Assessment of BHB Concentration Agreement Among Sampling Locations and the Impact of Ketosis on Erythropoietin Levels: A Two-Part Study

Aim and perspective

This study's main goal (part 1) is to determine

i. agreement in beta-hydroxybutarate (BHB) of capillary and venous blood estimates and subsequently whether agreement is dependent on BHB level, and
ii. agreement in BHB of finger and earlobe capillary samples, and
iii. agreement in BHB of venous estimates (estimated by point-of-care device) and full blood (estimated by use of hydrophilic interaction liquid chromatography tandem mass spectrometry (HLCMS))

The main goal (part 2) is to determine

i. effects of ketosis on erythropoietin(EPO) short-term and long-term
ii. derived effects on erythropoiesis and iron metabolism from two-week intermittent ketosis

This study will establish (part 1) whether it is appropriate to select a specific sampling site for measuring BHB in patients and performing research, and whether there is a difference between measurements capillary and venous blood. The agreement between the KetoSure, a point-of-care-test (POCT) device, and the gold standard will also be examined, offering insight into any discrepancies with point-of-care estimates obtained by electrochemical estimations and HLCMS. High BHB measurement validity is required in both clinical and experimental contexts. First, BHB quantification may aid in clinical decision-making for suspected hyperinsulinemia, hypoglycemia of uncertain etiology, diabetic ketoacidosis, and other diseases. Second, a substantial amount of research has been conducted on BHB inference and ketones in general in recent years, and the validity of the interpretation of these studies is highly dependent on measurement precision in regards to which sample type and sampling site is applied.
In addition, the effects of ketosis on EPO concentrations will be investigated (part 2) from blood samples obtained during part 1 (acute effects) and at day 7 and day 14 where participants are intermittently ketotic allowing also for investigations on EPO-derived effects on erythropoiesis and iron metabolism.

Background

During an experiment in which samples were obtained from two distinct places, namely the finger pulp and the earlobe, the KetoSure ketonometer disclosed a discrepancy in BHB estimates, prompting us to review the relevant literature on discrepancy between sampling sites.

In glucose determination, such discrepancy between capillary and venous blood concentration is well-known[1]. In contrast to our incidental findings, capillary glucose estimations are higher than venous plasma estimates. Several hypotheses, with variances in hematocrit being the most prevalent, have been suggested as explanations for these discrepancies; however, conclusions change depending on the analyte of interest[2]. In addition to the comparator, the POCT detection method, kind of enzymatic reaction, and pH value are of utmost importance[3].
As reported in a 2016 study, the accuracy of capillary BHB measurement has been tested in various contexts and with various ketonometers[4]. Primarily with the intent of improving its ability to identify diabetic ketoacidosis. Similarly, the accuracy of glucose estimates from different capillary sample sites has varied in response to both glucose concentration and capillary sampling site[5].

To our knowledge, neither the agreement between venous blood and full blood+HLCMS values nor the disparity between different sampling sites for the extraction of capillary samples have been investigated.

Two investigations have indicated a link between ketosis and EPO[6,7]. Both, however, had other primary endpoints, and thus were not meant to answer this particular research question. In addition, only the correlation between ketosis and EPO levels has been studied. Thus, erythropoietic effects of EPO in the bone marrow (and elsewhere) have not been studied. The incorporation of ambulatory blood testing, and the extension of the ketosis, such effects can be investigated over an appropriate time frame and provide the opportunity to investigate not only the relationship between exogenous ketosis in general, but also any temporal aspects thereof, as the study design includes both an "acute" (part 1) and a "chronic" (part 2) factor.

References

1. Boyd R, Leigh B, Stuart P. Capillary versus venous bedside blood glucose estimations. *Emerg Med J*. 2005;22(3):177-179. doi:10.1136/emj.2003.011619
2. Osteresch B, Cramer B, Humpf HU. Analysis of ochratoxin A in dried blood spots – Correlation between venous and finger-prick blood, the influence of hematocrit and spotted volume. *Journal of Chromatography B*. 2016;1020:158-164. doi:10.1016/j.jchromb.2016.03.026
3. Kotwal N, Pandit A. Variability of capillary blood glucose monitoring measured on home glucose monitoring devices. *Indian J Endocrinol Metab*. 2012;16(8):248. doi:10.4103/2230-8210.104052
4. Brooke J, Stiell M, Ojo O. Evaluation of the Accuracy of Capillary Hydroxybutyrate Measurement Compared with Other Measurements in the Diagnosis of Diabetic

Ketoacidosis: A Systematic Review. *Int J Environ Res Public Health*. 2016;13(9):837. doi:10.3390/ijerph13090837
5. Lightbody TO, Nightingale P, Reddy Kolanu V, Sapey E. The Benefits and Limitations of Point of Care Testing for Patients with Diabetes Who are Acutely Unwell. *J Diabetes Metab*. 2017;08(09). doi:10.4172/2155-6156.1000761
6. Evans E, Walhin JP, Hengist A, Betts JA, Dearlove DJ, Gonzalez JT. Ketone monoester ingestion increases post-exercise serum erythropoietin concentrations in healthy men. *American Journal of Physiology-Endocrinology and Metabolism*. Published online November 30, 2022. doi:10.1152/ajpendo.00264.2022
7. Lauritsen KM, Søndergaard E, Svart M, Møller N, Gormsen LC. Ketone Body Infusion Increases Circulating Erythropoietin and Bone Marrow Glucose Uptake. *Diabetes Care*. 2018;41(12):e152-e154. doi:10.2337/dc18-1421
8. Topping J, Reardon M, Coleman J, et al. A Comparison of Venous versus Capillary Blood Samples when Measuring Blood Glucose Using a Point-of-Care, Capillary-Based Glucometer. *Prehosp Disaster Med*. 2019;34(5):506-509. doi:10.1017/S1049023X19004850

Methods

Design

The study is performed with an analytical cross-sectional design, i.e., participants are sampled during three different exposures (different degrees of ketosis) on a single study day(part 1) and on two out-patient occasions on day 7 and day 14 (part 2).
Part 2 is a participant-blinded randomized controlled design where participants receive in a 1:1 fashion either the intervention (ketone monoester drink) daily or a taste- and volume-matched placebo drink

Study day (part 1)

The study day duration will be approximately four hours with participants reporting in at Klinik for Diabetes & Hormonsygdomme, Viborg Regional Hospital at 8 A.M. in the overnight fasted state. A standard 18G venous catheter is inserted in a median cubital, basilic, or cephalic vein for blood sampling. Baseline and following, blood samples are drawn on the hour for 3 hours. Subsequently, participation is over, the catheter is removed with observation and compression 5-10 min. upon which the study participation is over for the part of the participants.

Ambulatory investigations (part 2)

Following the study day (part 1) participants are provided the full amount of either the ketone monoester drink or the comparator (placebo-drink). The drinks are provided in small plastic bottles

Methods

In order to obtain ketosis, participants ingest a common sports drink, KE4, which contains a ketone monoester. Upon this, ketosis is established with a substantial increase in BHB for estimation in the venous and whole blood, along with capillary measurements from the earlobe and finger pulp. The dosage will be 500 mg/kg for the study day (part 1) and daily, divided in two or three dosages as preferred, for the following 14 days (part 2)

Feasibility

No substantial practical challenges are expected – all equipment and competences are present in the Department of Internal Medicine and Department of Clinical Biochemistry.
No recruitment issues are expected – this is a small-scale study with minimal impact in relation to participation.
The study is fully funded by independent research funds within departments involved although external funding will be sought for.

Analytic methods

BHB is estimated by the Ketosure™ for capillary measurements (finger pulp and earlobe), for venous blood samples, and HLCMS for full blood estimation of BHB as the reference standard.

Statistical considerations

Analytical approach

Following a visual examination of the graphical representation for linearity, differences are calculated using the paired t-test, agreement is determined using the Bland-Altman plot, and Pearson's r is used to determine correlations. Supporting calculations are performed using Lin’s concordance correlation coefficient of absolute agreement. When comparisons over observations from part 1 and 2 are performed an repeated measurements anova equivalent analysis is applied.

Sample size and power calculation

No previous studies with data on BHB agreement have been performed. Based on our incidental findings, only very few subjects would be needed. The data is, however, far too small to base sample size calculations on.

Based on Boyd et al.[1] with highly correlated estimates of glucose and the acceptance of a type 1 error (alpha) of 0.05, type 2 error of 20% (beta), a sample size of 13 participants is required to provide adequate statistical power to detect a difference of 0.58 mM in glucose estimates between capillary and venous blood samples with the standard deviation of 0.68 mM. Others have similarly found a clinically meaningful difference of 1 mM between sampling sites[8]. With alpha and beta set as mentioned, a sample size of 20 participants is

required to provide adequate statistical power to detect a difference of 1 mM (SD = 1.5), should such a difference exist.

As such, we find it reasonable to include 15 patients in our study resulting in a power of 91% if expecting a meaningful difference of 1 mM and an average standard deviation of the two studies mentioned of 1.1 mM. Similar number of participants have been used elsewhere with regards to part 2 of this project and sample size as suggested is fully justified to test hypotheses of part 2.

Participants

Recruitment

Subjects will be recruited through notices on specific websites (sundhed.dk, forsøgsperson.dk) and social media (Facebook) along with board notice on selected local educational institutions, hospitals, and in newspapers.
First contact will be by telephone with the primary investigator(s) followed by the formal information on study participation (se section on recruitment and informed consent)

Inclusion criteria

Age 18-60 years
BMI 19-30 kg/m^2
Expected ease of catheter insertion
Considered of sound health
Oral and written informed consent

Exclusion criteria

Inability to fully understand the consent including consent forms
Inability to cooperate to the study
Any electrolyte disorders, kidney disease or otherwise compromised renal function including excess risk hereof, e.g. hypertension, albuminuria, autoimmune disease, family history of kidney disease
Any liver or bile disease, or an increased risk of getting one, such as from hepatotoxic drugs, alcoholism, gallstones, pancreatitis, an autoimmune disease, or a history of liver disease in the family.
Diabetes mellitus or any metabolic and/or hormonal disease including diagnosed/undiagnosed reactive hypoglycemia or similar disorders. This includes treatment with drugs, and dietary supplements with inference on key metabolic or hormonal markers, e.g. insulin, glucagon, lipids, and GLP-1
Any use of illegal or otherwise use of medicinal products without prescription
Anemia or other know disease of the hematopoietic system
Previous bariatric surgery
Previous myocardial infarction or uncontrolled myocardial ischemia
Recent intended/unintended weight loss

Allergies to catheters or adhesives

Safeguards to minimize pain, discomfort, fear and other risks

Short-term and long-term risks and side effects

There are neither short-term nor long-term serious hazards or adverse effects to concern. KE4 is a bitter sports drink that can cause moderate, temporary nausea and bloating. Appetite may be slightly reduced over the full study period (part 2). Compared to sweet, salty, or umami-flavored beverages, the digestive adverse effects are equivalent to those of bitter-flavored beverages in general. This is a well-known side effect of bitter-tasting beverages in certain individuals.
As with the pricking of the fingers and earlobes, there is a small risk of infection following catheter placement, as well as a slight degree of discomfort.

Risk of radioactive radiation

There is no radiation involved in this study

Collection of new biological material

What and how much material

A total of 150 mL of blood is drawn in this study. This includes unavoidable spill from repeated sampling from indwelling catheter in part 1. No spillage is associated with part 2 since blood sampling is performed only twice in an outpatient setting (at the hospital laboratory for blood sampling)

Purpose

For analysis in bulk.

How long and what is the purpose of storage

All biological samples are stored for the full period of data collection and 18 months after in order to analyse all samples in bulk. Samples are stored at -80 ºC.

Biobank

A research biobank is developed for the bulk analysis of all samples not analyzed on the day of the study, i.e. measurements using the Ketosure™ POCT device. The analyses are conducted within a few months at the earliest opportunity and surplus material is preserved as a biobank for future yet undetermined research.

Information from patient records

Access to participants' electronic patient records (exclusively and limited to solely laboratory results section) is included in study participation consent and necessary for practical reasons, i.e., information on medication, blood pressure readings. Only information otherwise routinely obtained as part of participants' treatment and are registered with the purpose of analysis within the frame of the study. Any information for use in the study given prior to oral and written consent are forwarded to the investigator.
Consent gives the study investigators as well as any control authority direct access to obtain information in the patient's health records, etc., including electronic health records, in order to see information about the participant’s health conditions necessary for the implementation of the research project and for the purposes of any and all controls, including self-monitoring, quality control, and monitoring, which these are required to perform.

Handling of personal data in the study

The study is reported to and registered within Region Midt’s research projects registration.
The Data Protection Act is upheld in accordance with The General Data Protection Regulation in all aspects of the study.
All data are pseudonymized by de-identification when entered in data analysis sheet. A copy of the de-identification code kept only by primary investigator.
Access to data is equally open only to investigators until final analysis. Hereafter other investigators have access by which time all data are completely anonymized. After publication all data are open to public scrutiny.

Financial information

The study is initiated by primary investigator Henrik Holm Thomsen
The study is financed from independent research funds within the Department of Internal Medicine, Regional Hospital Viborg. Additional external funding will be sought. All funding will be related to study expenses only, excluding any investigator salary which is cared for according to individual agreements with respective employers. All financial transactions are administered according to the research practices of the Central Denmark Region and Regional Hospital Viborg.

Research investigators and collaborators have no financial interests in the study.

Remuneration and/or other benefits to participants

Participants will be offered an inconvenience allowance of DKK 750 for completed study day participation (part 1) and DKK 750 for participation in part 2 (outpatient), thus DKK 1500 in total, with proportionate remuneration in the event of premature withdrawal. Requirements of the Committee Law §20, stk. 1, nr. 3 are upheld.

Recruitment of participants and informed consent

Potential participants are recruited through post on social media (Facebook and Twitter).

The first formal contact will be by telephone with the primary investigators (Henrik Holm Thomsen or Birgitte Sandfeld Paulsen) who are contacted by potential participant through notice of the study. Following this, the mandatory information about study participation, the interview, and the examination will be conducted as either a face-to-face meeting conducted with regard to privacy or as a video call, per request of the potential study participant. Potential study participants are informed that they are allowed to bring a companion to the interview.

Potential participants are always approached in a friendly and professional manner with a focus on minimizing any coercion.

After receiving information about study background and participation, potential participants are given reasonable time (one to two weeks or as deemed necessary by potential participant) to consider participation and allowing for further questions. Hereafter, they submit the signed consent form and give their oral consent.
It is emphasized to the potential participants that participation can be halted or stopped at any time upon request. Participants are not obliged to give the reason(s) for withdrawing consent and/or participation.
Special consideration will also be on ensuring that potential participants fully understand the study procedures.

Dissemination of results

The aim is to publish results, be them 'negative', 'positive' or inconclusive, in an internationally recognised peer-reviewed medical journal. If not accepted for publication, results are disseminated through the registration at clinicaltrials.gov where the study will be registered.

Ethics

Participation is associated with minute risks and discomfort and obtaining blood samples are done on routine visits to outpatient clinic as already planned. Hence, the study is believed to be justified with obtaining novel knowledge to better treatment options for future patients.

Information about the compensation scheme

The study is covered by the Danish Compensation Association