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**THE ACCUMULATING DATA TO OPTIMALLY PREDICT OBESITY TREATMENT (ADOPT):  
RECOMMENDATIONS FROM THE BIOLOGICAL DOMAIN**

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## **Abstract**

### **Background**

The responses to behavioral, pharmacological, or surgical obesity treatments are highly individualized. The Accumulating Data to Optimally Predict obesity Treatment (ADOPT) project provides a framework for how obesity researchers, working collectively, can generate the evidence base needed to guide the development of tailored, and potentially more effective, strategies for obesity treatment.

### **Objectives**

The objective of the ADOPT biological domain subgroup is to create a list of high-priority biological measures for weight-loss studies that will advance the understanding of individual variability in response to adult obesity treatments. This list includes measures of body composition, energy homeostasis (energy intake and output), brain structure and function, and biomarkers, as well as biobanking procedures, which could feasibly be included in most, if not all, studies of obesity treatment. The recommended high-priority measures are selected to balance needs for sensitivity, specificity, and/or comprehensiveness with feasibility to achieve a commonality of usage and increase the breadth and impact of obesity research.

### **Significance**

The accumulation of data on key biological factors, along with behavioral, psychosocial, and environmental factors, can generate a more precise description of the interplay and synergy among them and their impact on treatment responses, which can ultimately inform the design and delivery of effective, tailored obesity treatments.

## **Introduction**

Weight loss provokes coordinated changes in multiple energy homeostatic systems which culminate in disproportionately increased energy intake (EI) and decreased energy expenditure (EE) 1, 2. The extensive interindividual variability in responses to weight loss 3 and treatments differentially targeting these systems 4, 5 suggests that treatment could be improved via better understanding of the biological factors mediating energy balance 6, 7.

The NIH-sponsored Accumulating Data to Optimally Predict obesity Treatment (ADOPT) Core Measures Project 8 aims to advance adult obesity medicine in the face of this individual variability in treatment responses 9. ADOPT is designed to provide investigators with tools to generate an evidence base consisting of common measures across the following four domains: behavioral, biological, environmental, and psychosocial, which can enhance interdisciplinary research and advance understanding of the sources of response variability, depicted in the ADOPT Working Model.

The biological domain subgroup of the ADOPT working group was tasked with recommending core measures relevant to the “constructs” of body composition, energy balance, biomarkers, brain structure and function, and biobanking, as previously designated by the full ADOPT working group. The uniform reporting of common measures can increase the impact and generalizability of the combined research body. Further information regarding each construct and measure described below is available at the ADOPT Core Measures Workspace in the Grid-Enabled Measures (GEM) database (<https://www.gem-measures.org/workspaces/ADOPT>), which is also a venue for discussions that help to build consensus around common measures.

## **Identification of Biological Measures**

In an effort to create a list of measures of relevant biological constructs that could be employed across most, if not all, clinical human obesity studies, several factors were considered in the selection of the methods (designated as “measures”) best designed to evaluate each construct given the constraints of human weight-loss trials. These included 1 the strength of the current evidence relating each measure to relevant constructs and weight-loss outcomes, 2 the measure's validity and reliability, 3 the feasibility of widespread use of the measure, 4 the measure's burden to investigators (cost, administration, availability) and subjects (time and invasiveness), and 5 the measure's applicability to small (<50 participants), moderate (50-200 participants), and large (>200 participants) studies.

In many cases, the “gold standard” measures were not the most practical within the feasibility constraints of many weight-loss trials. Recognizing the frequent dichotomy between precision and practicality, we provide two classifications of measures within the constructs. “Recommended” measures are those that should be feasible and cost-effective in all obesity-related clinical trials. “Suggested” measures are those that should be feasible and cost-effective in many, if not most, clinical trials and, if possible, should be performed in addition to the recommended measures. Except as otherwise noted (e.g., for targeted genetic studies), all measures outlined below should be obtained at baseline and at other time points (e.g., during and after an intervention) in each study.

## **Body Composition**

Within the area of body composition, we included both anthropometric and bioelectrical impedance measures to allow for the assessment of body fat content, central versus peripheral fat distribution, and visceral versus subcutaneous fat distribution in a manner that can be integrated across previous

and future studies 10. It is likely that this recommendation will change going forward, as more investigators use recommended measures of body fat content (see below).

### **Anthropometry**

Anthropometry includes the assessment of height, weight, and calculated BMI (recommended) as well as central fat mass (FM) and the relative distribution of fat in central and peripheral fat depots by waist and hip circumferences and calculated waist-to-hip ratio (WHR) (recommended). The feasibility of anthropometry permits easy frequent measurement (at least every 1 to 3 months in the initial phases of weight-loss trials), which is necessary to document early responses (1 to 2 months) to surgical 11, 12 and nonsurgical 13, 14 interventions as predictors of long-term success.

However, together BMI, sex, and age only explain approximately 50% to 60% of the variance in percent body fat as measured by dual energy x-ray absorptiometry (DXA) (suggested), which is the “gold standard” 15, and anthropometry is clearly less accurate than bioelectric impedance spectroscopy (BIS), DXA, magnetic resonance imaging (MRI), and BodPod (see below). The accuracy of BMI is further diminished in participants with increased fractional lean body mass, such as athletes with very high muscle mass, (e.g., weightlifters) 16, or increased fractional FM, such as the elderly. BMI does not assess FM and fat-free mass (FFM), both of which are important determinants of EE and EI, and different interventions may differentially affect body composition (FM and FFM) and weight 16.

It is recommended that both waist circumference and WHR are collected in all adult studies 17 because both the absolute amount of central fat 17, 18 and the relative amount of fat distributed in central versus peripheral fat depots 19, 20 have been shown to be predictive of multiple adiposity-related comorbidities and may affect intervention response 21-23. Both of these measures have been reported to correlate closely with visceral and subcutaneous adipose tissue measured by DXA (suggested) or MRI 24, 25. To minimize variability in WHR, it is critical that investigators utilize uniform landmarks, with waist circumference measured at the iliac crest and hip circumference measured at the level of the trochanters as utilized by the National Center for Health Statistics in National Health and Nutrition Examination Survey studies 26. It should be noted that the abdominal circumference measured at the midpoint between the inferior border of the ribcage and the superior aspect of the iliac crest has been reported to be a better correlate of central adiposity in some studies 27. However, the NIH method is recommended to allow for better comparisons with existing National Health and Nutrition Examination Survey and other data.

### **Anatomic fat storage**

Anthropometry fails to distinguish subcutaneous from visceral fat, which has been reported to be correlated with the risk for metabolic syndrome, cardiovascular disease, and several malignancies 28, and the relative distribution of visceral and subcutaneous fat varies significantly by gender and between ethnic and/or racial groups 29. Specifically, it is recommended that all studies include more direct measures of FM by BIS 30, which is a noninvasive and inexpensive type of bioimpedance to assess body composition that can be utilized in studies of any size or duration. BIS is comparable with the single-frequency devices used in bioimpedance analysis (conventionally denoted as BIA) in terms of subject burden and cost. The multisegmental, multifrequency BIS device allows for the identification of more components of bioimpedance (capacitance, resistance, etc.) than single-frequency bioimpedance analysis devices, and BIS measures of extracellular/intracellular resistance have been reported to be significantly correlated with intraabdominal FM measures by computed tomography scans 31. BIS has also been reported to correlate better with FM than BMI and with

visceral and/or intraabdominal fat (measured by MRI) than waist circumference or WHR 32. Overall, BIS has been reported to explain 80% to 90% of the variance in FM and visceral fat and 50% to 55% of the variance in intraabdominal fat by DXA and/or MRI 32, 33. Despite the advantages of BIS, it has been reported to underestimate FM and overestimate FFM, especially in males 33, and BIS can be less reliable when the hydration status of FM and FFM are uncertain (e.g., in children, individuals with edema, and postbariatric surgery patients). In addition, there are multiple BIS systems available that have not been crossvalidated. It is therefore essential to always report the exact BIS system utilized.

The addition of BIS will allow for the integration of data from multiple studies and further validation of this approach. Though BIS provides the best option for the assessment of body composition, when balancing the value of the data with the cost and participant burden in large clinical trials, this recommendation does not preclude the use of more accurate or precise suggested measures of body composition such as DXA, MRI, or quantitative magnetic resonance spectroscopy. It is anticipated that the acquisition of BIS data along with other suggested measures of body composition in some studies will provide a large data set that can be utilized to better define the precision of BIS in different populations.

### **Energy Balance**

The relative long-term constancy of body weight and the overall lack of success of nonsurgical interventions in long-term weight reduction suggests that, at usual weight, EI and output are “coupled” and vary directly to maintain energy stores. Once weight is perturbed, this coupling is lost, and EI and energy output now vary inversely to “defend” previous body energy stores 2, 34. There is large interindividual variability in EI and EE (including cardiorespiratory fitness [CRF], which is an important determinant of exercise recommendations) and, of course, adherence before, during, and after weight change 3, 23, 35-37. A better understanding of this variability is likely to identify certain energy homeostatic phenotypes that are predictive of individualized best practice recommendations.

### **EE**

Total EE (TEE) is composed of resting EE (REE), the thermic effect of feeding (TEF), and nonresting EE (NREE) 38, each of which is likely to change in weight-loss studies. Direct and indirect objective measures for these variables present challenges for large clinical weight-loss trials because of their expense, participant burden, and feasibility. Because of these limitations, it is recommended that measures of TEE and its components be calculated.

Calculated REE should be acquired with the Mifflin-St. Jeor equation (recommended) 39, which is best correlated ( $R^2 = 0.80-0.85$ ) with calorimetric measures of REE 39 and superior to other less-studied or more population-specific equations 40. The following are the Mifflin-St. Jeor equations: for males,  $REE \text{ (kilocalories per day)} = 10 \times \text{weight (kilograms)} + 6.25 \times \text{height (centimeters)} - 5 \times \text{age (years)} + 5$ ; for females,  $REE \text{ (kilocalories per day)} = 10 \times \text{weight (kilograms)} + 6.25 \times \text{height (centimeters)} - 5 \times \text{age (years)} - 161$ .

It should be noted that the Mifflin-St. Jeor equation may also have limitations in its generalizability. Equations for individuals who are transgender, intersex, have abnormal numbers of X or Y chromosomes, or who have undergone or are undergoing surgical or hormonal therapy relevant to gender have not as yet been derived 41. The increasing attention to gender medicine and gender-specific biological variation in human metabolic disease should yield an expanded list of calculations going forward.

In lieu of directly measuring NREE, it is recommended that a questionnaire-based assessment of Physical Activity Level (PAL) be acquired 42. PAL is defined as TEE/REE, and the questionnaire-derived PAL was chosen because of its simplicity, its validation by correlation with PAL measured by calorimetry, and its applicability across multiple studies. This questionnaire provides a self-evaluation of physical activity during work and leisure activities. The calculated REE and PAL can then be used to derive a calculated TEE ( $TEE = PAL \times REE$ ).

The working group readily acknowledges that other direct and indirect measures can provide more accurate and precise measures of TEE, its components, and metabolic adaptation in response to treatment 43 but are not feasible in many weight-loss trials because of the expense and required specialized equipment 38, 44. If possible, the calorimetric measurement of REE in the overnight fasted state by using a metabolic cart or room indirect calorimeter to measure rates of carbon dioxide production (VCO<sub>2</sub>) and oxygen consumption (VO<sub>2</sub>) is suggested. The VCO<sub>2</sub>/VO<sub>2</sub> ratio provides an index of the relative mixture of metabolic fuels being utilized. Similarly, doubly-labeled water can be used to measure TEE in the free-living environment, and whole-room indirect calorimetry chambers can be used to provide robust objective estimates of TEE, NREE, and REE. Though these approaches can also be utilized to acquire TEF with the appropriate feeding study design, TEF is neither recommended nor suggested across all studies because of the necessary complexity of these designs coupled with the relatively small contribution of TEF to TEE and adaptive thermogenesis 3.

## EI

Under static conditions of body weight and composition stability, EI must be equal to TEE. EI steady state (recommended) refers to EI when body composition and mass are not changing over time and can be presumed equal to the calculated TEE 45. It should be noted that this does not include or account for variations in diet composition or the possible effects on the partitioning of stored calories (see the body composition section) that might occur, for example, as a result of weight loss with a resistance training component (increased partitioning of stored calories as FFM) versus aerobic training 46. During dynamic periods of weight change ( $EI \neq EE$ ), measuring EI is more difficult. Self-reported EI measures have been shown to be inaccurate 47 but changes in EI (recommended) during such periods can be mathematically modeled with measures of EE and body composition dynamics 33. This model has recently been validated against the intake-balance method in a 2-year calorie-restriction experiment in 140 people of varying age and BMI 48, and it requires only baseline anthropometric and demographic information along with repeated body weight measurements over the course of the intervention. There are assumptions underlying this model, such as the stability of REE and PAL. More frequent body weight measurement increases the precision of the calculated changes in EI over time, and physical activity time course data (e.g., from actigraphy measurements) can also be incorporated to account for changes in NREE. The full description of this calculation, including MatLab Code information, can be found at the GEM website (<https://www.gem-measures.org/workspaces/ADOPT>).

The estimation of the average EI over an extended duration is ideally calculated by using the intake-balance method involving body composition measurements along with repeated TEE measurements by using doubly labeled water 49; these procedures are, unfortunately, impractical for larger weight-loss studies. Depending upon the duration and types of weight-loss intervention, and participant age and gender, calculated EI based on TEE and weight or body composition change has accounted for between 20% and 60% of the variance in EI calculated by using the intake-balance method (suggested) as described above 48.

## **CRF**

CRF varies significantly between individuals and may modify responses to obesity treatment either directly or via compliance with exercise recommendations 50. CRF is also significantly correlated with mortality and comorbidity risk, independent of body fatness 51. Though the gold standard for CRF is an ergometric VO<sub>2</sub>max test, an approximation can be made from resting heart rate or an alternative submaximal fitness test. Resting heart rate was selected as a recommended measure because it is easy and inexpensive to perform and has been reported to explain about 20% of the variance in CRF 52 using multiple different equations and calculations. The 3-minute step test has been reported to explain 50% to 90% of the variance in fitness by treadmill testing but may not be practical for larger studies, and it is therefore suggested rather than recommended 53, 54. For subjects who are unable to perform the step test because of orthopedic or fitness issues, a simpler version using a corridor walk may be substituted 55.

## **Brain Structure and Function**

Obesity-associated alterations in brain structure and function 56, 57 have been correlated with weight loss and regain in behavioral and bariatric surgery trials 56, 58, with limited data regarding other treatments. Incentive motivation, reward learning, and executive function (including working memory) 58 are the neurocognitive constructs most predictive of treatment outcomes. These functions are largely mediated by the dopaminergic fronto-striatal (reward/motivation), fronto-parietal (executive function), and hippocampal-amygdala (learning and memory) systems, which operate as both independent and interdependent networks 59. Emerging data has also indicated that related neural systems (interoceptive and salience networks) could be important for weight outcome prediction 60.

This manuscript focuses on brain structure and function rather than the affected neurocognitive constructs discussed in the ADOPT psychosocial domain 61. The current gold standard measures are MRI to assess structure and blood oxygen-dependent functional MRI (fMRI) to assess function. fMRI can identify the 1 neural substrates that execute behaviors in response to environmental demands and 2 functionally linked intrinsic neural networks that can be assessed in the absence of external, environmental demands (i.e., resting state fMRI). The measurement of resting-state connectivity is feasible in large multisite studies and, when integrated with other measures (e.g., cognitive, psychosocial, other biomarkers), can define mechanisms and neuropsychological subtypes that may predict responses to treatment 62 even though correlations of resting-state fMRI in specific single brain regions with behaviors are relatively low 63-69.

Because of the cost and burden to both the participants and researchers, resting-state fMRI scans are only suggested when feasible. Analyses of these fMRI data should include the assessment and comparison across several networks representing the neural systems described above. The suggested MRI/fMRI protocol includes 1 anatomical MRI for structure (volume, density, shape, cortical thickness) and to aid in preprocessing of the fMRI data, 2 diffusion MRI (white matter tractography or structural connectivity), and 3 resting-state functional connectivity MRI. Given that resting-state functional connectivity MRI may be sensitive to the internal state, collecting data about the participant's last meal and the subjective experience of the internal state (hunger, satiety) is suggested 61. Resting-state studies in fasting and fed states will provide insights into brain areas related to meal initiation and cessation. The value of fMRI is significantly increased through the integration with neurocognitive and psychosocial measures 61 and biomarkers of EI (e.g., gut peptides) and EE (e.g., leptin and thyroid hormones) described below.

## **Humoral Biomarkers**

Biomarkers can provide information about the potential mechanisms by which information regarding nutrient availability, energy stores, and energy balance are communicated to central nervous system tracts regulating energy homeostasis as well as provide valuable insights into adiposity-related comorbidities. Biomarker significance is influenced by whether subjects are at weight homeostasis, are weight-reduced, or are in the process of weight gain or loss. Some of the recommended biomarkers (e.g., leptin) predictably and coordinately change in response to calorie-restricted weight loss in a manner that would elevate appetite and suppress EE (1). Baseline measures for some recommended biomarkers (e.g., leptin, total ghrelin) are predictive of weight regain 70, 71 in some, but not all, studies 72. The potential value and/or cost of these measures at baseline, after weight loss, and after a period of weight maintenance is considered high, given the low subject burden (i.e., a blood draw) and commercial availability of the assays.

## **Energy homeostasis**

A number of adipocyte-derived hormones yield valuable data regarding factors that may affect or represent energy stores and balance. As exemplified by leptin (recommended) 73, response to any intervention may depend upon whether the participant is being treated to promote weight loss or prevent weight regain. Therefore, biochemical assessments relevant to energy balance and/or affecting EI and energy output are extremely relevant.

Leptin is secreted by adipose tissue, reflects both adipose tissue stores and energy balance, and is recommended. At baseline weight, circulating leptin concentrations are directly proportional to FM, while during caloric restriction, the leptin/FM ratio may be severely decreased versus only mildly diminished following weight loss 73. A low ratio of leptin to FM may therefore be an indicator of undernutrition, while an unusually high ratio may suggest leptin resistance.

The measurement of fasting levels of the pancreatic peptide amylin is recommended, not only for its effects on nutrient utilization by inhibition of postprandial glucagon, but also because it reduces EI by promoting satiation and attenuates the disproportionate decline in EE that typically occurs during and following weight loss; it thus serves as a marker of weight response 74. In rodent studies, amylin receptors are located within the brainstem as well in multiple other organs, and exogenous amylin acts synergistically with leptin as well as glucagon-like peptide 1 (GLP-1), peptide YY (PYY3-36), and other anorexiants 75. In human studies, coadministration of amylin with leptin has been shown to enhance weight loss during caloric restriction 76.

Adiponectin, particularly high-molecular-weight (HMW) adiponectin, is recommended because of its positive association with cardiovascular fitness and insulin sensitivity and its negative association with the secretion of multiple proinflammatory cytokines. During caloric restriction, adiponectin levels increase disproportionately to the decrease in FM. The multifunctional nature of adiponectin and its potentially pivotal role in mediating comorbidity risk make it a worthwhile and relatively inexpensive test to perform; analyses of high-molecular-weight and low-molecular-weight adiponectin is recommended in all studies 77, 78.

## **Thyroid hormones**

Measures of key components of thyroid hormones (thyroid-stimulating hormone, thyroxine (T4), and the free T4 Index) are recommended because of their known role in regulating energy balance. Diet-induced weight loss is accompanied by a decline in thyroid-stimulating hormone, Triiodothyronine (T3), and T4 similar to "sick euthyroid syndrome" 79, 80, and thyroid-hormone

repletion in weight-reduced individuals has recently been reported to resolve some of the peripheral adaptive responses thought to drive weight regain (Rosenbaum M, Goldsmith R, Haddad F, et al., unpublished data, 2018) in a manner similar to what is seen following thyroid repletion in hypothyroid individuals 81, 82. Other factors commonly measured when assessing thyroid status (T3, reverse T3) present a significant burden for researchers because of the expense of these assays and are suggested only when resources are available.

### **Hunger/satiety signals**

Hunger and/or satiety hormone levels change in response to meals and collectively provide surrogate signals for nutrient availability. A number of gut-derived peptides, including ghrelin, GLP-1, and PYY3-36, affect appetite 83 and coordinately change in response to calorie restriction 1. Baseline total ghrelin levels are predictive of weight loss 70, and meal responses of PYY3-36 and GLP-1 have been associated with successful reduced-weight maintenance 84. Such studies provide examples of how these peptides could be valuable in modeling treatment outcomes. Though postprandial responses of these hormones have provided the most pertinent information for predicting treatment responses, meal challenges were judged as too great of a burden on both participants and the researchers. The measurement of fasting total ghrelin, GLP-1, and PYY3-36 is recommended for all weight-loss studies as the best compromise between feasibility and informational value. Other gut peptides with similar actions were considered for predicting treatment outcomes, including cholecystokinin and glucagon inhibitory peptide, but the added value was deemed insufficient because of the greater assay difficulty and expense and the likelihood of collinearity with those already recommended. Plasma AgRP has been suggested as a biomarker of hypothalamic melanocortin activity, which could reflect downstream activity of the hunger and satiety signals even though there is only limited evidence for it as a biomarker. AgRP is linked to insulin sensitivity during and after weight loss 85, suggesting that this molecule may provide insights relevant to other biomarkers as well as brain function. For these reasons, measures of cholecystokinin, glucagon inhibitory peptide, AgRP, and postprandial responses of all of these hunger/satiety signals were suggested only when it is feasible and resources are available. It should also be noted that properly processed and stored samples can be biobanked (see below) for future analyses if cost limitations are prohibitive in a given study.

### **Nutrient status**

Nutrient-sensing systems in peripheral tissues and in specific regions of the hypothalamus exist that convey signals of nutrient availability for appetite regulation and, therefore, serve as indices of nutrient status. Fasting levels of glucose, nonesterified fatty acids (NEFA), and triglycerides (TG) are recommended at baseline, during weight loss, and after weight loss. Glucose, NEFA, and TG are the primary nutrients in circulation, and all three respond to calorie-restricted weight loss 1. TG are also thought to affect the sensitivity of the brain to peripheral hormones through their effects on blood brain barrier transport. These metabolites consistently change with diet-induced weight loss in a manner that could elevate appetite 1, and the assays are relatively inexpensive and commonly used in clinical research. As with the gut peptides, the measuring postprandial responses of these molecules is not feasible in all studies. For these reasons, acquiring postprandial responses in glucose, NEFA, and TG is suggested only when resources are available.

### **Metabolic function**

Metabolic (anabolic and catabolic) function, insulin sensitivity, and glucose control (recommended) could serve as mediators or moderators of treatment responses and have been shown to influence

patterns of weight loss and weight-loss maintenance 71, 86. Some studies have suggested that diet macronutrient content may influence weight-loss intervention efficacy according to the level of insulin sensitivity 87. The group recommends that fasting levels of insulin, glucagon, and glycosylated hemoglobin (HbA1c) be measured before, during, and after weight loss. Along with the metabolite data, these measures can be utilized to calculate indices of insulin sensitivity and secretion (homeostatic model assessment of insulin resistance and homeostatic model assessment for  $\beta$ -cell function; HOMA-IR, HOMA- $\beta$  recommended) 88. There was some discussion that an oral glucose tolerance test should also be included to provide a more accurate assessment of glucose control. However, there was insufficient evidence that the oral glucose tolerance test would provide sufficient added value over the other surrogate biomarkers of metabolic function for weight-loss outcomes, and it is suggested only when resources are available. There are more invasive measures of insulin secretion and sensitivity (hyperinsulinemic-euglycemic clamp, intravenous glucose tolerance test, etc.) and more comprehensive measures of key aspects of metabolic flexibility, but these tests are not feasible for all large-scale clinical weight-loss trials.

### **Inflammation**

We also recommend assessment of the inflammatory markers tumor necrosis factor-alpha, C-reactive protein, and interleukin 6 (IL-6) in the fasted state before, during, and after weight loss. These inflammatory factors could mediate or moderate the impact of metabolic dysfunction on treatment outcomes. Tumor necrosis factor-alpha is a true adipokine and is elevated in obesity 89. C-reactive protein is made in the liver, largely in response to IL-6, which is produced in liver and skeletal muscle. These molecules represent three different sources of inflammatory markers and are all associated with the subsequent risk for type 2 diabetes 90 and cardiovascular disease 91. By using network modeling that combined biological, gut microbiota, and environmental factors relevant to weight trajectories, baseline levels of IL-6 and plasma insulin most accurately classified individuals who did or did not lose weight and maintain weight loss 92. The global inflammatory status could be assessed by larger panels, but these biomarkers should be sufficient at present to examine the strength of the link between inflammation and treatment outcomes.

### **Biobanking Tissues**

The molecular mechanisms underlying the physiological opposition to weight loss and reduced weight maintenance (2), and the possibility that they mimic the “preobesity” state in which someone is genetically and physiologically “at risk” for weight gain, have not been comprehensively elucidated. A uniform methodology across weight-loss studies for collecting and storing biological samples to measure RNA and DNA from cells in the blood as well as exosomal and free RNAs would provide a valuable resource for interrogating the molecular underpinnings of weight gain, weight loss, and weight regain. For reasons discussed below, biobanking of whole blood and its components (plasma, serum, etc.) with appropriate stabilization (e.g., protease inhibitors) is recommended as the best balance of feasibility, cost, participant burden, expertise and/or equipment needed to collect and process the samples, and relevance to weight-loss physiology.

### **Whole Blood**

Though many different kinds of bodily fluids and tissues can be collected with varying degrees of difficulty, expense, and value (Figure 1), whole blood gives the greatest advantages for ease and the potential amount of relevant information it can provide. Though sample collection is invasive and can be challenging for individuals with obesity, participant burden and the need for expertise to collect whole blood samples is offset by the ability to use samples to interrogate circulating proteins,

metabolites, and noncoding RNAs, which have been identified as relevant to energy homeostasis or which may one day be identified. In addition, both red and white cells can be used to examine cellular processes related to a host of outcomes, including glycosylation, immune response, and metabolism.

Sample Type	Average Collection Cost	Difficulty of Collection	Need for Expertise/ Equipment	Subject Burden	Range of Markers Measured	Relevance to Multiple Biological Processes	Average Rating	Comments
Whole Blood	Yellow	Yellow	Yellow	Orange	Green	Green	Green	Subject burden and expertise offset by ability to measure multiple types of markers and high relevance to multiple biological processes
Buccal Cells	Green	Green	Light Green	Light Green	Red	Red	Light Green	Buccal cells are easy and inexpensive to collect via cytobrushes; however, the range and relevance of markers that can be measured is limited
Urine	Green	Yellow	Green	Yellow	Orange	Orange	Light Green	Urine is relatively easy and inexpensive to collect; but subjects are not always amenable to collecting this sample, and the range and relevance may be limited
Feces	Green	Orange	Green	Orange	Yellow	Yellow	Light Green	Fecal samples are relatively easy and inexpensive to collect, but subjects are not always amenable to collecting this sample, and the range and relevance may be limited.
Saliva	Red	Light Green	Light Green	Light Green	Yellow	Orange	Yellow	Collection of saliva can be relatively expensive; limited range of markers can be measured and the relevance of markers measured in saliva may be limited.
Tissue Biopsy	Red	Red	Red	Red	Light Green	Light Green	Red	Tissue biopsies are expensive to collect, require expertise and skill, and are heavily burdensome to subjects; may provide the most relevant physiological markers

Legend:  Poor      Excellent

Figure 1

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Review of selection criteria for different tissues that could be biobanked. Whole blood is clearly the best fit for these criteria, though other tissues should not be eliminated if available depending upon their relevance to the specific study. Collection costs include creation of sample aliquots; measured markers include metabolites, nucleotides, and proteins.

Caption

Review of selection criteria for different tissues that could be biobanked. Whole blood is clearly the best fit for these criteria, though other tissues should not be eliminated if available depending upon their relevance to the specific study. Collection costs include creation of sample aliquots; measured markers include metabolites, nucleotides, and proteins.

### Functional Analysis/Genetic Screening

In addition to the assessment of the biomarkers described above, both RNA and DNA can be extracted from whole blood for a global assessment of genomic variation, gene expression, and epigenetic modification to ultimately allow a uniform characterization of the contribution of genes

across weight-loss studies. This genetic screening would comprise a powerful resource for understanding the genetic and epigenetic molecular underpinnings of variability in weight loss. This type of screening is suggested at present because of resource limitations, but, as its feasibility and cost becomes more reasonable, we fully expect that it will become a recommended assessment. The biobanking recommendation of whole blood will ensure that tissues will be available to pursue future genetic studies, even if resources are not immediately available. Sequence variations in multiple genes have been tied to energy balance and treatment outcomes in weight-loss studies, which could serve to focus the analysis. These genes include BDNF, DRB3, FTO, GNPDA2, LYPLA, MC4R, MTCH2, and MTIF3, while other less-studied genes (NEGR1, PLIN, and RANK, LEP and LEPR) may also provide relevant information about the variability in treatment response. Studies demonstrating significant intrapair correlations ( $r = 0.75-0.85$ ) in response to weight-loss interventions among identical twins 93 and genetic predictors of weight-loss response to various interventions in large clinical trials 94, 95 suggest that different single-nucleotide polymorphisms (SNPs) may be predictive of the magnitude of weight loss versus regain depending upon the nature of the intervention and subject population. In the future, the use of array-based chips with highly informative dense SNP content, including genome-wide tag SNPs found across diverse world populations and customizable markers for use in large weight-loss studies, can be used as a cost-effective means of creating a well-powered cohort of individuals in which the genetic underpinnings of weight loss can be examined for multiple traits and outcomes.

### **Other Tissue Considerations**

Biobanking of other tissues is also suggested depending upon resource availability. Buccal (cheek) cells (suggested) are easy and inexpensive to collect via cytobrushes, which involves simply swabbing the cheeks and gutters of the mouth to collect sloughed cells. Both DNA and RNA can be extracted from buccal cells 96, though there is a limited range and relevance of markers that can be measured 96. Similar limitations are inherent in the use of saliva samples (suggested). Urine and stool samples (suggested) are relatively easy and inexpensive to obtain, though the relevance of urinary biomarkers to weight loss may be limited. Stool 97 can be used to examine the gut microbiome, gut absorption, and markers of metabolism. Although transplantation of the microbiome from obese or lean mice or humans to gnotobiotic (germ-free mice) clearly affects somatotype, the role of the microbiome as a cause or potential therapeutic target in human obesity is not clearly established 98. Current research in the NIH Human Microbiome Project (HMP) 99 directly addresses these issues, and investigators are suggested to contact the NIH directly for microbiome banking information.

Tissue biopsies allow for the unbiased genomic, epigenetic, and proteomic interrogation of key metabolic tissues (skeletal muscle, adipose) involved in the regulation of energy balance. Because of the burdens placed on subjects and investigators, the collection of specific tissue biopsies is suggested for studies with specific objectives directed at elucidating molecular mechanisms or generating the evidence as to how these tissues could serve as mediators or moderators of treatment outcomes.

### **Conclusions**

The ADOPT biological domain subgroup was tasked with identifying feasible measures of biological constructs (body composition, energy homeostasis, brain structure and function, blood biomarkers, and biobanking tissues) that, when used consistently in weight-loss trials, could serve to explain the variability in treatment outcomes and lay the foundation for genetic-, epigenetic-, and genomic-based analyses. Selections (Table 1) were made with the underlying goal of maximizing the potential to address knowledge gaps in obesity treatment in conjunction with the other ADOPT domains. It is

the intent of the ADOPT project that these recommendations and suggestions as well as the GEM website will be updated as new information becomes available.

It should be emphasized that the recommended measures are not always the “gold standard,” but they are the ones that are most feasible across numerous different study sizes and population. When integrated across studies and with the other ADOPT domains, the recommended measures will facilitate the development of a large comprehensive database that could be mined to evaluate, propose, and implement current and future obesity treatments with maximal efficacy. The recommended and suggested measures are intended to augment weight-loss intervention studies rather than replace planned measures.

The strengths and weaknesses of the ADOPT biological domain are closely intertwined. The main strength is the practicality of the measures that can be implemented in most, if not all, studies going forward at little additional expense and inconvenience to investigators and participants. An additional strength is the intentional malleability of the ADOPT recommendations and suggestions. Regular modifications of the ADOPT domain manuscripts are anticipated based on new data and techniques that become available and on input from the scientific community through the GEM website. The weaknesses of the recommended and suggested measures are that, to achieve necessary fiscal and feasibility goals, numerous more sensitive or specific measures are not included. To address these issues, we would encourage biobanking of serum, plasma, and buffy coats on as many participants as possible in anticipation of the decreasing costs and increasing understanding of relevant future assays.

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