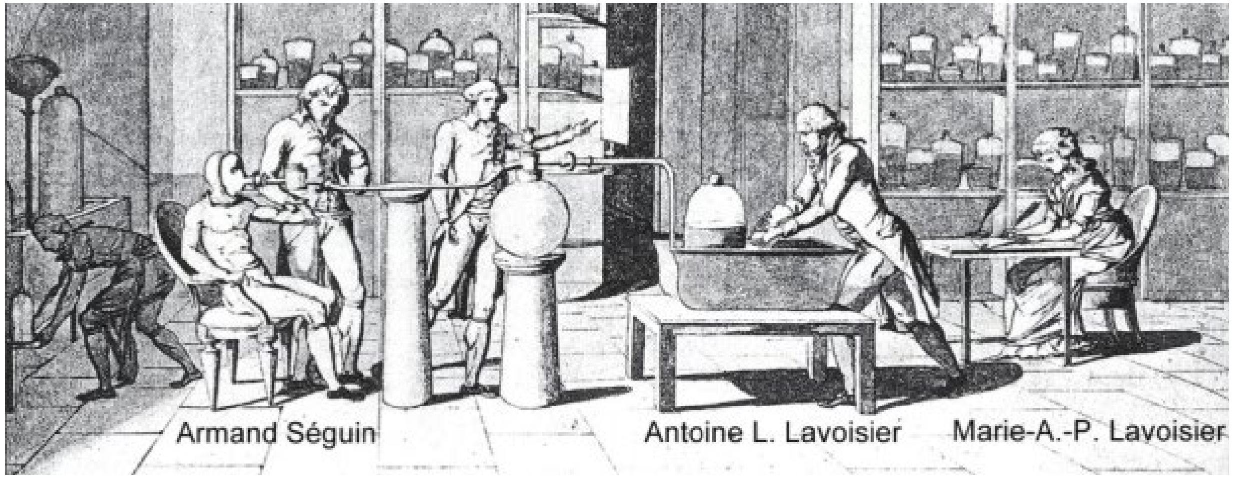
National Institute of Diabetes and Digestive and Kidney Diseases

International Standards and Data Sharing in Obesity and Energy Balance Research



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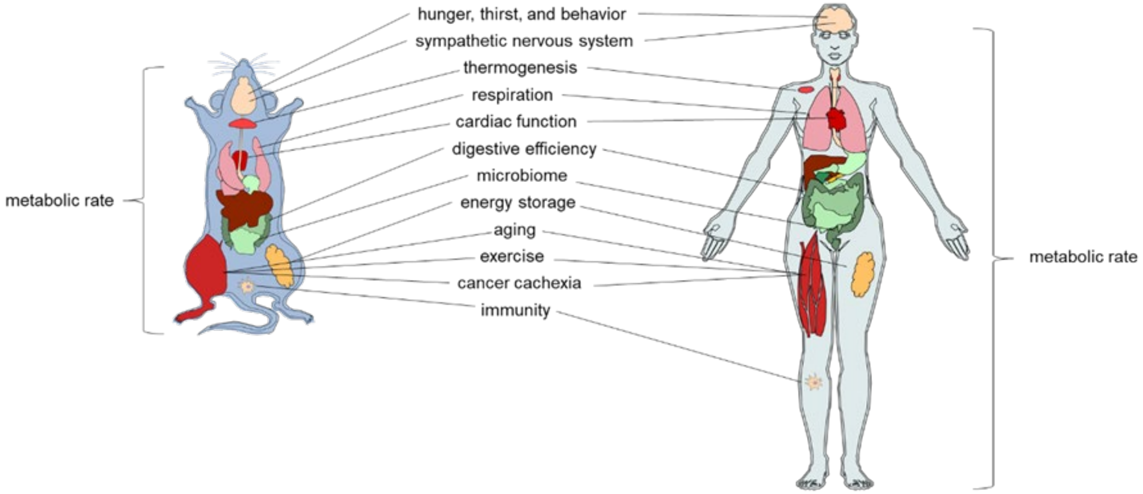
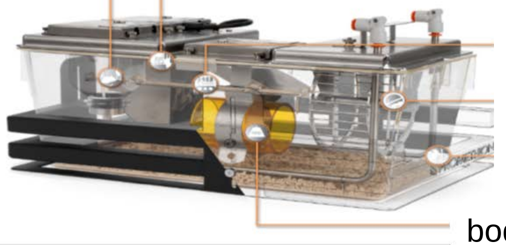
HARVARD MEDICAL SCHOOL

Many uses for preclinical indirect calorimetry





What are the experimental uses for indirect calorimetry?

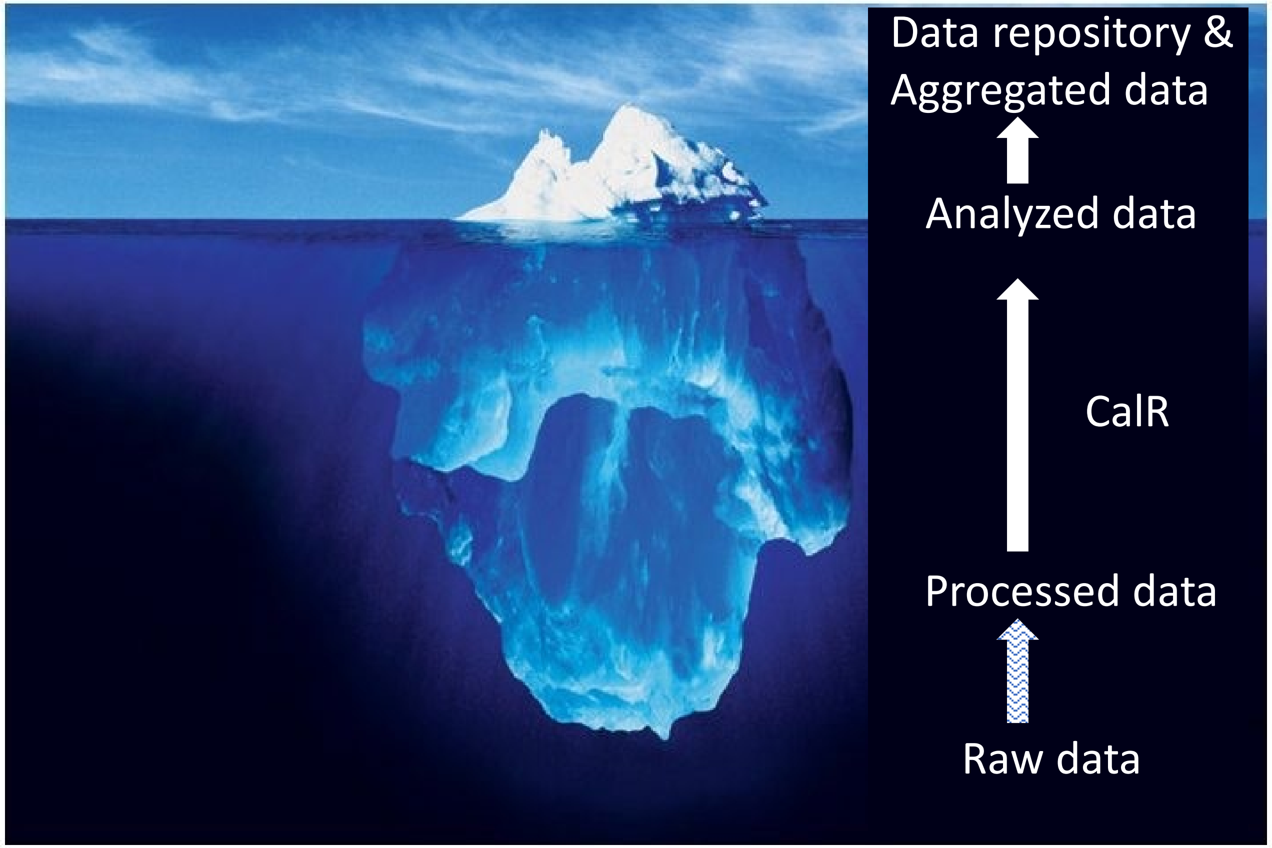
- Aging-related changes in metabolism
- Altered behaviors
- Bone disorders
- **Brain circuits to study feeding and dietary patterns**
- Cancer cachexia
- Cardiopulmonary disorders
- **Control of thermogenesis through activation of the sympathetic nervous system and brown adipose tissue**
- **Energy storage and release**
- Exercise and physical activity
- Inflammation and states of altered immunity
- Muscular dystrophies
- Neurological disorders
- Nutrition, digestion, and the microbiome
- **Obesity**
- Sleep patterns
- Surgical interventions
- Torpor
- Toxicology

<u>The simplified Weir equation to estimate energy expenditure</u>

Energy Expenditure (kcal/hr) = 0.06 x 3.94 VO_2 (ml/min) + 1.106 VCO_2 (ml/min)

JB Weir J Physiol 1949

The World's Metabolic Physiology Data

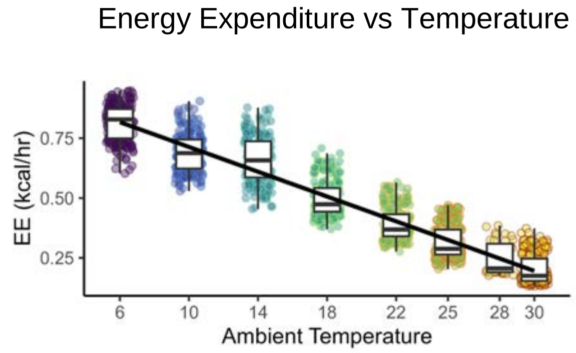


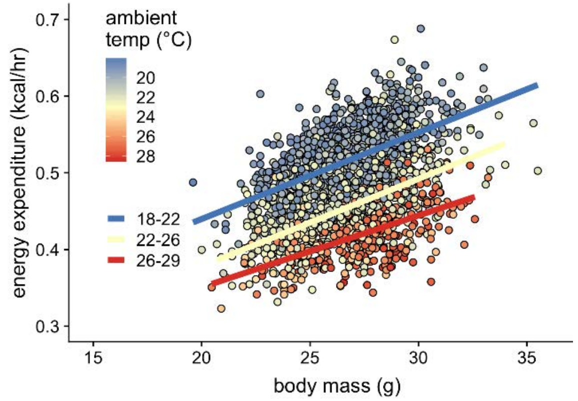
Large-scale trends,
pathway analysis,
fundamental insights

Data visualizations,
quality control visualizations,
group means, SEM,
Analysis of statistical significance

Calculated values
e.g., energy expenditure or
Respiratory exchange ratio

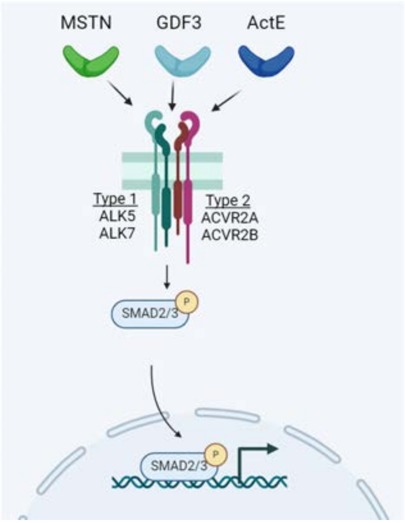
Sensor values





No fixed standard for reporting metabolic rates

What are the “right” or “wrong” ways to report data?



Activin receptor signaling in adipose tissue.

- Ligands include Myostatin (MSTN), GDF3, and Activin E (ActE)
- Receptors include ALK5, ALK7, ACVR2A, and ACVR2B
- Transcription factors SMAD2 and SMAD3

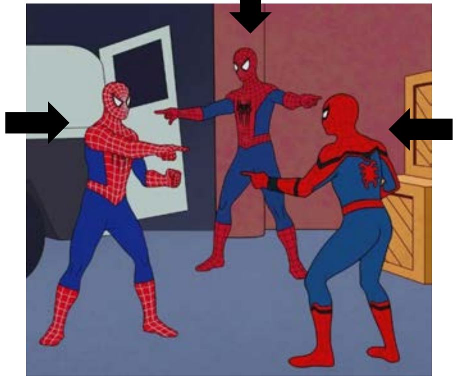
Table 1: studies, effects and units in analysis of activin receptor signaling in mice

Metabolic rate units	Metabolic rate phenotype	Body weight	Food Intake	Genetic modification	GoF LoF	Factor	Reference
mL/kg/min	↑VO_2	↓	= g/day	Alb-*Inhbe* OE	G	Ligand	Hashimoto 2018
mL/kg/min	↓VO_2	↑	= g/day	Fc-ACVR2B	L	Receptor	Koncarevic 2012
mL/kg^{lean}/h	↑VO_2	↓	= g/g/h	aP2-*Mstn* OE	G	Ligand	Feldman 2011
mL/kg^{lean}/h	↑VO_2	↓	= kJ/g/day	*Smad3* $^{-/-}$	L	TF	Yadav 2011
mL/kg/h	↓VO_2*	↑	= g/g	*Mstn* $^{-/-}$	L	Ligand	McPherron 2002
mL/kg/h	↑VO_2	=	↓ g/12h	*Mstn* $^{-/-}$	L	Ligand	Wilkes 2009
mL/kg/h	↑VO_2	↓	ND	*Gdf3* $^{-/-}$	L	Ligand	Shen 2009
mL/kg/h	↑VO_2	↓	ND	^{A} *Acvr1c* $^{-/-}$	L	Receptor	Yogosawa 2013
mL/kg/h	↑VO_2	↓	ND	*Acvr1c* I195T	L	Receptor	Tangseefa 2024
mL/kg/h	=VO_2	↓	ND	*Acvr1c* I482V	L	Receptor	Tangseefa 2024
mL/kg/h	↑VO_2	↓	ND	*Smad2/3* $^{fl/fl}$::*Adipoq*^{Cre}	L	TF	Kumari 2021
mL/kg/12 h	↑VO_2	↓	= g/day	ACVR1C mAb	L	Receptor	Zhao 2023
L/kg/h	↑VO_2	=	= g/day	*Acvr1c* $^{fl/fl}$::*Adipoq*^{CreERT2}	L	Receptor	Srivastava 2021
L/kg/day	↑VO_2	↓	= g/day	*Acvr1c* $^{-/-}$	L	Receptor	Guo 2014
L/kg/day	↑VO_2	↓	= g/day	*Acvr1c* $^{fl/fl}$::*aP2*^{Cre}	L	Receptor	Guo 2014
mL/h	↑VO_2*	↑	↑ g/day	*Mstn* $^{-/-}$	L	Ligand	McPherron 2002
mL/h	↑VO_2	=	= g/day	*Mstn* $^{-/-}$	L	Ligand	Choi 2011
mL/h	↓VO_2	↑	ND	*Mstn* $^{\Delta UCP1}$	L	Ligand	Wang 2024
mL/h	=VO_2	↑	↑ kcal/day	Fc-ACVR2B	L	Receptor	Akpan 2010
mL/2 h	↑VO_2	↓	= g/day	Alb-*Inhbe* OE	G	Ligand	Hashimoto 2018

Oxygen consumption, VO_2; kg refers to total body mass in kilograms unless otherwise specified. Gain-of-function GoF; Loss-of-function LoF, TF, transcription factor; ND, not determined or not reported; ^{A} TSOD vs T.B-Nidd5/3 is a model of *Acvr1c* deficiency.

Funding bodies

Academics



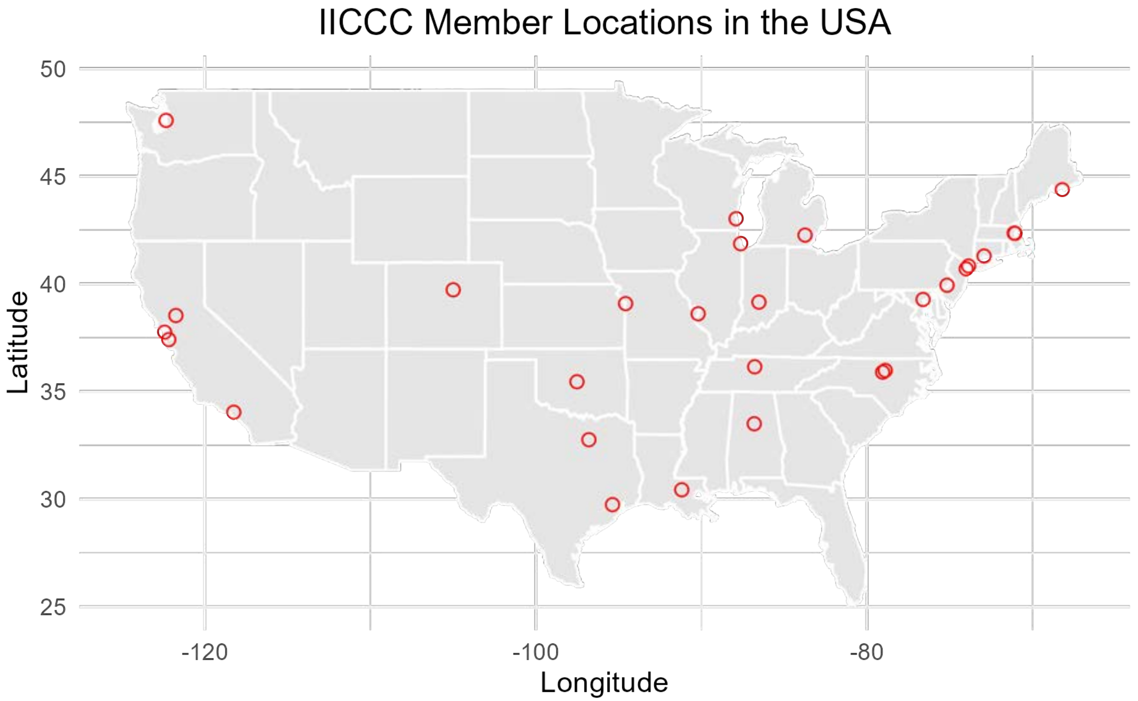
Journal editors

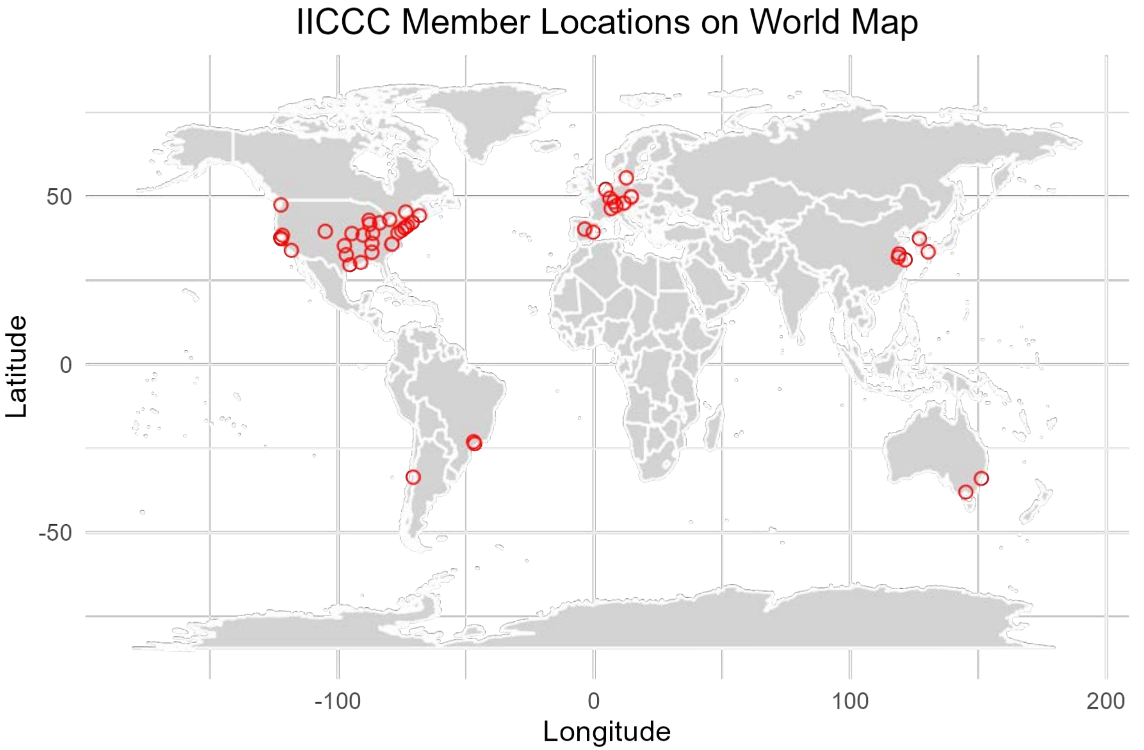
Who is responsible for establishing standards?

International Indirect Calorimetry Consensus Consortium (IICCC) Academic Members (n=79)

David B. Allison, Thierry Alquier, Ansarullah, Steven N. Austad, Johan Auwerx, **Julio E. Ayala**, Joseph A. Baur, Stefania Carobbio, Gary A. Churchill, Morten Dall, Rafael de Cabo, Jose Donato Jr., Nathalia R. V. Dragano, Carol F. Elias, Anthony W. Ferrante Jr., Brian N. Finck, Jose E. Galgani, Zachary Gerhart-Hines, Laurie J. Goodyear, Justin L. Grobe, Rana K. Gupta, Kirk M. Habegger, Sean M. Hartig, Andrea L. Hevener, Steven B. Heymsfield, Corey D. Holman, Martin Hrabě de Angelis, David E. James, Lawrence Kazak, Jae Bum Kim, Martin Klingenspor, Xingxing Kong, Sander Kooijman, Louise Lantier, K.C. Kent Lloyd, James C. Lo, Irfan J. Lodhi, Paul S. MacLean, Owen P. McGuinness, Gema Medina-Gómez, Raghavendra G. Mirmira, Christopher D. Morrison, Gregory J. Morton, Timo D. Müller, Yoshihiro Ogawa, David Pajuelo-Reguera, Matthew J. Potthoff, Nathan Qi, Marc L. Reitman, Patrick C.N. Rensen, Jan Rozman, Jennifer M. Rutkowsky, Kei Sakamoto, Philipp E. Scherer, Gary J. Schwartz, Radislav Sedlacek, Mohammed Selloum, Saame Raza Shaikh, Chen Shuai, Gerald I. Shulman, Vojtěch Škop, Alexander A. Soukas, John R. Speakman, Bruce M. Spiegelman, Gregory R. Steinberg, Katrin J. Svensson, John P. Thyfault, Tony Tiganis, Paul M. Titchenell, Nigel Turner, Licio A. Velloso, Antonio Vidal-Puig, Christopher S. Ward, Ashley S. Williams, Christian Wolfrum, Allison W. Xu, Ying Xu, Juleen R. Zierath,

Alexander S. Banks*



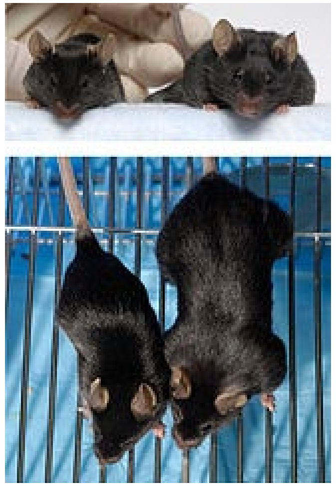


44 USA-based investigators:
All Mouse Metabolic Research Centers (MMPC)
All Diabetes Research Centers (DRC)
All Nutrition Obesity Research Centers (NORC)

35 non-USA investigators

Energy balance in myostatin (*Mstn*) KO mice

Mstn$^{+/+}$ *Mstn*$^{-/-}$



Abstract: "Here we show that Mstn-null mice have a significant reduction in fat accumulation with increasing age compared with wild-type littermates, even in the setting of **normal food intake** (relative to body weight), normal body temperature, and a slightly **decreased metabolic rate**."

McPherron & Lee 2002

Mstn	**VO_2 (ml/kg/hr)**	**Food Intake (g/g BW)**	**Body weight**
Mstn$^{+/+}$	2707 ± 52	0.17 ± 0.01	28.0± 3.0
Mstn$^{-/-}$	↓ 2134 ± 57 ***	↔ 0.16 ± 0.01 (ns)	↑ 36.2± 4.0

"wrong"

Mstn	**VO_2 (ml/hr)**	**Food Intake (g/day)**
Mstn$^{+/+}$	71.4 ± 1.7	5.0 ± 0.2
Mstn$^{-/-}$	↑ 81.4 ± 1.8 **	↑ 5.8 ± 0.2 *

"right"

How do we fix this?

Establish global standards for pre-clinical and clinical indirect calorimetry measurements.

- Data formats
- Analysis methods
- Graphical representation

Future goals: Create a large-scale data repository for aggregation and curation of indirect calorimetry data: a resource for the community

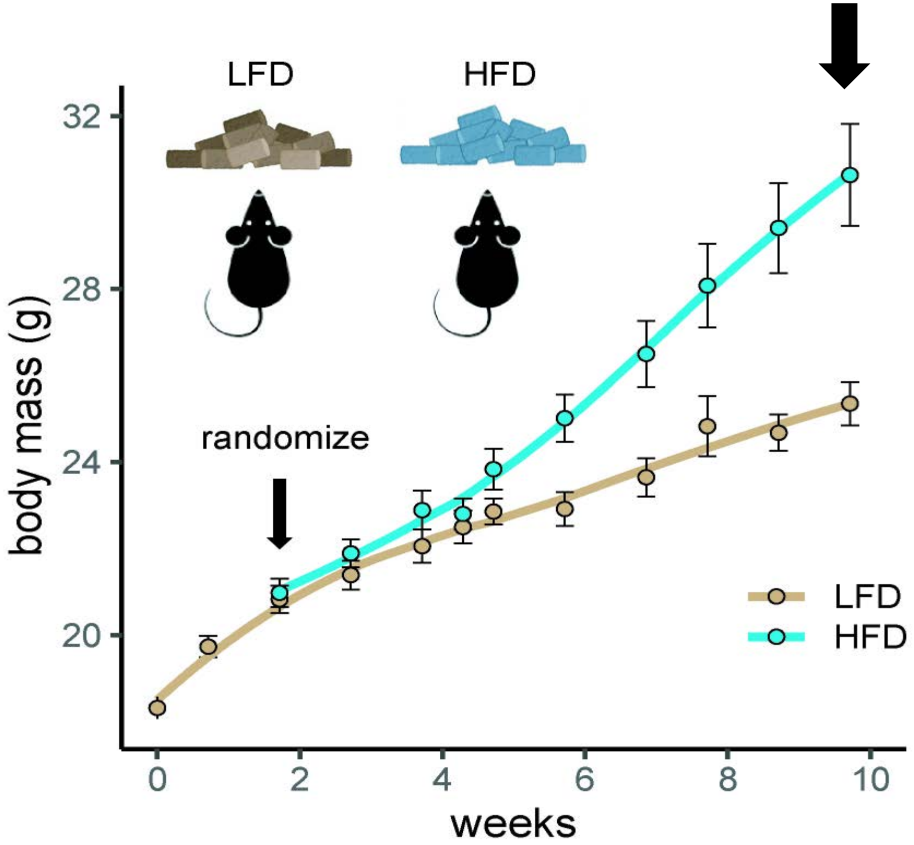
Proposed Standardized Units

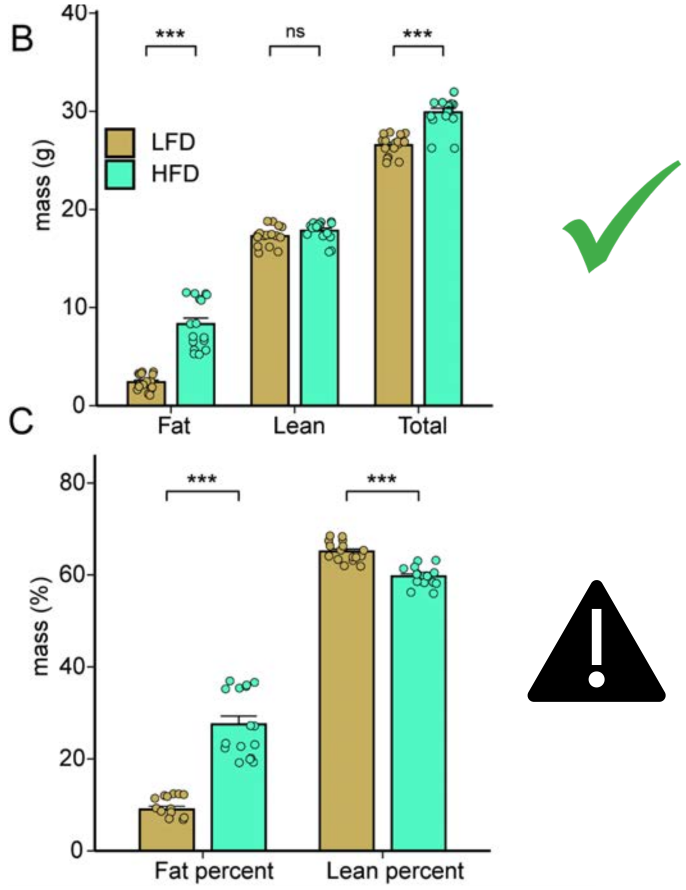
Metabolic Parameter	Standard Units	Non-standard units
Oxygen Consumption (VO_2) Carbon Dioxide Production (VCO_2)	**ml/hr** ✓	ml/min ml/kg/hr $ml/kg^{0.75}/hr$ $ml/kg^{2/3}/hr$ $ml/kg^{lean}/hr$ ✗
Energy Expenditure (EE)	**kcal/hr**	kcal/hr/kg Watts (J/s) kJ/hr
Energy Intake (EI)	**kcal/hr**	g
Water Intake (WI)	ml/hr	g/hr
Respiratory exchange ratio (RER)	(no units)	-
Physical activity (PA)	meters	beam breaks

These standards are less critical once a data repository exists and can convert available data files

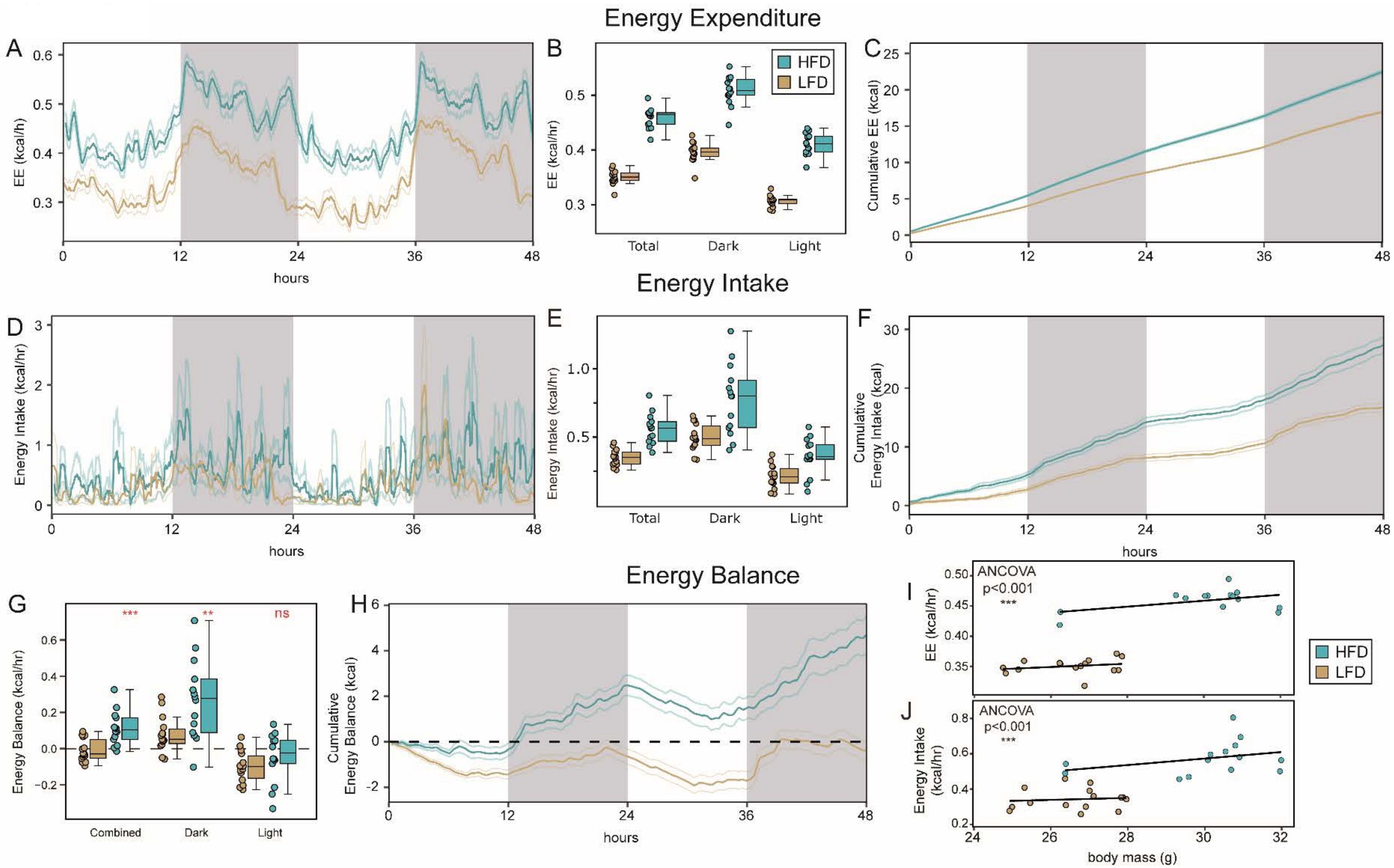
Standard visualizations of body composition in diet-induced obesity
Example: Weight gain—Low fat diet vs High fat diet

Why do mice on HFD become obese?

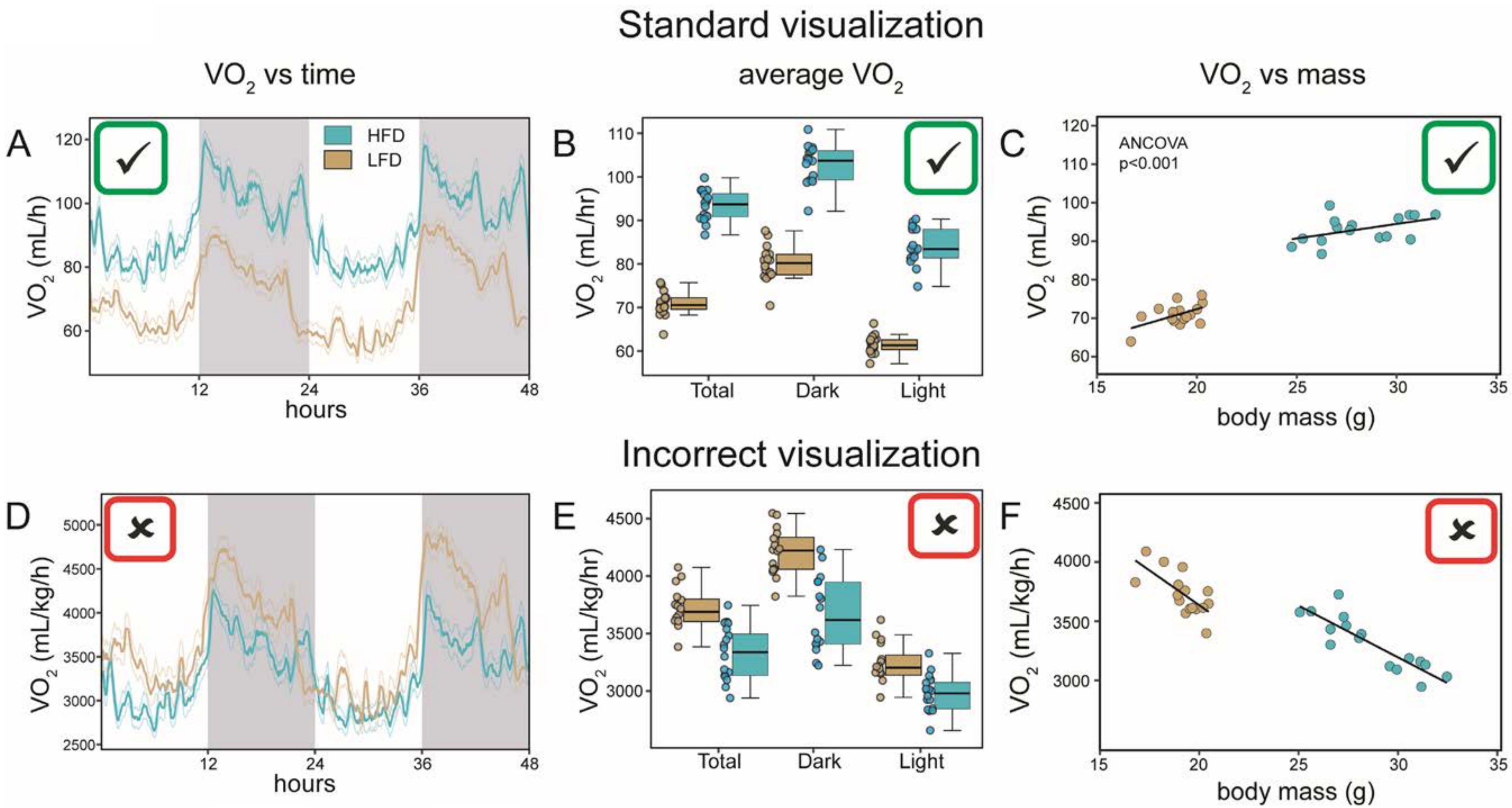




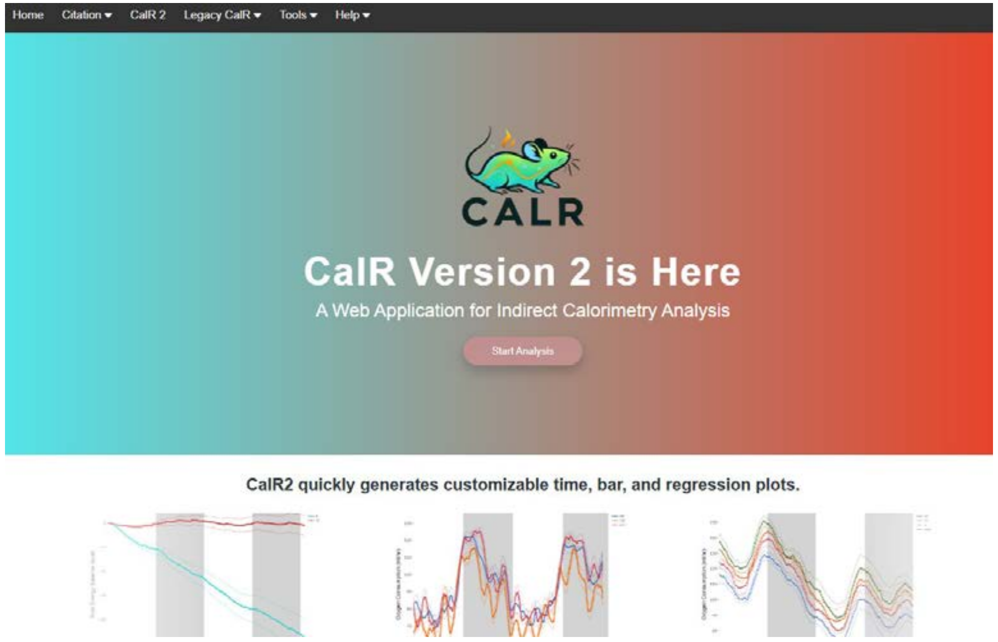
Visualizing Indirect Calorimetry Experiments

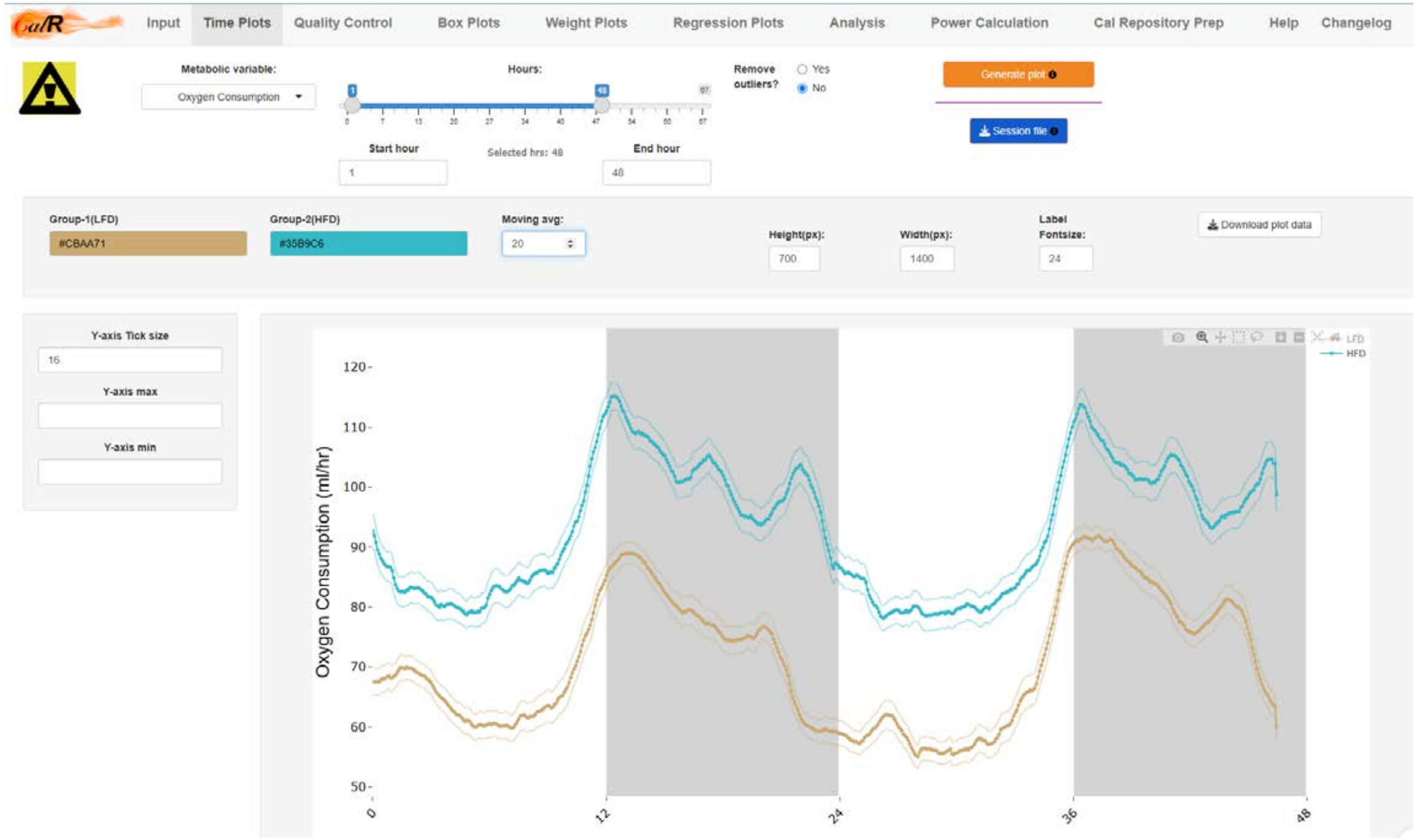


Correct and incorrect data visualizations



Creating data standards
The CalR analysis portal is the starting point



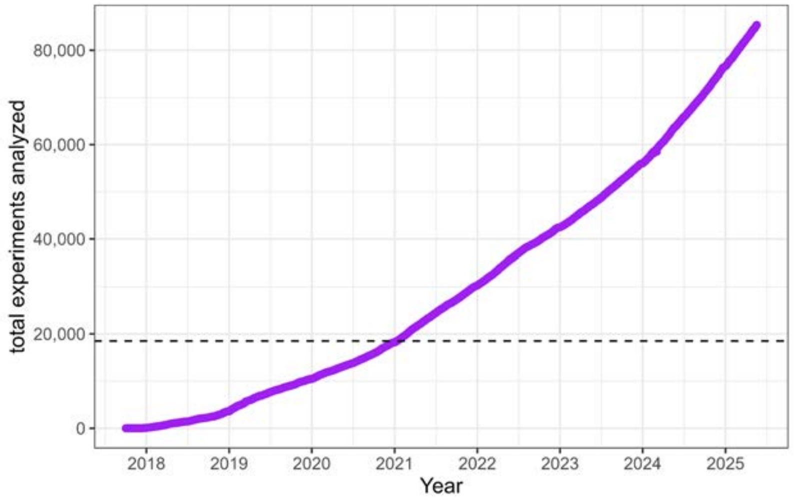


CalRapp.org

CalR 1.0 launched Aug 2017
CalR 2.0 launched Nov 2024

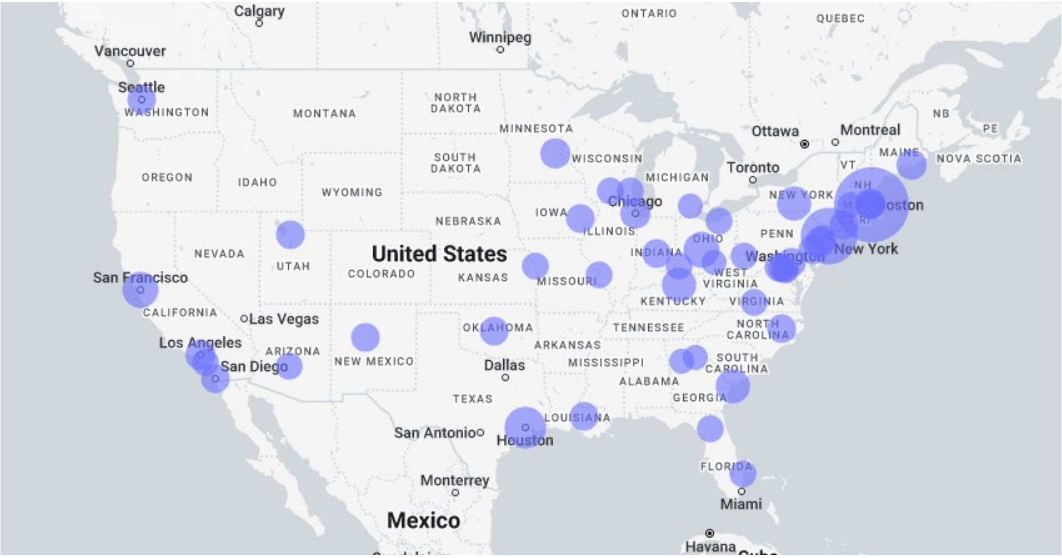
Summary of the CalR Project:
A statistical tool for metabolic scientists

of CalR experiments analyzed



Simplify the analysis of energy balance experiments using indirect calorimetry

- Establish standards for data, metadata, and analysis
  - Pre-define statistical approach and cutoffs/ remove statistical decisions from the hands of end users
  - Increase replicability, transparency, and rigor
  - Read and standardize data from all manufacturers of indirect calorimetry systems
  - Clean data sets (they are large and noisy)
- Promote collaboration and sharing of data with a standardized file format & compatibility
- Anonymous (no data is stored other than accession time and city)
- Limitations: cannot accommodate all experimental designs



Snapshot of CalR users by US location. 30-day accession history April 21-May 20, 2025, illustrating the impact of this tool across the US biomedical research landscape. The size of the circle represents the number of distinct IP addresses per site.

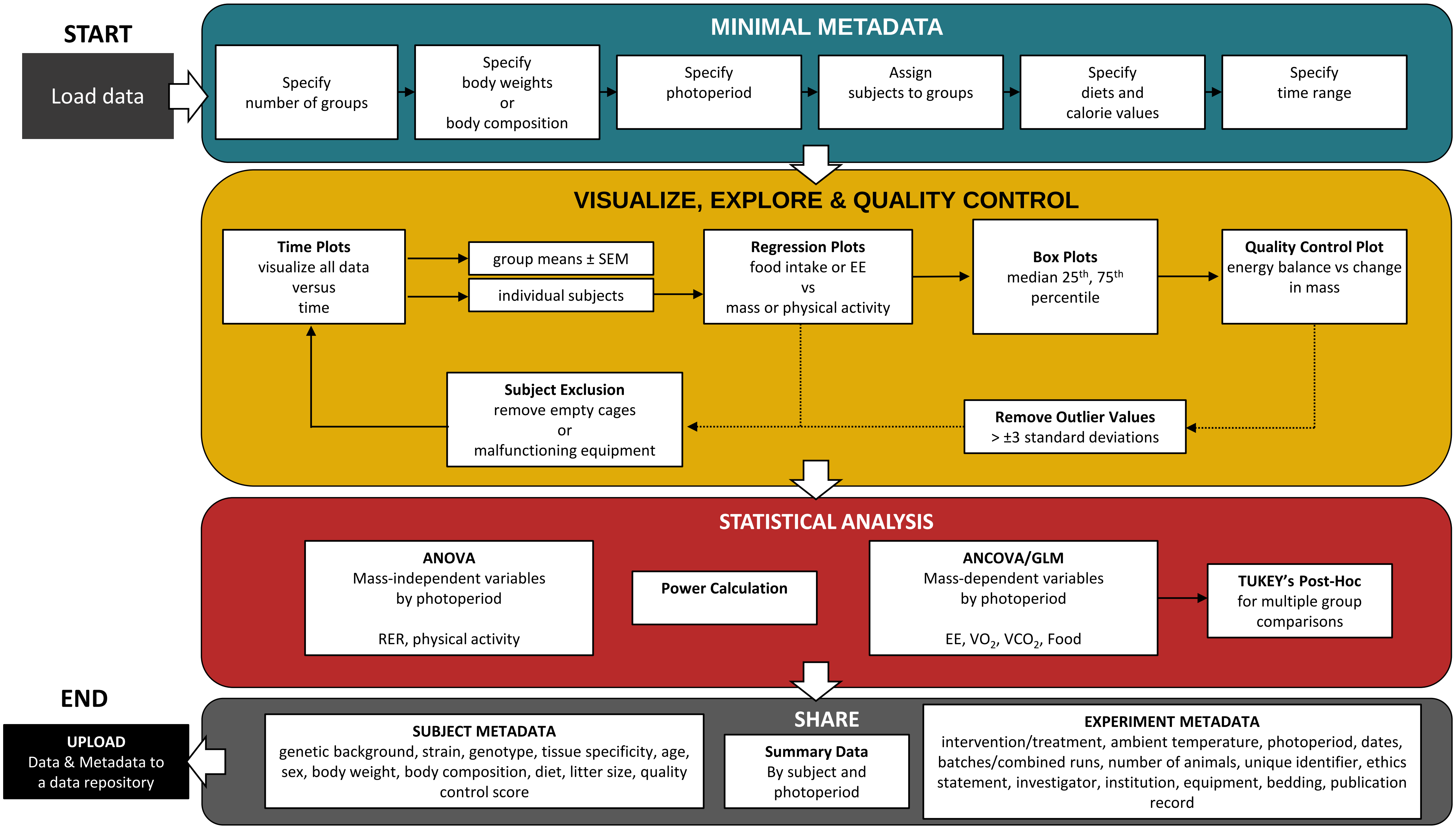
CalRapp.org/

A free web tool where investigators can upload their raw data, perform quality control, perform the appropriate linear regression statistical analysis, and generate publication-quality graphs and tables.

Metadata for data sharing

Animal information	Experimental Information
species	intervention/treatment
genetic background	ambient temperature
strain & genotype	photoperiod
tissue specificity	dates
age	batches/combined runs
sex	number of animals
body weight at start	unique identifier
body weight at end	ethics statement
body composition at start	investigator
body composition at end	Institution/location
diet	equipment
litter size	bedding
-	EE calculation method

Experimental Analysis with CalR



The solution to creating data standards for preclinical indirect calorimetric measurements of metabolic rate, food intake, and activity

- Accomplishments
  - Community-driven, large, multinational agreement of common standards: Creation of the International Indirect Calorimetry Consensus Consortium (IICCC)
  - Consensus paper is in revision at a high-visibility journal
- Realizations
  - Standards are not fixed and will evolve over time
- Downsides
  - Many, many emails
  - Global committees will have meetings in inconvenient times for some members
- Upsides
  - Finally getting everyone in agreement about how to analyze these experiments has cleared the path to data aggregation and creation of a bespoke data repository