Challenges in tackling energy expenditure as obesity therapy: From preclinical models to clinical application

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ABSTRACT

Background: A chronic imbalance of energy intake and energy expenditure results in excess fat storage. The obesity often caused by this overweight is detrimental to the health of millions of people. Understanding both sides of the energy balance equation and their counter-regulatory mechanisms is critical to the development of effective therapies to treat this epidemic.

Scope of review: Behaviors surrounding ingestion have been reviewed extensively. This review focuses more specifically on energy expenditure regarding bodyweight control, with a particular emphasis on the organs and attractive metabolic processes known to reduce bodyweight. Moreover, previous and current attempts at anti-obesity strategies focusing on energy expenditure are highlighted. Precise measurements of energy expenditure, which consist of cellular, animal, and human models, as well as measurements of their translatability, are required to provide the most effective therapies.

Major conclusions: A precise understanding of the components surrounding energy expenditure, including tailored approaches based on genetic, biomarker, or physical characteristics, must be integrated into future anti-obesity treatments. Further comprehensive investigations are required to define suitable treatments, especially because the complex nature of the human perspective remains poorly understood.

Keywords Obesity; Energy homeostasis; Energy expenditure; Methodology; Clinical translatability

1. PROLOGUE

Recidivistic weight gain is one critical complication in the treatment of obesity. Potent counter-regulatory mechanisms where changes in energy intake (EI) or energy expenditure (EE) act reciprocally lead to the failure of dieting and exercise as a treatment option for overweight and obesity. Understanding the mechanisms of these complex regulations to ensure a balanced energy homeostasis is therefore critical in developing more effective, persistent, and safe therapeutic interventions. Thus far, therapeutic approaches aiming at increasing EE have either failed to demonstrate efficacy or have been associated with severe side effects, including altered heart function or muscle wasting [1,2]. Therefore, the safe increase of EE remains critical for the development of future therapies. In this perspective review, we highlight key strategies and challenges that emerge when interfering with EE as a therapeutic goal.

In greater detail, we review the current knowledge about the regulation of energy homeostasis and possible attempts for modulation. Further highlighted are the major organs involved in this process, which are the brain, muscle, adipose tissue, and liver [3], as well as metabolic processes that are targeted to achieve meaningful changes in bodyweight and/or body fat. The pathophysiology of obesity is the result of an imbalance between EI and EE where a decrease in EE, one of the contributors to a positive energy balance, promotes this process. We further discuss past and present attempts at therapeutic strategies for the treatment of obesity as well as possible compensatory mechanisms that might contribute to the occurrence of bodyweight regain [5–7]. In addition, the technical challenges preventing accurate and precise measurements of EE in cells, disease-related animal models (typically rodents), and humans are discussed. Furthermore, approaches toward monitoring organ-specific activity using specific biomarkers can serve as an important basis for developing tissue-based therapies. Finally, it is imperative to investigate all mechanisms affecting EE and to figure out how interventions targeting EE can be translated from cellular systems to animal models and humans in order to ensure that they can be applied as therapeutics. Because of

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2. ENERGY HOMEOSTASIS AND BODY WEIGHT CONTROL

2.1. Regulation of energy homeostasis and control of EE

Maintenance of energy homeostasis (balance) and body weight requires a balance of EI and EE. Total EE (TEE) is made up of the sum of calories that an organism burns per day, and is composed of five major components: 1) resting metabolic rate (RMR), 2) thermic effect of food (TEF), 3) activity related EE (AEE), 4) adaptive thermogenesis, and 5) growth [10–12] (Figure 1). While EI varies greatly between different meals, changes in RMR are rather long-term. Increases in body mass cause an increase in RMR (due to increased tissue mass), TEF (due to greater food intake), and AEE (due to the increased cost of moving a larger body). Decreases in body mass result in opposite changes. In addition to these “passive” modifications of EE, there are “active” or “regulatory” components of EE changes, called adaptive thermogenesis, that occur due to feedback from the altered body mass. It comprises changes in resting and non-RMR, amounts to 10–15% of TEE, and poses a substantial limitation on changes in bodyweight [13]. TEF has been proposed to buffer the effect of abnormally large EI [14]. However, this appears counterintuitive from an evolutionary point of view [15], as evolution preserves traits ensuring reproductive success. A long-term substantial increase in AEE decreases RMR [16] and is associated with an increase in muscle efficiency (lower increase in AEE over time) and food intake without a major net change in TEE [17].

The composition of dietary macronutrients in the form of carbohydrates, fat, and protein further affects EE as well as energy homeostasis with a constant dietary energy content [18]. The synthesis and breakdown of proteins and carbohydrates are associated with changes in levels of water and extracellular fluids. Carbohydrates also affect extracellular fluid by influencing renal sodium excretion. Protein is built up from amino acids and is not “stored” like carbohydrates or triglycerides in higher-order structures that serve as a macronutrient, which requires more energy than the synthesis of glycogen or triglycerides. Low-carbohydrate diets can therefore increase TEE [19,20], but the initially beneficial effects on the body’s weight and composition appear to be relatively minor due to the long-term weight regain caused by rapid physiological adaptations [21,22]. Diet-induced changes in EE depend on the processing of the ingested nutrients and their partitioning into tissues and/or their mobilization [23].

A regulatory feedback loop between central and peripheral regions controls EE and EI. The brain receives long-term feedback signals from adipose tissue and lean body mass as well as information about short-term nutrient availability in relation to individual meals [24,25]. This also involves neuroendocrine signaling [26]. The integration of these signals operates to maintain the body’s weight and composition by modulating energy homeostasis [27,28].

The fat mass increases and lean mass decreases often associated with aging are observed even under conditions when no change in bodyweight occurs. Changes in RMR and macronutrient oxidation are potential causes for the observed changes in body composition [29]. In a large study, RMR varied between 1344.7 and 1927.49 kcal/day in males and between 1277.83 and 1542.94 kcal/day in females [30]. In addition to age, gender, and body size/composition, hormonal status and physical activity explain a large part of these differences in RMR among individuals. Of course, there is also a substantial genetic component [31] that is associated with the variations in individual RMR (at similar bodyweight and composition), TEF, and AEE.

2.2. Dissecting thermogenic contributions of key organs

To understand energy homeostasis on a body-wide level, it is important to dissect the thermogenic effects of different tissues as well as their degree of contribution. Therefore, in addition to body mass index (BMI), bodyweight, and height, it is also important to investigate the thermogenic activity and capacity of individual organs and tissues when estimating resting energy expenditure (REE). Values are available for specific organ-tissue oxygen consumption or energy flux rates, which is important to establish the basis of observed differences in energy requirements between individuals as well as between groups such as young, old, lean, and obese [3,32,33].
Specific organs, such as the adipose tissue (~5% EE), skeletal muscle (~20% EE), brain (~20% EE), liver (~17% EE), heart (~11% EE), and kidneys (~6% EE), are the greatest contributors to the basal metabolic rate (BMR) [3,33–35]. Skeletal muscle and adipose tissue, despite their lower metabolic rate per gram of organ weight compared to the liver, kidney, heart, or brain, are relevant to the whole-body EE due to their larger organ mass (Figure 2), with their contribution being even further elevated upon exposure to exercise and cold [2,36,37]. Therefore, it is important to consider the biochemical pathways that determine the metabolic rate of individual tissues with a larger size. Advances in organ-tissue prediction models to better assess human EE have been reviewed by Heymsfield et al. [38].

Clear differences are observed during cold-induced thermogenesis when measuring adipose tissue metabolic activity in brown, beige, and white adipocytes, indicating the importance of tissue localization [3]. Increasing the amount of thermogenic brown or beige adipocytes in humans is associated with a substantial contribution to whole-body EE and an improvement in metabolic parameters [39–44]. In addition, skeletal muscle is an important organ for thermogenesis due to its ability to induce energy-expending futile cycles [45,46]. During mild cold exposure, the relative contribution of heat-generating organs such as skeletal muscles can increase to account for at least 40% of EE [47]. A more detailed review on the molecular pathways of both thermogenic organs was published by Betz and Enerbäck [48]. Finally, a recent review highlights the importance of liver energy metabolism, especially in the context of metabolic associated fatty liver disease.

Based on preclinical studies performed with different small molecule mitochondrial uncouplers, the authors propose increasing EE via mitochondrial uncoupling specifically in the liver as an attractive therapeutic approach to treat metabolic associated fatty liver disease, non-alcoholic steatohepatitis, and type 2 diabetes (T2D) [49]. Knowing the relative thermogenic contribution of these organs and the scope for which their contribution can be modified is important for the identification of key organs or metabolic processes that target meaningful and safe changes in whole-body EE.

2.3. Obesity and EE
A positive energy balance leads to the development of obesity, with low RMR and low non-exercise activity thermogenesis (NEAT) representing two of the risk factors [50]. The lowering of bodyweight results from a negative energy balance and leads to a curve-linear ending in a new steady state as opposed to a continuous one. Thus, a new equilibrium between EI and EE is reached [13,51]. Individual responses to weight loss are thereby variable and appear to be related to the degree of adaptive thermogenesis. The different adaptive thermogenesis responses characterise the so-called thrifty [52] (favoring weight gain) and spendthrifty [13] (favoring weight loss) phenotypes, respectively. Interestingly, recent studies indicate that thriftier individuals have less capacity for cold-induced thermogenesis and that BAT activation seems to be indicative of those individuals’ predisposition to weight gain [53,54].

Adaptive thermogenesis occurs with short- and long-term weight reduction. A maintenance of bodyweight reduction ≥10% is
accompanied by an ~20–25% reduction in TEE [13]. This decrease corresponds with a 300–400 kcal reduction of daily caloric intake to maintain the same bodyweight and physical activity of an individual that did not undergo a weight loss intervention [13]. The difficulties experienced by individuals who reduce their bodyweight and aim to maintain said reduction reflect the potent counter-regulatory mechanisms. Those include adaptations in EI and adaptive thermogenesis, which are both controlled by redundant metabolic, neuroendocrine, and autonomic systems [55].

The adaptive changes in EE and EI associated with weight loss are highly persistent [56] and have been described in various models [57]. The traditional “set-point model” is influenced by control theory thinking and suggests a negative-feedback loop that defends bodyweight at a “pre-defined” level. It is still the prevailing model of bodyweight regulation depicted in textbooks, although it has been disputed for decades and is inconsistent with many experimental findings [5,58]. An alternative model compares bodyweight regulation to water in a lake. In this non-regulated system, the level of the lake (fat stores) passively “settles” at an equilibrium that is determined by the inflow (EI) matching the outflow (EE) [59]. Presumably, bodyweight regulation does not follow either of these models. Bodyweight is actively regulated, but a clear-cut defended bodyweight set point could not be established. Moreover, active compensatory responses in humans mainly occur after reductions in bodyweight and appear to be negligible after increases in bodyweight. The defense mechanism against potentially harmful weight loss is suggested to be more evolutionarily beneficial than the development of a resistance to increases in bodyweight. This theory led to the hypothesis that active compensatory mechanisms would only be activated at a lower and an upper intervention point, with no active regulation in between [59]. Again, the available evidence argues against such a model. For excellent in-depth discussions about these issues, see Geary [5] and Ravussin et al. [6]. Since Kennedy’s lipostatic hypothesis [60], models of bodyweight regulation have mainly focused on fat mass as the main targeted parameter, with the discovery of leptin as a major hormonal signal defending bodyweight loss promoting this “adipocentric” concept of bodyweight regulation. However, there is solid evidence that supports the existence of an independent active regulation of fat-free mass (FFM) in addition to fat mass [61–64]. This is practically relevant because the feedback loop that drives EI to restore FFM may lead to collateral fattening [64].

Most importantly, physiological adaptations that favor weight re-gain are triggered simultaneously with an initial weight loss, which can be achieved by a number of different therapeutic approaches, as mentioned below.

2.4. Anti-obesity strategies and EE

2.4.1. Lifestyle

Lifestyle changes made to reduce bodyweight through dietary restrictions and/or exercise have historically applied the so-called 3,500 kcals rule, i.e., the idea that eating 500 fewer kcals/day yields one pound of fat loss/week. This rule, however, does not account for dynamic changes in energy balance and hence underestimates the change in intake or expenditure required to achieve this loss [65]. Dieting reduces bodyweight more efficiently than exercise, but is accompanied by endocrine changes [66], reductions in EE, and an increased eating drive [65,67], all of which favor weight regain. According to the constrained TEE model proposed by Pontzer et al. [16], the total level of daily EE increases linearly alongside progressive increases in physical activity but eventually plateaus with higher rates of physical activity (>230 activity counts per minute per day). This might be explained by compensatory mechanisms like decreases in REE and increases in muscle efficiency [7], both of which limit the bodyweight lowering efficacy of exercise. Despite improving general health, particularly by reducing cardiovascular risk factors and increasing insulin sensitivity, exercise alone only moderately reduces bodyweight [68,69].

The combination of both dietary restrictions and exercise demonstrated a greater potential for weight loss compared to either of the two interventions alone, achieving and maintaining a weight loss ≥10% after 1 year [70] and ≥5% after 8 years [71]. However, long-term dietary restrictions in combination with exercise are also associated with metabolic adaptations, including a persistent reduction (about 500 kcal/d from 30 weeks to 6 years) in RMR despite substantial weight regain [72].
2.4.2. Bariatric surgery

Bariatric surgery achieves a long-term weight loss of ≥25% after 5 years [73,74], with minor increases of weight after up to 20 years post-surgery [75–77]. Gastric bypass patients showed a larger reduction in FFM and smaller reductions in RMR, plasma leptin, and thyroid hormones than patients who underwent lifestyle intervention. There is a transient decrease in RMR/FFM for 1–2 years after bariatric surgery, as the larger mass of organs with a high–metabolic rate leads to most of the significant weight loss during that time [78]. This indicates that the surgery somehow enhances eating inhibitory signals. The lower small intestinal gut peptides glucagon-like peptide-1 (GLP-1) and peptide tyrosine tyrosine (PYY) are likely candidates in this context, as has been discussed extensively by Holst and colleagues [79]. They are established satiation signals [80], and their release is massively stimulated in response to meals after bariatric surgery. Several lines of evidence [79] indicate that GLP-1 and PYY play a role in the beneficial effects of bariatric surgery on humans, although experiments with pertinent receptor knockout mice appear to indicate that endogenous GLP-1 is not necessary for the reduction in bodyweight that occurs after bariatric surgery in mice [81]. The mechanisms that cause the weight loss after bariatric surgery may therefore differ between humans and rodents. In any case, the weight loss observed following bariatric surgery is a complex phenomenon to which different factors contribute [81].

2.4.3. Pharmacotherapy

Pharmacotherapies directly manipulating EE to lower weight were already attracting attention in the 19th century. A variety of previous attempts at treating obesity have been reviewed in detail by Müller et al. [1] and Chen et al. [2]. Many of them were initially approved for their ability to decrease bodyweight by increasing EE but were withdrawn as safety concerns started to outweigh the pharmacological benefits [82,83]. The mitochondrial uncoupler 2,4-dinitrophenol has been suspended by the Food and Drug Administration, based on substantial side effects such as rashes and cataracts [1,2]. In particular, adverse cardiovascular events observed with the serotonin and noradrenaline reuptake inhibitor Sibutramine outweighed the benefits of weight loss and led to the withdrawal of the drug in 2010 [84]. Similarly, the beta-3 receptor agonist mirabegron leads to an increase in heart rate and blood pressure depending on the dose, despite its effects on increased EE [85].

Currently approved pharmacological interventions for obesity treatment rather support weight loss by decreasing EI through the reduction of food intake or energy absorption. Among those are the lipase inhibitor orlistat [86] or the GLP-1RA semaglutide [87], with GLP-1RA being recognized as the most effective and safe treatment for obesity to date. The EI-lowering principle of GLP-1RA showed clinical benefits not only for weight loss but also for cardiovascular risk reduction, despite an increase in heart rate [87–89], although the discrepancy between an increased heart rate and undesirable cardiovascular effects needs to be evaluated further. These evaluations may alter the popular view of such changes when developing new EE increasing approaches.

In addition, the simultaneous targeting of different biological mechanisms has garnered interest over the last few years. These combination approaches include different EE assets, as well as combining EI and EE to achieve a greater weight loss through either multitarget drugs or the combination of multiple independent therapies. Either way, these mixtures simultaneously target different mechanisms and thereby deliver synergistic or complementary pharmacology to aim for efficacies that are currently only achieved through bariatric surgeries. There are more detailed reviews about the history and benefits of polyagonists targeting the receptors for GLP-1, GIP, and glucagon [1,4,90].

2.5. Considerations on compensatory mechanisms

Regardless of the therapeutic intervention, it is important to identify potential compensatory responses by simultaneously quantifying the major contributing thermogenic processes and organs. For example, daily cold exposure in humans resulted in a nearly two-fold increase in brown adipose tissue (BAT) thermogenesis without eliciting any changes in shivering activity or whole-body heat production [91,92]. This was made possible by shifting the contribution of non-shivering thermogenesis (NST) from skeletal muscle (via proton leak) to BAT [91]. Similarly, several types of metabolically futile cycles may be recruited simultaneously but adapt differentially under varying metabolic conditions.

Our current understanding regarding these and other compensatory mechanisms in humans remain limited. However, the measurement of as many metabolic processes and components of total daily EE as possible remains critical in identifying energy compensations in humans as well as its therapeutic potential to treat or prevent the development of metabolic diseases.

3. MEASURING EE

Assessing EE, including the contributions of single organs, is important to better understand the role of EE in the context of obesity as well as from a drug discovery perspective. Therefore, the use of in vitro systems and preclinical models serves two main purposes: to generate a general mechanistic understanding of physiological and pathophysiological processes, and to determine whether this knowledge can ultimately be translated to influence human biology. The proper regulation of metabolic activity is crucial for cell survival and the healthy function of a tissue, whilst deregulation has been linked to the onset and progression of multiple diseases, such as obesity and metabolic syndrome. As described above, influencing muscle, fat, and/or liver to modulate EE remains an attractive option. Therefore, it is important to correctly assess EE in vitro, ex vivo, or in vivo.

3.1. Preclinical methodologies

3.1.1. Reliable methods to study EE in vitro

To elucidate tissue-specific biology and the pathways that lead to an increased level of EE, the ability to accurately measure increases in oxidative capacity, as well as the activity of the cells themselves, is important. Several methods are currently being used to define oxygen partial pressure, substrate utilization, and thermodynamics.

**Direct measures:** Cellular heat production directly reflects metabolic changes using thermodynamic approaches. Infrared thermography [93,94], microcalorimetry [95,96], and fluorescent thermal sensors [97–102] all offer appropriate physiological read-outs, as they measure real-time fluctuations in thermogenesis. However, these techniques are not frequently used in research as they are time-consuming, often require high cell counts, and do not inform about the substrates utilized. Furthermore, the heat signal is often used to monitor biological processes, but rarely allows for quantitative interpretation [103].

**Indirect measures:** Indirect measures are typically employed to quantitate the heat production-related phenomenon in cells using surrogate readouts, which are mainly respirometry-based. The XF Flux Analyzer (Seahorse Bioscience) has proven to be a workhorse in determining both aerobic and anaerobic metabolism of cultured cells/tissue through real-time measurements of oxygen consumption rate
throughput platform [106–108]. Another way to determine molecular oxygen is the usage of luminescence-based probes. The big advantage of this technology is the simplicity of the measurement procedure as well as the high-sample throughput, with the drawbacks being a lower sensitivity and the inability to measure several compounds [108–112]. The electron paramagnetic resonance spectroscopy is another technology where cells and oxygen sensors are distributed homogenously throughout the samples, enabling the precise detection of low-oxygen concentrations in solution, but it requires a closed and sealed set-up and cannot be used with several compounds [108,113]. Additional considerations, such as cellular ATP demand, signaling events, substrate availability, and utilization, as well as effects on mitochondrial membrane potential, mitochondrial content, and dynamics, must be considered [114–118].

Given the variety of possible methodologies, it is important to choose the most suitable method for assessing EE in vitro. One must consider the necessity of a high throughput for drug discovery in combination with a sensitive and reliable detection, as well as the identification of mechanistic insights. Therefore, the combination of multiple technologies is recommended. In addition to the right technology, it is also important to choose the correct cellular system for mechanistic studies. To achieve this, discrepancies between cell lines and primary cells must be considered in addition to translatable issues between animal models and humans. Immortalized cell lines offer a suitable model to generate valuable data [119]. Nevertheless, some molecules and mechanisms can only be investigated within human primary cells, e.g., certain microRNA (miRNA) and locked nucleic acids. However, humans are outbred, and strong clonal effects may occur with primary human cells. In addition, based on donor variability, it would be difficult to reach the standardization required for reproducibility. Therefore, cell lines provide a more stable model. To increase translation and relevance to the human physiology, primary cells, especially from humans, should still be considered [120]. Testing organoid cultures and ex vivo tissue biopsies for the improvement of the physiological relevance should also be taken into consideration when looking for the most appropriate technology to assess EE in vitro.

Key findings in vitro should be verified for their physiological relevance. Therefore, measuring EE in vivo is invaluable to biological interpretation and is vital for the physiological extrapolation of both health and disease progression. This includes characterization of the factors that influence EE and the dysregulation of energy balance that leads to obesity. Hereby, it is important to note that the organism behaves as an open thermodynamic system and thus the net energy balance is dictated by the EI and EE. In the following paragraph, we want to provide theoretical, practical, and analytical considerations one should be aware of when measuring EE and whole energy homeostasis.

### 3.1.2. EI (food) and metabolized energy

Foremost, it is important to keep in mind that not all food is metabolized. To calculate EI in absolute terms, one must calculate the metabolized energy [121,122]. The total energy of the ingested food and the total unabsorbed energy present in feces is experimentally measured using bomb calorimetry. Metabolized energy, as well as the energy equivalence of individual components, can be calculated using the derivatives of Atwater’s system [122,123].

However, several limitations should be considered for these calculations; they correspond to short-term measurements and assume that the bodyweight remains constant during the experimental period. Should there be a change in bodyweight, it must be accounted for as total energy lost.

In certain pathologies (e.g., diabetes and other kidney diseases), the urinary wastage of the energy could account for a larger proportion. Because the number of cells in the body and gut microbes are approximately equal [124] (with most of them being anaerobes), we must also contemplate the effect of inter-group interventions (such as diets or antibiotics) that could alter the gut microbiome to confound the data.

There is no information available on the significance of micronutrient availability and mobilization in these calculations. This should be considered especially when the environment/genetic intervention is expected to alter the biology of microorganisms.

As mentioned above, TEE is based on five components. In preclinical studies, a segregation of the TEE’s key components is used to generate insights into predominant metabolic pathways, therapeutic prospects, and physiological correlations of specific experimental interventions. There are several ways to experimentally measure TEE and derive its individual components using direct and indirect calorimetry. Besides these validated methodologies, there are additional methods for estimating TEE [125–127] that can be used to cross-validate the findings from indirect calorimetry.

#### 3.1.3. Methodologies to measure EE in vivo

**Direct calorimetry:** All components of TEE (except growth) culminate in heat loss through radiation, convection, evaporation, or conduction [128]. Direct calorimetry gives the most accurate quantitation of heat loss by an organism. However, because of (a) the very high cost, (b) the violable assumption that the entire EI is reflected in the form of heat loss during the measurement period, (c) the lack of information on the type of fuel being oxidized, and (d) the lower segregation capacity of an individual component of TEE, direct calorimetry has largely been replaced by respirometry-based measurements. The direct calorimetry methodologies and instrumentation have evolved considerably over time since Rubner’s first calorimetric measurement was made in 1894 [126]. A detailed description of the variants of the direct calorimeter and its principle are reviewed in detail by Kenny et al. [129], Kiyalya and Ramsay [128], and Webb [130]. Although recent decades have experienced a very limited use of direct calorimetry in preclinical studies, it still remains a gold standard [128].

**Doubly labelled water (DLW):** There is an isotopic equilibrium of oxygen between water and respiratory CO2 when oxygen is re-cycled as H2O or CO2. Hydrogen, in contrast, leaves the body mainly as water. The double-labeled water technique utilizes these differential elimination rates of oxygen and hydrogen (dERO,H) by using heavy isotopes of hydrogen (2H) and oxygen (18O). The dERO,H is based on the principle of indirect calorimetry and is applied to estimate the rate of CO2 production (rCO2) [12,131]. The rCO2 can be used to estimate the metabolic rate [132]. Over the years, the DLW method has been adapted for both preclinical and clinical studies [133,134]. The main advantage of this method is that it can be used within the social context of freely moving animals. A combination of indirect calorimetry and DLW can be used to consolidate the findings in cases where respirometry data is ambiguous.
Indirect calorimetry: The philosophical concept of the fire of life and the important role of oxygen has been around for centuries [135]. Originating in the 18th century, this concept was discovered by Lavoisier and Laplace, who were the first to use an ice calorimeter to measure animal heat [136]. In 1849, Regnault and Reiset performed oxygen consumption measurements on a closed-circuit system and on carbon dioxide absorbers [137]. They concluded that the ratio of CO$_2$ production to oxygen consumption during respiration is a function of the composition of ingested food. Later, Zuntz & Schumburg [138], as well as Lusk [139], established that one mole of molecular oxygen is utilized to oxidize one mole of carbohydrate and that this process liberates one mole of CO$_2$. The ratio of the volume of liberated CO$_2$ to utilized oxygen (VCO$_2$/VO$_2$) was derived and referred to as the respiratory quotient (RQ). The RQ for carbohydrate oxidation was found to be $\sim 1$, while the RQ for fat was $\sim 0.7$. In modern terminology, RQ is reserved for the direct measurement of moles of oxygen consumption and CO$_2$ released at tissue level. In contrast, the respiratory exchange ratio (RER) is calculated from the gas content of breath. Therefore, the favored term for organismal studies is RER, which is a reliable indicator of the fuel type being used and has been widely utilized in metabolic and nutritional research [140]. These findings helped found the starting point for the development of the modern indirect calorimeter, which is presently the most commonly used method for calculating EE. EE and RER are normally calculated from the volume of utilized oxygen and liberated CO$_2$. The estimation of EE is made using Weir’s formulae [141].

3.1.4. Technological advancements in indirect calorimetry

In recent years, there have been several technical advancements in the instrumentation and analysis capacity of commercial indirect calorimeters. One of the most significant developments was the addition of a carbon isotope sensor in-line with regular respiratory analysis. This enables the study of the oxidation of various metabolites with unprecedented convenience and precision. It is now possible to label the specific carbon atoms ($^{13}$C) of metabolites and analyze the rate of $^{13}$CO$_2$ production with a unique speed and accuracy. This leads to excellent in vivo mechanistic studies on the metabolism of exogenous versus endogenous substances and provides novel details via the tracing of specific labelled metabolites and substrates [142]. The second advancement is the extension of the commercial analyzers to integrate the sensor for other gases (e.g. CH$_4$ and H$_2$) along with oxygen and CO$_2$ sensors [142]. This provides a unique opportunity to dissect the effect of dietary intervention in preclinical models and has a huge potential in the field of microbiome and its crosstalk with systemic metabolism.

The third advancement in this field is the size-reduction and sensitivity of telemetry-based implantable sensors. With the availability of implantable telemetric sensors for electrocardiogram, electroencephalogram, electromyogram, tissue temperature, etc., a combination of indirect calorimetry and $^{18}$O$_2$ sensors with tissue-implanted temperature sensors can yield deep insight into the organ-specific thermogenesis along with RER, REE, and the contribution of a specific metabolic pathway through the use of the isotope sensor data.

Measuring thermogenesis: There are several ways to infer the magnitude of thermogenic response (reviewed by Speakman, 2013) [11]. The defense of core body temperature and OCR have been used as surrogates for thermogenic response upon cold exposure. Nonetheless, it is difficult to distinguish the NST from shivering thermogenesis. To measure NST, it is common to acclimatize the animal at thermoneutrality and inject noradrenaline to activate brown adipose NST through a direct stimulation of $\beta$-adrenergic receptors. In ideal cases, however, the NST component should be validated in uncoupling protein 1 (Ucp1) knockout animals by demonstrating the lack of the intervention’s effect on Ucp1 knockout animals [143]. It is also important to consider the history of the animal’s cold exposure, as it alters the NST quite drastically (reviewed by Speakman, 2013) [11].

Tissue level resolution into thermogenesis: It is difficult to precisely assign the expended energy to a certain tissue within the animal’s body. Nonetheless, by using the tissue-implanted telemetric temperature sensors, it is now possible to monitor slight changes in tissue temperature upon cold exposure without systemic interference. The real-time data monitoring advancement is gaining popularity over conventional methods not only due to the precision and resolution but also due to the non-ambiguity of the data.

3.1.5. General considerations for reliable indirect calorimetry analysis

Several excellent reviews have outlined the best practices for the design and execution of calorimetric experiments [144–146]. Most of these guidelines are subjective and vary according to the objective of a particular study. However, certain aspects that must be considered in any preclinical calorimetric experiment are discussed below.

Acclimation, Food intake, EE, and thermogenesis: As rodents are social animals, individual housing constitutes a stressful situation and impacts many important aspects, such as behavior, and often leads to reduced food intake and body weight [147,148]. Therefore, before experimental analysis, animals should be appropriately acclimatized to experimental conditions. It should be noted that changes in the housing conditions may also affect the results of EE measurements in mice. Further detailed studies comparing single-housed with group-housed mice using physiological paradigms will be required to address this point. Besides being caused by stress, changes in food intake may also be the consequence of experimental intervention (genetic, pharmacological, or nutritional). Therefore, one should carefully measure both the EI and EE to accurately attribute the change of increased EE or reduced EI. Another conceivable but often ignored aspect is the contribution of thermogenesis. Most calorimetric studies are performed at an ambient temperature of 22–25 °C [149,150]. These temperatures are below thermoneutral conditions for mice. Therefore, the mice actively regulate body temperature through NST. However, the intervention, even if it does not affect the thermogenesis directly, might alter the rate of thermogenesis. For example, effects on fur and skin might increase heat loss and thereby further increase the metabolic rate [151,152]. In such cases, it is important to carefully delineate the thermogenic contribution. In another scenario, an intervention might affect the BMR, but the calorimetry may not be able to discern the difference due to a compensatory increase in energy consumption for thermogenesis. Therefore, one should thoroughly investigate the origin of difference in EE or the lack thereof.

Body composition and normalization of data: To study a novel genetic model or intervention, it is prudent to first establish baseline data for food intake, bodyweight, and body composition during the anticipated study period. In such a pilot study, it is sensible to first employ a non-invasive body composition analysis (such as magnetic resonance imaging (MRI) based measurement, e.g., echoMRI) and perform endpoint analysis to determine tissue composition. Even if these background data are unavailable, it is recommended to assess the body composition of every experimental animal using non-invasive methods before and after the calorimetric measurement. To normalize the EE data, it is recommended that lean body mass be used. To circumvent the issue that division by lean mass overcompensates for
the mass effect, one should consider data analysis using relevant covariates in an ANCOVA, as it includes the mass of each individual organ separately [144].

Intervention-related alteration in absorption efficiency or gut microbiome: When the intervention is expected to cause considerable change to absorption of macronutrients (e.g., lipase inhibitors treatment) or the intervention causes alterations in the gut microbiome, it is necessary to calculate the metabolized energy of every experimental animal group. The lack of this might cause an artefact in data or could mask the actual effect of intervention. We therefore recommend running a pilot experiment, whenever appropriate, to first estimate the metabolizable energy before performing an indirect calorimeter analysis.

3.2. Clinical methodologies

3.2.1. Measuring whole-body EE in humans

For translational studies in humans, indirect calorimetry enables the non-invasive measurement of EE at a relatively low cost [140]. Current research calorimeters usually use the so-called canopy technique, which measures the amount of CO2 and oxygen in a constant flow of air. This technique is highly precise, with a variation coefficient of less than 2% [153].

Indirect calorimetry is generally confined to measuring EE during limited time periods, i.e., in the range of hours. Whole body calorimeters, which are basically small rooms equipped with exact measurements of airflow and devices to quantify oxygen and CO2 in the exhausted air, can theoretically be used to measure EE over longer periods of time; however, individuals cannot be studied in their usual environment. Mobile indirect calorimeters have also been developed but generally lack the precision of conventional devices and are uncomfortable to wear, thus precluding long-term measurements. A promising alternative to assess free-living EE is the use of DLW [134,154,155]. The levels of 18O and 2H in blood, urine, and exhaled air are measured with isotope ratio mass spectrometry. This method is used to quantify total daily EE, and therefore must be combined with indirect calorimetry and activity trackers to quantify the components that make up this expenditure, including the RMR, TEF exercise activity thermogenesis, and NEAT.

Activity trackers and mobile devices offer the possibility of counting steps and assessing physical activity by means of other sensors, such as GPS tracking. The devices often calculate estimates of EE which has been tested against calorimetry in some instances [156,157]. It should, however, be noted that these algorithms do not offer the accuracy and reliability necessary in the research setting, with R2 being in the range of 0.5—0.6. This is also the case with equations used to derive EE from anthropometric parameters such as the Harris Benedict [158] or Mifflin Formula [159].

3.2.2. Organ-specific metabolism and its contribution to whole-body EE

An important consideration when investigating whole-body EE in humans is understanding the specific metabolic rates of various organs and thermogenic processes. Some attempts have been made to estimate organ metabolic rates in humans and animals using tissue preparations [3,32,33]. However, these tissue preparations often lack the electrical activity, metabolites, or substrates required for their optimal function, thereby limiting their extrapolation to in vivo physiological conditions. More commonly, organ-specific oxygen consumption in vivo has been determined using an iteration of the Fick principle, which combines measurements of regional or organ blood flow with differences in arterial and venous concentrations of oxygen. While this historically required the catheterization of arteries and/or veins across regions encompassing the organ of interest [34,160], advancements in molecular imaging over the past decades have allowed for the metabolic rate and mass of specific organs to be directly quantified non-invasively in vivo [34].

The gold standard for the measurement of organ-specific oxygen consumption requires the use of quantitative positron emission tomography (PET) with 18O-labelled carbon monoxide (18O2), radiolabeled water ([18O]H2O), and inhaled oxygen gas ([18O]O2). This allows for the absolute quantification of organ blood volume, blood flow, and metabolic rate of oxygen, respectively. Other indirect measures of organ metabolic rate have been applied using PET with [11C]acetate, which can quantify tricarboxylic acid cycle turnover (or [11C]CO2 production) [161,162], or 31P-phosphorous-magnetic resonance spectroscopy (31P-MRS) to examine the relative changes in ATP, phosphocreatine, and inorganic phosphate [163,164]. From these direct in vivo measurements combined with measurements of organ weight, the relative contribution to whole-body EE of the most metabolically active tissues can be calculated.

3.3. Brown/beige adipose tissue biomarkers to estimate EE

The standard method for measuring BAT activity is 18F-fluorodeoxyglucose (FDG) uptake using PET combined with computed tomography (PET/CT). This technique was also the basis for the (re) discovery of BAT in adult humans by several groups around the world between 2007 and 2009 [165—169]. However, FDG-PET/CT exposes the patients to ionizing radiation from the 18F-labeled FDG glucose tracer and the PET scan radiation dose. Moreover, it requires exposure to cold in order to activate the BAT thermogenesis necessary to induce uptake of glucose [170]. Cold exposure induces norepinephrine release by the sympathetic nerves not only in BAT, but also in the cardiovascular system, and can therefore be problematic in patients with obesity and concomitant cardiovascular diseases. Finally, PET is generally a high-cost imaging tool and FDG-PET/CT is overall an expensive diagnostic tool requiring dedicated PET/CT scanners [171]. Taken together, new diagnostic tools that allow for the safe (non-radioactive, non-invasive) and affordable assessment of thermogenic adipose tissue activity in humans are required.

Beyond its well-recognized function in energy dissipation, BAT has been recognized as an endocrine organ that synthesizes and secretes different molecules, such as peptides, lipids, metabolites, and microRNA (miRNA), to regulate systemic metabolism [172—174]. Several groups have identified lipid species, referred to as lipokines, that exert signaling effects and act as metabolic messengers in regulating nutrient utilization, thermogenesis, and insulin sensitivity [175]. These lipid species can be detected in serum and tissue by liquid chromatography with tandem mass spectrometry (LC-MS/MS). In both mice and humans, there are marked changes in lipid compositions of adipose tissues and circulation upon a cold challenge. 12,13-dihydroxy-9Z-octadecenoic acid (12,13-dihOME) is one of the cold-induced lipokines found to be produced by BAT [176]. 12,13-dihOME is a metabolite derived from linoleic acid by the sequential enzymatic reactions of cytochrome P450 (CYP450) monooxygenase and soluble epoxide hydrolase. Circulating levels of 12,13-dihOME are significantly higher in human subjects acutely exposed to cold [176] or exercise [177]. 12,13-dihOME acts in an autocrine/paracrine manner to facilitate fatty acid utilization in BAT and reduce plasma triglyceride concentration in obese mice [176]. Besides 12,13-dihOME, cold exposure enhances the enzymatic activity of 12-lipoxygenase (12-LOX) in BAT, leading to elevated circulating levels
of 12-LOX metabolites [178]. The administration of one of these 12-LOX-derived lipids, 12(S)-hydroxyecosapentaenoic acid (12(S)-HEPE), into obese mice significantly improved their glucose tolerance and insulin sensitivity. In humans, circulating levels of 12(S)-HEPE are raised in response to the specific β3-adrenergic agonist mirabegron. Since the plasma levels of 12,13-diHOME and 12-HEPE are positively correlated with BAT activity, as measured by FDG uptake [176,178,179], it is suggested that these lipids could serve as surrogate biomarkers for BAT activities in humans. Recently, Kulterer et al. used [18F]-FDG PET/CT to define the presence or absence of detectable BAT activity and found that the presence of active BAT is associated with cold-induced EE. Their studies also revealed that circulating levels of 12,13-diHOME and 12-HEPE are higher in human subjects with detectable BAT activity compared to those with undetectable BAT activity [179]. The levels of both lipokines are elevated in subjects with active BAT in response to cold exposure, reinforcing the notion that 12,13-diHOME and 12-HEPE are cold-induced, BAT-derived lipokines. Notably, the levels of 12,13-diHOME and 12(S)-HEPE in human plasma correlate negatively with bodyweight and insulin resistance [176,178,180], indicating a potential role of these lipid mediators in modulating human obesity and diabetes.

miRNAs, small non-coding RNAs of approximately 25 nucleotide size, mediate the specific post-transcriptional gene silencing of their target mRNAs by binding to an untranslated region (prominently at 3’) and marking them for degradation [181]. Specific miRNAs could potentially serve as biomarkers. miRNAs are involved in many biological processes, including the regulation of differentiation of brown and beige/white adipocytes [182], with an important example being miR155, which regulates the transcription factor CAAT enhancer binding protein β [183]. Moreover, miRNAs are actively secreted/released by cells and are involved in inter-organ and -cellular communication. Thus, miRNAs can be found in the extracellular space and bodily fluids, including blood [184] and urine. These extracellular miRNAs are either packaged in extracellular vesicles or form protein-miRNA complexes [185]. Extracellular miRNAs have great diagnostic potential for several diseases including cancer [186,187], T2D, and obesity [185]. Adipose tissues have been shown to contribute to body-wide extracellular vesicle secretion [188,189]. Interestingly, brown adipocytes not only release extracellular vesicles but also significantly increase the extracellular vesicle release after stimulation with cAMP, the second messenger that mediates adrenergic- and cold-induced BAT activation [188]. Moreover, cold exposure in mice induces a nine-fold increase in extracellular vesicle release from BAT [188]. BAT-derived extracellular vesicles contain several hundred miRNAs, and the profiling of extracellular vesicles isolated from the serum of cold-exposed or CL-316,243-treated mice revealed that several miRNAs are either up- or downregulated after BAT activation [188]. The levels of one of these exosomal miRNAs, miR92a, in the serum reflected brown fat activity in mice [188]. Importantly, analysis of extracellular vesicles isolated from cold-exposed humans that were either BAT-positive or -negative — as measured by FDG PET/CT — revealed that miR92a levels in human serum inversely correlate with human BAT activity. Thus, exosomal miR-92a represents a potential first serum biomarker for BAT activity in mice and humans. Further studies are required to elucidate whether miR92a or other miRNAs can be used as diagnostic biomarkers for BAT activity in larger cohorts, including patients with obesity or diabetes, as well as in patients with weight loss. The usage of biomarkers could be helpful tools in predicting the success of EE approaches to treat obesity.

4. Clinical Perspective

A substantial proportion of individuals in today’s environment gain significant excess bodyweight during their lifetime. Tailored approaches are necessary to provide precision medicine catered to individual needs. Interactions between genetic, environmental, and lifestyle factors contribute to obesity and must be taken into account during the development of therapies [6,9].

4.1. Patient subpopulations

Genetic factors such as heritability and genetic variants, including epigenetics, play a role in the pathogenesis of obesity [190,191]. While a few causes of monogenic obesity have been identified, only a small amount of the apparent heritability of obesity has been explained until today (reviewed by Xia and Grant) [192]. Because the BMI distribution is very wide, indicating differences in individual responses to a common obesogenic environment, large genome-wide-association studies (GWAS) were performed (recent GWAS obesity reviews [193,194]) to investigate the genetic mechanisms underlying the development of extreme cases of obesity and to improve the understanding of the factors responsible for excessive weight gain in certain populations. A number of genetic loci were identified to be associated with BMI and adiposity traits, with prominent genes near loci enriched for central nervous system expression as well as for fat distribution in adipose tissue itself, confirming that these two tissues are important for bodyweight control. However, current associations explain only a minor percentage (2–3%) of the variation in adult BMI. Hence, the low level of variance in these results clearly calls into question the clinical relevance of GWAS-identified obesity genes [195]. Therefore, a relaunch of future obesity GWAS studies employing more biologically meaningful phenotypes (e.g., EE, in-depth body composition analyses, and longitudinal weight changes) could provide a better mechanistic understanding of how genetics influence bodyweight and could help to design personalized treatments. Moreover, only a few studies on the other side of the BMI range exist, investigating the physiology and genetic architecture of healthy thinness (defined as BMI < 18 in the absence of disease). In a recent publication, the heritability of thinness was found to be comparable with that of severe obesity [196]. The loci identified overlapped with those already identified in association with obesity. However, novel loci were also identified and could be studied in more detail to improve the understanding of the physiology of habitual leanness. Interestingly, a distinct molecular signature of adipose tissue indicating a potential role of adipose tissue and EE in human thinness was suggested in another recent study [197]. This demonstrates that obesity and leanness being opposite phenotypes provide interesting starting points for assessing causal gene variations related to bodyweight and composition as well as understanding differences between individuals and identifying subpopulations.

4.2. Environmental factors and lifestyle

Environmental changes such as a sedentary lifestyle may further explain the mean weight gain observed in a population [198,199]. However, despite said environmental changes as well as the physiological adaptations outlined above, about 25% of any given population can maintain a stable bodyweight overtime without adhering to caloric restriction or excessive exercise [200,201]. Conversely, various large studies show that the lower rates of occupational-related (non-exercise) physical activity observed over the past few decades are associated with an increase in obesity rates (USA [198]; UK [202]). In addition, availability of energy-dense food [199], chronic stress [203], and disrupted sleep [204] add to the dysregulation of energy balance.
One explanation of the changes in energy balance is centered in the central nervous system. As mentioned above, there is a constant crosstalk between the brain and adipose tissue that controls body fat mass and energy homeostasis \cite{27,28}. Furthermore, changes in hormone levels throughout life also favor bodyweight gain \cite{205}. Drug-induced weight gain is a common side effect of medications such as beta-blockers, sulfonylureas, or centrally active substances \cite{206} and needs to be taken into account during weight lowering approaches in these individuals.

These contributors to bodyweight gain already imply that the pathophysiology of the disease varies among patients and that it is important to capture these factors and provide treatments that match each individual patient’s needs.

Taken together, the basis for non-response to certain treatments is complex and poorly understood. Therefore, there is an urgent need for a chronically effective pharmacotherapy that increases EE and/or decreases EI to manage the disease long-term.

### 4.3. Outlook

In the clinical context, different phenotypes of patients with obesity require tailored therapeutic approaches. In some patients with a high degree of physical activity, e.g., relatively young males who work physically, a reduction in EI quickly leads to considerable weight loss. Thus, an approach using a calorie-reduced diet or a GLP-1 agonist which reduces appetite and EI may be a highly effective strategy. Conversely, another common phenotype in obesity clinics presents relatively low amounts of RMR. As reviewed earlier in chapter 2, this phenomenon is mostly present following very low calorie diets \cite{72} or bariatric surgery \cite{207}. The further reduction of EI proves difficult in these patients and is often unsuccessful. While physical exercise can increase the TEE, its efficacy is often minimal because patients are either limited in the time they can spend exercising or in their exercise capacity. Disease conditions such as hyperthyroidism and pheochromocytoma demonstrate that an increase of RMR can lead to significant weight loss \cite{208–210}. Prior experience with the energy-expending drug 2,4,-dinitrophenol also underscores that the therapeutic targeting of EE is essentially feasible \cite{211}. This indicates that future obesity therapies should target RMR, an important component of the calorie balance. Furthermore, patients in clinical and observational trials are not phenotyped to an extent that allows for the definition of the different subtypes. Differences in treatment duration, with some studies investigating effects after several weeks and others after a year, a huge BMI range, and a rather small sample size make it difficult to compare results.

Moreover, patients that experienced weight regain and weight cycling driven by a reduction in RMR would benefit from new approaches to increase EE \cite{72} the most. However, current data on personalized obesity treatments that also address EE is extremely limited. Most weight loss trials used low-calorie diets (e.g., diabetes remission clinical trial (DIRECT)). In addition, weight loss programs with a significant amount of exercise are associated with decreases in RMR \cite{212} that make it mostly impossible to maintain a stable bodyweight. Therefore, it might be speculated that, in patients with low EE due to a sedentary lifestyle and/or decreases in RMR due to weight loss attempts, increasing EE by activating BAT or other uncoupling mechanisms could not only promote weight loss but, more importantly, weight maintenance.

Together, the challenge for all weight loss attempts seems to be the physiological response to a negative energy balance as metabolic efficiency is increased, which results in a decrease of RMR, at least in the short-term. Therefore, attempts to increase RMR to complement reduced EI are urgently needed, especially in obesity patients who struggle with weight maintenance and weight cycling.

### 5. EPILOGUE

Energy mobilization and expenditure are subjective but decisive factors for chronic bodyweight homeostasis. Through this perspective, we put forth a concise discussion on key considerations during the experimental data analysis from preclinical \textit{in vitro}, \textit{in vivo}, and clinical setups. We project that, in the future, patient-centric care must include not only BMI, but also the evaluation of disease history (weight cycling history, concomitant conditions, medication, eating behavior, environment, physical functioning, and chronic pain that enables dedicated chronic therapy in patients with obesity) as well as individuals’ energy homeostatic and metabolic features. Clinical measurement of EE might also help to decide if it is better for a given patient to target EI or to focus on intervention of EE. Through precision medicine, an optimized therapeutic benefit is given by targeting an individual patient’s needs based on genetic, biomarker, phenotypic, or psychosocial characteristics.

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### CONFLICT OF INTEREST

None declared.

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