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Özgün araştırma

Hidrasyonun Sıçanlarda Distorsiyon Ürünü Otoakustik Emisyon Üzerine Etkisi

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Öz

Amaç; Bu çalışmanın amacı dehidrate ve aşırı hidrate edilen ratların iç kulak akustik özelliklerinde oluşacak değişimlerin distorsiyon ürünü otoakustik emisyon (DPOAE) ile değerlendirmektir.
Gereç ve Yöntem; Çalışmamız, 24 adet erkek, Sprague Downey cinsi sıçan üzerinde yapılmıştır. Sıçanların genel anestezi altında otoskopik muayeneleri yapıldıktan sonra, kan örnekleri alınmış, kiloları ve DPOAE değerleri ölçülmüştür. Daha sonra ratlar dehidrasyon grubu, aşırı hidrasyon grubu ve kontrol grubu olmak üzere üç gruba ayrılmıştır. 72 saat sonra tüm ölçümler tekrarlanarak sonuçlar karşılaştırılmıştır.
Bulgular; İlk ölçümlerde üç grup arasında istatistiksel fark saptanmamıştır ($p>0.05$). Sıvı alımı değişikliği sonrasında kilo ve vücut sıvı miktarları karşılaştırıldığında dehidrasyon grubunda ve aşırı hidrasyon grubunda istatistiksel olarak anlamlı bir değişiklik olurken ($p<0.05$), kontrol grubunda anlamlı bir değişiklik olmamıştır ($p>0.05$). DPOAE SNR (dB) değerleri karşılaştırıldığında, dehidrasyon grubu ve kontrol grubunda anlamlı bir değişim olmazken, aşırı hidrasyon grubunun 4004 Hz, 7998 Hz ve 9854 Hz frekanslarındaki dB(SNR) değişimleri istatistiksel olarak anlamlı bulunmuştur ($p<0.05$).
Sonuç;Bu çalışmanın sonuçları sıçanlarda oluşturulan aşırı hidrasyonun DPOAE değerlerinde değişiklik yapabileceğini göstermiştir.

Anahtar kelimeler: *dehidrasyon, aşırı hidrasyon, iç kulak, otoakustik emisyon*

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Original Research

The Effect of Hydration on Distortion Product Otoacoustic Emission Values in Rats

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Abstract

Objectives: This study aimed to evaluate the changes that would appear in the inner ear acoustic characteristics of dehydrated and overhydrated rats through distortion product otoacoustic emission (DPOAE).
Materials and Methods: The study was conducted with 24 male Sprague Dawey rats. After otoacoustic examinations of the rats were made under general anesthesia, their blood samples were taken, weights were measured. Then, the rats were divided into three groups as dehydration group, overhydration group and control group. Distortion product otoacoustic emission measurements were repeated after 72 hours, and the results were compared.
Results: There were no significant differences in the first measurements between three groups in terms of ($p>0.05$). Weight and osmolarity values differed significantly in the dehydration and overhydration groups following the fluid intake change ($p<0.05$), but in the control group ($p> 0.05$). There was a significant decrease in the SNRs at 4004 Hz, 7998 Hz and 9854 Hz frequencies of the overhydration group ($p<0.05$). On the other hand, there were no significant changes in the DPOAE SNR dB values of the dehydration and control groups ($p>0.05$).
Conclusions: The results of this study indicate that overhydration in rats may change DPOAE values.

Keywords: Dehydration, overhydration, inner ear, otoacoustic emissions.

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Introduction

Dehydration due to water loss leads to decrease in blood and plasma volume while increased hematocrit causes an increase in plasma osmolarity and aggregation of red blood cells (Pereira et al.,2002). In conditions where fluid intake is excessive or insufficient excretion by the kidneys, it can cause severe hyponatremia water poisoning, convulsions and sometimes death (Salvatoni et al.,1990). Anatomical and physiological water and ion balance is quite importance for the structures that form the ear to function. Outer, middle and inner ear is not only connected to one another anatomically and functionally, and any problem in one of them affects the others, too. The water and ion balance of ear is of great importance. The three semicircular organs that form the membranous labyrinth, otolith organs and the scale in the cochlea show continuity through the endolymph with media content. Information transfer occurs as a result of the hydromechanical properties of the environment in which ductus cochlearis outer hair cells (OHCs) are located, and the movement of their stereocilia (Lee, 2012; Lawrence, 1991). The mechanic function of the ear is to obtain and analyze the sound of wide frequency and intensity in the air and to convey this to brain with nerve fibers (Wilson 1987).

Distortion product otoacoustic emissions (DPOAE), which is noninvasive, fast and inexpensive measurement method for evaluating hearing function, occur with the formation of two advancing wave by two stimuli with different frequency and intensity, and with the formation of a response that occurs in the cochlear region where they overlap. DPOAE provides information specific to frequency in the cochlea (Uzun et al.,2000; Brown et al.,1989).

This study aimed to evaluate the changes that would appear in the inner ear acoustic characteristics of dehydrated and overhydrated rats through DPOAE.

Materials and Methods

The study was conducted in Baskent University Animal Experiments Laboratory after the approval of Baskent University Animal Experiments Local Ethics Committee (2016, DA 16/27) was obtained. The study complied with the rules related to animal care and use reported in the international Declaration of Helsinki. Animals were housed in cages in a room with an ambient noise level <50 dB, a 12-h light/dark cycle, and a room temperature of 20-22^{0}C. Animals had free access to water and food during the day. General anesthesia was achieved using ketamin HCL (Ketalar, Pfizer, İstanbul) 60 mg/kg and Xylazine HCL (Rompun, Bayer, İstanbul) 6 mg/kg.

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There were different dehydration models with different methods and time periods regarding test animals in the literature. A pilot study was conducted with two rats to ensure that test animals are least affected. Weight measurements and osmolarity measurements with intracardiac blood samples were made at the 24th, 48th and 72th hours after fluid restriction in this pilot study. It was determined based on the measurements and results of this study that the most suitable period was 72 hours, and the pilot study was terminated. The study included 24 male Spraquey Dawey rats (350-400 gr).

The first group (dehydration group) (n=8 rats) was in an environment where they can have unlimited food, but water restriction was applied for 72 hours. Thus, the dehydration model was formed. In the measurements made after 72 hours, 5% increase in osmolarity and 10% decrease in weight were regarded as dehydration.

The second group (overhydration group) (8 rats) was in an environment where they can have unlimited food and was given 600 mM (milimol) sugar water as for 72 hours. Thus, the hydration model was formed. In the measurements made after 72 hours, 5% decrease in osmolarity and 10% increase in weight were regarded as overhydration.

The third group (8 rats) was the control group. No food or fluid restrictions were applied to the control group.

DPOAE Measurements;

The measurements of the rats were made under general anesthesia. Otoscopic examinations of all rats were performed, debris and plugs in the external auditory canal were cleaned and a normal tympanic membrane image was obtained before the measurements were made. After the osmolarity values of the rats with normal ear structure were measured with the intracardiac blood samples (0.5 cc), and weight measurements were made using precision weighing. Osmolarity measurements were made using the Advanced ® Model 3320 Micro-Osmometer device in Baskent University Faculty of Medicine Biochemistry Laboratory.

ILOv6 EchoPort (Otodynamics® Ltd, London, England) device was used to determine the otoacoustic emission values. The measurement was made on the general diagnostic mode of the device to obtain the distortion product otoacoustic emission values, and the stimulus intensity was taken as L1 for f1 frequency and L2 for f2 frequency in a way that the ratio between f2 and f1 frequencies was (f2/f1) 1.22, and L1-L2 was kept at 10 dB SPL (L1=65, L2=55 dB SPL) level. The values where the signal/noise ratio was above 6 dB in at least three frequencies were regarded as positive. The measurements were made in an environment where the noise level was not over 50 dB.

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The measurements were made on both ears of the rats. After the measurements were made, the rats were divided into three groups of eight rats.

All measurements were repeated for the rats in all groups after 72 hours under general anesthesia.

The data obtained were analyzed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) v22.0. The descriptive statistics were given as mean and standard deviation in the evaluation of the study data. The study used nonparametric tests for the analysis of DPOAE signal noise ratios, the Kruskal Wallis test for intergroup comparisons and the Wilcoxon rank test for the intragroup comparisons of measurements made at different times. When a statistically significant difference was found in intergroup comparisons, MannWhitney U test was used for the evaluation between two groups. The statistical significance level was $p<0.05$.

Results

The Study Groups' Weight and Osmolarity Values

Weight and osmolarity values of the rats in all groups were compared with the values before the implementation of the study. While the osmolarity value between the study groups was p=0.109, weight measurement value was p=0.216. Accordingly, it can be stated that the groups distributed normally, and there was no significant difference between the groups in terms of weight and osmolarity values (Table 1).

Table 1: The groups in terms of weight and osmolarity values.

Groups	Weight (gr)	Osmolarity (mOsm/kg)
Dehydrated	361,38±38,663	302,50±4.140
Overhydrated	348,25±24,558	305,13±5,194
Control	377,88±34,540	299,63±4,173

It was also statistically found that the intended model was formed as a result of the dehydrated, overhydrated and control groups' weight and osmolarity measurements made after 72 hours (weight p= 0.023, osmolarity p=0.000) (Table 2).

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The Study Groups' Inner Ear Distortion Product Otoacoustic Emission Values

No response was obtained from four rats in the control group, two rats in the dehydration group and one rat in the overhydration group in the DPOAE test, and one rat in the overhydration group died. Therefore, the measurements were made on eight ears (4 rats) in the control group, twelve ears (6 rats) in the dehydration group and twelve ears (6 rats) in the overhydration group. There were no significant differences between the groups in terms of SNR (Signal-Noise 214 Ratio) values at each frequencies in all rats during first measurements ($p>0.05$). No significant difference was found in the comparison of the first and last measurement 216 DPOAE SNR values of the dehydration group.

Table 2: The last measurement weight and osmolarity values of the groups.

Groups	Weight (gr)	Osmolarity (mOsm/kg)
Dehydrated	314,63±41,593	318,38±2,774
Overhydrated	346,25±24,335	294,13±4,190
Control	369,38±30,166	299,63±2,875
P	0,023	0,000

*p<.05

A significant decrease was found in the comparison of the first and last measurement DPOAE SNR (dB) values at 4004 Hz, 7998 Hz, 9854 Hz frequencies of the overhydration group (respectively, p=0.012, p=0.015, and p=0.015) (Table 3).

No significant difference was found between the first and last measurement DPOAE SNR (dB) values of the control group (Graph 1, Graph 2).

Table 3. Comparison of DPOAE results of all groups.

	Dehydration			**Hydration**			**Control**		
	First DPOAE	Last DPOAE	P value	First DPOAE	Last DPOAE	P value	First DPOAE	Last DPOAE	P value
Frequency									
2002	5,55±5,89	5,02±8,06	0,937	1,37±5,56	1,47±5,49	0,695	2,60±3,58	7,43±22,92	0,779
4004	10,94±4.42	14,69±12,43	0,239	9,41±7,43	3,37±6,30	0,012*	9,58±2,90	8,81±2,76	0,123
6064	19,86±12,63	22,75±17,13	0,433	17,18±14,63	13,21±15,64	0,388	17,75±14,01	6,72±8,76	0,093
7998	29,51±11,95	28,23±12,29	0,433	27,84±12,79	12,75±9,78	0,015*	26,22±12,56	25,35±11,45	0,092
9854	32,13±15,06	30,63±13,42	0,695	28,74±11,92	18,66±10,25	0,015*	27,21±12,98	17,70±14,02	0,093

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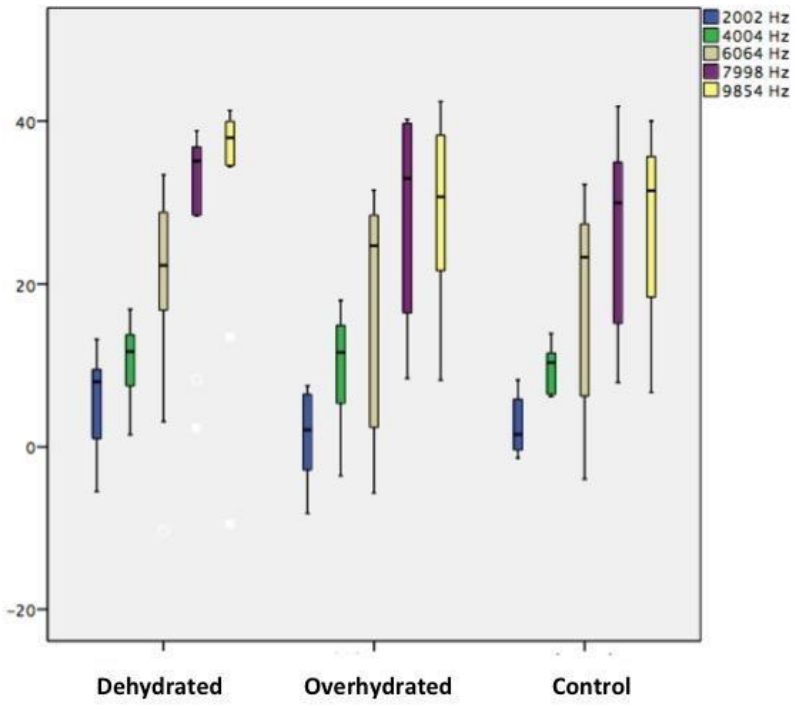
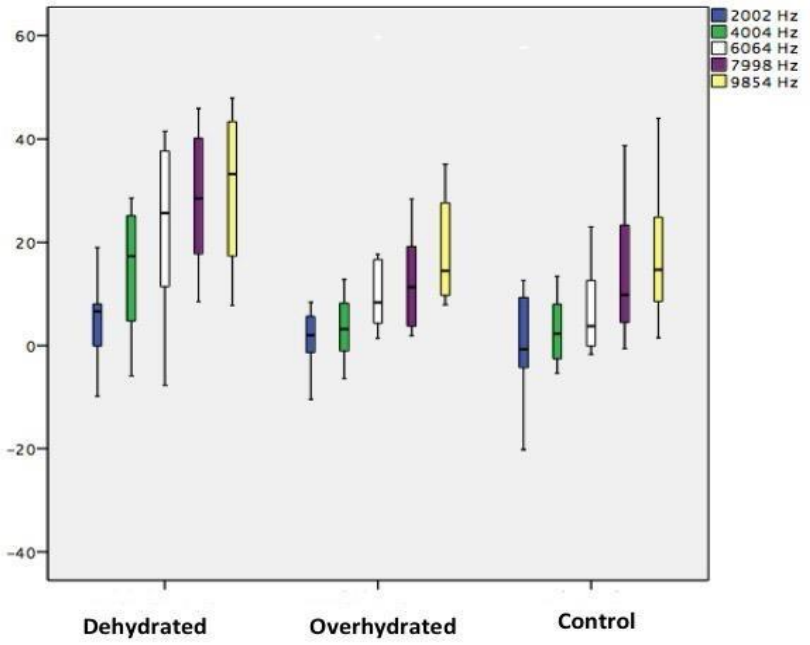


Graph 1: The first measurements Otoacoustic Emission Values (SNR) of the groups.



Graph 2: The last measurements Otoacoustic Emission Values (SNR) of the groups

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Discussion

This study, which was conducted to examine the changes on cochlear functions due to body fluid intake amount, compared the SNR values obtained with DPOAE in dehydration and overhydration models formed with rats. The results indicated that cochlear functions were affected in the overhydration model.

Electro-osmosis is a motion of flow made by the electric field that occurs between extracellular and intracellular including the cell wall. It provides the required pressure to match the hydrodynamic motion in the membrane for fluid motion to occur. According to this model, there is an electric field between extracellular fluid and intracellular fluid, and active and passive transport of ions, inorganic, organic materials and water occur there. It is considered that hydraulic movements of ions cause the osmotic movement of water (Nakagawa et al.,2006). OHCs, which are located in inner ear in mammals, act as a biological sensor. It is also stated that changes in osmolarity cause a decrease in endolymph in scale media; thus, affect the activities of OHCs (Salt et al.,1995).

Although there are some structural and functional differences between humans and rats, hearing system is generally similar in all mammals. In rats, the cochlea turns 2.5 turns as in humans. Tympanic membrane does not fill the entire outer ear path. There is an opening named incus and malleus that enables the entrance to middle ear in the tympanic membrane of rats. Eustachian tube length is approximately 4.5 mm. The inner ear structures of the rats are similar to that of human, but the absence of Hensen cells creates the difference. Hearing sensations of rats are well developed at high frequencies due to the anatomical features of the cochlea in addition to the anatomical and functional features of the head and pinna. (Li et al.,2015). The current study included male rats, and no distinction between right or left ear was made.

A pilot study was conducted for the dehydration and overhydration model with two rats to ensure that test animals are least affected. Water restriction was applied on the test animals and weight measurements and osmolarity measurements with intracardiac blood samples were made in 24, 48 and 72-hour periods. It was determined that the most suitable period was 72 hours to form the model as a result of this study. In the measurements made after 72 hours, 5% increase in osmolarity and 10% decrease in weight were regarded as dehydration while 5% decrease in osmolarity and 10% increase in weight were regarded as overhydration.

Dehydration occurs as a result of the decrease in the total amount of fluid in the body. There are limited number of studies on the effects of dehydration on inner ear in the literature. Barbara et al. conducted an experimental study with gerbils and found that dehydration did not

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cause a damage in cochlea while endolymphatic duct and sac got negatively affected by this condition and matrix accumulation occurred in subepithelial tissue (Barbara et al., 1989).

Salt et al. measured endolymph fluid changes during osmotic dehydration with two different methods and found an increase in endolymph fluid, but they did not have sufficient data on the physical capabilities of endolymphatic system related to osmotic dehydration (Salt et al.,1995). In the comparison of the effects of the hyperosmotic agent glycerol and urographin on cochlear blood flow and serum osmolarity, it was determined that urographin infusion had a higher intensity and longer duration of action than glycerol infusion. This study found that both agents contributed to the recovery of hearing loss occurred in endolymphatic hydrops. However, since glycerol passes more easily from the interstitial space to the inner ear fluids, the blood pressure in the cochlea drops rapidly. In conclusion, these agents have direct effects on vascular wall and contribute to recovery of hearing by affecting different mechanism (Noi et al.,1998). OHC mobility is considered to be based on a hydromechanical principle, and the changes in the osmolarity of the media and an increase in serum osmolarity cause the functions of the OHCs in the inner ear to deteriorate. Suckfull et al. examined the effect of the changes in serum osmolarity on the functions of OHCs, determined these effects through DPOAE and addressed the relationship between sudden sensorineural hearing loss and dehydration. Accordingly, it was reported that mild to medium hearing loss can be explained with changes in serum osmolarity (Suckfüll et al.,1999). Brownell et al. conducted an experimental study and determined that the dynamic and mechanical characteristics of OHCs caused osmolarity changes and reversible changes and that otoacoustic emissions decreased (Brownell et al.,1990). Baskabadi et al. tried to answer the question whether newborn hyponatremia dehydration changes hearing condition and found that temporary hearing loss was higher among newborns with hyponatremia in their experimental study (Boskabadi et al.,2014). In the experimental study by Choi, the effects of osmotic changes on cochlear function was examined and the action potential, DPOAE, cochlear microphonic and endocochlear potential values were measured. This study found an increase in cochlear microphonic otoacoustic emission values after hypotonic perfusion and a decrease after hypertonic perfusion. There were no changes in the stimulant threshold of low frequency cochlear microphony and the endocochlear potential was not affected by perilymphatic osmolarity (Choi & Oghalai 2008).

Duration of dehydration changed between 72 and 120 hours or various chemicals were given to test animals to cause dehydration in previous studies. The researchers found that 72 hours of dehydration was sufficient in the pilot study conducted, and used this period of time in

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the current study. However, decrease in DPOAE SNR was not statistically significant in this study unlike other studies. There are different models in the literature, but the tests were made within the shortest time that dehydration occurred in this study. The researchers are in the opinion that it is not correct to comment about the effect of being dehydrated for a longer time based on these findings. There might be differences between the dehydration model in this study and available models in the literature.

There is also no study on the effect of overhydration on hearing in the literature. The present study found that overhydration affected cochlear functions. It is considered that changes in bodyweight affect ear tissues based on this information obtained. It is known that body mass index is related to the increase in intracranial pressure, and water and Na ratios. Increasing bodyweight might affect the inner ear mechanics by increasing intracranial pressure (Sözen et al.,2018).

The present study found that dehydration caused changes in inner ear acoustic characteristics, but these changes were not statistically significant while overhydration affected especially the functions of OHCs. Additionally, there is a decrease in emission responses, in other words, a negative effect on the functions of OHCs in the overhydration model. According to the data of this experimental study, supportive histopathological studies that examine the effect of dehydration and overhydration on inner ear should be carried out. Another weakness of this study is that the effect of hydration and dehydration on hearing can be revealed more comprehensively with the ABR test with which synaptic and postsynaptic area can be evaluated, in addition to DPOAE test.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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