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Does the Duration of Type 2 Diabetes Correlate with Changes in Taste Deficits?

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PHILADELPHIA COLLEGE OF OSTEOPATHIC MEDICINE
THE GRADUATE PROGRAM IN BIOMEDICAL SCIENCES

DOES THE DURATION OF TYPE 2 DIABETES CORRELATE WITH
CHANGES IN TASTE DEFICITS?

A Thesis in Biomedical Science Research by Ann T. McLaughlan

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Submitted in Partial Fulfillment of the Requirements for the Degree of
Master of Biomedical Sciences

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ABSTRACT

The loss of insulin sensitivity in Type 2 diabetes interferes with cellular utilization of glucose. The underlying down-regulation of insulin receptors and the resulting insulin resistance is wide-spread throughout the body. The cardiovascular consequences may be indirectly responsible for decreased taste sensitivity because of diminished perfusion of the taste buds in this patient population. This study utilized an inexpensive, non-invasive technique, electrogustometry, to directly stimulate the taste buds by applying a variable, direct-current stimulus to measure taste receptor thresholds in newly-diagnosed (< 2 years) and long-standing diagnosed (> 6 years) male and female Type 2 diabetes mellitus subjects. Taste thresholds were elicited by application of an anodal current to the taste receptors. An increased taste threshold to the anodal current, indicative of a loss of taste sensitivity, was detected in those Type 2 diabetes mellitus subjects with long-standing disease compared to newly diagnosed subjects. Although the differences were not statistically significant a consistent trend suggestive of a significant difference was demonstrated. The results of this pilot study suggest that a future study utilizing a larger number of subjects may result in statistical significance of this approach to managing these two groups.

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Introduction

The complications of Type 2 diabetes are associated with numerous pathologies related to the duration of this disease. The monitoring of these complications such as diabetic retinopathy, neuropathy, and nephropathy present a difficult problem. The current clinical evaluation of the diabetic patient includes several routine laboratory tests. Perhaps changes in taste thresholds may provide an additional monitoring resource in the future. This study was designed to determine whether taste thresholds could be affected as a consequence of the progression of the disease process or a predictor of disease onset. It is presently unclear whether the evolution of the taste threshold differences and disease process in Type 1 and Type 2 diabetics occurs in a similar manner. This study utilized an inexpensive, non-invasive technique, electrogustometry, to directly stimulate the taste buds by applying a variable, direct-current stimulus to measure taste receptor thresholds in newly-diagnosed (< 2 years) and long-standing diagnosed (> 6 years) male and female Type 2 diabetic subjects. A better understanding of the declining changes in taste perception is important because individuals with diabetes often compensate for decreased taste sensitivity (impairment) by consuming foods higher in salt and sugar content. This life-style behavior may place the individual at risk for increased blood pressure and labile glucose status. The increased susceptibility to hyperglycemia and hypertension has been shown in observations using chemical taste testing and electrogustometry (1, 2).

Two previous studies were carried out at the Philadelphia College of Osteopathic Medicine (1, 3). The first explored taste threshold differences among non-diabetics, Type 1 and Type 2 diabetics. Significant differences were found between non-diabetic and diabetic subjects, but no significant differences were found between Type 1 and 2

diabetic subjects. The goal of the second study was to determine whether taste thresholds are altered in subjects with a history of diabetes among their immediate relatives. Using an electrogustometer, taste thresholds of healthy 20-30 year old volunteers with and without a primary family history of type 2 diabetes were studied using an electrogustometer. The study also compared results of the PTC (phenylthiocarbamide) taste test of healthy 20-30 year old volunteers with and without a primary family history of type 2 diabetes. A positive primary family history of type 2 diabetes was considered to be parents and/or grandparents with a diagnosis of the disease. Study participants in both studies were non-smokers without a history of tongue pathology. The present study is designed to investigate whether there are differences in threshold taste sensitivity relative to the duration of the Type 2 diabetes mellitus disease.

Background

Diabetes mellitus

Diabetes mellitus is a disease of altered carbohydrate, fat, and protein metabolism caused by a lack of insulin secretion from pancreatic beta cells (Type 1) or the decreased sensitivity of the other cells in the body to respond to insulin (Type 2) (4). Insulin, a hormone secreted from the beta cells of the pancreas is responsible for glucose uptake from the bloodstream into insulin sensitive cells such as adipose tissue, liver and muscles (4). Despite their different etiologies, both types result in hyperglycemia (high blood sugar) because glucose cannot be transported into the cells and remains in the blood stream. The characteristics of Type 2 diabetes mellitus are hyperinsulinemia (excess insulin in the blood) with insulin resistance of all cells and decreased beta cell secretion of insulin. However, in Type 2 diabetes mellitus, insulin resistance begins with a defect in the initial response to high concentrations of blood glucose. Insufficient insulin is released in proportion to the concentration of plasma glucose, which results in a compensatory and exaggerated response resulting in increased insulin release and hyperinsulinemia. If the hyperinsulinemia is sustained then the insulin receptors are down-regulated which causes the tissues to become less sensitive and more resistant to insulin action and decreases glucose uptake (5). The glucose remains in the bloodstream following a meal, causing hyperglycemia as well as fasting hyperglycemic levels.

Two other components necessary for establishing a diagnosis are excessive glucose production by the liver and abnormal fat metabolism (5). A hypoglycemic state will cause the brain to stimulate the adrenal glands to release epinephrine and cortisol, pancreatic alpha cells to secrete glucagon, and the pituitary gland to release growth

hormone. All three responses cause the liver to convert glycogen to glucose and the glucose to be released into the blood (4, 6, 7, and 8). In regards to fat metabolism, insulin promotes fat synthesis and storage. However, in the absence of insulin, fat metabolism is increased as an alternative form of energy since the body cannot utilize the glucose from the bloodstream. There is an increase in breakdown of the stored fat into fatty acids which are released into the bloodstream. The liver can convert the excess fatty acids into phospholipids and cholesterol which can be released into the bloodstream as lipoproteins. Excess lipoproteins can cause atherosclerosis, one of many complications, to develop over time.

Diabetic Complications

Type 2 diabetes mellitus is a chronic disease and it is important that the patient assume responsibility for controlling their glucose levels. Control is difficult under even the best circumstances and hyperglycemia will occasionally occur, so that after five to ten years of the disease, complications are usually apparent. Microvascular complications such as neuropathy, retinopathy, and nephropathy are predominantly mediated by periods of uncontrolled hyperglycemia. According to the Diabetes Control Complications Trial (DCCT) (10), United Kingdom Prospective Diabetes Study (UKPDS) (11), and Kumamoto studies (12), hyperglycemia is the major cause of microvascular complications.

Microvascular Complications

Neuropathy

Neuropathy is present in about 50% of individuals with long-standing diabetes. The development of neuropathy is strongly correlated with the duration of the disease and the degree of glycemic control. The two main types are polyneuropathy and autonomic neuropathy. Distal symmetric polyneuropathy is the most common form of diabetic neuropathy. Symptoms are a sensation of numbness, tingling, sharpness, or burning that begins in the feet and spreads proximally. These symptoms usually involve the lower extremities, are present at rest, and worsen at night (5). These symptoms eventually disappear in six-twelve months, but leave a sensory deficit in the lower extremities (13). Physical examination reveals sensory loss, loss of ankle reflexes, and abnormal position sense (5). Autonomic neuropathy develops in patients who have had the disease for twenty years or more, and involve, the sympathetic, parasympathetic, and enteric nerves,

which affect the blood vessels, digestive, urinary, and reproductive systems (13).

Clinical symptoms of autonomic neuropathy include: gustatory sweating (sweating after eating), low blood pressure, erectile dysfunction, bladder dysfunction, and diarrhea.

Neuropathic complications can be minimized with effective control of blood glucose levels (14).

Retinopathy

Retinopathy is a progressive eye disease that results from damage to blood vessels at the back of the eye (15). There are two types: nonproliferative and proliferative diabetic retinopathy. Nonproliferative diabetic retinopathy is the first phase of the progression. It occurs late in the first decade of Type 2 diabetes mellitus or early in the second decade. Symptoms of the disease are retinal vascular microaneurysms, blot hemorrhages, and cotton wool spots. As the retinopathy progresses there is loss of retinal pericytes, increased retinal vascular permeability, alterations in retinal blood flow, and abnormal retinal microvasculature. All of these lead up to retinal ischemia, which is a reduced supply of blood to the retina. If nothing is done to stop or slow the progression, the condition turns into proliferative diabetic retinopathy, the primary sign of which is neovascularization; the growth of new blood vessels. Neovascularization is stimulated by the decreased blood flow and the retina's requirement for oxygen and nutrients. These new blood vessels occur near the optic nerve or macula, and are problematic because they rupture easily and can lead to retinal detachment. The best predictors for the development and progression of retinopathy are the duration of diabetes and the degree of glycemic control (5).

Nephropathy

Nephropathy, also known as kidney disease, is another microvascular complication. Diabetic nephropathy is the leading cause of end stage renal disease as well as diabetes related morbidity and mortality. Once again hyperglycemia is the source of change in function and structure of the kidney. The progression of diabetic nephropathy begins at the diagnosis of diabetes. Within the first few years, after the onset of diabetes, glomerular hyperperfusion and renal hypertrophy occur, especially associated with a high glomerular filtration rate. The three key points to be considered with Type 2 diabetes mellitus and nephropathy pertain to microalbuminuria (a small amount of albumin in the urine), soluble factors, and structural changes:

1. Microalbuminuria or macroalbuminuria may be present at diagnosis when there is a long asymptomatic period. Hypertension commonly accompanies the microalbuminuria and macroalbuminuria. Microalbuminuria may be less predictive of diabetic nephropathy and the progression to macroalbuminuria in Type 2 diabetes mellitus. Albuminuria may be due to other health issues unrelated to diabetes mellitus, including hypertension, congestive heart failure, prostate disease, or infection. The progression of microalbuminuria can be slowed and controlled with increased glycemic control, but once macroalbuminuria sets in, the glomerular filtration rate (GFR) steadily declines and there is a slight rise in blood pressure. These pathologic effects are irreversible; and usually result in end stage renal disease. Strict blood pressure control (<130/80 mmHg) and angiotensin converting enzyme (ACE) inhibitors help reduce albumin excretion. ACE inhibitors help to decrease blood pressure by dilating blood

vessels, but they are also helpful in delaying the progression of microalbuminuria to macroalbuminuria and the related decline in GFR (5).

2. Soluble factors such as growth factors, angiotensin II, and endothelin cause structural and functional changes to the kidney. Decline in function is caused by glomerular hyperfiltration and glomerular hyperperfusion, which do not allow enough time for the kidney to adequately regulate the body's concentrations of sodium and water. Because the flow through the kidney is increased, glomerular capillary pressure also increases (5).

3. Structural changes occurring in the glomerulus include increased extracellular matrix, with basement membrane thickening, mesangial expansion and fibrosis (5). All of these factors together result in an abnormal functioning and eventual loss of kidney function.

Treatment

The treatments for Type 2 diabetes are to learn meal planning, as well as exercise and weight loss methods to control glucose levels. When these efforts are not sufficient, insulin injections and/or medication such as, sulfonylureas, meglitinides, biguanides, alpha-glucosidase inhibitors, and thiazolidinedione agents are required (9). They all work to decrease blood glucose but have different mechanisms of action.

Taste

Taste is a special sense. The organs for taste are the taste buds located in the mucosa of the epiglottis, palate, pharynx, and tongue. This study focuses on the taste buds on the tongue because of their accessibility and their distinct pattern which enables accurate repeated testing of specific sites. The taste buds are found within the fungiform and circumvallate papillae. The fungiform papillae are rounded structures and most numerous near the tip of the tongue as shown in Figure 1.

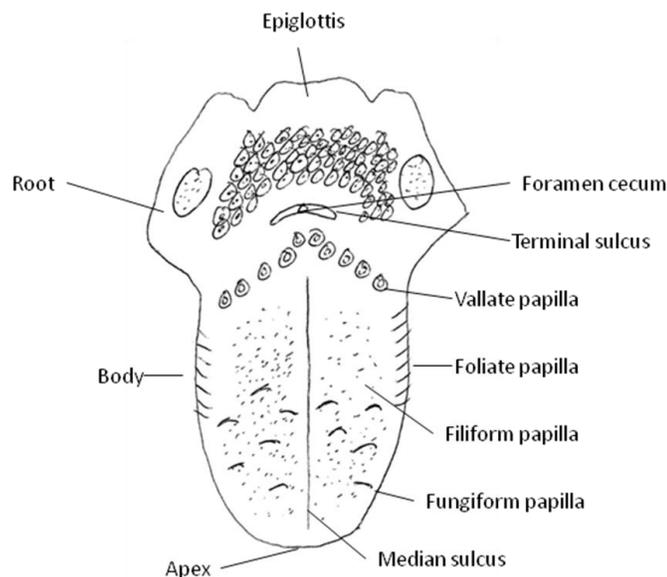


Figure 1. Papillae on dorsum of the tongue (16).

Each papilla has 5 taste buds which are located on top of the papillae. The circumvallate papillae are larger, prominent structures arranged in a V near the back of the tongue. The circumvallate papillae can have up to 100 taste buds, which are located along the sides of the papilla as shown in Figure 2.

Each taste bud is composed of four different cell types. Basal cells, Type 1, and Type 2 sustentacular cells, and Type 3 cells. Type 3 cells are the gustatory receptor cells

that make connections with the sensory nerve fibers that propagate the signals to the brain. The Type 3 cell has microvillus that project into a taste pore, the opening of the taste bud to the oral cavity and the fluids present there (Figure 3).

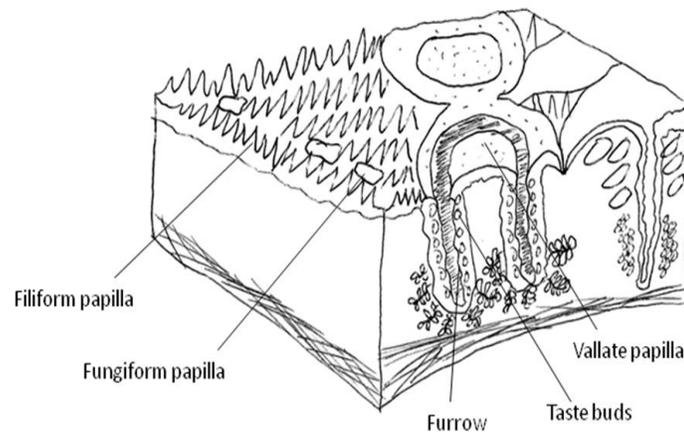


Figure 2. Schematic of the tongue and a cross section of a taste bud (16).

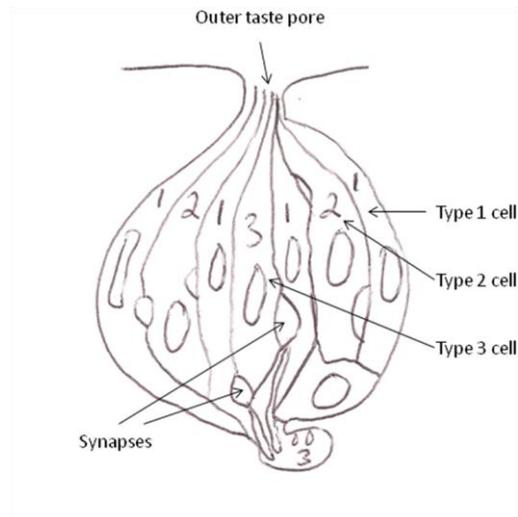


Figure 3. Taste pore detail (17).

Each taste bud is innervated by about 50 nerve fibers and each fiber receives input from 5 taste buds (17). Taste signals are conveyed by nerves to the brain where the specific qualities are interpreted. The nerves involved with the special sense of taste

include: the chorda tympani branch of the facial nerve which innervates the anterior 2/3 of the tongue, the glossopharyngeal nerve which innervates the posterior 1/3 of the tongue, and the vagus nerve which innervates the other areas of the tongue. The nerves responsible for general sensations are the lingual nerve which innervates the anterior 2/3 of the tongue and the glossopharyngeal nerve which innervates the posterior 1/3 of the tongue (Figure 4).

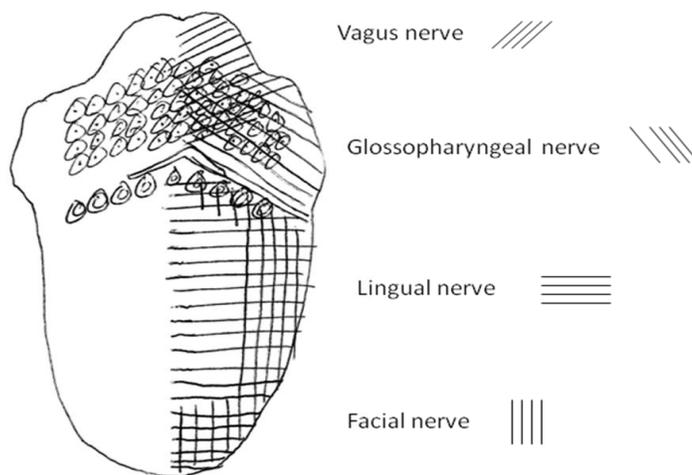


Figure 4. Innervations of the tongue (16).

There are five types of taste qualities: sweet, sour, salt, bitter, and umami.

Umami is also known as savory and when translated from Japanese means “delicious flavor” (18). The five sensations of taste result from the reactions of substances with the chemical receptors in the taste cells; there are sodium, potassium, chloride, adenosine, inosine, sweet, bitter, glutamate, and hydrogen ion receptors. Sodium, potassium, and chloride receptors are activated for salty foods, hydrogen ion receptors by sour foods, sweet receptors by sugars, and organic substances containing nitrogen activate the bitter

receptors. Foods containing glutamate, such as broth and aged meats and cheeses are responsible for the umami taste sensation (19). Substances must be dissolved in the fluid (saliva) to be sensed by the taste buds. The fluid part of the saliva comes from the microvasculature surrounding the taste buds and other parts of the vascular tongue especially the salivary glands, parotid, submandibular, and sublingual. Substances dissolved in the saliva flowing over the taste buds activate the various receptors which then transmit signals to the brain to be interpreted. Contrary to previous thought, all taste qualities are sensed from all areas of the tongue; there is little localization of taste to a specific area of the tongue (17). Since the tongue has extensive microvasculature, and the taste receptors depend upon stimulation by substances dissolved in an aqueous environment and saliva is derived from plasma, any disease affecting the microvasculature will affect taste. The microvasculature of the tongue is almost identical to the retinal microvasculature and this is the reason why we hypothesize that taste can be used as an indirect monitor of the progression of diabetes and in particular diabetic retinopathy.

Electrogustometer

The electrogustometer (Figure 5) is a clinical tool that utilizes the topical application of weak anodal, DC current that can be applied to lingual and other taste buds in the oral cavity (20). These electrical stimuli can be precisely applied and localized on the tongue and provide a good measure of taste sensitivity thresholds (21). The application of anodal current generated by the electrogustometer produces a sour-metallic sensation. The weak anodal DC current is powered by 4 AA batteries, and stimulates the taste buds directly with less than 400 μA . To complete and ground the electrical circuit, a plastic clamp with a metal strip is placed on the subject's non-dominant forearm with a water-soluble gel to increase conductivity. The mechanism of electric taste is thought to involve hydrogen ions liberated at the anode which acidifies the saliva in a localized area of the tongue due to their combining with the chloride ions in the saliva and thereby activating the ionic taste receptors responsible for the sour taste sensation (21). The electrogustometer activates all types of taste buds that have hydrogen ion receptors.



Figure 5. TR-06 Rion Electrogustometer (22).

Hypothesis

The specific hypothesis being tested is presented is: the mean current detected in quadrant x for subjects with long-standing diagnosed Type 2 diabetes mellitus is equal to the mean current detected in quadrant x for subjects with newly diagnosed Type 2 diabetes mellitus.

Goal

The goal of this proof-of-concept/feasibility study was to determine whether differences in taste threshold perception in subjects with newly-diagnosed or long-standing diagnosed Type 2 diabetes mellitus can be detected and associated with the duration of their disease.

Objectives

1. Identify appropriate subjects by reviewing the patient records.
2. Administer subject questionnaire to rule out confounds that would preclude participation in this study.
3. Administer the electrogustometry test to determine taste thresholds in each of four quadrants of the dorsal surface of the tongue.

Methods and Materials

This study was approved by the Philadelphia College of Osteopathic Medicine IRB.

Subjects

The inclusion criteria for the subjects in this study were newly-diagnosed (< 2 years) and long-standing diagnosed (> 6 years) male and female Type 2 diabetes mellitus subjects greater than 21 years of age and of all ethnic and racial backgrounds.

The exclusion criteria for subjects in this study were smoking, less than 21 years of age, and Type 1 insulin requiring diabetes. No patients diagnosed with Type 2 diabetes mellitus of a duration from 2 to 6 years were recruited for this study.

Procedures

Recruitment

Jeffrey Freeman, DO., Chairman, Division of Endocrinology, Department of Internal Medicine, Philadelphia College of Osteopathic Medicine, screened and recruited appropriate subjects from his patient population based upon their diagnostic records. He presented the study to the prospective patients and invited them to participate in the investigation. When a patient indicated their willingness to participate; a testing session was scheduled. At the beginning of the testing session, the investigators explained the procedure to the subject and answered any questions while obtaining informed consent. A personal history/health questionnaire was completed prior to commencing the testing.

Test Procedures

1. The dorsal surface of the tongue was divided into four test-quadrants by first dropping an imaginary perpendicular line through the midline of the tongue along the median sulcus extending from the far proximal edge of the circumvallate region to the

anterior tip of the tongue. An imaginary horizontal line was drawn at the midpoint of the vertical line while noting landmark features on the tongue's surface that enabled the recognition of these four divisions, so that these boundaries could be preserved, and the probe placement sites in each quadrant could be returned to with accuracy for repeated measurements.

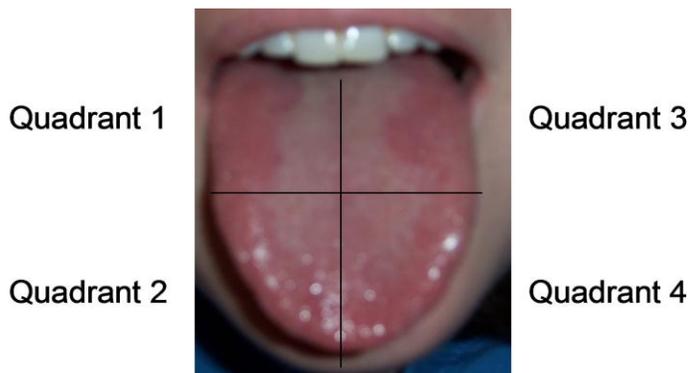


Figure 6. A tongue depicting the four test quadrants.

2. Twenty-four hours prior to testing each subject, a test probe was sterilized (Cidex, Johnson and Johnson Medical Incorporated, Arlington, TX) according to standard procedures.

3. Prior to initiating the actual test, the probe was placed at a location on the surface of the tongue excluded from the test area (distal edge of the tongue tip), and the highest decibel current was induced so that the patient would be aware of the nature of the stimulus (i.e. a dissatisfying sour, metallic-like taste) that they were asked to recognize by pressing a hand-held buzzer to indicate their response.

4. The test was administered by placing a stimulus probe, 2mm in diameter, sequentially in each of the four predetermined quadrants on the tongue surface.

5. In the first series of measurements, the administration of the current signal was controlled by first moving from the lowest intensity (-6 dB) to the highest intensity (34 dB) with a duration of 2 seconds for every increment of 2 dB. Each intensity was measured in each quadrant, for a total of 84 measurements. The subject indicated perception of the signal (i.e. a dissatisfying sour, metallic-like taste) by pressing a hand-held buzzer that was electronically coupled to the machine. In the next series of measurements the current signal was administered beginning at the highest intensity to the lowest intensity in decrements of 2 dB until the subject indicated the disappearance of the stimulus signal (i.e. a dissatisfying sour, metallic-like taste) by pressing a hand-held buzzer that was electronically coupled to the machine. Each intensity was measured in each quadrant, for a total of 84 measurements. In the cases where the subject was unable to discern either the presence or disappearance of the signal at any decibel level they were assigned as having a taste threshold greater than 34 dB for statistical purposes.

Random, sham responses consisting of no electrical stimulus through the probe were interspersed randomly to validate the accuracy of the subject's response. The electrogustometer was turned off for the sham response without the subject's awareness, and the correct response from the subject would be that he or she could not detect a taste sensation. A measurement consisted of placing the probe on a particular site on the tongue and turning the machine to a specific intensity where the subject indicated perception by pressing a hand-held buzzer. A mark was made on a table listing the intensities perceived. Each of the four tongue test sites were evaluated in this manner with both increasing and decreasing signal intensities. The subject was provided with a

cup of water to rinse their mouth out at will. Each subject participated in one test session of approximately 30 minutes duration.

There was no normal or control group. Prior studies have established the range of normal responses of 5-10dB and would be redundant to repeat (1, 3, 23, 24). The investigators used sham responses to prove that the subject actually had the ability to taste or not.

Statistical Analysis

Descriptive statistics were used to summarize the threshold level of current that was perceived by each subject as the intensity was increased and then decreased. Descriptive statistics included the number of subjects, arithmetic average current setting, standard deviation, median, maximum, and minimum current values, and the lower and upper 95% confidence limits. All of the descriptive statistics are presented by quadrant and the duration of diabetes, categorized into 2 mutually-exclusive groups (existing diagnosis for >6 years and newly diagnosed within <2 years). Comparisons of the level of current by duration of disease, quadrant, and increasing or decreasing intensity were all analyzed using a 1-Factor Analysis of Variance Model (ANOVA). The intra-subject threshold level of detection of increasing and decreasing current served as the dependent variable. Probability values <0.05 were considered significant; probability value ≤ 0.1 but ≥ 0.5 were considered indicative of a consistent 'trend' towards significance (Appendix A, Table 1 and Appendix B, Table 1 and 2). A two sample T-test Power Analysis was also completed on the data to determine how many subjects would be needed in a larger study to obtain statistically significant differences in taste thresholds at the probability level of $p \leq 0.05$ (Appendix C).

Results

Type 2 diabetes mellitus complications are associated with numerous pathologies related to the duration of the disease. This study was designed to determine whether taste thresholds could be affected as a consequence of this disease process. It would be expected that those individuals whose Type 2 diabetes mellitus is long-standing (>6 years) would have higher taste thresholds indicative of a decreased taste sensitivity.

Although no significant difference in taste thresholds was found between newly diagnosed and long-standing diagnosed diabetics, when quadrant by quadrant comparisons were made between the two groups, a definite trend was apparent ($p \geq 0.05$ but ≤ 0.10). With the exception of decreased current application in quadrant 3, every other measurement reflected a higher threshold in taste sensitivity for every quadrant of the tongue in the long-standing diagnosed diabetic subjects. However, when the data for increasing current application was combined for all 4 quadrants in each group, a significant difference was found for these subjects with long-standing disease having the higher taste thresholds ($p=0.005$)(Figure 7).

These results reflect determinations obtained from 11 subjects, grouped according to the duration of their disease i.e. whether their Type 2 diabetes mellitus had been diagnosed longer than 6 years ago (6 subjects) or whether their Type 2 diabetes mellitus had been newly diagnosed less than 2 years ago (5 subjects). Table 1 contains the raw data obtained for each individual quadrant and threshold response for both increasing and decreasing threshold determinations from the subjects in the study. Each subject's taste threshold is listed according to the increasing or decreasing current that was applied, with means and standard deviations for the groups long-standing and newly diagnosed

diabetics included in order to show each subject's relative placement with respect to the group mean.

COMBINED ELECTROGUSTOMETRY RESULTS IN PATIENTS WITH NEW (<2 YEARS) AND EXISTING (>6 YEARS) TYPE II DIABETES

Graphic No. 1.0: CURRENT AT THE TIME OF PERCEPTION (mean with 95% confidence limits)

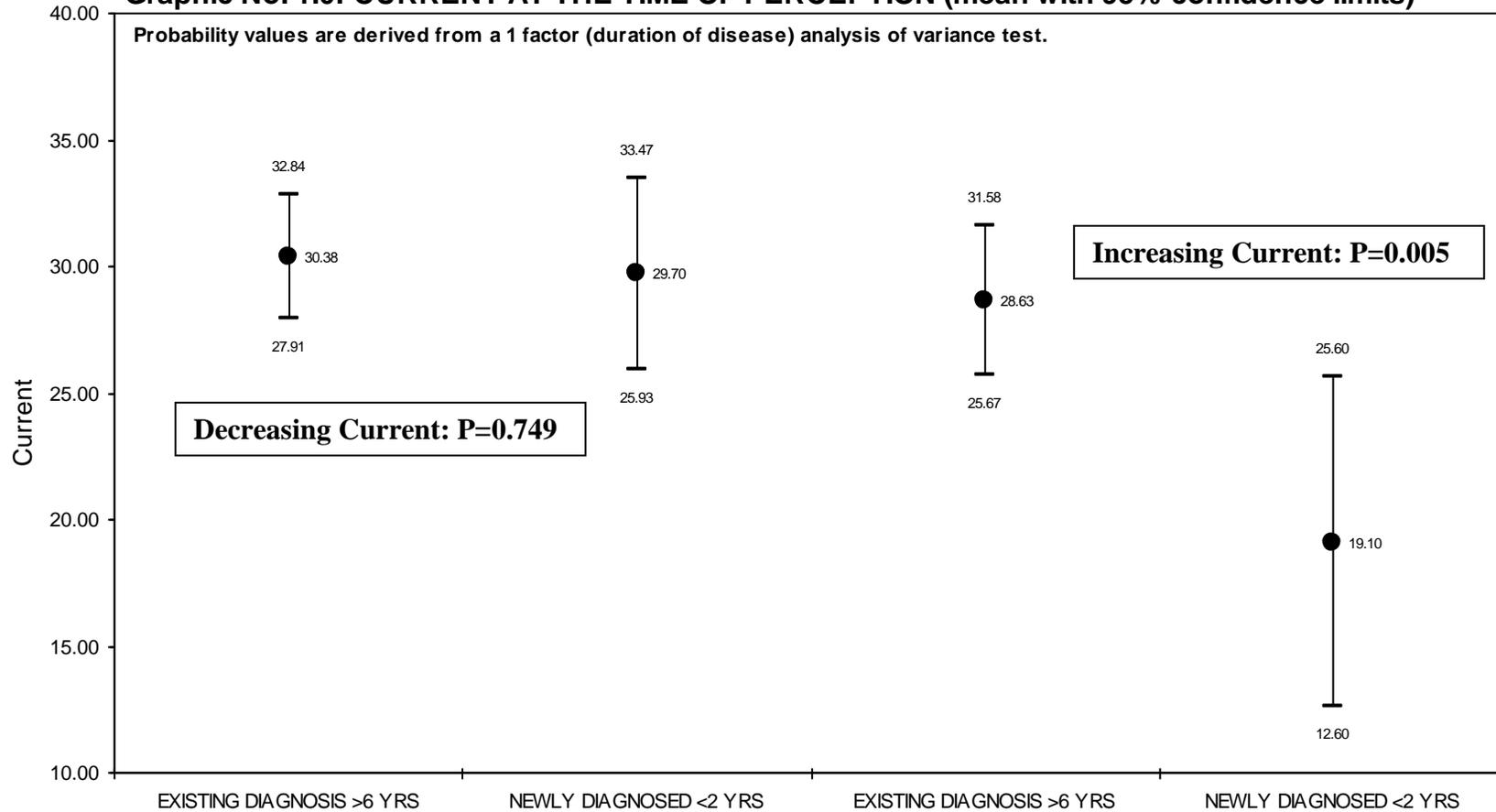


Figure 7. Electrogustometry results in subjects with newly diagnosed (< 2 years) and long-standing diagnosed (>6 years) Type 2 diabetes mellitus subjects. The current was at time of perception with mean at 95% confidence intervals.

Electrogustometry Raw Data Obtained from Type 2 Diabetic Subjects

Table 1. Subject raw data obtained from study with relative comparison to overall means and standard deviations of each quadrant for increasing and decreasing input current.

		Subject	↑Current Threshold (dB)	Mean	Standard Deviation	↓ Current Threshold (dB)	Mean	Standard Deviation
Quadrant 1	Newly Diagnosed (<2 yr)	1	34 dB (no taste)	22.8	9.86	34 (no taste)	29.6	6.19
		2	24 dB			30		
		3	16 dB			20		
		4	30 dB			34 (no taste)		
		5	10 dB			28		
	Existing Diagnosis (>6 yr)	1	18	30.17	6.91	26	33.17	3.54
		2	26			34		
		3	34 (no taste)			34 (no taste)		
		4	34 (no taste)			34 (no taste)		
		5	34 (no taste)			34		
6		32	34					
Quadrant 2	Newly Diagnosed (<2 yr)	1	34 (no taste)	16.8	16.69	34 (no taste)	25.6	13.13
		2	6			18		
		3	2			6		
		4	34			34 (no taste)		
		5	6			34		
	Existing Diagnosis (>6 yr)	1	18	27.83	6.46	26	29	7.21
		2	26			16		
		3	34 (no taste)			34 (no taste)		
		4	34			34 (no taste)		
		5	24			32		
		6	30			30		
		Quadrant 3	Newly Diagnosed (<2 yr)			1		
2	4			34				
3	2			30				
4	34			34 (no taste)				
5	10			34				
Existing Diagnosis (>6 yr)	1		24	29.83	5.71	22	28	6.9
	2		24			34 (no taste)		
	3		34 (no taste)			34 (no taste)		
	4		34 (no taste)			30		
	5		34 (no taste)			18		
	6		26			28		
	Quadrant 4		Newly Diagnosed (<2 yr)			1		
2		20		28				
3		4		18				
4		34		34 (no taste)				
5		4		34				
Existing Diagnosis (>6 yr)		1	26	26.67	9.67	22	31.33	4.97
		2	16			34 (no taste)		
		3	34 (no taste)			34 (no taste)		
		4	34 (no taste)			32		
		5	14			30		
		6	34			34		

The mean taste thresholds for Quadrant 1 are shown below in Fig. 8. For the increasing output currents, the long-standing diagnosed diabetics had a higher mean taste threshold value of 30.17 decibels (dB) compared to 22.80 dB mean taste threshold value for the newly-diagnosed diabetic subjects. For decreasing output current, the long-standing diabetics had a mean taste threshold value of 33.17 dB which was higher than the 29.60 dB mean taste threshold value for the newly-diagnosed diabetic subjects.

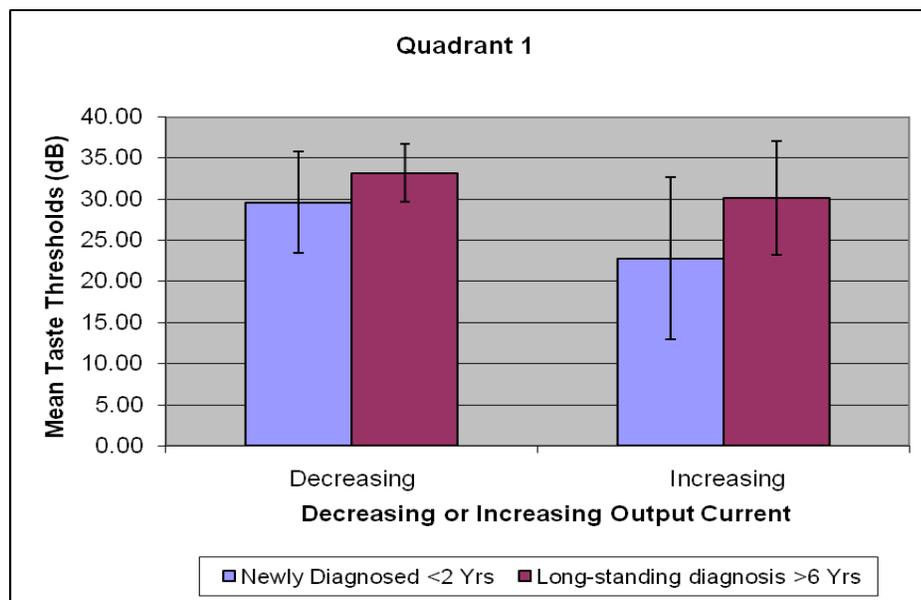


Figure 8. Quadrant 1 Mean Taste Thresholds Measured in Decibels (dB) with Decreasing and Increasing Output Current for both Newly-Diagnosed (< 2 years) and Long-standing Diagnosed (> 6 years) Type 2 diabetes mellitus subjects.

The mean taste thresholds for Quadrant 2 are shown below in Fig. 9. For the increasing output currents, the long-standing diagnosed diabetics had a higher mean taste threshold value of 27.83 dB compared to 16.80 dB mean taste threshold value for the newly-diagnosed diabetic subjects. In the decreasing output current, the long-standing diabetics had a mean taste threshold value of 29.00 dB which was higher than the 25.60 dB mean taste threshold value for the newly-diagnosed diabetic subjects.

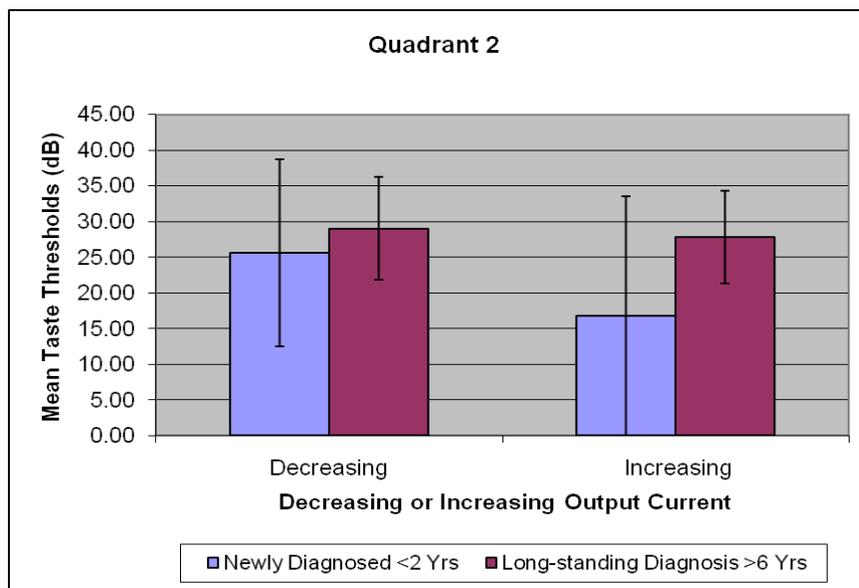


Figure 9. Quadrant 2 Mean Taste Thresholds Measured in Decibels (dB) With Decreasing and Increasing Output Current for both Newly-Diagnosed (< 2 years) and Long-standing Diagnosed (> 6 years) Type 2 diabetes mellitus subjects.

The mean taste thresholds for Quadrant 3 are shown below in Fig. 10. For the decreasing output current, the long-standing diagnosed diabetics had a lower mean taste threshold value of 28.00 dB compared to 33.60 dB mean taste threshold value for the newly-diagnosed subjects. It was the opposite case when increasing output current; the long-standing diabetics had a mean taste threshold value of 29.83 dB which was higher than the 17.20 dB mean taste threshold value for the newly-diagnosed diabetic subjects.

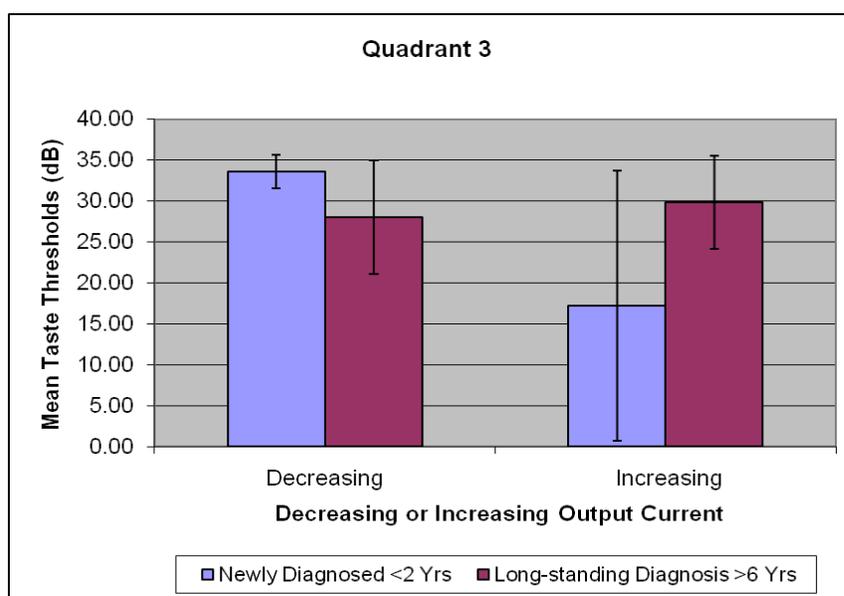


Figure 10. Quadrant 3 Mean Taste Thresholds Measured in Decibels (dB) With Decreasing and Increasing Output Current for both Newly-Diagnosed (< 2 years) and Long-standing Diagnosed (> 6 years) Type 2 diabetes mellitus subjects.

The mean taste thresholds for Quadrant 4 are shown below in Fig. 11. For the increasing output currents, the long-standing diagnosed diabetics had a higher threshold value of 26.67 dB compared to 19.60 dB mean taste threshold value for the newly-diagnosed diabetic subjects. In the decreasing output current, the long-standing diabetics had a mean taste threshold value of 31.33 dB which was higher than the 30.00 dB mean taste threshold value for the newly-diagnosed diabetic subjects.

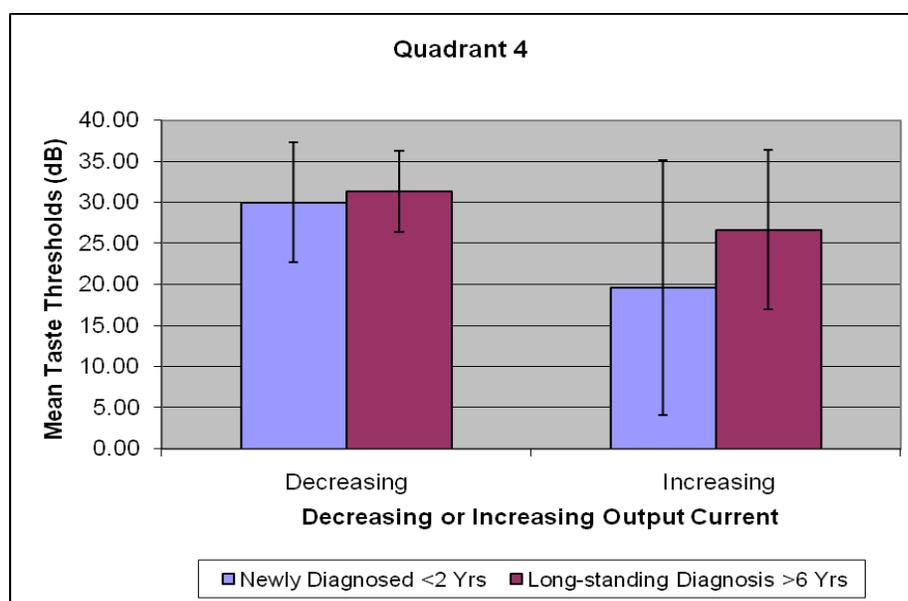


Figure 11. Quadrant 4 Mean Taste Thresholds Measured in Decibels (dB) With Decreasing and Increasing Output Current for both Newly-Diagnosed (< 2 years) and Long-standing Diagnosed (> 6 years) Type 2 diabetes mellitus subjects.

Summary: Although no significant differences in taste thresholds were found between newly-diagnosed and long-standing diagnosed diabetics when comparisons were made quadrant by quadrant using a univariate analysis, a definite trend towards decreasing sensitivity was seen from these comparisons (Appendix A). With the exception of threshold determination by decreased current application in Quadrant 3 every other threshold determination reflected a higher threshold in taste sensitivity in the

long-standing diabetic subjects in every quadrant of the tongue. An additional univariate analysis was performed after grouping the data for all four quadrants of each subject into results for either increasing or decreasing current application. No consistent distribution of the subjects in either group towards an increased taste threshold was detected (Appendix B Table 1). However, when the threshold data for increasing current application was combined for all 4 quadrants of each subject in the newly diagnosed group and compared to a similar combination for the long-standing diagnosed group a significantly higher taste threshold was detected in the latter group ($p=0.005$).

Initial analysis of the data was performed by completing a one Factor Analysis of Variance comparing each quadrant individually for both increasing and decreasing current application in subjects with newly diagnosed and long-standing diagnosed Type 2 diabetes mellitus (Appendix B Table 1). The results of this analysis did not confirm a significant difference between the two populations. However when a one Factor Analysis of Variance was applied to the combined data for each group a significantly elevated taste threshold was demonstrated, $p=0.005$ (Appendix B Table 2).

Based upon the number of subjects included in this proof of concept/feasibility study a Two-Sample T-Test Power Analysis was applied to determine how many additional subjects would be needed to obtain statistical significance for comparisons between groups for each quadrant (Appendix C):

1. Decreasing current application - 32 subjects for Quadrant 1,
153 subjects for Quadrant 2,
21 subjects for Quadrant 3, and
364 subjects for Quadrant 4.

2. Increasing current application - 22 subjects for Quadrant 1,
23 subjects for Quadrant 2,
17 subjects for Quadrant 3, and
54 subjects for Quadrant 4 (Appendix C).

Discussion

This study was undertaken to address the lack of non-invasive, cost-effective methods to assess the status and progression of Type 2 diabetes mellitus. Presently, blood tests for glucose levels and glycosylated hemoglobin are the standard methods used to monitor patients.

Changes in taste threshold sensitivity using electrogustometry may provide an alternative approach to monitor the progression of Type 2 diabetes since these results suggest that differences in taste threshold sensitivity may be related to the duration of the disease. This study was a proof-of-concept study, with a small number of subjects enrolled and evaluated and the results warrant further investigation. The rationale for increasing the number of test subjects would be to facilitate subject matching by factors such as ethnicity and lifestyle which all may be variables that contribute to changes in taste threshold sensitivity.

Confounds

Several confounds of communication, severity of the disease, demographics, uncertain date of disease onset, and unanticipated events were present in this study.

Communication

Despite initial queries of each potential subject regarding their smoking habits – patients were later found by the other investigators to be smokers, immediately prior to testing. One probable explanation for the withholding of this information could have been their reluctance to admit this behavior to their physician. These subjects were removed from the study since smoking has been shown to affect taste and was part of the exclusion criteria for subjects to participate in this study (25).

Severity of the disease

Some subjects with long-standing Type 2 diabetes were unable to detect the stimulus delivered by the electrogustometer. Apparently, their taste thresholds exceeded the ability of the instrument to generate a large enough signal to be detected. The electrogustometer only has a maximum current output of 34 decibels, and some longstanding diagnosed subjects could not perceive this stimulus. Anecdotal information indicated that they probably did have much higher thresholds. This was evidenced by comments such as “having trouble tasting on occasion,” and one of these subjects also had wound healing difficulties. Both factors point towards possible compromised circulation that may have contributed to their inability to detect the stimuli.

Demographics

The subjects were chosen solely on the basis of disease duration; less than 2 years or greater than 6 years. None of the subjects in either group were matched according to age, sex, ethnicity, lifestyle, or any other variable. Individual subjects were taking medications to treat conditions other than those directly related to their Type 2 diabetes mellitus. These additional medications also could have affected taste sensitivity. Most subjects had hypertension and hypercholesterolemia and were on medications for these conditions.

Uncertain date of disease onset

Perhaps the diabetes in “newly diagnosed” subjects went undetected for an extended period of time before they were officially diagnosed by a physician. Their taste sensitivity may have already been compromised by their disease.

Unanticipated events

Two incidents involved the use of chairs with metal arms in which the subjects were seated during testing. One subject evidenced a unique response to the testing. The subject mentioned a tingling sensation on the arm, provoked by the delivery of the stimulus to the tongue, at the site on his arm where the ground clamp was placed. When questioned, the subject stated that there was a metal plate placed in his forearm when a bone break was repaired many years prior to this test. The ground clamp was moved to the opposite arm, but a similar sensation was evoked in that same location as before when the current was applied through the probe to the tongue. It was hypothesized that the metal plate provided a pathway of lesser resistance for the current and dissipated the energy directed at the tongue site. Due to the unique nature of the result with this subject, testing was ceased immediately, and the data was not included in this study.

Another subject also evidenced a unique response to the testing. The subject mentioned a tingling sensation on the arm, provoked by the delivery of the stimulus to the tongue, at the site on her arm where the ground clamp was placed. The subject did have a metal watch and jewelry on the same arm as the ground clamp. The subject was required to remove the watch and jewelry, and the testing resumed. Again, the subject stated there was a tingling sensation on the arm with the ground clamp when the current was turned on. The subject then was instructed to place the arm with the ground clamp on their lap instead of on the metal arm of the chair they were seated in. The testing resumed from the beginning, and finally no more sensations were experienced in the arm with the ground clamp. The resulting data was included in the analysis. After the second incident occurred, it was determined that metal arm of the chair was causing interference,

grounding the subject, so all future testing had the subjects seated on a wood based examination table.

Conclusions

Even with all of the variables factored into the study, we were still able to detect a trend, that the long-standing diagnosed diabetics had a decreased taste sensitivity compared to the newly-diagnosed diabetics. This trend was similar for Quadrants 1, 2, and 4 in the delivery of decreasing and increasing output currents and in Quadrant 3 only with the increasing output. For the delivery of decreasing output current, the trend was opposite; newly-diagnosed subjects had a higher mean taste threshold value in Quadrant 3 than the long-standing diagnosed subjects. What is unusual is that this was a unilateral difference not bilateral. Both posterior quadrants 1 and 3 are innervated by the chorda tympani nerve, why only one side was affected is not apparent.

As stated previously, reasons for only the increasing current data being statistically significant and not the decreasing current was most likely due to the small subject population, and the inability to match the two groups of diabetics to one another. Repeated testing on each subject would give a better understanding of the precision of the observations in each quadrant.

Continuing this study with an enhanced number of more closely matched subjects as well as repeated testing of the same subject is certainly feasible as it is both cost and time-effective. Statistically significant results from a larger study would confirm the value of this test for physicians managing their Type 2 diabetic patients in their office environment and would complement the current routine laboratory tests used to monitor the severity of the disease.

Although the duration and limited number of subjects enrolled in this preliminary study limited the number of assumptions that could be confirmed, sufficient evidence of a correlation between taste thresholds and duration of disease was obtained to warrant an expanded investigation.

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APPENDIX A**ELECTROGUSTOMETRY RESULTS IN PATIENTS WITH NEW (<2 YEARS) AND EXISTING (>6 YEARS) TYPE 2 DIABETES
UNIVARIATE ANALYSIS BY QUADRANT, INCREASING OR DECREASING CURRENT, AND DURATION OF DISEASE**

QUADRANT	INCREASING/ DECREASING CURRENT	DURATION OF DISEASE	N	ARITHMETIC AVERAGE	STANDARD DEVIATION	MEDIAN	MIN. VALUE	MAX. VALUE	95% CONFIDENCE INTERVALS	
									LOWER	UPPER
QUADRANT 1	DECREASING	EXISTING DIAGNOSIS >6 YRS	6	33.17	3.54	34.50	26.00	35.00	29.45	36.89
QUADRANT 1	DECREASING	NEWLY DIAGNOSED <2 YRS	5	29.60	6.19	30.00	20.00	35.00	21.92	37.28
QUADRANT 1	INCREASING	EXISTING DIAGNOSIS >6 YRS	6	30.17	6.91	33.50	18.00	35.00	22.91	37.42
QUADRANT 1	INCREASING	NEWLY DIAGNOSED <2 YRS	5	22.80	9.86	24.00	10.00	34.00	10.56	35.04
QUADRANT 2	DECREASING	EXISTING DIAGNOSIS >6 YRS	6	29.00	7.21	31.00	16.00	35.00	21.43	36.57
QUADRANT 2	DECREASING	NEWLY DIAGNOSED <2 YRS	5	25.60	13.13	34.00	6.00	35.00	9.30	41.90
QUADRANT 2	INCREASING	EXISTING DIAGNOSIS >6 YRS	6	27.83	6.46	28.00	18.00	35.00	21.05	34.62
QUADRANT 2	INCREASING	NEWLY DIAGNOSED <2 YRS	5	16.80	16.69	6.00	2.00	35.00	-3.93	37.53
QUADRANT 3	DECREASING	EXISTING DIAGNOSIS >6 YRS	6	28.00	6.90	29.00	18.00	35.00	20.76	35.24
QUADRANT 3	DECREASING	NEWLY DIAGNOSED <2 YRS	5	33.60	2.07	34.00	30.00	35.00	31.03	36.17
QUADRANT 3	INCREASING	EXISTING DIAGNOSIS >6 YRS	6	29.83	5.71	30.50	24.00	35.00	23.84	35.82
QUADRANT 3	INCREASING	NEWLY DIAGNOSED <2 YRS	5	17.20	16.51	10.00	2.00	35.00	-3.30	37.70
QUADRANT 4	DECREASING	EXISTING DIAGNOSIS >6 YRS	6	31.33	4.97	33.00	22.00	35.00	26.12	36.55
QUADRANT 4	DECREASING	NEWLY DIAGNOSED <2 YRS	5	30.00	7.31	34.00	18.00	35.00	20.92	39.08
QUADRANT 4	INCREASING	EXISTING DIAGNOSIS >6 YRS	6	26.67	9.67	30.00	14.00	35.00	16.52	36.81
QUADRANT 4	INCREASING	NEWLY DIAGNOSED <2 YRS	5	19.60	15.50	20.00	4.00	35.00	0.35	38.85

Appendix B.

ELECTROGUSTOMETRY RESULTS IN PATIENTS WITH NEW (<2 YEARS) AND EXISTING (>6 YEARS) TYPE II DIABETES
TABLE 1: UNIVARIATE ANALYSIS BY INCREASING OR DECREASING CURRENT, AND DURATION OF DISEASE

INCREASING/ DECREASING CURRENT	DURATION OF DISEASE	N	ARITHMETIC AVERAGE	STANDARD DEVIATION	MEDIAN	MIN. VALUE	MAX. VALUE	95% CONFIDENCE INTERVALS	
								LOWER	UPPER
DECREASING	EXISTING DIAGNOSIS >6 YRS	24	30.38	5.83	33.00	16.00	35.00	27.91	32.84
DECREASING	NEWLY DIAGNOSED <2 YRS	20	29.70	8.06	34.00	6.00	35.00	25.93	33.47
INCREASING	EXISTING DIAGNOSIS >6 YRS	24	28.63	7.00	31.00	14.00	35.00	25.67	31.58
INCREASING	NEWLY DIAGNOSED <2 YRS	20	19.10	13.90	18.00	2.00	35.00	12.60	25.60

ELECTROGUSTOMETRY RESULTS IN PATIENTS WITH NEW (<2 YEARS) AND EXISTING (>6 YEARS) TYPE II DIABETES
**TABLE 2: RESULTS FROM THE ONE-FACTOR [DISEASE] ANALYSIS OF VARIANCE TESTS PERFORMED ON THE
 THRESHOLD VALUES BY INCREASING OR DECREASING CURRENT**

INCREASING OR DECREASING CURRENT	DEGREES OF FREEDOM	TYPE III SUMS OF SQUARES	F-VALUE	PROBABILITY VALUE
DECREASING	1	4.970455	0.1036	0.749
INCREASING	1	989.734091	8.6648	0.005

Appendix C. Two-Sample T-Test Power Analysis

Table 2. Two-sample T-test for decreasing and increasing current. The number of subjects needed to attain a power 80%; meaning 80% of the time it would be possible to detect a significant difference if a significant difference existed. N1 and N2 are the sample sizes needed. Alpha = default significance level of 0.05. Beta is used in calculating the power of the test. S1 and S2 are the standard deviations for mean 1 and 2 respectively.

For both Decreasing and Increasing Currents and all quadrants the following applies:

Null Hypothesis: Mean1=Mean2,

Alternative Hypothesis: Mean1<>Mean2,

The standard deviations were assumed to be unknown and unequal.

The following tables list the numeric results for Two-Sample T-Test.

DECREASING CURRENT										
				Allocation						
	Power	N1	N2	Ratio	Alpha	Beta	Mean 1	Mean 2	S1	S2
Quadrant 1	0.80098	32	32	1.000	0.05000	0.19979	30.4	29.7	5.8	8.1
Quadrant 2	0.80012	153	153	1.000	0.05000	0.19988	29.0	25.6	7.2	13.1
Quadrant 3	0.80074	21	21	1.000	0.05000	0.19926	29.0	33.6	6.9	2.1
Quadrant 4	0.80029	364	364	1.000	0.05000	0.19971	31.3	30.0	5.0	7.3
INCREASING CURRENT										
				Allocation						
	Power	N1	N2	Ratio	Alpha	Beta	Mean 1	Mean 2	S1	S2
Quadrant 1	0.80094	22	22	1.000	0.05000	0.19906	30.2	22.8	6.9	9.9
Quadrant 2	0.81206	23	23	1.000	0.05000	0.18794	27.8	16.8	6.5	16.7
Quadrant 3	0.80848	17	17	1.000	0.05000	0.19152	29.8	17.2	5.7	16.5
Quadrant 4	0.80592	54	54	1.000	0.05000	0.19408	26.7	19.6	9.7	15.5

Appendix D. Subject Health History Questions and Answers

All subjects were non-smokers and never had any injuries/diseases of the tongue and/or oral cavity.

Subject	Gender	Age	Type 2	Diagnosed	Family History	Glucose study done?	Suffer from metabolic diseases?	Ethnicity	Height	Weight (lbs)	BMI
1	M	49	Yes	3 months	Yes (Father)	No	No	Caucasian	5'11"	235	32.8
2	M	57	Yes	2 yrs 3 months	No	No	No	African-American	6'1"	230	30.3
3	M	60	Yes	6 yrs	No	No	No	Caucasian	6'2"	260	33.4
4	M	50	Yes	10 years	Yes (Father, sister, brother)	Yes	No	Caucasian	6'0"	240	32.5
5	M	71	Yes	15 years	Yes (Mother)	No	No	African-American	6'1"	194	25.6
6	M	37	Yes	1.5 years	No	No	Yes (Metabolic syndrome)	Caucasian	5'9"	270	39.9
7	F	48	Yes	20 years	Yes (Mother)	No	No	African-American	5'3"	228	40.4
8	F	73	Yes	8 years	No	No	No	African-American	5'5"	142	23.6
9	F	52	Yes	6 months	Yes (Father & Mother)	Yes	No	African-American	5'5"	202	33.6
10	F	69	Yes	6 years	Yes (Mother, Father, and Siblings)	No	No	Caucasian	5'6"	217	35.0
11	F	63	Yes	1 year 4 months	Yes (Father, Grandmother father's side)	No	No	Caucasian	5'0"	195	38.1