Brief Cutting Edge Report
OBESITY BIOLOGY AND INTEGRATED PHYSIOLOGY

Obesity

Breath Acetone to Monitor Life Style Interventions in Field Conditions: An Exploratory Study

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Objective: To assess whether breath acetone concentration can be used to monitor the effects of a prolonged physical activity on whole body lipolysis and hepatic ketogenesis in field conditions.
Methods: Twenty-three non-diabetic, 11 type 1 diabetic, and 17 type 2 diabetic subjects provided breath and blood samples for this study. Samples were collected during the International Four Days Marches, in the Netherlands. For each participant, breath acetone concentration was measured using proton transfer reaction ion trap mass spectrometry, before and after a 30-50 km walk on four consecutive days. Blood non-esterified free fatty acid (NEFA), beta-hydroxybutyrate (BOHB), and glucose concentrations were measured after walking.
Results: Breath acetone concentration was significantly higher after than before walking, and was positively correlated with blood NEFA and BOHB concentrations. The effect of walking on breath acetone concentration was repeatedly observed on all four consecutive days. Breath acetone concentrations were higher in type 1 diabetic subjects and lower in type 2 diabetic subjects than in control subjects.
Conclusions: Breath acetone can be used to monitor hepatic ketogenesis during walking under field conditions. It may, therefore, provide real-time information on fat burning, which may be of use for monitoring the lifestyle interventions.

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Introduction

Obesity results from an imbalance between energy and fat intake on one hand, energy expenditure and fat oxidation on the other hand. It is frequently associated with insulin resistance and type 2 diabetes mellitus (T2DM), non-alcoholic fatty liver disease, and dyslipidaemia. A low fat oxidation rate, possibly related to impaired mitochondrial function, and intracellular accumulation of lipid metabolites, such as diacyl-glycerol and ceramides, may play a role in the pathogenesis of all these conditions (1). Diet and physical activity are cornerstone treatments for obesity and T2DM (2); both aim at promoting a negative energy balance and increasing lipid oxidation, thus alleviating tissue lipotoxicity. Their success rate in clinical practice is unfortunately low. Their effectiveness may be enhanced if health professionals and patients could rely on a sensitive marker of lipid oxidation to adjust diet and exercise on a day-to day basis (3). For this purpose, breath acetone concentration may be a suitable marker, since physiological variations of hepatic ketogenesis are known to occur during physical activity and energy restriction.

Here, we present preliminary evidence that an activation of whole body lipolysis and hepatic ketogenesis induced by prolonged physical activity can be detected by monitoring breath acetone concentration in field conditions.

Methods

The Dutch Walking Organization (KNBLO-NL) organized a large annual walking event in July 2012; the International Four Days Marches (the Netherlands) (http://www.4daagse.nl/en/), during which more than 40,000 people engaged in a daily 30-50 km walk for four consecutive days. Among them, 11 subjects with type 1 diabetes mellitus (T1DM), 17 with T2DM, and 23 non diabetic subjects (controls, CT) were recruited. Their characteristics are shown in Table 1. The experimental protocol was approved by Medical Ethical Committee of the Radboud University Nijmegen Medical Centre.

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TABLE 1 Participant's characteristics

	CT ($n = 23$)	T1DM ($n = 11$)	T2DM ($n = 17$)
Age (mean ± SD)	56 ± 13	40 ± 12	56 ± 12
Gender (M/F)	13/10	4/7	11/6
Walking distance per day (30/40/50 km)	9/12/2	0/8/3	6/9/2
BMI (mean ± SD)	25.2 ± 3.3	28.1 ± 4.9	29.1 ± 4.1

Data collection

End-expiratory breath samples (0.5-0.8 l) were collected in Mylar bags using a hand-held breath sampler (4) from every study participant before starting the walk (3.30-8:00 am, after subjects had breakfast), and at the end of the walk (10:30 am-5:30 pm) on all four days of the event. Blood samples were collected on EDTA and immediately centrifuged, and plasma was aliquoted and stored at −80°C.

Sample analysis

Breath acetone measurements were performed within 8 h of collection, using a custom built proton-transfer-reaction ion trap mass spectrometer (PIT-MS) (5). This technique enables detection of a large variety of volatile organic compounds below parts per billion volume (ppbv) concentration (6). The mass spectrometer was calibrated with gas samples of known acetone concentration ranging from 35 ppbv to 1,000 ppbv. Preliminary experiment had shown that breath acetone concentration decreased by less than 8% in samples stored up to 8 h.

Blood non-esterified free fatty acid (NEFA) and beta-hydroxybutyrate (BOHB) concentrations were measured with an automated clinical chemistry analyzer from Randox RX series, RX Imola. Blood glucose concentrations were measured by Siemens Rapidpoint 405.

Statistical analysis

The normality of the data distribution was evaluated by the Lilliefors test. Breath acetone measurements were normally distributed, and were evaluated using parametric statistics. In contrast, blood glucose, NEFA, and BOHB concentrations were not normally distributed and were analyzed by nonparametric statistics. Changes in breath acetone levels were assessed using three-way ANOVA (ANalysis Of VAriance) with interaction terms, with intervention (walking/exercise), time (repetition of analysis for four successive days), and groups (CT, T1DM, and T2DM) entered as independent variables. Between-group's differences were assessed with *t*-tests for breath acetone, and with Mann–Whitney test for blood parameters.

Pearson's correlation coefficient was used to search for relationships between breath acetone levels and blood glucose, NEFA, and BOHB at the end of the walk on day 1 and day 0. All calculations were performed using Matlab R2013a (Mathworks, Natwick, MA, USA).

Results

The effect of walking on breath acetone for all participants is shown in Figure 1A. The three-way ANOVA analysis showed significant effects for intervention ($P < 0.0001$) and group (CT, T1DM, and T2DM) ($P < 0.0001$) and significant interaction between intervention and group ($P = 0.0003$), indicating that breath acetone was increased after the walk compared to before walking values, and that the effect of walking differed significantly between T1DM, T2DM, and non-diabetic subjects. There was no significant effect of time, indicating that the increase in breath acetone did not wane when

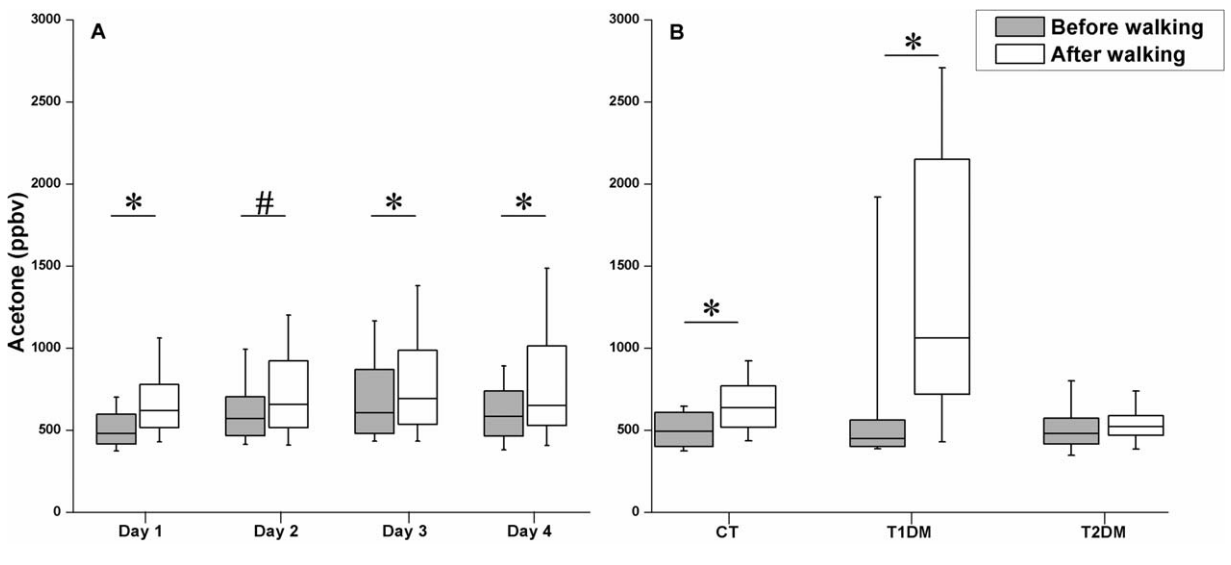

Figure 1 Breath acetone concentrations. **A:** Breath acetone concentrations measured before and after the walk over four consecutive days for all participants. **B:** Breath acetone concentrations measured before and after the walk in CT, T1DM, and T2DM on the first day. Data are displayed as box plots showing the median, interquartile ranges (25%, 75%); whiskers indicate the 10–90% values. *$P < 0.05$, # $P < 0.1$.

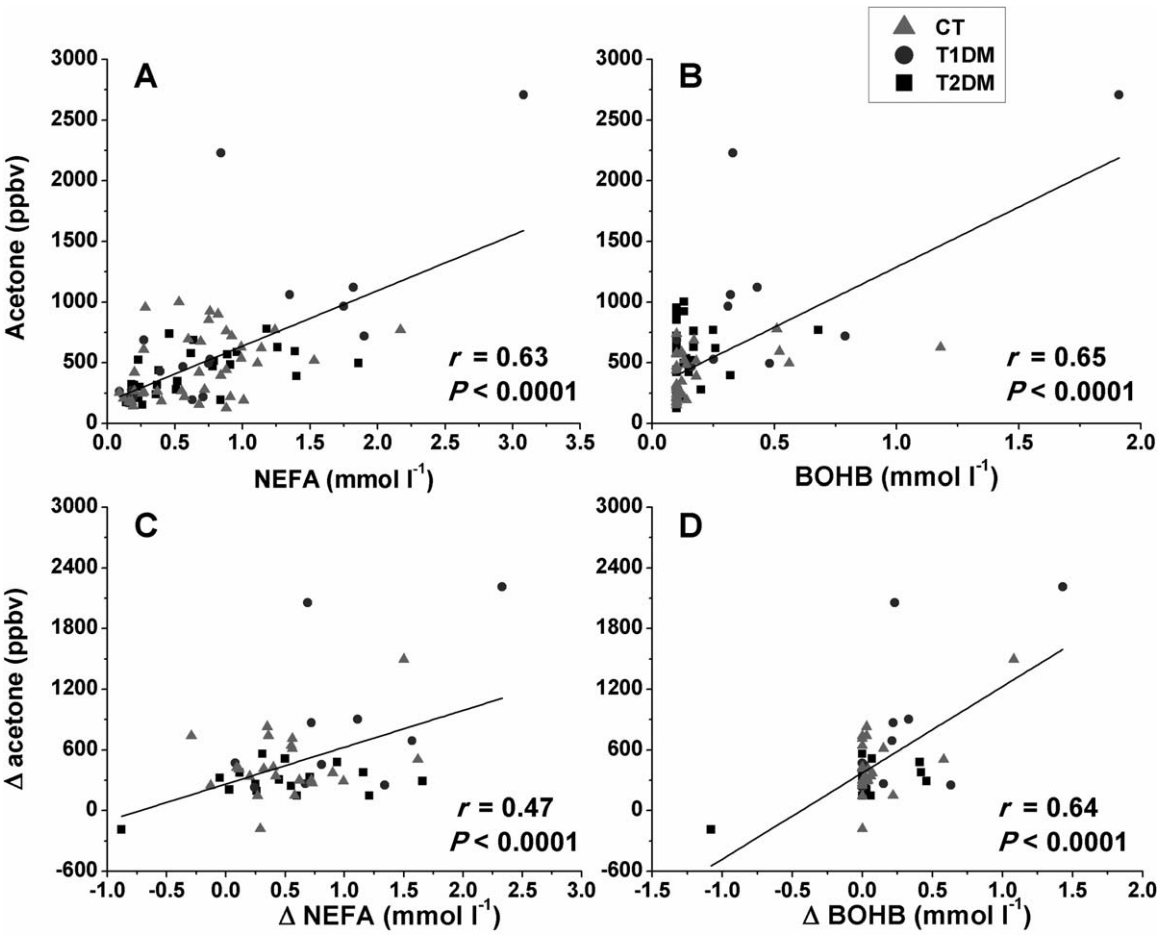

Figure 2 Relationships of breath acetone with NEFA and BOHB. Correlation of breath acetone (ppbv) with serum NEFA (mmol l^{-1}) **(A)** and BOHB (mmol l^{-1}) **(B)**. Difference between post- and pre-walking NEFA (Δ NEFA, **C**) and BOHB (Δ BOHB, **D**) vs. changes in breath acetone (Δ acetone).

walking was repeated day after day. Breath acetone levels observed in T1DM, T2DM, and CT subjects before and after the walk on day 1 are shown in Figure 1B. It increased significantly in T1DM and CT subjects after the walk, while the increase in T2DM failed to reach statistical significance. Blood NEFA and BOHB concentrations after walking as compared to before increased from 0.39 (± 0.30) to 0.98 (± 0.58) mmol l^{-1} ($P < 0.0001$) and from 0.14 (± 0.20) to 0.26 (± 0.30) mmol l^{-1} ($P = 0.02$), respectively. When the responses were assessed according to subjects subgroups, walking increased BOHB by 220% in T1DM ($P = 0.04$) and by 110% in CT ($P = 0.04$) and tended to increase it by 15% in T2DM ($P = 0.07$) respectively. NEFA concentration was significantly higher after walking than before in CT, T1DM, and T2DM with 113% ($P < 0.0001$), 257% ($P = 0.003$), and 132% ($P = 0.005$), respectively.

Breath acetone concentrations measured at the end of the walk on day 1 and day 0 were significantly correlated with blood NEFA concentrations (Figure 2A, $r = 0.63$, $P < 0.0001$) and BOHB concentrations (Figure 2B, $r = 0.65$, $P < 0.0001$), but not with blood glucose concentrations ($r = 0.28$, $P = 0.23$). The correlation between breath acetone and BOHB or NEFA remained significant ($r = 0.38$, and $r = 0.58$, $P < 0.0001$) when two outliers were removed from the analysis. Furthermore, differences in breath acetone before and after walking were correlated with differences in NEFA ($r = 0.47$, $P < 0.001$) and BOHB ($r = 0.64$, $P < 0.0001$).

Discussion

Breath acetone has been known for decades to increase several fold in diabetes ketoacidosis (7). Recent improvement in gas metabolites analysis has allowed the detection of smaller increases in breath acetone, which may correspond to changes in hepatic ketogenesis within the physiological range, in healthy subjects during strenuous physical exercise (8-10). Since hepatic ketogenesis closely parallels whole-body lipid oxidation (11), we propose that breath acetone may be used to monitor lifestyle interventions targeted to stimulate fat oxidation. Consistent with this hypothesis, we observed that a prolonged physical exercise, performed over consecutive days, repeatedly increased breath acetone in breath samples collected in "field conditions." We further observed that breath acetone concentrations were correlated with both blood BOHB and NEFA concentrations, but not with blood glucose. This supports that breath acetone changes reflect changes in lipolysis, fat oxidation, and

ketogenesis within the physiological range, rather than being linked to a worsening of metabolic control in diabetic subjects. Furthermore, breath acetone sensitively reflected changes in blood BOHB. While BOHB concentrations were below the assay detection limit in a significant number of participants, breath acetone concentrations were readily detected in all subjects.

Changes in breath acetone concentrations showed the same pattern in T2DM and T1DM as in CT, suggesting that it may be further evaluated as a marker for lipid oxidation in future studies on diabetic subjects. Breath acetone was however significantly higher in T1DM, most likely reflecting the lower suppression of hepatic ketogenesis with subcutaneous exogenous insulin. In T2DM, breath acetone and blood BOHB tended to be lower, and showed only a trend to increase after exercise. This suggests a lesser stimulation of ketogenesis in this subgroup.

This study relied on PIT-MS as a powerful analytical tool for measuring breath acetone. The type of equipment is obviously not adequate for large-scale field studies and self-monitoring by patients. However, simpler breath acetone analyzers, using other recent technological developments (8) are likely to become available in a near future.

In summary, this preliminary study demonstrates robust increases in breath acetone after a prolonged exercise in a small group of subjects including healthy, T1DM, and T2DM subjects. We therefore propose that breath acetone may reflect instantaneous lipid oxidation. Our study however did not control for several confounding factors, such as diet, energy intensity, effects of medications, and changes in blood pH. Further proof-of concept studies, performed in well controlled conditions will be needed to evaluate its validity as a marker of lipid oxidation and energy balance. O

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