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Comparison of Electrogustometrically Determined Taste Threshold and Phenylthiocarbamide Sensitivity between Non-Diabetic Subjects with First Degree Relatives with Type 2 Diabetes and Non-Diabetic Subjects without Type 2 Diabetic First Degree Relatives

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Philadelphia College of Osteopathic Medicine
The Graduate Program in Biomedical Sciences
Department of Neuroscience, Physiology, and Pharmacology

Comparison of Electrogustometrically Determined Taste Threshold and Phenylthiocarbamide
Sensitivity between Non-diabetic Subjects with First Degree Relatives with Type 2 Diabetes and
Non-Diabetic Subjects without Type 2 Diabetic First Degree Relatives

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

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Abstract

Diabetes is a systemic degenerative disease, having adverse effects on many different organ systems. Nerve conduction specifically related to taste is one of the affected functions and can lead to altered taste perceptions and taste thresholds. Electrogustometry was used in this study to evaluate taste thresholds, and taste sensitivity was tested using phenylthiocarbamide-impregnated testing strips. Healthy non-diabetic subjects were divided into two experimental groups: One with a first degree relative with Type 2 diabetes and the other matched controls having no family history of Type 2 diabetes. The hypotheses: 1). There is a significant difference in taste threshold values and sensitivity to phenylthiocarbamide in non-diabetic subjects with a first degree Type 2 diabetes family history; 2) There is a significant difference in the ability to taste phenylthiocarbamide in these same subjects. The data did not indicate a significant difference ($p > 0.05$) between groups for either of these hypotheses, however a trend towards significance ($p > 0.05 < 0.10$) was detected in taste thresholds in quadrant four when current was applied in increasing increments for subjects with a first degree Type 2 diabetes family history. Since the subject population was small and all were young and healthy, it is possible that small differences might not have been possible to discriminate. Another confounding factor, the perception of taste threshold by the subject, may have influenced the outcome of the study and future investigations with repeated measures on different testing days/time before or following meals etc are required to confirm or disprove these findings.

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Introduction

The goal of this study was to determine if changes in electrogustometric taste thresholds (the stimuli at which taste sensation is detected and below which no taste sensation can be elicited), and sensitivity to the taste of phenylthiocarbamide (a dominant genetic trait) in non-diabetic subjects with a first degree family history of Type 2 diabetes differed from those of normal subjects. If changes in these parameters could be detected, they might lead to the early identification of subjects at risk for Type 2 diabetes, and encourage clinicians to make similar evaluations on their patient populations using these relatively low cost methods.

Background

Diabetes mellitus includes a number of dysfunctional metabolic processes that produce an abnormally elevated blood sugar (hyperglycemia) that arise from a failure to properly secrete or utilize insulin. The form of this disease that is most prevalent and steadily increasing in the general population is Type 2 diabetes mellitus. It is an insidious disease because it is often not diagnosed in its early stages before it produces lasting and harmful changes in the body (6).

Delay in diagnosing Type 2 diabetes can lead to many complications in various organ systems that increase the risk for hypertension and stroke or coronary heart disease, dyslipidemia and obesity, peripheral vascular disease and disrupted nerve conduction, especially common in the lower extremities. Yet another manifestation of Type 2 diabetes mellitus is decreasing taste sensitivity. Le Floch, et al. demonstrated that the taste thresholds increase (an indication of loss of taste sensation) as a patient ages and develop further complications from Type 2 diabetes mellitus (1). Brownlee working in our laboratory, demonstrated that Type 2 diabetes mellitus

patients had higher taste thresholds (less taste sensitivity) than non diabetics and suggested that the decreased sensitivity might contribute to a tendency to add extra sugar, salt, etc to increase the palatability of food and thus indirectly lead to detrimental weight gain (2).

The American Diabetes Association recommends testing for type 2 diabetes mellitus following the screening of at risk populations. Presently, screening methods are not carried out routinely on the specific at risk population studied here (those with relatives with Type 2 diabetes mellitus) and the initiative to do so often rests on an individual clinician's judgment or patient preference (6). The fasting plasma glucose test is an appropriate method for diagnosing and/or screening for Type 2 diabetes mellitus and regular testing of Hemoglobin A1C levels (glycosylated hemoglobin) is an accepted method for following the course of the disease. This test measures blood sugars over the span of three months, so at times it can further delay diagnoses of type 2 diabetes. Given that neurodegenerative changes in taste thresholds and sensitivity to certain chemical compounds have been shown to decrease in Type 2 diabetic patients, it is plausible that evaluation of electrogustometrically determined taste thresholds and sensitivity to phenylthiocarbamide would be an inexpensive, less invasive way to screen for undiagnosed Type 2 diabetes mellitus (3,4,5). Advances in diagnosing and screening for Type 2 diabetes mellitus may also lead to earlier detection of at risk populations.

Diabetes Mellitus

Type 2 Diabetes mellitus disease progression is normally the result of many contributing factors. These include a resistance to insulin action and a deficiency of insulin secretion. It has many clinical complications. Not all Type 2 patients are insulin dependent; however some do eventually progress to insulin dependency (6). Type 2 diabetes was previously an adult disease

in the years prior to 2000, predominately occurring in adults aged 40 and over. The increased obesity and inactivity in children has been associated with the rise in Type 2 diabetes seen in younger populations (6). It is more common in Hispanics, Native Americans and African Americans compared to Caucasians. Although decrease in weight may help alleviate insulin resistance in these populations, there are no guarantees that it will completely reverse insulin resistance in these patients (6, 7).

Several studies have evaluated screening methods for Type 2 diabetes. Lu et al. evaluated the diagnostic efficacy and efficiency of Hemoglobin A1C to rule in or rule out diabetes (8). They found that, while it is efficient to rule out diabetes at a lower level ($<5.5\%$), a higher value of 7.0% is needed to efficiently rule in diabetes, although current recommendations are for $>6.5\%$ (18). The test is highly accurate, but it is also an expensive blood test that requires repeat testing every few months to accurately evaluate changes. Balion et al. demonstrated poor reproducibility of impaired glucose tolerance tests and impaired fasting glucose tests in the prediabetes group and in patients at risk for developing diabetes Type 2. Therefore, multiple tests are needed to confirm a diagnosis (9).

Patients with diabetes, especially Type 2 are at an increased risk for developing microvascular and macrovascular complications and diabetes mellitus is a major cause of blindness. Diabetic retinopathy is a contributor to blindness in adult patients aged starting as early as 20 going up to 74. Diabetes mellitus, more specifically Type 2, is the leading cause of end stage renal disease in the United States. It also is a risk factor for coronary artery disease (6,7). As of 2007, diabetes mellitus is the leading cause of nontraumatic lower limb amputations in the United States (7).

Electrogustometry

Although a correlation has been shown between Type 2 Diabetes and electrogustometry, but there is a lack in evidence related to electrogustometry as a screening tool for diabetes. Electrogustometry is a useful method for evaluating taste thresholds in the clinical setting (10). An electrogustometer operates on 4 AA batteries and emits weak electrical currents (4 μ A- 100 μ A) into the tongue, which stimulates the gustatory receptors. It is used to quantitatively measure taste thresholds. When the taste bud receptor is stimulated electrically the subject perceives a metallic displeasing taste unlike the more familiar taste qualities evoked by chemical stimulation of the taste buds. Taste testing using chemical tastants applied to the tongue (sweet, sour, salty, bitter, umami) is another alternative to electrogustometry and has been used to evaluate the linkage between obesity, and Type 2 diabetics (11). However, electrogustometry offers several practical advantages over chemical testing: the electrical stimulus can be localized to a very small area, sensitivity to the stimulus can be quantitatively measured by patient feedback response to a precisely controlled electrical current intensity scale, and the electrogustometer is portable, relatively inexpensive, non-invasive, and can be used at the patient's bedside or in the office (12). In the past, the electrogustometer has been used to evaluate regional differences in supra-threshold (i.e., taste sensitivities above the normal threshold) sensitivity in diabetic patients both in our laboratory and by others (1,2).

Phenylthiocarbamide Testing

The PTC (phenylthiocarbamide) taste sensitivity test is also a clinical tool has been used in clinical research as well as in lower grade and high school biology classes (4,13). Studies have indicated that subjects with Type 2 diabetes mellitus are not able to taste phenylthiocarbamide (5). Studies on PTC taste blindness (inability to taste chemical PTC) and

effects of diabetes mellitus on taste thresholds of certain chemical compounds has been done in the past (14). However, research is lacking on PTC taste ability of non-diabetic relatives of diabetics, and on this association with alterations in electrogustometry- determined taste thresholds in this same population. Therefore, one aim of this research is to evaluate this response to see if differences can be detected that may eventually serve as early indicators of the future development of overt diabetes.

Taste Physiology

The gustatory receptors detect each of the five different senses of taste. They are located in the papillae of the tongue and the mucosa of the epiglottis, soft palate, and pharynx. These receptors are responsible for translating molecules (tastants) into taste signals. There are taste receptors located in the taste buds that are distributed to the four types of papillae: foliate, filiform, fungiform, and the circumvallate.

Neurons that make up the chorda tympani branch off the cranial nerve VII and innervate taste buds located on the anterior 2/3 of the tongue. This includes the anterior palate mucosa and the fungiform papillae. The posterior 1/3 of the tongue is innervated by the glossopharyngeal nerve (IX). This includes the foliate and circumvallate papillae. The oral pharyngeal taste bud field, with taste buds occurring in mucosa of larynx and epiglottis is innervated by the vagus nerve (cranial nerve X) but are not included in the electrogustometric testing in this study (15).

The receptor stimulation of the taste buds leads to the activation of the first order neurons of cranial nerves VII, IX, and X. The axons of these neurons terminate in the nucleus of the solitary tract. Second order fibers ascend ipsilaterally and terminate within the ventral posterior medial (VPM) nucleus of the thalamus. Lastly third order fibers terminate from the VPM to the

primary taste cortex (postcentral gyrus, frontal operculum, and the rostral insula) and centers in the forebrain including the amygdala (16).

There are five different types of taste qualities, sweet, sour, salty, bitter, and unami. Sour and bitter are located at the posterior of the tongue, while the other three are located more toward the anterior of the tongue to the tip (17).

Purpose of Study

To evaluate a clinical screening method to predict the onset of diabetes mellitus prodromally in patients with a family history of Type 2 diabetes mellitus but who are not currently diabetic.

Specific Aims

- A. To compare the electrogustometrically determined taste thresholds of subjects who have a first degree relative with Type 2 diabetes mellitus to the taste thresholds of matched controls who do not have relatives with Type 2 diabetics.
- B. To compare the sensitivity to the taste of Phenylthiocarbamide in subjects who have a first degree relative with Type 2 diabetes mellitus to the taste thresholds of matched controls who do not have relatives with Type 2 diabetes.
- C. To determine whether either method is effective to use as a less invasive and cost efficient way to screen individuals who possibly may be at risk of developing Type 2 diabetes mellitus

Specific Hypotheses

1. There is a significant difference in taste threshold values of healthy subjects who have first degree Type 2 diabetic relatives and those who do not have first degree Type 2 diabetic relatives.

Null Hypothesis: There is not a significant difference in taste threshold values between healthy subjects who have first degree Type 2 diabetic relatives and those who do not have first degree Type 2 diabetic relatives.

2. There is a significant difference in taste sensitivity of Phenylthiocarbamide of healthy subjects who have first degree Type 2 diabetic relatives and those who do not have first degree Type 2 diabetic relatives.

Null Hypothesis: There is not a significant difference in taste sensitivity of Phenylthiocarbamide between healthy subjects who have first degree Type 2 diabetic relatives and those who do not have first degree Type 2 diabetic relatives.

Materials and Methods

Subjects

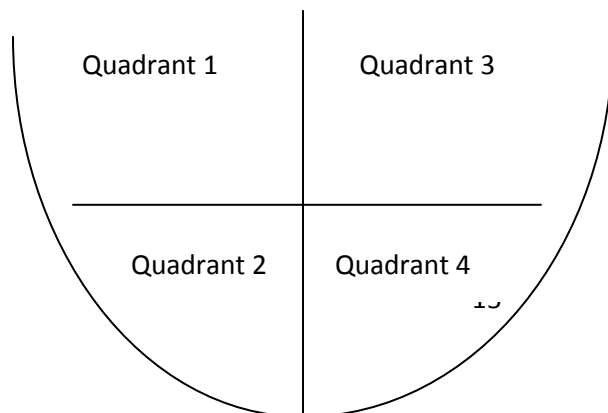
Volunteers were students aged 21-32 at Philadelphia College of Osteopathic Medicine. They volunteered to be a part of the study in response to general email announcements, written announcements during class, as well as presentations about the study. Participation requirements included: being non-diabetic, without a positive fasting blood glucose test within the past 5 years, no personal history of metabolic diseases, and either having or not having an immediate (not more than one degree removed) relative with Type II diabetes. Subjects were not required to produce a normal fasting blood glucose level, and not all of the subjects actually had a fasting blood glucose drawn. Smokers and users of tobacco based products also were excluded from participation because taste differences have been noted in heavy smokers in previous studies involving glucose load taste differences (18). Volunteers who have had injuries or serious,

debilitating pathologies of the tongue were also excluded from the study due to the possibility of permanently decreased taste ability.

Qualified volunteers were divided into two groups. Group I consisted of control subjects, volunteers with no previous history of Type II diabetes mellitus in themselves or primary relatives. The second group contained those with primary relatives (parents, grandparents, siblings) who have been diagnosed with Type II diabetes mellitus. Volunteers who have relatives that were not parents, grandparents, or siblings with Type II diabetes mellitus or those who had Type I diabetes were excluded from the study.

Test Quadrant Determination

Test quadrants were determined via the method used previously by Brownlee (2). The tongue was divided by an imaginary line drawn vertically through the median sulcus and extended from the proximal edge of the circumvallate region to the anterior tip of tongue. Another imaginary line was drawn from the midpoint of the vertical line to divide the tongue into four quadrants. quadrant 1: the right posterior quadrant, quadrant 2: the anterior right quadrant, quadrant 3: the posterior left quadrant and quadrant 4: the anterior left quadrant. Any distinguishing anatomy was noted in each quadrant that would facilitate repeated, accurate probe placement. The foliate and circumvallate papillae are located in quadrants 1 and 3, while the fungiform are located in quadrants 2 and 4. Cranial nerve VII innervates quadrants 2 and 4, while quadrants 1 and 3 are innervated by cranial nerve IX.



Schematic 1: Test Quadrant Tongue Schematic

Procedure

Subjects were contacted via telephone or email and a testing session was scheduled. The procedure was explained to the subject prior to testing, informed consents were signed, and each patient filled out a questionnaire that included diabetes family history, smoking/tobacco use history, and history involving any traumatic injuries to the tongue.

Stimulus probes were sterilized using Cidex solution (Johnson and Johnson Medical Incorporated, Arlington, TX) for 24 hours prior to use.

A Rion TR-06 electrogustometer was used to deliver low-amperage direct current (DC) via the contact tip of the stimulus probe directly to the dorsal surface of the tongue of the subjects. The probe causes neither a shock nor pain. The electrogustometer has an output current between -6dB and 34 dB (corresponding to $4\mu\text{A}$ - $100\mu\text{A}$). A single probe, circular steel, electrode plate 2 mm in diameter was used to deliver the current to the subject's tongue. When an appropriate taste threshold was reached, most subjects indicated a metallic unpleasant taste quality. The first round of testing began with a current of 34 dB. Using a switch on the electrogustometer that was invisible to the volunteer, the current was decreased by 2dB decrements until reaching a level where the volunteer could no longer detect the metallic sensation delivered by the electrogustometer, using a single staircase method. The single staircase method included a start at a high or suprathreshold level (34dB) and decreasing the level of current by 2 dB. The volunteer indicated to the researchers when that point was reached by use of a hand signal switch also attached to the electrogustometer that emitted an audible tone indicating a threshold measurement, expressed in decibels (dB). Researchers would interject sham signals to confirm validity of subject's ability to discriminate the signal. This was continued in each of four quadrants of the tongue. The second round of testing was identical to

the first round in procedure, except that the lowest or -6dB was the starting current. The investigators used a single staircase method of two db increasing increments until the subject indicated perception of the stimulus. Throughout this trial “sham currents” were inserted where no current would be emitted from the electrogustometer but the probe was still placed on the patients tongue. Application of the stimulus and control of stimulus intensity were accomplished by two different investigators working in concert with each other.

A filter paper strip was impregnated with PTC (phenylthiocarbamide) was placed on the dorsal aspect of the tongue of each subject following the electrogustometry testing. The subject was asked if he/she could detect a taste associated with the strip of PTC paper. During the PTC taste test, a dissatisfying sour, metallic-like taste may be briefly noted. This effect only lasts a few seconds. If that flavor was noted, it was concluded to be a positive test meaning that the subject was sensitive to PTC. One test was performed per subject.

Statistical Analysis

The least square mean values were compared between subjects with and without a family history of diabetes. Covariates were added to the analysis to determine if they affect the precision of the model. Significance of the least square mean changes is based on the type III sums of squares from the analysis of variance test.

To determine the appropriate sample size for this pilot investigation, an effect size of 1.3 was used to calculate the number of subjects that would need to be evaluated within the 2 arms of the study. These estimates are based on performing a two-sided test of significance to assess the detectable magnitude of the observable difference in the primary endpoint based on the standard deviation. The parameter of interest is a continuous variable that is assumed to be normally distributed, or can be transformed using a variance-stabilizing transformation to

normalize the distribution. Based on the proposed target sample size, if the standard deviation is less than or equal to 7/10ths of the observed difference, sufficient power will exist (>80%) to reject the null hypothesis in favor of the alternative. The table presented below provides additional estimates assuming a type I error rate of 5% and a homoscedastic population with equal variance. The Experimental Group is composed of subjects who have a family history of diabetes. The Control Group is composed of subjects who do not have a family history of diabetes (22, 23).

Table 1: Additional Estimates Assuming a Type I Error Rate of 5% and a Homoscedastic Population with Equal Variance

Power	Experimental Group (N)	Control Group (N)	Experimental Group Mean	Control Group Mean	Experimental Group Standard Deviation	Control Group Standard Deviation
0.80000	10	10	10.0	20.0	7.5	7.5
0.80000	10	10	10.0	21.0	8.3	8.3
0.80000	10	10	10.0	22.0	9.1	9.1
0.80000	10	10	10.0	23.0	9.8	9.8
0.80000	10	10	10.0	24.0	10.6	10.6
0.80000	10	10	10.0	25.0	11.3	11.3

Results

There were 41 subjects included in the data analysis. This included 21 subjects from the experimental group (has a primary relative with type 2 diabetes mellitus) and 20 subjects from the control group (no family history of type 2 diabetes mellitus). There were an additional three

subjects that were not included in the study. Two subjects had several distant family members with Type 2 diabetes mellitus; therefore they did not meet the inclusion criteria for the study. The third excluded subject had a brother with type 1 diabetes.

The results of the study were compared using a univariate analysis because the only differing factor between the control and experimental groups is the family medical history of Type 2 diabetes mellitus. Table 1 shows the data from the univariate analysis which summarizes the results by control and experimental groups. Probability values were derived from a 1-factor (gender) ANOVA. Significant differences in taste thresholds were considered to be present for probability values $p < 0.05$ and a trend towards significance considered to be present for probability values $p > 0.05 < 0.01$. Data for each trial (trial 1=high to low current; trial 2=low to high current.) was analyzed separately and comparisons were made between male and female group members for each quadrant of the tongue.

Location Description	Group	N	Mean	Standard Deviation	Median	Minimum Value	Max Value	95% CI Upper	95% CI Lower
Quad 1 DC	Cont.	20	22.50	11.62	27.00	0.00	34.00	17.06	27.94
Quad 1 DC	Exp.	21	20.76	10.54	18.00	8.00	34.00	15.97	25.56
Quad 2 DC	Cont.	20	24.80	10.45	29.00	8.00	34.00	19.91	29.69
Quad 2 DC	Exp.	21	22.95	11.86	28.00	4.00	34.00	17.55	28.35
Quad 3 DC	Cont.	20	25.30	9.21	27.00	4.00	34.00	20.99	29.61
Quad 3 DC	Exp.	21	20.86	10.95	18.00	4.00	34.00	15.87	25.84
Quad 4 DC	Cont.	20	26.70	9.91	33.00	4.00	34.00	22.06	31.34
Quad 4 DC	Exp.	21	20.76	9.77	20.00	8.00	34.00	16.32	25.21
Quad 1 IC	Cont.	20	18.00	9.08	19.00	2.00	34.00	13.75	22.25
Quad 1 IC	Exp.	21	13.52	10.31	14.00	-4.00	34.00	8.83	18.22
Quad 2 IC	Cont.	20	16.50	10.66	16.00	0.00	34.00	11.51	21.49
Quad 2 IC	Exp.	21	13.05	11.15	6.00	-4.00	34.00	7.97	18.12
Quad 3 IC	Cont.	20	13.10	7.77	16.00	2.00	24.00	9.46	16.74
Quad 3 IC	Exp.	21	13.90	11.37	14.00	-2.00	34.00	8.73	19.08
Quad 4 IC	Cont.	20	15.90	11.47	15.00	2.00	34.00	10.53	21.27
Quad 4 IC	Exp.	21	10.00	11.35	8.00	-4.0	34.00	4.83	15.17

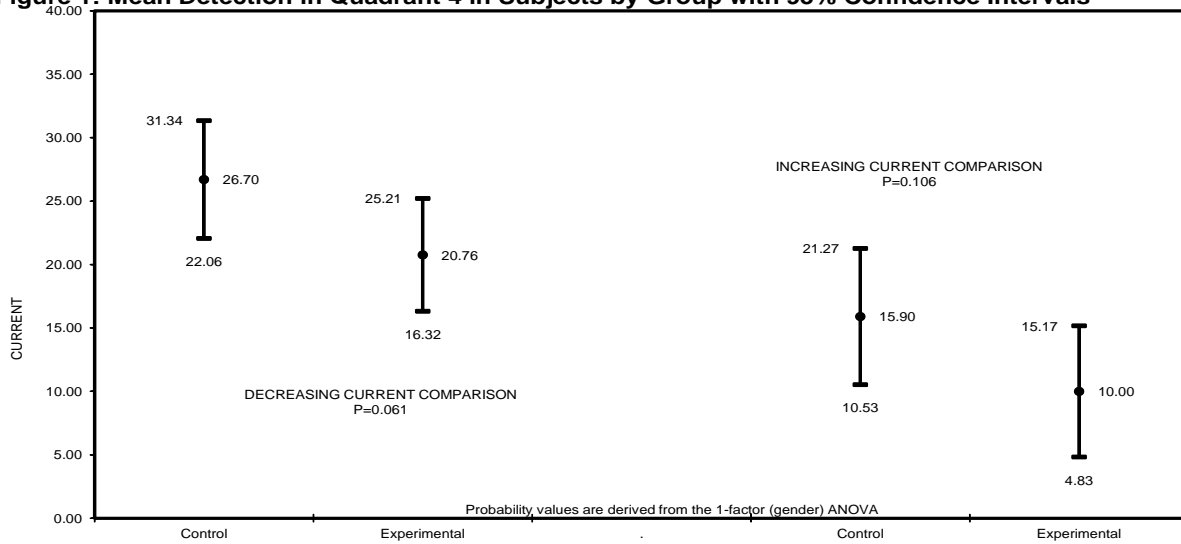
Table2: Univariate Analysis Comparing Control and Experimental Groups By Quadrant

Key: Quad – quadrant; IC – increasing current; DC – decreasing current; Cont – control; Exp – experimental; N- number of subjects; Max – maximum; CI – confidence interval; shaded areas signify statistical significance and will warrant further discussion

Table 2 presents the mean threshold for the subjects in each group, the minimum and maximum value threshold in each group and the 95% confidence intervals. Although the control and experimental group means did not differ very much, further analyses were undertaken to examine whether differences could be demonstrated between male and female subjects within both the control and experimental groups for each quadrant and for either trial 1 or 2. There was

one quadrant that showed a trend towards significant difference in taste thresholds: quadrant 4 for trial 1; going from high to low current. Figure 1 is a graphical representation of quadrant 4.

Figure 1: Mean Detection in Quadrant 4 in Subjects by Group with 95% Confidence Intervals



Location	Group	Gender	N	Mean	Standard Deviation	Min Value	Max Value	95% CI Lower	95% CI Upper
Quad 1 DC	Cont.	Female	12	20.33	12.41	0.00	34.00	12.45	28.22
Quad 1 DC	Cont.	Male	8	25.75	10.22	8.00	34.00	17.20	34.30
Quad 1 DC	Exp.	Female	12	21.33	11.55	8.00	34.00	14.00	28.67
Quad 1 DC	Exp.	Male	9	20.00	9.64	8.00	34.00	12.59	27.41
Quad 2 DC	Cont.	Female	12	22.67	10.49	8.00	34.00	16.00	29.33
Quad 2 DC	Cont.	Male	8	28.00	10.20	8.00	34.00	19.47	36.53
Quad 2 DC	Exp.	Female	12	23.50	12.48	4.00	34.00	15.57	31.43

Quad 2 DC	Exp.	Male	9	22.22	11.68	6.00	34.00	13.24	31.20
Quad 3 DC	Cont.	Female	12	22.00	10.23	4.00	34.00	15.50	28.50
Quad 3 DC	Cont.	Male	8	30.25	4.33	24.00	34.00	26.63	33.87
Quad 3 DC	Exp.	Female	12	20.50	11.82	4.00	34.00	12.99	28.01
Quad 3 DC	Exp.	Male	9	21.33	10.34	6.00	34.00	13.38	29.28
Quad 4 DC	Cont.	Female	12	23.00	11.00	4.00	34.00	16.01	29.99
Quad 4 DC	Cont.	Male	8	32.25	4.20	22.00	34.00	28.74	35.76
Quad 4 DC	Exp.	Female	12	22.67	10.03	8.00	34.00	16.29	29.04
Quad 4 DC	Exp.	Male	9	18.22	9.35	8.00	34.00	11.03	25.41
Quad 1 IC	Cont.	Female	12	15.17	8.84	2.00	34.00	9.55	20.78
Quad 1 IC	Cont.	Male	8	22.25	8.17	6.00	34.00	15.42	29.08
Quad 1 IC	Exp.	Female	12	12.00	9.98	-4.00	34.00	5.66	18.34
Quad 1 IC	Exp.	Male	9	15.56	10.99	2.00	34.00	7.11	24.00
Quad 2 IC	Cont.	Female	12	15.50	9.77	0.00	34.00	9.30	21.70
Quad 2 IC	Cont.	Male	8	18.00	12.42	0.00	34.00	7.62	28.38
Quad 2 IC	Exp.	Female	12	12.67	12.57	-4.00	34.00	4.68	20.65
Quad 2 IC	Exp.	Male	9	13.56	9.63	4.00	30.00	6.15	20.96
Quad 3 IC	Cont.	Female	12	11.67	7.38	2.00	24.00	6.98	16.35

Quad 3 IC	Cont.	Male	8	15.25	8.35	2.00	24.00	8.27	22.23
Quad 3 IC	Exp.	Female	12	10.50	10.76	-2.00	34.00	3.66	17.34
Quad 3 IC	Exp.	Male	9	18.44	11.13	6.00	32.00	9.89	27.00
Quad 4 IC	Cont.	Female	12	14.50	11.19	2.00	34.00	7.39	21.61
Quad 4 IC	Cont.	Male	8	18.00	12.33	2.00	34.00	7.69	28.31
Quad 4 IC	Exp.	Female	12	7.67	11.99	-4.00	34.00	0.05	15.29
Quad 4 IC	Exp.	Male	9	13.11	10.25	2.00	34.00	5.23	20.99

Table 3: Univariate Analysis By Quadrant, Group, and Gender

Key: Quad – quadrant; IC – increasing current; DC – decreasing current; Cont – control; Exp – experimental; N – number of subjects; Max – maximum; CI – confidence interval; Shaded areas will be explored in further discussion

The means in Table 3 were lower for the female subjects than the male subjects in every quadrant and trial except for quadrant 4 decreasing current (trial 1) in the experimental group. In almost every male group the control mean was higher than the experimental mean for taste threshold, meaning the experimental groups had higher taste acuity. The only exception was in quadrant 3, increasing current (trial 2). The female means demonstrated that in 6 out of 8 trials the experimental group's mean was higher than the control group. This would translate to a higher taste threshold for the experimental group which means they have lower taste acuity. The results would then also concur with the lower the taste threshold, the higher taste function acuity, which is what would be expected based on the original specific aims and hypothesis. The other two groups' results including quadrant 1 and 2 decreasing (both trial 1) were reversed with the control's mean being lower than the experimental mean. In general the female groups average taste thresholds were lower than the male groups taste thresholds.

Table 4: One Factor (Gender) analysis of Variance Test by Quadrant and Group

Quadrant	Group	Degrees of freedom	Probability Value
1- Decreasing Current	Control	1	0.320
1- Decreasing Current	Experimental	1	0.782
2- Decreasing Current	Control	1	0.275
2- Decreasing Current	Experimental	1	0.814
3- Decreasing Current	Control	1	0.046
3- Decreasing Current	Experimental	1	0.868
4-Decreasing Current	Control	1	0.037
4- Decreasing Current	Experimental	1	0.314
1-Increasing Current	Control	1	0.087
1-Increasing Current	Experimental	1	0.448
2-Increasing Current	Control	1	0.621
2-Increasing Current	Experimental	1	0.862
3-Increasing Current	Control	1	0.326
3-Increasing Current	Experimental	1	0.115
4-Increasing Current	Control	1	0.519
4-Increasing Current	Experimental	1	0.288

Table 4 contains the p-values related to a variance analysis by quadrant and group. Shaded areas would translate to areas of further discussion. Shaded areas signify areas that will be discussed further. A One Factor Analysis of Variance (ANOVA) was performed to compare the means for the Control and the Experimental Groups by gender in each quadrant and trial. The degrees of freedom are the number of areas in an analysis that are free to vary. There is only 1 here (that being gender). The significant p-values are determined by those less than 0.05. Significant differences were found in the control group for quadrant 3, trial 1 (decreasing current) and in the control group quadrant 4, trial 1 (decreasing current). There were also two p-values that were approaching significance; both were in trial 2 (increasing current). They were in quadrant 1 (control group) and quadrant 3 (experimental group).

Figure 2: Mean Detection of Decreasing Current in Control Subjects by Gender with 95% Confidence Intervals

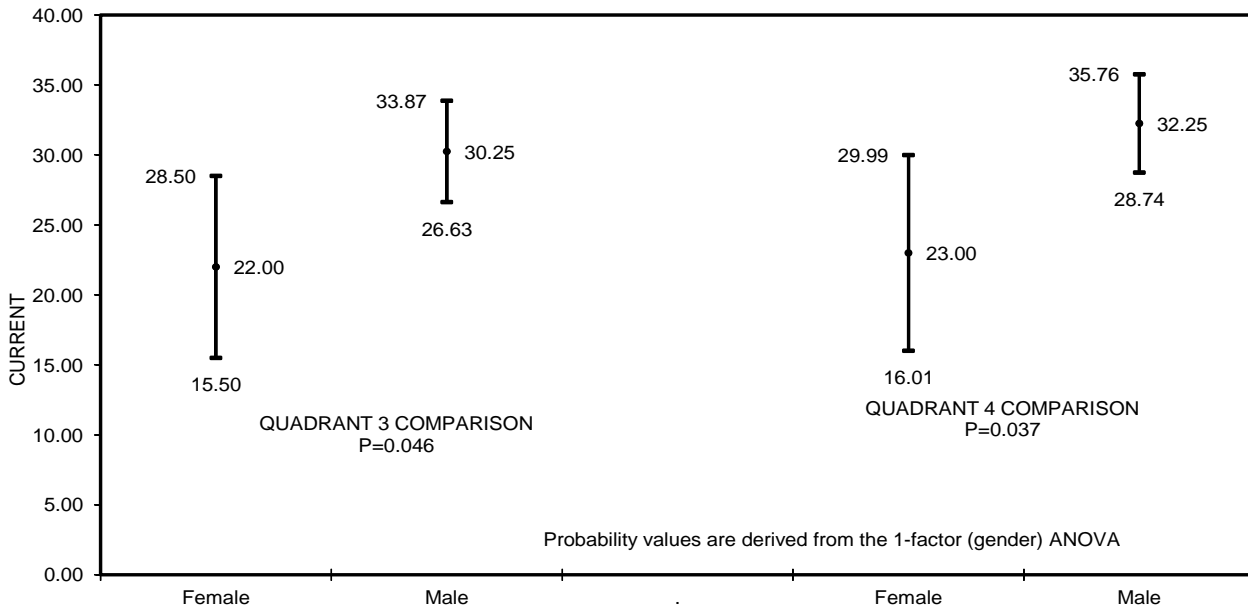


Figure 2 represents the mean and associated confidence intervals. The control group males demonstrated a significantly higher taste threshold than control group females in both

quadrants 3 and 4 for trial 1 (high to low current). This would translate to higher taste acuity in female subjects in both these groups for that quadrant specifically.

Figure 3: Mean Detection of Increasing Current in Subjects by Gender with 95% Confidence Intervals

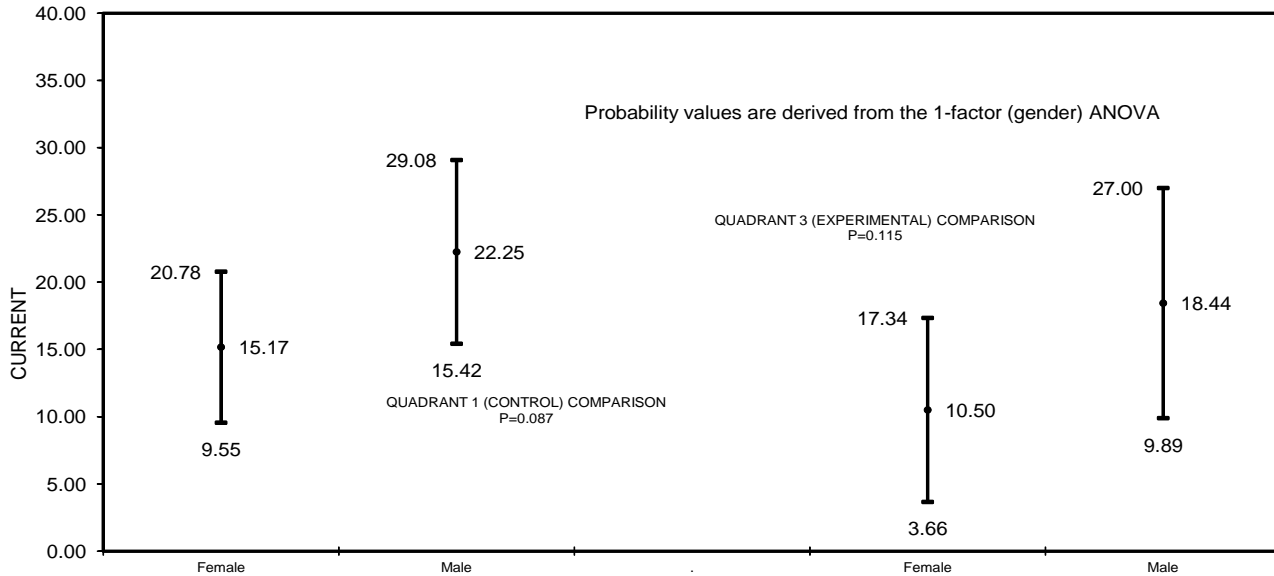


Figure 3 represents the mean and associated confidence intervals. The control group males demonstrated a trend towards significantly higher taste thresholds compared to the control group females in quadrant 1 for trial 2 (low to high current). Again, the male taste threshold means were higher than the female means for both quadrants, so female subjects have a higher taste acuity. In quadrant 3, it was the experimental group that had a p-value trending towards significance.

Quadrant	Degree of Freedom	Probability Value
1-Decreasing Current	1	0.618
2-Decreasing Current	1	0.600
3-Decreasing Current	1	0.169
4-Decreasing Current	1	0.061
1-Increasing Current	1	0.149
2-Increasing Current	1	0.317
3-Increasing Current	1	0.794
4-Increasing Current	1	0.106

Table 5: Results of One Factor (Group) Analysis of Variance Test by Quadrant; shaded areas will be discussed further

Table 5 contains the probability values determined by the Analysis of Variance (ANOVA) comparing quadrants by trial for the Control vs. the Experimental groups. No significant differences were found.

Despite the failure to reach the level of statistical significance, the following general observations could be made: The means were lower for the both the female control and experimental subjects than for the male subjects in every quadrant for trial 2 (low to high). This pattern also held true for the results of trial 1 (high to low) when comparing females to males in the control group. However, the reverse was true for the arithmetic means in trial 1 (high to low) for the experimental group where male taste thresholds were lower than the female taste thresholds except in quadrant 3. A summary of these observations is shown in Table 6.

Increasing Current	Male Control Means	Decreasing Current	Male Control Means
Quadrant 1	22.25	Quadrant 1	22.75
Quadrant 2	18.00	Quadrant 2	28.00
Quadrant 3	15.25	Quadrant 3	30.25

Quadrant 4	18.00	Quadrant 4	32.25
Increasing Current	Male Experimental Means	Decreasing Current	Male Experimental Means
Quadrant 1	15.56	Quadrant 1	20.00
Quadrant 2	18.00	Quadrant 2	22.22
Quadrant 3	18.44	Quadrant 3	21.33
Quadrant 4	13.11	Quadrant 4	18.22
Increasing Current	Female Control Means	Decreasing Current	Female Control Means
Quadrant 1	15.57	Quadrant 1	20.33
Quadrant 2	15.50	Quadrant 2	20.67
Quadrant 3	11.67	Quadrant 3	22.00
Quadrant 4	14.50	Quadrant 4	23.00
Increasing Current	Female Experimental Means	Decreasing Current	Female Experimental Means
Quadrant 1	12.00	Quadrant 1	21.33
Quadrant 2	12.67	Quadrant 2	23.50
Quadrant 3	10.50	Quadrant 3	20.50
Quadrant 4	7.67	Quadrant 4	22.67

Table 6: Means by Quadrant, Group and Trial

The PTC testing yielded varied results. For the control group 50% of the male subjects tasted the chemical (bitter, sour taste); while 50% male subjects did not taste the chemical. 66% (8/12) of the female subjects in control group tasted the chemical, while 33% did not. The experimental subjects yielded much different results. For the male subjects, seven out of nine (77.78%) tasted the chemical. Ten out of twelve (83.33%) of the female subjects in the experimental group tasted the chemical.

Discussion

The goal of this study was to determine whether subjects with primary relatives of Type 2 Diabetics could be identified by an increase in their taste thresholds and/or their ability to taste phenylthiocarbamide when compared to control subjects. The underlying rationale was to explore non-invasive, easily administered, and cost effective procedures that could provide markers that lead to earlier identification of individuals at risk for Type 2 diabetes mellitus.

The specific hypotheses for this study were that there would be a significant difference in taste threshold values and phenylthiocarbamide (PTC) sensitivity of healthy subjects with first degree Type 2 diabetic relatives compared to those without first degree Type 2 relatives.

The feasibility of this approach was supported by previous studies such as those of LeFloch, who demonstrated increased taste thresholds in Type 1 diabetes mellitus subjects, and suggested that "electric gustometry" could be an interesting test for early screening for diabetes complications (1). Likewise Bhatia and Sharma found that PTC taste-blind diabetics showed more impairment for sweet preferences which they interpreted as a decreased taste acuity in individuals with diabetes and suggested that this could be an important marker to chart the course of the disease (4).

Statistically higher thresholds were found for male control subjects in both quadrants 3 and 4 (quadrant 3 located in the posterior left of the tongue and quadrant 4 located on the anterior left) compared to females. Quadrants 3 and 4 are innervated by the glossopharyngeal and chorda tympani nerves respectively. This means that female subjects have higher taste acuity than males for those specific quadrants. In general the female groups' mean taste thresholds were lower than the male groups' mean taste thresholds although they did not reach the level of statistical significance. This is possibly due to enhanced taste acuity in female subjects as compared to

male subjects in general for this population of Americans in the age range of the study.

Yamauchi et al. also showed a correlation between increased taste thresholds in male subjects compared to female subjects. Specifically, he showed that male subjects have lower taste acuity than females, especially in sour, salty, and bitter (19). It is important to understand that higher taste thresholds translate to lower taste acuity, while lower taste thresholds indicate higher taste acuity.

A trend toward significance was similarly found in quadrants 1 and 3. These quadrants are both located posteriorly on the tongue, 1 being on the right and 3 being on the left. They are both innervated by the glossopharyngeal nerve. Again, the male taste threshold means were higher than the female means for both quadrants. In quadrant 3, the experimental groups' comparison was trending towards, but did not reach statistical significance. In quadrant 1, the control groups' comparison was trending towards, but did not reach statistical significance. Interestingly enough the special sense of taste occurs in the anterior two thirds of the tongue, an area innervated by the chorda tympani, which would correlate to quadrants 2 and 4. A follow up study could delve deeper into this relationship between the quadrants and the more significant research results. Overall, the most compelling data from the research was that which supported the observation that male subjects have lower taste thresholds than female subjects.

In both trials (decreasing and increasing) the taste threshold mean was higher in the control group, meaning the control subjects had the lower taste acuity. This is contrary to what was expected to be the results as well as the results from literature. The similarities between the experimental and control groups could be related to their actual ability to detect the threshold where the subject would no longer appreciate the taste sensation being delivered to the tongue. Ambiguity may weigh heavily on final determination of numbers. Or another likely explanation

could be an effect of the actual patient demographics. The subjects in our study consisted of 21 – 32 year old healthy, non-diabetic subjects. There are a number of studies that actually showed larger increases in taste thresholds when actual diabetic subjects were used (2, 4, and 5). In the other studies, older subjects were used, which also show a decline in taste may function in general, even in non-diabetics. Yamauchi et al. demonstrated an age related increase in taste threshold in male subjects, especially in salty, sour, and bitter (19). Lawson, et al. also showed that first degree relatives of diabetics had increased glucose threshold and increased sucrose thresholds (14). That study did not use electrogustometry, but did show a decrease in an aspect of taste acuity in healthy volunteers with diabetic first degree relatives.

The PTC value determinations demonstrated that the majority of the subjects tasted the chemical, based on the results from the study. This may be due to the taste acuity being better in younger subjects versus older subjects. No everyone could taste the material, which is not uncommon either. Some studies have shown decreases in PTC taste ability in diabetic patients (4, 5). The sweet taste area is located near the anterior portion of the tongue, where in general the PTC paper was placed on the subjects tested. This could relate to a correlation between an increased glucose taste threshold and an inability to detect the PTC chemical on the paper. The inability to detect the PTC then may be related to the decreased taste acuity in general in diabetics, or it could be due to a genetic inability to detect PTC. An interesting follow-up could include determining PTC testing ability in different stages of type 2 diabetes, including controlled, newly diagnosed, to uncontrolled, end stage patients.

Strengths of Study

The study's focus is to improve patient care and outcomes using less invasive and cost effective methods to determine diabetes development. The results could benefit patients at risk

for developing Type 2 diabetes mellitus, especially those with first degree relatives with Type 2 diabetes mellitus.

Limitations of Study

The study size (41 total subjects) itself may have not been large enough. A larger study size may also contribute to different outcomes. The age limitations (21-32) may have been a factor preventing different outcomes. Our subjects have not had a positive fasting blood glucose study in the past 5 years to their knowledge, but that may have been due to not being tested. The subjects could have had their fasting blood glucose tested to assure subjects did not fall into diabetic range.

Future Considerations

The correlation between electrogustometry and diabetes is one that could still use further research. There are many groups of patients and age ranges that can be explored. Perhaps an interesting correlation could include subjects of a more advanced age, with first degree relatives with diabetes compared with healthy subjects in a similar age range. Phenylthiocarbamide correlations could also lead to interesting results. Newer studies have shown correlations between handedness and taste function, so if that was taken into account perhaps the results may have been different or brought about new trends for research. Further explorations could include just looking at subjects that did not taste the chemical, then comparing subgroups that fall into that category. Our subjects were not divided by BMI (body mass index) or by heritage. Further explorations could include dividing the subjects by heritage and by BMI and exploring differences in PTC sensitivity or taste thresholds. Studies with this material may have to include a larger volume of subjects.

Summary

While the study did not show correlation between taste threshold and family history of type 2 diabetes, it may have opened up new avenues for research involving evaluation of genetically at risk individuals. It also supported the claim brought forth in previous studies that male subjects in general have lower taste acuity than female subjects. Diabetes is an extremely difficult disease to live with, manage, and recover from, but perhaps better screening methods can help to delay progression and implement the necessary changes needed in a patient's life before diabetes becomes their diagnosis.

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