weighed 65 pounds and was about to sink into coma. He first received Banting’s and Best’s extract but he developed allergic reaction. Twelve days later, he received a second dose purified by James Collip. Thomson’s symptoms began to disappear; his blood sugar returned to normal and he was brighter and stronger. He lived for 13 additional years by taking insulin. He died at the age of 27 years due to diabetic complications.

One of the first people in Britain to benefit from the discovery of insulin was Sir Norman Purvis Walker, Treasurer of the Royal College of Physicians of Edinburgh. Walker was suffering from diabetes and by 1922 he was reduced to extreme emaciation and muscular weakness. When Walker received insulin, the effect was immediate.
Marie Krogh, wife of August Krogh, a nobel laureate in physiology and medicine in 1921 was found to have maturity-onset diabetes. While in the USA in November 1922, together with the Danish physician H.C. Hagedorn, Krogh founded the Nordic Insulin Laboratory and Novo Nordisk Fund. Marie Krogh’s diabetes was successfully treated with insulin. In 1923, Eli Lilly & Co. in Indianapolis, Indiana started commercial production of insulin naming their product Isletin insulin.

In 1936, Zinc-protamine insulin was developed by the Canadians DA Scott and AM Fisher which presented a longer-acting insulin source. The Nordisk group in 1946 developed Neutral Protamine Hagedorn (NPH or Isophane insulin) which was neutral insulin with longer duration of action and which unlike the early protamine insulin, could be mixed with regular insulin. This was marketed in 1950. The same company researchers in 1953 developed the Lente insulins – Ultra lente, Lente and Semilente. In 1944, the standard insulin syringe was developed; helping to make diabetes management more uniform.¹

SMALL OR LARGE EXAMPLE OF THE BENEFIT OF THE USE OF INSULIN

The worst part of diabetes care is non-compliance in adhering to prescribed diet and insulin therapy. On the other hand, compliance of the patient and the support system in following prescribed diet and insulin therapy along with better understanding of the seriousness of diabetes complications without insulin treatment and taking full responsibility of the care along with the professionals will go long way in averting the complications of diabetes and staying healthy.

Here is an example of great compliance of the support system in rigidly following medical advice which has made it possible for healthy living of this 73 years old white male. He and his wife are residents of Montana, a northern state of the USA, lives in Florida during the winter months. He was first seen in March, 2014 by the author in consultation in a local hospital and thereafter followed regularly in one of author’s offices. In March 2014 he was found very lethargic and poorly conversing. He was noted to have uncontrolled diabetes and acute or chronic renal failure. He was treated with oral anti-diabetic agents consisting of glipizide and a DPP-4 inhibitor and angiotensin receptor blocker Losartan. These therapies were discontinued and he was started on detemir insulin (Levemir) 15 units after breakfast and dinner. Laboratory studies during hospital stay is shown in Table 1. After discharge he was seen in author’s office in April 2014. Medications consisted of detemir insulin 15 units after breakfast and dinner. Losartan 50 mg daily, paroxetine 20 mg daily, feroxousfate 300 mg PO TID, DPP-4 inhibitor (ONGLYZA) 5 mg daily, glipizide 10 mg daily, furosemide 20 mg PO as required and spironolactone 25 mg PO as required.

Author’s action was to discontinue glipizide and DPP-4 inhibitor and Angiotensin Receptor Blocker (ARB) Losartan. Continue insulin detemir (Levemir) 20 units after breakfast and dinner and hydralazine PO 25 mg BID was added. His next visit was in November 2014. Medications consisted of Novolin N 25 units morning and evening (could not afford Levevemir), calcitriol 0.25 mcg daily, ferrous sulfate as before, Vitamin C 500 mg daily, diprydamole 75 mg 2 tablets morning and evening, hydralazine 25 mg PO BID. He did his laboratory. The results are shown in Table 2.

Glucose control has improved to normal levels with insulin. Renal function has improved to near normal levels, as well as haemoglobin levels. All these improvements are due to insulin therapy and exclusion of the use of ARB drugs, Losartan.

Author’s action at this time was to discontinue dipyridamole as an additional cause of anaemia and double the dosage of iron. His next visit was in January 2015. He was very much conversing, feels happy as his wife. His medications were same as in the previous visit except dipyridamole. He did his laboratory. The results are shown in Table 3.

Glucose control is normal with Novolin N 25 units twice daily. Blood pressure was 110/70 mmHg. Postprandial renal function is normal thus the goal is met. Haemoglobin is still low but increasing. However, iron reserve is normal and there was no evidence of occult gastrointestinal bleeding. Author’s action was to continue current therapy.

People with established diabetes whose blood glucose levels are higher than 200 mg/dL (>11.1 mmol/L) without treatment with insulin are at a high risk to develop complications acutely or in a protracted (chronic) fashion. It is important to remember that while acute complications reverse with intensive insulin therapy, chronic complications do not necessarily reverse even with intensive insulin therapy.
Thus the chronic complications of diabetes are reiterated here and how do these complications develop from pathobiological standpoint? The microvascular system is affected singularly and the vascular endothelial cells are the target organ for serious damage by high circulatory glucose milieu. These chronic complications are enumerated here but they are not in any particular order

- Retinopathy leading to impaired vision;
- Nephropathy leading to End Stage Renal Disease (ESRD) and dialysis;
- Unhealed foot ulcer or gangrene of toes or feet and amputation of a foot or a limb;
- Sexual dysfunction;
- Coronary heart disease leading to myocardial infarction and sudden death;
- Neurogenic bladder leading to urinary retention and recurrent urinary tract infection;
- Gastroparesis and paralytic ileus leading to recurrent vomiting, diarrhea, loss of nutrition and cachexia.

Thus, when patients present to a doctor’s office with one complication, careful examination may reveal additional complications. A variable degree of renal function impairment is a common accompaniment of many of the diabetic complications listed above. The renal failure most commonly defined by eGFR<60 ml/min is not necessarily and entirely due to uncontrolled diabetes but more often due to other concomitant therapy.

An important question is about the threshold of glucose level above which complications are likely to develop and below which complications are unlikely to develop. No definite answer is available to that effect. The reason is most outcome studies used glycosylated Haemoglobin (HbA1c) as the only glycemic parameter to validate the results. However, studies from Europe and other countries used 2h postprandial glucose above 200 mg/dL to determine cardiovascular morbidity and mortality. Here is a hypothetical question and fundamental laboratory research of author is presented later to clarify the hypothesis. Hypothesis is if glucose particles are injurious why doesn’t normal glucose level (70-99 mg/dL or 4.4-5.5 mmol/L) produce Endothelial cell (Ecs) damage but Ecs are damaged when they are treated with high glucose (above 200 mg/dL or 11.1 mmol/L). However, Ecs damage is largely mitigated when Ecs are treated with high glucose but insulin is added in the culture plate.?

Thus, this research finding of the author validates the clinical scenario that insulin treatment prevents renal failure and perhaps other diabetes complications. However, it should be stressed here that all author’s patients are treated with insulin and they all are living complication-free life.

The most important therapy for diabetes is obviously replacement therapy, which is insulin. Early insulin therapy with resultant satisfactory glucose control appears to spare or delay beta-cell damage and might even spare beta-cell function. Glucose control can be graded into three categories, according to this study.3

2-hour postprandial glucose levels can be easily obtained by ordering 2-hour postprandial Basic Metabolic Panel (BMP) after eating normal breakfast or lunch whichever is patient’s preferable meal.

- **Satisfactory** = <200 mg/dL (<11.1 mmol/L)
- **Fair** = 200-300 mg/dL (11.1-16.6 mmol/L)
- **Poor** = >300 mg/dL (>16.6 mmol/L)

Thus, satisfactory glucose control and frequent office visits to maintain the glucose control and adequate blood pressure and electrolyte and acid-base controls are prerequisites to prevention of complications strategy.4

**Principle of Insulin Therapy**

A variety of insulin preparations are available in the market. They are short (fast) acting, intermediate-acting and long acting insulin. It is important to understand that insulin therapy begins with long-acting insulin. There are two types of long-acting insulin available. They are Insulin Glargine (Lantus®) and Insulin Detemir (Levemir®) A recombinant DNA analog of human insulin, forms micro precipitates in subcutaneous tissue, delaying its absorption and prolonging its duration of action. Both have similar onset of action in about 4 hours and reach peak action in 8-9 hours. Effect decreases after 12 hours. There is no data to support or refute that either Lantus or Levemir will be effective for 24 hours in those with normal kidney function. Author prefers Lantus because he has more experience with that product. While Lantus or Levemir should be prescribed after breakfast and dinner (12 hours apart) in those with normal kidney function, once daily after breakfast is appropriate in those with reduced kidney function. Author typically prescribes 25 units after breakfast and dinner at the outset and increases dosage to achieve satisfactory glucose control. Although Lantus or Levemir alone can be used to achieve glycemic control and stabilize kidney function in some patients, other patients will need coverage of fast acting or regular insulin.

**Fast-acting insulins**

1) Insulin aspart (Novolog®)
2) Insulin Lispro (Humalog®)
3) Novolin®
4) Humulin®

While aspart or lisproinsulin are very rapid acting (action starts within 20 minutes), Humulin or Novolin R are fast and action starts within 30-40 minutes.
Intermediate-acting

1) NPH (Neutral Protamine Hagedorn)
2) Novolin N
3) 70/30 mixed insulin

NPH is uncommonly used in the USA, however, it may be prevalently used in other countries. A combination of Novolin R and Novolin N is effective in achieving glycemic control but the efficacy of mixed insulin is undocumented. The mixed insulin preparations are turbid which decreases efficacy, thus mixed insulin is not prescribed. It is important to know that insulin must be water color to be efficacious.

Satisfactory Glucose Control: Insulin Therapy

For patients with 2h postprandial or random glucose of less than 200 mg/dL, author recommends a trial of diet control, avoid excesses (buffet lunch or dinner, parties) and regular exercise to reduce weight if overweight. Author does not recommend oral anti-diabetic agents as they reduce fasting blood glucose levels.

Here is an isolated example of effect of insulin in lowering all the glycemic parameters: fasting glucose, 2h postprandial glucose and HbA1c. To that effect, metformin even in large doses is ineffective compared to insulin.

A 54 year old Cambodian female gave long history of diabetes and was treated with metformin 1000 mg twice daily. The results are shown in Table 4.

<table>
<thead>
<tr>
<th>Glucose (mg/dL)</th>
<th>BUN (mg/dL)</th>
<th>Scr (mg/dL)</th>
<th>eGFR (ml/min)</th>
<th>HbA1c %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting</td>
<td>132</td>
<td>17</td>
<td>0.61</td>
<td>103</td>
</tr>
<tr>
<td>2hPP</td>
<td>292</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Scr- Serum Creatinine eGFR- Estimated glomerular filtration rate

Table 4: Shows serum fasting, 2h postprandial (2hPP) glucose, renal function parameters, and glycated hemoglobin (HbA1c) levels.

It should be noted that metformin didn’t affect HbA1c or 2h postprandial glucose. All glycemic parameters are high. Thus metformin was discontinued and she was started on Glargine insulin (Lantus) 25 units subcutaneously after breakfast and after dinner (12 hours apart). Her next office visit was in February 2011. The laboratory is shown in Table 5.

<table>
<thead>
<tr>
<th>Glucose (mg/dL)</th>
<th>BUN (mg/dL)</th>
<th>Scr (mg/dL)</th>
<th>eGFR (ml/min)</th>
<th>HbA1c %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting</td>
<td>113</td>
<td>21</td>
<td>0.56</td>
<td>113</td>
</tr>
<tr>
<td>2hPP</td>
<td>204</td>
<td>19</td>
<td>0.60</td>
<td>104</td>
</tr>
</tbody>
</table>

Scr- Serum Creatinine eGFR- Estimated glomerular filtration rate

Table 5: Shows serum fasting and 2hPP glucose, renal function parameters and glycated hemoglobin (HbA1c) levels.

Table 6: Shows serum fasting, 2hPP glucose, renal function parameters, and electrolytes levels.

He was discharged from the hospital and followed by a primary care physician. He did not receive insulin after discharge from hospital until he reported to author’s office.

He gave history of uncontrolled diabetes which was first detected in year 2002. He was treated with metformin 1000 mg PO daily. He also gave history of hypertension. Medications at this visit included: hydralazine 25 mg x 3 TID, clonidine 0.1 mg x 2 TID, atenolol 50 mg daily, Lisinopril 40 mg daily, doxazosin 2 mg x 2 daily, calcitriol 0.25 mcg daily, amlodipine 10 mg daily, furosemide 40 mg daily, simvastatin 40 mg daily, Humulin N 10 units subcut at bed time just started. On examination he showed slight edema, blood pressure was 120/80 mmHg. A limited laboratory done in early December, 2014 showed random glucose of 421 mg/dL (23.3 mmol/L), serum creatinine of 3.14 mg/dL and eGFR of 25 ml/min. BUN was 53 mg/dL. Hemoglobin 9 g/dL.

Thus, he was found to have uncontrolled diabetes, Chronic Kidney Disease (CKD) stage 4, and anemia probably due to CKD and Lisinopril. Also he showed low vitamin D level.

Therefore, actions included 1) Discontinue Lisinopril 2) reduce atenolol to 25 mg/ PO daily 3) start glargine insulin (Lantus) 25 units after breakfast and 25 units after dinner 4) increase Humulin N 10 units before each meal 5) order laboratory in one week. He returned to office in one week. He complained of erectile dysfunction but otherwise doing well. Laboratory is shown below (Table 7).
Glucose control improved, renal function remained low and anemia persisted. Authors action included 1) Increase Lantus insulin to 35 units after breakfast and 35 units after dinner and switch Humulin N to Humulin R 20 units before each meal and at bedtime. 2) Change furosemide to bumetanide 2 mg PO daily 3) Laboratory found. His serum CO2 is 26 mmol/L, calcium is 9.3 mg/dL, phosphorus 4.2 mg/dL and uric acid decreased to 8.8 mg/dL. He returned to office in 2 weeks. He states he feels well. Slight edema was noted. Medications consist of Lantus insulin 35 units after breakfast and dinner, Humulin R 20 units before each meal, clonidine 0.2 mg PO TID, hydralazine 75 mg TID, chlorothalidone 50 mg daily, amloidipine 10 mg daily, Kcl 10 mcq PO TID, simvastatin 10 mg daily. Laboratory (Fasting) done on February 9, 2014 showed hemoglobin 9.3 g/dL. Glucose 258 mg/dL, serum creatinine 3.34 mg/dL with eGFR 23 ml/min. HbA1c 10.2% and uric acid 7.4 mg/dL. Thus his diabetes is still uncontrolled, renal function is low, CKD stage 4 with slight or no improvement. However, uric acid decreased to near normal level. Allopurinol is decreased to 150 mg PO daily and simvastatin is discontinued. Novolin R changed to aspart insulin 15 units before each meal and 10 units at bedtime. He will return to office, a laboratory will be done before office visit.

In those with difficult to control glycemia, fixed dose of Novolin R or aspart of 15-20 units 15-30 minutes before each meal, and at bed time by sliding scale is prescribed. Author recommends insulin by injection and not by flexpen for less pain. Experiences indicate poor glycemic control in those resorting to pen technique. Also fundamentally, there is no difference between regular insulin and rapid acting insulin such as aspart. A study from United Kingdom showed that glycemic control was actually similar during treatment with human regular insulin (HbA1c 6.2±0.8%) and insulin lispro (6.0%±0.9%).

Uncontrolled hyperglycemia is most commonly due to noncompliance in adhering to a prescribed diet intentionally or otherwise. However, even in best of circumstances, uncontrolled hyperglycemia could be due to urinary tract infection, flu or pneumonia in and of itself as well as a result of failure to take prescribed doses of insulin due to the illness. Treatment of these incidental conditions along with aggressive insulin therapy help to restore good glycemic control.

As insulin therapy is initiated oral anti-diabetic drugs are discontinued one to two in each visit until they are all discontinued. Frequent office visits at 4 to 8 week intervals are an integral part of diabetes care to ensure and maintain glycemic control, blood pressure control, renal function control, fluid, electrolyte and acid base control. Compositely these controls ascertain healthy living and complication-free life as well these enhance the ability to cope with the disease and yet thrive.

**Monitoring of Glycemic Control**

There are two ways to monitor day to day glucose con-
trol. These are 1) finger stick practice using a glucometer and glucose strips 2) urine glucose testing. Although urine glucose testing is simple and cheap, but quantitative assay has not been developed to adjust insulin dosage based on urine glucose readings. On the other hand finger stick is painful and expensive, but it is the prevalent methods of glucose monitoring at home to adjust insulin dosage. Most patients are educated to check sugar in the morning and night. These random levels may be important to determine glycemic level and adjust insulin dosage ups and downs according to their levels of glucose readings. Sugar testing is preferable 1-2 hours after a meal and at bedtime in order to adjust regular or fast acting insulin up or down. It is important to know that dosage of Lantus or detemir remains unchanged. If bed time glucose is high it will increase further through the night and will lead to nocturia and frequent wake up for bathroom visits, resulting in tiredness in the morning. This is a common feature in uncontrolled diabetes. Thus a bed time dose of regular insulin will minimize nocturia and ensure a restful night. Early morning glycemic surge giving rise to fasting hyperglycemia is not uncommon even in the most compliant patients. It is important to understand that this early morning hyperglycemic surge is conductive to good sleep; whereas an extra dose of insulin at bedtime may increase the risk of hypoglycemia, and waking up in early morning. However, nocturnal hypoglycemia may be minimized by eating a bed time snack consisting of a small sandwich and a glass of milk or juice.

PREVAILING COMMERCIALISM HINDERING TREATMENT OF DIABETES WITH INSULIN AND IS THE ROAD TO DIABETIC COMPLICATIONS

Given the discovery of insulin’s efficacy for treating diabetes, unfortunately therapy for diabetes has taken a different turn. This turn for the worse has been made possible by the influence of commercialism on our medical practices. Commercialism seems to prevail in “Diabetes care” more than any other illness and is orchestrated by drug companies in collaboration with professionals which sadly promotes profit over patients. Prevailing commercialism consist of promoting oral anti-diabetic drugs instead of insulin as a first line therapy for diabetes. This commercialism seems to be magnified by greedy industry and different medical societies.

In the current practice of diabetes, the most commonly drugs prescribed include metformin (antidiabetic agent) and Angiotensin Converting Enzymes Inhibitors (ACEI) or Angiotensin Receptor Blockers (ARB). Thus the worst commercialism is attributed to diagnosis of every adult with diabetes as Type 2 diabetes and automatic prescription of metformin and an ACEI lisinopril or an ARB drug losartan. Seldom does a provider discuss insulin treatment with the patient even though the glucose level may be 300 to 400 mg/dL (16.6 to 22.2 mmol/L). Lack of the knowledge of the professionals and overriding influence of the industry to prescribe metformin and state and federal regulations to prescribe ACEI/ARB drugs for renal protection in Type 2 diabetes continue to victimize the adult patients with diabetes.8 Regrettably, Los Angeles Times as of March 22, 2009 reported that over the last 15 years, the US rate of foot amputations from complications of diabetes has soared approaching 100,000 annually, according to studies and government statistics. To that effect, both parties are responsible. Professionals are not emphasizing adequate glucose control with insulin therapy. A recent report indicates that only 17% of diabetes patients take insulin; another 17% take a combination of insulin and oral agents; whereas 54% of diabetes patients are treated with oral agents.9

FUNDAMENTAL LABORATORY RESEARCH ATTEST TO BETTER UNDERSTANDING OF UTILITY OF INSULIN THERAPY IN DIABETES

In the laboratories of the author and colleagues, vascular endothelial cells were cultured for growth of the cells and then treated with normal concentration of glucose (90 mg/dL or 5 mmol/L) or high concentration of glucose (540 mg/dL or 30 mmol/L) for a period of 2 days, 6 days or 10 days. Additional cultured cells were treated with glucose of the same concentrations as above and insulin or with glucose, insulin and heparin.10 High glucose levels bathe all the cells in the body. But why does damage occur in some cell types? The answer is that most cells are able to reduce the transport of glucose inside the cells when they are exposed to high glucose levels, so that their internal glucose concentration remains constant. In diabetes, endothelial cells and mesangial cells cannot reduce transport of glucose inside the cells in state of high glucose levels in the blood. Therefore, complications that develop in diabetes must involve mechanisms of excessive amount of glucose inside the cells, rather than outside of the endothelial cells.11 We have shown that vascular endothelial cells are progressively damaged with increasing duration of exposure of the cells to high concentrations of the glucose but the damage is mostly mitigated with insulin treatment.10

CONCLUSIONS

1) Define diabetes or diabetes mellitus by obtaining fasting and 2h Postprandial (PP) metabolic panel. 2hPP glucose greater than 200 mg per dL establishes diabetes.
2) Insulin is the cornerstone of therapy in established diabetes. Insulin is a gift of life for diabetes, it prevents complications and allows subjects to live a healthy life except a few needle pricks a day
3) Blood pressure control is an integral part of diabetes therapy which reduces the risk of complications. Blood pressure control can be achieved with safe antihypertensive drugs
4) Use of ACEI/ARB drugs should be avoided. These drugs have no place in diabetes care.
5) Adequate glycemic control with insulin reduces proteinuria. Manipulation of proteinuria with ACEI/ARB invariably results in acute renal failure or chronic renal failure with progression to end stage renal disease.
REFERENCES


Case Report

Glycemic Variations after Ingestion of Different Carbohydrate-Containing Foods Assessed by Continuous Glucose Monitoring in Healthy and Diabetic Individuals in Daily Life

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ABSTRACT

Carbohydrate counting is a meal planning approach for diabetic patients, but individual variations of the effect of glycemic index do not seem to be well taken into account. Here we assessed glycemic variations after the ingestion of different carbohydrate-containing foods by continuous glucose monitoring in healthy and diabetic individuals in daily life. After an overnight fast, seven healthy persons and four patients each of type 1 and 2 diabetes were instructed to eat three different kinds of 40 g carbohydrate-containing breakfast within 10 minutes for three consecutive days. The meals consisting of foods chosen from starchy foods, fruits and dairy foods were 100 g cooked rice with Japanese green tea seasoning (183 kcal), 300 g apple (162 kcal), and 35 g cereal with 180 ml milk (253 kcal), respectively. The glycemic profiles were measured every 5 minutes over three hours before and after the breakfast meals using a continuous glucose monitoring system (iPro2®). Sulfonylurea was not taken, and insulin dose was reduced at the breakfast to avoid hypoglycemia, which was fixed during the study. The respective profiles of glycemic responses to the breakfast meals varied largely among individuals, especially in diabetic patients. The sums of glycemic increments over three hours after the ingestion of apple and cereal+milk (322 ± 123 (mean ± SD) and 181 ± 275 mg/dl) were significant (p<0.05) lower than those after the rice ingestion (543 ± 209 mg/dl) in healthy persons, but there was no significant difference among those (528 ± 471, 766 ± 1374 and 1442 ± 1025 mg/dl, respectively) in diabetic patients. Postprandial glucose levels were suppressed in the cereal+milk meal, but the elevated glucose levels appeared to persist in patients with type 1 diabetes. Medical staff should be aware of controlling carbohydrate intake with glycemic index, keeping in mind large individual variations of glycemic responses to the same amount of carbohydrate. It may be wise for diabetic patients to develop their own personal glycemic indexes in their respective situations.

KEYWORDS: Carbohydrate counting; Glycemic index; Rice; Apple; Milk; Continuous glucose monitoring.

INTRODUCTION

Diet therapy remains the foundation of any diabetes treatment program. Carbohydrate counting is a meal planning approach to help diabetic patients adequately count and control their carbohydrate intake, in order to achieve and maintain glycemic control.1 In addition to the amount of carbohydrate, the type or source of carbohydrate is recognized to affect postprandial glucose levels. From this point of view, glycemic indexes of foods were developed in an attempt to systematically classify different carbohydrate-containing foods according to...
the incremental area under the curve for blood glucose responses relative to that of a reference food (glucose, white bread, or rice).2-5 Postprandial hyperglycemia has been suggested to affect oxidative stress and inflammatory responses leading to diabetic complications.6-12 Clinical usefulness of glycemic index has been reported in studies on people with diabetes and coronary heart disease.13-16 However, it is said that it can be difficult for diabetic patients to use glycemic index for glycemic control, because it usually evaluates one food at a time, not the combination of foods in a meal. In addition, individual variations of the effect of glycemic index do not seem to be well taken into account. In this study, we evaluated glycemic variations after the ingestion of different carbohydrate-containing foods by continuous glucose monitoring in healthy and diabetic individuals in daily life, to better guide diabetic patients by diet therapy with carbohydrate counting.

SUBJECTS AND METHODS

Study design

The present study was approved by the Ethical Review Board of National Hospital Organization Matsumoto Medical Center. Seven healthy persons (3 males, 4 females; age, 40 ± 12 (mean ± SD) years; Body Mass Index (BMI), 23.7 ± 4.7) (Table 1) and eight patients (4 males, 4 females; age, 40 ± 9 years; BMI, 22.7 ± 2.5) with type 1 and type 2 diabetes mellitus (T1DM and T2DM, four patients each) (Table 2) were studied with their written consent to the study. T1DM or T2DM was diagnosed by a physician in consideration of patient’s clinical course, insulin secretion capacity and serum test for glutamic acid decarboxylase auto antibodies. T2DM 1 was treated with 20 mg glimepiride, and 250 mg metformin twice; T2DM 2 was treated with 20 mg glimepiride; T2DM 3 was treated with 50 mg sitagliptin, and 250 mg metformin twice; and T2DM 4 was treated with 40 mg gliclazide; T2DM 1 and T2DM 2 were instructed to pass three hours after the breakfast similarly. T2DM patients took prescribed oral hypoglycemic agents excluding sulfonylurea (SU) after breakfast to avoid hypoglycemia, and the excluded SU was taken after lunch during the study. Similarly, to avoid hypoglycemia T1DM patients reduced their bolus insulin doses for breakfast to 40-50% of usual doses, according to the amount of carbohydrates diminished than usual. The subjects were instructed to pass three hours after the breakfast similarly during three days of the study in daily life. However, T1DM 2 explained that he had a hard labor for one hour due to the heavy snow, starting 30 minutes after the cereal+milk ingestion on the 3rd day of the study, and felt symptoms of hypoglycemia without snacks before lunch.

Measurements

Blood glucose levels were assessed by using a continuous glucose monitoring system (iPro2®, Medtronic Inc., Tokyo, Japan), which was attached to the subjects’ abdominal region on the day before the first day of the study, and was removed on the last day of the study. The continuous glucose monitoring system was used according to the manufacture’s instruction. The system monitored glucose levels in subcutaneous fluids every 5 minutes, and blood glucose levels for calibration were measured 3 times or more a day by self-monitoring of blood glucose with a glucometer (Medisafe Fit®, Terumo Corp., Tokyo, Japan) at home. The iPro2® device stored the sensor signal information, and was retrospectively calibrated.17

Statistical analysis

The data are expressed as the mean ± SD. Statistical
analysis was performed using repeated measures ANOVA with Bonferroni correction.

RESULTS

Figure 1 shows individual glycemic profiles after the ingestion of three different kinds of, but the same amount (40 g) of carbohydrate-containing foods (rice, apple and cereal+milk) for breakfast in 7 healthy individuals (A to G) as in Table 1. The glycemic profiles were assessed every 5 minutes over 3 hours by a continuous glucose monitoring system at home. Similarly, Figure 2 shows those in four T2DM patients (A to D) and those in four T1DM patients (E to H). The patients A to D consistently took 50 mg sitagliptin, nothing, 50 mg sitagliptin + 250 mg metformin, and 50 mg sitagliptin + 250 mg metformin, respectively, after the breakfast meal intake. The patients E to H injected bolus insulin before the meal intake, doses of which were reduced and fixed at 2U, 5U, 5U and 3U, respectively. The respective profiles of blood glucose responses to three different meals for breakfast varied largely among individuals, especially in diabetic patients. However, as expected, a rise in blood glucose levels was, on the whole, higher after the rice ingestion than after ingestion of the other two meals (apple and cereal+milk).

Incremental area of postprandial glucose levels after the ingestion of three different meals shown in Figures 1 and 2 is depicted as a mean± or –SD of sums of every 5-minute blood glucose level minus baseline level at time 0 in 7 healthy persons (Figure 3A) and in 8 diabetic patients (Figure 3B). At the time interval of 0 to 180 minutes, the sums for the rice, apple and cereal+milk in healthy persons were 543 ± 209, 322 ± 123 and 181 ± 275 mg/dl, respectively. In diabetic patients, those were 1442 ± 1025, 528 ± 471 and 766 ± 1374 mg/dl, respectively. Similarly, those at the time interval of 0 to 60 minutes were 154 ± 108, 148 ± 69 and 121 ± 122 mg/dl in healthy persons, and 420 ± 197, 360 ± 264 and 184 ± 196 mg/dl in diabetic patients; those at the time interval of 60 to 120 were 286 ± 108, 163 ± 92 and 92 ± 155 mg/dl in healthy persons, and 680 ± 390, 363 ± 306 and 442 ± 645 mg/dl in diabetic patients; those at 120 to 180 were 104 ± 132, 11 ± 59 and -31 ± 105 mg/dl in healthy persons, and 342 ± 612, -193 ± 275 and 141 ± 675 mg/dl in diabetic patients, respectively.

In healthy persons, glycemic increments were significantly (p<0.05) smaller after the ingestion of apple and cereal+milk than after the rice ingestion at the time interval of 0 to 180 minutes. At the time interval of 60 to 120, the increments after the cereal+milk ingestion were significantly (p<0.05) smaller than those after the rice ingestion. In diabetic patients, the increments after the cereal+milk ingestion at the time interval of 0 to 60 minutes and after the apple ingestion at the time interval of 120 to 180 minutes were smaller than those after the rice ingestion at the respective time intervals. The mean value of the sums after the cereal+milk ingestion in healthy persons and that after the apple ingestion in diabetic patients at the time interval of 120 to 180 minutes were below the baseline. When
glycemic indexes were calculated with rice as a reference (100) using the mean values at the time interval of 0 to 180 minutes in this study, those of apple and cereal+milk were 59 and 33 in healthy persons, and 37 and 53 in diabetic patients. Using the mean values at the time interval of 0 to 120 minutes, those were 71 and 48 in healthy persons, and 66 and 57 in diabetic patients.

When diabetic patients were divided into two groups (T1DM and T2DM, n=4 each), mean values of incremental blood glucose levels (ΔMean Blood Glucose) after the ingestion of three different meals (rice (A), apple (B) and cereal+milk (C)) in three groups of T1DM, T2DM and Control (7 healthy persons) are depicted in Figure 4. The mean value in A was the highest in T1DM, intermediate in T2DM, and the lowest in Control at every 5-minute time point. Compared with this, the mean value in B was higher in T1DM and T2DM than in Control during a shorter period of time. The mean value of postprandial glucose levels after the ingestion of three different kinds of food (rice, apple and cereal+milk) was significantly smaller than those after the ingestion of rice. *p<0.05 vs rice at the same time interval (repeated measures ANOVA with Bonferroni correction).
Figure 4: Mean values of incremental blood glucose levels (Δ Mean Blood Glucose) after the ingestion of three different foods (rice (A), apple (B) and cereal+milk (C)) in healthy persons (Control, n=7), type 2 (T2DM, n=4) and type 1 (T1DM, n=4) diabetic patients.

DISCUSSION

In the present study, we assessed glycemic variations after the ingestion of three different kinds of, but the same amount of carbohydrate-containing meals, chosen from starchy foods, fruits and dairy foods, by using continuous glucose monitoring in healthy and diabetic individuals in daily life. The rice meal contained protein, and the cereal+milk meal contained protein and fat as a combination of foods. Individual variations of glycemic responses to these meals were considerably large, especially in diabetic patients, although less accuracy of continuous glucose monitoring at home should be taken into account. However, the average glycemic excursions appeared to be more or less similar to those in previous reports in view of glycemic index. In both the groups, it was indicated that glycemic indexes of apple and cereal+milk were approximately 35 to 70.

Fruits generally belong to low glycemic index foods, as they are rich in dietary fiber and contain fructose and glucose moieties. It has been demonstrated that glycemic indexes of several kinds of fruits were approximately the same between healthy persons and type 2 diabetic patients. According to International Tables of Glycemic Index, 2008, the glycemic indexes of fructose, sucrose and apples are 15, 65 and 36, respectively. In our study, postprandial glucose levels after the apple ingestion were lower than those after the rice ingestion, and returned to or often decreased below the baseline, as had been shown in the other reports. Such a pattern of postprandial glucose levels is suggested to be linked to increased appetite and weight gain, which seems to be one of hints on nutrition education. However, the restriction of fruit intake had no effects on HbA1c level or weight loss in type 2 diabetic patients. The consumption of fruit juice seems to have no overall effects on fasting blood glucose and insulin levels.

Glycemic indexes of milk and yogurt are 39 and 41, respectively, in the International Tables. In addition to lactose providing fewer glucose moieties than the same amount of maltose or starch, dairy protein, especially the whey protein, can stimulate glucagon-like peptide 1 and insulin secretion, and dairy fat and organic acids can delay gastric emptying. Along with these observations, the present study showed sums of glycemic increments after the ingestion of common breakfast cereal (corn flakes) of a high glycemic index together with milk (or yogurt in T1DM) were significantly lower than those after the rice ingestion. Interestingly, persistent elevated glucose levels were seen in type 1 diabetic patients, excluding one who had hypoglycemia after the cereal+milk ingestion due to an accidental hard labor. Oestman et al. previously presumed that milk may produce a higher glycemic index when tested in individuals with diminished or absent β cell function than in healthy subjects capable of responding to the insulinitropic components in milk. Our finding seems to be consistent with this presumption.

Thus, it was clearly demonstrated that inter-individual variations of postprandial glucose levels after the ingestion of
different carbohydrate-containing foods were large in healthy and, especially, in diabetic individuals in daily life. Nevertheless, the average profiles of the glucose levels approximately accorded with those previously reported. The glucose levels slowly rose after dairy meals, and the elevated levels persisted in type 1 diabetes. Medical staff should be aware of controlling carbohydrate intake with glycemic index, keeping in mind large individual variations of glycemic responses to the same amount of carbohydrate, due to the impact on glycemia of dietary components and non-dietary factors. It may be wise that diabetic patients are advised to develop their own personal glycemic indexes in their respective situations.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

ACKNOWLEDGEMENTS

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Glycemic Variability: Clinical and Prognostic Significance

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ABSTRACT

Minimizing development or progression of chronic diabetic micro and macro-vascular complications has been always the goal of glycemic control. In recent years, much attention has been focused on the possibility that glycemic variability confers an additional risk factor for diabetic complications independent of glycated hemoglobin (HbA1c). Evidence suggests that fluctuating glucose levels produce endothelial dysfunction as well as an increase in free radicals, the key link between hyperglycemia and diabetic complications and that these changes are greater than those produced by sustained hyperglycemia in in vitro and in animal studies. In humans studies, experimental setting also support the hypothesis that plasma glucose fluctuations produce a higher increase in oxidative stress as well as endothelial dysfunction than those produced by sustained hyperglycemia in type 2 diabetes. Moreover, glycemic variability may have a role in the prediction of severe hypoglycemia, which may act as a precipitating factor of diabetic complications. Based on review of available evidence, we advocate decreasing hyperglycemia and diminishing glycemic variability as well as avoiding hypoglycemia in diabetic patients as targets of diabetic therapy. Future trials targeting the influence of the control of plasma glucose fluctuations on the development of diabetic micro-and macro-vascular complications are needed to further strengthen the evidence base.

KEYWORDS: Type 1 diabetes; Type 2 diabetes; Glycemic variability; Diabetic complications.


INTRODUCTION

Diabetes is ranked among the leading causes of morbidity and mortality, and is a tremendous cost burden in medical care. Although glycated hemoglobin (HbA1c) has been considered the surrogate endpoint for long-term glycemic control; recent evidence has raised the question that plasma glycemic variability, irrespective of HbA1c level, may confer an addition risk for the development of diabetic complications.

Several large prospective clinical studies suggest the possibility of glycemic variation may be the explanation for microvascular complication difference between intensively treated and conventionally treated type 1 diabetes patients. Furthermore, there were no significant associations between tighter HbA1c control and cardiovascular risk reduction in the landmark studies in patients with type 2 diabetes: Action to Control Cardiovascular Risk in Diabetes (AC-
CORD), Action in Diabetes and Vascular Disease (ADVANCE), and Veteran Administration Diabetes Trial (VADT).8–10 It has been suggested that the one of these determining factors is the frequency and magnitude of glycemic variation.11 Moreover, there is increasing evidence in recent studies suggesting a positive link between glycemic variability, as measured by Coefficient of variation (CV) of Fasting Plasma Glucose (FPG), and the risk of developing ischemic stroke as well as all-cause, cancer and cardiovascular mortality in type 2 diabetic patients.12–19

In this review article, we firstly provide an overview of the various methods to measure glycemic variability. Secondly, we intend to assess the published evidence investigating glycemic variability and the development of microvascular complications (e.g. retinopathy, neuropathy, and nephropathy) and macrovascular complications (e.g. cerebrovascular disease, coronary artery disease, and peripheral artery disease) in type 1 and type 2 diabetes patients. Thirdly, we review laboratory studies investigating the issue of glycemic variability. Lastly, key issues regarding glycemic variability will be summarized in the conclusion.

MEASUREMENTS FOR GLYCEMIC VARIABILITY

To quantify glycemic variability, several methods have been suggested but no “gold standard” has been universally accepted. More detailed descriptions of various methods have been described elsewhere in one review article, and we just briefly introduce commonly used measurements herein.20 Overall, glycemic variability may be determined as interday or intraday variation. Glycemic intraday variation reflects the swings of plasma glucose in a diabetic patient as a consequence of diminished or absent auto regulations of sugar control within-day. The simplest and most common way to measure glycemic variability is to calculate the Standard Deviation (SD) of mean blood glucose and Coefficient of variation (CV), if one wishes to correct for the mean.20 It is possible to calculate SD and CV from seven-point glucose curves by Self-monitoring blood glucose (SMBG).20–23 However, obstacles arose from using seven-point glucose curves because certain peak or bottom values will always be missing between the two measurements, making it less accurate.20 Another widely deployed method, Mean Amplitude of Glycemic Excursions (MAGE), was first proposed from Service, et al. measuring the differences between peaks and bottoms around a mean glucose value greater than one SD of mean blood glucose for 48 hours.24 It was designed to disregard small fluctuations and focus on major glucose fluctuations.

The easiest way to measure interday variability is to calculate the SD of mean blood glucose and CV. However, it requires data from at least 2 consecutive days to calculate and it neglects within-day blood glucose variations. The other method used to measure interday glucose variability, Mean of daily differences (MODD), was proposed by Molnar, et al. to calculate the mean of absolute differences between glucose levels at corresponding time on two consecutive days.22 However, it appears to be a challenge in daily practice as mealtime variation will affect interfering the measurement.

Each of these tools has its own advantages and disadvantages; while some are easy to apply, others are more complex and are not practical for clinical use.23 Future research must compare different methods in assessing glycemic variability and investigate predictive capacity of glucose variance for medical outcome in diabetes patients.

LABORATORY EVIDENCE FOR GLYCEMIC VARIABILITY

Evidence has suggested endothelial dysfunction and formation of reactive oxygen species as the key link between hyperglycemia and diabetic complications.24 In vitro studies have shown that exposure of cell cultures to rapid glucose fluctuations produced more severe cellular damage as compared with continuous high glucose.25,26 Evidence also suggests that oscillating plasma glucose proves more deleterious than constant high glucose on oxidative-stress generation, important factors in micro- and macroangiopathy.27–30 Experiments in animals also support the hypothesis that fluctuating glucose significantly induced monocyte-endothelial adhesion and atherogenesis as compared with sustained hyperglycemia.31–33 Production of free radicals, accompanied by inadequate intracellular anti-oxidant defenses seems to account for this phenomenon.34 Similarly, in humans, two studies assessed the effect of fluctuations of plasma glucose producing on oxidative stress among patients with type 2 diabetes. These authors found that oscillating glucose was more damaging to endothelial function and resulted in higher levels of oxidative stress markers as compared with sustained hyperglycemia in type 2 diabetes.35,36 On the other hand, it is somewhat amazing and difficult to explain that such a relationship between glycemic variability and elevated levels of oxidative stress could not be confirmed in patients with type 1 diabetes.

More specifically, glucose fluctuations appear more relevant to atherosclerosis progression in type 2 diabetics than those with sustained hyperglycemia.37,38 This is also supported by the evidence that change in intima-media thickness, a marker of endothelial dysfunction, was associated with a reduction in daily glucose excursions, but not indices of chronic sustained hyperglycemia.39,40

GLYCEMIC VARIABILITY AND MICROVASCULAR COMPLICATIONS IN TYPE 1 DIABETES

Bragd, et al. initiated a cohort study to examine the linkage between glycemic variability measured by Standard Deviation of Blood Glucose (SDBG) for 4 weeks and the development of microvascular complications in type 1 diabetes. After 11-years followed up, authors concluded that glycemic variabili-
ity was a predictor of prevalence but not incidence of peripheral neuropathy. No significant relationship toward to the development of retinopathy and nephropathy was found.43 Kilpatrick, et al. began series of studies using DCCT data to access the correlation between glycemic variability measured by Mean Blood Glucose (MBG) and risk of developing diabetic retinopathy and nephropathy complications during the 9-years follow up as well as risk in developing cardiovascular diseases, which will be discussed later in this article.42,43 Results revealed that MBG was associated significantly with the development of retinopathy but not nephropathy. Similarly, Service, et al. utilizing DCCT data to assess the development of diabetic retinopathy by 7-point capillary glucose profiles collected at quarterly intervals for a period of minimal 4-years.21 Cox regression analysis revealed MBG was significantly associated with retinopathy. Moberg, et al. investigated the association between twenty-four different clinical parameters and glycemic variability in type 1 diabetes patients with intense insulin therapy, significant association between glycemic variability and the presence of nephropathy was found.44 However, retinopathy and nephropathy were not associated with glycemic variability in this study.

GLYCEMIC VARIABILITY AND MACROVASCULAR COMPLICATIONS IN TYPE 2 DIABETES

As for macrovascular complications in male patients with type 1 diabetes, Gordin, et al. assessed the association of glycemic variability toward arterial stiffness, an early sign of macrovascular complications.45 Interestingly, arterial stiffness was correlated with MBG but not with MAGE at baseline. Furthermore, none of measurements were associated with arterial stiffness during the hyperglycaemic clamp. As mentioned previously, Kilpatrick, et al. using DCCT trial data to investigate whether there are relationship between glucose control (MBG, HbA1c and intraday SDBG) and the risk of developing cardiovascular diseases. Results showed significantly relationship between MBG, pre-prandial, and postprandial blood glucose to the risk of developing cardiovascular diseases but not with HbA1c and glucose variability.42,43 Hence, there is no evidence between glycemic variability as well as HbA1c to macrovascular risk among DCCT trial.

GLUCOSE VARIABILITY AND MICROVASCULAR COMPLICATIONS IN TYPE 2 DIABETES

Gimeno-Orma, et al. investigated in a 5-year follow up prospective cohort study whether glycemic variability (measured by the FPG-CV quartiles) can be an independent predictor for diabetic retinopathy.46 The results showed that patients suffered from diabetic retinopathy had higher FPG-CV values and there was an increased trend of diabetic retinopathy with increased FPG quartiles. In the Verona Diabetes Study conducted in Italy, during the cross-sectional analysis, FPG-CV was significant associated with the presence of diabetic retinopathy. However, after a mean interval of 1.6 years between the first and second eye evaluation, this study found the magnitude of hyperglycemia (M-FPG) and HbA1c not the FPG-CV as a strong independent predictor for the development and progression of diabetic retinopathy in type 2 diabetes patients.47

GLYCEMIC VARIABILITY AND MACROVASCULAR COMPLICATIONS IN TYPE 2 DIABETES

In recent year, the Verona Diabetes Study focused on whether long-term glucose control as assessed by fasting plasma glucose is a predictor of all-causes mortality and cardiovascular diseases related mortality among a cohort of patients with type 2 diabetes who are aged≥75 years.12,13 Results revealed that glucose variability has a greater effect on survival in elderly patients with type 2 diabetes and authors concluded that the glucose instability measured by FPG-CV was the predictor of cardiovascular diseases related mortality. Furthermore, two extensions of the same study assessing among patients with different age group (aged between 56-74 years, aged<65 year, and aged≥65 years) further proved that variability of FPG independently increased the risk of all-cause mortality in patients with type 2 diabetes.14,15 Similarly, the Taichung Diabetes Study conducted in Taiwan, have demonstrated that FPG-CV is a independent predictor of all-cause or cause specific mortality in type 2 diabetic patients.16,17 Furthermore, the same group initiated the Taiwan Diabetes Study, a large population-based retrospective study, 28,354 type 2 diabetic, aged≥30 years and free of ischemic stroke patients were selected from National Diabetes Care Management Program (NDCMP). The patients were grouped into four quartiles according to FPG-CV measurements.18 The results revealed that compared to patients with the first quartile, higher associated risk of developing ischemic stroke in patients who fell into second, third, and fourth FPG-CV quartile, independent of HbA1c level.

GLYCEMIC VARIABILITY AND HYPOGLYCEMIA COMPLICATIONS

Severe hypoglycemia can lead to severe consequences, such as coma and death. Many harmful events could be avoided if it were possible to predict severe hypoglycemia. Both Cox, et al. and The Diabetes Outcome in Veterans Study (DOVES) suggested that glycemic variability may be a potential predictor of future hypoglycemia occurrence.48,49 Furthermore, glycemic variability if accompanied with severe hypoglycemia episodes may adversely alter the prognosis of acutely ill patients.50,51 It has also been shown that hyperglycemia after hypoglycemia could be more hazardous than that when hypoglycemia is followed by normoglycemia.52 One can argue that much of risk in long-term microvascular and macrovascular complications could be avoided by focusing merely in reduction of hypoglycemia episodes. However, in the Taiwan Diabetes Study, FPG-CV still showed links with incidence of ischemic stroke after excluding patients with hypoglycemia in sensitivity analysis; this association cannot
be explained by hypoglycemia in patients with high glucose variability. Further studies need to investigate the effects of glycemic variability whether coexistence of hypoglycemia or not for diabetic complications.

CONCLUSION

For the past two decades, glycemic variability is of great interest to the diabetes community. Studies have focused on correlation between glycemic variability and diabetic complications, methods to measure glycemic variability, and understanding mechanisms driving glycemic variation. There is increasing evidence in in vitro, animal studies and in an experimental setting in type 2 diabetes patients suggesting that glycemic variability is closely associated with the pathogenesis of diabetic complications. In clinical settings, accumulating evidence suggests that, in addition to and independently of HbA1c, glycemic variability may play an important part in the development of diabetic complications. However, No ‘gold standard’ currently exists to rate glycemic variability. Setting universally accepted measurements, methodologies is mandatory. Lowering HbA1c and diminishing glycemic variability as well as avoiding hypoglycemia should be crucial steps in reducing complications or mortality in diabetes patients. We advocate following the 2015 revision of American Diabetic Association (ADA) guidelines for a tighter glycemic control in early disease course as well as avoidance of extreme blood glucose fluctuations to effectively prevent or delay the development of diabetic complications both in type 1 and type 2 diabetes.

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