A PRACTICAL METHOD FOR TRACING KETOSIS: BREATH ACETONE MEASUREMENT

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Introduction

The ketogenic diet was first discovered in the 1920s, and it was thought that ketosis occurring in individuals on diet may be effective in some diseases, especially drug-resistant epilepsy (glucose transporter protein 1 deficiency, mental and neurological diseases) (1-2). The ketogenic diet which was used as an anticonvulsant therapy for drug-resistant epilepsy has become popular in weight control in recent years. The basis of the ketogenic diet is that it contains high fat, low carbohydrates and protein. Although there are several types, the most common type is high fat low carbohydrate ketogenic diet (3). While the fat content of this diet, which is defined as the classical ketogenic diet, constitutes 80-90% of the energy, carbohydrates and proteins constitute 10-20% (4-5).

In individuals who follow a ketogenic diet, a measurement of ketone levels in urine and blood is used to evaluate the state of ketosis. The American Diabetes Association reported that urine and blood ketone measurements are standard in diagnosis and evaluation (6). Urine acetone/acetoacetate detection method is also preferred because it is more practical and noninvasive compared to detecting betahydroxybutyrate in the blood (7-9). However, urine collection throughout the day is a difficult process. Urine dipstick analysis, which is another method, is less invasive but also less reliable (10). Additionally, in case of dehydration, the value of urine ketone analysis may be limited in the evaluation of ketosis (11). In a study conducted in adult individuals following a ketogenic diet, the levels of betahydroxybutyrate of the individuals determined in the breath sensors were correlated with the levels detected in the blood and urine (12). Breath acetone analysis is another method used to detect acetone since acetone leaves the body via the lungs (13). It has been reported that the breath acetone measurement method can also be used to monitor weight control as an indicator of body fat loss. In recent years, measurement of the amount of acetone in breath has been shown to be reliable in determining the degree of ketosis (14).

From a clinical point of view the measurement of breath acetone levels is important, but there are some limitations such as the storage of samples, standardization of clinical settings, challenges in using clinical devices and the price of these devices. It is stated that the sensors will become an important tool for the detection of breath acetone in the future due to their small size and low price (12). Until now, studies of the accuracy of breath acetone sensors have been inadequate (15). In this study, it was aimed to evaluate the effectiveness of breath acetone measurement with a portable and economical sensor in determining ketosis in short-term ketogenic diet. In addition, the effects of the short-term ketogenic diet on weight loss and ketosis with the inclusion of the control group were also examined.

Methods

Participants

Ethical approval of the study was prepared according to the ethics standards of the Helsinki Declaration and approved by the ethics committee of the local university (Decision No: 2019-12 / 04). An informed consent was obtained from the participants. Eighteen individuals were included in the study, 10 of whom were in the treatment group and 8 of whom were controls. Individuals under the age of 18 and over 65, those who exercise heavily, who have chronic disease (hypertension, diabetes, kidney, liver diseases, etc.) and / or who use regular medications are excluded from the study. The control group consisted of 8 adults whose age, gender, and anthropometric measurements were matched with the treatment group.

Study plan

A ketogenic diet list was created by the Faculty of Nutrition and Dietetics to apply to the participants in the treatment group for a total of 1 week. The energy content of the diet applied to men is 1200 kcal (13% carbohydrate, 21% protein, 64% fat), and the energy content of the diet applied to women is 1100 kcal (10% carbohydrate, 22% protein, 68% fat). Urine ketone and breath acetone measurements were obtained from the treatment group for 5 days, as well as from the control group without a ketogenic diet.

Anthropometric measurements

Body compositions of the individuals who participated in the study were determined by bioelectrical impedance analysis method at the beginning and end of the study. All measurements were made in the Anthropometry Laboratory of the Nutrition and Dietetics Department. Height (cm) was measured using a stadiometer (Leicester Height Measure, Seca 214, UK). Body composition data [weight (kg), body mass index (BMI; kg / m2), fat mass (kg), body fat ratio (%)] were obtained using bioimpedance analysis via Inbody 270 (InBody USA, Cerritos, CA, USA). Waist circumference (cm) was measured using a non-stretching measuring tape.

Breath acetone and urine ketone measurements

Breath acetone measurements of both the treatment and control groups were taken at the same time every day and when the individuals were fasted. Avocadio Ketone Testing Kit (Mp303a; semi-conductor metal oxide gas sensor) device was used for the measurement of breath acetone. This semiconductor metal oxide-based gas sensor obtained by nanotechnology has been preferred for its advantages such as fast and precise detection, portability and low cost compared to other traditional techniques (16). The gases in the breath react with the sensor and change the sensor resistance, while the changing resistance value indicates the gas concentration. To increase the accuracy of breath measurements, each measurement was applied three times and averaged.

In addition, urine ketone measurements of all participants were made using the Laboquick urine strip at the same time of the day with breath measurement. The reagent tip of the strip resulted in color change following contact with urine. The resulting colors were evaluated 15-30 seconds after the reaction compared to the color chart supplied with the product.

Statistical analysis

All analyzes were evaluated in SPSS software (version 21.0, Inc., Chicago) package program. Statistical significance was accepted as $p < 0.05$ in all analyzes. Descriptive statistics include mean and standard deviation values. Wilcoxon test for the difference between repeated measurements; Mann Whitney U test was used for comparison of independent groups and Spearman correlation test was used for those who constantly changed.

Results

A total of 18 adults, 16 females and 2 males with a mean age of 27.5 ± 4.4 years, were included in this study. There were no significant differences in age and anthropometric measurements between the intervention and control groups at the beginning of the study and the two groups were homogeneous. As a result of the 7-day ketogenic diet program implemented in the intervention group, significant reductions were observed in weight (*P*=0.005), BMI (*P*=0.007), waist circumference (*P*=0.020), fat mass (*P*=0.005) and body fat ratio (*P*=0.066) (Table 1).

Table 1: Change of anthropometric measurements by intervention and control groups

Anthropometric measurements		Intervention group (n=10)	P*	Control group (n=8)	P*	P**
Weight (kg)	Beginning	66.7±17.1	**0.005**	63.8±13.9	0.400	0.633
	End	65.3±16.6		64.2±14.7		0.829
BMI (kg/m²)	Beginning	23.6±4.5	**0.007**	22.2±3.3	0.574	0.408
	End	23.1±4.3		22.3±3.5		0.829
Waist circumference (cm)	Beginning	77.3±15.8	**0.020**	72.2±13.9	0.564	0.146
	End	76.3±15.4		72.3±14.3		0.315
Body fat mass (g)	Beginning	21.2±7.8	**0.005**	18.1±5.4	0.752	0.122
	End	20.5±7.6		18.1±5.7		0.633
Body fat ratio (%)	Beginning	31.2±6.0	0.066	28.0±4.3	0.574	0.829
	End	30.8±6.1		27.9±4.2		0.122

Average and standard deviation values were included. *Wilcoxon test was used, statistical significance level p <0.05. **Mann Whitney U test was used, statistical significance level p <0.05.

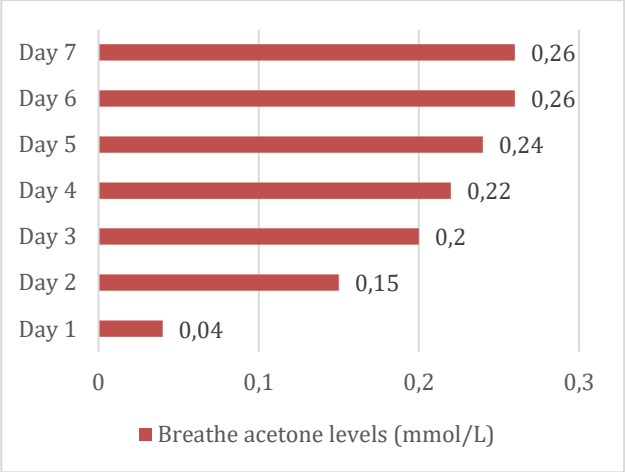
Breath and urine measurements of the intervention group for 7 days and the control group for 5 days are shown in Table 2. After the ketogenic diet intervention, on day 1, there was no significant difference between the groups (*P>0.05*); on day 2, only significant difference was detected in the measurement of breath acetone (*P<0.05*). However, the measurements at 3rd, 4th and 5th days were significantly higher than the control group (*P<0.05*). The change of the breath acetone and urine ketone levels of the intervention group following the ketogenic diet are also summarized in Figure 1.

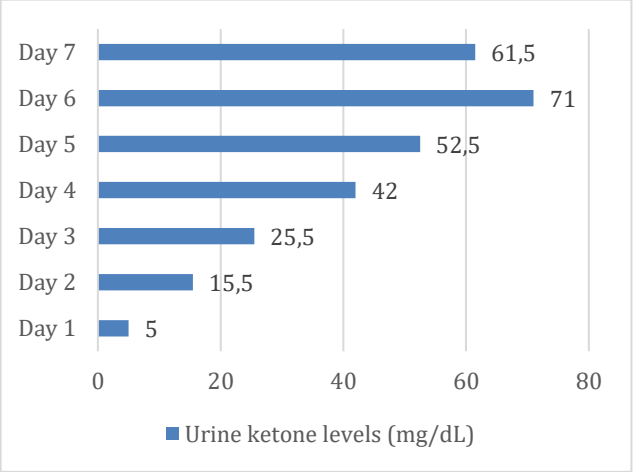
Table 2: Distribution of breath acetone and urine ketone levels by groups

Days	Breath and urine tests	Intervention group (n=10)	Control group (n=8)	P*
1	Breath (mmol/L)	0.04±0.0	0.0±0.0	0.315
	Urine (mg/dl)	5.0±3.3	5.0±0.0	1.000
2	Breath (mmol/L)	0.15±0.1	0.0±0.02	**0.004**
	Urine (mg/dl)	15.5±13.8	6.2±2.3	0.083
3	Breath (mmol/L)	0.20±0.09	0.0±0.0	**0.001**
	Urine (mg/dl)	25.5±22.9	5.0±0.0	**0.001**
4	Breath (mmol/L)	0.22±0.09	0.0±0.0	**0.001**
	Urine (mg/dl)	42.0±23.5	5.0±0.0	**0.001**
5	Breath (mmol/L)	0.24±0.04	0.0±0.0	**0.000**
	Urine (mg/dl)	52.5±30.3	5.0±0.0	**0.000**
6	Breath (mmol/L)	0.26±0.08	-	-
	Urine (mg/dl)	71.0±41.6	-	-
7	Breath (mmol/L)	0.26±0.03	-	-
	Urine (mg/dl)	61.5±24.9	-	-

Average and standard deviation values were included.
*Mann Whitney U test was used, statistical significance level p <0.05

Figure 1: Distribution of breath acetone and urine ketone levels in intervention group





When the relation between breath acetone levels and urine ketone levels in the intervention group was analyzed, significant correlations were found in all 7 days *(P<0.05).* On days 4 and 5, positive moderate correlations were observed; on the other days, high level of positive correlations were observed *(P<0.05)* (Table 3).

Table 3: The relationship between breath acetone and urine ketone levels of the intervention group

		1	**2**	**3**	**4**	**5**	**6**	**7**
Correlation	R	0.745	0.710	0.854	0.641	0.697	0.869	0.868
	P*	**0.013**	**0.021**	**0.002**	**0.046**	**0.025**	**0.001**	**0.001**

*Spearmen correlation was used, statistical significance level $p<0.05$

Discussion

The ketogenic diet is a diet model that contains high amounts of fat, low amounts of carbohydrates and sufficient protein (17). It is an effective non-pharmacological treatment method for 'drug-resistant epilepsy' patients (18). In addition, the use of the ketogenic diet in the management of diseases such as obesity and type 2 diabetes has been discussed in recent years (19-20). In a meta-analysis which included 13 studies that examined long-term effects of very low-carb ketogenic diets on weight loss; very low carbohydrate ketogenic diets provided significantly more weight loss than low-fat diets ($p = 0.02$) (20). Similar to the literature findings, in our study anthropometric measurements were found to be significantly lower in the ketogenic diet group after treatment. Despite the consistent results, considering the long-term possible side effects of the ketogenic diet, it states that it is important to set the diet correctly, to determine the time to be followed and regular follow-ups are important (21).

Ketone bodies are mainly produced by ketogenesis in the mitochondrial matrix of liver cells, and then exported to other organs to meet the energy demands of cells in the body through the blood (17). After a few days of fasting or carbohydrate-restricted (<20 g / day) diet, the body's glucose reserves become insufficient for normal fat oxidation and providing glucose to the central nervous system.The central nervous system requires an alternative energy source other than glucose, which leads to the production of ketone bodies such as acetoacetate, hidro-hydroxybutyric acid and acetone. These ketone bodies rising in the blood are removed from the body through urine and respiration (21). In the case of ketoacidosis, it was first determined by John Rollo in 1978 that individuals had a smell of acetone in their breath and that this condition may be a symptom of ketoacidosis (22). Measurement of these ketone bodies gives information about the presence and severity of ketoacidosis. Breath acetone and urine ketone methods are

used to measure ketone bodies. But breath acetone measurement has been determined to be more reliable than urine ketone measurement (10).

Due to the advancement of modern measurement technologies, breath measurements provide a non-invasive method for disease diagnosis and metabolic status monitoring (23). In a study conducted on individuals with type 2 diabetes (n=58), a significant relation was found between breath acetone and blood acetoacetate (R=0.89) and β hydroxybutyrate (R=0.82) (24). In another study conducted on individuals with diabetes (n=99), it was determined breathe acetone levels correlated with blood and urine ketone levels in the case of ketosis, and that breath sensors were reliable for the use of diabetic ketoacidosis (25). Prabhakar et al. (2014) showed that breath acetone levels are more effective compared to blood and urine methods in the early detection of ketosis (26). In another study, it has been determined that it is safe to determine acetone level in adult individuals (n=11) who received classical ketogenic diet (27). These results showed the effectiveness of using breath sensors in determining/monitoring ketone levels. In most of these studies, breath acetone levels were determined using methods such as gas chromatography (GC-MS/FID) and selected ion flow tube mass spectrometry (SIFT-MS) (24-26). Although these clinical tools are extremely sensitive, they do not allow personal monitoring due to their size, cost and the need for trained personnel (15). In our study, we found a correlation between urine ketone levels and breath acetone levels measured by semi-conductor metal oxide gas sensor technology. The rapid progress of nano technological semiconductor technologies has made it easier to measure breath (28). The non-invasive technologies, more especially the nanosensor breath technologies which are portable, cheap to fabricate, highly sensitive and easy to use, have potential in determination of the amount of volatile gases such as acetone, carbon dioxide (28,29). In this context, since the method of determining ketone levels in the blood is invasive and difficult to reach, determination and monitoring of ketone level with breath sensors becomes important.

In contrast to our study, the population of most studies in the literature that determined this relation constituted in individuals with diabetes (24,25). Considering that there are many factors (stress, exercise, drug use) other than dietary factors affecting the ketosis in diabetic individuals, the population of our study was healthy, and those who did not use exercise and did not use

drugs. In addition, in our study, there was no significant difference in the anthropometric measurements of individuals who participated in the control and treatment group at the beginning of the study. This situation minimizes environmental factors other than diet that affect individuals' ketosis status.

There are some limitations to this study. Firstly, the number of patients included in our study was relatively low. Secondly, blood ketone levels, which are a more reliable measurement method, could not be measured and compared with breath measurements. For this reason, breath acetone levels were compared only with urine ketone levels. Despite all these limitations, this study will contribute to the literature by evaluating the accuracy of breath acetone measurement among individuals on ketogenic diets.

Conclusion

In our study, it was determined that acetone levels were correlated with urine ketone levels in individuals on a ketogenic diet, and breath acetone levels were a suitable biomarker for monitoring ketosis. Given the long-term possible side effects of the ketogenic diet, regular follow-ups are important in the process of applying the diet. Breath acetone test is a method that can be used in the follow-up of ketosis due to its noninvasive nature, easy application and reliability. In our study, we emphasized that nanosensors measuring breath acetone are more reliable and useful compared to the urine method**.** It is also important for patients with epilepsy and diabetes, such as ketogenic diets for the purpose of weakening ketosis with a practical method. In the future, additional studies conducted on various patient groups (epilepsy and other diseases related with ketogenic diet) will provide more information about the clinical utility of the breath acetone sensors.

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Conflict of interest statement

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