Investigation of glycosylation effects on skin-to-fat tissue water content in persons with diabetes mellitus assessed by skin tissue dielectric constant (TDC)

BACKGROUND:
It has been estimated by the International Diabetes Foundation that there are about 285 million people around the world living with diabetes and that approximately one third of this population undergo some form of skin manifestations. While patients with type I diabetes are more likely to suffer from autoimmune related lesions, patients with type II diabetes are more prone to cutaneous infections. Previous research has established that the diabetic condition often entails changes in blood flow and blood vessels in the skin (1-3) however there is not much explanation offered as to the reasons for the biophysical changes in the skin. It has been thought that skin homeostasis could be disrupted through either diabetic-induced changes in skin metabolism or through associated complications such as vasculopathy and neuropathy. Recent research using ultrasound has shown that persons with diabetes that have ulcers have thinner skin (epidermis plus dermis) and less subcutaneous fat than age-matched people without diabetes (4). These results suggest that structural changes within the skin may affect tissue water content, either directly or indirectly. Literature supports the view that hyperglycemia-induced non-enzymatic glycation of structural and regulatory proteins play a major role in the pathogenesis of diabetic complications (5). This is known as the Maillard reaction in which the excess supply of glucose in the blood plasma leads to the non-enzymatic chemical reaction between the carbonyl group of glucose and the amino acid of proteins (5). However the influence of excess glucose in the diabetic condition on the hydration of the stratum corneum is still heavily controversial. While Sakai et al. (6) discovered reduced stratum corneum hydration levels, a lower level of skin surface lipids, and decreased sebum secretions in diabetic mice, Seirafi et al. (7) did not detect a difference in stratum corneum hydration between diabetic and healthy patients. Thinning of the dermis decreases relative water content whereas subcutaneous fat loss increases skin-to-fat water content since the water content of fat is low. Additionally excess glycation of proteins tends to create and accumulate advanced glycation end products (AGEs), which contribute to the pathogenesis of various diabetic disorders (8-10). In addition, glycosylation of structural proteins strongly adheres glucose molecules to the protein; hence, a plausible hypothesis is that diabetic persons with higher HbA1c values will have less tissue water content compared with persons with lower HbA1c values.

OBJECTIVE:
Our goal is to determine the correlation between skin-to-fat tissue water as measured by tissue dielectric constant (TDC) and HbA1c amongst patients with diabetes. This study may be viewed as a pilot investigation of the possible correlation between these two important parameters. The non-invasive TDC method will be the basis for measuring skin tissue water. This method has already been successfully implemented to evaluate the levels of local tissue water in the arms and legs of patients with breast cancer, patients with lymphedema, as well as healthy patients (11-16). As an additional component of this study, we will also examine the potential impact gender and age impacts on TDC values in persons with diabetes.
METHODS:

Experimental
The study will take place at NSU, specifically at the Ziff Clinic. The main source of subjects will be patients of Dr. Pandya from the Geriatrics department diagnosed with type II diabetes. As such there will be a predominance of elderly patients aged over 65 years in the study. All of the measurements will be taken on the same day, in the office. Patients will be seen either before or after the official physician visit. The study will take no longer than 15 minutes not including the time to deliver consent. Total time allotted is 20 minutes.

During a scheduled clinical visit at the HPD Geriatrics Clinic, Dr. Pandya, the physician co-investigator will inform the subjects as to the existence of the research study. If a subject is interested she/he will meet with a co-investigator, who will explain the study in details and administer the consent form.

Each subject will be asked to remove her/his shoes and socks and then be asked to step onto a Bioimpedance scale (Ironman InnerScan Body Composition Monitor, Tanita BC-558, Tokyo, Japan) and grip two attached handles for a period of about 20 seconds. This will allow obtaining the subject’s weight, and percentages of body water and fat. This measurement takes about one minute in total. Next the subject will be asked to lie supine on an examination table. While lying, the target sites for the subsequent TDC measurements will be marked on the dominant side of the subject’s body on the forearm, lower leg and foot. These TDC measurements are to be performed in triplicate at the following locations; on the forearm—6 cm below the antecubital fossa, on the leg—10 cm proximal to the lateral malleolus, and on the dorsum of the foot at a site close to the junction of the 1st and 2nd toes. Each TDC measurement will be made to effective depths of 0.5, 1.5, 2.5, and 5.0 mm as further described in section B. Approximate time required for the TDC measurements is nine minutes. Following the TDC measurements, the subject’s blood pressure will be made in triplicate with the subject still supine. Blood pressure will be measured simultaneously in both arms using a dual pressure-measuring device (MicroLife Watch BP office Twin 200). This measurement takes about two minutes. The total measurement time is estimated to be about 15 minutes. Each subject will be measured during only one visit.

The process of assessing local tissue water is based on the principle that TDC is directly related to the amount of free and bound water contained in a measuring volume (17-25). The TDC is a dimensionless quantity that is the ratio of the absolute tissue dielectric constant to that of free space. The TDC value of a specific target area is determined using a coaxial probe that gently contacts the skin for approximately 10 seconds; the probe which is connected to a control and display device measures the TDC value at a frequency of 300 MHz. Pure water has a TDC value of about 78.5. For the purpose of this study, the TDC measurements will be taken at effective depths of 0.5, 1.5, 2.5, and 5.0 mm. Measurements will be made at three anatomical sites; the forearm 6 cm below the antecubital fossa, 10 cm proximal to the lateral malleolus, and between the first and second toes on the dorsum of the foot. All three sites will be measured only on the dominant side of the body. Each measurement with each probe will be made in triplicate starting with the 5.0 mm probe and progressing towards the 0.5 mm probe. The time required for completing a triplicate tissue water measurement is about 45 seconds. This means that the total time requirement to complete TDC measurements for all four probes, taking into account the time needed to change probes, is approximately nine minutes.
**Analytical**

The main goal of this study is to test the hypothesis that in persons with diabetes mellitus TDC values, used as index of skin-to-fat tissue water, significantly correlates with the level of HbA1c. Consequently the main analysis procedures will be based on the following null and alternate hypothesis. The null hypothesis (H0) is that there is no correlation between the two variables and the alternate hypothesis (Ha) is that there is a correlation. Based on the judgment that a fundamentally meaningful and potentially clinically useful relationship would need a correlation coefficient (r) of at least 0.45 in magnitude, this effect size (r=0.45) has been chosen for the present study. The composite of the Individual TDC values and corresponding HbA1c values will constitute the paired values for the correlation analysis, with a Bonferroni correction. Although TDC values will be measured at multiple depths (0.5, 1.5, 2.5 and 5.0 mm), previous work has shown that these values are highly correlated (p<0.001). Thus, in order not to reduce statistical power of the primary correlation analysis, it is a priori planned to use only the 2.5 mm depth TDC in the correlation analysis. The depth data will be used separately to characterize the depth dependence of TDC values in this diabetic population and to compare values between genders, and different age groups. TDC data from the three anatomical sites (forearm, lower leg and foot) are viewed as independent assessments since prior work has shown significant differences in TDC values among sites in persons without diabetes. Thus separate correlation analysis of HbA1c vs. TDC at 2.5 mm and will be done for each site. Depth dependence of TDC values will be determined separately at each anatomical site. This is achieved by characterizing the TDC versus depth characteristic for each site using regression analysis. The comparisons among the three sites will be done by comparing the regression coefficients that essentially represent the TDC-depth slope using analysis of variance (general linear model) for repeated measures. To investigate possible gender differences, these slopes and absolute TDC values will be compared between genders with each site analyzed separately. The possible impact of clinical parameters, including blood parameters, blood pressure and ABI on TDC-HbA1c relationships will be examined using stepwise regression methods.

Some of the additional information that we are planning on obtaining from patients chart will be the following, age, height, blood pressure (as measured that day by a nurse), duration of diabetes, basic metabolic panel, most recent HbA1c values (< 6 months), most recent blood glucose values, current diabetes medication usage, history of lower extremity arterial disease, current medication list and presence of renal arterial disease.

If the hypothesis holds true, we will be able to show a yet unknown relationship between non-enzymatic glycosylation and tissue water. This can have a huge impact on preventive care; if a positive correlation is established then physicians will be able to prescribe preventative skin care regimens to patients with diabetes based on their trend of HbA1C values. It will also effect patient education, and open new research possibilities.

The acquired information will be disseminated to both patients and physicians. We are planning to have our work published; some of the potential journals would be Diabetes, Diabetes Care, Clinical Diabetes, Diabetes Technology & Therapeutics, Skin Research and Technology. We are also planning on presenting our research at various conferences.
REFERENCES: