Validation of the doubly-labelled water method to determine total energy expenditure

Anita Eberl[1], Simon Schwingenschuh[1], Albrecht Leis[2], Christoph Magnes[1]

CONTACT

[1]JOANNEUM RESEARCH
Forschungsgesellschaft mbH

HEALTH
Institute for Biomedicine
and Health Sciences

Anita Eberl

Neue Stiftingtalstraße 2
8010 Graz, Austria

Phone +43 316 876-42 09

anita.eberl@joanneum.at

health@joanneum.at
www.joanneum.at/health

[2]JR-AquaConSol GmbH

Steyrergasse 21
8010 Graz, Austria

Introduction

Doubly labelled water (DLW) consists of hydrogen (D-deuterium) and oxygen (^{18}O) isotopes and is commonly used to measure total energy expenditure in animals and humans.

After a bolus dose of DLW, hydrogen leaves the body primarily via water turnover, whereas oxygen is depleted both via water turnover and via CO_2-exhalation. CO_2-production rate is estimated using the excess disappearance rate of ^{18}O relative to deuterium and thus serves as an indirect measure of **total energy expenditure**.

This study aimed to implement DLW measurements for a correlation of total energy expenditure with CO_2 production measured in metabolic cage experiments. Mass (IR-MS) and laser spectrometry were used to determine ^{18}O and deuterium-enrichments.

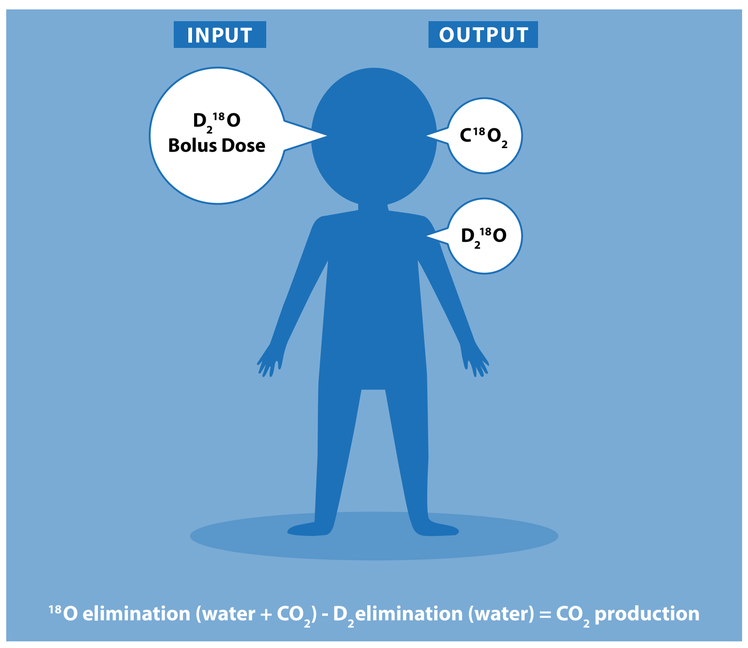


Figure 1: *Doubly-labelled water method*

Methods

Plasma samples were collected 5 days after intraperitoneal injection of a mixture of $H_2{}^{18}O$ and D_2O in rats housed in metabolic cages. Sample preparation methods were assessed in urine and in low-volume rat plasma samples (spiked to three enrichment levels) by testing a simple dilution method as well as 10, 30 and 50 kDa cut-off membranes.

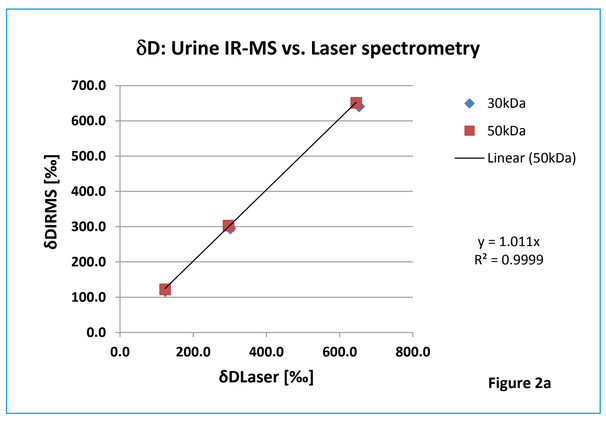
Mass spectrometry measurement (DI-IRMS) was carried out without sample preparation for plasma samples as the gas phase was used and hence a sample preparation is not essential. For laser spectrometry (WS-CRDS) low-volume plasma samples were pre-treated with ultrafiltration and results were compared to IR-MS measurements.

Rate constants and pool sizes were calculated using the slopes and intercepts of the log-transformed laser spectrometric data to calculate CO_2-production.

Results

A first initial correlation of spectrometric CO_2-production and metabolic cage data was not accurate enough with only 50% of the rats showing %diff of ≤ 15%, most likely due to the dilution sample preparation method.

Subsequently, ultrafiltration sample preparation yielded reliable correlation data. Independent of the used membrane (10, 30 and 50 kDa), mass spectrometry and laser spectrometry data were well correlated for three enrichment levels which cover the expected enrichment range in our typical study setups, indicating ultrafiltration as an efficient sample preparation method for low-volume (10 – 100 µl) plasma samples.



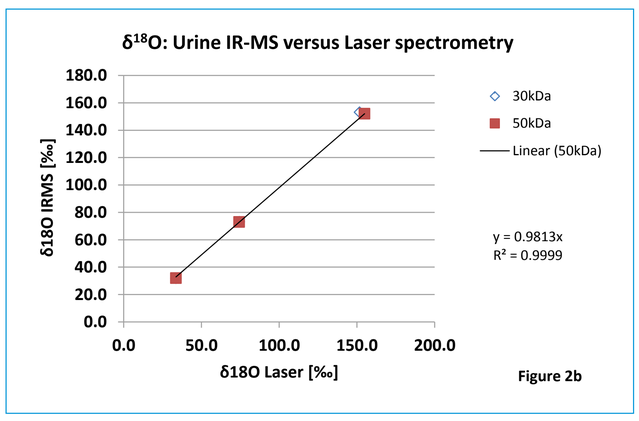


Figure 2a, 2b: *Urine samples - Correlation coefficients were > 0.9999 for both isotopes and 30kDa and 50 kDa membrane cut-offs. The slopes are almost at 1, with deviations of maximum ±0.02.*

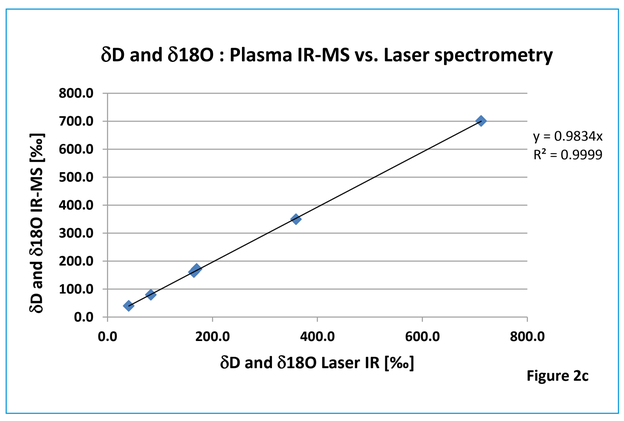


Figure 2c: *Plasma IR-MS without sample preparation and laser-IR with ultrafiltration correlated well very for both isotopes.*

Conclusion

We successfully established a sample preparation method for low-volume plasma samples. The DLW method can thus be used for reliable energy expenditure measurements covering typical observation times of one or more weeks.

The method is also a promising tool to assess the effect of routine activities or exercise on total energy expenditure in a home environment in obese patients.

The knowledge about total energy expenditure is the basis for the fight against obesity which is still one of the major health challenges with a global increase.

Acknowledgement

This work was supported financially by BMVIT

Federal Ministry
Transport, Innovation
and Technology