Calopy – An Advanced Framework for the Integration and Analysis of Indirect Calorimetry Data

Stefan Loipfinger[1,2], Matthias Grosholz[3], Santhosh Kumar[2], Helin Erbilir[2], Kenneth Allen Dyar[4,5], Timo Dirk Müller[5,6,7], Stephan Grein[8], Jan Rozman[9], Martin Klingenspor[10.11], Carola Meyer[12], Dominik Lutter[2,5,13]

[1] Department of Medical Oncology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

[2] Computational Discovery Research Group. Institute for Diabetes and Obesity, Helmholtz Munich, Germany

[3] Free Contributor

[4] Metabolic Physiology, Institute for Diabetes and Cancer, Helmholtz Diabetes Center, Helmholtz Munich

[5] German Center for Diabetes Research (DZD), Neuherberg, Germany

[6] Institute for Diabetes and Obesity, Helmholtz Zentrum München, Neuherberg, Germany

[7] Walther Straub Institute of Pharmacology and Toxicology, Ludwig-Maximilians-University, Munich, Germany

[8] Life and Medical Sciences (LIMES) Institute and Bonn Center for Mathematical Life Sciences, Rheinische Friedrich-Wilhelms-Universität Bonn, Germany

[9] Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, Esch-sur-Alzette, Luxembourg

[10] Chair for Molecular Nutritional Medicine, TUM School of Life Sciences, Research Department of Molecular Life Sciences, Technical University of Munich, Freising, Germany

[11] EKFZ—Else Kröner Fresenius Center for Nutritional Medicine, Technical University of Munich, Germany

[12] Institute of Pharmacology, University of Marburg, Germany

[13] Corresponding Author: Dominik Lutter, dominik.lutter@helmholtz-munich.de

Here, we introduce Calopy, an innovative software suite for the intuitive and comprehensive analysis of indirect calorimetry (IC) data. Calopy is an open-source, web-based Shiny for Python application, accessible online or locally, platform-independent, and available via any web browser at https://www.calopy.app.

IC is a widely used technique for measuring energy metabolism in both humans and animals[1]. At its core, IC tracks oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) to analyze energy metabolism through parameters like energy expenditure and resting metabolic rate (RMR)[2] or respiratory exchange ratio (RER), a key indicator of substrate utilization[3]. In comprehensive animal systems IC systems, additional relevant metabolic parameters such as locomotor activity, food and water intake, and body temperature can additionally be measured at high temporal resolution, along with environmental factors like cage temperature, lighting and humidity. Continuous or categorical phenotypic variables, such as body weight, genotype, and treatments, are also recorded. Noteworthy IC data may further be affected through factors that entail mutual dependencies like circadian rhythms, food intake, environmental conditions, and data noise which adds further complexity[4]. Together, these data form a complex set of time-resolved, continuous, and categorical variables, demanding sophisticated statistics and methods for their analysis.

Over the years, the research community has developed several tools to analyze and interpret IC data with CalR being the most prominent[5]. However, despite the widespread use of IC and standardized software, flexible and accessible open-source tools for easy use and integration of advanced statistical methods to help harness the full potential of IC data remain lacking [6-9].

Calopy addresses this gap by providing an open-source, transparent, and reliable platform for IC data analysis with an intuitive reactive and easy-to-use interface (Fig1a). In contrast to other software, Calopy offers advanced preprocessing tools, including outlier detection and removal, removal of individual subjects/animals ensuring robust and reproducible results. In addition, Calopy provides a unique optional filtering for time-resolved variables (Fig1b) allowing users to extract meaningful data features that are robust against noise, such as global and daily maxima and minima, amplitude, area under the curve (AUC), and more. Additionally, Calopy is unique in offering a global and time-resolved estimation method for RMR and basic metabolic rate (BMR) based on a linear model incorporating activity and food intake, as introduced by Klinken et al.[10] (Fig1c-e). All settings in Calopy are non-destructive and can be reset or changed at any time, and all data created and analyzed can be downloaded for further use.

Building on these data processing methods, Calopy includes a comprehensive suite of exploratory data analysis tools, enabling statistics on both raw data and estimated features while incorporating all types of metadata. This allows for an in-depth exploration of IC data through Between-Group Comparison (Fig1f-h), Temporal-Condition Analysis (Fig1i), and Time-Window Comparison (Fig1j). A comprehensive user guide can be found in the Supplementary Information, with the most up-to-date version available through Calopy Help section.

In summary, Calopy assists the scientific community in easily performing state-of-the-art and extended IC data analysis. Calopy provides a flexible and robust framework to handle and analyze IC data which can be easily extended with novel features and methods as they are implemented by us or the community.

Code availability

All source code is available from the Gitlab repository: https://gitlab.com/computational-discovery-research/calopy

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Author Contributions

SL, MG, SK, HE and DL wrote the code and designed the software. KAD, JR, CM and TDM contributed data and helped design the application. JR, MK, SG and CM helped design the application. DL wrote the manuscript and conducted the whole project.

Conflict of interest

TDM holds stocks from Eli Lilly, and receives research funding from Novo Nordisk. TDM further received speaking fees within the last 3 years from Merck, AstraZeneca, Boehringer Ingelheim, Eli Lilly and Novo Nordisk. All remaining authors declare no competing interests.

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Figure 1. Indirect calorimetry data analysis with Calopy. a) Overview of Calopy's data analysis framework, from data handling to processing and exploratory analysis. b) Filter based feature extraction. Various filter can be applied and fitted to the metabolic variables for extraction of additional data features such as maxima, minima, amplitude and more. c-e) Data analysis examples. Estimation of resting metabolic rate (RMR) in an individual mouse, by removing locomotor activity related energy expenditure (EE) from total EE (c). Locomotor activity counts (d) are used to train a regression model to predict EE (e). The intercept (β_0) can be used as a basic measure of individual RMR whereas the regression coefficient is used to create a time resolved estimation of the RMR (c). For details see Calopy documentation. f-h) Between group comparison of the respiratory exchange ratio (RER) as a metabolic variable. Shown are group wise mean and standard error of the mean for two inbred mouse strains C57Bl6n and C57Bl6j (n = 6 mice per group). Global median is compared for day, night and total (g). Comparing 24h mean EE between two genotypes using an ANCOVA model (h). i-j) Temporal-Condition comparison of oxygen consumption (VO_2) for three temperature conditions between a wildtype (WT) and a knockout (KO) mouse model (n = 16 mice per genotype). A mixed effect model is applied to test for differences in VO2 (j).

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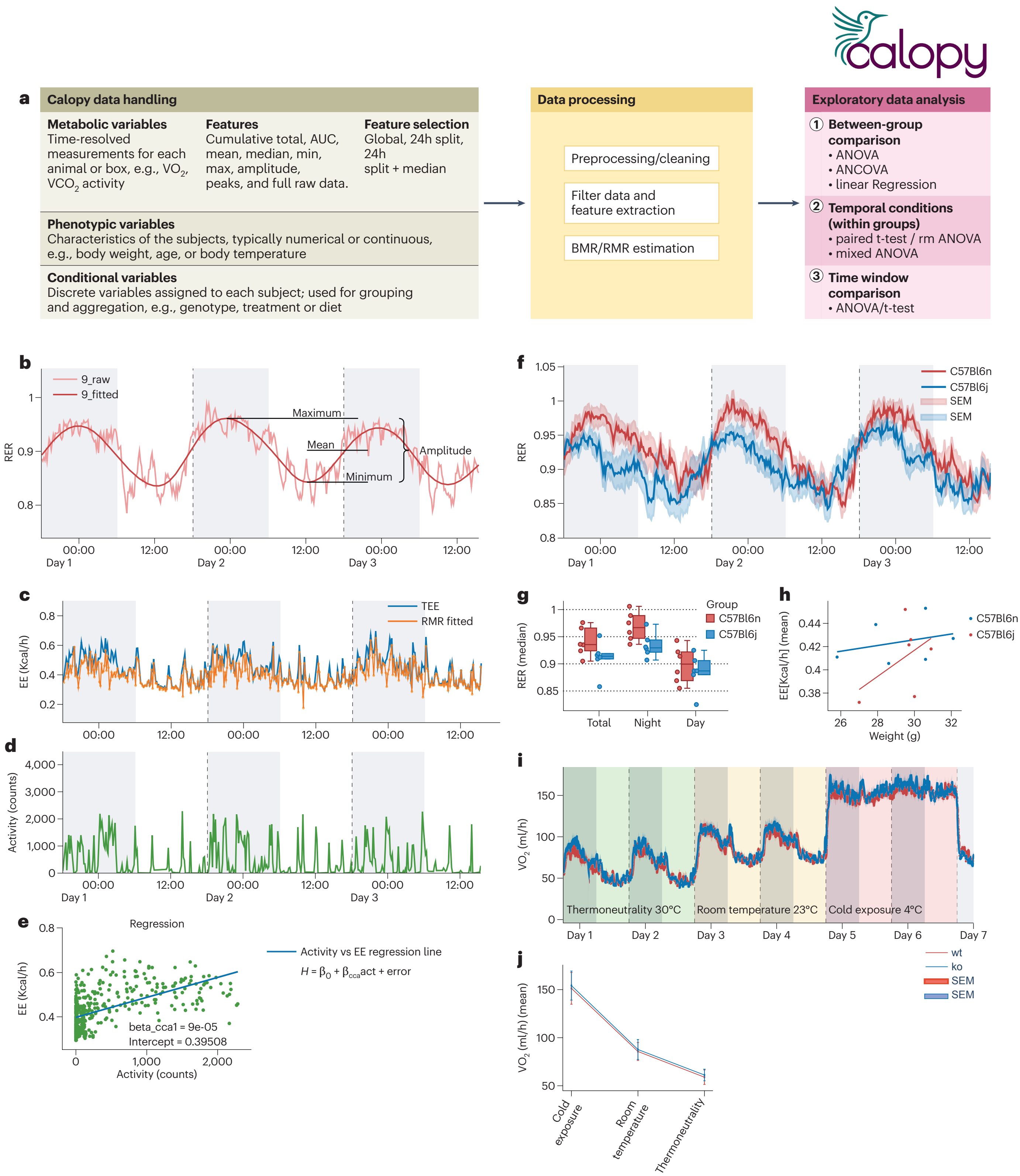

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