Influence of acute hyperglycemia on otoacoustic emissions and the medial olivocochlear reflex^{a)}

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Stimulus-frequency (SF) otoacoustic emission (OAE) amplitude and the amplitude of medial olivocochlear (MOC) inhibition of SF OAEs for ipsilateral, contralateral and bilateral MOC reflex elicitors were recorded in six subjects with type 2 diabetes during a glucose tolerance test (GTT). Five of the six subjects were tested twice for a total of 11 trials and three subjects were tested in a control experiment. During the GTT experiment, the subjects' blood glucose was elevated from a euglycemic level below 150 mg/dL to a hyperglycemic level above 160 mg/dL following the consumption of a bolus of 80 g of sugar. A subset of three subjects were tested in a control experiment during which SF OAE and MOC reflex measurements were made while blood sugar levels remained constant within the euglycemic region. Mean SF OAE amplitudes were elevated following glucose consumption. A statistically significant increase in MOC inhibition amplitude was observed during elevated sugar levels for the 11 GTT trials. Maximum inhibition occurred about an hour after glucose consumption when blood glucose levels peaked. Results indicate that acute hyperglycemia influences efferent control of the cochlea in people with type 2 diabetes. [DOI: 10.1121/1.3676609]

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I. INTRODUCTION

Diabetes mellitus is a metabolic disease that affects one in 12 adults in the U.S (Centers for Disease Control and Prevention, 2011). Individuals with diabetes are unable to regulate the level of glucose in their bloodstream. As a result, blood glucose levels can become severely elevated, which can be harmful to the body's tissues and organs, and levels can also become dangerously low at other times. There are two forms of diabetes: type 1 diabetes is characterized by the pancreas' beta cells inability to generate insulin and type 2 diabetes is characterized by the body's resistance to insulin absorption. According to the American Diabetes Association, more than 90% of people with diabetes are those with type II (Centers for Disease Control and Prevention, 2011).

Low blood sugar (hypoglycemia) can lead to confusion, loss of consciousness, seizures, and in rare cases brain damage or death. High blood sugar (hyperglycemia) is not an immediate danger, but in the long term can damage the

eyes, kidneys, and blood vessels and can impair the functioning of neural pathways. Recent results show that diabetes is also associated with hearing loss among adults with the disease (Bainbridge, Cheng, and Cowie, 2010). Although less well studied, results in a rodent model show that diabetes may cause noise-induced temporary threshold shifts to persist longer (Wu, Cheng, Tan, Guo, and Hsu, 2009) and olivocochlear activity to be compromised (Wu, Guo, Cheng, and Hsu, 2010). Even less is known about how acute swings in blood glucose, such as those experienced by diabetic patients on a daily basis, might impact cochlear function. This study examines cochlear and olivocochlear function during experimentally induced hyperglycemia among individuals with diabetes in order to provide much needed information about if and how changes in glucose impact cochlear homeostasis.

Otoacoustic emissions (OAEs) are used in this study to provide a noninvasive test of the cochlear mechanical response to sound. OAEs are low-level sounds emitted from the ear caused by motion of the cochlear outer hair cells (Kemp, 1978), and they are already widely used in humans and animals to study cochlear function and hair cell micro-mechanics in both normal and damaged ears (Berlin, 2002). OAEs are inhibited (reduced in amplitude) by activation of efferent nerve pathways that descend from the brainstem near the superior olivary complex, through the medial olivocochlear (MOC) bundle, and back onto the outer hair cells

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(Guinan, 2006). This inhibition is believed to be caused by changes to the cochlear amplifier gain (Mountain, 1980; Siegel and Kim, 1982). The auditory efferent system has been hypothesized to play a role in protecting the ear from intense sounds (Rajan, 2000) and also in improving signal-to-noise ratio, specifically in enhancing speech perception in a noisy environment (de Boer and Thornton, 2008). Several extensions to cochlear models (Messing, Delhorne, Bruckert, Braidá, and Ghitza, 2009; Brown, Ferry, and Meddis, 2010) have been proposed for mimicking the MOC reflex, and these models have shown that better automatic speech recognition performance in noise can be achieved when the MOC model is incorporated into the cochlear model. However, there have been reports by others who have not observed a correlation of speech intelligibility with the olivocochlear reflex (Wagner, Frey, Heppelmann, Plontke, and Zenner, 2008; Mukari and Mamat, 2008).

Transient evoked (TE) and stimulus frequency evoked (SF) OAEs at low and moderate stimulus levels are thought to be generated by a linear coherent reflection of the forward traveling wave near the tonotopic peak region (Zweig and SHERA, 1995; Kalluri and SHERA, 2007). Distortion product (DP) OAEs at moderate levels are thought to arise from intermodulation distortion and combine with a linear reflection component to produce a two (or more) source OAE measured in the ear canal (Kim, Molnar, and Matthews, 1980; SHERA and Guinan, 1999).

Perhaps the most common MOC assay involves generating a TE OAE and presenting white noise to the contralateral ear to serve as an elicitor of the MOC reflex (Collet, Kemp, Vuillet, Duclaux, Moulin, and Morgon, 1990; Liberman, 1989). This protocol provides a rapid test of reflex effects over a range of basilar membrane characteristic frequency locations. However, transient stimuli used to generate TE OAEs are themselves effective elicitors of efferent activity, which can diminish observable changes with MOC activation (Guinan, Backus, Lilaonitkul, and Aharonson, 2003). SF OAEs were used in the current study to assess auditory efferent system effects on cochlear function because tonal stimuli produce minimal or no efferent activity (Guinan, Backus, Lilaonitkul, and Aharonson, 2003) and based on recent experimental results suggesting that the reflection component may be more sensitive to changes in cochlear amplifier gain compared with the distortion component (Rao and Long, 2011).

The pathway for eliciting the MOC reflex is different for ipsilateral and contralateral elicited MOC reflexes. The ipsilateral MOC reflex pathway involves ventral cochlear nucleus (VCN) projections that cross the brainstem to innervate MOC neurons on the contralateral side; the contralateral MOC fibers project to the cochlea in the crossed olivocochlear bundle. The contralateral pathway is similar with the exception that sound enters from the contralateral ear, crossing the brainstem to innervate MOC neurons on the ipsilateral side which then extend to the ipsilateral cochlea through the uncrossed olivocochlear bundle. Results from animal models indicate there are more fibers in the crossed MOC bundle than in the uncrossed bundle (Warr, 1992), leading to a larger ipsilateral MOC reflex. These anatomical distinctions have not been

observed in humans. The contralateral and ipsilateral MOC reflexes in humans have been shown by some groups to have different sizes, for example Berlin, Hood, Hurley, Wen, and Kemp (1995) showed that the ipsilateral MOC is generally larger than the contralateral MOC. Lilaonitkul and Guinan (2009) have shown that for narrow-band elicitors, the ipsilateral MOC is larger than the contralateral MOC, but for broad-band elicitors, the ipsilateral and contralateral MOC are of comparable size and the bilateral reflex is close to additive. In the current study, MOC reflexes were elicited ipsilaterally, contralaterally, and bilaterally to observe individual differences between the reflex strengths and how they were affected during acute blood glucose changes.

The purpose of this work is to test whether efferent MOC inhibition of the OAE increases consistently during hyperglycemia, and whether the SF OAE amplitude also changes during hyperglycemia. The primary importance of this research is that it will establish the extent that acute changes in blood glucose impact cochlear function, and provide information about potential mechanisms for this action. Investigation of the relationship between OAEs and blood sugar levels can also help answer the question of whether OAEs can be used to noninvasively estimate blood sugar.

Self-monitoring of blood glucose is as an important component of the care plan for both type 1 and type 2 diabetic patients. Glucose monitoring is particularly important for patients on multiple insulin injections per day or on pump therapy, but it has potential roles in other groups as well. Monitoring contributes to patients' understanding of their response to therapy and helps them achieve glycemic targets. Access to blood glucose information enables patients to adjust medications, diet, and activity to avoid dangerous hypoglycemia events and to prevent the microvascular complications associated with chronic hyperglycemia (American Diabetes Association, 2011). While technology for glucose monitoring has improved dramatically over the years, current tools require blood samples, usually obtained from the fingertip with a lancing device. This is invasive, and compliance with monitoring can suffer because of pain associated with finger-sticks (Burge, 2001). For these reasons, a method for noninvasively measuring blood glucose is critical. The FDA has approved several continuous glucose monitors which measure glucose in interstitial fluid using a subcutaneous sensor changed every 3–7 days. These devices are useful only as adjuncts to blood glucose monitoring and require calibration with standard blood glucose meters up to several times per day (Mazze, Strock, Borgman, Wesley, Stout, and Racchini, 2009). A truly noninvasive glucose monitor is not currently commercially available.

There are two potential mechanisms by which glucose could be influencing OAEs and the MOC. The first potential mechanism is based on the fact that glucose is the energy source for the cochlea; if this energy source is disrupted, it affects the function of the cochlea (Mendelsohn and Roderique, 1972; Wing, 1959; Koide, 1958).

The second potential mechanism that could explain how OAEs are impacted by glucose is based on work done by Bobbin *et al.* (1978) that adenosine tri-phosphate (ATP) not only provides energy for the cochlea, but is also an important

neurotransmitter in hair cells and other supporting cells within the cochlea. ATP is generated when cells break down glucose for energy storage and it has been shown that outer hair cells have ion channel receptors that are sensitive to ATP as a neurotransmitter (Bobbin and Thompson, 1978; Munoz and Thorne, 1995; Munoz, Thorne, and Housley, 1999). Kujawa *et al.* (1994a) demonstrated that when ATP and ATP analogs were applied to the cochlear perilymph, reductions were observed in OAE amplitudes and auditory nerve compound action potentials. Since the ATP impact on OAEs is dependent on Ca^{++} , and since Ca^{++} is released by MOC activation, this is a possible mechanism for how ATP-related inhibition of OAEs could be modulated by MOC activation. This prior work led us to hypothesize that the MOC inhibition of OAEs will increase during hyperglycemic conditions. Results presented in this paper show that the MOC inhibition of SF OAEs increases during hyperglycemia consistently across subjects. SF OAE amplitudes also increased on average, but the increase was not as large as the MOC inhibition amplitude increase and the SF OAE amplitude change was not statistically significant.

II. METHODS

Two experiments were done to evaluate the influence of blood glucose on SF OAEs and MOC reflexes. The first experiment tested whether subjects' SF OAE and MOC reflex changed when the subjects' blood glucose went from a euglycemic baseline to a hyperglycemic period. Subjects were forced to become hyperglycemic through the consumption of sugar. The second experiment was a control test, in which a subset of three of the subjects was evaluated under the same test conditions, except that they were not given sugar to consume. The control experiment tested whether the SF OAE and MOC reflex changed over the course of the 3–4 h testing period under euglycemic conditions.

A. Hyperglycemia experiment: Measuring SF OAE and MOC reflex during hyperglycemia

Six diabetic subjects (diagnosed as type 2) were given a 4-h glucose tolerance test (GTT) while their OAEs and MOC inhibition amplitudes were measured at the Portland VA Medical Center. Subjects had a mean age of 48.5 and all were female. Subjects with type 2 diabetes were recruited from the Portland VA Medical Center and Oregon Health and Science University as approved by the local IRBs. Subjects were required to have been diagnosed with diabetes less than 15 years prior, a hemoglobin A1C of 5.5%–8%, normal hearing (auditory thresholds below 20 dB HL for frequencies under 3 KHz), no kidney disease, no congestive heart failure, no coronary artery disease, no diabetes-induced foot or limb ulcerations, no alcohol or drug abuse, and no history of psychiatric illness. Subjects fasted for at least 8 hours prior to participation in the GTT. Subjects sat in a quiet sound-proof room and had their MOC and SF OAE elicited during a 3.5–4 h test period. The MOC was elicited using procedures described by Guinan (2006) and further discussed below. SF OAEs were elicited using a double-evoked method (Keefe, 1998; Keefe and Ling, 1998) also discussed further below. SF OAE meas-

urements and MOC inhibition of SF OAEs were recorded in test subjects for a 1 hour euglycemia baseline period (glucose <150 mg/dL). After the baseline period, the subjects consumed 80 g of cane sugar mixed with 8 oz of water. This amount of sugar is equivalent to about two candy bars or two cans of soda. This amount of sugar would cause the blood glucose of a person with diabetes to exceed 200 to 300 mg/dL. The same amount of sugar given to a person without diabetes would also cause an increase in blood glucose, but the change would typically not exceed 200 mg/dL and would return to a level below 100 mg/dL within an hour. MOC and SF OAE recordings were continued for approximately 3 h after consumption of the sugar. Approximately every 10 min, subjects were asked to prick their finger with a sterile needle, acquire a small sample of blood, and enter it into an Abbott brand blood glucose meter. These recordings were used to determine the true blood glucose level of the subject throughout the experiment. Five of the subjects repeated the GTT on a separate day. Figure 1 shows the subjects' blood glucose increase during the experiment.

1. Data acquisition system

The OAE data acquisition system consisted of a desktop PC with an internal Lynx2B 24-bit 6 channel sound card recording and presenting data at a sample rate of 44 100 samples/s, an Etymotic ER-2 ultra low-noise insert speaker phone, a custom built low-noise audio amplifier, and an ER10B + sensitive insert microphone for recording the responses. Custom software developed in c# and running on a desktop PC was used for managing the presentation and recording of the audio waveforms. A custom written adaptive in-ear calibration routine was used to adjust the level of the audio stimuli to account for the different size ear canals of the test subjects. The amplitude of the stimuli delivered by the speaker was automatically adjusted to be larger in level for larger volume ear canals, and smaller in level for smaller volume ear canals. In this way, the sound levels, as measured at the plane of the microphone, was automatically adjusted such that it was identical for all subjects tested.

2. Procedures for evoking and measuring the SF OAE

In this experiment, SF OAEs were recorded using a three-interval nonlinear residual technique described by

FIG. 1. Increase in blood sugar during the glucose tolerance test for all individual test runs (light gray) and the mean value increase (black).

Keefe and Ling (Keefe, 1998; Keefe and Ling, 1998). The SF OAE was estimated as a measure of the cochlear suppression of the basilar membrane response to a probe tone (S_2) by a suppressor tone (S_1). The frequency of the suppressor tone (F_1) was chosen to be spectrally close to the frequency of the probe tone ($F_1 = 0.95 \cdot F_2$). Tones S_1 and S_2 were outputted by two separate channels in the DAC, routed through separate headphone buffers, to separate ER-2 speakers, and finally through separate ports in the ear tip of the ER10 B + probe-mic assembly. The cochlear responses to these tones (P_1 and P_2 , respectively) were measured using the ER10B + microphone. Next, tones S_1 and S_2 were presented simultaneously (S_{12}) and the cochlear response to this two-tone stimulus (P_{12}) was measured using the microphone. The SF OAE was the nonlinear residual difference (P_d) between ($P_1 + P_2$) and P_{12} as calculated using the equation $P_d = P_1 + P_2 - P_{12}$.

The probe tones were chosen to be 55 dB SPL in level with a frequency of either 1000, 1025, 1050, or 1075 Hz. The subject-dependent frequencies were selected based on a preliminary screening exam in which each subject's SF OAE and MOC inhibition of the SFOAE were measured. The probe frequency that yielded the most robust SFOAE and the best MOC inhibition (procedures for measuring MOC inhibition are discussed in the next section) were selected as the test frequency for that subject. The suppressor level was selected to be large enough that complete suppression was assumed; a 75 dB SPL suppressor was used. Complete suppression is when the SF OAE is completely inhibited by the suppressor tone. The measurement window was 1 second. During this window, the suppressor was presented for 800 ms starting 200 ms after the start of the window. The probe was presented for 300 ms starting 400 ms after the start of the window. During the time when the probe and suppressor were both presented simultaneously, An FFT at the probe frequency was taken of the SFOAE residual (P_d) to get the SF OAE amplitude. A total of 20 presentations were done and outliers were removed if amplitudes (1) were further than one standard deviation outside of the mean SF OAE amplitude or (2) had a signal-to-noise (SNR) ratio of less than 6 dB. The final SFOAE amplitude was the mean value of the remaining valid SFOAE amplitudes.

3. Procedures for evoking and measuring the MOC

The activation of the MOC affects both the amplitude and phase of the SF OAE (Guinan, 2006). To estimate the MOC, a tone was presented to the ipsilateral ear to evoke the SF OAE. The total pressure was measured in the ear canal in response to this tone and this was called the baseline window measurement. Next, while the tone was still being presented, a noise was presented to either the ipsilateral ear, the contralateral ear, or bilaterally to elicit the MOC reflex. The amplitude and phase of the total pressure measured in the ear canal during MOC elicitation (for contralateral elicitors) or within the post-elicitor window (for ipsilateral and bilateral elicitors) was vector subtracted from the total pressure measured in the ear canal during the baseline window. Vector subtraction involved calculating the real and imaginary com-

ponents of the measured acoustical signal when the MOC was active and subtracting the real and imaginary components from the measured acoustical signal in the baseline window to calculate a complex magnitude. The merits of this method are discussed in other work (Guinan, Backus, Lilaonitkul, and Aharonson, 2003) and Fig. 2 shows the method graphically. Calculating the complex magnitude of the response (i.e., including phase) can yield a more robust MOC inhibition signal than when using amplitude alone.

In this study, a 55 dB SPL audio tone was presented to the ear of the test subject for 12 s to evoke the SF OAE in the subject's ear. During the 12-s recording period, the MOC elicitor was presented ipsilaterally, contralaterally, and bilaterally. The MOC elicitor was broadband noise presented at a level that varied for each subject. The level was set to 65 dB SPL or less to avoid middle ear muscle activation. All subjects were pre-screened and determined to not have a middle ear muscle reflex at the level at which the noise elicitor was presented. The noise was presented in 1-s bursts to elicit the MOC. An analysis window was used either during elicitor presentation (for contralateral elicitors) or immediately following the presentation of the elicitor signal (for ipsilateral and bilateral elicitors) to monitor the effect of the elicitor on the cochlear response to the tone in the ipsilateral ear. During the analysis window, the pressure changes within the ipsilateral ear canal were measured and the effect that the elicitor had on the OAE was determined. For the ipsilateral and bilateral elicitors, the post-elicitor window was 80 ms long and began 40 ms following the end of the elicitor to allow time for two-tone suppression effects to subside; these affects are known to end in less than 10 ms while the time constant of the MOC is on the order of hundreds of milliseconds (Guinan, 1990). For the contralateral elicitor, the analysis window lasted for 1 s and occurred at the same time that the MOC elicitor was turned on. See Table

FIG. 2. Method for measuring MOC inhibition of OAE using vector subtraction (Guinan, Backus, Lilaonitkul, and Aharonson, 2003). Total pressure in the ipsilateral ear is first measured in a baseline window, while presenting a tone to the ear, which evokes the SF OAE. While the tone is still being presented, an MOC elicitor is presented (noise) either ipsilaterally, contralaterally, or bilaterally. If the MOC elicitor is presented contralaterally, then the effect of the MOC elicitor on the SF OAE can be measured during the presentation of the elicitor. If the MOC is presented ipsilaterally or bilaterally, then the MOC must be measured after the elicitor has turned off—within a post-elicitor window. The MOC complex amplitude (V_{MOC}) and phase angle (Φ) are calculated by vector-subtracting the total pressure measured in the elicitor or post-elicitor measurement window from the total pressure measured in a baseline window.

TABLE I. Timing summary for the MOC baseline window, the elicitor presentation window, and the analysis window for evoking and measuring MOC inhibition of SF OAEs. The total length of the presentation window was 12 s. Notice that the contralateral analysis window occurs during the presentation of the elicitor. This is because effects of the contralateral MOC elicitor on the SF OAE measured in the ipsilateral ear can be measured simultaneously. Whereas effects of the ipsilateral and bilateral MOC elicitor on the SF OAE evoked in the ipsilateral ear must be measured after the elicitor has been turned off

Elicitor	Baseline window (s)	Elicitor active (s)	Analysis window (s)
Ipsilateral	0–1	1–2	2.04–2.12
Contralateral	3–4	5–6	5–6
Bilateral	7–8	9–10	10.04–10.12

I for a summary of the timing for each elicitor, analysis window, and baseline window.

To obtain an accurate estimate of the MOC amplitude and phase, it is necessary to (1) remove outliers caused by patient motion, (2) and average responses over multiple presentations. The 12-s presentation was done 50 times sequentially to enable removal of outlier data and averaging of the responses. Outliers were removed by taking the FFT of the total pressure measured in the ear canal during the baseline window and the analysis window for each of the 50 presentations, and measuring the mean complex magnitude and the standard deviation of the response at the frequency of the probe. An outlier was defined as having complex magnitude of more than one standard deviation outside of the mean and also having a signal-to-noise ratio of less than 3 dB. These outliers were removed from further analysis. After the outliers were removed, the remaining post-elicitor waveforms were averaged. The measurements were repeated every 10 min during the course of the GTT.

B. Control experiment: Measuring SF OAE and MOC reflex during euglycemia

A control experiment was done in which three of the subjects followed the same procedures described above with

the only exception being that rather than consuming sugar 1 h into the test, the subjects consumed 8 oz of water. The hypothesis for this experiment was that there would be no statistical difference between the MOC measurements acquired during the control experiment and the MOC measurements acquired during the glucose tolerance test prior to the subjects' consumption of the sugar. The hypothesis was also that there would be a statistically significant difference between the MOC measurements acquired during hyperglycemia and those measurements acquired during the control experiment and prior to the consumption of sugar in the GTT.

III. RESULTS

Results from the hyperglycemia experiment show that the ipsilateral MOC reflex increased in amplitude significantly during hyperglycemia. The SF OAE and the contralateral MOC inhibition amplitude also increased on average during hyperglycemia, but the change was not statistically significant. The control experiment revealed that when sugar was not given to the subjects during the experiment (and euglycemia was maintained during the testing) then the MOC and SF OAE remained stable over time in most, but not all cases. Each case is described in detail in the results that follow.

A. Results from the hyperglycemia experiment

Figure 3 shows how the mean SF OAE amplitude value and the mean complex magnitude of the MOC inhibition of the SF OAE increased during hyperglycemia. The data displayed in Fig. 3 were divided into two groups: (1) data measured during euglycemia (blood glucose less than or equal to 160 mg/dL) and (2) data measured during hyperglycemia (blood glucose greater than 160 mg/dL). The data acquired during euglycemia were termed the euglycemic baseline period and a euglycemic baseline average was calculated for each run. The change in MOC inhibition and OAE amplitude during hyperglycemia was measured as a percent change

FIG. 3. Change in SF OAE amplitude (a) and MOC inhibition of SF OAE amplitude during a glucose tolerance test for three MOC elicitation methodologies; (b) ipsilateral, (c) contralateral, and (d) bilateral stimulus presentation. Results are shown for each individual test run (light gray traces) and the mean across all runs (black traces). Notice that while there is variability for individual subject runs, the average SF OAE amplitude and the average MOC inhibition amplitude measured using each of the three elicitors all demonstrated an increase shortly after the consumption of sugar. The light grey shaded region of the plot represents the standard error (SE) of the control runs where the subjects consumed only water, and no sugar.

TABLE II. MOC inhibition amplitude and SF OAE amplitude during euglycemia and hyperglycemia.

Metric	Euglycemia	Hyperglycemia	% SF OAE change	Kruskal–Wallis p -value	Bonferroni adjusted p -value
Ipsilateral MOC inhibition amplitude (fraction of SFOAE inhibited)	0.43	0.69	26.0	0.0064 ^a	0.0257 ^a
Contralateral MOC inhibition amplitude (fraction of SF OAE inhibited)	0.25	0.40	15.0	0.0278 ^a	0.1113
Bilateral MOC inhibition amplitude (fraction of SF OAE inhibited)	0.46	0.68	22.0	0.028 ^a	0.1113
SF OAE amplitude (dB SPL)	16.0 dB	17.8	11.3%	0.2786	0.9999

^a Statistical significance ($p < 0.05$).

from the euglycemic baseline average. Individual data were averaged during the euglycemic period and during the hyperglycemic period; these averages were then combined into the euglycemic and hyperglycemic group data, respectively. The y-axis on Fig. 3 shows the change in SF OAE amplitude and MOC inhibition amplitude as a percentage of the euglycemic baseline average. No change in these metrics relative to euglycemic levels would give a value of zero; any increase in these metrics relative to euglycemic levels would give a positive value on the graph. For certain test subjects the SF OAE and MOC inhibition amplitudes were variable due to noise, even during euglycemia, and this is why for some of the individual test runs, the amplitudes do not display at 0% during euglycemia. The average SF OAE and MOC inhibition traces shown in Fig. 3, generally are at 0% during euglycemia, and then rise after consumption of glucose.

Also shown in Fig. 3 is a shaded region that indicates the standard error of the control subjects' change from baseline. During the control trials, the subjects did not consume glucose during their testing and we would therefore not expect to see a large change in SF OAE or MOC inhibition amplitude.

A Kruskal–Wallis one-way analysis of variance was used to assess the statistical significance of the difference between the SF OAE and MOC inhibition amplitudes measured during euglycemia and during hyperglycemia. The Kruskal–Wallis statistical result of the increase in MOC inhibition amplitude was $p = 0.0064$ for MOC inhibition elicited ipsilaterally, $p = 0.0278$ for MOC inhibition elicited contralaterally, and $p = 0.0280$ for MOC inhibition elicited bilaterally. The result of the increase in SF OAE amplitude during hyperglycemia was not found to be significant ($p = 0.2786$). A Bonferroni adjustment was made to the p -values; the MOC inhibition elicited ipsilaterally remained statistically significant after this adjustment ($p = 0.0257$). These results are summarized in Table II.

Figure 4 shows how the MOC inhibition amplitude increased during hyperglycemia compared with the euglycemia baseline both on average and across nearly all individual runs for the three types of MOC elicitors. The SF OAE amplitude also tended to increase during hyperglycemia, but the increase was not statistically significant and it was not as consistent as was observed with the MOC inhibition amplitude.

Five out of the six subjects were tested twice on separate days using the identical procedures. The box and whisker plot in Fig. 5 shows how the MOC inhibition elicited using an ipsilateral stimulus increased during hyperglycemia for each of the subjects and for each of the subjects' test runs.

Figure 6 shows the change in SF OAE and change in MOC inhibition for each of the three MOC elicitors (ipsilateral, contralateral, and bilateral) plotted as a function of blood glucose. The strongest correlation was found to be between the MOC inhibition elicited ipsilaterally ($R^2 = 0.29$), consistent with the finding discussed earlier that the ipsilaterally elicited MOC inhibition was found to change significantly during hyperglycemia relative to euglycemia.

While prior work has shown that the bilaterally elicited MOC is approximately equivalent to the sum of the contralaterally and ipsilaterally elicited MOC in humans (Lilaonitkul and Guinan, 2009; Guinan, Backus, Lilaonitkul, and Aharonson, 2003), the results shown in Table II do not follow this trend. This is partially explained by the methods used for eliciting the MOC in this experiment. Specifically, different windows were used to evaluate the MOC effects for the contralateral (window coincided with elicitor presentation) compared with ipsilateral and bilateral (window lagged the elicitor presentation). A post-elicitor window was required for measuring both the ipsilateral and bilateral MOC since the ipsilateral elicitor interferes with the measurement if it is presented simultaneously with the measurement window. If the purpose of the experiment had been to examine differences in elicitor types, a post-elicitor window could have been used for measuring the contralateral MOC. However, the objective of this study was to evoke and

FIG. 4. Results showing how MOC inhibition of SF OAEs increased across all three elicitors during hyperglycemia compared with euglycemia on average (bars) and for every individual trial ($\times$). Results also show that the SF OAE amplitude increased on average 11.3% (1.8 dB) during hyperglycemia relative to the baseline euglycemia measurement period, however several subjects showed a reduced SF OAE amplitude during hyperglycemia or no change at all. Data are plotted as a percent change from the euglycemic baseline average.

FIG. 5. MOC inhibition amplitude (elicited ipsilaterally) measured during euglycemia and hyperglycemia for all subjects. Results above show box and whisker plots displaying the mean value (line) and variance of MOC inhibition amplitude both under euglycemic conditions and hyperglycemic conditions. The horizontal axis groups identical subject IDs to indicate that five of the six subjects were tested twice. Results from the first test are shown, and re-test results taken on a different day are also shown for these five subjects.

measure a robust MOC and then determine if there is an influence of glucose on this MOC. The MOC effect is diminished over time relative to its level during the elicitor presentation and there were concerns that the use of a post-elicitor window may have diminished the contralateral MOC effect to a level that was too small to reliably manipulate through glucose uptake. Guinan, Backus, Lilaonitkul, and Aharonson (2003) showed that the size of the MOC inhibition measured in the post-elicitor window was approximately 3 dB smaller than when measured at the time of the elicitor. We adjusted for the fact that the MOC elicited in a post-elicitor window is 3 dB smaller than the MOC elicited during the elicitor window by multiplying the contralaterally elicited MOC by 0.7 and then adding it to the ipsilaterally elicited MOC. We then plotted this summed value versus the MOC elicited bilaterally as shown in Fig. 7. Results indicate a high correlation

FIG. 7. Relationship between MOC inhibition elicited ipsilaterally, contralaterally, and bilaterally during euglycemia. The MOC elicited contralaterally was recorded during the elicitor window while the MOC elicited ipsilaterally and bilaterally was elicited in a post-elicitor window 40 ms after the MOC elicitor turned off. Guinan, Backus, Lilaonitkul, and Aharonson (2003) showed that the size of the MOC inhibition measured in the post-elicitor was approximately 3 dB smaller than when measured at the time of the elicitor. For this reason, we multiplied the contralaterally elicited MOC by 0.7, added it to the ipsilaterally elicited MOC, and then plotted that value versus the MOC elicited bilaterally. They should correlate exactly and have nearly a 1-to-1 correspondence, which is shown as the solid line on the plot.

tion between the combined ipsilaterally and contralaterally elicited MOC and the MOC elicited bilaterally, with a nearly 1-to-1 correspondence.

B. Results from the control experiment

Results from the control experiment indicate that the MOC inhibition amplitude remains relatively constant under euglycemic conditions (i.e., when the subjects were given water alone instead of sugar water during the testing). Table III shows the results obtained from the three subjects who participated in both the GTT testing and also the control test during which they did not consume any sugar during the test. The p -values shown in Table III were calculated using a single-tail t -test. For the case where the subjects underwent the GTT, the t -test tests the null hypothesis that the SF OAE

FIG. 6. Change in SF OAE amplitude (a) and MOC inhibition for three elicitor types, ipsilateral (b), contralateral (c), and bilateral (d) during hyperglycemia plotted as a function of glucose. The lines on the plots show the least-squares line fit. The correlation coefficients (R^2) are shown on the individual plots.

amplitude and MOC inhibition amplitude during hyperglycemia were the same as during euglycemia (*p*-value indicated in column labeled “GTT”). For the case where the subjects underwent a control experiment, the *t*-test tests the hypothesis that the SF OAE and MOC inhibition amplitude before consumption of water were the same as after the consumption of water (*p*-value indicated in column labeled “control”).

Results shown in Table III indicate that for all three subjects, the MOC inhibition amplitude during hyperglycemia is statistically different (for subject 62’s contralateral MOC reflex, the result was marginally significant) compared with during euglycemia. For subject 46, there was a statistically significant difference between the MOC inhibition amplitude and SF OAE amplitude during hyperglycemia compared with euglycemia. As was expected, during the control experiment there was not a significant difference between the MOC inhibition amplitude or the SF OAE amplitude before the subject consumed water and after the subject consumed the water.

The other two control subjects did not exhibit a statistically significant change in SF OAE amplitude during hyperglycemia. They did exhibit a change in MOC inhibition amplitude for all three ipsilateral MOC reflexes during hyperglycemia. However, note that for subject 63, the MOC inhibition elicited by a bilateral elicitor changed during the control experiment as well as during the GTT.

The standard error for all three control subjects is included as the light-gray shaded region in Fig. 3. In this figure, it is evident that the mean change in SF OAE and MOC inhibition for all three elicitors during hyperglycemia increased above the standard error of the control subjects.

IV. DISCUSSION

In this paper, results indicate that acute hyperglycemia (blood sugar levels exceeding 160 mg/dL) in patients with diabetes cause an increase in the MOC inhibition of SF OAEs relative to the amplitudes measured during the euglycemic state. During hyperglycemia, an average increase in SF OAE amplitude was also observed, but the increase in amplitude was not statistically significant. Furthermore, the SF OAE amplitude increase was proportionally smaller than the increased MOC inhibition of the SF OAE. This is evident from Table II and Fig. 4, which show that during hyperglyce-

TABLE III. Data are shown for three subjects comparing glucose tolerance test (GTT) results with control test results. The columns labeled with “GTT” show the *p*-value from a single-tail *t*-test that tests the null hypothesis that the SF OAE or MOC inhibition amplitude during hyperglycemia is the same as the SF OAE or MOC inhibition amplitude during euglycemia. The columns labeled “control” show the *p*-value from a single-tail *t*-test of the null hypothesis that the SF OAE/MOC inhibition amplitude before consumption of water is the same as after consumption of water.

	Subject 46		Subject 62		Subject 63	
	GTT	Control	GTT	Control	GTT	Control
Ipsilaterally elicited MOC	0.009 ^a	0.838	0.012 ^a	0.316	0.001 ^a	0.905
Contralaterally elicited MOC	0.003 ^a	0.193	0.056	0.157	0.001 ^a	0.072
Bilaterally elicited MOC	0.001 ^a	0.113	0.001 ^a	0.144	0.004 ^a	0.008 ^a
SF OAE amplitude	0.009 ^a	0.507	0.656	0.200	0.099	0.858

^aStatistical significance (*p* < 0.05).

mia, the MOC elicited by ipsilateral, contralateral, and bilateral noise resulted in a 26%, 15%, and 22% increase, respectively, in the amount of SF OAE that was inhibited by the MOC as compared with during euglycemia. The effect of glucose on the ipsilateral MOC reflex was similar compared with the bilateral MOC reflex as measured during a post-elicitor window. A paired two-tail *t*-test showed that the mean MOC inhibition elicited bilaterally was equivalent to the mean MOC inhibition elicited ipsilaterally and this was true both during euglycemia and hyperglycemia (*p* = 0.13, rejecting the null hypothesis that the means are different during euglycemia, and *p* = 0.6499, rejecting the null hypothesis that the means are different during hyperglycemia). This observation suggests that the contralateral MOC reflex contributed little to the bilateral response. Further, the ipsilateral MOC reflex was the only one which changed significantly during hyperglycemia when a Bonferroni adjustment was made to the *p*-values. This implies that the crossed olivocochlear bundle (the pathway activated by the ipsilateral MOC reflex) appears to be affected more significantly than the uncrossed olivocochlear bundle (the pathway activated by the contralateral MOC reflex).

The SF OAE amplitude increased during hyperglycemia (11.3%) but the increase was proportionally less than the MOC inhibition of the SF OAE during hyperglycemia. And the SF OAE amplitude increase was not statistically significant. Our original hypothesis that SF OAE amplitudes would increase during hyperglycemia was based on the fact that glucose is the energy for the cochlea, and that when the cochlea is deprived of its energy source, there should be a decrease in the EP, which would result in a reduced OAE amplitude measurement. Wing (1959) and Koide (1958) and more recently Angelie *et al.* (2009) showed that hypoglycemia reduces the endocochlear potential (EP) and cochlear microphonic amplitudes. A reduction in cochlear microphonics during hypoglycemia may be caused by the lack of available energy required for maintaining the active transport of sodium and potassium ions into and out of the endolymph in the cochlea. Within the endolymph, there is a high concentration of potassium and a low concentration of sodium, as first shown by Smith *et al.* (1954). This concentration gradient is responsible for maintaining the large potential difference between outer hair cells and the endolymphatic fluid (i.e., the EP), and when the cochlea is deprived of glucose, the EP drops (Mendelsohn and Roderique, 1972). A reduction in EP also causes a reduction in OAE levels (Mills, Norton, and Rubel, 1993). A reduction in DP OAEs during hypoglycemia was verified by Zuma e Maia *et al.* (2006, 2008) who found that when sheep were given a bolus of insulin, thereby lowering their blood sugar levels, DP OAE levels declined. This reduction in level of the DP OAE occurred primarily for higher frequency measurements and the decrease occurred about 60 min after induction of hypoglycemia. Zuma e Maia *et al.* did not look at DP OAE changes during hyperglycemia as was done in the current study. Their study was also different in that they were measuring DP OAEs which have a different generator mechanism than SF OAEs. However, not all prior work supports the hypothesis that OAEs will be larger during hyperglycemia and smaller during hypoglycemia. Results of a

study by Sasso *et al.* (1999) indicate that DP OAEs do not change with blood sugar. Another study reports that DP OAE level is inversely proportional to glucose level. Specifically, Suckfull *et al.* (1999) found that by perfusing a rabbit's cochlea with a solution of saline and sugar, DP OAEs were reduced in amplitude; this result is opposite to what was observed in the current experiment and what was observed by Zuma e Maia's group. Suckfull hypothesized that the DP OAE amplitude changes observed in his experiments were caused by changes in serum osmolarity, not glucose elevation per se, and serum osmolarity has been shown to change outer hair cell function (Chertoff and Brownell, 1994). However, neither of these groups investigated SF OAEs specifically and neither examined correlations of blood sugar with MOC inhibition.

In addition to affecting the EP, increased glucose in the blood results in more ATP within the perilymph, and Kujawa *et al.* (1994a) showed that increases in ATP in the perilymph resulted in smaller DP OAE amplitudes. Activation of ATP-sensitive ion channels can change the polarization of outer hair cells, which could lead them to be less sensitive to acoustical signals and reduce the gain of the cochlear amplifier (Yu and Hong-Bo, 2008), which would then lead to smaller DP OAE amplitudes. Kujawa *et al.* showed that reductions in DP OAE amplitudes were due to ATP-sensitive ion channels in the outer hair cells by presenting ATP antagonists and showing that the effects could be abolished (Kujawa, Fallon, and Bobbin, 1994b). Importantly, it has been shown that calcium ions (Ca^{++}) are necessary for ATP-induced effects to occur (Yu and Zhao, 2008). The efferent neurotransmitter, acetylcholine (ACh) is released by the MOC terminals and acts on $\alpha 9\alpha 10$ AChRs subunit terminals that allow entry of Ca^{++} ions into the outer hair cell (Elgoyhen, Vetter, Katz, Rothlin, Heinemann, and Boulter, 2001). The MOC is known to exhibit an inhibitive effect on OAE amplitudes and this same inhibitive effect has been demonstrated by perfusion of the cochlea with ATP and ATP agonists in the presence of calcium. The possibility that there are two mechanisms that could be impacting OAE amplitudes during hyperglycemia (metabolic vs neural), and that these two mechanisms act in opposing directions on the outer hair cells, could potentially explain how the body maintains consistent cochlear function during glucose changes. For example, when glucose levels are elevated, the EP and cochlear microphonics become elevated which makes the cochlea more sensitive to sound; however, the ATP-sensitive ion channels also become activated which causes a reduction in the gain of the cochlear amplifier, thereby reducing the sensitivity of the cochlea to sound. Perhaps these two opposing mechanisms cancel each other out. This could explain why Sasso *et al.* (1999) showed no change in DP OAE amplitudes in his experiments.

If there are indeed two mechanisms that cause glucose-related changes to the cochlea but in opposite directions, the MOC perhaps provides the tie-breaker by mediating and enhancing the mechanism in which ATP is acting as a neurotransmitter. ATP-related effects on ion channels are reliant on the presence of calcium ions (Ashmore and Ohmori, 1990; Yu and Zhao, 2008), and it is the activation of the

MOC which enables release of this calcium. When the MOC is activated, there is a release of the neurotransmitter acetylcholine (Elgoyhen, Vetter, Katz, Rothlin, Heinemann, and Boulter, 2001) which has been shown to lead to the release of extracellular calcium ions (Frolenkov, Mammano, Belyantseva, Coling, and Kachar, 2000; Blanchet, Erostequi, Sugawara, and Dulon, 1996; Evans, 1996). Since ATP increases in the endolymph during a hyperglycemic event as shown by Puschner and Schacht (1997), and availability of calcium ions increase when the MOC is activated as discussed above, one might expect to observe a larger MOC inhibition amplitude during hyperglycemia than during euglycemia. This could be an explanation for why an increase in MOC inhibition amplitude during hyperglycemia across elicitors in all subjects as shown in Fig. 4 was found.

In addition to providing some insight about how glucose affects cochlear homeostasis, results from this study help address the question of whether OAEs and the MOC inhibition of OAEs can be used to noninvasively estimate blood sugar. Results presented are encouraging in that there was a measurable change in MOC inhibition during hyperglycemia, especially when the MOC was elicited ipsilaterally. However, the results also show a wide difference in the amount of change observed for each individual subject tested. This is clear in Fig. 5, which shows that while the MOC inhibition amplitude was generally repeatable for each subject, the amount of increase in MOC inhibition during hyperglycemia was different, sometimes markedly, across subjects. Furthermore, the size of the MOC reflex in the euglycemic state also differed widely across subjects (Fig. 5). In addition, about half of potential subjects were excluded from participating in the present experiment based on pre-screening measures that showed they did not have an MOC reflex that could be measured accurately within the time-frame required (less than 10 min) for the GTT (see Sec. II for more details). If MOC reflex measurement methods can be improved in terms of both speed and accuracy, and in the proportion of test subjects that exhibit the reflex, then it may eventually be possible to use this metric as a clinical tool for estimating blood sugar.

One way to improve accuracy of the test involves addressing the large inter subject variability in the MOC reflex (at least as measured in the present experiment). This could be done by calibrating the OAE-based glucose measurement device to each patient using the device. Such a calibration procedure would require a person with diabetes to have their OAEs and MOC inhibition recorded at multiple blood glucose levels, perhaps during a glucose tolerance test in a doctor's office. These recordings would then be used to calibrate that person's MOC levels to their specific blood glucose levels. In the future, we may also consider using TE OAEs as an alternative to SF OAEs for recording the MOC more quickly and for observing a wider spectrum of the influence of glucose on the MOC reflex. TE OAEs have been used in the past to quickly measure the MOC reflex (Collet, Kemp, Veuillet, Duclaux, Moulin, and Morgon, 1990; Liberman, 1989). However, transient stimuli have the disadvantage of being good elicitors of the MOC reflex, thereby potentially confounding the influence of the MOC elicitor on the TE OAE response. Other factors would also need to be overcome

including the fact that a disproportionate percentage of people with diabetes have hearing impairment and reduced or non-existent OAEs (Sasso *et al.*, 1999; Ottaviani, Dozio, Neglia, Riccio, and Scavini, 2002). Exposure to loud noises or other ototoxic agents can also affect OAEs, which could further confound the use of OAEs and the MOC for monitoring blood glucose. It may be that a device such as this would only be useful for a subset of the diabetes community with good hearing and robust OAEs. In summary, there are many confounding factors which must be overcome if OAEs and MOC inhibition are to ever be used as a clinical test to measure blood glucose. This study serves as a preliminary evaluation of the potential of the method.

V. CONCLUSIONS

MOC inhibition of SF OAE amplitudes have been shown in this paper to increase during hyperglycemia in patients with diabetes. SF OAE amplitudes generally increased during hyperglycemia as well, but the increase was not statistically significant and was not as proportionally large as the change in MOC inhibition amplitude. Two mechanisms have been proposed for why acute changes in blood glucose impacts SF OAEs and the MOC efferent reflex. Results indicate that the crossed efferent pathway is significantly affected by blood sugar levels; and this may be a result of ATP-sensitive ion channels influencing the MOC inhibition reflex. Results further suggest that OAEs and the MOC inhibition of OAEs could potentially provide a noninvasive means for estimating blood glucose. However, a large inter-subject variability was observed which would require individual calibration of such a device.

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