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Original Research

Antihyperglycemic Mechanisms of *Allium sativum*, *Citrus sinensis* and *Persea americana* Extracts: Effects on Inhibition of Digestive Enzymes, Glucose Adsorption and Absorption on Yeast Cells and Psoas Muscles

Boris Gabin Kingue Azantsa, PhD[1*]; Guy Roussel Takuissu, MSc[1]; Etienne Junior Tcheumeni, MSc[1]; Martin Fonkoua, PhD[1]; Edwige Ruth Kemadjou Dibacto, MSc[1,2]; Judith Laure Ngondi, PhD[1]; Julius Enyong Oben, PhD[1]

[1]*Laboratory of Nutrition and Nutritional Biochemistry, Department of Biochemistry, Faculty of Sciences, University of Yaounde 1, Yaounde 812, Cameroon*
[2]*CRAN, Ministry of Scientific Research and Innovation, Yaounde, Cameroon*

[*]***Corresponding author***
Boris Gabin Kingue Azantsa, PhD
Senior Lecturer, Department of Biochemistry, Faculty of Sciences, University of Yaounde 1, Yaounde 812, Cameroon; Tel: +237677920184;
E-mail: borisazantsa@yahoo.fr

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ABSTRACT

Background
Mechanisms by which some plants with antihyperglycemic effects reduce postprandial hyperglycemiaare not fully elucidated. This study was designed to investigate some action mechanisms of extracts from stem bark of *Citrus sinensis*, seeds of *Persea americana* and bulbs of *Allium sativum* including *in vitro* inhibition of α-amylase and invertase; glucophagic capacity, absorption capacity on yeast cells and psoas tissues.

Methods
Ethanolic (EE) and aqueous (AE) extracts were tested on α-amylase and invertase activities. Glucose remaining in the medium was measured after direct interactions of: Glucose-extracts; Glucose-yeast-extracts and Glucose-psoas-extracts at different doses 0, 5, 7.5, 10 mg/ml.

Results
All extracts inhibited invertase with IC_{50} varying from 1.92 to 4.81 mg/ml for *Allium sativum* extracts. α-amylase was inhibited by EE *C. sinensis* (IC_{50}=0.063 *vs* 2.73 mg/ml for arcabose) and not by EE of *A. sativum* and EE of *P. americana*. Glucophagic capacity of extracts varied signicanty from 47.55 % of AE *P. americana* (5 mg/ml) to 100% with *C. sinensis* (5 mg/ml). All extracts stimulated glucose uptake (p<0.05) from 2.62 % AE *C. sinensis* (2.5 mg/ml) to 54.74% for EE of *Persea americana* (10 mg/ml). All extracts enhanced glucose uptake by psoas tissues increasing absorption capacity to up to 38.56 % with *A. sativum* (10.45% insulin, p<0.05).

Conclusion
Cumulative actions of each plant extract on inhibition of carbohydrates' digestive enzymes, adsorption of glucose in intestine and blood, stimulation of glucose uptake and insulin action on yeast cells and psoas tissues, contribute to lower hyperglycemia and diabetes related complications. Therefore, extracts from the plants could be good candidates for diabetes therapy.

Keywords
Mechanism; Absorption; Yeast cell; Psoas; Enzymes inhibition; Antihyperglycemic Plants.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder of carbohydrates, fats and proteins resulting in an inability of insulinaction and/or secretion.[1] T2DM is characterized by chronic hyperglycaemia with perturbations of glucose homeostasis due to absence of regulation of postprandial glycaemia. Postprandial glyceamia is at the onset of disturbances of glucose tolerance which appears earlier before high-levels of fasting blood glucose.[2] Several factors influence postprandial glycaemia: foods rich in carbohydrates, glucose digestion and absorption, insulin secretion as a result of rise of glucose levels, incretins actions and glucose intake by cells.[3] Postprandial glucose is also associated to protein glycosylation to the generation of reactive oxygen species which attack DNA and membrane lipids, to increase plasma lipid.[4,5] It is also a risk factor for cardiovascular diseases.[6]

Disturbances related to the glucose homeostasis as well as complications associated to dyslipidemia complexify management of diabetes, leading to alarming and increasing prevalence. In fact, 451 million individuals aged 18-99 years were reported to suffer from diabetes with more then 693 million of patients projected by 2045.[7] Such increasing prevalence is a public health concern and constitutes economic burden for governments. It is why efforts are made everyday to improve health of diabetic patients. Several oral drugs developed are used to reduce blood sugar. These include inhibitors of digestive enzymes, which blocked alpha glucosidases (amylase and invertase), inhibitors of intestinal absorption of glucose which inhibit membrane transporters: inhibitors of Dipeptidyl peptidase-4 (DPP4) which promote insulin secretion and molecules involved in the capture of peripheral glucose.[8,9] Thiazolidinediones, sylfonylureas, Metformin are oral antidiabetic drugs that play a prominent role in the treatment algorithm of T2DM.[10,11] Unfortunately, despite the efficacy of those drugs side effects like hypoglycemia, nausea, vomitting, headache and constipation are still reported.[12-14] Exploration of novel bioactive molecules from plants against diabetes has been intensified mainly with the availabilityof cell and tissue models like yeast cells and psoas mucles.[14-16]

Cameroonian pharmacopea is very rich in plants with medicinal action including *Citrus sinensis*, *Persea americana* and *Allium sativum*.[17] They have been reported to have antihyperglycemic effects on rats in an oral glucose tolerance test.[18,19] However, few studies on their action mechanisms exist to justify their efficacy. This study was designed to evaluate and compare effects of ethanolic and aqueous extracts of *C. sinensis* stem bark, *P. americana* seeds and *A. sativum* bulbson digestive enzymes (invertase and alpha amylase); to determine their adsorptive/glucophagic capacity; to evaluate their capacity to enhance glucose uptake by yeast cells and psoas musleas models.

MATERIALS AND METHODS

Chemicals and Reagents

Krebs solution, insulin and acarbose (GLUCOR®) was purchased in local pharmacy. Baker's yeast, and *Chronolab* test kits for Glucose andamylase assays were purchased locally. Amylase Kit contained reagent R[(2-chloro-4-nitrophenyl-α-D-maltotrioside (CNPG3; 2.25 mmol/L)+sodium Chlorure (NaCl, 350 mmol/L)+calcium acetate $(CH3COO)_2Ca$, 6 mmol/L)+potassium Thiocyanate (KSCN, 900 mmol/L)]. Alpha amylase and invertase were purchased from Sigma Co., Louis, MO, USA.

Plant Materials

Stem bark of *C. sinensis*, seeds of *P. americana* and bulb of *A. sativum* were used. *C. sinensis* was harvested in February 2017 in Yaounde, City capital of Cameroon. *A. sativum* and *P. americana* fruits were bought at Mokolo, a local market in Yaounde, Cameroon. *C. sinensis* L (voucher N° 25859/SRF), *P. americana* mill (voucher N° 31940 HNC), *A. sativum* (voucher N° 44810 HNC) were identified at the National Herbarium as belonging to the families of Rutaceae, Alliaceae and Lauraceae respectively. Stem bark were isolated from *C. sinensis* trees. Peels of *A. sativum* bulbs were removed and the bulbs were isolated. Seeds of *P. americana* fruits were removed. Each isolated part was cut into small pieces and dried in open air (till constant weight). The dried materials were ground finely to obtain powder, from which different extracts were prepared.

Preparation of Ethanolic and Aqueous Extracts

One hundred grams (100 g) of each powder were placed in 800 mL of ethanol (95%) for maceration during 48 hours. The resulting supernatant was filtered using Whatman #1 filter paper (Whatman International Limited, Kent, England) through a funnel and concentrated to about 10% of the original volume by a rotavapor (BUCHI Rotavapor R-114, Switzerl) and at 45 °C, before complete drying in an oven at 50 °C. The same procedure was followed to prepare aqueous extract; ethanol being replaced by distilled water. After 24 h of maceration, the mixture was submitted to rotavapor. Extracts collected were stored in polyethylene bags to avoid moisture.

Assessment of Antihyperglycemic Mechanisms

Three mechanisms were evaluated: inhibition of carbohydrates digestion through the α-amylase and invertase assays, glucophagic effects through the glucose adsorption assay and stimulation of insulin-sensitivity through glucose uptake by yeast cells and muscles (psoas) assays.

Invertase inhibition Assay

Invertase activity was evaluated after hydrolysis of sucrose to form glucose. Reaction mixture was made up with 290 µL phosphate buffer, 40 µL invertase and 250 µL of sucrose was pre-incubated at 370 °C for 10 min. Then 40 µL of plant extracts at different concentrations(0.5; 1; 1.5; 2; 2.5; 5 and 10 mg/ml) was added in all tubes except in the control containing 40 µL of phosphate buffer. After incubation at 37 °C for 15 min, all the tubes were heated 100 °C in a water bath to stop the reaction. Glucose formed in each tube was measured as described by Trinder.[20] Absorbance (OD) was read at 505 nm. Another test tube containing no enzyme was

used as blank for calibration. Tests were done in triplicate. Results were expressed as inhibition percentage:

$$\text{Inhibition (\%)} = \frac{\text{((OD control-OD sample))}}{\text{(OD control)}\times 100}$$

α-Amylase Inhibition Assay

α-amylase activity was evaluated according to protocol described by Foo et al[21] with modifications. *Chronolab* test kit was used. α-amylase (AMS) hydrolysis 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNPG3) to yield 2-chloro-4-nitrophenol (CNP),2-chloro-4-nitrophenyl-α-D-maltoside (CNPG2), maltotriose (G3) and glucose (G). The initial velocicity measuring the formation of CNP was proportional to the enzyme catalytic concentration. Absorbance was read at 405 nm. In presence of extacts at different concentration, a reduction of absorbance was interpreted as inhibition of enzyme activity. Briefly, 75 µL of reagent Rwas diluted in 6 mL of distilled water. Then , 550 mL of reagent Rwas mixed with 100 µL of extracts at 1.25; 2.5; 5; 7.5 and 10 mg/ml. Another tube containing 100 µL of distilled water served as control. All the tubes were pre-incubated for 3 min at 37 °C, and 20 µL α-amylase at (0.31 mg/mL) was introduced in the reaction mixture. All the tubes were incubated again at 37 °C for 10 min and the reaction was stopped by placing them 5 min in a water bath at 100 °C. Another test tube containing no enzyme, but 40 µL phosphate buffer served as blank. Tests were done in triplicate. Results were expressed as inhibition percentage.

$$\text{Inhibition (\%)} = \frac{\text{((OD control-OD sample))}}{\text{(OD control)}\times 100}$$

Glucose Adsorption Assay

Glucose adsorption capacity of the extract was determined according the method of Ou et al.[22] Briefly, 1 ml of extracts prepared at different concentrations (5; 10; 15 and 20 mg/ml) was added to 1 mL of glucose solution of increasing concentrations (12.5, 25, 37.5 and 50 mM), the mixture was stirred well, incubated in a shaker water bath at 37 °C for 1 hr, centrifuged at 4,000 g for 20 min. The glucose content in the supernatant was determined according to the method of Trinder.[20] Results were expressed as inhibition concentration. Glucose bound was calculated using the following formula:

$$\text{Glucose adsorption (\%)} = \frac{\text{(([Glucose]initial -[Glucose final))}}{\text{[Glucose]initial}\times 100}$$

Glucose Uptake by Yeast Cell Assays

Yeasts were prepared according to the method of Cirillo.[23] Commercial baker's yeast was washed by repeated centrifugation (4,000 g for 5 minutes) in distilled water until the supernatant became clear. Then 10% (v/v) suspension was prepared in distilled water. Extracts prepared at various concentrations (5; 10; 15 and 20 mg/ml) were added to 1 mL of glucose solution (25 mmol/L) and incubated together for 10 minutes at 37 °C. The reaction started by adding 100 µL of yeast suspension into the medium. The mixturewas vortexed and further incubated at 37 °C for 60 minutes. After 60 minutes, the tubes were centrifuged (3,000 g for 5 minutes) and glucose in the supernatant was measured according to the method of Trinder.[20] A tube containing amixture without extract was used as control. Glucose uptake by yeast cells was expressed in percentage and calculated using the following formula:

$$\text{Glucose uptake (\%)} = \frac{\text{((Absorbance Control-Absorbance sample))}}{\text{(Absorbance Control)}\times 100}$$

Glucose Uptake in Rat Psoas Muscle Assay

Glucose uptake in rat psoas muscleof the extract was determined according to the method of Al-Awadi et al[24] with modifications. Modifications included point measurements at 2h 30 min only. Psoas muscle was isolated from two anaesthetized adult rats and placed immediately in Krebs solution containing glucose (11.1 mm). Muscle tissue was cut into pieces of equal mass (0.25 g), and preincubated for 5 min in a CO_2 incubator. Triple sets including muscle tissue alone (control), muscle tissue with insulin (50 mU/L), muscle tissue with both insulin and extract (5, 7.5 and 10 mg/ml) were incubated for 2.5 hrs in CO_2 incubator under 95% O_2 and 5% CO_2 atmosphere. Changes in glucose concentrations were measured according to the method of Trinder.[20] The percentage of glucose uptake was calculated using the following formula:

$$\text{Glucose uptake (\%)} = \frac{\text{((Absorbance Control-Absorbance sample))}}{\text{(Absorbance Control)}\times 100}$$

Statistical Analysis

All experiments were performed in triplicate. Results obtained were expressed as mean±SD from three distinct observations and as percentages. The software SPSS 20.0 (Chicago-Illinois Inc., IL, USA) was used for analyses. Chi square test were performed to compare percentages. One-way ANOVA followed by post-hoc Tukey's test was performed to compare variable amongst groups. Using Graph Pad Prism 6.0 (GraphPad Prism INC., CA, USA), the inhibition percentages *versus* logarithm of the extract concentrations were plotted and inhibitory concentration 50 (IC_{50}) were

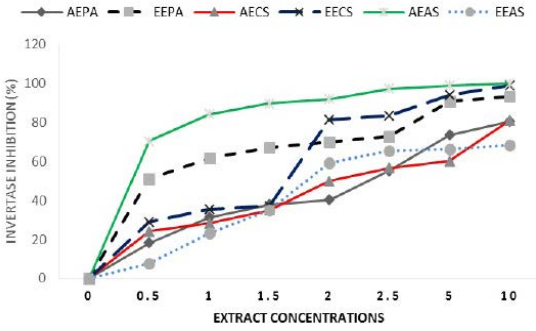
determined by regression. Analyses were done at 95% confidence interval.

RESULTS

Inhibition of Invertase Activity by Extracts

All the extracts inhibited invertase in a dose-dependent manner (Figure 1). Ethanolic extracts of *P. americana* and *C. sinensis* were the most efficient wtih values of IC_{50} of 2.69 and 2.35 mg/ml respectively, while aqueus extract of *A. sativum* was the most efficient of the aqueous extracts, with an $IC_{50}$of 1.92 mg/ml (Table 1).

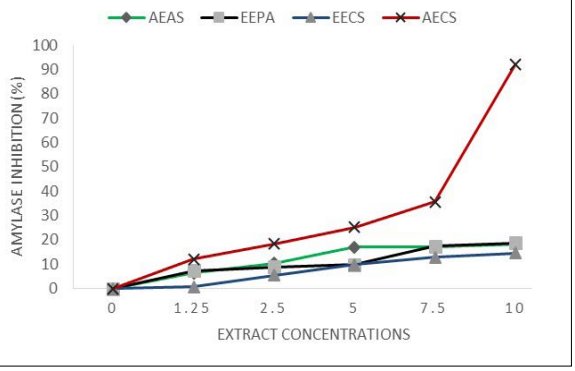
Figure 1. *EEPA: Ethanolic Extract of P. americana; AEPA: Aqueous Extract of P. americana; AECS: Aqueous Extract of C. sinensis; EECS: Ethanolic Extract of C. sinensis; AEAS: Aqueous Extract of A. sativum; EEAS: Ethanolic Extract of A. sativum. Results Expressed in Percentage*



Inhibition of α-amylase activity by extracts

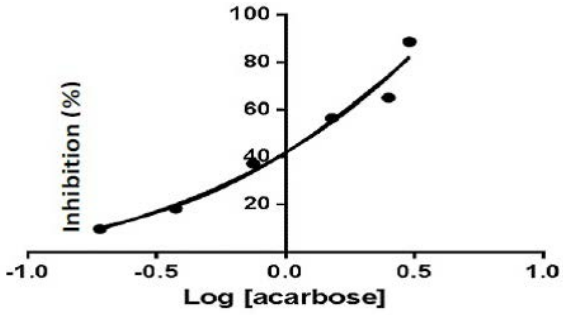
All extracts partially and weakly inhibited α-amylase in a dose dependant manner except ethanolic extracts of *A. sativum* and aqueous extracts of *P. americana* that showed no inhibition (Figure 2A). Regression analysis revealed that aqueous extracts of *C. sinensis* was the most efficient and provided upto 91.94% inhibition of enzyme activity and an IC_{50}=4.96 mg/ml. All other extracts had inhibitions lower than 17.06%.

Figure 2A. *EEPA: Ethanolic Extract of P. americana; AECS: Aqueous Extract of C. sinensis; EECS: Ethanolic Extract of C. sinensis; AEAS: Aqueous Extract of A. sativum. Results Expressed as Percentage*



Under similar conditions acarbose used as reference drug gave an IC_{50} of 2.73% (Figure 2B and Table 1), higher than that of AE C.sinensis.

Figure 2B. *Inhibition of alpha Amylase by Acarbose as Reference Drug*



Invertase activity was reduced by extracts. IC_{50}s vary from 1.92 (AE *A. sativum*) to 4.81 (EE A.sativum). Inhibition of alpha-amylase activity by *C. sinensis* varies from IC_{50}=0.068 mg/ml (thanolic extracts)to 58.09 mg/ml (aqueous extract) (Table 1). Aqueous extract of *P. americana* and ethanolic extracts of *A. sativum* showed null inhibition.

Table 1. *Inhibitory Concentration 50 of Different Extracts (IC_{50})*

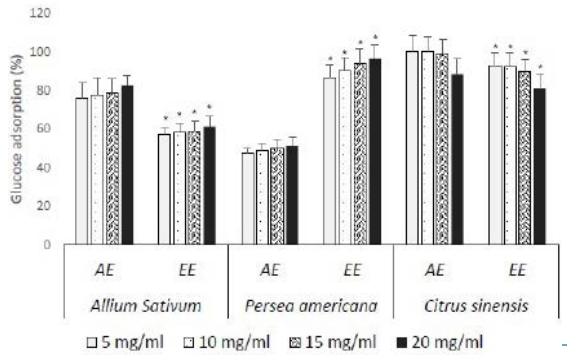
Extracts	P. americana		Citrus sinensis		Allium sativum		Acarbose
Ezymes	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic	
Invertase	4.66	2.69	4.76	2.35	1.92	4.81	/
α-amylase	0	6.08	58.09	0.063	2.85	0	2.73

IC50 : Inhibitory Concentration 50.; Results expressed in mg/ml of extracts

Glucose Adsorption Capacity of Extracts

All extracts of different plants presented a minimum of 47.55% of glucose adsorption at the tested doses (Figure 3). Glucophagic activity observed was dose dependent. Aqueous extracts of *A. sativum* and *C. sinensis* were the most efficient with percentages varying from 75.87% to 82.68% ; 88.21% to 100% respectively, while ethanolic extractof *P. americana* was the most efficient with adsorption pourcentages of glucose from 86.71% to 96.35%. Aqueous extracts of *C. sinensis* presented the best glucophagic activity at low concentrations (<15 mg/ml).

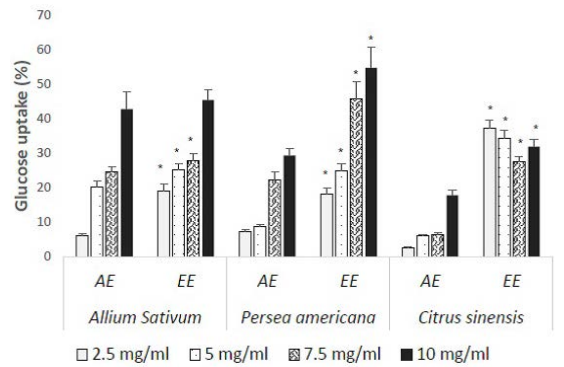
Figure 3. *EA : Ethanolic Extract ; EE : Aqueous Extract; Results expressed as Mean±SD in Percentages *: p<0.05 when Compared to Aqueous Extracts of each Plant at the Same Concentration*



Glucose Uptake Capacity of Extracts on Yeast Cells Model

All extracts increased glucose capture by yeasts in a dose dependent manner (Figure 4). Ethanolic extracts were the most efficient with values varying from 19.14% to 45.46%, 18.24% to 54.74% and 27.56% to 37.19% respectively for *A. sativum*, *P. americana* and *C. sinensis*. The lowest efficacy was observed with AE of *C. sinensis* while EE of *P. americana* seeds presented the best absorption on yeast cells.

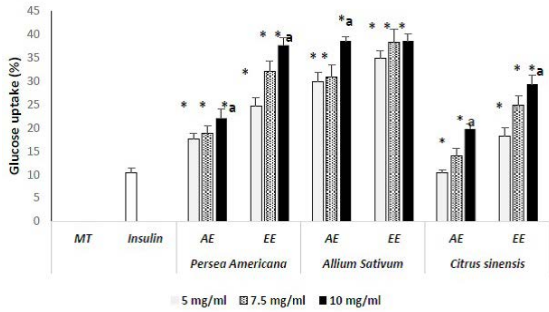
Figure 4. *EA : Ethanolic Extract ; EE : Aqueous Extract; Results expressed as Mean±SD in percentages *: p<0.05 when Compared to Aqueous Extracts of each Plant at the Concentration*



Glucose Uptake Capacity in Rat Psoas Muscle

Using insulin as reference drug, psoas tissues were capable to uptake 10.45% of glucose. It appears that all extracts were capable to enhance glucose uptake at increasing concentrations ($p<0.05$ *vs* insulin) between 14.03% to 38.56 %; above insulin performance (Figure 5). Ethanolic extracts were the most efficient with uptake varying from 24.66% to 37.66%, 34.90% to 38.56% and 18.26% to 29.29% at increasing concentrations respectively for *P. americana*, *A. sativum* and *C. sinensis* compared to aqueous extracts. Futhermore, increment estimated as portion of glucose uptake by muscle tissue intrinsically attributable to extracts varied from 4.21% to 27.21%; 24.45% to 28.11 % and 7.81% to 18.84 % respectively for *P. americana*, *A. sativum* and *C. sinensis*. Bulbs of *A. sativum* presented the best lowering effects on blood glucose by mediation of glucose uptake by muscle tissues.

Figure 5. *EA : Ethanolic Extract ; EE : Aqueous Extract; MT: muscle tissues; Results expressed as Mean±SD in percentages;*: p<0.05 when compared to insulin; a: p<0.05 when Compared to the Lowest Concentration of each Plant Extract*



DISCUSSION

Hyperglycemia is a major symptom of type 2 diabetes and a risk factor of cardiovascular diseases. However, many bioactive molecules derived from plants like polyphenols have proved their efficacy in the treatment of metabolic disorders.[25] Polyphenols are highly soluble in polar sovents like water and ethanol, reason why those two extracts were prepared from stem bark of C sinensis, bulbs of *Alium sativum* and seeds of *Persea americana*. After consumption of foods, action of digestive enzymes α-amylase and invertase in the gastrointestinal tract produces smaller molecules.[15] Monosaccharides resulting from digestion are absorbed into enterocytes to reach the blood stream, where they enter cells to supply energy and perform other biological functions.[26] Treatments administred in conditions of metabolic syndrome or diabetes target interaction with one or several of the abovementioned steps. In this study, the action of different plants have been reported on digestive enzymes. Starch and saccharose are the main polysaccharides found in meals.

An evaluation of invertase activity in the presence of both extract and sucrose revealed that all the extracts inhibited invertase (Figure 1) with IC_{50} varying from 1.92 (AE *A. sativum*) to 4.81 (EE A.sativum) (Table 1). Sucrase or invertase is a bifunction-

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al enzyme also called sucrase-isomaltase which hydrolysis sucrose and isomaltose substrates.[15] If aqueous extract of *P. americana* and ethanolic extracts of *A. sativum* showed no inhibition (null) of alpha amylase at the tested concentrations (Figure 2A), EE of *Citrus sinensis* inhibited α-amylase instead with an IC_{50}=0.063 mg/ml (*vs* 2.73 with acarbose) (Figure 2B and Table 1). α-amylase hydrolyses the alpha bond linked polysaccharides of starch producing di- and mono-saccharides. Partial inhibition of the enzyme by *C. sinensis* extracts reduces its activity and prevents formation of monosaccharides.[12-15] Inhibition of amylase and invertase by extracts delays absorption of consumed carbohydrates and reduces plasma glucose.[12]

All the extracts strongly inhibited invertase (Figure 1), but only the AE of *P. americana* and EE extract failed to inhibit the two enzymes. Inhibitors of those enzymes surely posssess in their structures functions similar to their substrates starch and sucrose although plant extracts do not possess such complex structures in their contents. Acarbose used as reference drug under the same experimental conditions provided an IC_{50} of 2.73 mg/mL, higher than *C. sinensis* extracts. Acarbose has a structure similar to oligosaccharides and therefore inhibits α-glucosidases in a dose dependent manner, preventing the formation of glucose.[27] Flavonoids and alkaloids present in *Allium sativum* and americanaextracts can justify their efficiency as well.[18,19] In fact, myricetin, quercetin, kaempferolare inhibitors of α-amylase and α-glucosidase while alkaloids like berberin are reported to be able to bind to α-glucosidases and prevent sucrose fixation.[28-31] Their use to inhibit carbohydrates digestion is therefore beneficial in the management of diabetes because absorption of glucose is reduced and and postprandial hyperglycemia obviously.[32,33]

The residual glucose resulting from digestion can be trapped in the intestine limiting its absorption by adsorption, reducing its availibility and diffusion into blood; reducing glucotoxicity as well. Adsorptive capacity through glucophagic activity of extracts revealed high percentages of glucose adsorption (Figure 3). Flavonoids and fibers content of extracts can justify such results. Insoluble fibers are capable of binding to glucose reducing its availibility and therefore its intestinal absorption.[34] Also, they can reduce viscosity of secretions in intestial lumen slowing or preventing diffusion. Absorbed glucose present in the blood can condense with hydroxyl groups of flavonoids to form complex molecules like glycosyl-flavonoids.[35] Decrease in glucose concentration contributes to reduce harmful effects of high plasma glucose on hemoglobin and other proteins, preventing glycation and complications such as retinopathy, neuropathy, and nephropathy observed in diabetic patients.[36-38]

Finally, the extracts were tested for their absorption capacity at the cellular levels. Glucose transport mechanism through cell membranes using yeast cells and psoas culture as models have been of great interest in the understanding of physiological processes of hypoglycemic activities of extracts.[39,40] Results revealed that all the extracts stimulted glucose entry in yeast cells (Figure 4). In fact, extracts are capable of stimulating glucose membrane transporters, just like many oral antidiabetics drugs.[39,40] On yeast membrane, glucose is transported *via* specialised stereospecific groups of tranporters called hexose transporters (HXT1 to HXT17) and specific molecules Snf3 and Rgt2, present on the membrane and involved in its capture. Binding of glucose to Snf3/Rgt2 located on yeast cell membrane permits phosphorylation of two co-receptors Mthl and Std 1 of Rgt1 (transcriptional repressor in charge of down regulation of the *HXT* gene) exposing Rgt1 to phosphorylation by a protein kinase A, causing glucose to diffusion accross the membrane.[41,42]

Effectiveness of glucose absorption on yeast membrane mediated by extracts was confirmed by muscle cells tested *ex-vivo*, on extracts capacity to boost insulin action (Figure 5). Glucose absorption by cells is very important in glusose homeostasis. Upto 80% of glucose fixation depend on insulin action.[43] This study revealed that psoas in the presence of insulin produced 10.45% of glucose uptake. Insulin in the presence of each plant extract produced values higher than 10.45% in a dose dependent manner (p<0.05). Similar observation was done with A. aspera extract.[44] Ability of extracts to boost insulin action can be attributed to polyhenols and alkaloids content of plants which act through stimultion of GLUT4 and/or AMPK activation or insulin secretory action.[45] Polyhenols stimulate glucose capture by muscle *via* activation of AMPK by mechanisms similar to thiazolinediones.[46,47]

The enhanced action of extracts alongside with insulin on rat psoas muscles has been reported with maltitol.[48] Alkaloids like berber in stimulates direct glucose absorption mediated by AMPK without involvement of GLUT4.[49] Ability of extracts mainly ethanolic extracts to boost insulin action can be attributed to polyphenols known to stimulate binding of insulin to IRS receptors, to increase the number of insulin receptors or/and to increase insulin sensitivity.[44-50] Enhanced uptake of glucose by cells *via* joint action of insulin and extracts may stimulate glucose clearance from blood, through improved insulin resistance. Enhanced uptake of glucose also promote post absorptive utilisation in glycolysis for energy supply.[44] Absorption values above 10.45% and increments due to extracts varying from 4.21 to 28.11 % respectively for *P. americana* and *A. sativum* (Figure 5) cannot be intrinsically attributed to the stimulation of insulin action, because *in vitro*, extracts could adsorb part of glucose before insulin action, lowering its concentration in the medium. This study however demonstrated that, the cumulative actions of extracts on enzyme digestion, glucose adsorption and insulin action lead to reduced hyperglycemia, targeted in the first treatment outcome in type 2 diabetes. Reduced hyperglycemia also limits progression to complications.

LIMITATIONS OF THE STUDY

Limitation of this study includes measurement of glucose concentration at 2 h 30 min only instead of different time intervals for better follow-up of absorption. Also, no reference drugs were used in invertase and glucose adsorption assays. Their use will have permitted better appreciate of the extracts' efficacy.

CONCLUSION

Extracts from bulbs of *A. sativum*, seeds of *P. americana* and stem bark of *C. sinensis* exert their antihyperglycemic effects *via* inhibition of carbohydrate digestive enzymes, adsorption of glucose and stimulation of glucose absorption by cells. Stem bark extracts of *C. sinensis*, mainly ethanolic extracts are more effective on inhibition of digestion and glucose adsorption while bulbs of A sativums timulates glucose absorption in cells than other plant extracts. Bulbs of *A. sativum*, seeds of *P. americana* and stem bark of *C. sinensis* based on their efficiency and action mechanisms act like oral diabetic drugs. Therefore their use should be encouraged in the management of metabolic disorders.

ACKNOWLEDGEMENTS

Not applicable.

ETHICAL ISSUE

Not applicable.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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DIABETES RESEARCH
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Editorial

Recent Tendency of Therapeutic Medical Agents for Diabetic Peripheral Neuropathic Pain

Hiroshi Bando, MD. PhD, FACP[1,2*]

[1]*Medical Research, Tokushima University, Nakashowa 1-61, Tokushima 7700943, Japan*
[2]*Shikoku Island Division, Integrative Medicine Japan (IMJ), Tokushima University, Nakashowa 1-61, Tokushima 7700943, Japan*

[*]***Corresponding author***
Hiroshi Bando, MD. PhD, FACP
Medical Research, Tokushima University, Nakashowa 1-61, Tokushima 770-0943, Japan; Phone. +81-90-3187-2485; Fax. +81-88-603-1030;
E-mail: pianomed@bronze.ocn.ne.jp

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ABSTRACT

Recently, elder patients tend to have neuropathic pain such as lower back and joints pain, stiff shoulders, besides diabetic neuropathy. Typical peripheral neuropathic pain includes diabetic peripheral neuropathic pain (DPNP), postherpetic neuralgia (PHN) and chronic pain due to herniated disc. Three analgesic agents are described. Pregabalin (Lyrica®) has been prevalent worldwide. However, it has been provided for several diseases for off-label administration, which has been one of the clinical problems.Mirogabalin (Tarlige®) has revealed efficacy for DPNP in a dose-dependent manner. Duloxetine hydrochloride (Cymbalta®) has efficacy for pain and also depression as serotonin and noradrenaline reuptake inhibitor (SNRI).

Keywords
Neuropathic pain; Pregabalin; Mirogabalin; Duloxetine hydrochloride; Diabetic peripheral neuropathic pain (DPNP).

Abbreviations
DPNP: Diabetic peripheral neuropathic pain; PHN: Postherpetic neuralgia; CRPS: Complex regional pain syndrome; FDA: Food and Drug Administration.

In primary care medicine, the number of elderly outpatients has increased in recent years. Regarding common health problems, general medicine region includes non-communicable diseases (NCD) such as diabetes mellitus, and orthopedic region includes low back pain, knee joint pain and shoulder stiffness.[1] Statistic survey of lifestyle revealed the health and medical complaints of people. They include several popular symptoms in order as follows: man showed lower back pain, stiff shoulders, joints pain and woman showed stiff shoulder, lower back pain, joints pain, respectively.[1]

Patients often complain of numbness. This includes several situations as follows: i) tingling abnormal sensations that are close to pain, ii) the sensation of the skin is dull, and iii) the movement of extremities are stiff and rigid. Thus, various conditions are observed for the combination of numbness and neuralgia. There is some difference in the perception between patients and medical professionals.[2]

The progress situation of numbness and pain has been important.[3] For example, low back pain is classified into three types due to persisting period, which are acute less than 4-weeks, subacute for 4-weeks to 3-months, and chronic for more than 3-months. As described above, detail medical interviews, diagnosis and treatment would be crucial for patients with diabetic neuropathy, orthopedic neuropathy and other impaired states.

Recently, comprehensive medical term "neuropathic pain" has been widely prevalent.[2,3] It is also used in the documents in U.S. Food and Drug Administration (FDA), such as management of neuropathic pain associated with diabetic peripheral neuropathy and spinal cord injury.

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Neuropathic pain has a variety of causes, including traumatic, infectious, nutritional metabolism, toxic, neoplastic and compression/strangulation.[3] They are also classified according to whether the affected nerves are central (brain or spinal cord) or peripheral (terminals such as limbs). The typical examples of peripheral neuropathic pain would be shown as follows: i) diabetic peripheral neuropathic pain (DPNP), ii) postherpetic neuralgia (PHN), iii) chronic pain due to herniated disc.

In the category of neuropathic pain, there are rare but important diseases. One is allodynia, where pain is caused by a stimulus that does not usually elicit pain. The other is complex regional pain syndrome (CRPS), also known as reflex sympathetic dystrophy (RSD).[4] CRPS is characterized by continuing regional pain that seems disproportionate in time, degree or region. Regarding the analgesics for reducing pain, three kinds of medicine would be described in this article.

The first medicine is Pregabalin (trade name: Lyrica®) (D02716). The indication has been neuropathic pain whose effects were demonstrated in fibromyalgia, postherpetic neuralgia, and pain after spinal cord injury. However, in daily medical practice, it has been often used for low back pain, sciatica and joint pain.[5] From 58 available literatures, clinical abuse potential of pregabalin was suggested and prescribers had to pay attention to this situation especially for patients with abuse of some medicine.[5]

Patients having pain deserve attention, empathy, time and understanding.[6] Some patients may receive efficacy from trial of gabapentin or pregabalin for off-label indications. However, prescribers do not have to think that these medicines show effect for pain of most pain syndromes.[6] The comparative study for groups of gabapentin (n=362) and pregabalin (n=362) was conducted.[7] As a result, clinicians providing these medicines for pain should recognize the limited evidence and explain the patients uncertain benefits for off-label administration.[7]

There have been increasing of intentional gabapentin misuse.[8] In order to determine the pharmacovigilance abuse signals for gabapentin, FDA adverse events reports from 2005 to 2015 (6 million) were investigated with gabapentin reports (0.1 million). Compared to duloxetine, gabapentin had significantly greater odds of a co-report for an abuse-related and abuse-specific adverse event (AS-AE).[8]

The second is Mirogabalin. This is also ligand for the α2δ subunit of voltage-gated calcium channels as Pregabalin.[9] It has been developed to reduce several pains associated with DPN (diabetic peripheral neuropathy) and postherpetic neuralgia.[9] Regarding the adequate therapy of Mirogabalin, various doses of 5, 10, 15, 20, 30 mg/day was provided for patients with DPNP.[10] Treatment effect least squares (LS), adverse events (AEs) and other biomarkers were studied. As a result, doses of 15, 20, 30 mg/day had statistically significant reductions in average daily pain score (ADPS) and Mirogabalin may become a promising new option for DPNP.[10] Mirogabalin revealed efficacy for DPNP in a dose-dependent manner.[11] Mirogabalin given 30 mg/day showed statistically significant reduced pain in Asian patients. All doses of Mirogabalin tested were well tolerated.[11]

This medicine Mirogabalin has been known and used widely as Tarlige® (DS-5565). This name is from the combination of targeting and ligand. As a matter of fact, a high prevalence of painful DPN has been observed with about one-third of diabetic patients.[12] According to the study of maximum observed effect (Emax) for Pregabalin and Mirogabalin, therapeutic doses of the latter showed limited evidence of abuse potential.[13]

The third is duloxetine hydrochloride (D01179) (Cymbalta®).[14] As a standard treatment for administration of the analgesics, the recommended agents for neuropathic pain have been Pregabalin (evidence level 1A), duloxetine hydrochloride (Cymbalta) (1A), and Amitriptyline Hydrochloride (Tryptanol) (1B).[15] Duloxetine is categorized as serotonin and noradrenaline reuptake inhibitor (SNRI), that is effective for depressive state. It has been widely used for diabetic neuropathy.[16]

The indication includes diabetic neuropathy, fibromyalgia, chronic low back pain, osteoarthritis and others.[15] In contrast, its contraindications include severe liver and kidney impairment, hypersensitivity, glaucoma, and so on.[17] As for the method of intaking medicine, Pregabalin and Mirogabalin are administered twice a day, while duloxetine is administered once a day. Therefore, compliance and adherence seem to be better in duloxetine.

Pregabalin has been very common medicine in terms of drug sales. In Japan, pregabalin is the number one sales agent among all medical agents sold in Japan. Behind this situation, however, there have been many off-label administrations that deviate from conventional rules. Consequently, drug abuse has been continued for diseases with indeterminate efficacy.

Furthermore, cost-effectiveness analyses (CEAs) would be important in this region. There have been several CEAs for neuropathic pain, in which heterogeneous factors are present such as methodology, design, treatments and perspectives for influencing cost-effectiveness of health condition and pain relief.[18] For initial treatment for DPNP, National Institute for Health and Care Excellence (NICE) shows the recommendation of amitriptyline, duloxetine, pregabalin or gabapentin.[19] However, there was not clear consensus concerning the treatments for neuropathic pain.[20,21] In the light of CEAs for DPNP, clinically beneficial and tolerated treatment choice has been studied by optimal pathway for treating neuropathic paiN in diabetes mellitus (OPTION-DM) study.[22] Recent CEAs include systematic reviews focusing on strength and limitation of data and modeling practices.[23]

In summary, current topics concerning neuropathic pain and its related medical agents were described in this article. Among them, proper diagnosis and treatment of medical agents would be necessary. This comment will be hopefully useful for adequate therapy in the medical practice.

ETHICAL CONSIDERATIONS

As regard to this report, author has established an ethical committee in the Integrative Medicine Japan, Shikoku island division. It included the director, vice-director, expert in the pharmacology, nursing and legal specialties. We have discussed and made confir-

mation that current report was valid and agreed with all members without any problems.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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Case Report

Profile of Blood Glucose in Diabetic Patient Suffered from Diabetic Foot Osteomyelitis with Effective Low Carbohydrate Diet

Hiroshi Bando, MD, PhD, FACP[1,2,3*]; Yoshiro Abe, MD[3,4]; Kazuki Sakamoto, MD[3]; Shigeki Hatakeyama, MD[3]; Keisuke Yagi, MD[3]; Toshiharu Kobayashi, MD[3]; Tomoya Ogawa, MD[3]; Noboru Iwatsuki, PharmD[3]; Mitsuru Itagaki, RN[3]; Kaori Ashikaga, RN[3]; Yukari Matsumoto, RN[3]

[1]*Medical Research, Tokushima University, Nakashowa 1-61, Tokushima 7700943, Japan*
[2]*Japan Low Carbohydrate Diet Promotion Association, Kyoto, Japan*
[3]*Sakamoto Hospital, Higashi Kagawa City, Kagawa, Japan*
[4]*Department of Plastic and Reconstructive Surgery, Tokushima University, Tokushima, Japan*

[*]***Corresponding author***
Hiroshi Bando, MD, PhD, FACP
Medical Research, Tokushima University, Nakashowa 1-61, Tokushima 770-0943, Japan; Phone. +81-90-3187-2485; Fax. +81-88-603-1030;
E-mail: pianomed@bronze.ocn.ne.jp

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ABSTRACT

The case was 52-year-old female with type 2 diabetes mellitus (T2DM) for 10-years. She complained of the decreased sensation of right lower foot, and revealed diabetic foot infection (DFI) and/or diabetic foot osteomyelitis (DFO) at right 1st proximal phalanx. Various data included body mass index (BMI) 33.3 kg/m^2, HbA1c 11.4%, blood glucose 430 mg/dL, WBC 12100 /μL, C-reactive Protein (CRP) 13.5 mg/dL. On admission (day 1), she was started by 4 times of injection (Aspart and Glargin) with glucose profile 200-500 mg/dL. Surgical amputation of the right toe was performed between 1st metatarsal and proximal phalanx (day 17). Then, blood glucose profile decreased moderately. After discharge of the hospital, super-Low Carbohydrate Diet (LCD) was started without Aspart (day 37). Consequently, glucose profile was normalized with HbA1c 6.3% on (day 77). Consequently, LCD was evaluated to be effective for glucose variability in this case and some related discussion was described.

Keywords
Diabetic foot infection (DFI); Diabetic foot osteomyelitis (DFO); Low Carbohydrate Diet (LCD); Japanese LCD promotion association (JLCDPA); International Working Group on the Diabetic Foot (IWGDF).

Abbreviations
DFI: Diabetic foot infection; DFO: Diabetic foot osteomyelitis; LCD: Low carbohydrate diet; JLCDPA: Japanese LCD promotion association; IWGDF: International Working Group on the Diabetic Foot.

INTRODUCTION

Diabetes mellitus is chronic and crucial non-communicable disease (NCV) across the world.[1] It may give various influence in social, medical and economic aspects.[2] Then, some diabetic standard guidelines were presented for adequate diagnosis and treatment for better glycemic control.[2] Chronic complications related to diabetes mellitus include both microvascular and macrovascular influences.[1] Among them, a variety of mechanism has been involved in the development and aggravation of macroangiopathy, including vascular damage, neuronal dysfunction, interstitial injury, metabolic damage and others.[1]

Diabetes mellitus brings more incidence of various dia-

betic complications of foot.[3] Among them, diabetic foot infection (DFI) has been crucial problem for actual diabetic practice. The reason includes persisting infection for long, necessary frequent visits, treatment of antibiotics, daily wound care, sometimes surgical procedures.[4]

DFI has been one of the macroangiopathy and complication to be necessary for hospitalization.[5] It may show complex clinical course, with several exacerbation such as the amputation of lower extremities and economically high health costs.[4] When patients have some degree of DFI, the prognosis would be poor from several reports.[6] According to a prospective study, the prognosis of DFI showed that foot ulcer healed in 46% at 1-year, 15% of patients were dead and 17% had the amputation of lower extremity.[6]

For clinical practice and research of diabetes, the topic concerning DFI has been frequently discussed for long.[7] Further, physicians majoring on diabetes pay attention to the prevention of DFI for long.[7]

On the other hand, the basic treatment of diabetes has been adequate diet. In recent years, the transition tendency has been found from the calorie restriction (CR) to low carbohydrate diet (LCD). LCD was formerly started by Bernstein and Atkins in the health and medical fields.[8] Clinical effect was reported in comparison with CR and Mediterranean diet.[9] After that, lots of comparison reports were found resulting in the predominant efficacy of LCD.[10] Then, LCD has been more prevalent in European and North American region.

In Japan, the authors and colleagues have initiated the method of LCD, and developed LCD movement through Japanese LCD Promotion Association (JLCDPA).[11] We have continued clinical research on LCD such as the comparison of blood glucose profiles in CR and LCD, Morbus (M) value, continuous glucose monitoring (CGM), meal tolerance test (MTT), elevated ketone bodies in the axis of fetus, placenta, pregnant mother and newborn, and so on.[12-14] We have proposed three types of LCD meal pattern for everyone to understand LCD well and continue LCD easily. They are petite-, standard-, super-LCDs with 40%, 26%, 12% of carbohydrate ratio included.[15,16]

As mentioned above, we have continued clinical research on diabetic patients with various problems. Among them, there was a diabetic case who had diabetic foot osteomyelitis (DFO) in the category of DFI, operation of osteotomy and satisfactory effect on LCD. In this article, the impressive and suggestive case would be reported and discussed.

CASE REPORT

Present History

The case was 52-year-old female patient with T2DM for 10-years of duration. She had received the treatment of oral hyperglycemic agents (OHA) without no history of insulin treatment. For last 6-months, she had not treated enough for the diabetic control. During this period, her HbA1c value was persisted around 11-12% for half year. She had an outpatient consultation in January, 2020. At that time, she complained of the decreased sensation of right lower foot. Rapid exam of blood glucose and HbA1c revealed 430 mg/dL and 11.4% in the out clinic.

Physical Examination

Her physicals were as follows: consciousness was alert, the vitals were pulse 76/min, BP 187/90 mmHg, SpO 2 98%, BT 36.8 °C, respiration was normal, body weight 73 kg, stature 148 cm, body mass index (BMI) 33.3 kg/m^2, unremarkable of lung, heart, abdomen. Her neurological reflexes of patellar (PTR) and Ankle (ATR) were decreased. There was remarkable change in the right 1st toe, which was a diabetic ulcer to gangrene in moderate to severe degree (DFO). This situation was evaluated to treat as soon as possible for save her life. Then, she was admitted to the hospital for further evaluation and treatment for the hyperglycemia and DFO in the right 1st toe.

Laboratory Test

Laboratory tests on the first visit were performed. The standard peripheral blood and biochemical data were: WBC 12100 /μL, RBC 4.72×10^6/μL, Hb 13.0 g/dL, PLT 34.9×10^4/μL, C-reactive protein (CRP) 13.5 mg/dL (5+), blood glucose 453 mg/dL, C-peptide 4.1 ng/mL (0.8-2.5), AST 13U/L, ALT 14 U/L, r-GT 35 U/L, ALP 338 U/L (104-338), LDH 197 U/L (106-211), T-Bil 0.4 mg/dL, CPK 50 U/L (30-200), BUN 15 mg/dL, Creatinine 0.6 mg/dL, Uric acid 5.7 mg/dL, HBs Ag(-), HCV-Ab (-).

Examination on Admission

She received fundamental screening for several tests. Chest X-ray and electrocardiogram (ECG) were negative. Abdominal X-ray was unremarkable. Abdominal CT scan showed fatty liver. X-ray of right foot showed that right 1st proximal phalanx was damaged due to severe degree as osteomyelitis, and 1st metatarsal was unremarkable. Urinalysis showed that glucose (3+), protein (2+), urobilinogen (+). In ophthalmic detail examination, she has vague visual function with bilateral proliferative retinopathy. Her intraocular pressure was RT 23 mmHg, LT 18 mmHg. She showed that RV=0.08 (0.3x-1.25D)=CYL -2.75 DAX 50°, LV=0.1 (0.4x-2.25 D)=CYL -1.25 DAX 90°.

Diagnosis and Problem Lists

According to her medical history, she had not been treated diabetes enough for half year. Such situation has brought her current distress situation. She was diagnosed as the following problem lists. They were #1: Type 2 diabetes mellitus (T2DM), #2: osteomyelitis of 1st proximal phalanx in the right foot (DFO), #3 obesity (BMI 33.3), #4: hypertension, #5, diabetic neuropathy, #6 diabetic proliferative retinopathy, #7 diabetic nephropathy.

Concerning #1 and #3 mentioned above, her long his-

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tory will be summarized. The body weight was 52 kg at 20-years old (yo) and 64 kg at 24 yo. She kept weight about 50 kg for a while after marriage. After that, she came back to working as a nurse, and gradually increased up to 68 kg at 40 yo and 75 kg at 50 yo. The weight was 73 kg at this first visit (53 yo). Diabetes was pointed out about 10 years ago (43 yo) and has been receiving treatment intermittently since then.

Treatment for DFO

She has diagnosed to have osteomyelitis in the right 1st phalanx. According to the result of the culture from the infected area, detected bacteria was *Streptococcus agalactiae* (B group) (++). Then, sensitive kind of antibiotics was selected and the case was given cefmetazole sodium (D00911) 2 g per day, and continued. On the 5th day after admission, the data were rather improved with WBC 8800/μL, Hb 12.4 g/dL, CRP 1.5 mg/dL (++).

Nutritional Therapy

As for the nutritional therapy for diabetes, she was on the diabetes meal regimen for modified protocol of regular Japanese standard meal for CR by Japan Diabetes Association (JDA).[17] JDA has proposed 50-60% of carbohydrate ratio of total energy per day, but our hospital has modified diabetic meal with 40% of carbohydrate ratio.

The detail of the meal and its calculation methods were as follows: i) stature of the case was 148 cm, then standard body weight becomes 48.2 kg (1.48×1.48×22=48.2), ii) standard calorie per day becomes 1205 kcal/day with (48.2 kg×25 kcal/kg=1205 kcal/day) (25 kcal/kg means sedentary lifestyle in the hospital), iii) the meal per day included 1250 kcal, protein 50 g, fat 48 g, carbohydrate 125 g, NaCl 7 g, water more than 1500 ml.

Table 1. *Changes in Daily Profile of Blood Glucose Before and After the Operation*

Month	Date	Day		Daily Profile of Blood Glucose (mg/dL)							Aspart Glargine	
				700 h		1100 h		1700 h		2100 h	3 times	once
				0 min	60 min	0 min	60 min	0 min	60 min		units/day	units/day
January	20	1	Admission					396		451		p
	21	2		396		524		378		273	26	6
	22	3		263		359		313		367	32	7
	24	5		196		343		251		234	29	8
	27	8		172		256		238		233	29	9
	30	11		190		231		220		225	28	10
February	2	14		161		202		140		242	24	10
	5	17	Operation	202		239		167		129	29	10
	6	18		136		185		193		128	26	10
	7	19		154		153		125		183	26	10
	10	22		185		155		137		144	26	10
	14	26		142		176		125		118	22	10
	18	30		128		170		141		134	22	10
	24	36		159		102		127		105	22	10
March	25	1	Discharge	126		150		130		131	22	10
	26	2		132	142		133		143		0	10
	27	3		110	132	122	138	110	162	154	0	10
	29	5		117		119	145	123	158	147	0	8
	2	7		116	151	127	135	162	148	149	0	8
	5	10		106	140	107	144	113	141	113	0	6
	10	15		112			150		154	136	0	6
	15	20			132		207	160	169	166	0	4
	25	25			142		164	125	127	120	0	4
	26	31		110	133		136		169	149	0	0
	31	36		114			166		116	118	0	0
April	12	48			133		118		114	106	0	0

Insulin Treatment

She has started ACE Self-Assessment Program (ASAP) multiple daily insulin injection (MDI) with four times of insulin per day. She was given 2 kinds of insulin. One is Novo rapid (Novo Nordisk, Bagsværd, Denmark) three times a day just before three meals. Another is insulin Glargine (Eli Lilly and Company, Indiana, USA) at night at 2100 h. These two kinds in brand names were Insulin Aspart by pre-filled pen with 100 units/mL, and Insulin Glargine by BS injection kit FFP with 300 units/mL.

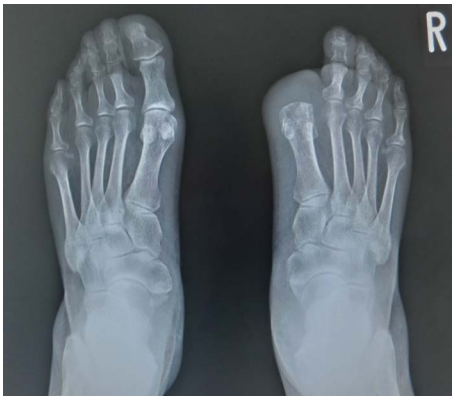
In this case, the control of blood glucose and insulin administration were shown in Table 1. The doses of Novo rapid three times a day were decided by the sliding scale method, according to the pre-prandial blood glucose before three meals. We used two kinds of sliding scale methods, which were middle degree and high degree. Due to 8 levels of blood glucose [<100, 101-, 151-, 201-, 251-, 301-, 351-, 401<mg/dL], the former indicates insulin doses [0, 4, 6, 9, 12, 14, 16, 19 units] and the latter indicates insulin doses [2, 6, 10, 12, 14, 16, 18, 21 units] by insulin aspart. High degree was used from day 4 to day 16 after admission.

Table 1 (upper) showed the changes in blood glucose after admission. From admission to the operation, the profile of blood glucose profile during day 1-10 revealed higher values (Table 1, upper). They were almost 200-500 mg/dL and gradually decreased from day 11-17. After the operation, glucose profile decreased rapidly and kept stable situation during day 18-36.

Progress Note

The general status and DFO were gradually improved. On 17th day from the admission, she received the surgical operation of an amputation at the right toe. The osteotomy was performed between 1st metatarsal and proximal phalanx (Figure 1). The operation was successfully achieved and the progress condition after the operation showed improvement. After the operation, blood glucose showed improvement than before the operation (Table 1, middle). After 18-days of operation, she was discharged from the hospital.

Figure 1. *X-ray of the Bilateral Feet After the Operation*



LCD without Insulin

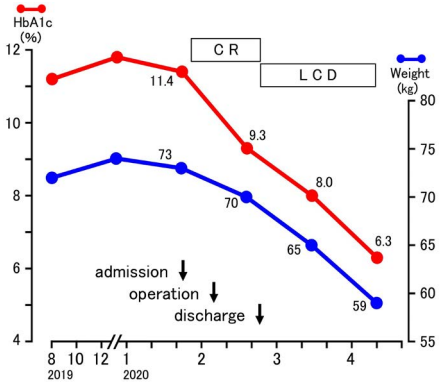
Just after the discharge, she started LCD. Daily profile of blood glucose without insulin is shown (Table 1, lower). Postprandial blood glucose was checked just after 60-minutes, when the max value of hyperglycemia is usually observed. Without Novo rapid insulin, postprandial glucose 1-hour after meal was stable around 130-160 mg/dL. Her glucose variability persisted satisfactory after discharge on day 1-48 (Table 1, lower).

After her blood glucose rapidly dropped, there was no progression of retinopathy. She has no hypoglycemic episode so far, and her visual acuity has been not changed. Further, the degree of the proteinuria has reduced from ++ to +/-. The blood level of ketone bodies were not determined, and will be followed as the meal of LCD continues.

Changes in HbA1c and Weight

Changes in HbA1c value were observed in the following: 11-12% during August 2019- December 2019, 11.4% in January 2020, 9.3% in February, 8.0% in March, 6.3% in April (Figure 2). Changes in body weight were as follows: 72-74 kg during August 2019-December 2019, 73 kg in January 2020, 70 kg in February, 65 kg in March and 59 kg in April (Figure 2).

Figure 2. *Changes in HbA1c and Weight Associated with Operation and LCD*



Current Medication

During the admission, she was provided insulin treatment. After her discharge from the hospital, she discontinued insulin therapy, and started metformin 500 mg a day for diabetes mellitus, and Aimix® Combination Tablets LD or HD (amlodipine 5 or 10 mg, irbesartan 100 mg) a day for hypertension. After discharge, her diabetic and hypertensive condition has been stable without any problems.

DISCUSSION AND CONCLUSION

In this article, a diabetic case was presented who suffered from

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DFI and DFO, received the osteotomy of right toe, and showed remarkable improvement of glucose variability by LCD. Discussion would be described from some points of view.

Firstly, the background of the patient was a registered nurse working hard for years. Possible causes related to current serious condition were as follows: i) the work has been irregular due to day shift and night shift, ii) the stress of work was persisted for long, iii) necessary care of her two children at home, iv) general malaise from various stresses physically and psychologically, v) irregular medications with poor compliance or resilience. These factors might become exacerbating influence to the clinical course of this case.

As regard to her diabetic control, hyperglycemia seemed to be continued more than half a year. During that time, she did not have any consciousness disorder due to hyperglycemia, and was able to perform normal nurse duties. The body seemed to be accustomed to long-term hyperglycemia.

The weight was probably maintained about the same level for half a year. There were weakened tendon reflexes, impaired perception, and neuropathy. As to visual acuity, she cannot hold a clear visual field because of the proliferative retinopathy. She has some numbness in hand and foot at the extremities as neuropathy. Proteinuria as nephropathy has also persisted. Consequently, the existence of three types of microangiopathy were bound. On the other hand, detail presence of macroangiopathy would be investigated such as brain, heart, and feet.

Secondly, the case suffered from DFI. There has been some development in the light of DFI. From international necessity of management of DFI, there have been several guidelines. The standard one is from International Working Group on the Diabetic Foot (IWGDF). It has continuously published the guidelines every 4-years since 2004.[18] The prevalent edition includes the format of standard content.[18] Furthermore, it incorporates necessary information of systematic reviews about the interventions for DFI.[19]

Recently, new IWGDF Guideline on the diagnosis and treatment of foot infection for diabetic patient was presented in 2019.[20,21] It has 7 guidelines, which was structured using the patient-intervention comparison-outcome (PICO) format.[22,23] IWGDF has also produced systematic review on DFO.[22] In 2019 updated IWGDF guideline is supported by reviews for diagnosis[24] and interventions of DFI.[25]

For current case, Streptococcus was identified in bacterial culture from an infected wound on the foot. There are known studies of this in foot infections for diabetes. In the category of DFI, rather severe DFO is present. Regarding DFO, narrative review was conducted, in which 65 papers were included from 756 related reports.[26] Formerly in the pre-antibiotic era, DFO by staphylococcus had 50% of mortality. Recent situation shows usually polymicrobial with gram-negative and positive bacilli can be identified by the modern molecular techniques.[26]

Regarding the epidemiology for osteomyelitis, DFO shows rarely mono-microbial organism. There are often polymicrobial results, including *Staphylococcus aureus* (at most 50%), *Staphylococcus epidermidis* (around 25%), *Streptococci* (around 30%) and *Enterobacteriaceae* (at most 40%). These bacteria have been usually detected in DFO.[18,27] In contrast, the proportion of the anaerobes has been usually low. Gram negative bacteria usually include *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus*, and *Pseudomonas aeruginosa*.[18,27]

Long-term prospective study was conducted for limb amputation and mortality after the first neuropathic diabetic foot ulcer (DFU).[28] Subjects were 2880 patients with neuropathic DFU group (DFU) and diabetic patients without DFU group (nDFU), and followed-up 14-years. For median follow-up 7-years, death rate was 17.4% (n=501) *vs* 3.1% (n=89).[28] The 5-/10-year mortality was 22%/71% with survival of 7.72-years, in DFU group, while 3%/5% with 12.6-years in nDFU group (all showed significant difference). DFU group showed 29.3% of limb amputations. Odd ratio (OR) was 1.31 in DM duration >10-years, 1.47 in nephropathy, 1.85 in minor amputation, 2.96 in major amputation, respectively, which could predict mortality.[28]

Thirdly, an impressive clinical course was found associated with DFI and/or DFO. After admission, blood glucose control was performed by the sliding scale method. Although the sliding scale method revealed stronger level than usual, the blood glucose level remained high at around 200-500 mg/dL during continuous administration of insulin. Blood glucose control had been resistant, and its cause seemed to be due to strong inflammatory state from foot infection.

After the operation, the daily profile of blood glucose became rapidly stable. Preprandial glucose was 100-200 mg/dL. This process was supposed to be from reduced inflammatory state from DFO. As to the changes in CRP value, it decreased to 13.5 mg/dL (day 1) was decreased to, 1.5 mg/dL (day 6) and 1.5 mg/dL (day 10). There are reports on the extent of CRP increase.

In the clinical diabetic practice, differential diagnosis of osteomyelitis and soft-tissue infection has been clinically important.[29] Because the former needs the possibility of more antibiotic administration, operation and amputation. For distinguishing osteomyelitis, optimal cut off values for ESR and CRP were investigated. The threshold of 60 mm/h in ESR showed sensitivity of 74% sensitivity and specificity of 56%. In contrast, threshold of 7.9 mg/dL in CRP had 49% sensitivity and 80% specificity.[29]

Fourthly, clinical effect of LCD was demonstrated in this case. During her stay in the hospital, she was on CR with 125 g of carbohydrate associated with insulin.[30] During this period, her weight reduction was mild. The reason was that both carbohydrate intake and insulin administration were present. On the other hand, after discharge from the hospital, neither intake of sugar nor increase in blood insulin concentration was continued. Therefore, body weight seemed to be decreased rapidly.

After discharge from the hospital, the meal was switched from CR to LCD immediately. During admission, it is difficult to cut staple foods for regular meals. On the other hand, outpatients can delete carbohydrate food completely, while much amount of vegetables and protein can be taken. Therefore, fast-acting Aspart insulin was immediately discontinued, and only metformin 500 mg was given as oral hyperglycemic agent (OHA). Generally speaking, postprandial blood glucose is highest at 30-60-minutes. This case showed stable blood glucose level 60 min after meal, indicating satisfactory efficacy of LCD.

In this case, the body weight was reduced from 73 kg to 59 kg and HbA1c was reduced from 11.4 to 6.3% in less than 3-months. We have already reported concerning the effect of weight reduction by LCD.[31] As a result of LCD for 2699 cases, weight loss of 10% or more was observed in 25.6%, and weight loss of 5% or more was 57.6%, which showed enough clinical effect of LCD. It is effective to teach the LCD method for obese and diabetic patients and to continue LCD. We have developed LCD movement by English papers, books and seminars through JLCD-PA. Depending on the situation, petite-, standard-, super-LCD can be applied and treated.[16,32]

Regarding the limitations of this paper, we report on the surgical treatment of foot infection in diabetic patients (DFI, DFO) and the effect on LCD. For foot infections, soft tissue can be mild and osteomyelitis can be severe. As future work, it may be necessary to examine the severity of infectious diseases, the types of bacteria that cause the infections, the effects of antimicrobial agents, and the response of other biomarkers. With respect to LCDs, it was shown that the increase in postprandial blood glucose was suppressed by LCD. Furthermore, detailed intake of food and postprandial blood glucose should be examined in the future.

In summary, we reported a case of a diabetic patient who underwent surgery for diabetic foot osteomyelitis (DFO) and was able to control blood glucose with LCD. We hope that this report will be useful for the management of diabetic complications and blood glucose control.

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CONSENT

The authors have received written informed consent from the patient.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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Case Report

Deep Venous Thrombosis in an Amputated Limb Stump of a Diabetic Patient: A Case Report

Taoreed A. Azeez, MBChB, MSc, MWACP[1*]; Arionola Esan, MBBS, FWACP[1]; Taiwo R. Kotila, MBBS, FMCPath[2]

[1]*Endocrinology Unit, University College Hospital, Ibadan, Nigeria*
[2]*Haematology Department, University College Hospital, Ibadan, Nigeria*

[*]***Corresponding author***
Taoreed A. Azeez, MBChB, MSc, MWACP
Senior Registrar, Endocrinology, Metabolism and Diabetes Unit, Department of Medicine, University College Hospital, Ibadan, Nigeria;
E-mail: adegokegalaxy@yahoo.com

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ABSTRACT

Deep venous thrombosis (DVT) in an amputated stump is potentially life-threatening but rarely diagnosed and there are limited data in sub-Saharan Africa. This is aimed at demonstrating an additional vascular risk in patients with lower limb amputation and diabetes. A 74-year-old man who had a right above knee amputation done on account of grade 5 right diabetic foot with post-operative prophylactic anticoagulation. Doppler ultrasound done before the surgery showed bilateral multiple lower limb arteries atherosclerosis but no evidence of deep venous thrombosis. He was discharged home on Zimmer frame. Three months after, he was noticed to have differential swelling of the right amputation stump. Thigh circumference measured at 15 cm below the anterior superior iliac spine was 55 cm and 50 cm on the right and left respectively there was but no differential warmth or tenderness. The vital signs were relatively stable. Doppler ultrasound scan of the lower limbs showed an echogenic thrombus in the right deep femoral vein. He was commenced on therapeutic dose of subcutaneous enoxaparin. DVT in an amputated stump is uncommonly encountered. It may not have classical clinical findings. Poor mobility and pooling of venous blood in the amputated stump are some of the risk factors that have been reported. DVT in an amputated stump in a patient with diabetes is rare and may not present classically. It may be one of the potential reasons for the increased mortality after lower limb amputation.

Keywords
Deep venous thrombosis (DVT); Amputated stump; Diabetes patient.

INTRODUCTION

Diabetes mellitus is the commonest reason for non-traumatic amputation.[1] Deep venous thrombosis (DVT) is the formation of blood clot in a deep vein. DVT affects the lower limbs most commonly. Major amputation procedure in a patient with diabetes is associated with an increased risk of deep venous thrombosis in the immediate post-operative period.[2] DVT complicating an amputated limb in patients with diabetes is not a commonly reported phenomenon after the immediate post-operative period. The incidence of DVT in amputated stumps is so rare that the initial redness and swelling may be considered as features of an infectious process like cellulitis.[3] The classical risk factors are represented by the Virchow's triads-stasis, hypercoagulability and damaged endothelium.[4] DVT in the amputation stump is not frequently encountered and there is limited data in sub-Saharan Africa.

The objective of this case report is to demonstrate that DVT in an amputated stump is an additional vascular risk in patients with lower limb amputation and diabetes.

CASE PRESENTATION

The patient is a 74-year-old man who was diagnosed with type 2 diabetes mellitus (T2DM) 20-years earlier. He was not adherent with his oral glucose lowering agents (Metformin and glimepiride). The short-term and long-term glycemic control were suboptimal.

He presented with a right foot ulcer. He was walking in his house barefooted and he had a small nail puncture injury to the right sole. He did not feel pain but he noticed the bleeding.

The ulcer progressed within a few days, got swollen and started discharging pus. The ulcer later extended to involve the whole of the right sole and the distal part of the dorsum. The tissue turned darkish, involving the whole right foot, with foul-smelling purulent discharge. There was associated high grade fever, some episodes of postprandial vomiting, reduced appetite and generalized body weakness. There was no loss of consciousness.

The essential findings on general physical examination were pallor, fever (38.4 °C) and moderate dehydration. Cardiovascular examinations showed tachycardia (pulse rate of 106 beats per minute) and elevated systolic blood pressure (164/80 mmHg). The left foot showed some clawing of toes, dystrophic nails and loss of protective sensation using 10 g-monofilament testing. Dorsalis pedis artery and posterior tibial artery pulsations were felt. The right foot showed an extensive ulcer involving the whole sole and the dorsum. It was covered with necrotic tissue with foul-smelling discharge. There was ascending fasciitis up to the right knee.

The assessment was Meggitt-Wagner grade 5 right diabetic foot and sepsis. Among other investigations done, doppler ultrasound before the surgery showed bilateral multiple lower limb arteries atherosclerosis. There was no evidence of deep venous thrombosis prior to the surgery.

He eventually had a right above knee amputation done on account of grade 5 right diabetic foot and he had post-operative prophylactic anticoagulation. The recovery was uneventful and he was discharged home on Zimmer frame. He was seen twice at the Endocrinology and Orthopedic Clinics and there was no remarkable development. The healing was satisfactory and glycemic control had improved. The pre-operative HbA1c was 11.4 while the HbA1c 3-months after was 8.6%.

Three months later, he presented with differential swelling of the right amputation stump. He had first noticed it 2-days prior to presentation. There were no respiratory symptoms, chest pain or syncopal attack. Thigh circumference measured at 15 cm below the anterior superior iliac spine was 55 cm and 50 cm on the right and left respectively but there was no differential warmth or tenderness. The vital signs were relatively stable. A clinical suspicion of deep venous thrombosis involving the amputated stump was entertained. Doppler ultrasound scan of the lower limbs showed an echogenic thrombus in the right deep femoral vein. He was commenced on therapeutic dose of subcutaneous enoxaparin. He was later changed to Warfarin and he was followed-up at the Hematology Clinic. There were no further complications.

DISCUSSION

Foot ulceration and amputation which in the presence of common predisposing factors such as peripheral neuropathy, peripheral arterial disease and poor glycemic control, is a common cause of morbidity and mortality in people with diabetes.[5] Despite the presence of risk factors, uncertainty still exists about the incidence of DVT after lower extremity amputation.[6] However, an incidence rate of about 50% has been reported in the literature.[4] It has also been found that DVT in an amputated stump is more common following above knee amputation, as found in this case report also.[4] Classical symptoms and signs of DVT are often absent which may lead to an underestimation of the problem.[7] Therefore further examination of the patients with duplex scanning is often required to ascertain the diagnosis. Suggested explanations for DVT in an amputated stump include immobility and increased venous pooling of blood in the amputated limb as a result of loss of soleal pump that assists venous drainage of the lower limbs.[6]

CONCLUSION

Deep venous thrombosis in an amputated stump in a patient with diabetes may not present with classical symptoms and signs and a high index of suspicion is needed to make a diagnosis. It may be also account for the well-documented increased cardiovascular mortality after lower limb amputation in patients with diabetes.

CONSENT

The authors have received written informed consent from the patient.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Mini Review

The Interrelationship of Menopause and Type 2 Diabetes Mellitus

Tejal Lathia, DM[1]*; Sanjay Kalra, DM[2]

[1]*Endocrinology Unit, Fortis and Apollo Hospitals, Navi Mumbai 400703, Maharashtra, India*
[2]*Endocrinology Unit, Bharti Hospital, Karnal 132001, Haryana, India*

Corresponding author
Tejal Lathia, DM
Consultant Endocrinologist, Endocrinology Unit, Fortis and Apollo Hospitals, Navi Mumbai 400703, Maharashtra, India; E-mail: drtblathia9748@mail.com

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ABSTRACT

Menopause is a critical time in a woman's life which heralds the cessation of reproductive competence. There is body fat redistribution which increases the risk of type 2 diabetes mellitus (T2DM). There is a complex interrelationship between menopause and T2DM; several variables like the timing of the menopause, the type of menopause and the symptomatology impact this relationship. The treatment of vasomotor symptoms with hormone replacement therapy may also impact glycemia both in women with and without pre-existing T2DM. We tried to examine this relationship based on current scientific evidence. We also suggested strategies to reduce the burden of T2DM in menopausal women.

Keywords
Menopause; T2DM; Hormone replacement therapy.

INTRODUCTION

Menopause is the cessation of menses for a year or more. The years preceding and succeeding this event are fraught with medical and psychological issues which greatly impact a woman's quality of life. Most women struggle with weight gain during this period. Whereas weight gain per se cannot be attributed to the menopause transition, the change in the hormonal milieu at menopause is associated with an increase in total body fat and an increase in abdominal fat.[1]

A retrospective study showed that weight increase around pregnancy and menopause correlated significantly with higher odds for the diagnosis of type 2 diabetes mellitus (T2DM) and/or hypertension, irrespective of the number of children.[2] Midlife women are at significant T2DM risk due to the high prevalence of excess adiposity, insulin resistance and disorders that contribute separately to T2DM risk such as sleep disorders and depression.[3]

It is well-known that there are gender differences in the burden of complications of T2DM. Large-scale meta-analyses, summarizing all the evidence available to date from the best quality epidemiological studies globally, have provided compelling evidence that T2DM confers a 44% greater excess risk of coronary heart disease (CHD)[4] and a 27% greater excess risk of stroke in women than in men, independent of sex differences in other major risk factors.[5]

Thus, weight redistribution with increase in total body fat may predispose a woman to T2DM; and the complications of T2DM tend to be more severe for women. Menopause is a golden window where early diagnosis of T2DM is feasible and this window must not be missed.

CHARACTERISTICS OF MENOPAUSE AND ITS TREATMENT WHICH INFLUENCE TYPE 2 DIABETES MELLITUS

The timing of menopause, the type of menopause, the treatment of vasomotor symptoms with hormonal therapy all impact T2DM. In the article, we address several of these questions.

1. Does early menopause increase the risk of T2DM?

2. Does type of menopause influence risk of T2DM- surgical *vs.* natural?

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3. Does T2DM lead to early menopause?

4. Does the severity of menopausal symptoms help predict T2DM?

5. What is the impact of hormone replacement therapy on T2DM?

6. What are the strategies for prevention of T2DM in menopausal women?

Does Early Menopause Increase the Risk of T2DM?

In the prospective, population-based Rotterdam Study,[6] Three thousand six hundred thirty-nine (3639) post-menopausal women were followed for a median duration of 9.2-years. Of these, 348 women were identified with incident T2DM. After adjustment for confounders, hazard ratios (HRs) for T2DM were 3.7 (95% confidence interval (CI) 1.8, 7.5), 2.4 (95% CI 1.3, 4.3) and 1.60 (95% CI 1.0, 2.8) for women with premature (<40-years), early (40-45-years) and normal (>45-years) menopause, respectively, relative to those with late menopause (p trend <0.001). The HR for T2DM per 1-year-older at menopause was 0.96 (95% CI 0.94, 0.98).

Further adjustment for body mass index (BMI), glycemic traits, metabolic risk factors, C-reactive protein, endogenous sex hormone levels or shared genetic factors did not affect this association. Thus, the early onset of natural menopause is an independent marker for T2DM in post-menopausal women.

The Dongfeng-Tongji cohort study[7] examined the association of earlier menopause (<45-years) with the prevalence of T2DM in 16,299 women. Seventeen percent of the study population had T2DM. The average age at menopause was 49.5±3.3 years. For each 1-year delay in menopausal age, the presence of T2DM was reduced by 2% (OR: 0.98, 95% CI: 0.97-0.99) after adjusting for potential confounding factors. Compared with those whose menopausal age was 46-52-years, the OR for T2DM was 1.20 (95% CI: 1.03-1.39) for those with an earlier menopausal age (≤45-years). The risk was small but discernible.

Findings from the China Kadoorie Biobank study in the Zhejiang area[8] in 17,076 post-menopausal women showed that 1,288 (7.54%) of the participating women had T2DM. In comparison with those with menopause at 46-52-years, women with menopause at a later age (≥53-years) were 1.21-fold (95% confidence interval 1.03-1.43) more likely to have T2DM.

An early age at natural menopause/surgical menopause was associated with a higher risk of T2DM, which exhibited a linear relationship (p for trend= 0.009) with HRs of 1.83, 1.02, 0.89, and 0.64 for ages <40, 40-44, 45-49, and >55-years, respectively.[9] This study did not find an increased risk of T2DM with later age at menopause, women > 55-years showed the least risk of T2DM.

Additionally, the duration of natural menses was positively associated with T2DM, with women who had fewer years of menstrual cyclicity having elevated risks, yielding HRs of 14.89, 5.41, 2.09, 0.51, and 0.31 for reproductive life span of <20, 21-25, 26-30, 36-40, and >40-years, respectively (p for trend <0.001).

In contrast, a study by Qiu et al[10] found no association of age at menarche or menopause with T2DM though higher menopause age was associated with decreasing cardiovascular disease (CVD) risk (p for trend 0.020) and earlier menopause (46-years) with significantly higher osteoporosis risk (odds ratio 1.59, 95% confidence interval, 1.072.36; p 0.023).

Thus, there is a definite trend of increased risk of T2DM with earlier menopause (<45-years). Some evidence suggests greater osteoporosis risk with early menopause. The evidence for risk of T2DM at a later age (>45-years) at menopause and lesser years of menstrual cyclicity is sparse and needs more study.

Women with earlier menopause require focused screening, appropriate counselling of this risk and strategies for prevention must be in place to prevent T2DM. It is well-known that early intensive glucose lowering reduces the risk of microvascular and macrovascular complications of T2DM.[11]

Does the Type of Menopause Affect Risk of T2DM-Surgical *vs.* Natural?

Data from a cohort of 2,597 post-menopausal women enrolled in the National Health and Nutrition Examination Survey I Epidemiologic follow-up study[9] with a median follow-up time of 9.2-years, found the incidence of T2DM (in cases/1,000 person-years) was 7.4 for women with no hysterectomy or bilateral salpingo-oophorectomy (BSO), 8.2 for hysterectomy alone, and 8.5 for hysterectomy with BSO. Hysterectomy status was associated positively with T2DM (HR 1.66, 95% CI 1.23-2.23). However, the elevated risk was restricted to women with both hysterectomy and BSO after adjustment for relevant confounders (HR 1.57, 95% CI 1.03-2.41).

Of 437 post-menopausal women, who participated in the Tehran Lipid and Glucose Study,[12,13] women with surgical menopause and 39 age-matched controls with natural menopause were selected. During the follow-up period, changes in metabolic and biochemical profiles were compared between surgical and natural menopause women. Odds of incidence of metabolic syndrome in surgical menopause women compared to natural menopause women, was 9.7 (95% CI 1.8-51.8).

The authors concluded that metabolic disturbances after menopause are highly influenced by type of menopause and are more prevalent in those undergoing surgical menopause. However, the study was small and definite conclusions are difficult.

Thus, surgical menopause confers a greater risk of T2DM with hysterectomy and bilateral salpingo-oophorectomy both as compared with hysterectomy alone or no hysterectomy. The preservation of ovaries if feasible mitigates the risk of T2DM to a certain extent and must be given due consideration prior to planning surgery.

Does T2DM Lead to Early Menopause?

In a study in a teaching, tertiary care hospital in Southern India,[13]

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600 post-menopausal (300 had T2DM, 300 did not) women were recruited over a period of 1-year. Average age of menopause among diabetic women was 44.65-years which is much earlier than the menopause in non-diabetic women (48.2-years). Out of the 600 women, 212 women had an early menopause (<45-years). Among them, 54 were non-diabetic and 158 were diabetic. The authors concluded that T2DM increases the risk of early menopause but patients with T2DM had greater BMI.

In the longitudinal and multiethnic Study of Women's Health Across the Nation (SWAN) bone study[13] (n=2171), women with T2DM near the beginning of the study experienced a significantly earlier age at their final menstrual period (FMP) than women without T2DM (at age 49.1 *vs.* 52.4; p=0.002). The study further noted that although all women in SWAN were premenopausal (54%) or early peri-menopausal (46%) at baseline, a significantly higher proportion of women with DM of any kind transitioned to early peri-menopause as compared to those without T2DM (58% *vs.* 45%, p<0.001)

These results are consistent with the findings of a study of women in Latin America[14] that reported a more than two-fold higher prevalence of early menopause among 40-44-year-old women with T2DM (n=410) as compared with women without (n=5669) (29% *vs.* 13.2%; OR 2.76: CI 1.32-5.34).[15]

In contrast, Lopez-Lopez et al[16] found that Mexican women with T2DM (n=409) experienced a similar age at menopause as compared with women without T2DM (n=404) (49.8 *vs.* 49.6; p not provided).[15]

The European Prospective Investigation into Cancer and Nutrition (EPIC)[17] investigated the impact of T2DM on age at natural menopause (ANM) in 258,898 women enrolled between 1992 and 2000.

Overall, no association between T2DM and ANM was found (HR=0.94; 95% CI 0.89-1.01). However, women with T2DM before the age of 20-years had an earlier menopause (10-20-years: HR=1.43; 95% CI 1.02-2.01, <10-years: HR=1.59; 95% CI 1.03-2.43) compared to non-diabetic women, whereas women with T2DM at age 50-years and older had a later menopause (HR=0.81; 95% CI 0.70-0.95). None of the other age groups were associated with ANM.

Thus, the question of T2DM predisposing to early menopause is not yet clear at this time.

Do Patients with T2DM Experience Different Symptomatology as Compared with Non-diabetic Women during Menopause?

A study of 100 women in Mexico[18] aged 45-72-years of age, 51 with and 49 without non insulin dependent diabetes mellitus (NIDDM) compared the physical characteristics, emotional symptoms and metabolic conditions of menopausal women with and without NIDDM. They found greater scores for depression and empty nest syndrome in NIDDM women.

A high quality cohort study by Herber-Gast et al[19] assessed the association between four distinct VMS profiles, (including the early severe profile characterized by symptoms reported in pre-menopause with a peak at menopause) in a population of 4895 healthy peri-menopausal women, with a baseline age of 45-50-years. Results show that women with an early severe VMS profile are more likely to have T2DM across a period of 15-years (odds ratio, 1.55; 95% CI, 1.11-2.17). This association is not explained by body mass index or other potential confounders.

There are several studies that reflect the greater severity of menopausal symptoms in diabetic women compared with non-diabetic women.

What is the Impact of Hormone Replacement Therapy on Type 2 Diabetes Mellitus?

Without pre-existing T2DM: Hormone replacement therapy is used to treat the severe vasomotor symptoms that accompany the menopause transition. It is now clear that hormone replacement therapy (HRT) can be safely given to early (<10-years) menopausal women, less than 60-years of age with low risk of breast cancer and cardiovascular disease and are willing to take HRT.[20] However, the Endocrine society guidelines for the treatment of symptoms of menopausere commend using systemic HRT with caution in women with T2DM.

In women without T2DM, use of menopausal hormone therapy (MHT) appears to reduce the risk of self reported T2DM and glycated haemoglobin (HbA1C).[21,22] In a meta-analysis by Salpeter et al[23] of 107 randomized trials comparing MHT to placebo or no treatment in women without T2DM, MHT was associated with a reduction in fasting glucose and fasting insulin that led to a 13% drop in insulin resistance, as calculated using the homeostatic model assessment of insulin resistance (HOMA-IR). This was associated with an estimated reduction of 30% in new-onset T2DM. In most randomised controlled trials (RCTs), the beneficial effects of estrogen on metabolism were attenuated by the addition of a progestogen.[23]

These RCTs confirm results from large observational studies such as the Nurses' Health Study, in which current users of MHT showed a 20% reduced incidence of T2DM compared with past users and women who had never used MHT, after adjustment for age and BMI.[24] The post-menopausal estrogen/progestin interventions (PEPI) study showed a small increase in post-challenge glucose but it did not affect the overall glycemic control.[25]

With pre-existing T2DM: In two placebo-controlled, randomized, cross-over trials of oral CE or Estradiol (E2) treatment in post-menopausal women with T2DM, estrogens reduced fasting glucose, HbA1c, and insulin resistance without affecting post-prandialglycemia.[26,27]

Similarly, in an RCT of oral E2 in post-menopausal women[28] with T2DM, E2 produced a decrease in HbA1C and significantly increased insulin suppression of hepaticglucose production (HGP). It should be noted, however, that these studies indiabetic post-menopausal women had fewer subjects, used estrogens alone,

and were performed over a shorter duration of time than studies in women without T2DM.[29]

Oral *vs.* transdermal? Oral estrogen therapy and CE, in particular, results in a stronger beneficial effect on insulin resistance (as assessed by HOMA-IR) than does transdermal E2 delivery.[23] The stronger effect of oral therapy on blood glucose probably results from the first-pass liver metabolism leading to a better suppression of HGP. Surprisingly, in the Kronos Early Estrogen Prevention Study (KEEPS),[30] an RCT to assess the effects of early initiation of oral or transdermal MHT *vs.* place boon rates of progression of atherosclerosis in post-menopausal women, serum insulin and the HOMA-IR score decreased significantly with transdermal E2 (50 mcg), but not with oral CE (0.45 mg) probably because of the smaller dose of CE used. In summary, both oral and transdermal E2 can lower blood glucose and improve insulin sensitivity, although oral CE demonstrates the more powerful effect at equivalent doses.[29]

Though HRT is not recommended for the prevention of T2DM, if HRT is needed for a woman for severe vasomotor symptoms, it can be safely used without fear of worsening T2DM.

The cardiovascular risk needs to be considered in women with T2DM prior to prescribing HRT and women with previous or current CVD, strong family history of cardiovascular disease, current or past smokers, HRT should be prescribed after weighing risks *vs.* benefits. There is also very little data on the effect of HRT on complications of T2DM. It may be prudent to use other non-hormonal alternatives like selective serotonin reuptake inhibitors (SSRI's) for the treatment of vasomotor symptoms.

Strategies for Prevention of T2DM in Menopausal Women

A unique opportunity presents itself when a woman consults her doctor for vasomotor symptoms. This visit can be used to sensitize a woman about the need for screening herself for T2DM, cautioning her about expected increase in weight and adiposity and what she can do to prevent the weight gain and T2DM.

Three randomized controlled trials have shown that systematic intervention with diet and lifestyle can offset much of the risk of T2DM in midlife women. The Da Qing study enrolled 557 middle-aged women and men with impaired glucose tolerance and followed them for 6-years.[31] Participants were assigned to control group or one of three interventions: dietary therapy, physical activity or a combination of the two. The cumulative incidence of T2DM was 68, 44, 41 and 46%, respectively, with a significant difference between each of the intervention groups and the control group, but no significant difference between each of the intervention groups.[31]

The next randomized trial to be conducted was the finnish diabetes prevention study.[32] Women participating in this study, most of whom were middle-aged, also had impaired glucose tolerance and were also overweight. If randomized to the intervention, participants received dietary counseling aimed towards reduction of total caloric content, particularly saturated fat content and increased fiberin take, along with 30 min of exercise per day. After 3-years, participants in the intervention group had a cumulative incidence of T2DM of 14 *vs.* 6% in the control group ($p<0.05$), even though less than half of the participants in the intervention group achieved their weight loss goals.[32]

The most recent study was the aforementioned diabetes prevention program (DPP), which enrolled 3,819 adults, approximately two-thirds of whom were women, and the majority of whom were middle-aged. Women participating in this study also had impaired glucose tolerance and were overweight, and if randomized to lifestyle change, were given weight loss targets, dietary counseling for calorie reduction and healthy calorie consumption, and moderate physical activity goals.[33]

The menopause transition is the perfect time to sensitize a woman to the hazards of weight gain, risk of T2DM and its complications. Regular exercise and dietary modification are clearly effective in reducing the risk of T2DM. Stress reduction strategies like yoga, meditation and deep breathing exercises not only help to better manage vasomotor symptoms but also help to control glycemia.

Menopause is a golden window of opportunity and all health care professionals attending women in this period of life must use it to actively increase awareness of T2DM and its prevention.

CONCLUSION

Women with earlier age at menopause (<45-years) have greater risk of T2DM. Women who undergo hysterectomy with BSO also have a greater risk of T2DM. Type 2 T2DM may predispose women to an earlier age at menopause, though this is not yet definite. Women with T2DM may possibly have more severe vasomotor symptoms. In women without T2DM, HRT initiation is found to reduce the insulin resistance, fasting plasma glucose and HbA1c. In women with pre-existingT2DM, there is no worsening of T2DM with either E alone or E+P. Oral estrogen used in appropriate doses possibly has a more favorable impact on insulin resistance and glycemia compared with transdermal estrogen. However, in women with T2DM, those with current or risk of cardiovascular disease or existing complications of T2DM need to evaluated more carefully prior to initiation of HRT. Non-hormonal alternatives would be better treatment options. Early intervention with lifestyle modification helps to prevent T2DM in midlife in women and offers a unique opportunity that should not be missed.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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TABLE 1

Table 1. *Summary–Interrelationship of Menopause with Type 2 Diabetes Mellitus*

Early menopause increases the risk of type 2 diabetes mellitus
Surgical menopause increases the risk of T2DM which is partially mitigated by the preservation of ovaries
There is conflicting evidence as to whether T2DM leads to early menopause
In women with severe vasomotor symptoms, it is safe to give hormone replacement therapy especially if they are less than 60-years of age within 10-years of menopause and are low risk of VTE, CVD and breast cancer
Hormone replacement therapy reduces the risk of T2DM in women without pre-existing T2DM
HRT does not worsen T2DM and may improve glycemia in women with pre-existing T2DM