

Breath Acetone Correlates with Capillary β -hydroxybutyrate in Type 1 Diabetes

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Abbreviations: (BrACE) Breath Acetone, (BOHB) β -hydroxybutyrate, (T1D) Type 1
Diabetes, (SGLT2i) Sodium Glucose Cotransporter-2 inhibitor, (DKA) Diabetic
Ketoacidosis, (VOC) volatile organic compound, (MDI) Multiple Daily Injections,
(CSII), Continuous Subcutaneous Insulin Infusion, (HbA1c) Hemoglobin A1c, (eGFR)
Estimated Glomerular Filtration Rate, (MOS) Metal Oxide Sensor, (CGM) Continuous
Glucose Monitor, (BKA) Biosense Ketone Analyzer, (CTRU) Clinical Translational
Research Unit.

Keywords: Type 1 Diabetes, Ketone Monitoring, Diabetic Ketoacidosis, Sodium glucose
co-transporter 2 inhibitors

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Tables: 2

Figures: 5

60 **Abstract**

61 Background

62 Breath acetone (BrACE) is an end product of ketone metabolism that is measurable by
63 noninvasive breath ketone analyzers. We assessed the correlation between capillary blood
64 β -hydroxybutyrate (BOHB) and BrACE in people with type 1 diabetes (T1D) during 14
65 days of outpatient care with and without dapagliflozin treatment and during supervised
66 insulin withdrawal studies with and without dapagliflozin.

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68 Methods

69 In this randomized crossover study, participants completed 14-day two outpatient periods
70 with or without dapagliflozin 10 mg daily. Each 14-day unsupervised outpatient period
71 was followed by a one-day supervised insulin withdrawal study. Paired BOHB and
72 BrACE measurements were obtained three times daily during outpatient periods, then
73 hourly during supervised insulin withdrawal. The correlation between BrACE and
74 BOHB was assessed by Spearman's ρ .

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76 Results

77 Twenty people with T1D completed the study. During outpatient periods, BrACE and
78 BOHB were moderately correlated ($n=1425$ paired readings; $\rho = 0.41$; 95% CI: 0.36 to
79 0.45; $P < 0.0001$). However, BrACE and BOHB were strongly correlated during insulin
80 withdrawal ($n=246$ paired values, $\rho = 0.81$; 95% CI: 0.77 to 0.85). In ROC analysis,
81 BrACE > 5 ppm demonstrated optimal sensitivity (93%) and specificity (87%) for
82 detecting capillary BOHB ≥ 1.5 mmol/L. No serious adverse events occurred.

83

84 Conclusions

85 In adults with T1D, measurement of breath acetone provides a noninvasive estimate of
86 blood BOHB concentration. The correlation between BrACE and BOHB was suboptimal
87 during unsupervised outpatient care, but was strong during supervised insulin withdrawal.

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89 Trial registration: clinicaltrials.gov (NCT05541484)

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108 Introduction

109 Despite progress in developing insulin analogs and insulin delivery devices, the
110 frequency of hospitalizations due to diabetic ketoacidosis (DKA), the most critical acute
111 complication of type 1 diabetes (T1D), has risen.¹ Furthermore, the mortality risk
112 associated with DKA has not improved over the past decade.¹⁻³ Risk factors for DKA
113 include poor adherence to insulin regimens, errors in insulin administration, chronic poor
114 glucose control, acute illness and use of sodium glucose co-transporter 2 inhibitors
115 (SGLT2i).^{4,5} Individuals with T1D are instructed to measure ketones in the setting of
116 symptoms of ketosis, during other illnesses, and when severe hyperglycemia occurs.^{4,6,7}
117 Although routine ketone monitoring during usual care is generally not recommended, this
118 has been shown to identify individuals at higher risk of DKA.⁸ In practice, ketone
119 monitoring remains suboptimal; over 50% of adults do not test for ketones during periods
120 of illness.⁹⁻¹¹

121 Sodium-glucose cotransporter 2 inhibitors (SGLT2i) improve glycemic control,
122 decrease insulin requirements, and reduce glycosylated hemoglobin levels in T1D.¹²⁻¹⁸
123 However, multiple randomized trials and meta-analyses have revealed a greater incidence
124 of DKA and higher ketone levels in individuals using SGLT2i.^{13,14,19,20} Ketone
125 monitoring has been proposed as a strategy for DKA mitigation in the setting of SGLT2i
126 use.^{12,21-24}

127 Ketone monitoring refers to the routine surveillance of one of the three major
128 circulating ketone bodies: acetoacetate, β -hydroxybutyrate (BOHB), or acetone.²⁵
129 Acetoacetate is measured in the urine using semi-quantitative test strips, whereas BOHB
130 can be measured quantitatively by point-of-care capillary blood ketone meters. Urine
131 acetoacetate testing has major limitations: it measures the average ketone concentration
132 in urine stored in the bladder since the previous void, and urinary ketone levels can
133 misleadingly increase when treatment for ketosis is initiated as BOHB is oxidized to

134 acetoacetate.²⁶ Capillary blood ketone meters that measure BOHB are commercially
 135 available but the technology is not widely used due to expense, inconvenience, and
 136 possibly inexperience with ketone meters.²⁴

137 Measurement of breath acetone (BrACE), the highly volatile metabolic end
 138 product of ketone metabolism excreted through the lung, has not been thoroughly
 139 evaluated in persons in T1D. Breath ketone analyzers (BKA) have been employed in non-
 140 diabetic individuals to monitor adherence to ketogenic diets. Previous studies have
 141 demonstrated linear relationships between BrACE and plasma acetone during episodes of
 142 DKA²⁷, and between BrACE and blood BOHB in various settings, suggesting potential
 143 utility of BrACE in risk prediction for ketosis and DKA.²⁸⁻³⁰ Breath acetone meters are
 144 subject to potential interference from volatile organic compounds (VOCs) including
 145 alcohols, acetic acid, propionic acid, and butyric acid. Commonly used household items
 146 like cleaning products, nail polish removers, hand sanitizer, lip balm and fermented foods
 147 contain VOCs which can lead to falsely elevated readings of breath acetone. Recent food
 148 or alcohol ingestion or smoking can also falsely elevate BrACE readings.

149 The Biosense breath ketone analyzer (Readout Health) has previously been
 150 studied in people without diabetes who were instructed to follow either a standard diet or
 151 a ketogenic diet and collected paired capillary BOHB and BrACE measurements five
 152 times daily. A moderate correlation ($r = 0.75$) between BrACE and BOHB was observed
 153 in this setting.³¹ In a study investigating the use of a breath acetone analyzer in adults and
 154 children with T1D, a significant correlation between BrACE and capillary blood ketones
 155 was observed in adults but not in children.³² BrACE >3.9 ppm yielded optimal
 156 performance for detecting BOHB >0.6 mmol/L, with sensitivity of 94.7% but specificity
 157 of just 54.2%.³² In this context, breath ketone analyzers are not currently recommended
 158 by professional society guidelines for ketone monitoring in those with T1D.

The goal of this study was to assess the utility of BKA to detect mild to moderate ketosis in persons with T1D and to compare BrACE levels with capillary BOHB. The correlation between capillary BOHB and BrACE was assessed in unsupervised outpatient settings with and without an SGLT2i and during insulin withdrawal to induce mild to moderate ketosis. To further evaluate the utility of BrACE in identifying a threshold level of ketosis that would require intervention, we interrogated the data using receiver-operating characteristic (ROC) analysis to identify the BrACE level that best predicted capillary blood BOHB concentration ≥ 1.5 mmol/L. This point-of-care capillary blood BOHB threshold has been validated to identify impending DKA, demonstrating a sensitivity and specificity ranging from 93-100% and 85-98%, respectively.³³⁻³⁵

Methods

Study Design

This was an open-label, single-center (Washington University School of Medicine, St. Louis, MO) randomized crossover trial in which study participants completed two study periods in random order: usual care (UC) and usual care plus dapagliflozin (DAPA). During UC, participants continued their usual insulin regimen, either multiple daily injections (MDI) or continuous subcutaneous insulin infusion (CSII), for 14 days while performing paired measurements of capillary BOHB and BrACE 3 times per day. This was followed by a one-day supervised insulin withdrawal study. During DAPA, participants added dapagliflozin 10 mg daily to their usual care for 14 days and continued to perform paired measurements of capillary BOHB and BrACE 3 times per day. A supervised insulin withdrawal study followed immediately after a dose of dapagliflozin in the morning. The study was unblinded to both participants and investigators.

185 *Study Participants*

186 The study was approved by the Washington University Human Research
187 Protection Office (IRB #202206078) and all study participants provided written informed
188 consent. The trial was registered in clinicaltrials.gov (NCT05541484). Inclusion criteria
189 included: i) T1D of >1 year duration, ii) age 18 to 75 years, iii) HbA1c $\leq 10\%$, iv) stable
190 insulin delivery method for the past 30 days, v) vision of 20/40 or better, vi) insulin
191 delivery by either continuous subcutaneous insulin infusion (CSII) or multiple daily
192 injections of insulin (MDI), and vii) use of Dexcom G6 or G7 continuous glucose
193 monitor (CGM). . Key exclusion criteria include: i) history of DKA within 6 months of
194 study entry or more than 1 episode of DKA in the past 2 years, ii) use of SGLT2i or prior
195 intolerance of SGLT2i, iii) eGFR $< 30 \text{ mL/min/1.73 m}^2$, iv) adherence to a very-low-
196 carbohydrate ($< 90 \text{ g/day}$) or ketogenic diet, v) pregnancy, plan to become pregnant within
197 the next 3 months, or lack of contraceptive use in premenopausal women, vi) history of
198 urinary tract infection within 3 months of study entry, vii) unwilling to avoid alcohol
199 ingestion during the study period, viii) vomiting within the past 30 days.

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201 *Breath Ketone Analysis*

202 The Biosense® breath ketone analyzer uses a metal oxide semiconductor (MOS)
203 sensor which is selective for acetone. A specific breathing pattern is recommended that
204 includes prolonged forced exhalation because breath acetone concentration increases
205 during exhalation. The MOS sensor is housed inside a sealed flow cell to decrease the
206 entrance of surrounding environmental air.³¹ Results from this device are reported as
207 units of parts per million (ppm). Each device is calibrated using laboratory gas standards
208 certified by the National Institute of Standards and Technology, with acetone
209 concentrations of 0, 5, and 20 ppm.

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211 *Procedures*

212 Study participants completed a screening evaluation including demographics,
 213 medical history, physical examination, assessment of insulin administration (doses and
 214 routes), download of continuous glucose monitor (CGM) data and insulin pump data if
 215 applicable. Participants were instructed in the use of the Precision Xtra® ketone
 216 monitoring system and the Biosense® breath ketone analyzer (BKA), and the need for
 217 paired (within 30 minutes) measurements three times every day. Specifically, participants
 218 were instructed to use the BKA device prior to eating, smoking, drinking soda, using
 219 toothpaste, mouthwash, or lip balm and to avoid environmental contaminants such as
 220 cleaning solutions. The device was to be used only in indoor spaces only. Study
 221 participants were educated on a ketone action plan and provided individual insulin dosing
 222 recommendations for capillary blood BOHB ≥ 1.5 mmol/L. During the DAPA period,
 223 insulin dose adjustments were recommended based on insulin delivery method and level
 224 of glucose control. The standard recommendation was to reduce bolus doses by 10-20%.

225 For the insulin withdrawal study, participants were admitted to the Clinical
 226 Translational Research Unit (CTRU) after an overnight fast. Participants on continuous
 227 subcutaneous insulin infusions were instructed to stop insulin delivery 1 hour prior to
 228 arrival. Participants using multiple daily injections (MDI) were transitioned to morning
 229 basal insulin for the study period, which was held the morning of the insulin withdrawal.
 230 Participants were required to have blood glucose level between 90-250 mg/dL and
 231 capillary blood BOHB <1.5 mmol/L before leaving home on the morning of the
 232 supervised insulin withdrawal study visit or the visit was cancelled. Participants remained
 233 fasting with serial venous blood sampling for plasma BOHB and basic metabolic panel
 234 every 2 hours and paired measurements of capillary blood glucose, capillary blood
 235 BOHB, and BrACE every hour. A plastic cap was applied to the BKA device when not in
 236 use to minimize VOC interference. The supervised insulin withdrawal study was

terminated when one or more stopping criteria were met (capillary blood glucose > 400 mg/dL, capillary blood BOHB > 4 mmol/L, symptoms of DKA, more than 8 hours elapsed from insulin withdrawal, or participant request).

240

241 *Outcomes*

242 The primary outcome for the study was the correlation coefficient between
243 capillary blood BOHB and BrACE in the outpatient periods with and without
244 dapagliflozin. Other outcomes included the correlations between capillary blood BOHB
245 and BrACE levels during the insulin withdrawal days. Adverse events were actively
246 collected for safety and included the daily ketone measurements, severe hypoglycemia
247 events, sensor glucose <54 mg/dL for more than 1% of time, diabetic ketoacidosis,
248 genitourinary infections, gastrointestinal distress, or dehydration.

249

250 *Statistical Analysis:*

251 The prespecified primary outcome of the study was the correlation of capillary
252 blood BOHB and BrACE during the 2-week outpatient periods. We calculated using the
253 pwr R package that at least 84 ketone measurements above baseline (~0.1 mmol/L or 1
254 BrACE ppm would provide >80% power to detect a weak correlation of 0.3 using a two-
255 sided significance level of 0.05 and estimated that 20 participants would need to be
256 enrolled to achieve this sample size.³¹ Descriptive statistics were examined for normality
257 by using histograms and Kolmogorov-Smirnov tests and are reported as mean ± SD or
258 median (interquartile range) as appropriate. Median capillary blood BOHB or BrACE
259 concentrations between UC and DAPA periods were compared by using the Mann-
260 Whitney U test. Correlations of blood and breath ketone concentrations were calculated
261 as Spearman's ρ given non-normal variable distributions. For ROC curves, Youden's J
262 statistic was used to determine the optimal BrACE threshold. Values were excluded from

analysis if study participants described reason for BrACE >10 ppm that included food or drink prior to measurement, use of lip balm, environmental contaminants or measurements taken outside. Paired values were compared by using paired two-tailed t-tests or Wilcoxon matched-pairs signed-rank test as appropriate. Statistical analyses were completed in SPSS 29 (IBM), R version 4.3.2 and GraphPad Prism 10.

Results

Participant characteristics are shown in **Table 1**. Twenty-one adults with T1D were screened, and 20 participated in the study, including 11 males and 9 females. The average age was 48 ± 18 years and mean BMI was 29.5 ± 5.2 kg/m². CSII in hybrid closed loop systems were used by 16 (80%) of the participants. At baseline, CGM time-in-range (70-180 mg/dL) was $61 \pm 18\%$, with mean hemoglobin A1c of $7.0 \pm 0.9\%$.

Table 1. Baseline characteristics

<i>N</i>	20
Age (yr)	48 ± 18
Sex (Male/Female)	11/9
Race (White/Black)	19/1
BMI (kg/m ²)	29.5 ± 5.2
Diabetes duration (yr)	26 ± 16
History of DKA (yes/no)	7/13
Hemoglobin A1c (%)	7.0 ± 0.9
Time in range 70-180 mg/dL (%)	61 ± 18
Insulin delivery (CSII/MDI)	16/4
Total daily insulin (units/kg)	0.70 ± 0.32

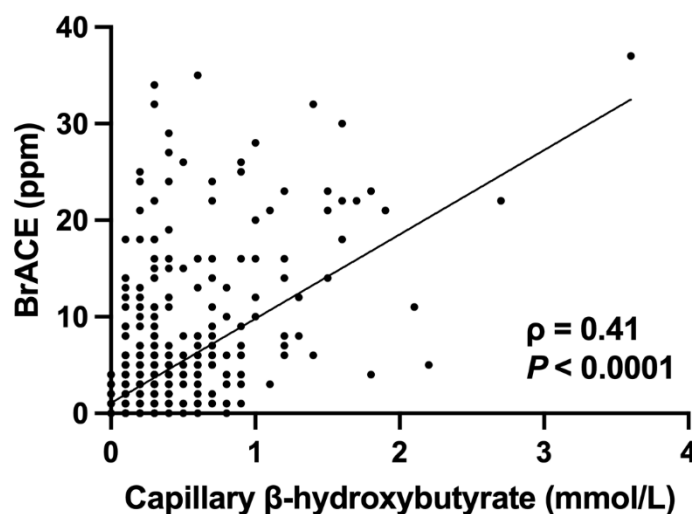
Estimated GFR (mL/min/1.73 m ²)	90 (83-94)
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277 BMI, body mass index; DKA, diabetic ketoacidosis; CSII, continuous subcutaneous
278 insulin infusion; MDI, multiple daily injections; GFR, glomerular filtration rate. Data are
279 expressed as mean $\pm$ SD or median (IQR) as appropriate.

280

281 The correlation between BrACE and BOHB during unsupervised outpatient care
282 was moderate ($\rho = 0.41$; 95% CI: 0.36 to 0.45; $P < 0.0001$; **Figure 1**). During the DAPA
283 period, the median (IQR) capillary blood BOHB concentration was greater than in the
284 UC period [0.2 (0.1 – 0.3) mmol/L DAPA vs. 0.1 (0.1 – 0.2) mmol/L UC, $P < 0.001$].
285 Median (IQR) BrACE was also greater during the DAPA period than in the UC period [2
286 (1 – 4) ppm DAPA vs. 1 (1 – 3) ppm UC, $P < 0.001$]. During unsupervised outpatient UC
287 periods, 3 of 718 BOHB readings were ≥ 1.5 mmol/L.

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289

290 **Figure 1. Correlation of blood and breath ketones during unsupervised outpatient**
291 **periods.** Paired capillary BOHB and BrACE measurements during unsupervised
292 outpatient periods (n =1425 paired measurements). Linear trendline is shown.

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296 During unsupervised outpatient DAPA periods, 10 of 707 BOHB readings were

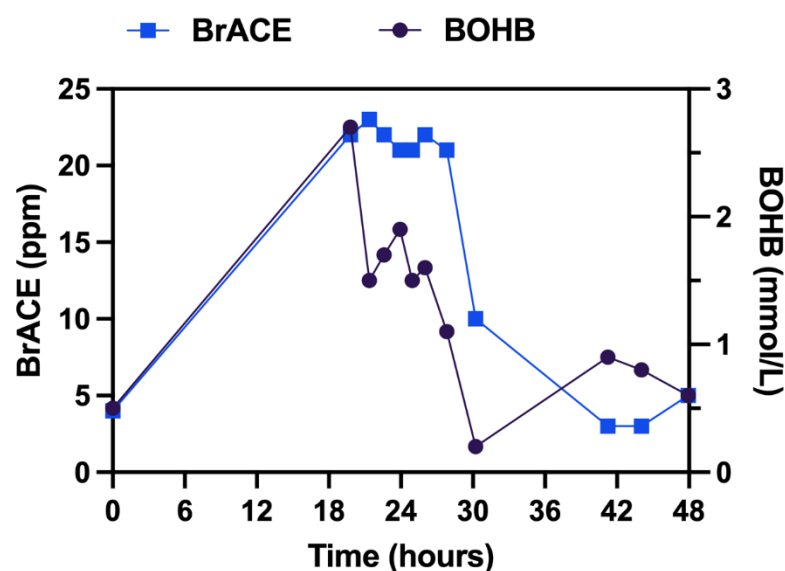
297 ≥ 1.5 mmol/L, but 6 of these occurred in a single bout of ketosis in one participant

298 **(Figure 2)**. In this individual, BrACE identified clinically significant ketosis during the

299 outpatient dapagliflozin treatment period. Repeated paired BrACE and BOHB

300 measurements showed that BrACE values were slower to normalize compared to

301 capillary BOHB **(Figure 2)**.



302

303 **Figure 2. Paired BrACE and capillary blood BOHB values during a bout of**

304 **outpatient ketosis**. Paired values collected over a 48-hour period in a participant who

305 developed and treated ketosis while taking dapagliflozin.

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During the supervised insulin withdrawal visits, 341 paired capillary blood BOHB and BrACE measurements were collected. A strong correlation of capillary blood BOHB and BrACE during supervised insulin withdrawal was observed ($\rho = 0.81$; 95% CI: 0.77 to 0.84; $P < 0.0001$; **Figure 3A**). The correlation of 254 paired plasma BOHB and BrACE measurements during supervised insulin withdrawal was similar ($\rho = 0.82$; 95% CI: 0.78 to 0.86; $P < 0.0001$; **Figure 3B**).

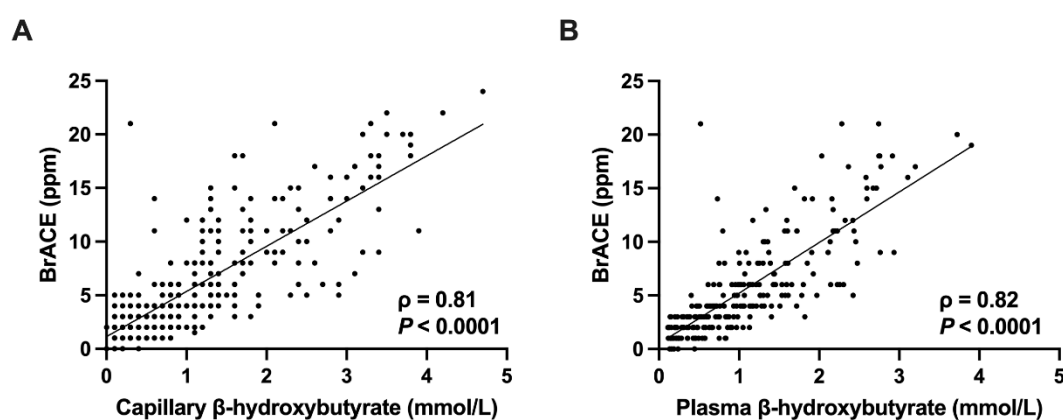


Figure 3. Paired BOHB and BrACE measurements during supervised insulin withdrawal. (A) Correlation of capillary blood BOHB and BrACE (n=341 paired measurements). (B) Correlation of plasma BOHB and BrACE (n=254 paired measurements). Study visits with and without dapagliflozin treatment were pooled (n=38 visits). Linear trendlines are shown.

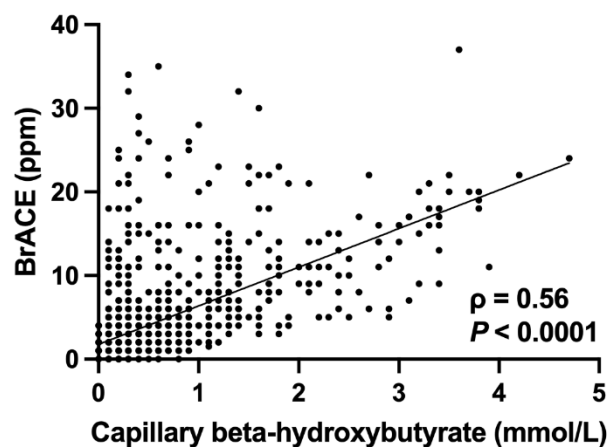
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When the data from the two-week unsupervised outpatient care and supervised insulin withdrawal study periods were combined, the correlation of capillary blood BOHB and BrACE was moderate ($\rho = 0.56$; 95% CI: 0.53 to 0.59; $P < 0.0001$; **Figure 4**).



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Figure 4. Correlation of capillary BOHB and BrACE during unsupervised outpatient care and supervised insulin withdrawal. Data from Fig. 1 and Fig. 3A were pooled (n=1766 paired measurements). Linear trendline is shown.

ROC analysis including data from both the unsupervised outpatient periods and the supervised insulin withdrawal visits with and without dapagliflozin was performed to determine the optimal BrACE value for identification of ketosis, defined as capillary BOHB ≥ 1.5 mmol/L (**Figure 5**). Optimal sensitivity (92%) and specificity (83%) were obtained at BrACE values ≥ 5 ppm.

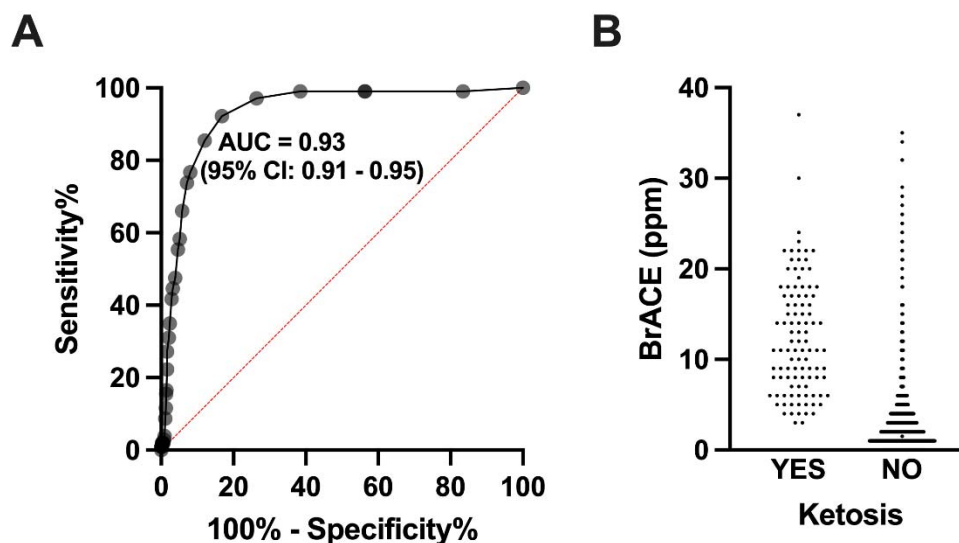


Figure 5. ROC curves for BrACE identification of ketosis. Pooled data from 2-week outpatient periods and supervised insulin withdrawal studies with and without dapagliflozin treatment were analyzed. Ketosis status was defined as capillary blood BOHB ≥ 1.5 mmol/L (YES) or < 1.5 mmol/L (NO). n=1743 non-ketotic and 103 ketotic paired measurements.

As expected, dapagliflozin use in those with T1D was associated with a reduction in insulin requirements and an improvement in blood glucose metrics (Table 2). As compared to UC, DAPA was associated with a ~11% reduction in total insulin requirement, a ~5% decrease in average CGM glucose level and a ~8% increase in time in range (70-180 mg/dL) (Table 2).

Table 2. Effect of dapagliflozin on insulin requirement and glycemia.

	UC (n=18-19)	DAPA (n=18-19)	P value
Daily basal insulin (units)	27.2 (17.4 – 55.4)	21.9 (15.4 – 40.9)	0.0134
Daily bolus insulin (units)	31.4 ± 15.8	26.7 ± 11.6	0.0401
Total daily insulin (units)	47.9 (36.2 – 85.5)	44.7 (35.0 – 76.3)	0.0082
Total daily insulin (units/kg)	0.66 ± 0.30	0.59 ± 0.24	0.0104
Average CGM glucose (mg/dL)	154 (138 – 165)	143 (139 – 147)	0.0016
Time-in-range 70-180 mg/dL (%)	70.1 ± 10.7	75.6 ± 9.5	0.0111

Data are from each 14-day outpatient period, with or without dapagliflozin. CGM, continuous glucose monitor. UC, usual care. DAPA, usual care plus dapagliflozin. Data are mean ± SD or median (IQR) as appropriate. 18 of 20 subjects had paired insulin data and 19 had paired CGM data from both study periods for analysis. P values by Wilcoxon matched-pairs signed-rank test or paired two-tailed t-test.

381 Discussion

382 The increased ketoacidosis risk associated with SGLT2i has prevented their
383 regulatory approval and widespread use in people with T1D. To alleviate this concern
384 and potentially allow those with T1D to benefit from the established cardiovascular and
385 renal benefits of SGLT2i, expert consensus panels have recommended the development
386 of noninvasive ketone monitoring technologies.²⁴ Handheld BrACE analyzers represent
387 one such technology with potential clinical utility. BrACE has been shown to correlate
388 well with capillary blood ketone measurements in free-living people with T1D, but the
389 performance of breath ketone analyzers has not been examined during insulin
390 withdrawal—a scenario closely mimicking real-world ketogenesis associated with insulin
391 pump failure or insulin omission.³²

392 In this study, to explore the utility of BrACE measurement in T1D, participants
393 monitored ketones with paired blood and breath assessments both during 14 days of their
394 usual care at home, and during 14 days of usual care plus dapagliflozin treatment, which
395 we hypothesized would enhance ketone production. Further, participants underwent
396 supervised insulin withdrawal during usual care and immediately after 14 days of
397 dapagliflozin treatment. We observed a strong correlation between capillary blood BOHB
398 and BrACE during supervised insulin withdrawal conditions but a moderate correlation
399 in the unsupervised outpatient settings.

400 The lower correlation between capillary blood BOHB and BrACE observed
401 during the unsupervised outpatient periods may be related to several factors. First, most
402 ketone values collected during the unsupervised outpatient periods were very low,
403 generating the problem of restricted range.³⁶ Second, proper use of the breath ketone
404 analyzer including the correct breathing pattern and the use of a filter cap was enforced
405 by supervision during the insulin withdrawal study. Third, VOC interference may have
406 occurred in the outpatient setting. VOCs are released from many items used in daily life

407 and can result in falsely elevated BrACE readings. During the insulin withdrawal study,
408 all measurements were done during fasting and under supervision, so the technical
409 validity of the measurements and the range of ketones produced were both greater.

410 In ROC analysis, a BrACE level of ≥ 5 ppm had 92% sensitivity and 83%
411 specificity for detecting a capillary BOHB of ≥ 1.5 mmol/L. This level of BrACE was
412 therefore considered the actionable level for prevention of ketoacidosis. A recent study of
413 SGLT2i treatment in people with T1D demonstrated that DKA mitigation strategies
414 including ketone monitoring with capillary BOHB or urinary ketones can reduce DKA
415 episodes despite higher average BOHB levels in those taking sotagliflozin.¹⁹ BrACE
416 measurement using a BKA is non-invasive, allows repeated measurements without
417 additional cost and may represent a viable method for ketone monitoring that can be
418 included in DKA mitigation strategies for persons with T1D using SGLT2i and those at
419 increased risk of episodic DKA.

420 One individual experienced a clinically significant bout of ketosis during the
421 unsupervised outpatient DAPA period. This individual did not eat breakfast and lunch,
422 thus reducing his automated insulin delivery. He did not have hyperglycemia, did not
423 manually change insulin doses, and did not report symptoms during the ketosis episode.
424 He reported the rise in ketones to the study team and was instructed in a treatment
425 strategy that led to resolution of the ketosis. He did not skip any further meals and did not
426 have additional ketosis episodes during the remainder of the study period despite
427 continuing SGLT2i treatment. Identifying persons at risk for episodic ketosis may require
428 trial periods of SGLT2i use with frequent ketone monitoring and underscores the
429 importance of appropriate patient selection for SGLT2i use in people with T1D.

430 Notable aspects of this study's design include the collection of over 1700 paired
431 BrACE and BOHB measurements and testing in the participants' home environment. The
432 data from the supervised insulin withdrawal study is relevant to scenarios such as insulin

433 pump failure or loss of insulin access, in which absolute insulin deficiency gradually
 434 develops.³⁷ This is important as CSII remains a risk factor for development of DKA.³⁸
 435 This study has important limitations including the small sample size. Further, in
 436 the unsupervised outpatient care periods, the range of values used for correlation
 437 calculations was relatively narrow and the risk for interference of VOC with BrACE
 438 measurements was higher. The restricted range likely contributed to the weaker
 439 correlation between BrACE and capillary BOHB observed in the outpatient setting. In
 440 addition, the strong correlation between BrACE and BOHB measurements during
 441 supervised insulin withdrawal may have been enhanced by the controlled environment of
 442 the research unit, in which paired measurements were supervised by a nurse and the BKA
 443 device was capped when not in use to reduce the risk of VOC interference. Additionally,
 444 this study has the limitation of excluding individuals with history of frequent DKA,
 445 uncontrolled diabetes (HbA1c >10%) or use of a ketogenic diet, who may be at increased
 446 risk for DKA with use of SGLT2i especially during insulin withdrawal. Future studies
 447 should consider investigating BrACE monitoring in patients at greater risk for
 448 ketoacidosis.

449 Our study demonstrates that BrACE measurement using a noninvasive, handheld
 450 breath ketone analyzer can detect clinically actionable ketosis but is more reliable in
 451 supervised settings as compared to unsupervised outpatient care. A critical limitation for
 452 more widespread real-world use in T1D is that currently available BKA devices remain
 453 highly susceptible to VOC interference. Whether repeated training on proper use of the
 454 device or deployment of a filter cap in the outpatient care setting would have reduced
 455 presumed interference in the BrACE measurements is unknown. Additional BKA device
 456 development is needed to explore modifications that could address these issues, making
 457 them more viable for home use in people with T1D. Additionally, the occurrence of
 458 ketosis in more than one patient while at home reminds health care providers that persons

459 with T1D are at risk for asymptomatic ketosis that may lead to serious adverse
460 consequences. The advice to test for ketones after symptoms develop may be too late for
461 early detection and effective treatment at home.

462

463 **Conclusions**

464 BrACE monitoring in adults with T1D offers a noninvasive assessment of
465 circulating ketones. A BrACE reading of ≥ 5 ppm had 92% sensitivity and 83%
466 specificity for a capillary blood BOHB concentration ≥ 1.5 mmol/L. The efficacy of the
467 breath ketone analyzer is limited by common interfering VOC found in the home and by
468 the need for proper operating technique. Larger studies are needed to further evaluate the
469 utility of BrACE measurements as a substitute for capillary BOHB measurements in
470 persons with T1D at risk of ketosis.

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474 **References**

- 475 1 McCoy RG, Herrin J, Galindo RJ, Sindhu Swarna K, Umpierrez GE, Hill Golden S *et al.* All-cause mortality after hypoglycemic and hyperglycemic emergencies among
476 U.S. adults with diabetes, 2011–2020. *Diabetes Res Clin Pract* 2023; **197**: 110263.
477
- 478 2 Shaka H, Aguilera M, Aucar M, El-Amir Z, Wani F, Muojieje CC *et al.* Rate and
479 Predictors of 30-day Readmission Following Diabetic Ketoacidosis in Type 1
480 Diabetes Mellitus: A US Analysis. *J Clin Endocrinol Metab* 2021; **106**: 2592–2599.
- 481 3 Benoit SR, Zhang Y, Geiss LS, Gregg EW, Albright A. Trends in Diabetic
482 Ketoacidosis Hospitalizations and In-Hospital Mortality — United States, 2000–
483 2014. *MMWR Morb Mortal Wkly Rep* 2018; **67**: 362–365.
- 484 4 Viridi N, Poon Y, Abaniel R, Bergenstal RM. Prevalence, Cost, and Burden of Diabetic
485 Ketoacidosis. *Diabetes Technol Ther* 2023; **25**: S75–S84.
- 486 5 Rosenbloom AL. The management of diabetic ketoacidosis in children. *Diabetes Ther*
487 *Res Treat Educ Diabetes Relat Disord* 2010; **1**: 103–120.
- 488 6 American Diabetes Association Professional Practice Committee, ElSayed NA,
489 Aleppo G, Bannuru RR, Bruemmer D, Collins BS *et al.* 6. Glycemic Goals and
490 Hypoglycemia: *Standards of Care in Diabetes—2024*. *Diabetes Care* 2024; **47**:
491 S111–S125.
- 492 7 “Ketone Testing.” Joslin Diabetes Center, [www.joslin.org/patient-care/diabetes-](http://www.joslin.org/patient-care/diabetes-education/diabetes-learning-center/ketone-testing-0)
493 [education/diabetes-learning-center/ketone-testing-0](http://www.joslin.org/patient-care/diabetes-education/diabetes-learning-center/ketone-testing-0). Accessed 28 Feb. 2024. .
- 494 8 Song C, Dhaliwal S, Bapat P, Scarr D, Bakhsh A, Budhram D *et al.* Point-of-Care
495 Capillary Blood Ketone Measurements and the Prediction of Future Ketoacidosis
496 Risk in Type 1 Diabetes. *Diabetes Care* 2023; **46**: 1973–1977.
- 497 9 Larsson CR, Januszewski AS, McGrath RT, Ludvigsson J, Keech AC, MacIsaac RJ *et al.*
498 Suboptimal behaviour and knowledge regarding overnight glycaemia in adults
499 with type 1 diabetes is common. *Intern Med J* 2018; **48**: 1080–1086.
- 500 10 Foster NC, Beck RW, Miller KM, Clements MA, Rickels MR, DiMeglio LA *et al.*
501 State of Type 1 Diabetes Management and Outcomes from the T1D Exchange in
502 2016–2018. *Diabetes Technol Ther* 2019; **21**: 66–72.
- 503 11 Albanese-O’Neill A, Wu M, Miller KM, Jacobsen L, Haller MJ, Schatz D *et al.* Poor
504 Adherence to Ketone Testing in Patients With Type 1 Diabetes. *Diabetes Care* 2017;
505 **40**: e38–e39.
- 506 12 Goldenberg RM, Berard LD, Cheng AYY, Gilbert JD, Verma S, Woo VC *et al.*
507 SGLT2 Inhibitor–associated Diabetic Ketoacidosis: Clinical Review and
508 Recommendations for Prevention and Diagnosis. *Clin Ther* 2016; **38**: 2654–2664.e1.
- 509 13 Haidar A, Yale J, Lovblom LE, Cardinez N, Orszag A, Falappa CM *et al.* Reducing
510 the need for carbohydrate counting in type 1 diabetes using closed-loop automated

- 511 insulin delivery (artificial pancreas) and empagliflozin: A randomized, controlled,
512 non-inferiority, crossover pilot trial. *Diabetes Obes Metab* 2021; **23**: 1272–1281.
- 513 14 Pasqua M-R, Jafar A, Kobayati A, Tsoukas MA, Haidar A. Low-Dose Empagliflozin
514 as Adjunct to Hybrid Closed-Loop Insulin Therapy in Adults With Suboptimally
515 Controlled Type 1 Diabetes: A Randomized Crossover Controlled Trial. *Diabetes*
516 *Care* 2023; **46**: 165–172.
- 517 15 Tomasoni D, Fonarow GC, Adamo M, Anker SD, Butler J, Coats AJS *et al.* Sodium-
518 glucose co-transporter 2 inhibitors as an early, first-line therapy in patients with
519 heart failure and reduced ejection fraction. *Eur J Heart Fail* 2022; **24**: 431–441.
- 520 16 Dandona P, Mathieu C, Phillip M, Hansen L, Griffen SC, Tschöpe D *et al.* Efficacy
521 and safety of dapagliflozin in patients with inadequately controlled type 1 diabetes
522 (DEPICT-1): 24 week results from a multicentre, double-blind, phase 3, randomised
523 controlled trial. *Lancet Diabetes Endocrinol* 2017; **5**: 864–876.
- 524 17 Mathieu C, Dandona P, Gillard P, Senior P, Hasslacher C, Araki E *et al.* Efficacy and
525 Safety of Dapagliflozin in Patients With Inadequately Controlled Type 1 Diabetes
526 (the DEPICT-2 Study): 24-Week Results From a Randomized Controlled Trial.
527 *Diabetes Care* 2018; **41**: 1938–1946.
- 528 18 Boeder SC, Thomas RL, Le Roux MJ, Giovannetti ER, Gregory JM, Pettus JH.
529 Combination SGLT2 Inhibitor and Glucagon Receptor Antagonist Therapy in Type 1
530 Diabetes: A Randomized Clinical Trial. *Diabetes Care* 2025; **48**: 52–60.
- 531 19 Peters AL, McGuire DK, Danne T, Kushner JA, Rodbard HW, Dhatariya K *et al.*
532 Diabetic Ketoacidosis and Related Events With Sotagliflozin Added to Insulin in
533 Adults With Type 1 Diabetes: A Pooled Analysis of the inTandem 1 and 2 Studies.
534 *Diabetes Care* 2020; **43**: 2713–2720.
- 535 20 Boeder S, Davies MJ, McGill JB, Pratley R, Girard M, Banks P *et al.* Beta-
536 Hydroxybutyrate Levels and Risk of Diabetic Ketoacidosis in Adults with Type 1
537 Diabetes Treated with Sotagliflozin. *Diabetes Technol Ther* 2024; : dia.2023.0605.
- 538 21 Rodbard HW, Peters AL, Slee A, Cao A, Traina SB, Alba M. The Effect of
539 Canagliflozin, a Sodium Glucose Cotransporter 2 Inhibitor, on Glycemic End Points
540 Assessed by Continuous Glucose Monitoring and Patient-Reported Outcomes
541 Among People With Type 1 Diabetes. *Diabetes Care* 2017; **40**: 171–180.
- 542 22 Rosenstock J, Marquard J, Laffel LM, Neubacher D, Kaspers S, Cherney DZ *et al.*
543 Empagliflozin as Adjunctive to Insulin Therapy in Type 1 Diabetes: The EASE
544 Trials. *Diabetes Care* 2018; **41**: 2560–2569.
- 545 23 Garcia-Tirado J, Farhy L, Nass R, Kollar L, Clancy-Oliveri M, Basu R *et al.*
546 Automated Insulin Delivery with SGLT2i Combination Therapy in Type 1 Diabetes.
547 *Diabetes Technol Ther* 2022; **24**: 461–470.
- 548 24 Danne T, Garg S, Peters AL, Buse JB, Mathieu C, Pettus JH *et al.* International
549 Consensus on Risk Management of Diabetic Ketoacidosis in Patients With Type 1

- 550 Diabetes Treated With Sodium-Glucose Cotransporter (SGLT) Inhibitors. *Diabetes*
551 *Care* 2019; **42**: 1147–1154.
- 552 25 Laffel L. Ketone bodies: a review of physiology, pathophysiology and application of
553 monitoring to diabetes. *Diabetes Metab Res Rev* 1999; **15**: 412–426.
- 554 26 Dhatariya K. Blood Ketones: Measurement, Interpretation, Limitations, and Utility in
555 the Management of Diabetic Ketoacidosis. *Rev Diabet Stud RDS* 2016; **13**: 217–225.
- 556 27 Owen OE, Trapp VE, Skutches CL, Mozzoli MA, Hoeldtke RD, Boden G *et al*.
557 Acetone metabolism during diabetic ketoacidosis. *Diabetes* 1982; **31**: 242–248.
- 558 28 Amlendu P, Ashley Q, Di W, Haojiong Z, Mirna T, David J *et al*. Breath Acetone as
559 Biomarker for Lipid Oxidation and Early Ketone Detection. *Glob J Obes Diabetes*
560 *Metab Syndr* 2014; **1**: 012–019.
- 561 29 Anderson JC. Measuring breath acetone for monitoring fat loss: Review. *Obesity*
562 2015; **23**: 2327–2334.
- 563 30 Güntner AT, Kompalla JF, Landis H, Theodore S, Geidl B, Sievi N *et al*. Guiding
564 Ketogenic Diet with Breath Acetone Sensors. *Sensors* 2018; **18**: 3655.
- 565 31 Suntrup Iii DJ, Ratto TV, Ratto M, McCarter JP. Characterization of a high-resolution
566 breath acetone meter for ketosis monitoring. *PeerJ* 2020; **8**: e9969.
- 567 32 Akturk HK, Snell-Bergeon J, Pyle L, Fivekiller E, Garg S, Cobry E. Accuracy of a
568 breath ketone analyzer to detect ketosis in adults and children with type 1 diabetes. *J*
569 *Diabetes Complications* 2021; **35**: 108030.
- 570 33 Naunheim R, Jang TJ, Banet G, Richmond A, McGill J. Point-of-care test identifies
571 diabetic ketoacidosis at triage. *Acad Emerg Med Off J Soc Acad Emerg Med* 2006;
572 **13**: 683–685.
- 573 34 Charles RA, Bee YM, Eng PHK, Goh SY. Point-of-care blood ketone testing:
574 screening for diabetic ketoacidosis at the emergency department. *Singapore Med J*
575 2007; **48**: 986–989.
- 576 35 Brooke J, Stiell M, Ojo O. Evaluation of the Accuracy of Capillary Hydroxybutyrate
577 Measurement Compared with Other Measurements in the Diagnosis of Diabetic
578 Ketoacidosis: A Systematic Review. *Int J Environ Res Public Health* 2016; **13**: 837.
- 579 36 Bland JM, Altman DG. Correlation in restricted ranges of data. *BMJ* 2011; **342**:
580 d556.
- 581 37 Herring RA, Shojaei-Moradie F, Garesse R, Stevenage M, Jackson N, Fielding BA *et*
582 *al*. Metabolic Effects of an SGLT2 Inhibitor (Dapagliflozin) During a Period of
583 Acute Insulin Withdrawal and Development of Ketoacidosis in People With Type 1
584 Diabetes. *Diabetes Care* 2020; **43**: 2128–2136.
- 585 38 Wersäll JH, Adolfsson P, Forsander G, Hanas R. Insulin pump therapy is associated
586 with higher rates of mild diabetic ketoacidosis compared to injection therapy: A

587 2□year Swedish national survey of children and adolescents with type 1 diabetes.
588 *Pediatr Diabetes* 2022; **23**: 1038–1044.

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