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Calopy — an advanced framework for the integration and analysis of indirect calorimetry data

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

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

Over the years, the research community has developed several tools to analyse and interpret indirect calorimetry data, with CalR being the most prominent[5]. However, despite the widespread use of indirect calorimetry 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 indirect calorimetry data remain lacking[6–9].

Calopy addresses this gap by providing an open-source, transparent and reliable platform for indirect calorimetry data analysis with an intuitive, reactive and easy-to-use interface (Fig. 1a). In contrast to other software, Calopy offers advanced preprocessing tools, including outlier detection and removal, and removal of data for individual subjects, ensuring robust and reproducible results. In addition, Calopy provides unique optional filtering for time-resolved variables (Fig. 1b), enabling 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. Calopy is unique in offering a global and time-resolved estimation method for RMR and basal metabolic rate (BMR) that is based on a linear model incorporating activity and food intake, as introduced by Klinken et al.[10] (Fig. 1c–e). All settings in Calopy are non-destructive and can be reset or changed at any time; all data created and analysed can be downloaded for further use.

Building on these data processing methods, Calopy includes a comprehensive suite of exploratory data analysis tools, enabling statistics to be performed on both raw data and estimated features while incorporating all types of metadata. This allows in-depth exploration of indirect calorimetry data through between-group comparison (Fig. 1f–h), temporal-condition analysis (Fig. 1i) and time-window comparison (Fig. 1j). A comprehensive user guide can be found in the Supplementary Information, with the most up-to-date version available through the Calopy Help section.

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

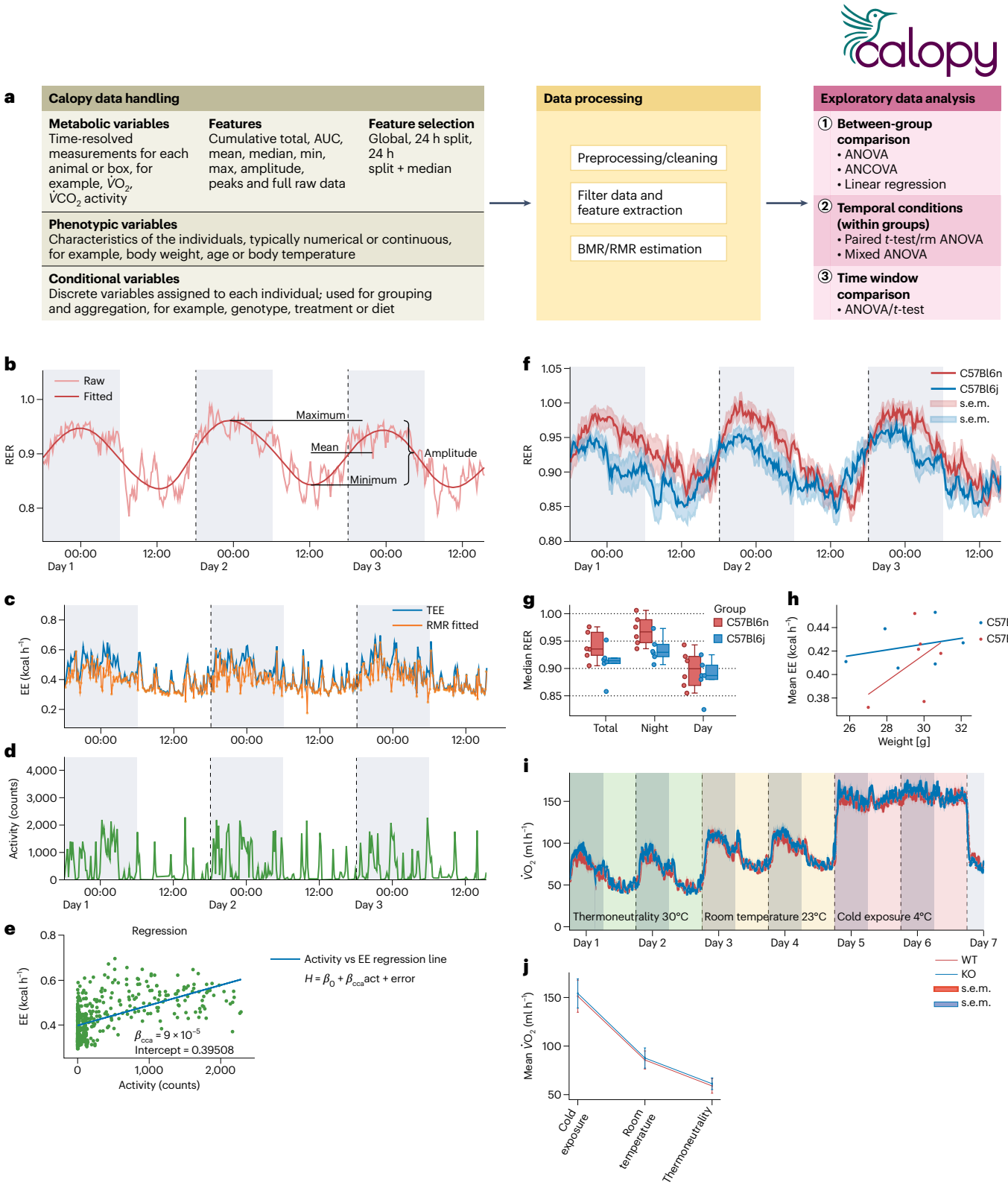
Code availability

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

Stefan Loipfinger[1,2], Matthias Grosholz[13], Santhosh Kumar[2], Helin Erbilir[2], Kenneth Allen Dyar[3,4], Timo Dirk Müller[4,5,6], Stephan Grein[7,8], Jan Rozman[9], Martin Klingenspor[10,11], Carola Meyer[12] & Dominik Lutter[2,4]

[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, Neuherberg, Germany. [3]Metabolic Physiology, Institute for Diabetes and Cancer, Helmholtz Diabetes Center, Helmholtz Munich, Neuherberg, Germany. [4]German Center for Diabetes Research (DZD), Neuherberg,

Fig. 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 filters 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 RMR in an individual mouse by removing locomotor activity-related energy expenditure (EE) from total energy expenditure (TEE) (**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**). See Calopy documentation for details. **f**–**h**, Between-group comparison of the RER as a metabolic variable. Shown are group-wise mean and s.e.m. for two inbred mouse strains, C57Bl6n and C57Bl6j ($n = 6$ mice per group) (**f**). Global median is compared for day, night and total (**g**). Comparison of 24-h mean EE between two genotypes using an ANCOVA model (**h**). **i**,**j**, Temporal-condition comparison of oxygen consumption ($\dot{V}O_2$) for three temperature conditions between a wild-type (WT) and a knockout (KO) mouse model ($n = 16$ mice per genotype). A mixed effect model is applied to test for differences in $\dot{V}O_2$ (**j**).


a
Calopy data handling
Metabolic variables
Time-resolved measurements for each animal or box, for example, V̇O2, V̇CO2 activity
Features
Cumulative total, AUC, mean, median, min, max, amplitude, peaks and full raw data
Feature selection
Global, 24 h split, 24 h split + median
Phenotypic variables
Characteristics of the individuals, typically numerical or continuous, for example, body weight, age or body temperature
Conditional variables
Discrete variables assigned to each individual; used for grouping and aggregation, for example, genotype, treatment or diet
Data processing
Preprocessing/cleaning
Filter data and feature extraction
BMR/RMR estimation
Exploratory data analysis
① Between-group comparison
• ANOVA
• ANCOVA
• Linear regression
② Temporal conditions (within groups)
• Paired t-test/rm ANOVA
• Mixed ANOVA
③ Time window comparison
• ANOVA/t-test
b
Raw
Fitted
Maximum
Mean
Minimum
Amplitude
RER
Day 1
Day 2
Day 3
c
TEE
RMR fitted
EE (kcal h⁻¹)
d
Activity (counts)
e
Regression
Activity vs EE regression line
H = β0 + βccaact + error
βcca = 9 × 10⁻⁵
Intercept = 0.39508
f
C57Bl6n
C57Bl6j
s.e.m.
g
Median RER
Total
Night
Day
h
Mean EE (kcal h⁻¹)
Weight [g]
i
V̇O2 (ml h⁻¹)
Thermoneutrality 30°C
Room temperature 23°C
Cold exposure 4°C
j
WT
KO
Mean V̇O2 (ml h⁻¹)
Cold exposure
Room temperature
Thermoneutrality

Germany. [5]Institute for Diabetes and Obesity, Helmholtz Zentrum München, Neuherberg, Germany. [6]Walther Straub Institute of Pharmacology and Toxicology, Ludwig Maximilian University, Munich, Germany. [7]Life and Medical Sciences (LIMES) Institute, Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn, Germany. [8]Bonn Center for Mathematical Life Sciences, Rheinische Friedrich-Wilhelms-Universität Bonn, 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]Else Kröner-Fresenius Center for Nutritional Medicine, Technical University of Munich, Freising, Germany. [12]Institute of Pharmacology, University of Marburg, Marburg, Germany. [13]Unaffiliated: Matthias Grosholz.
✉e-mail: dominik.lutter@helmholtz-munich.de

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Author contributions

S.L., M.G., S.K., H.E. and D.L. wrote the code and designed the software. K.A.D., J.R., C.M. and T.D.M. contributed data and helped design the application. J.R., M.K., S.G. and C.M. helped design the application. D.L. wrote the manuscript and conducted the whole project.

Competing interests

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

Additional information

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