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Distortion Product Otoacoustic Emissions in an Animal Model of Induced Hyperinsulinemia

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Abstract: The existence of a relationship between abnormal insulin levels and the occurrence of labyrinth disorders has been demonstrated in several works. Among many metabolic alterations, such studies indicate that hyperinsulinemia is one of the most frequent causes of cochlear and vestibular syndromes. In this study, we monitored distortion product evoked otoacoustic emission thresholds during induced acute hyperinsulinemia in sheep so as to identify the occurrence of electrophysiological changes in cochlear outer hair cells. In the study group, seven sheep received a bolus of 0.1 U/kg of regular human insulin. In the control group, seven sheep received saline solution. We measured insulin and glucose levels simultaneously with the recording of distortion product otoacoustic emissions at 10-minute intervals over 90 minutes. We successfully induced hypoglycemia and hyperinsulinemia. We detected no changes in distortion product thresholds in the control group during the 90 minutes of the experiment. In the study group, we recorded a reduction in distortion product thresholds in relation to the control group at frequencies above 1,500 Hz and after 60 minutes ($p < .001$). We observed significant electrophysiological changes in cochlear outer hair cells reflected in the variation of distortion product thresholds at high frequencies after 60 minutes.

Key Words: carbohydrate metabolism disorder; cochleovestibular syndrome; distortion product otoacoustic emission; hyperinsulinemia

The increased prevalence of carbohydrate metabolism disorders (CMD) in individuals presenting with cochlear and vestibular dysfunction as compared to the general population provides strong evidence for the existence of an association between these clinical entities [1]. Data from the literature indicate that CMDs affect between 42% and 80% of those who suffer from tinnitus and dizziness [2], and many studies show that hyperinsulinemia is one of the most frequent causes of cochleovestibular syndromes [3–8].

In 1864, Jordao was the first to correlate labyrinth disorders with metabolic alterations in a group of patients with sensory hearing loss and diabetes mellitus. In the 1960s, Jorgensen [9] observed histopathological alterations in the stria vascularis of 32 temporal bones from diabetic patients, and Goldman [10] reported improvement in 90% of 75 patients with Ménière's syndrome and hypoglycemia with the use of adrenocortical extract associated with a diet. A similar observation was later reported by Powers [11]. The importance of glucose levels for the production of adenosine triphosphate, required by the inner ear for maintenance of the cochlear potential, has also been demonstrated [12–14]. D'Avila and Lavinsky [15], who studied the carbohydrate profile of individuals with Ménière's disease, showed that 72% of their patients had varying degrees of hyperinsulinemia, detectable in 5-hour glucose and insulin tolerance tests. In a study of 100 patients with clinical signs of hypoglycemia and cochleovestibular symptoms, Lavinsky et al. [3] observed that hyperinsulinemia and hypoglycemia were the most prevalent findings, with a 96% positive predictive value for the occurrence of cochleovestibular disorders. Another study [6] found that 82% of the patients with tinnitus and a clinical history suggesting dysglycemia had abnormal 5-hour glycemia and insulin curves. Among

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those patients, hyperinsulinemia was the most frequent finding.

Evidence of cochlear and vestibular involvement is apparent even in early stages of metabolic alterations associated with glucose and insulin [9,15]. Research with animal models focusing on endocochlear alterations has revealed that the inner ear stores practically no energy and depends on the supply of oxygen and on glucose perfusion to maintain its intense level of activity. Thus, changes in metabolism or blood flow have a great potential to alter inner-ear homeostasis, and the study of such alterations can probably bring to light important aspects to explain the relationship between CMDs and inner-ear disorders. In that sense, the use of a sheep as an animal model is especially useful. As previously shown [16], significant similarities are seen between sheep and humans in terms of ear anatomy, histology, and morphometry, especially concerning the size of structures.

In the last decade, evoked otoacoustic emissions (OAEs) have been successfully employed for the detailed study of mechanical aspects of cochlear function. Of two basic types of OAEs, transient and distortion product, distortion product OAEs (DPOAEs) are more frequently employed in clinical settings, as they are capable of showing early cochlear alterations not detected by pure-tone audiometry and other conventional tests in Ménière's and other inner-ear disorders [17]. However, no study has focused on DPOAE measurements in the presence of hyperinsulinemia.

On the basis of the assumption that the presence of CMDs will cause changes in DPOAEs, the objective of our study was to measure these emissions to evaluate function of cochlear outer hair cells (OHCs) during induced acute hyperinsulinemia in sheep.

SUBJECTS AND METHODS

Fourteen male Textel sheep with a mean live weight of 40 kg and a mean age of 18 months were randomly assigned to one of two groups (control or study group). The following were exclusion criteria: impossibility of performing DPOAE measurements owing to the anatomical shape of the external auditory canal; dislocation of the OAE probe during the procedure; and death of the animal during anesthesia. The animals in which we were able to achieve a 50% reproducibility in transient OAEs and that presented DP at experimental time zero were included in the study.

None of the experiments caused any discomfort to the animals, as all procedures were performed under sedation and general anesthesia. Because this was an acute-type observational study, the animals were not sacrificed and were returned to their place of origin as soon as possible after the end of the experiment.

Induction of Acute Hyperinsulinemia and Anesthesia

After a 48-hour fast, each animal in the control group (n = 7) received 20 ml of endovenous saline infusion. We measured OAEs, at zero and at 10-minute intervals over 90 minutes, simultaneously with the collection of blood samples for glucose and insulin determinations. After a 48-hour fast, the animals in the study group received an endovenous insulin bolus (0.1 U/kg diluted in 10 ml saline solution). This dose is the standard used in the insulin tolerance test [18]. In this group, OAEs were also measured, at zero and at 10-minute intervals over 90 minutes, simultaneously with the collection of blood samples for glucose and insulin determinations.

We achieved sedation using 500 mg/kg intramuscular acepromazine (Univet, São Paulo, Brazil). For anesthesia induction, we used 15 mg/kg of endovenous sodium thiopental and achieved maintenance with continuous pump infusion of 600 mg/hr sodium thiopental (B-Braum Nutrimat II, São Gonçalo, RJ, Brazil). We performed local block of the auricular region at the tragus using 75 g of 0.75% bupivacaine chlorhydrate (Astra, Cotia, SP, Brazil).

Ten minutes after premedication, we induced anesthesia by introducing thiopental into an No. 8 or 9 tracheal tube attached to an anesthesia cart (Narcosul Modulus 4000, Porto Alegre, RS, Brazil). We supplied 100% oxygen throughout the procedure and monitored the following parameters: oxygen saturation, pulse (by oximeter, Ohmeda Biox 3700e, Louisville, CO), current volume, respiratory rate (Takaoka Venticare, São Paulo, Brazil), and rectal temperature (CONTEMP 400/700, São Caetano do Sul, Brazil).

Surgical Exposure of the External Auditory Canal

After local block with 0.75% bupivacaine chlorhydrate, we performed a surgical incision of approximately 2 cm with an electric scalpel (Medecir BMO-870, Medical Cirúrgica, São Paulo, Brazil) in the preauricular region. This incision was aimed at improving both visualization of the external auditory canal and tympanic membrane and placement of a probe to measure OAEs.

Recording of OAEs

During the recording, mean body temperature was maintained at 37 ± 0.5°C using a Termway thermal mattress. Ambient noise level did not exceed 65.5 dBA during the entire procedure.

We recorded transient OAEs using a Madsen Capella Cochlear Emission Analyzer attached to a Toshiba Sat-

ellite notebook. We employed the parameters in fast-screen mode. The stimulus consisted of a 40-μsec click with an intensity of 80-dB peak equivalent sound pressure level and condensation polarity. For data analysis, we considered only the segments recorded in the 3- to 12.50-msec window.

We recorded DPOAEs using the same equipment described earlier. The parameters we employed were DP1 = 2F1 – F2 with a ratio of F1:F2 = 1.22. F1 and F2 intensity levels were equal to 65 and 55 dB, respectively. We employed the decibel thresholds for the following frequencies: 750, 1,000, 1,500, 2,000, 3,000, 4,000, 6,000, and 8,000 Hz.

Collection of Blood Samples for Glucose and Insulin Determinations

We performed a jugular vein puncture for collection of blood samples. We employed an enzymatic colorimetric method (glucose-oxidase) for glucose determination (LABTEST kit, Labtest Diagnóstica, Lagoa Santa, Brazil) and determined insulin using an electrochemiluminescence assay (Roche Modular Analytics E170 Analyzer, Roche Diagnostics, Basel, Switzerland) with measurements performed at 10-minute intervals for a total of 90 minutes.

Statistical Analysis

We analyzed data using the Statistical Package for the Social Sciences (SPSS) 13.0. We used the Student's *t*-test for comparison of two groups with an effect size of 2 standard deviations ($\alpha = .05$) and power of 90% in a pilot study to estimate the sample size of 6 animals per group (in the actual study, 7 animals were used in each group). During the experiment, we employed the binomial test to compare the minimum DP detection thresholds at 750, 1,000, 1,500, 2,000, 3,000, 4,000, 6,000, and 8,000 Hz for the study and control groups, at 60, 70, 80, and 90 minutes.

This experimental study was carried out in the Animal Facility at the Hospital de Clínicas de Porto Alegre Research Center and was approved by the Hospital's Research Ethics Committee. The study followed *Guiding Principles in the Care and Use of Animals*, DHEW Publication, NIH, 80–230.

RESULTS

All the animals were docile during preoperative procedures (anesthesia induction). No case of hemorrhage occurred, and we encountered only one case of death due to aspiration in the control group. Total recovery from anesthesia took approximately 2 hours and was uneventful. We returned the animals to their place of origin for recovery from the surgical wound.

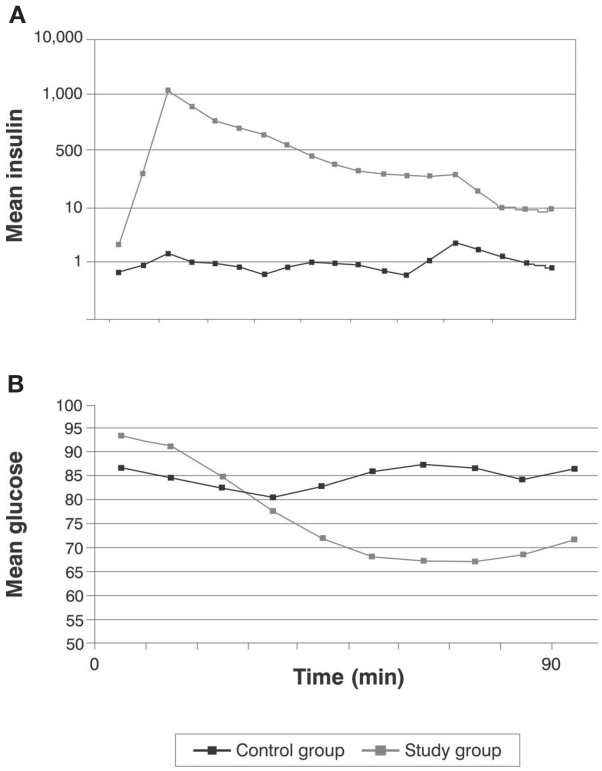

Figure 1. (A) Mean insulin levels and (B) mean glucose levels in the control and study groups during the 90 study minutes.

We successfully induced hypoglycemia and hyperinsulinemia (Fig. 1). We observed no change in DP thresholds (expressed in decibels) in the control group during the 90 study minutes (Fig. 2). Figure 2 also shows a progressive dispersion in DP thresholds over time in the study group.

Table 1 shows the minimum thresholds (expressed in decibels) observed for DP at four different moments starting at 60 minutes after the injection of insulin in the study group in the eight frequencies (Hz) studied. The study group presented a much higher occurrence of minimum DP events than did the control group (28 versus 4), especially in frequencies above 1,500 Hz ($p < .001$; Fig. 3) and after 60 minutes. Stimulation with insulin did not affect the median number of events in the study group.

DISCUSSION

The inner ear is influenced by small variations in blood glucose and insulin levels due to the presence of insulin receptors in the endolymphatic sac [17] and glucose carriers in the stria vascularis [19]. Hypoglycemia and

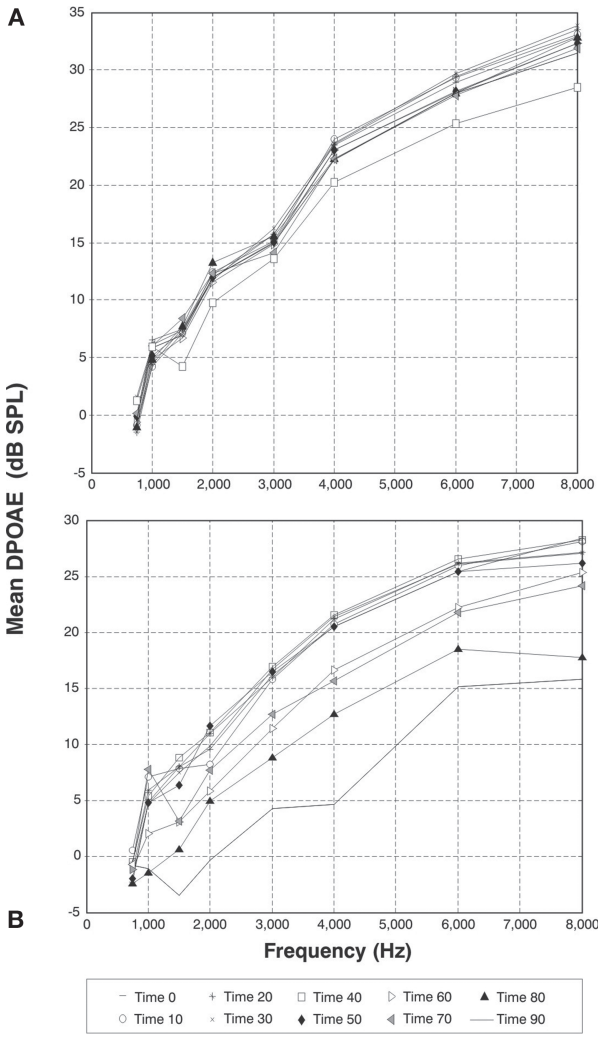

Figure 2. Mean distortion product otoacoustic emission (*DPOAE*) during the 90 study minutes. (A) Control group. (B) Study group.

high levels of insulin interfere with the ionic and energetic parameters required for adequate functioning of the inner ear.

The main objective of our study was to identify the occurrence of electrophysiological alterations in cochlear OHCs through the study of DPOAEs after induced acute hyperinsulinemia in an animal model. To demonstrate the occurrence of damage in the cochlear OHCs during acute hyperinsulinemia, we used a 0.1-U/kg bolus injection of insulin [18], such as that employed in the insulin tolerance test to evaluate the glucose reduction rate in the 15-minute interval after the injection of insulin. In our study, this procedure successfully promoted acute hypoglycemia and hyperinsulinemia. The mean blood glucose and insulin levels in our control group were similar to those described in other studies with animals using a commercial kit also employed in human beings. One limitation of our study is that the experiment ended after 90 minutes, so that we were unable to verify whether the DPs returned to normal with a simultaneous return to normal of the insulin and glucose levels. However, continuing the experiment beyond 90 minutes was not possible owing to the anesthetic limitations of the animals [16], and further studies have been planned to investigate this point.

Despite the importance of the topic, a literature review did not reveal any experimental work that could be compared to our study. Only one case report [20] describes a patient who had primary migraine and suspicion of hypogonadism and was treated with insulin-induced hypoglycemia for diagnostic purposes. In that patient, OAEs disappeared in the presence of hypoglycemia, which provides evidence for the hypothesis that hypoglycemia does indeed affect inner-ear functioning.

The use of sheep for experimental purposes was initially proposed by Lavinsky and Goycoolea [21] for otological research. Since then, this animal was adopted as

Table 1. Minimum Thresholds (dB) for Distortion Product Otoacoustic Emissions at Four Moments after Injection of Insulin in the Study Group and of Saline in Controls

Frequency (Hz)	Control Group				Study Group			
	60 min	70 min	80 min	90 min	60 min	70 min	80 min	90 min
750	−6.2	−16.5	−19.8	−19.1	−8.2	−6.3	−16.9	−20.0
1,000	−1.6	−4.4	−2.3	0.5	−23.1	−0.1	−9.3	−19.5
1,500	−4.6	−3.7	−7.4	−4.0	−26.6*	−21.6*	−17.2*	−30.2*
2,000	−5.5	−0.5	2.2	−1.8	−32.9*	−21.9*	−22.9*	−31.5*
3,000	5.2	3.8	5.9	6.1	−25.1*	−17.8*	−18.7*	−34.3*
4,000	10.6	8.1	8.4	7.8	−22.7*	−27.7*	−24.3*	−50.5*
6,000	23	19.5	21.4	22	−11.8*	−14.6*	−8.6*	−18.3*
8,000	18.9	18.4	23.8	25.8	−6.3*	−12.9*	−19.9*	−25.5*

* Indicates statistical significance for the comparison between control and study animals.
Note: Binomial test for homogeneous occurrence of minimum values for the study and control groups: $p < .001$.

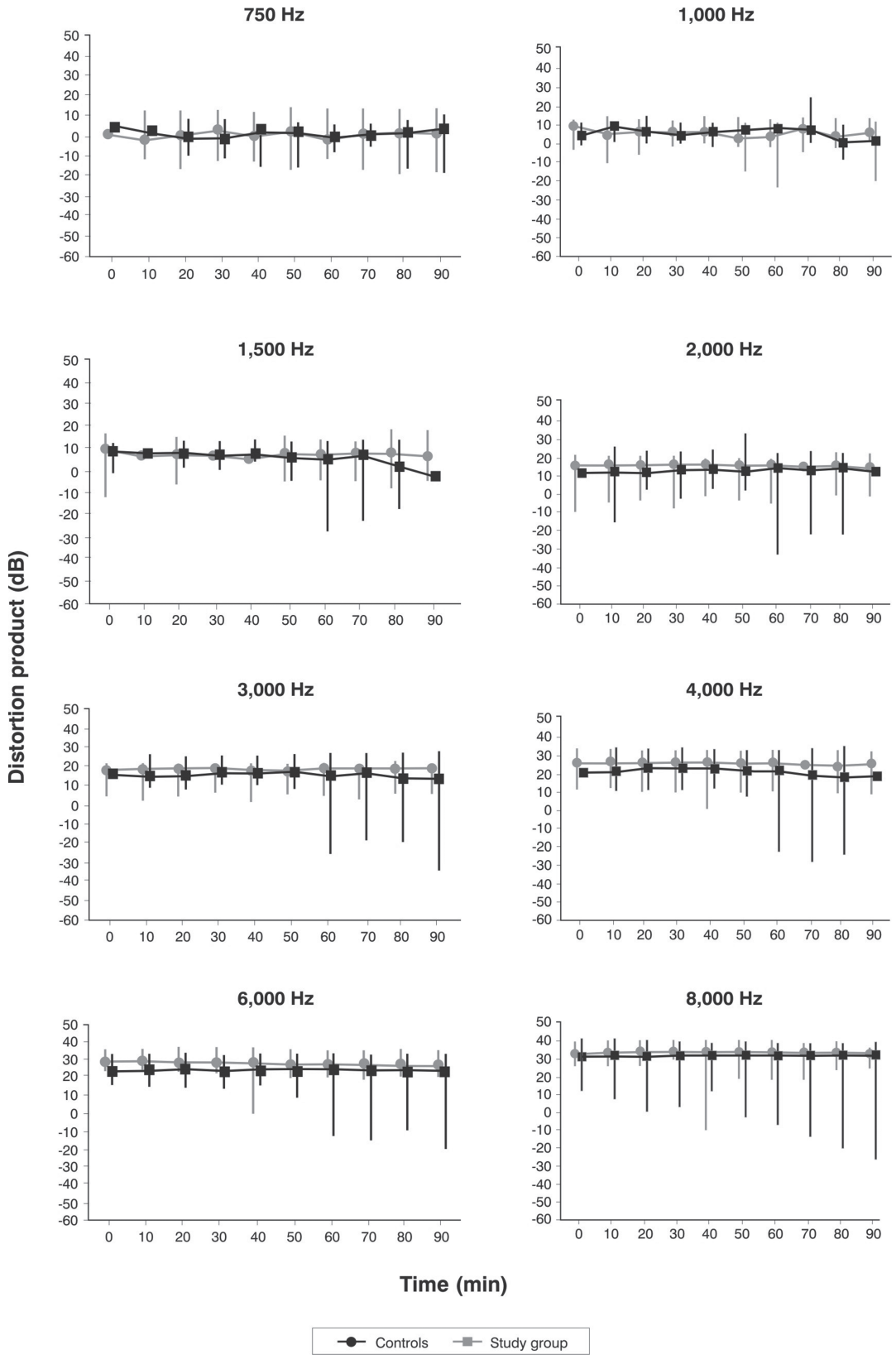

Figure 3. Median, minimum, and maximum thresholds (in decibels) between 750 and 8,000 Hz for distortion product in the control group and study group during the 90 study minutes. Note the dispersion of minimum values in high frequencies (≥1,500 Hz) after 60 minutes.

the model of choice by our research group. Many of the animals currently employed in research, such as dogs, cats, and monkeys, are different from human beings in terms of size, and managing them in confined spaces is difficult owing to their aggressiveness and susceptibility to disease. In addition, these animals are often expensive and difficult to obtain. Finally, as some are seen as pets, their use in research and training may cause unwanted conflict with animal protection organizations. Previous work from our group [16] has revealed significant similarities between sheep and humans in terms of ear anatomy, histology, and morphometry, especially concerning the size of structures. Owing to such similarities, sheep are especially useful for surgical studies and investigation of otological and neurophysiological aspects.

In our study, the exposure of the external auditory canal through a preauricular incision was crucial for visualization of the tympanic membrane and for perfect adaptation and isolation of the external auditory canal during the OAE test, ensuring that cochlear monitoring was free of interference during the entire experiment. This interference-free cochlear monitoring resulted in consistent and homogeneous OAE recordings and results and allowed the performance of the first DP-grams in sheep. The fact that the minimum thresholds for DPOAEs were not stable for the study group (see Table 1) is probably due to electrophysiological involvement and not to any recording instability. This should be further investigated in similar studies using the same animal model.

Several techniques are currently employed to monitor auditory function. Transient and distortion product OAEs, which reflect the functioning of cochlear OHCs, are a recent noninvasive, easy-to use alternative that has been gaining importance. Although they cannot determine the auditory threshold and do not replace pure-tone audiometry, immitance testing, or auditory evoked potentials, OAEs are capable of detecting early signs of cochlear damage.

We defined a 50% reproducibility parameter in transient OAEs as inclusion criterion for the study of experimental time zero for each animal. This reproducibility level rules out artifacts and renders the results reliable.

The amplitude of DPOAEs showed a tendency toward growth in high frequencies. This larger amplitude in high frequencies could be associated with tonotopical distribution, as high frequencies are located at the basis of the cochlea, proximal to the location where OAEs are measured. Gorga et al. [22] suggested some explanations for the reduced response obtained with low-frequency stimulation. One would be the low signal-to-noise ratio for grave tones. A second explanation could be the mode of energy transfer through the middle-ear system, which has less amplification capacity for grave tones. According to those authors, this characteristic of the middle ear interferes with the transmission of grave sounds, both from the middle ear to the cochlea (stimulus) and in the opposite direction (response). The sum of these factors seems to result in smaller amplitude in lower frequencies for DPOAEs measured at the level of the external auditory canal, complicating the distinction between emissions and background noise.

Our study raised several points. No threshold change (expressed in decibels) in the control group DPs was seen during the entire experiment (90 minutes). Control DP-grams remained stable during the experimental period, establishing this result as the normality standard in sheep. Also, our most relevant finding was the observation that the study group experienced a significant reduction in DP thresholds as compared to controls, especially in frequencies >1,500 Hz and after 60 minutes ($p < .001$). This provides evidence of electrophysiological changes in the OHCs, especially at the base of the cochlea.

OHCs are responsible for the amplification of sound at specific frequencies, a process known as *electromotility*, which results from the variation in membrane fluid in these cells. Thus, our findings probably reflect the acute action of insulin, which after 60 minutes starts to influence the electromotility of OHCs. The work of Horner [23] revealed that induced hydrops in guinea pigs led to changes in OHCs at the apical cochlea, whereas the cells at the base of the cochlea remained intact. Horner proposed that this outcome is owing to the size of the cell hair, which increases progressively toward the apex. The changes observed could have resulted from the damage to cell neural connections: The presence of hydrops in the endolymphatic space might force the basal membrane down or the tectorial membrane up, introducing tension and breaking neural connections. A second mechanism that could explain the alterations in OHCs is the efferent feedback mechanism that controls and selectively protects the base of the cochlea, where most medial fibers end. Seemingly, the changes observed in high frequencies in our study result exclusively from the acute nature of the insulin stimulation, which would interfere with cellular electrophysiology, however without enough time to generate a hydropic process, which is the basis for the study by Horner [23].

Although establishing correlations between physiological alterations in the cochlea and disturbances in carbohydrate metabolism is difficult, DPOAEs could eventually become a useful tool for that.

The study of OAEs in sheep during induced acute hyperinsulinemia revealed the occurrence of significant electrophysiological changes in cochlear OHCs, rendered evident through the observed variation in DP thresholds at high frequencies (>1,500 Hz) after 60 minutes.

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