Skin Acetone as a Clinical Diagnostic Biomarker of Ketosis

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Abstract

Background/Objectives: We present the first receiver operating characteristic (ROC) diagnostic analysis of skin-excreted acetone as a biomarker of ketosis.

Participants/Methods: In a pilot study involving 16 healthy participants, we investigated the ability of skin-excreted acetone to differentiate between ketosis and non-ketosis states. Non-ketosis conditions were established under a normal diet and energy balance, while ketosis was induced through dietary and energy manipulations, including a ketogenic diet with energy balance, a normal diet with negative energy balance, and a ketogenic diet with negative energy balance. Alongside skin acetone concentration and excretion rate, we quantified a comprehensive set of ketone body parameters for comparative diagnostic purposes, including breath acetone concentration and excretion rate, blood beta-hydroxybutyrate concentration, urine beta-hydroxybutyrate concentration and excretion rate, urine acetoacetate concentration and excretion rate. Blood beta-hydroxybutyrate concentration (in mM) was included as a benchmark since it is a well-established clinical biomarker of ketosis.

Results: ROC curves were generated to evaluate the diagnostic performance for each biomarker in distinguishing ketosis. Both breath and skin acetone excretion rates achieved an area under the

curve (AUC) of 0.83, outperforming several other ketone biomarkers. Similarly, breath acetone concentration and skin acetone concentration also exhibited AUCs of 0.82 and 0.83, respectively. In comparison, blood beta-hydroxybutyrate concentration and urine acetoacetate excretion rate both showed an AUC of 0.81, while urine beta-hydroxybutyrate excretion rate and concentration achieved AUCs of 0.66 and 0.68, respectively. Furthermore, the excretion rates of breath acetone and skin acetone were systematically compared within individual participants and across different participants. The correlation between the two reinforces the significance of skin acetone as a body ketone biomarker that diffuses through the skin representing the body acetone concentrations.

Conclusions: Overall, the results highlight the potential of skin acetone excretion rate as a reliable, non-invasive biomarker for ketosis, offering significant promise for clinical and health monitoring applications.

Keywords: ketogenesis, lipids/fat oxidation, carb oxidation, energy balance, obesity, nutrition, ketosis, beta-hydroxybutyrate, acetone, acetoacetate.

Introduction

Ketosis is a metabolic state characterized by elevated levels of circulating ketone bodies, typically defined as when blood beta-hydroxybutyrate concentrations exceeding 0.5 mM.[1] This metabolic state arises as a result of accelerated fat oxidation, which stimulates the production of ketone bodies—acetoacetate, β-hydroxybutyrate, and acetone—through a process known as ketogenesis. The accumulation of these ketones subsequently leads to their excretion ~~in~~ through different body fluids (**Figure 1A**).

Over the years, various strategies to induce ketosis have garnered significant attention for their potential roles in weight management[1, 2], enhancing healthy aging,[3, 4] optimizing human performance,[5] and treating conditions such as epilepsy[6] and diabetic kidney disease.[3, 7] As interest in ketosis continues to grow, validating the diagnostic accuracy of non-invasive biomarkers becomes critical for developing advanced technologies and improving the understanding of individual metabolic responses to dietary and physical activity interventions.[8, 9]

Ketosis biomarkers are traditionally measured through the detection of ketone bodies in blood (beta-hydroxybutyrate)[10], urine (acetoacetate), and breath (acetone).[11] While blood and urine ketones have been widely used for ketosis detection via finger-prick blood analyzers[12] and urine test strips, breath acetone has emerged over the past decade as a preferred non-invasive biomarker for early ketosis detection.[7, 11, 13-16] Notably, studies have shown that breath acetone can detect increases in ketone levels earlier than blood or urine analyses, underscoring its potential as an ideal biomarker for identifying the early activation of lipid metabolism.[11, 14] Additionally, breath acetone has proven valuable for public health applications, such as screening undiagnosed cases of type 1 diabetes and ketosis during community health events.[17] Compared to urine acetoacetate[16] and blood beta-hydroxybutyrate,[14] breath acetone has demonstrated superior diagnostic accuracy, further solidifying its value in assessing ketosis stages.

In this study, we present the first comprehensive analysis of the diagnostic accuracy of skin-excreted acetone as a biomarker for ketosis (**Figure 1B**). Skin acetone originates from the diffusion of acetone through the dermis and epidermis, driven by concentration gradients established between blood vessels in the skin and the external environment. According to Fick's first law of diffusion[18] and our observations, the skin acetone concentration (C_s) or excretion rate (i.e., the

amount of acetone accumulated over a defined period on a specific skin area) should be directly proportional to the blood acetone concentration (C_B).[19, 20]

To evaluate the potential of skin acetone as a reliable ketosis biomarker, we systematically compared its diagnostic accuracy against other ketone bodies, including blood, urine, and breath (**Figure 1A**). This comprehensive analysis provided an overall perspective on the diagnostic performance of skin acetone relative to these established biomarkers. Additionally, we investigated the correlation between skin acetone and breath acetone, both for the entire cohort and on an individual basis. These comparisons aim to validate skin acetone as a robust, non-invasive biomarker for detecting and monitoring ketosis.

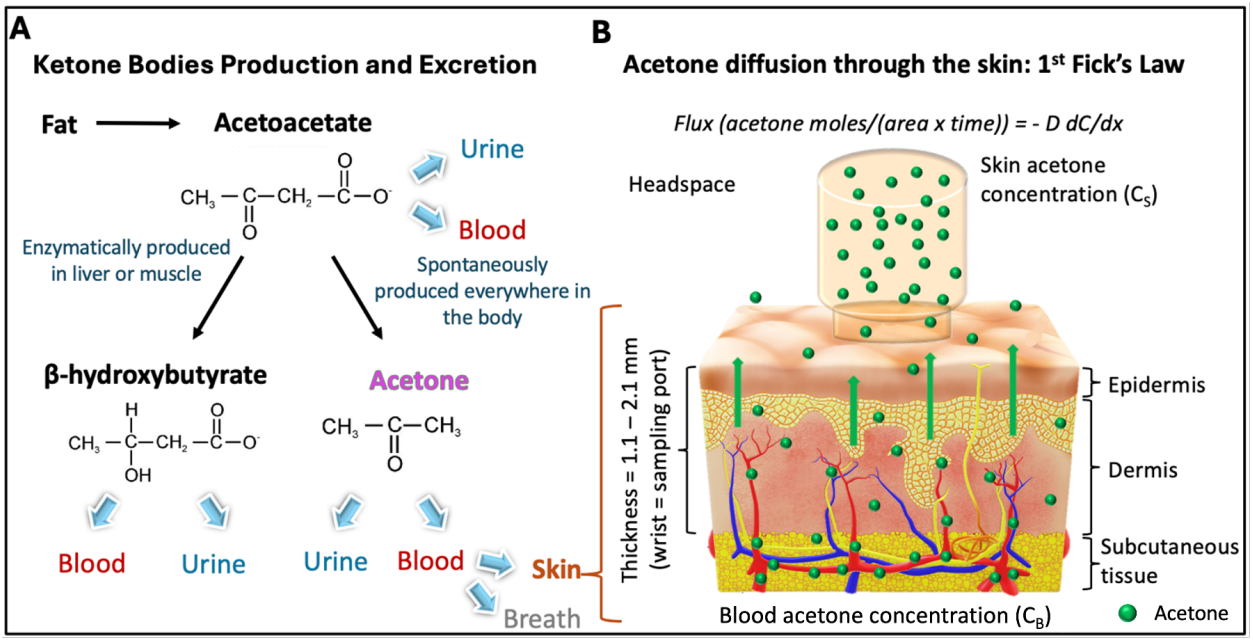


Figure 1. A- Schematic representation of ketone bodies production and excretion, including acetoacetate, beta-hydroxybutyrate and acetone. **B-** The skin plays a significant role in acetone excretion, with acetone diffusing through the dermis and epidermis according to Fick's First Law of Diffusion.[18] In this study, acetone excretion was specifically sampled from the inner wrist. The schematic representation illustrates the approximate skin thickness (note: the epidermis and dermis are not shown to scale).

Methods and Procedures

Human Subject Study

We studied and performed systematic statistical analysis of the ketone bodies profiles obtained from a study comprised of 16 healthy participants (9 males and 7 females).[21] The study spanned an 8-week duration and involved 4 combinations of diet type and caloric intake: 1- normal diet/energy balance (NDEB), 2- ketogenic diet/energy balance (KDEB), 3- normal diet/negative balance (NDNB), and 4- ketogenic diet/negative balance (KDNB). Sixteen participants were chosen based on a power calculation (0.80 power, 0.05 alpha) with the aim to detect increase of ketones from two mean values differing by 20% in average.[22]

Each dietary and energy condition lasted for 2 weeks. The first week was dedicated to the subject's conditioning, and the second week was focused on assessment with 3 appointments scheduled every other day for interventions of exercise, fasting, and meal. **Figure 2** summarizes the subject's interventions during the conditioning and assessment weeks.

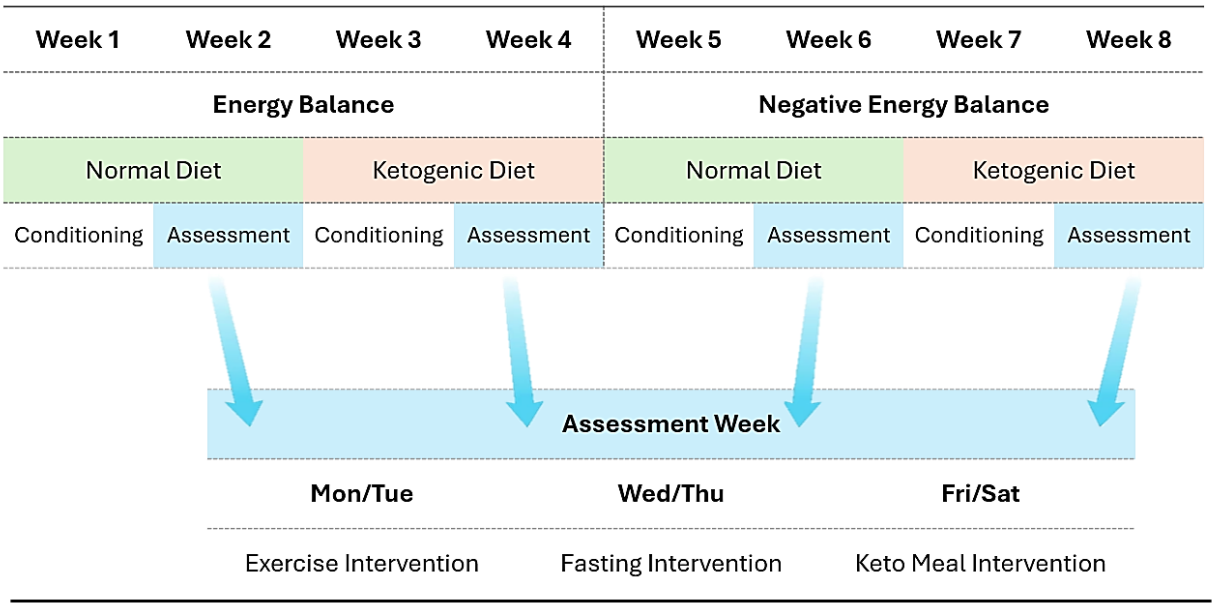


Figure 2. Study design: subject's conditions during the 8-week study.

At each intervention, the subject was assessed both before (pre-intervention) and after (post-intervention). Pre-intervention assessment conditions included 12-hour overnight fasting. Exercise intervention included 1 hour of running on a treadmill at ~60-80% heart rate maximum and the assessment was taken after 1 hour of finishing the exercise. The fasting intervention included an additional 8-hour fasting period to the 12-hour overnight fasting. Ketogenic meal intervention included the intake of a single ketogenic meal with a caloric intake represented by 25% of the subject's measured REE (assuming a person daily consumes 3 meals and one snack, and 25% would represent the calorie intake average in a single meal). The assessment was conducted 2 hours after the meal completion. The ketogenic meal was provided as breakfast on the last day of each condition period, specifically at week 2, week 4, week 6, and week 8.

The normal diet comprised a macronutrients distribution of approximately 50-60% carbohydrates, 20-30% fat, and 10-20% protein. In contrast, the ketogenic diet (KD) consisted of 3-10% carbohydrates, 65-80% fat, and 20-30 % protein. The energy balance condition entailed a caloric intake equal to each subject's personal total energy expenditure.[23] The negative energy balance had a caloric intake of 500 kcal/day lower than the personal energy expenditure.[24] The personal total energy expenditures were assessed through indirect calorimetry (Medical Graphics Metabolic Cart, MN or Breezing Med™ Wearable Metabolic Rate Analyzer, Phoenix, AZ, both FDA-cleared instruments) at the beginning and periodically throughout the study to ensure personalized dietary caloric intake (see details below in **Description of the diet meals**).[21]

We assessed the ketone bodies from different sources in the 16 healthy participants for pre- and post-exercise, pre- and post-fasting, and pre- and post-ketogenic single meal interventions performed at 1- normal diet/energy balance (NDEB), 2- ketogenic diet/energy balance (KDEB),

3- normal diet/negative balance (NDNB), and 4- ketogenic diet/negative balance (KDNB) conditions.

Participants Physical Characteristics

All the participants were educated about the study design and purpose. An informed consent form approved by the Institutional Review Board at Arizona State University (IRB protocol number: STUDY00016374) was obtained from each subject before the study. All methods were performed in accordance with the relevant guidelines and regulations.

The sixteen healthy participants participating in the study included: 9 males and 7 females. The participants' mass and body composition were assessed with a 4-electrode bioimpedance scale (Omron, model HBF-514C, Kyoto, Japan). The subject's height was assessed with a stadiometer (Seca, model 213, Hamburg, Germany). The subject's body surface area was estimated based on principles of weight and height published elsewhere.[25] Physical activity levels (PAL) of the participants were classified based on reported frequency, duration, and type of physical activities, and were determined to be sedentary (low physical activities) for all the participants. The 16 participants of the study had an average BMI of 25 ± 4 kg/m^2. More specifically, the males had a body mass index (BMI) average of 26.4 kg/m^2 (SD: 4.3 kg/m^2), and the females had a BMI average of 22.5 kg/m^2 (SD: 3.9 kg/m^2). The participants were between 20 and 60 years old. For males, body fat percentage was 23.9% (SD: 7.2%), muscle mass percentage was 36.9% (SD: 4.6%), and body surface area was 2.0 m^2 (SD: 0.2 m^2). For females, body fat percentage was 31.2% (SD: 9.9%), muscle mass percentage was 28.6% (SD: 4.3%), and body surface area was 1.7 m^2 (SD: 0.1 m^2).

None of the participants was on regular medication, nor had any history of respiratory diseases or diabetes, which enabled the comparison of ketone buildup capabilities under conditions of healthy acid-base balance and alveolar gas exchange.[25]

Description of the diet meals

The calorie intake for each subject was determined based on Total Energy Expenditure (TEE), which was estimated from the measured Resting Energy Expenditure (REE) (Ultima CCM^{TM}, Medical Graphics, Saint Paul, MN, USA; or Breezing Med Wearable Metabolic Rate Analyzer, Phoenix, AZ, USA) and the Sedentary Physical Activity Level coefficient (PAL) as TEE = REE × PAL.[25]

The diet caloric intakes were determined at the beginning of week 1 (Normal Diet – Energy Balance condition), week 3 (Keto Diet – Energy Balance Condition), week 5 (Normal Diet, Negative Energy Balance), week 7 (Keto Diet, Negative Energy Balance), as well as multiple point in times during the study to ensure accuracy of the intake caloric condition.[21]

Food from Healthy Choices (Conagra Food Services, Chicago, IL) and Factor75 (Factor75 LLC, Batavia, IL) was provided to the participants for the entire period of the study to sustain a normal diet and a KD as well as to meet the caloric intake goals. The allowed caloric intake was distributed with meals consisting of breakfast, lunch, dinner and snacks. The participants were instructed to eat only the provided food, and they were followed by the study coordinator every few days to ensure they had everything they needed for dietary needs and to answer questions they might have. This procedure ensured adherence to caloric intake under the free-living conditions of the study.[21]

Ketone Bodies Measurements

The assessment of the ketone bodies was as follows:[21]

Urine acetoacetate concentration and excretion rate: The urine acetoacetate concentration in the collected volume of urine at the appointment was measured with urine strips (Fisherbrand, 10SG Urine Reagent Strips, Fisher HealthCare, Pittsburg, PA, USA). Urine acetoacetate excretion rate was assessed by measuring urine volumes excreted during the assessment appointment. The participants were instructed to empty the bladder at the beginning of the appointment, and the exact period of the excretion was timed when they urinated at the end of the appointment.

Skin acetone concentration and excretion rate: It was measured from the skin acetone concentration determined by gas chromatography-mass spectrometry (GC-MS) (see below details of the method), taking into account the time required for the skin acetone to accumulate in the skin headspace for 45 minutes.

Breath acetone concentration and excretion rate: It was measured by determining breath acetone concentration using gas chromatography-mass spectrometry (GC-MS) (see below for details of the method: **Gas Chromatography-Mass Spectrometry Method**) and assessing the breath exhalation rate while collecting the breath sample in a Mylar bag, using an adapter connected to an indirect calorimetry system (Ultima CCM^{TM}, Medical Graphics, Saint Paul, MN, USA or Breezing Med Wearable Metabolic Rate Analyzer, Phoenix, AZ, USA).

Blood beta-hydroxybutyrate concentration: Blood beta-hydroxybutyrate concentration (in mM), has been used as the historical clinical biomarker of ketosis.[1, 5, 26] It was determined with a Precision Xtra blood ketometer (Abbott, USA). It should be noted that, in this study the change in blood beta-hydroxybutyrate concentration between pre- and post-intervention was insufficient to

evaluate the changes in excretion rates and therefore blood beta-hydroxybutyrate excretion rates are not used for comparison.

Urine beta-hydroxybutyrate concentration and excretion rate: It was assessed from the urine samples obtained during the assessment appointment using the same samples and collection method described for urine acetoacetate excretion rate. The urine beta-hydroxybutyrate concentration was assessed in a Beckman Coulter Instrument, using beta-hydroxybutyrate 21 FS assay (Vidan Diagnostics, LLC 24123 Boerne Stage Rd Ste 205 San Antonio, TX 78255 USA).

Gas Chromatography-Mass Spectrometry Method

Overall method: Measurements from skin acetone and breath acetone were assessed using gas chromatography-mass spectrometry (GC-MS) (Agilent, 6890N Network GC System, 5973 Network Mass Selective Detector, USA) with conventional gas chromatography method using a solid phase micro-extraction (SPME) fiber modified with hydroxylamine derivative.[27]

Modification of the SPME fiber: We used SPME fiber assemblies with 65 um PDMS/DVB, fused silica 24Ga, and Manual Holder (Supelco, Sigma-Aldrich, USA). The SPME fibers were modified with O-2,3,4,5,6-(Pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA) (Sigma-Aldrich) in HPLC-grade deionized water (Sigma-Aldrich), which was initially adsorbed on the SPME fiber of polydimethylsiloxane–divinylbenzene (PDMS–DVB) for 10 minutes at room temperature, using a septum vial with 1 mL of the solution under stirring conditions.

Skin samples' collection method: Skin acetone samples were collected from the inner wrist area, which has typically thickness reported between (1.1 and 2.1) millimeter.[28] (**Figure 1B**). Customized clean 5-mL glass vials (Fisherbrand, autosampler vials, Fisher Scientific, USA) with a septum positioned by an adapter were fixed with an elastic band onto the clean surface of the

skin. The entire glass-adapter-elastic band system was degassed with heat treatments at 100 ^{o}C (glass vial), and 50 ^{o}C (adapter and elastic band) for at least overnight before use.

The skin was cleaned with a deionized water-impregnated wipe (Kimwipe, Kimberly Clark®) and dried with a Kimwipe as well. The vials were tightly sealed to prevent excreted acetone leaks and purged with ultra-high purity nitrogen for one minute to clean the headspace. (Matheson, NJ, USA) to clean the headspace gas right before the skin acetone sample collection. The skin-excreted acetone was collected for a total time of 45 minutes. The last 15 minutes of the 45 minutes were used to collect the sample, using the PFBHA–modified SPME fiber. The modified SPME was inserted through a septum located on the top of a 5-ml glass vial (with the bottom part removed) that was attached in tight contact with the skin. **Fig. 5Q** shows a schematic representation of this skin acetone collection gadget, which was customized and developed in our lab.

Breath samples' collection: The collection of breath acetone required the subject to breathe inside a 40 L aluminum Mylar bag for ~10 minutes while measuring the exhalation rate from the indirect calorimeter. The modified SPME fiber was then exposed in the bag through the septum for 5 minutes for acetone collection and it was quantitatively analyzed by GC-MS as described for skin acetone.

Results and Discussion

Skin Acetone Diagnostic Accuracy of Ketosis

To define clinical stages of true ketosis and no ketosis, we apply the criteria described as follows. We defined clinical ketosis as the metabolic state in which ketone levels are elevated due to accelerated fat oxidation.[1, 29] Therefore, ketosis state was defined for the participants under

Negative Energy Balance (NEB) conditions, e.g. participants experiencing a caloric deficit of approximately 500 kcal/day relative to their personalized total energy expenditure.[30] According to the First Principle of Thermodynamics,[24] under NEB, the body utilizes its tissues, including adipose tissue, as an energy source, leading to increased fat oxidation and, consequently, ketosis.[7, 8] The NEB was created for either a Normal Diet (ND) or a Ketogenic Diet (KD).

Additionally, ketosis state was also defined when participants followed the Ketogenic Diet under Energy Balance (EB) conditions. This is because the high-fat content of the KD provided a significant amount of fat as energy source, promoting accelerated fat oxidation and ketone production.[31, 32]

In contrast, the no-ketosis state was defined as the condition where participants were in Energy Balance (EB) while following a Normal Diet (ND).[24] Under these conditions, caloric intake matched total energy expenditure, and no significant fat oxidation and ketone production was expected.[25]

To evaluate the diagnostic performance of various ketone biomarkers in differentiating between ketosis and no-ketosis states, we generated Receiver Operating Characteristic (ROC) curves.[33] Each ketone measurement was treated as a continuous test variable, while the reference classification of ketosis (true positive) or no-ketosis (true negative) was based on the dietary and energy balance conditions described above. ROC curves were constructed by plotting sensitivity (true positive rate = (true positives + false negatives) / true positives) against 1-specificity (false positive rate = 1 – (true negatives + false positives) / true negatives)) at multiple thresholds for each biomarker. A data point was considered as a true positive if it correctly classified a participant as being in ketosis (e.g., NDNB, KDEB, or KDNB), or as a false positive if it incorrectly labeled a no-ketosis condition (NDEB) as ketosis. To determine the optimal threshold for each biomarker,

we selected the point on the ROC curve that was closest to the ideal classifier point (0, 1; e.g. 100% specificity, and 100% sensitivity).[33] This method identifies the threshold that simultaneously maximizes sensitivity and specificity by minimizing the Euclidean distance to (0,1) on the ROC plot.[33] This threshold was then used to define the concentration limit from which diagnostic classification of ketosis will be applied.

The ROC analysis was performed for each measured ketone (breath acetone, skin acetone, urine beta-hydroxybutyrate, urine acetoacetate, and blood beta-hydroxybutyrate) using both excretion rates and concentration units. **Figures 3A** and **3B** illustrate the corresponding ROC curves. On these plots: the y-axis represents the true positive rate (sensitivity), and the x-axis represents the false positive rate (1-specificity).[33] The Area Under the Curve (AUC) of each ROC curve was used to quantify the overall diagnostic ability of the biomarker, reflecting how well the biomarker can discriminate between ketosis and no ketosis, independent of any particular threshold.[33] A higher AUC value (closer to 1) indicates better discrimination between ketosis and no-ketosis states. **Tables 1A and 1B** summarize the characteristics of the ROC curves, including AUC, the threshold of best diagnostic sensitivity and specificity, and the sensitivity, selectivity, and accuracy ((true positive + true negative)/total number) at the specific threshold[33] for all the analyzed biomarkers.

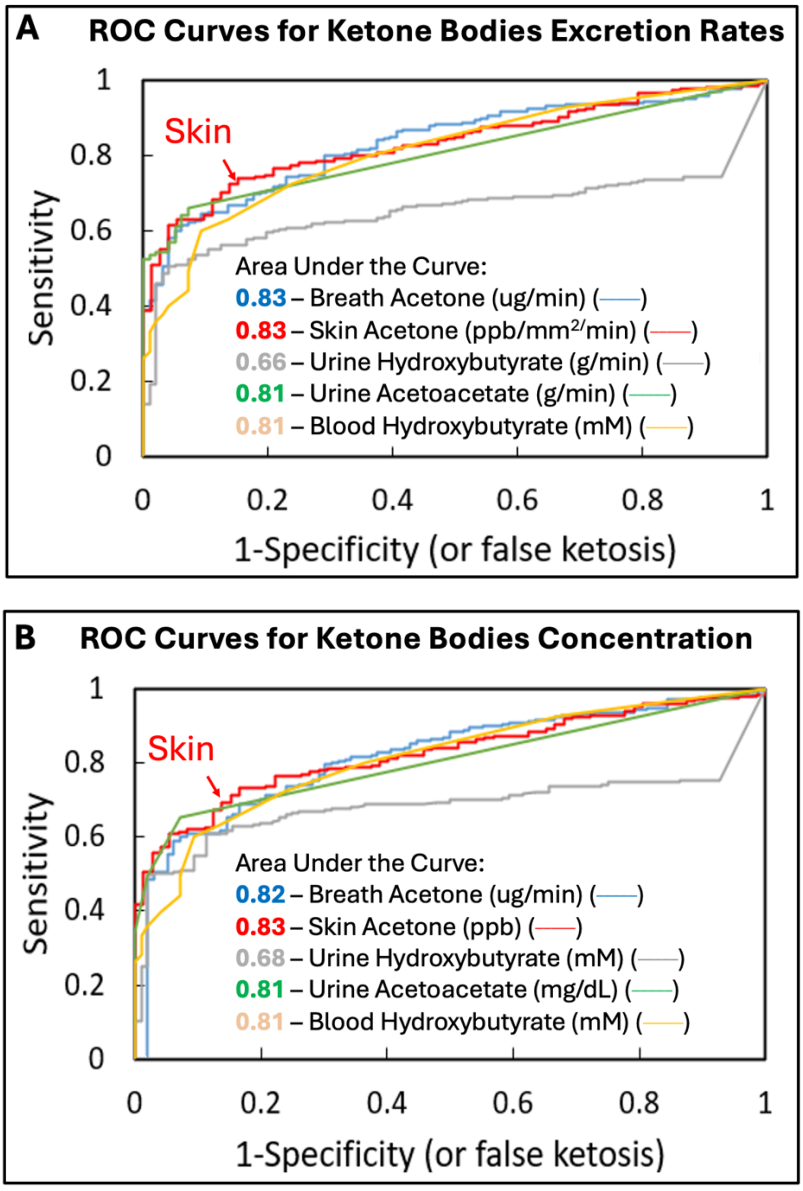


Figure 3. Ketosis State Clinical Diagnostic Receiving Operating Characteristic (ROC) curves for: **A-** ketones' excretion rates and **B-** concentrations from different sources. Data from 16 healthy human participants for 8-weeks (N=384). True No Ketosis condition was defined for the participants with normal diet and normal energy balance (normal caloric intake) condition (e.g. NDEB). True Ketosis condition was defined for participants with negative energy balance (food caloric intake deficit) or/and ketogenic diets (rich fat diets) (e.g., NDNB, KDNB, KDEB). Bold numbers represent diagnostic accuracy from Area Under the Curve.

Table 1A. Characteristics of ROC curve for five different ketone sources based on excretion rates*.

	AUC	Threshold	Sensitivity**	Specificity**	Accuracy**
Breath Acetone (µg/min)	0.83	13.6	0.74	0.77	0.75
Skin Acetone (ppb/mm^2/min)	0.83	0.011	0.74	0.85	0.77
Urine Beta-hydroxybutyrate (g/min)	0.66	$5.4x10^{-6}$	0.60	0.80	0.65
Urine Acetoacetate (g/min)	0.81	$2.1x10^{-5}$	0.66	0.93	0.73
Blood Beta-hydroxybutyrate (mM)*	0.81	0.40	0.73	0.76	0.73

*We use blood beta-hydroxybutyrate concentration for comparative purposes.

**Sensitivity, Specificity and Accuracy at the specific threshold (show in the threshold column).

Table 1B. Characteristics of ROC curve for five different ketone sources based on concentration units.

	AUC	Threshold	Sensitivity**	Specificity**	Accuracy**
Breath Acetone (ppb)	0.82	1318	0.69	0.83	0.72
Skin Acetone (ppb)	0.83	69	0.73	0.83	0.76
Urine Beta-hydroxybutyrate (mM)	0.68	0.028	0.63	0.84	0.68
Urine Acetoacetate (mg/dL)	0.81	5.0	0.66	0.93	0.72
Blood Beta-hydroxybutyrate (mM)	0.81	0.40	0.73	0.76	0.73

**Sensitivity, Specificity and Accuracy at the specific threshold (show in the threshold column).

From **Figure 3A** and **Table 1A**, it can be observed that breath acetone excretion rate and skin acetone excretion rate achieved the highest AUC of 0.83 among all the ketone biomarkers, including urine beta-hydroxybutyrate, urine acetoacetate excretion rate, and blood beta-

hydroxybutyrate concentration, among which blood beta-hydroxybutyrate concentration is the commonly used ketosis biomarker.[34, 35] Notably, breath acetone excretion rate and skin acetone excretion rate performed equivalently to the blood beta-hydroxybutyrate concentration, which exhibited a AUC of 0.81 in this study. The urine acetoacetate excretion rate demonstrated an AUC of 0.81 comparable to the blood beta-hydroxybutyrate concentration, while the urine beta-hydroxybutyrate excretion rate exhibited lower diagnostic performance than other ketone biomarkers, with an AUC of 0.66.

Similar trends were observed in the ketone biomarker concentration comparison. From **Figure 3B** and **Table 1B**, it can be seen that breath acetone concentration and skin acetone concentration performed well, with AUCs of 0.82 and 0.83, respectively. In comparison, blood beta-hydroxybutyrate concentration and urine acetoacetate concentration showed AUCs of 0.81, while urine beta-hydroxybutyrate concentration achieved an AUC of 0.68.

Overall, the results demonstrate that breath acetone and skin acetone, whether measured as excretion rates or concentrations, consistently achieved the highest AUC values among the ketone biomarkers analyzed. Breath acetone has already been FDA-approved as a biomarker for fat oxidation[15, 36] and is widely used to assess an individual's capacity to oxidize fat.[1, 2] However, skin acetone has not been previously characterized as a biomarker for fat oxidation and ketosis. This study demonstrated skin acetone measurements' potential as reliable, non-invasive fat oxidation indicators for identifying ketosis.

Building on these findings, we further examined the correlation between breath acetone and skin acetone to evaluate the feasibility of using skin acetone as a non-invasive diagnostic tool for ketosis.

Comparative Analysis of Skin Acetone with Breath Acetone

Figure 4 presents the correlation plot between breath acetone excretion rate and skin acetone excretion rate, derived from 16 participants under four distinct assessment conditions: normal diet/energy balance (NDEB), ketogenic diet/energy balance (KDEB), normal diet/negative energy balance (NDNB), and ketogenic diet/negative energy balance (KDNB). The data, comprising a total of 384 observations, exhibit a regression coefficient (R^2) of 0.75, indicating a strong positive correlation between these two biomarkers.[37] This result aligns closely with previous findings reported by Naitoh et al.[38], who observed a similar correlation with $R^2 = 0.81$ based on a smaller dataset of 22 data points assessed from 15 participants, and by Yamai et al.[39], who observed a correlation of $R^2 = 0.75$ in 5 male participants subjected to exercise conditions.

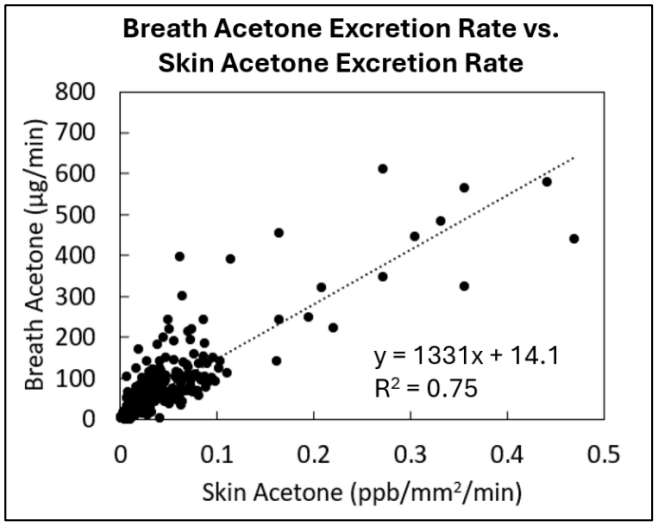
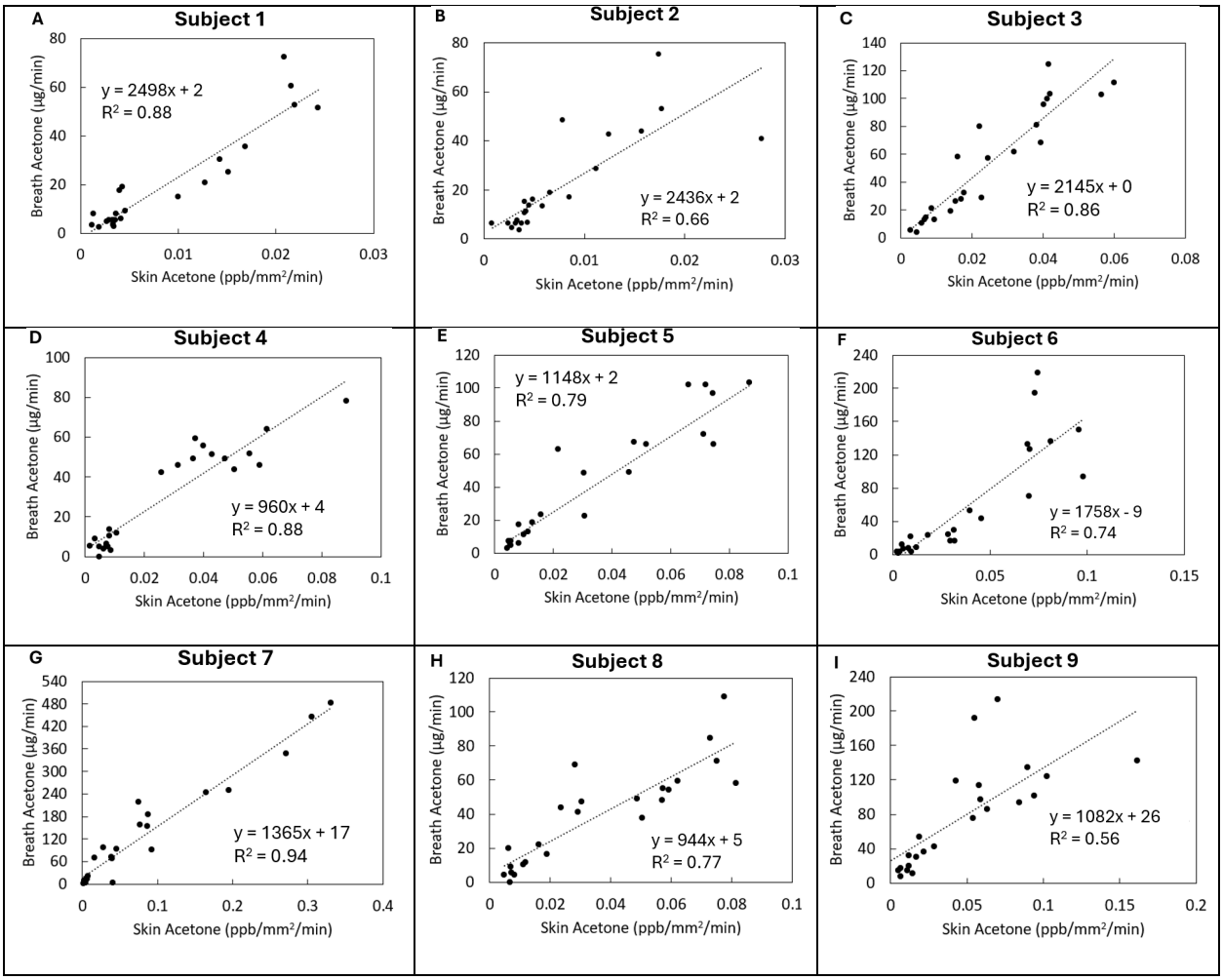


Figure 4. Relationship between breath acetone and skin acetone for 16 participants with ketosis and no ketosis throughout the study.

A detailed subject-specific analysis of the correlation between breath acetone and skin acetone is presented in **Figure 5A-P**. For the majority of the participants (e.g., 10 out of 16 participants), the regression coefficients are greater than **0.74**, indicating a strong linear relationship between these

two biomarkers.[37] However, some participants, (e.g. participants 2, 9, 10, 11, 14, and 15) exhibit lower correlation values, which may be attributed to potential leaks during the skin acetone collection process. These findings underscore the critical need for designing leak-free and robust sampling devices for skin acetone. Future research could prioritize refining sampling methodologies for skin acetone to ensure robustness of skin acetone collection.



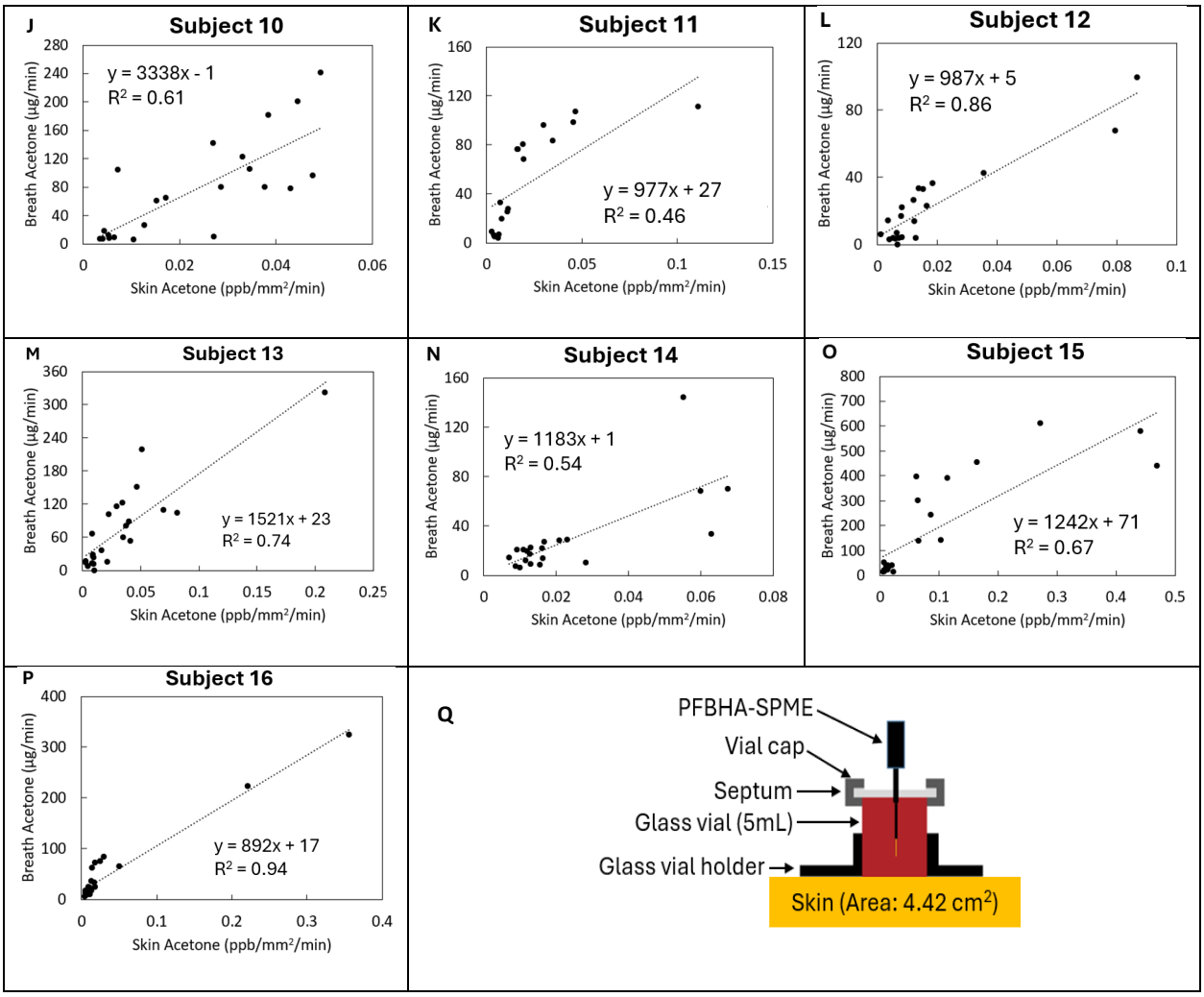


Figure 5. A-P- Intra-individual correlation of breath acetone excretion rate and skin acetone excretion rate for the 16 participants of the study. **Q-** Schematic method for skin acetone collection used in GC-MS analysis.

One of the key differences between this study and those by Naitoh et al.[38] and Yamai et al.[39] lies in the methodology used to measure skin acetone. In our study, skin acetone concentration was quantified using GC-MS with a SPME fiber, which allows for the quantification of acetone-derived oxime with lower vapor pressures than acetone, enabling control of the acetone quantification. In addition, we captured acetone from a well-defined volume on the skin surface

using an inert material—a customized, clean 5-mL glass vial with a septum (Fisherbrand autosampler vials, Fisher Scientific, USA). Finally, we secured the vial with a custom-made 3D printed support that enabled us to secure the vial firmly onto the cleaned skin surface (**Figure 5Q**) using an elastic band (not shown). This setup enabled the collection of a well-defined headspace volume and a consistent skin exposure area, enhancing both the precision and reproducibility of the measurements. On the other hand, Naitoh et al.[38] used a homemade polyacrylic plate attached to the skin with a continuous nitrogen stream for collection in a cold trap. Meanwhile, Yamai et al.[39] employed a skin headspace bag for skin headspace gas collection. Despite differences in methodology and measurement conditions, all three studies support the conclusion that skin acetone levels are associated with systemic ketone metabolism, demonstrating the relationship between skin and breath acetone. Our study is the first one to our knowledge to evaluate and perform an analysis of skin acetone in the context of its clinical diagnostic value for ketosis and comparative analysis of skin acetone with other ketone biomarkers.

Conclusions

This study is the first to establish the significance of skin acetone excreted by diffusion as an accurate diagnostic biomarker for ketosis, with diagnostic accuracy levels comparable to breath acetone and the traditional ketosis biomarker, i.e. blood beta-hydroxybutyrate, using ROC diagnostic analysis.

In addition, we validated the strong correlation between skin acetone and breath acetone. Given that breath acetone has been increasingly established as a ketosis biomarker such observed correlation demonstrated the reliability of skin acetone as a non-invasive ketone biomarker.

The above-mentioned findings mark an important step toward expanding the use of skin acetone for monitoring fat oxidation metabolism via passive sampling methods and provide potential opportunities for continuous ketone monitoring.

Acknowledgments

This research was supported by Amazon Corporation through a Sponsored Research Project (Amazon), Wear Tech Research Center via Partnership for Economic Innovation, Arizona Commerce Authority, A. J. and Sigismunda Palumbo Charitable Trust, and Discretional Funding of the Principal Investigator (E.F.). The authors thank Mrs. Serafina Barbero (Dr. Forzani's mother) for her support in preparing the custom-made elastic devices for the skin samples collection.

Author contributions

A.N., O.O., D.J., M.E.T., A.P. participated in the assessment of the participants' measurements. D.J. determined the energy requirements of the participants and provided tailored caloric intakes to each subject. E.F. and D.J. participated in the study design. H.Y., C.C., C.S., and E.F. participated in the discussion and data analysis. F.C. provided diagnostic software for data analysis. A.N. and R.E.D. corroborated the data collection, analysis and presentation. A.N., A.P., O.O., and D.J. participated in data collection, calculations, and data quality controls. All authors participated in weekly meetings including result discussions, and considerations for data interpretation.

Competing interests

The author declares no competing interests or conflict of interest.

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