ISSN: 2469-5718

Abadie and Fernández. Int J Sports Exerc Med 2024, 10:264
DOI: 10.23937/2469-5718/1510264
Volume 10 | Issue 1
Open Access

International Journal of
Sports and Exercise Medicine

ORIGINAL RESEARCH

Enhancing Precision Nutrition: Investigating the Interplay of Breath Acetone and Body Composition in Division I Athletes

Allyn Abadie* and José R Fernández

Department of Nutrition Sciences, The University of Alabama at Birmingham, Birmingham, Alabama, USA

***Corresponding author:** *Allyn Abadie, MA, ATC, The University of Alabama at Birmingham, Webb Nutrition Sciences Building, 1675 University Boulevard, Birmingham, AL 35233, USA, Tel: (205) 934-3006.*

Abstract

Background: Precision nutrition requires the discovery and understanding of quantitative biomarkers with sufficient between person variability. In healthy individuals, glucose is used as the preferential energy substrate and is not readily stored. Circumstances such as disease, fasting, or injury, can cause glucose to be unable to be used or not readily available. In these circumstances the body can utilize alternative energy sources such as ketones derived from stored fatty acids. Acetone, which is produced when the primary ketone acetoacetate is converted to beta-hydroxybutyrate, is commonly considered a proxy measure of fat utilization for energy. Breath acetone holds potential as a biomarker for monitoring these changes in energy substrate preference and is both non-invasive and quantitative offering sufficient variability to provide unique individual measures. Understanding the factors influential to breath acetone is important when characterizing an individual for clinical and performance purposes. The effect body composition and size have on the utilization of ketones for energy at restis relatively unknown. Though glucose is the preferential energy substrate, body fat detected in body composition and size measures may impact breath acetone measures and must be fully realized before use of the marker in decision-making. The purpose of this study is to determine the relationship of body composition and body size metrics with breath acetone concentration as measured by portable breathalyzer.

Methods: Division I football players (N = 36) were recruited for a one-time measure of their body composition [through body impedance analysis] and breath acetone [through portable breathalyzer] at rest. Correlation between breath acetone and body composition and size metrics were completed with a Pearson Correlation. A logistic regression was conducted to assess the influence of body-related factors.

Results: Breath acetone was not correlated with body composition or body size metrics ($P > 0.05$). Body composition and size influence breath acetone little (08%); however, when playing position, a qualitative external factor, was added to the model the influence rose substantially (59%).

Conclusion: Breath acetone, and thus fat utilization, was not significantly related to body composition and size measurements. Future research should include more factors when characterizing an individual and their production of breath acetone. Understanding the production of breath acetone may be advantageous as a clinical marker and for precision performance.

Keywords

Breath acetone, Ketone, Body composition, Body size

Abbreviations

ATP: Adenosine Triphosphate; BMI: Body Mass Index; PPM: Parts Per Million

Introduction

The implementation of personalized medicine, a term used interchangeably with precision medicine, has grown in popularity among researchers and clinicians. Personalizing medicine is the act of characterizing an individual uniquely based on a variety of factors (genomic, biochemical, behavioral, and environmental) and providing specialized interventions based on that unique makeup rather than using a general approach [1]. In sports medicine, this approach gained early popularity due to the close interprofessional relationships between physicians, athletic trainers, dietitians, and other sports performance professionals. These close direct relationships allow professionals to

Citation: Abadie A, Fernández JR (2024) Resistance Exercise Order Does Not Affect Training Intensity in Middle Aged and Older Women. Int J Sports Exerc Med 10:264. doi.org/10.23937/2469-5718/1510264

Accepted: April 27, 2024; **Published:** April 29, 2024

stay informed with changes that occur in external and internal factors and adapt the personalized treatments. Efforts have been made to personalize treatments in sports through hydration, playing time, player load, and overall nutrition [2-5]. Additionally, the growing use of wearable devices has allowed clinicians to individualize care for performance and injury risk based on real-time biometrics [6]. As the personalization of medicine broadens, new methods emerge within physiologic domains that were previously thought of as generalizable.

Energy metabolism is one area of health that may present potential as an opportunity to personalize treatment. In some endurance sports, athletes use training and nutrition to enhance their performance and use muscle fat stores to their advantage in what is called “metabolic flexibility” [7]. Thus, training their bodies to preferentially use muscle fat after exercise for a specific amount of time and intensity. As advancements have been made in epigenetics, scientists have started exploring whether there are developmental or physiologic factors that may be targeted for intervention as pertains to the role of energy metabolism in disease [8,9]. Energy metabolism can be summarized as the generation of adenosine triphosphate (ATP) from nutrient-derived substrates [9]. These substrates can be produced from carbohydrates, fats, or proteins. As a default, the body typically uses glucose from carbohydrates as its main energy source [9]. A new continuous-glucose monitor developed in 2020 by Abbott Laboratories, *The Libre Sense Glucose Sport Biosensor*, points to the sports industry adapting to the monitoring of glucose to personalize training and performance [10]. When glucose is unavailable or unable to be used, the body can alternatively produce ketones for energy from free fatty acids through oxidation [11] (Lee et al., 2017). There are three ketone bodies: Acetoacetate, beta hydroxybutyrate, and acetone. Acetoacetate is the primary ketone body from which the other two are derived [11]. Beta hydroxybutyrate is the preferred ketone body for energy utilization. When acetoacetate breaks down into beta hydroxybutyrate, acetone is created and released [12]. Since acetone is created through fat oxidation, acetone can be seen as a marker of fat lipolysis and can shed light on fat utilization for energy rather than carbohydrates [13].

Acetone has advantages as a marker of metabolism, even over the other ketone bodies. Acetone is the smallest ketone. The small size volatility of the ketone body allows it to be transported to the lungs and exhaled in breath [12]. This means it can be tracked non-invasively through an affordable and portable breathalyzer. Though acetone reflects fat lipolysis, little is known regarding the relationship of body mass index (BMI) or other body composition metrics such as body fat percentage to the concentration of breath acetone in healthy individuals. Previous literature has suggested BMI and acetone may be related, but conflicting results remain [11]. It is unknown whether individuals with higher body fat percentages have a higher predisposition to usage off at for fuel than those with lesser fat, or vice versa. Football is a sport that includes a diversity of athletes in terms of body size and body composition. Understanding the effect body composition has on a resting breath acetone concentration is a vital component to determining breath acetone’s potential as a marker for performance, disease, or injury in sport.

The objective of this study is to determine if there is an association between breath acetone and body composition and size measurements (BMI, body fat percentage, muscle percentage, and visceral fat level) in Division I football players. The primary hypothesis is that there will not be a significant association between breath acetone and body composition and size measurements. Breath acetone reflects the utilization of fatty acids for energy, and though an individual may have a lower or higher body fat percentage, they may not utilize fat for energy at rest. The study results will help guide future studies investigating the potential of energy substrates as precision medicine markers.

Methods

Experimental overview

The purpose of this study is to determine if body composition and size metrics are associated with breath acetone concentration. Healthy football players from a Division I university located in the southeastern United States were recruited for this study. Participants had body composition, body size, and three breath acetone measurements taken at rest. All participants were male and were recruited by convenience sampling. A variety of body sizes and football positions were present in this sample. Only football players aged 18-26 were included in the study. Players were excluded if they were currently concussed, following a low-carbohydrate or ketogenic-like diet, or had an underlying medical condition such as asthma that might affect the breathalyzer protocol. Additionally, some participants were heavier than the maximum capacity of the scale (330lbs.) and thus were excluded from those aspects of the investigation.

Dependent variable

Breath acetone: Breath acetone concentration was measured with a portable breathalyzer (Ketonix® Professional, Ketonix®, Stockholm, Sweden). The device can detect breath acetone concentration between 0 and 200 ppm. Breath acetone measures were taken consecutively three times while the participant remained in a resting seated position, and the average of those measurements was used for assessment. Measures were taken according to device instructions and protocols, and the same devices were used throughout the duration of the investigation.

Independent variables

Body composition and size variables: All body measures of size and composition were assessed by the OMRON® Full Body Sensor Body Composition Monitor and Scale (HBF-510), a bioelectrical impedance analysis system. These measurements included body weight (0-330 lbs.), body fat percentage (5.0-60.0%), skeletal muscle percentage (5.0-50.0%), BMI (7.0-90.0), and visceral fat level (0-30 relative levels). Height was self-reported for the BMI calculation. Measurements were taken abiding with equipment manufacturer protocols. Two players had weight measured but were unable to be measured for the other body composition metrics (body fat percentage, skeletal muscle percentage, and visceral fat level), as they weighed more than the equipment specifications. These players were included in association tests using weight, height, and BMI but were excluded for the other association tests by the statistical analysis software because of missing measurements.

Statistical analysis

Analysis for this investigation was completed using XLSTAT v.3.1 (Statistical and Data Analysis Solution, Addinsoft, New York, NY, USA, 2023). The continuous dependent variable of breath acetone was initially assessed for outliers using Grubb's test. A single outlier, the maximum (71.067 ppm), which was more than 4 z-scores from the mean, was removed including all of the participant's results, and a second Grubb's test that demonstrated no outlierswas completed. The continuous variable of breath acetone was normalized using a box-cox transformation, and normality was verified with the Shapiro-Wilk test (W = 0.957; P = 0.187), the Anderson-Darling test (A2 = 0.496; P = 0.199), and the Jarque-Bera test (JB [observed] = 1.719, JB [critical] = 5.991, P = 0.423). The associations between independent and dependent variables were assessed by the Pearson Correlation test. A logistic regression model was completed by transforming breath acetone into a binary categorical variable (higher or lower than the sample mean) to assess the influence body composition and size variables have on fat utilization. Predictor variables of body fat percentage, skeletal muscle percentage, visceral fat level, and BMI were included in the model. –An exploratory categorical predictor variable (self-reported playing position) was added to the model to assess the impact of an external factor.

Results

Demographics

Division I male football players were recruited for this investigation. Characteristics of the sampled population of 35 players (outlier removed) are demonstrated in Table 1. A wide distribution of playing positions was included. The positions most included in this study were safeties (N = 6) and offensive linemen (N = 5). The defensive back and long snapper positions each included only one player. The body composition variable of weight had the most variation among players with a standard deviation of 50.2 lbs. Body fat percentage had a standard deviation of only 4.0%, while skeletal muscle percentage varied by 11.7% among players. Height did not have wide variability, with the standard deviation being 2.8 inches.

Relationship of breath acetone and body composition

The primary aim of the investigation was to assess the association of breath acetone to body composition and body size metrics. A Pearson Correlation test was performed (Table 2). All body metrics were significantly associated with each other. However, no body metrics were significantly associated with breath acetone. Weight had the most relation with breath acetone (P = 0.329) with all other factors demonstrating higher probability values. The influence of body composition and size were assessed with logistic regression modeling.

Table 1: Participant demographics.

	Division I Football Players
N	35
Position (N, %)	
Defensive Line	3, 8.6%
Offensive Line	5, 14.3%
Offensive Linebacker	3, 8.6%
Defensive Linebacker	2, 5.7%
Defensive Back	1, 2.9%
Corner Back	2, 5.7%
Safety	6, 17.1%
Receiver	4, 11.4%
Tight End	2, 5.7%
Running Back	4, 11.4%
Kicker	2, 5.7%
Long Snapper	1, 2.9%
Height (in)	
	73.0 ± 2.8
Weight (lbs)	
	221.4 ± 50.2
BMI (kg/m^2)	
	28.2 ± 4.0
Body Fat (%)	
	22.6 ± 5.4
Skeletal Muscle (%)	
	38.3 ± 11.7
Visceral Fat Level	
	8.9 ± 2.9
Breath Acetone (ppm)	
	9.9 ± 8.9

Data are mean ± SD unless noted

The first model conducted only considered those quantitative factors and overall demonstrated poor influence on breath acetone as a dependent variable (P > 0.05), accounting for 8% of the variability (Table 3). An exploratory model was completed adding in an external factor, playing position, to the model. The addition of playing positionincreased the complexity of the model, but also accounted for more of the variation in breath acetone (59%) (Table 4).

Discussion

The current investigation explored whether there was an association between body composition in Division I football players and breath acetone concentration. Acetone is an intriguing biomarker because it has between person variability and can be measured affordably, portably, and non-invasively. In the sports community, those characteristics are extremely valuable.

The primary aim of this study was to investigate the correlative relationship between resting breath acetone values and body size and composition metrics. Based on the Pearson Correlation statistical test, there were no significant relationships seen between breath acetone and body metrics. To understand what influence body size and composition measures have on breath acetone, a logistic regression was completed to estimate the influence of the factors. The results demonstrated that body composition and size metrics had little predictive power in breath acetone. None of the body metrics were statistically significant in their influence on breath acetone. Thus, it is reasonable to assume based on both the Pearson correlation and the logistic regression analyses that there is little relationship between body composition and size and breath acetone.

The results of this study demonstrate the lack of relationship between breath acetone and body

Table 2: Pearson correlation matrix.

Variables	Breath Acetone (ppm)	Weight (lbs)	Body Fat (%)	Visceral Level (Scaled Interval)	BMI (kg/m^2)	Skeletal Muscle (%)
Breath Acetone (ppm)	**1**^{a} **0**^{b}	-0.170^a 0.329^b	0.059^a 0.745^b	0.131^a 0.469^b	0.139^a 0.441^b	-0.045^a 0.805^b
Weight (lbs)	-0.170^a 0.329^b	**1**^{a} **0**^{b}	**0.797**^{a} **< 0.0001**^{b}	**0.909**^{a} **< 0.0001**^{b}	**0.938**^{a} **< 0.0001**^{b}	**-0.879**^{a} **< 0.0001**^{b}
Body Fat (%)	0.059^a 0.745^b	**0.797**^{a} **< 0.0001**^{b}	**1**^{a} **0**^{b}	**0.864**^{a} **< 0.0001**^{b}	**0.841**^{a} **< 0.0001**^{b}	**-0.982**^{a} **< 0.0001**^{b}
Visceral Level (Scaled Interval)	0.131^a 0.469^b	**0.909**^{a} **< 0.0001**^{b}	**0.864**^{a} **< 0.0001**^{b}	**1**^{a} **0**^{b}	**0.989**^{a} **< 0.0001**^{b}	**-0.884**^{a} **< 0.0001**^{b}
BMI (kg/m^2)	0.139^a 0.441^b	**0.938**^{a} **< 0.0001**^{b}	**0.841**^{a} **< 0.0001**^{b}	**0.989**^{a} **< 0.0001**^{b}	**1**^{a} **0**^{b}	**-0.873**^{a} **< 0.0001**^{b}
Skeletal Muscle (%)	-0.045^a 0.805^b	**-0.879**^{a} **< 0.0001**^{b}	**-0.982**^{a} **< 0.0001**^{b}	**-0.884**^{a} **< 0.0001**^{b}	**-0.873**^{a} **< 0.0001**^{b}	**1**^{a} **0**^{b}

^{a}Correlation coefficient, ^{b}Correlation p-value

Table 3: Logistic regression model 1 statistics.

Goodness of Fit Statistics					
Observations	**-2 Log (Likelihood)**	**R^2 (McFadden)**	**AIC**	**SBC**	**Interpretation**
33	39.627	0.084	49.627	57.109	The low R^2 value reflects the lack of ability of the model to predict whether a breath acetone value will be higher or lower than the sample mean. The model accounts for 8% of the variation in breath acetone.

Type II Analysis Chi-Square (LR) | Pr > LR					
Dependent Variable	**Body Fat (%)**	**Visceral Level**	**BMI (kg/m^2)**	**Skeletal Muscle (%)**	**Interpretation**
Breath Acetone (Higher/Lower than sample mean 9.95 ppm)	0.045 | 0.832	0.399 | 0.527	0.007 | 0.932	0.673 | 0.412	The explanatory variables have low Chi-Square (LR) statistic values and high p-values indicating each variable contributes little to the predicting model.

Table 4: Logistic regression model 2 statistics.

Goodness of Fit Statistics					
Observations	**-2 Log (Likelihood)**	**R^2 (McFadden)**	**AIC**	**SBC**	**Interpretation**
33	17.595	0.593	49.595	73.539	The model accounts for 59% of the variation in breath acetone. It is an adequate fit of the model and has more complexity with a higher SBC than Model 1.

Type II Analysis Chi-Square (LR) | Pr > LR						
Dependent Variable	**Body Fat (%)**	**Visceral Level**	**BMI (kg/m^2)**	**Skeletal Muscle (%)**	**Position**	**Interpretation**
Breath Acetone (Higher/Lower than sample mean 9.95 ppm)	1.427 | 0.232	2.134 | 0.144	0.866 | 0.352	0.408 | 0.523	22.031 | 0.024	The position variable accounts for most of the predictive value in this model and different positions have a statistically significant impact on the likelihood of the outcome.

metrics at rest. Previous literature had suggested conflicting results of a relationship between BMI and acetone, but body composition has not be as widely considered [11]. It is unknown whether the percentage of body fat influenced the utilization of fatty acids (and ketones) at rest. In healthy individuals not adhering to a low-carbohydrate or ketogenic diet, glucose is the preferred energy substrate. However, individuals may induce their body to switch from using glucose to using ketones either unintentionally through injury response or intentionally through behavioral changes such as fasting, consuming a ketogenic diet, or intaking ketone salt or ester supplements [14]. Behavior changes such as these lead to increases in fat oxidation and/or circulating ketones [15]. The theory being an increase in circulating ketones causes a metabolic shift to preferentially utilize ketones for energy rather than glucose. The utilization of ketones can provide metabolic benefit (by consuming less NAD+) and neuroprotective benefits (reduce oxidative stress and regulate inflammatory responses) to individuals [16]. The results of this study emphasize that while at rest, having an increased number of fatty acids stored as body fat does not relate to an increase in circulating ketones or a preference in the utilization of ketones. Conversely, those with less body fat also do not have circulating ketones or a preference to the utilization of ketones. While body composition and size did not account for much variability in breath acetone at rest other internal factors and external factors could be at play.

An exploratory logistic regression model was completed adding an external variable, self-reported playing position, to the previous regression model. This model demonstrated a lower -2 Log (Likelihood) value (17.595) and a higher McFadden R2 (0.593) indicating a better model fit and more predictive power. The second model provided more insight into breath acetone as a marker than the first, only including the body metrics. Though both models indicated adequate fit to the data collected, considering additional variables both internal and external may provide more predictive power to this potential biomarker for precision medicine.

In rat models, enzymes that facilitate the utilization of ketones, and thus the production of breath acetone, were shown to be dependent on muscle fiber type and muscle training status [17]. It is possible that although skeletal muscle percentage was not influential to breath acetone concentration, overall muscle fiber composition could account for more of the variability. A meta-analysis including studies on other sports teams (soccer, rugby, handball, and volleyball) expressed that some sports teams have up to 27.5% variation in their muscle fiber typology within the team [18]. This variation was suggested to be associated with high intensity running performance and short distance sprint times. Though no studies included in the meta-analysis focused on American football players, running performance and sprint times are known to differ drastically by playing position and could account for the increased predictive power of the second regression model. In future research, muscle fiber composition may need to be explored more fully for its possible influence on energy substrate preference.

Conclusion

In this sample of healthy Division I football players, breath acetone was not significantly associated with body composition and size measurements at rest. Having more or less body fat did not correlate to having an increased utilization of fat for energy at rest in this sample. Playing position influenced breath acetone at rest more than body metrics. The results of this study suggest future studies evaluating the potential of energy substrates as markers for precision medicine should include both internal and external factors in their analyses.

Acknowledgments

The authors would like to acknowledge the University of Alabama at Birmingham's Athletic Department for their continuous commitment to research advancement.

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