NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

NCT02939404

Abbreviated Title: EIR

NIH IRB #: 17DKN004.

Version Date: March 23, 2023

Title: **Energy intake response to short-term alterations of energy expenditure in humans**

Principal Investigator: Douglas Chang, MD
ODCRS, PECRB, NIDDK
4212 N 16th St
Phoenix, AZ 85016
Tel: 602-200-5312
douglas.chang2@nih.gov

Table of Contents

Abbreviations

DXA:	dual-energy x-ray absorptiometry
EE:	energy expenditure
FGF21:	fibroblast growth factor 21
GLP-1:	glucagon-like peptide 1
IGF-1:	insulin-like growth factor 1
IL-6:	interleukin-6
OGTT:	oral glucose tolerance test
PYY:	peptide YY
PP:	pancreatic polypeptide
RMR:	resting metabolic rate
RQ:	respiratory quotient
SPA:	spontaneous physical activity
T3:	triiodothyronine
T4:	thyroxine
TSH:	thyroid stimulating hormone
WMEN:	weight maintaining energy needs

STATEMENT OF COMPLIANCE

The protocol will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and trial site staff who are responsible for the conduct, management, or oversight of NIH-funded trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

PRÉCIS

More than 30% of adults are considered overweight. In general, lifestyle changes (diet and exercise) or current weight loss drugs only lead to about 5 to 10% weight loss. This may be because a person's energy expenditure, aka the number of calories the body uses, leads to hunger and may increase the amount of food a person eats. Cold exposure is known to increase metabolism but it may not lead to weight loss if appetite and the desire for food are also increased. The primary goal of this study is to evaluate whether changing energy expenditure by cool temperature exposure results in changes in food intake. This study will involve a stay on our clinical research unit where we will determine the energy requirements (at 24°C) of 68 healthy, adult volunteers without evidence of diabetes. Exposure to cool temperatures (19°C) will be used to increase the number of calories a person's body uses in a day. Participants will spend 24 hours in a room that measures energy expenditure while the temperature in the room is turned down, once with a fixed diet and once with a buffet of food choices. After the fixed diet, volunteers will self-select how much food they wish to eat for one day from a vending machine. Volunteers will also spend one day fasting followed by a day self-selecting their food from the vending machine. Findings from this study will provide knowledge about a possible causal link between energy expenditure and eating behavior. This information may shed light on why many weight loss interventions that increase energy expenditure do not work as well as expected, and may eventually lead to new weight loss approaches and therapies.

INTRODUCTION

Energy Expenditure and Energy Intake Link

The fundamental principle of energy balance states that energy intake and energy expenditure (EE) are the counterbalancing factors that determine body weight change. Both components are likely influenced by environmental and genetic factors; however, while energy intake can vary largely from day to day due to psychological and social influences, daily sedentary EE is largely determined by body size and composition. An increased energy intake leads to a small increase (~10% on average) in EE known as the diet-related energy expenditure [1]. In contrast, we have found in a previous study that larger individuals have both a higher absolute EE due to a larger amount of fat-free mass and also, eat a greater amount of food in an *ad libitum* setting both as total kilocalories and as a percentage of weight maintaining energy needs [2]. As the 24-h EE measures done in this study were done during a eucaloric diet (achieved energy balance within 20%), this correlation is likely because body energy requirements modulate a physiological signal for hunger that results in increased food intake [2, 3]. Of note, during the *ad libitum* portion of the study, participants ate more than their weight maintaining energy requirements on average, suggesting that relatively higher energy demands may lead some people to overeat to a greater degree in a food rich environment. A previous study by Caudwell *et al.* also found a direct correlation between resting EE (RMR) and food intake as well as hunger sensations measured during a 3-month trial [3]. Interestingly, after accounting for fat free mass, fat mass is negatively associated with subsequent food intake [2]. The effects of EE on food intake may act, in part, through central pathways that modulate appetite as fat free mass, the primary determinant of resting EE, has been associated with food intake [4] and reduced gray matter volume in regions implicated in energy homeostasis [5].

From an evolutionary viewpoint, a link between energy expenditure, i.e. energy needs, and food intake would ensure that an organism would experience a physiologic drive to find enough food to maintain both life and reproduction. Although some studies have found an inverse association between lower-than-predicted EE and future body weight change [6, 7], others have found that a greater-than-predicted EE predicts future weight gain [8], including preliminary results from one of our own studies with an ethnically diverse study population. The former results may be limited to certain ethnicities (Native American) or may reflect differences between a population with a high prevalence of obesity versus not. It is possible that a greater-than-predicted EE may increase hunger and food-seeking behavior in most individuals, often leading to a greater-than-necessary intake, and eventual weight gain. What is unknown is whether an increase in daily EE in humans would promote a compensatory caloric intake either because of the creation of a negative energy balance or because there is a homeostatic mechanism directly linking energy expended with hunger and food intake. It is known that intermittent cold exposure, which leads to increases in basal metabolic rate in rodents, also increases food intake, [9] resulting in weight changes similar to controls. These findings in rodents lend support to the hypothesis of a homeostatic link between EE and energy intake, potentially due to a direct signal reflecting the energy requirements of the organism. More detailed and specific studies are warranted to elucidate whether directly increasing EE will alter dietary intake.

In our previous study, the 24-h RQ, as an estimate of carbohydrate-to-fat oxidation, and particularly the carbohydrate oxidation rate during energy balance were both positively associated with *ad libitum* food intake and weight change during three days when subjects self-selected their food using the vending machine system [10]. These results imply that a negative carbohydrate balance due to higher oxidation may trigger hunger sensations, possibly due to a depletion of

glycogen stores [11, 12]. In a recent reanalysis of the same EE-intake data [13], both 24-h RQ and 24-h EE were independent determinants of ad libitum food intake (Table 1). This finding indicates that substrate oxidation rates may influence dietary intake independently from the total amount of calories expended. Nevertheless, it is not clear whether an altered EE and RQ would independently influence food intake.

Table 1. Determinants of *ad libitum* food intake using data from a previous protocol.

Predictors	**Total energy intake (kcal/day)**	**Total energy intake (% WMEN)**
Age (years)	−21.0 ± 11.6 (*p*=0.073)	−0.6 ± 0.5 (*p*=0.195)
Gender (female)	949.1 ± 505.2 (*p*=0.064)	**44.6 ± 19 (*p*=0.021)***
Ethnicity (Nat. Am.)	27.0 ± 212.6 (*p*=0.899)	2.6 ± 8.2 (*p*=0.752)
Fat mass (kg)	**−71.0 ± 20.1 (*p*=0.001)***	**−2.9 ± 0.8 (*p*<0.001)***
Fat free mass (kg)	10.6 ± 23.2 (*p*=0.649)	−0.1 ± 0.9 (*p*=0.926)
Energy balance (kcal)	1.01 ± 1.27 (*p*=0.426)	0.03 ± 0.05 (*p*=0.470)
SPA (%)	30.7 ± 35.4 (*p*=0.389)	1.0 ± 1.3 (*p*=0.460)
24-h EE (100•kcal/day)	**316.7 ± 123.5 (*p*=0.012)***	**10.5 ± 4.6 (*p*=0.026)***
24-h RQ (%)	**183.7 ± 44.3 (*p*<0.001)***	**6.7 ± 1.7 (*p*<0.001)***
Intercept	−16,076 ± 3,989	−525 ± 150
Total R^2	**54%***	**42%***

Abbreviations: EE, energy expenditure; RQ, respiratory quotient; SPA: spontaneous physical activity; WMEN, weight maintaining energy needs.

Total energy intake and macronutrients intake during a 3-day ad libitum period on the vending machine is expressed both as the average kcal/day and as percentage of WMEN on the metabolic ward.

Beta coefficients in each cell are reported as mean ± SE. Beta coefficients for 24-h EE and 24-h RQ are expressed per 100 kcal-increase and per 0.01-unit increase, respectively. *: *P*<0.05

We now propose to clarify the cause-and-effect relationship between EE and energy intake with a proof-of-concept study wherein we alter EE and assess the subsequent effect on energy intake. We will also ask participants to return at 6 months and 1 year to assess whether any observed EE-energy intake link is associated with change in weight over time. An increased understanding of the ways in which changes in EE may alter energy intake may provide insight into the limited efficacy of obesity treatments directed solely at increasing EE.

Cold Exposure Increases Energy Expenditure

Thermoregulation to maintain core body temperature is a fundamental homeostatic drive in homeotherms. In previous studies, cold exposure increased EE by approximately 5-15% depending on the temperature exposure and body weight of the study population [14-17]. The individual maximum increase in EE due to cold exposure can already be detected after one day, as shown in a previous 4-month study assessing cold-induced thermogenesis over 24 hours in a whole-room calorimeter after one-month overnight acclimation at both 24°C and 19°C [18]. In this study [18], no difference in the magnitude of 24-h cold-induced thermogenesis was observed after one month spent at thermoneutrality (24°C) versus after one month with overnight cold-exposure at 19°C, suggesting that the full increase in EE due to cold exposure can already be detected after 24 hours. This increase in EE is due to activation of adaptive thermogenesis mechanisms to maintain core body temperature, and include both activation of brown adipose tissue [15] and non-shivering thermogenesis in skeletal muscle. However, in at least one study, lean subjects had a greater increase in 24-h EE and a greater decrease in distal skin temperature (measured by iButtons®) in response to cool temperature exposure than obese subjects [17]. Cold exposure is known to increase food intake in rodents [9] and even with prolonged exposures to cold temperatures, rodents maintain body weight [19]. Weight loss due to prolonged cold temperature exposure does not begin to occur until about −8°C [19]. This

increase in both EE and energy intake with cold exposure has also been observed in other animal species including pigeons [20], which lack the UCP1 gene responsible for heat generation in the brown adipose tissue. This finding suggests that the mechanisms underlying the positive feedback between increased EE and energy intake may involve pathways beyond the activation of brown adipose tissue. Nevertheless, the effects of cold exposure on food intake have not been adequately assessed in humans, including a potential correlation between the degree of increase in EE with cold exposure and any change in subsequent food intake. In a previous pilot study of 9 subjects residing for 24-h in a whole-room calorimeter once at 22°C and once at 16°C with free access to food [16, 21], subjects overate approximately 30% of energy requirements in both conditions , but there was no difference in the degree of overeating at 22°C (+32%) and at 16°C (+34%) in this small study group [16]. Nevertheless, this study included only 9 lean White males, a study population not representative of the current US population in terms of body size, ethnicity and gender. Therefore, studies in a larger and more diverse cohort employing an objective method to measure *ad libitum* food intake are warranted to clearly establish whether or not an association exists between increased EE and food intake. Furthermore, it is unknown how long any effect of increased EE due to cold exposure on energy intake would persist. For example, the energy deficit created by the cold-induced thermogenesis may lead to a hunger signal that persists even with a return to thermoneutral temperatures. Alternatively, the increase in EE from the cold-induced thermogenesis may directly stimulate food-seeking behavior. If there is a direct effect of an EE increase, it is unclear if it would exist only during cold exposure or if it would persist even after a return to more comfortable temperatures.

Possible Mediators of the Energy Expenditure to Energy Intake Link

The signals from lean tissue that might indicate energy needs to the orexigenic centers of the brain are largely unknown. One possibility is hormonal signals. Hormones that may be potential transmitters of energy needs include myokines released from skeletal muscle or other factors released from non-fat tissue. Interleukin-6 (IL-6) is a multifunctional cytokine secreted by a number of tissues including skeletal muscle. IL-6 deficient mice develop obesity late in life but also, many of the beneficial effects of brown fat are not present if the brown adipose tissue is missing the *IL-6* gene. Interestingly, when the calcium release channel of the sarcoplasmic reticulum (RYR1), a protein implicated in non-shivering thermogenesis of skeletal muscle, is activated, the myotubes secrete IL-6 [22]. In addition to local effects, IL-6 can also bind to a circulating, soluble form of the IL-6 receptor (IL-6R) and activate cells that do not express the membrane form of the receptor. The soluble form of IL-6R has been reported to be correlated with EE and both IL-6R and IL-6 are reported to increase with exercise [23] but any effect of the IL-6 pathway on food intake is not clear. Studies have identified fibroblast growth factor 21 (FGF21) as a hormone involved in energy homeostasis, which increases in response to fasting, low protein diets, and intense exercise, and stimulates appetite. As noted above, thyroid hormone is known to act through independent pathways to increase both EE and appetite and, is therefore, also a potential candidate to explain any EE-energy intake link.

More traditional anorexigenic and orexigenic hormones may also be important if the creation of an energy deficit is the cause of increased energy intake after increased EE. These include adiponectin and leptin, hormones secreted from adipocytes to indicate the current status of energy stores in the body [24-26]. A fall in leptin with calorie restriction or weight loss activates the appetite-stimulating pathways in the body, including the melanocortin system. Leptin concentrations might also moderate any EE-energy intake link, i.e. by blunting the link if the body already has adequate

energy stores or by providing the signal that leads to increased food intake. Cold exposure is known to induce a decrease in plasma leptin up to 20% [27, 28], suggesting that leptin may play a role in the EE response to cold by increasing appetite and, subsequently, energy intake. Concentrations of gastrointestinal hormones known to stimulate hunger, i.e. ghrelin, or act as satiety agents, including glucagon-like peptide 1 (GLP-1), glucagon, insulin, peptide YY (PYY) and pancreatic polypeptide (PP) are other potential mediators. In a previous study, acute cold exposure induced a rise in glucagon concentrations in a setting of unchanged glucose and insulin levels as well as increased concentrations of free fatty acids and β-hydroxybutyrate [29], indicating increasing needs for glucose and ketone bodies production by the liver. The thyroid hormone, triiodothyronine (T3), has been associated with EE [30], increases and decreases with weight gain and weight loss, and changes in free T3 related to weight changes are associated with changes in urinary norepinephrine, resting EE, and 24-h EE [31]. Variations in free T3, free thyroxine (T4) and in the thyroid stimulating hormone (TSH) with cold exposure may partly explain the EE-energy intake link. In a previous study, exposure to cold temperatures resulted in an increase in urinary norepinephrine, cortisol and serum TSH without significant changes in serum free T4, T3, or cortisol levels [15], while another study reported an increase in T4 [14]. Conversely, other studies report no significant changes in thyroid hormones and TSH compared to thermoneutral conditions [18, 32, 33]. This current study will help to increase understanding of the role these hormones may have in signaling energy requirements to the body.

Behavioral Aspects

There are many cognitive and behavioral components of food intake that may mediate or moderate the effects of EE on energy intake. Of note, although EE was correlated with energy intake in our past studies [2], individuals still tended to eat more than their energy requirements. Behavioral factors and executive function measures may also independently influence food intake in this study.

Dietary restraint, disinhibition, sensations of hunger, stress and emotional related eating behaviors, impulsivity, perseveration, cognitive flexibility and decision making processes may all influence eating behavior. For example, a prior study in lean weight-stable women found that dietary restraint was greater in those with lower relative EE (despite similar BMIs between restrained and unrestrained individuals), potentially indicating that the dietary restraint was a cognitive response to reduced calorie requirements [34]. Assessing how such characteristics may affect or moderate any link between EE and energy intake, as well as understanding the independent effects of these factors will increase knowledge of determinants of eating behavior.

Summary

Although there is data indicating that 24-h EE is associated with food intake, a direct link between EE and energy intake has not been clearly established. In this study, we propose to explore whether increases in 24-h EE lead to increases in *ad libitum* food intake. We will explore this potential relationship by increasing 24-h EE in humans using cold exposure and secondarily comparison also with fasting, and then assess whether concomitant and/or subsequent 24-h food intake also increases. Given the dynamic nature of human physiology, it is highly likely that these acute increases in EE will exert an almost immediate effect on *ad libitum* food intake to insure that the body has enough fuel to meet daily energy needs. We will also explore potential physiologic mechanisms underlying an EE-energy intake link including assessment of behavioral mediators and hormones.

AIMS OF THE STUDY

The overarching goal of this study is to better understand the short-term association between EE and energy intake by altering EE through environmental means (cold exposure) and assessing the degree to which subsequent and concomitant *ad libitum* energy intake changes. The specific aims of the study are as follows:

Primary Aims

1. To investigate whether increases in 24-h EE by cold exposure lead to increased *ad libitum* food intake, as measured using a vending machine paradigm.
2. To determine whether the effects of increased EE due to cool temperature exposure on energy intake are only concomitant with the increase in EE or persist until the following day. This will be explored by repeating the cool temperature exposure intervention twice: i) once inside the whole-room calorimeter with an *ad libitum* diet; and, ii) once inside the whole-room calorimeter during a fixed diet, followed by one day of *ad libitum* food intake on the vending machines.

Secondary Aims

1. To determine whether 36 hrs of fasting will result in a change in energy intake on the following day, and to determine whether the decrease in 24-h EE during fasting will correlate with amount of food consumed the following day.
2. To determine if cold exposure will alter the 24-h respiratory quotient (RQ, an approximation for the ratio of carbohydrate-to-fat oxidation rates), and test whether the induced RQ changes independently contribute to the altered energy intake.
3. To determine if the 24-h EE responses to cool temperature exposure correlate with core body temperature or changes in peripheral skin temperatures, and to determine if these changes are related to body adiposity or associated with food intake.
4. To assess if behavioral testing addressing dietary restraint, stress related eating behaviors, and executive function or the responses to visual analogue scales assessing hunger modify any observed EE to energy intake association.

5. To assess whether the above behavioral testing as measured by previously validated questionnaires and tasks are associated with measured food intake or hormone concentrations.
6. To assess if concentrations of appetitive or exercise-induced hormones will correlate with the observed changes in EE and energy intake.
7. To determine if any observed association between EE and energy intake predicts future weight change at 6 months and 1 year.

HYPOTHESES

Primary Hypotheses

1. An increase in 24-h EE, as induced by 24 hours of exposure to 19°C, will be associated with an increase in *ad libitum* food intake, which will exceed the increase in 24-h EE, on the day of the intervention and the effect will persist for at least 24 hours.
2. The effect of increasing EE by cool temperature exposure on *ad libitum* food intake will be evident both on the day of the intervention and the following day.

Secondary Hypotheses

1. Thirty-six hours of fasting will induce compensatory overeating when the subject is re-introduced to food, and the decrease in 24-h EE during fasting will positively predict the degree of energy consumption.
2. The creation of an energy deficit will lead to a decrease in RQ, but the observed changes in energy intake due to changes in EE will be independent of any change in RQ.
3. Subjects with less body adiposity will have a greater increase in 24-h EE with cool temperature exposure and a greater decrease in distal skin temperatures while more obese subjects will have a smaller increase in 24-h EE with cool temperature exposure and a greater decrease in

proximal skin temperatures indicating greater reliance on insulating mechanisms rather than adaptive thermogenesis.

4. Measures of executive function and results of questionnaires related to eating behaviors will be associated with the amount of *ad libitum* caloric intake, but the effects of EE will be independent of these relationships.
5. Induced increases in EE will be associated with changes in hormone concentrations, including cold-induced increases in adiponectin, insulin, free T4, TSH, FGF21, glucagon, ghrelin, urinary free cortisol, urinary catecholamines and decreases in leptin, GLP-1, PP and PYY, and at least some of these changes will be associated with the change in food intake.
6. The amount of energy intake increase after an increase in EE or the ratio of Δenergy intake/ΔEE will be directly associated with weight change at 6 months and 1 year.

STUDY DESIGN AND METHODS

Study Design

This is a inpatient natural history, observational cohort study of healthy human research volunteers with research interventions designed to understand the relationship between the physiologic sensing of EE and food intake behavior. This is primarily a cross-sectional study but with a longitudinal component to assess the association of baseline measurements with future weight change.

Subjects

Sixty-eight healthy participants of all body sizes representing both genders, without evidence of diabetes mellitus [35], aged 18-55 years will be recruited from the greater Phoenix area for assessment of EE and *ad libitum* food intake before and after environmental intervention. All subjects will be asked to reside for an approximately 23-day stay on the clinical research unit.

Pre-Screening

Prior to the official screening appointment, prospective subjects will be interviewed in person or by phone. Both general and protocol-specific recruitment scripting will be used to guide pre-screening interviews. Limited identifiable information pertaining to demographics, general health status, and contact information will be collected. We are requesting a waiver of consent for the pre-screening interviews since, per 45 CFR 46.116(d) (pre-2018 requirements):

1. The collection of limited identifiable information for pre-screening purposes involves no more than minimal risk to the subjects.

2. The waiver will not adversely affect subjects' rights or welfare. Pre-screening interviews will not impact subjects' patient care or ability to decline participation before or during the study.

3. Pre-screening interviews could not practicably be carried out without a waiver of consent as recruiting services, utilized in the ODCRS (Obesity & Diabetes Clinical Research Section), perform pre-screening activities for multiple studies and obtaining consent for each is beyond their resources.

4. It is unlikely that any pertinent information about the pre-screening process will be discovered after subjects have completed it, but should that occur, we will provide subjects with that new information as appropriate.

Following pre-screening interviews, all prospective subjects will be fully informed of the aims, nature, and risks of the study and written informed consent will be obtained. Informed consent will be obtained by either the principal investigator or one of the associate investigators who will be well informed of the nature, risks and procedures of the study.

Inclusion Criteria

1. Premenopausal women and men <55 years of age
2. Body weight <204 kg (<450 pounds) and ≥36 kg (≥80 pounds)
3. Stable weight (±5% within past 6 months) as determined by volunteer report
4. Healthy, as determined by medical history, physical examination, and laboratory tests

Exclusion Criteria

1. Age <18 years
2. Weight ≥204 kg (≥450 pounds, maximum weight of the iDXA machine as per manufacturer's manual), or weight <36 kg (<80 pounds, minimum weight allowed based on the NIH guidelines of blood drawing for research purposes)
3. Use of medications affecting metabolism and appetite in the last three months
4. Expresses unwillingness to consume all food given during the weight maintaining diet portions of the study (e.g., due to strict dietary restrictions including allergies or vegetarian or kosher diet)
5. Current use of tobacco products, marijuana, amphetamines, cocaine, or intravenous drug use
6. Current pregnancy, pregnancy within the past 6 months or lactation
7. History or clinical manifestation of:
    - Type 1 and Type 2 diabetes mellitus
    - History of surgery for the treatment of obesity
    - Endocrine disorders, such as Cushing's disease, pituitary disorders, and hypo and hyperthyroidism
    - Pulmonary disorders, including chronic obstructive pulmonary disease

- Cardiovascular diseases, including coronary heart disease, heart failure, arrhythmias, and peripheral artery disease
- High blood pressure by sitting blood pressure measurement using an appropriate cuff higher than 140/90 mmHg on two or more occasions, or current antihypertensive therapy
- Liver disease, including cirrhosis, active hepatitis B or C, and AST or ALT ≥2x normal.
- Gastrointestinal disease including Crohn's disease, ulcerative colitis, celiac disease or other malabsorptive disorders
- Abnormal kidney function (eGFR <60 mL/min/1.73m^2)
- Central nervous system disease, including previous history of cerebrovascular accidents, dementia, neurodegenerative disorders or history of severe head trauma
- Cancer requiring treatment in the past five years, except for nonmelanoma skin cancers or cancers that have clearly been cured
- Infectious disease such as active tuberculosis, HIV (by self report), chronic coccidiomycoses or other chronic infections that might influence EE and weight
- Diagnosis of binge eating disorder, anorexia and major psychiatric disorders based upon the DSM-IV including depression, schizophrenia and psychosis, which may impact the ability of the participant to be in the respiratory chamber for 24 hour time periods
- Chronic ethanol use (more than 3 drinks/day)

Strategies for Recruitment and Retention

Subject recruitment for this study will be through IRB-approved advertisements in local newspapers and through postings of approved flyers and pamphlets in various public places such as,

but not limited to, libraries, public universities, hospitals, health clubs, and local health fairs. Additionally, advertisements may be placed in some local and national newspapers or websites (e.g. Craigslist) and distributed via ResearchMatch and OPR and NIH Listservs to reach a wider audience. The study will also be listed on the webpage created by the NIH Clinical Center Office of Communications; this webpage will offer a brief synopsis of studies recruiting at NIDDK in Phoenix, AZ. In addition, volunteers who had previous contact with the Recruitment Office of NIDDK in Phoenix and had agreed to be contacted about possible participation in future studies may be contacted.

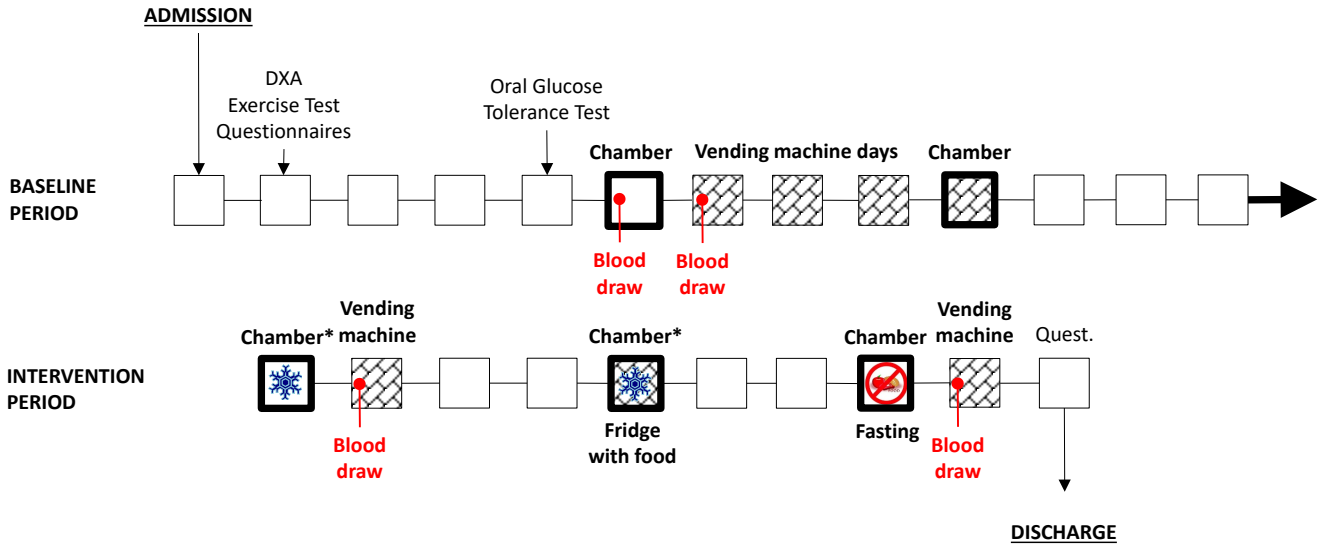
Monitoring Subjects and Criteria for Withdrawal of Subjects from the Study

Volunteers who are unable to follow study and unit policies and procedures, are discovered to have a diagnosis consistent with the exclusion criteria following admission, or who develop an illness unrelated to the study will be withdrawn from the study. Volunteers who are withdrawn may be considered for re-enrollment.

Experimental Design

The study consists of two phases: a baseline and an intervention phase. The baseline phase will be for assessment of EE during energy balance as well as *ad libitum* food intake following energy balance. We will also determine 24-h EE with *ad libitum* food intake in the respiratory chamber during this phase. The intervention phase will include two 24-h EE measurements during 19°C exposure, as well as any associated changes in concomitant and subsequent food intake. Finally, we will determine 24-h EE during fasting as well as *ad libitum* food intake the following day. The fasting assessment will occur last to prevent any residual effects of fasting on other interventions. Subjects will undergo the cool temperature exposure assessments in random order. A summary study diagram of the protocol is shown in Figure 1.

Figure 1. Study diagram of the clinical trial.



*: *These two chamber sessions will be done in a random order*

: 24 hours on the floor (weight-maintaining diet)

: 24 hours inside the metabolic chamber (chamber diet)

: 24 hours on the floor using the vending machines (*ad libitum* diet)

: 24 hours inside the metabolic chamber (*ad libitum* diet)

Quest.: self-administered questionnaires and computerized tasks
OGTT: oral glucose tolerance test
DXA: dual energy X-ray absorptiometry for body composition assessment
VO_2 max: graded-cycle ergometry
Chamber: 24-h measure of EE in the metabolic chamber
Vending: computerized vending machines for *ad libitum* food intake measurement

Volunteers will provide informed consent prior to entering the study. Subsequently, they will be screened and interviewed for eligibility. During screening, a medical history will be obtained as well as a physical exam and resting 12 lead electrocardiogram. Blood will be drawn for screening tests including complete blood cell count, platelet count, glucose, electrolytes, urea nitrogen, creatinine, liver function tests (total protein, albumin, bilirubin, AST, ALT, gamma-GT, alkaline phosphatase), thyroxine stimulating hormone (TSH), calcium and phosphorus. Urine will be collected for complete urinalysis, urinary hCG (pregnancy test for females only) and drug testing. All subjects who meet inclusion and exclusion criteria and agree to participate will be admitted to the clinical research unit for about 23 days to complete the study. Initial demographic information will be collected on all subjects including comorbidities, race, gender, weight, height and waist, thigh and neck circumferences. There is nursing staff available 24 hours a day.

Randomization

Randomization will be done using a permuted block randomization design with a block size of 6 stratified by sex to account for potential order effects of cold interventions (described below). Specifically, half the subjects will undergo first the assessment of *ad libitum* food intake on the vending machines the day following the cold intervention inside the metabolic chamber (as shown in Figure 1,), with the other half undergoing first the *ad libitum* food intake assessment the day of the cold exposure inside the metabolic chamber (Figure 1). The random allocation sequence will be created by a statistician who does not have subject contact.

Admission

A description of the inpatient phase of the study is provided below. The specific day numbers on which procedures occur represent a common scenario. The precise day may vary due to normal subject scheduling variations or technical issues associated with sampling that will not impact the health of the subject or the

interpretation of the data. Specifically, all tasks scheduled prior to the first chamber session can occur in any order, so long as the revised order does not compromise the scientific integrity of those procedures or compromise participant safety. Additional weight-maintaining days or washout days may be inserted before or between chamber sessions throughout the inpatient stay.

Baseline Phase

Day 1: Upon admission, subjects will be given a standard weight maintaining diet (50% carbohydrates, 30% fats and 20% proteins) for three days. The weight maintaining energy needs of the floor diet will be calculated on the basis of weight and gender [10] using the following unit specific equations (kcal/day): Female volunteers = weight (kg) × 9.5 + 1745; Male volunteers = weight (kg) × 9.5 + 1973. Morning weight will be checked daily and the weight maintaining diet will be adjusted as necessary throughout the stay to maintain a stable weight within ±1%.

Subjects will be asked to complete a food-selection questionnaire (FSQ) to rank 80 different food items presented in random order on a 9-point Likert scale with the following anchors: never tasted; dislike extremely (=1); neutral (=5); like extremely (=9). These breakfast, lunch, dinner, and snack food items are based on the model developed by Geiselman *et al.* [36] but with modifications which reflect intake patterns common to the Southwestern United States. These 80 food items are categorized as high in fat (>45%) or low in fat (<20% kcal), and then further subdivided as high in simple sugar (≥30% kcal), complex carbohydrate (≥30% kcal), or protein (≥13% kcal). In addition, subjects are asked to indicate how often they eat an item, and how often they would prefer to eat an item given a lack of financial and other constraints.

Volunteers will also be asked to complete the self-administered international physical activity questionnaire (IPAQ) [37], which consists of 27 questions to assess the degree of physical activity during the subject's everyday life. Questions address job-related physical activity, methods of

transportation, home-related physical activity, leisure-time physical activity and time spent sitting. This questionnaire has been validated across more than 12 countries to estimate metabolic equivalents (MET) per day.

Day 2: Body composition will be determined in the morning before breakfast using dual-energy x-ray absorptiometry (DXA) for the estimation of percent body fat, fat mass and fat free mass by using the General Electric Lunar iDXA machine (Madison, WI). DXA is an X-ray device which non-invasively assesses both skeletal density and regional soft tissue composition with a precision of <1% for bone and 4-5% for soft tissue densities. The total amount of x-ray exposure is a fraction of that received during a standard chest x-ray. Total body scan requires 5-10 minutes depending on the stature and thickness of the subject. The Lunar iDXA machine allows patients up to 204 kg (450 pounds) to be scanned. The software calculates body composition in grams of fat tissue and lean tissue and percentage of body fat, along with volume estimates of subcutaneous and visceral adipose tissue in the abdomen (CoreScan, GE [38]). The operator remains in the room with the subject during the entire scan.

Cycle ergometry will be used to determine subject's aerobic capacity during a submaximal graded exercise test. Subjects will sit comfortably on the cycle ergometer (Monark 839 E), which has a maximum user weight of 250 kg, and the mouthpiece for indirect calorimetry (Parvo Medics' TrueOne® 2400) will be placed on the subject. Heart rate and rhythm will be continuously monitored throughout. Measurement of oxygen consumption and CO_2 production will be measured for 2 minutes while the subject cycles at a comfortable pace (about 50 to 60 rpm) with no resistance to become familiarized with the machine. The power output will then be set to 25W for a 2-minute warm up. The workload will progressively be increased by 25W every minute until the heart rate will

reach 85% of the age-predicted value (=220 bpm−age [years]). The ergometer provides a constant workload during each interval that is independent of pedal speed. Upon completion, the power will be decreased to 25W and the subjects will continue to pedal for 10 minutes or until their heart rate is less than 100 bpm. The aerobic capacity measured during the submaximal graded exercise test will provide a measure of physical fitness and exercise capacity that may be an important confounder in understanding the 24-h EE relationship with energy intake.

Eating-related psychological constructs will be assessed using validated self-administered questionnaires. In addition, executive functioning will be assessed using validated computerized tests. Questionnaires include the following:

1. Stunkard's three-factor eating questionnaire (TFEQ) [39]: a self-administered questionnaire comprised of three subscales measuring dietary restraint, i.e. the cognitive control of eating, disinhibition, i.e. the tendency to eat food without inhibition, and perceived hunger, i.e. the susceptibility of eating in response to subjective feelings of hunger.
2. Power of Food (PFS) [40]: a 15 item self-administered questionnaire to assess feelings of being controlled by food and susceptibility to food-abundant environments, independent from feelings of hunger.
3. Emotional Appetite Questionnaire (EAQ) [41]: a self-administered questionnaire to examine eating response to both positive and negative emotions and situations.
4. Food frequency questionnaire (FFQ) [42, 43]: a measure of habitual dietary intake over the past 6 months.
5. Perceived Stress Scale (PSS) [44]: a questionnaire to assess stress and an individual's *perceived* ability to cope with such stress over the past month.

6. Household Food Security Scale: Six-Item Short Form (FS) [45]: a 6 item assessment of food insecurity over the past year. It classifies households into 3 categories of food security status: food secure, food insecure without hunger, or food insecure with hunger.
7. Physical Anhedonia Scale (PAS) [46]: a scale to assess sensitivity to reward. It is designed to reflect the degree to which individuals take pleasure from and are motivated to engage in rewarding behaviors including food consumption.
8. Barratt Impulsivity Scale-11 (BIS-11) [47]: a self-administered, 30 item scale that categorizes impulsivity into 3 main aspects: motor (acting without thinking), cognitive (quick decisions), and present orientation (non-planning).
9. Reward-Based Eating Drive Scale (RED) [48]: 9-item scale to assess excessive drive to eat due to feelings of lack of control, diminished satiety and preoccupation with food. It is used to assess the more normative reward-based eating in non-clinical samples and on a non-pathological continuum.
10. MacArthur Scale of Subjective Social Status [49]: contains 2 ladders measuring subjective socio-economic status (SES). Studies have indicated that ladder rankings are more powerful determinants of health-related outcomes than traditional measures of SES.
11. The Social Problem-Solving Inventory - Revised (SPSI-R) [50] is a 52-item self-report measure that uses a 5-point Likert scale to assess problem solving abilities. It has 5 subscales: 1) Positive Problem Orientation; 2) Negative Problem Orientation; 3) Rational Problem Solving; 4) Impulsivity/Carelessness Style; and 5) Avoidance Style.
12. The Alexithymia Scale (TAS-20) [51]: is a 20-item instrument that classifies people who have trouble identifying and describing emotions and who tend to minimize emotional

experience and focus attention externally. It has 3 subscales: 1) Difficulty Describing Feelings; 2) Difficulty Identifying Feelings; and 3) Externally-Oriented Thinking.

13. Hollingshead Four Factor Index of Social Status [52]: The Hollingshead scale was selected due to its wide scale use in public health measures of socioeconomic status. The Hollingshead index provides a score based on a combination of an adult's marital status, education, and occupation. Education level is assigned a value of 1 to 7 and occupation title is assigned a value from 1 to 9. The total score is determined by weighting and summing the scaled education and occupation title values. Total scores range from 8 to 66, with higher scores indicating higher socioeconomic status. Based on the total score individuals fall into one (score 8-19, unskilled laborer) of five (score 55-66, higher executive and professional) social strata categories where higher groups represent higher ranking in social position. For homes with multiple sources of income (i.e. marital status) scores for an individual are averaged to obtain a single score.
14.

The computerized tasks include:

1. Iowa Gambling Task (IGT) [53]: A computerized test to evaluate decision-making. Subjects are instructed to gain as much fake money as possible by drawing 100 selections from a choice of 4 decks of cards. The decisions to choose from the decks should be motivated by gain and loss schedules inherent in the task.
2. Stroop Color Word test and Food-related Stroop Word Test [54]: Tests used to assess cognitive flexibility, selective attention and, for the Food Stroop, food specific distractibility. Names of colors are presented in either the same color as the word or in a

different color than the color named. The subject has to either read the words or name the ink colors as quickly as possible within a time limit. The food-related Stroop test presents food words and neutral words in varying colors. The subject has to name the color of the word, rather than read the word.

3. Wisconsin Card Sorting Test [55]: A computerized test to examine executive functioning related to flexibility and perseveration. Subjects are presented with 4 stimulus cards and 128 response cards. They are instructed to respond with response cards to match each of the 4 stimulus cards (based on color, number or shape) and told if they are right or wrong. Once a number of correct answers are made, the sorting principle is changed without warning and the subject must deduce the new sorting strategy.
4. Go/No-go Test [56]: A task assessing the executive functions of sustained attention and self-control. Participants are asked to make a binary decision for each stimulus. They must either press a button in response to one stimuli or inhibit that action under a different set of stimuli. Accuracy and reaction time are measured.

Day 3 and 4: Subjects will be fed a weight maintaining diet.

Day 5: Glucose tolerance and confirmation of non-diabetic status will be assessed by a 75g oral glucose tolerance test (OGTT) after at least two full days on the weight maintaining diet. After an overnight fast, an intravenous catheter will be placed in the forearm vein for blood withdrawal. Volunteers will then ingest 75 g of glucose over 2 minutes. Blood will be drawn at −15, 0, 30, 60, 120, and 180 minutes for plasma glucose and insulin. Fasting samples for DNA will be drawn at time −15 minutes to screen for known and future discoveries of genetic changes associated with human obesity, EE, and food intake, as described in the “Genetic Studies” section.

To measure subjects' activity during the inpatient stay as a potential covariate for EE and energy intake changes, subjects will be asked to wear an omnidirectional accelerometer (Actigraph®). An Actigraph monitor will be placed on both wrists, both ankles and at the waist and thigh to monitor physical activity. Subjects will be asked to wear the monitors from day 5 to day 23 (last day of inpatient). The Actigraph is designed to be worn for about one week at a time so the monitors will be changed twice on about day 15 and day 17.

Day 6: Participants will spend approximately 23 hours and 15 minutes in the respiratory chamber for the assessment of energy metabolism during eucaloric conditions at an ambient temperature of 24°C, which is maintained by an adjustable air conditioning system. The respiratory chamber is a comfortable, air-tight room (10 feet x 8 feet x 7 feet, net volume ~20,000 liters) constructed as a large open-circuit whole-room indirect calorimeter. It is furnished with a toilet, sink, bed, desk, chair, color television, DVD player, and radio. Visual contact with the subject is possible through one of the windows and verbal contact is possible via the telephone. An emergency call bell is within reach of the bed and toilet. Food is provided into the chamber through an airtight interlock. Spontaneous physical activity (SPA) is continuously monitored by a radar system based on the Doppler Effect. The flow rate through the chamber and the CO_2 and O_2 concentrations of the out-flowing air are continuously computed, and calculations of oxygen consumption, carbon dioxide production, RQ [57], and SPA are made every minute. Volunteers will be asked to only engage in sedentary activities while in this chamber. Breakfast will be given immediately before volunteers enter the chamber. Total food intake for the day (four meals total) will be equivalent to 80% of the weight maintaining diet to account for the restricted physical activity in the confined environment of the chamber.

Prior to entering the respiratory chamber, fasting blood will be collected for measurement of insulin, glucagon, leptin, adiponectin, ghrelin, insulin-like growth factor 1 (IGF-1), active GLP-1, PP, PYY, IL-6, free T3, free T4, TSH, FGF21, estradiol (women only) and progesterone (women only), beta-hydroxybutyrate, lactate, pyruvate. A 24 hour urine specimen will be collected for measurement of nitrogen, urinary catecholamines, and urinary free cortisol. Carbohydrate and fat oxidation rates will also be calculated after accounting for protein oxidation, estimated from measurement of 24-h urinary nitrogen excretion [58].

During the approximately 23.25-h stay in the respiratory chamber, the subjects will complete a Visual Analogue Scale (VAS) for measurement of appetite sensations, mood and body temperature including hunger, anticipated food consumption, and desire to eat something fatty, salty, or sweet [59, 60]. Subjects will complete this VAS at 3 time points: 1) prior to entering the chamber; 2) at approximately 6:30 pm, prior to their evening snack; and, 3) immediately upon exiting the chamber.

We will use the CorTemp™ core body temperature monitoring system to assess body temperature during this eucaloric feeding chamber. The system uses an ingestible, wireless sensor to transmit a signal to a data recorder worn on the hip. The data recorder will be worn by volunteers for the duration of the EE assessment to provide a continuous measurement of body temperature change.

We will also ask the subjects to wear iButtons® to measure their skin temperature [61]. iButtons® are digital thermometers with a built-in real-time clock measuring 1.7 cm in circumference and 0.6 cm in depth. They record more than 2000 consecutive temperature measurements to a 0.5°C resolution which can then be downloaded to a computer. iButtons® are intended for monitoring of the body temperature of humans and have been used in other studies to monitor peripheral skin

temperature [61, 62]. Because they are lightweight, they can be attached to the skin simply with tape. Four iButtons will be used to measure distal skin temperature: two iButtons will be placed on the wrist of both arms, and two iButtons will be placed at the ankles of both legs. Five iButtons will be also used to measure proximal skin temperature: one cm below the navel (1°), near the left paravertebral on the upper back (2°), two iButtons on the left and right supraclavicular fossa (3° and 4°), and one iButton near the right paravertebral at the lower back (5°).

Day 7: Upon exiting the respiratory chamber, the subjects will complete the VAS. Fasting blood will then be collected for measurement of insulin, glucagon, leptin, adiponectin, ghrelin, IGF-1, active GLP-1, PP, PYY, IL-6, free T3, free T4, TSH, FGF21, beta-hydroxybutyrate, lactate, pyruvate, to assess for stability after a day of energy balance spent inside the metabolic chamber.

The participants will then be introduced to the vending machine system which they will use to self-select all their food for three consecutive days. Based on the FSQ results, their individualized, refrigerated vending machine will be stocked with 40 different foods that the subject has given an intermediate hedonic rating. In addition, a core group of condiments including butter, cream cheese, salad dressing, bread, water, milk, juices and a drink of the subject's choosing will be provided. The vending machine will require the use of a security code to access the shelves, and this allows us to track the time of day the shelves are accessed. The shelves can be accessed only twice per meal, once to retrieve an item and a second time to return the wrappings and leftovers for weighing and exact determination of caloric intake by the metabolic kitchen. This also provides information on the duration of the meal. Each volunteer will be asked to follow his typical eating pattern as closely as possible. Volunteers may eat as little or as much as they wish; however, they will be asked to eat all meals in the vending machine room, which is equipped with a table, chair, a microwave oven, and a

554 toaster. Volunteers will not be permitted to watch television or read during meals. Using the actual
555 weights of the foods, beverages, and condiments consumed, total energy intake and specific
556 macronutrient intake will be calculated using the CBORD Professional Diet Analyzer Program (version
557 4.1.11, CBORD Group Inc., Ithaca, NY, USA) based on the Food Processor SQL Edition database
558 (version 10.0.0, ESHA Research, Salem, OR, USA). On our unit, repeat measurements of food intake in
559 the same individual using this vending machine paradigm are very reproducible with an intraclass
560 correlation coefficient of 0.9 ($p<0.0001$) [63]. Upon completion of the vending day, subjects will be
561 asked to complete a vending machine exit questionnaire designed to assess the motivation to eat
562 while using the vending machines. It includes 12 items related to mood, food enjoyment and timing of
563 meals.
564 **Days 8 and 9:** Subjects will self-select all their food on the vending machines as described above.
565 **Day 10**: Subjects will again reside for approximately 23.25 hrs in the respiratory chamber at an
566 ambient temperature of 24°C, this time with *ad libitum* access to food choices similar to those
567 provided during the vending machine assessment on the floor. Snacks, drinks, condiments and food
568 items will be stocked in a refrigerator within the respiratory chamber. A small microwave oven will be
569 available inside the chamber, just as in the vending room, for preparation of hot food items. The
570 participant will be asked to keep a record of the timing of all food consumption, and to place all waste
571 and leftover food in the air lock for weighing by the metabolic kitchen. Total energy intake will be
572 calculated as for day 7. A 24-h urine collection will again be done for nitrogen, cortisol and
573 catecholamines. Subjects will complete the VAS at 3 time points during the approximately 23.25 hours
574 inside the calorimeter. The results of this assessment will provide a measure of *ad libitum* food intake
575 inside the confined environment of the metabolic chamber at 24°C. This assessment will be used as

the baseline comparator for the energy intake assessment at 19°C when subjects will again be confined within the metabolic chamber.

Days 11, 12 and and 13: After exiting the chamber, there will be a washout phase for any residual effects of the excess food consumption expected to occur with *ad libitum* food intake to wane. Subjects will be fed a weight maintaining diet.

Intervention Phase

Procedures and interventions described on days 14-15 and 18 will be conducted in random order.

Day 14: Subjects will again spend approximately 23.25 hrs inside the respiratory chamber as above, but this time the temperature will be set at 19°C. Prior to entering the respiratory chamber, fasting blood will be collected for measurement of plasma glucose and insulin. The energy intake provided during this chamber will equal the EE expended during the chamber assessment done on Day 6. Standardized clothing, including shorts, a t-shirt and flip-flops will be provided for this 24-h EE assessment. For sleep (from approximately 11 pm to 6 am), 3 blankets will be provided so that volunteers can create their own microenvironment to achieve a level of comfort conducive to sleep. If the subjects begin to shiver, they will be instructed to notify the nurse at the nurse's station and to use the provided blankets until the shivering ceases.

The cold-induced thermogenesis (CIT, kcal/day) will be calculated as the difference between the 24-h EE at 19°C and 24-h EE at 24°C measured on Day 6. The CorTemp™ core body temperature monitoring system will be used to assess changes in body temperature during this 24-h period. Subjects will again be asked to wear 9 iButtons® to measure skin temperature changes during cool temperature exposure. A 24-h urine collection will again be done for nitrogen, cortisol and catecholamines. Subjects will complete the VAS at 3 time points during the approximately 23.25 hours inside the calorimeter.

Day 15: Upon exiting the chamber, the subjects will complete the VAS. Fasting blood will be then collected for measurement of insulin, glucagon, leptin, adiponectin, ghrelin, IGF-1, active GLP-1, PP, PYY, IL-6, free T3, free T4, TSH, FGF21, estradiol and progesterone, beta-hydroxybutyrate, lactate, pyruvate. Subjects will again spend 24 hrs using the vending machine paradigm, as above, for the assessment of *ad libitum* food intake following the increased EE resulting from the cold-induced thermogenesis during Day 14.

Day 16 and 17: This will be a wash-out period for any residual effects of the overeating expected to occur during *ad libitum* food intake to wane [10]. Subjects will be fed a weight maintaining diet.

Day 18: Subjects will again reside for approximately 23.25 hrs in the respiratory chamber in a cool environment (19°C), this time with access to food choices similar to those provided during the assessment on Day 10. A 24-h urine collection will again be done for nitrogen, cortisol and catecholamines. Subjects will complete the VAS at 3 time points during the approximately 23.25 hours inside the calorimeter.

The amount of food intake on Day 18, as well as on Day 15, will help to determine the length of time in which the cold-induced increase in EE might affect food intake, i.e. whether any EE-effect on energy intake only occurs during cooler temperature exposure or whether the effect persists until the following day. The amount of food intake consumed inside the metabolic chamber on Day 18 at 19° C will be compared to that obtained on Day 10 at 24°C in the same setting to evaluate the difference in energy intake during the cool temperature exposure.

Days 19 and 20: This will be a washout period for any residual effects of the overeating expected to occur with *ad libitum* food consumption to wane. Subjects will be fed a weight maintaining diet.

Day 21: Subjects will spend approximately 23.25 hrs inside the respiratory chamber at 24°C but with no food intake, for assessment of 24-h EE while fasting. Water will be provided and volunteers will be encouraged to stay well-hydrated. Prior to entering the respiratory chamber, fasting blood will be collected for measurement of plasma glucose and insulin. A 24-h urine collection will again be done for nitrogen, cortisol and catecholamines. The CorTemp™ core body temperature monitoring system will be used to assess changes in body temperature during fasting. Subjects will again be asked to wear 9 iButtons® to measure skin temperature changes during fasting. Subjects will complete the VAS at 3 time points during the 24 hours inside the calorimeter.

Day 22: Upon exiting the chamber, the subjects will complete the VAS. Fasting blood will be then collected for measurement of insulin, glucagon, leptin, adiponectin, ghrelin, IGF-1, active GLP-1, PP, PYY, IL-6, free T3, free T4, TSH, FGF21, estradiol and progesterone, beta-hydroxybutyrate, lactate, pyruvate. Subjects will spend 24-h using the vending machine paradigm on the floor, as above. The results of this vending machine assessment will be used to ascertain the amount of intake following a maximal energy deficit, as a baseline comparison.

Day 23: Participants will complete the vending machine exit questionnaire, an 11-item questionnaire designed by our staff to assess motivation to eat while using the vending machines. This *ad hoc* questionnaire includes items related to mood, liking of food, and timing of meals. Subjects will then be discharged home.

Outpatient Visits

Volunteers will be invited to return after 6 months and 1 year from the date of discharge. On these visits, vital signs and weight data will be obtained. Volunteers will also have an assessment of body composition by DXA and will be administered the IPAQ and FFQ questionnaires to have measures of physical activity and dietary intake, respectively, during the follow-up period. Fasting

blood collection will be done for measurement of complete blood cell count, platelet count, glucose, electrolytes, plasma urea nitrogen, creatinine, liver function tests, thyroid profile as well as plasma estradiol and progesterone (women only). Urine pregnancy test (β-hcg) will be done in females only. The DXA scan will not be performed if the pregnancy test is positive.

Blood Withdrawal

Blood withdrawal will remain within the NIH guideline of <10.5 mL/kg or <550 mL (whichever is smaller) every 8 weeks for research purposes. The total amount of blood drawn is detailed below.

Tests	Amount
Screening lab work	32 mL
OGTT	151 mL
Fasting blood measurements	
EBL chamber (pre- and post-)	76 mL
CIT chamber (pre- and post-)	60 mL
FST chamber (pre- and post-)	60 mL
Total (baseline admission):	**379 mL**
Outpatient visits	
6 months	32 mL
1 year	32 mL
Total:	**443 mL**

Sample Size Calculation

The sample size calculation to answer the primary research question is based on the number of patients needed to demonstrate an association between any change in EE due to interventions and subsequent change in energy intake. In preliminary analyses using data from our clinical research unit [13], the cross-sectional Pearson correlation between unadjusted 24-h EE during energy balance and energy intake is 0.46 ($p<0.0001$). A target sample size of 46 participants will provide a power of greater than 0.80 with an alpha of 0.05 to detect a 0.40 correlation ($r^2=0.16$) using simple linear regression assuming that the association between change in EE and change in energy intake will be the same as that between EE and energy intake. However, any finding will subsequently be adjusted in multivariate linear regression analysis for age, sex, race, fat mass and fat free mass (5 covariates), which requires a minimum of 50 volunteers. In addition, given the number of analyses that will be done, an alpha of 0.01 is likely more appropriate. Therefore, to detect a correlation of 0.4 with a power >0.80 at an alpha = 0.01, a total of 68 subjects will be enrolled for the core study (Table 2).

Table 2. Sample size calculations achieving a statistical power ≥0.8.

	α=0.05	**α=0.01**	**α=0.005**	**α=0.001**
r=0.50	n=29	n=41	n=47	n=59
r=0.40	n=46	**n=68***	n=77	n=98
r=0.30	n=84	n=124	n=142	n=181

r: Pearson's unadjusted correlation between change in EE and change in energy intake
α: significance level (2-sided)
*: Sample size for the final analysis

An interim analysis will be conducted when a target sample size of 47 subjects complete the baseline admission. This sample size will provide 80% power (alpha=0.005) to detect a simple correlation of 0.5 between change in EE due to any intervention and change in energy intake. The alpha level for this interim analysis is set to 0.005 based on the alpha spending function approach [[64, 65]]. The alpha level for the final analysis will remain at 0.01. More importantly, the interim analysis will allow us to assess whether there is likely to be a significant correlation once the full sample size is reached or whether the null hypothesis is highly likely to be accepted. If the correlation within the first 47 subjects is <0.2, the study will be stopped as the null hypothesis will be highly likely to be accepted. However, if the correlation is much stronger than anticipated, the study will continue to obtain adequate sample size for adjusting for covariates and answering the secondary research questions. If the interim correlation is between 0.2 and 0.4, the final sample size will be increased from 68 to 124 subjects to provide 80% power at an alpha of 0.01 to detect a more moderate association. Achieved power as a function of final sample size, alpha and correlation value between change in EE and energy intake is reported in Table 3. Assuming a 30% dropout rate, we will ask for a total enrollment of 160 subjects.

Table 3. Power calculations based on different values of alpha (α), correlation value (r) and sample size (n).

α	r	n	Power
0.001	0.30	47	0.103
		68	0.208
		124	0.544
	0.40	47	0.308
		68	0.547
		124	0.915
	0.50	47	0.635
		68	0.873
		124	0.997
0.005	0.30	47	0.223
		68	0.378
		124	0.727
	0.40	47	0.503
		68	0.732
		124	0.969
	0.50	**47**	**0.803***
		68	0.949
		124	0.999
0.01	0.30	47	0.301
		68	0.470
		124	0.799
	0.40	47	0.598
		68	**0.804****
		124	0.982
	0.50	47	0.863
		68	0.969
		124	1.000

*: interim analysis

**: final analysis

Data Analysis

Our primary outcome will be the association between the change in EE and the change in energy intake induced by the interventions (cool temperature exposure and fasting). As the purpose of this protocol is to evaluate the effects of altered EE on food intake regardless of the type of intervention, only within-subject analyses rather than between-interventions analyses will be carried

698 out to evaluate the EE response to each intervention compared to energy balance. For descriptive
699 statistics, normally distributed variables will be described with means and standard deviations,
700 skewed variables will be described with medians and interquartile range. Baseline differences in
701 demographics between sexes and races will be assessed using Student's *t*-test or one-way ANOVA,
702 respectively. Determinants of the EE response to interventions will be determined using mixed
703 models to account for repeated measures and including the variables age, sex, race, percentage body
704 fat, aerobic capacity and type of intervention as fixed effects. The diagonal (DIAG), the compound-
705 symmetry (CS) and the first-order autoregressive (AR1) covariance structures will be considered for
706 estimation of the random effect of the within-subject repeated measures. The model fit of all
707 calculated models will then be compared using the Bayesian information criterion (BIC) statistic to
708 select the best model in terms of covariance structure for random effects and the number of
709 predictors (i.e., lowest BIC). Post-hoc tests comparing interventions to 24-h EE during energy balance
710 will be conducted using the Dunnett's test to correct for multiple comparisons.
711 The change in 24-h EE during each intervention will be calculated as the absolute change in
712 kcal as well as percent change compared to energy balance. Similarly, change in 24-h RQ,
713 carbohydrate and fat oxidation rates, sleeping EE and sleeping RQ will be also calculated and
714 considered in the analyses. Changes in *ad libitum* food intake due to interventions will be calculated
715 as absolute kcal change as well as percent change in weight maintaining energy needs. Associations
716 between continuous variables will be quantified by Pearson's r for data that are normally distributed
717 and Spearman's ρ for data not normally distributed. These analyses will include correlations among
718 changes in 24-h EE during fasting and cool temperature exposure to uncover those EE responses that
719 share common underlying mechanisms, and the association between the EE responses and

concomitant and subsequent *ad libitum* food intake. Multivariate regression models will be calculated to control for potential confounders including age, sex, race, aerobic capacity, fat mass and fat free mass. In evaluating the relationship between altered EE by cool exposure/fasting and *ad libitum* food intake, the concomitant RQ will also be included as further covariate in the multivariate model. Lastly, sensitivity analyses accounting for the SPA inside the metabolic chamber will be done. Although we anticipate that the wash-out period will remove any carry-over effect, potential order effects arising from the cross-over design of the study will be assessed by including a dichotomous variable for the sequence effect of interventions (e.g., 0 = "cold exposure & subsequent *ad libitum* food intake"; 1 = cold & concomitant *ad libitum* food intake) in the multivariate models assessing the association between change in EE and change in energy intake. Student t-tests will be also calculated to determine if the change in EE or the change in energy intake differ between these two groups. If no difference exists in the outcome variables between these 2 groups and the dichotomous variable is not significant in the multivariate model, we will determine that no evidence for an order effect exists and leave the dichotomous variable out of the final multivariate model. If there will be evidence of an order effect, we will include the dichotomous variable in the multivariate model to 'adjust' for the order effect.

To follow-up the regression analyses and assess potential causality linking EE to energy intake, mediation analysis based on hierarchical multiple regression models as outlined in [66] will be used to evaluate whether the effect of increased EE on energy intake is exerted through the influence of mediators, including hormonal changes, RQ appetite sensations, behavioral and neurocognitive scores. The Sobel test [67] will be used to test the significance of the mediation effect.

For all hormonal measures, the change in concentrations induced by the interventions will be compared to the baseline fasting concentrations drawn prior to the energy balanced 24-h EE assessment using paired t-tests for normally distributed data or the Wilcoxon nonparametric test for skewed data. A sample size of 68 subjects will provide greater than 80% power with an alpha of 0.05 to detect a minimum mean expected decrease in fasting leptin, as a representative hormone, of −1.8±6.0 ng/mL as observed in prior studies measuring leptin concentrations during 24-h fasting [1]. This sample size provides a similar power to detect a correlation of −0.30 at an alpha of 0.05 between the decrease in any of the hormones and cold induced thermogenesis or the expected increase in energy intake. Hormonal changes will be evaluated as possible contributors to the altered 24-h EE and subsequent energy intake first in correlation analyses stratified by type of intervention (i.e., cold exposure and 24-h fasting) and then using similar mixed models as described above. Additionally, behavioral and cognitive test scores will be evaluated as possible predictors of the EE-energy intake link, again using correlations and mixed models. The minimum expected correlation detectable in 68 subjects for any of the scores with changes in energy intake will equal 0.30, as reported above. Differences in core body temperature and skin temperatures from measures during energy balance will be calculated for fasting and cold exposure. The significance of these changes will be assessed using one-sample t-tests. The association between these changes and percent body fat as well as EE responses will be assessed using correlation coefficients as above, and again, the minimum detectable correlation with 68 subjects equals 0.30. Significant associations will be evaluated further with mixed models to account for repeated measures, sex, race and age and other potential confounders.

Associations between EE responses to each intervention and absolute weight change at each follow-up visit will be quantified by regression models accounting for age, sex, race, and baseline

763 weight. All longitudinal analyses for future weight change will be confirmed using percent change in
764 weight as the dependent variable instead of absolute weight change. Similar models will be calculated
765 for the absolute changes in fat mass and fat free mass including baseline measures as covariates. In
766 case of significant associations between the EE responses to different intervention and weight
767 change, full regression models will be calculated to determine independence of the identified
768 predictors. Mixed model analysis will be used to evaluate the predictive effect of baseline EE
769 measures in relation to the body weight trajectory including all the follow-up visits and accounting for
770 repeated measures. The Compound Symmetry (CR) and the AR(1) covariance structures will be
771 evaluated and compared by the BIC statistic to identify the best model for weight data over time.
772 Changes in EE after 24-h of fasting and its association with changes in body weight at 6 months and 1
773 year will be combined with data from prior studies (using the exact same methodology) to increase
774 the statistical power of the weight change analysis (currently we have >60 subjects with complete
775 data available at baseline).
776 Statistical analysis will be performed using SAS with E-guide (SAS Institute, Cary, NC). All tests
777 of primary outcome measures will have a 2-sided Type 1 error of p=0.01. For the secondary outcome
778 analyses, we will control for Type 1 error at the level of the comparison with p=0.05 as each
779 hypothesis is substantively important and stated *a priori*.

Informed Consent Process

Written informed consent will be obtained from the participant prior to any screening visits, study procedures or treatments. The person obtaining consent will explain the study in language understandable to the subject. Sufficient time and opportunity will be given for discussion of the research as well as to answer any questions they may have, taking care to minimize or eliminate the perception of coercion or undue influence. The participant and the investigator will sign the current IRB-approved informed consent document. A copy of the consent will be given to the subject for future reference. The signed documents will be sent to the Medical Records Department for placement in the subject's permanent record.

Participants will have the opportunity to carefully review the written consent form, either as a physical or electronic document, and ask questions prior to signing. Participants will be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice.

The consenting process may be performed using the NIH iMedConsent platform (which is 21 CFR Part 11 compliant). If the iMedConsent platform is used, then the participant and consenting investigator will review and sign the current IRB-approved electronic informed consent document using a hand signature (e.g., signing using a finger, stylus, or mouse). Electronic signatures (i.e., the "signature" and a timestamp are digitally generated) will not be used. If a paper copy of the IRB-approved consent document is used, the participant and consenting investigator will both sign using an ink hand signature. A copy of the fully signed consent will be given to the subject for future reference.

We do not plan or anticipate the enrollment of non-English speaking subjects. This is due to the use of questionnaires which are important for the study. These questionnaires have not been translated or validated other languages.

BENEFITS

There are no direct benefits to the individuals participating in this study other than identifying their current state of health, but this study is likely to yield generalizable knowledge to further society's understanding of the relationship of energy requirements and food intake, which will provide important knowledge about the development and prevention of obesity. Volunteers will receive information about some aspects of their health including the results of screening laboratory tests, a thorough physical examination, measures of body fat and EE and assessment of their glucose tolerance status. The results of these tests will be available to the participants and will be provided to their personal physician, if requested.

HUMAN SUBJECT PRECAUTIONS

Rationale for Subject Selection

The main purpose is to increase understanding of the normal physiology and regulation of energy balance in adult humans. Subjects will be recruited from the general Phoenix area via advertisement. All races and genders will be recruited. Due to changes in EE and hormone measurements that may occur with menopause, only premenopausal women will be admitted to the study. Men will be limited to age less than 55 years to prevent discrepancies in age range between men and women in the study population. Children are excluded as: 1) this study requires an inpatient stay and may interfere with school; and, 2) the energy expenditure and associated food intake during the active growth period of childhood is likely to be different than that in adults who have completed

linear growth. In addition, pregnant women are also excluded, again, because of changes in EE and hormones that occur with pregnancy.

Risks and Discomforts

1. *Urine drug screening*: If the results of the urine drug screen are positive, the volunteer will be ineligible for the study as per the inclusion/exclusion criteria. The volunteer will be informed confidentially of their results and referred to their primary care provider for further treatment. The results of the urine drug screen will be part of the volunteer's medical records. This fact will be emphasized during the consent procedure.

2. *Executive Function computer tests and questionnaires including food preferences, eating behavior, and psychological questionnaires:* There are no known risks associated with completing these questionnaires. Subjects will be informed that they may opt not to complete the questionnaires if they feel uncomfortable answering questions about appetite and their emotional relationship with food.

3. *Oral glucose tolerance test*: The risks of this test are those associated with a four hour indwelling catheter including hematoma, ecchymoses, and infection. These will be treated if they occur.

4. *ActiGraph GT9X Link activity monitor:* There are no risks associated with this procedure. The Actigraph GT9X Link activity monitor is 3.5 x 3.5 x 1 cm, weighs 14 grams, and can be worn on the wrist (comparable to a watch), ankle, waist or thigh. It is water resistant up to 1 meter. Per the manufacturer, "the Actigraph Link is an FDA cleared Class II medical device within the United States."

5. *Radiation exposure due to DXA*: The radiation exposure for a scan may be as much as 1 mrem, as indicated by a January 2014 internal radiation exposure review. For subjects who move excessively during the scan, a complete or partial repeat of the scan procedure may be necessary.

Thus, subjects could receive a maximum of 2 mrem per procedure, which is equivalent to 2.5 days of exposure from natural background sources, such as the sun or radioactive materials found naturally in the earth's air and soil. In our study, three DXA scans will be performed over the duration of the study (including follow-up visits) resulting in a possible maximum exposure of 6 mrem (equivalent to 7.5 days of exposure from natural sources). This is well within the NIH-Phoenix Radiation Safety Committee's Guidelines for research subjects of 3,000 mrem to any organ or tissue in a 13-week period and 5,000 mrem per year. The radiation exposure from each DXA scan increases the lifetime cancer risk from 25% to 25.00008%. With the 3 DXA scans over the duration of this study, the increase in lifetime cancer risk will be from 25% to 25.00024%. Pregnancy will be excluded using measurement of hCG in urine prior to the DXA scan.

6. *Cycle Ergometry to determine aerobic capacity*: Because we will carefully screen the subjects with an EKG, history and physical, and cardiovascular disease is an exclusion criterion, we anticipate that there will be minimal risk associated with this measurement. Blood pressure and heart rate/rhythm will also be monitored before, during, and after the cycle ergometer test. If there is significant abnormality on blood pressure or cardiac monitoring, we will stop the cycle ergometer test. If subjects develop extreme shortness of breath or chest pain, we will stop the cycle ergometer test immediately and do a 12-lead EKG. If the subject feels claustrophobic from the mouthpiece for indirect calorimetry, we will not do the measurement. Nursing staff present during the procedure will be certified in basic life support. A medical provider trained in advanced cardiac life support will directly supervise the cycle ergometer test. A hospital code cart is readily available on the clinical research unit if needed.

7. *Respiratory chamber*: There is minimal risk associated with this procedure. There is a telephone in the room so subjects can be in contact with the nurses or their local friends and family at any time. The nurses also monitor the room by checking the temperature as well as the different parameters listed on our computer screen every 4 hours. In addition, sound alarms are set for CO_2 concentration and air flow. The CO_2 alarm is triggered if the CO_2 concentration rises above 1%. The air flow alarm alerts if air flow drops below 45 L/min. These limits, which are far from being harmful to the subject, were chosen because measurements are not reliable when these limits are exceeded. In case of discomfort, the volunteer can ask to be taken out of the chamber at any time. In the event of an emergency, the subject can immediately leave the chamber of his/her own accord through the airtight interlock.

8. *Twenty-four hour urinary collection for nitrogen, free cortisol, and catecholamines:* There are no risks related to this procedure.

9. *Fasting blood collection*: Topical lidocaine cream will be used to minimize the pain associated with the procedure. The blood volume withdrawn will remain within the NIH guideline of <10.5 mL/kg or 550 mL (whichever is smaller) every 8 weeks, as previously described.

10. *CorTemp™ core body temperature monitoring system:* The system is intended for the measurement of continuous core body temperature. Contraindications for use include weight less than 80 lbs (36.4 kg); the presence of obstructive GI disease, previous GI surgery, felinization of the esophagus, or GI hypomotility; a history of gag reflex impairment; MRI scanning while the sensor is in the body (which will not occur in this study); a cardiac pacemaker or other implanted electromedical device. Prior to administration of the CorTemp sensor pill, the contra-indications will be reviewed to make sure the volunteer is an appropriate candidate. Per the device manual,

889 the CorTemp sensor is “registered and cleared by the FDA as a Class II Non-Invasive Medical
890 Device under 510(k) number (880639)”.
891 **11.** *iButtons®:* The only risk associated with wearing the iButtons would be a small risk of local skin
892 reaction from the stainless steel case or the tape. If the subject reports a rash or pruritis, they will
893 be free to remove the iButtons.
894 **12.** *Vending machine food intake assessment*: There are no known risks to performing this test.
895 **13.** *Exposure to 19℃ (66°F) for 1 day:* There are no known risks to the exposure to cool temperature.
896 Other studies have used similar temperature exposures within a respiratory chamber for as long
897 as 48 hours [17]. We will provide blankets as needed to prevent shivering and sleep deprivation.
898
899 **Genetic Studies**
900 The genetic studies are designed to screen for monogenic forms of obesity and to identify
901 novel genes involved in the metabolic pathways regulating food intake. The types of genetic analyses
902 for which DNA may be used are as follows:
903 1. Investigation of specific candidate genes selected by their known biology or association
904 with diabetes, obesity, and related medical problems.
905 2. Genome-wide association studies (GWAS).
906 3. Sequencing of all known exons or entire genome.
907 4. Epigenetic studies.
908 5. Other technologies that will be developed before this research is completed.
909 Results from genetic studies could theoretically lead to as yet unknown discrimination, for
910 example, difficulty obtaining medical insurance. All information on genetic markers in individuals is
911 maintained with the same safeguards that apply to medical records and other personal information.

Any information provided to other investigators who may receive DNA will be coded so that identifying information can be linked to specific individuals only by the investigators named in this protocol or employees working directly under their supervision. All participants will be informed that genetic information relating to them will not be divulged unless genetic variations are identified that could be important to their health care. Clinically actionable findings for the purpose of this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. If this is the case, participants will be asked to provide an additional sample for validation in a CLIA-certified laboratory and then informed if the finding is confirmed. Depending on the nature of the findings, which currently cannot be predicted with certainty, the recommendation may be to seek appropriate clinical care relevant to the finding or to receive professional genetic counseling. The investigators of this protocol and other senior investigators and clinicians at NIDDK will meet as needed to discuss potentially actionable findings (i.e. a finding of a genetic variant, the knowledge of which could help in prevention or treatment of a health condition). When appropriate, we will involve other clinicians, clinical geneticists, or ethicists in these discussions. Potentially actionable findings will also be discussed with others at NIH such as the Bioethics Core at the National Human Genome Research Institute.

Disclosure of Medical Conditions

The participants will be informed of any medical conditions uncovered by the screening process and referred to their primary care provider. The information will also be entered into their medical record. All information from the study will be made available to patients and their physicians at the patient's request. Individuals will not be directly informed of results from non-CLIA certified

laboratories, and such results will not be entered into the medical record. Study information will be treated with the same protection and confidentiality as all other medical records. If requested by an insurance company and the subject has signed a release of information, data from the study may be made available to insurance companies. This could theoretically affect future insurability and employment opportunities. These facts will be emphasized during the consent process.

Clinical Monitoring

Study procedures will be subject to audits and/or monitoring visits to ensure compliance with the protocol and applicable regulatory requirements consistent with the NIDDK quality assurance program plan. Audit and/or monitoring visits results will be reported to the Principal Investigator for further reporting as appropriate. Study documents and hospital records will be reviewed to verify that the conduct of the study is consistent with the protocol plan.

Data and Safety Monitoring

Since this is not a medical intervention trial, the principal investigator will act as the data and safety monitor. Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site manager. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. The PI and study team will review participant data on a monthly basis to review the data for safety and efficacy. Events meeting requirements for expedited reporting as described in HRPP Policy 801 will be submitted within the required timelines.

Event Reporting

Adverse events, non-compliance both serious or continuing, protocol deviations both major and minor, as well as unanticipated problems are defined & described by the NIH Office of Human Subjects Research Protection policy #801, and will be reported in accordance with this policy.

Research Use and Disposition of Human Samples and Data and Data Sharing

Blood (stored as plasma or serum) and urine samples not immediately used for study purposes will be stored for future measurements. All samples will be initially stored in freezers located on the premises at the NIH in Phoenix, AZ. Stored samples and specimens will be used only to measure factors that relate to diabetes, obesity, and their complications. All stored samples, specimens, and data will be coded so that when sent for measurements the identity of the volunteer remains confidential. Identification of coded samples will be kept in secure, password-protected database accessible only to investigators, but will be identifiable in case specific tests yield clinical information of importance to a particular volunteer or samples can be destroyed per volunteer request (see below). Samples will be used only for research and not for commercial purposes. Research volunteers will not be informed of individual results from analyses performed specifically for research purposes, unless there is clear evidence accepted by the medical community that these results would impact the volunteer's individual medical care or future health. Reports of samples lost due to technical issues or destroyed secondary to volunteer request will be included in the annual renewal report. Samples collected for DNA will be screened as described but then will be stored.

As part of the consent process, participants will be informed that their samples may made available collaborating investigators upon request and review of the research proposal to assure that it is within the research goals for the study under which the samples were originally collected. If

samples are sent to collaborators and participants request that stored samples be destroyed, every attempt will be made to assure this is done by collaborators as well, if they have stored samples but we will make it clear to participants that this may be difficult to verify.

As part of the consent process, participants will also be informed that their data may be shared through publicly accessible databases. For genomic data, this will involve de-identified data from this study will being made available by submission to dbGAP or a similar publicly available database, for which user registration is required. Data will be made available proximately following publication of findings that address the main and secondary aims of the protocol, and will include the relevant genomic data and corresponding phenotypic data. Users must agree to the conditions of use governing access to the public release data, including limitation of research to investigations consistent with the participants' consent, restrictions against attempting to identify study participants, destruction of the data after analyses are completed, reporting responsibilities, restrictions on redistribution of the data to third parties, and proper acknowledgement of the data resource. To protect privacy, the tribal affiliations of the participants will not be included in the submitted data. Some study participants may be members or residents of a small, well-defined Native American population, including the Gila River Indian Community and Navajo Nation. On several occasions, these community members and leaders have expressed that study data not be made available other than to the research team because of concerns with individual privacy and stigmatization of the Community. Individual privacy would be seriously threatened by data sharing for these study participants because of the extensive data collected on these individuals as well as data on their relatives. Therefore any submitted data will not include specific tribal affiliations and would only list race as Native American.

Remuneration

For those study participants who complete their stay on the clinical research unit, the payment will be a total of $3, 645.00. This includes $3,385.00 for the inpatient days and testing procedures; plus, $130.00 each for the two outpatient visits (at +6 months and 1 year).

If a volunteer is recruited and makes a good-faith effort to attend the screening, but at the time of the examination is found to be ineligible, the $50.00 payment for time and inconvenience will still be made. In such cases, any clinically relevant data that was obtained prior to determination of ineligibility will be reported to the participant, as indicated. A record will be kept that the volunteer was seen, along with any clinically relevant data that might have been obtained.

If a volunteer is deemed ineligible for reasons not disclosed during pre-screening/recruitment, such as positive for drug use or nicotine use, payment will not be provided. The urine drug and nicotine screen are performed upfront prior to the more thorough health exam.

Per local Ethics Committee guidelines, if early discharge occurs prior to completion of the planned inpatient stay, reimbursement will be at the rate of $50.00 per day, including the day of discharge, plus any procedures that have been completed. This reimbursement rate goes into effect if the study is not completed for any reason. Because we conduct these studies under highly controlled conditions, the scientific validity of our studies may be affected if participants leave the clinical research unit unsupervised. Thus, participants will generally not be allowed to return to complete the study if they need to leave the unit. However, this will be evaluated by the investigators on a case by case basis.

Table 4. Study Variables.

Variable	Time of Assessment*	Method of Ascertainment	Purpose
Demographics (age, gender, ethnicity)	Day 1	Patient Interview / Medical Record	Descriptive
Comorbidities, prior medication use	Day 1	Patient Interview / Medical Record	Descriptive
Physical Exam (blood pressure, heart rate)	Day 1, FU visits	Screening Exam	Descriptive
Weight	Days 1-23, FU visits	Measured by RN	Secondary Outcome
Height	Day 1	Measured by RN	Descriptive
BMI	Days 1-23, FU visits	Calculated	Descriptive
Waist Circumference	Day 1	Measured by RN	Descriptive
Thigh Circumference	Day 1	Measured by RN	Descriptive
Neck Circumference	Day 1	Measured by RN	Descriptive
Percentage of body fat, fat mass and fat free mass	Day 2, FU visits	iDXA	Potential Confounders /Effect Modifier
Glucose tolerance	Day 5	75g OGTT	Descriptive
DNA collection	Day 5	Fasting blood draw	Potential Mediator
Psychological Assessment			
Food-selection questionnaire (FSQ)	Day 1	Self-administered	To set-up Vend
Physical activity (IPAQ)	Day 1, FU visits	Self-administered	Descriptive
Dietary restraint (TFEQ)	Day 2	Self-administered	Effect Modifier/Potential Confounder
Power of food (PFS)	Day 2	Self-administered	Effect Modifier/Potential Confounder
Emotional overeating (EAQ)	Day 2	Self-administered	Effect Modifier/Potential Confounder
Food frequency (FFQ)	Day 2, FU visits	Self-administered	Potential Confounder

Variable	Time of Assessment*	Method of Ascertainment	Purpose
Perceived stress (PSS)	Day 2	Self-administered	Effect Modifier/Potential Confounder
Household Food Security (FS)	Day 2	Self-administered	Potential Confounder
Physical anhedonia (PAS)	Day 2	Self-administered	Effect Modifier/Potential Confounder
Impulsivity (BIS)	Day 2	Self-administered	Effect Modifier/Potential Confounder
Reward-Based Eating Drive (RED)	Day 2	Self-administered	Effect Modifier/Potential Confounder
MacArthur Scale of Subjective Social Status	Day 2	Self-administered	Effect Modifier/Potential Confounder
Social Problem-Solving Inventory - Revised (SPSI-R)	Day 2	Self-administered	Effect Modifier/Potential Confounder
Alexithymia Scale (TAS-20)	Day 2	Self-administered	Effect Modifier/Potential Confounder
Hollingshead Four Factor Index of Social Status	Day 2	Self-administered	Effect Modifier/Potential Confounder
Cognitive Assessment			
Iowa Gambling Task (IGT)	Day 2	Computerized test	Effect Modifier/Potential Confounder
Stroop tests	Day 2	Computerized test	Effect Modifier/Potential Confounder
Wisconsin test	Day 2	Computerized test	Effect Modifier/Potential Confounder
Go/No-go Test	Day 2	Computerized test	Effect Modifier/Potential Confounder
Physiologic and Metabolic Measures			
Aerobic capacity	Day 2	Cycle ergometer	Potential confounder / Effect modifier
Physical activity levels	Days 5-23	Actigraph®	Potential confounder / mediator
Core body temperature	Days 6, 14, 21	CorTemp™	Secondary Outcome
Skin temperature	Days 6, 14, 21	iButtons®	Secondary Outcome
24-h energy expenditure (EE)	Days 6, 10, 14, 18, 21	Metabolic Chamber	Primary Outcome
Respiration quotient (RQ)	Days 6, 10, 14, 18, 21	Metabolic Chamber	Secondary Outcome

Variable	Time of Assessment*	Method of Ascertainment	Purpose
Laboratory tests			
Hormones (insulin, glucagon, leptin, adiponectin, ghrelin, IGF-1, active GLP-1, PP, PYY, IL-6, FT3, FT4, TSH, FGF21)	Days 6, 7, 14, 15, 21, 22	Fasting blood draw	Secondary Outcome / Potential Mediator
Estrogens (estradiol and progesterone, women only)	Days 6, 14, 22, FU visits	Fasting blood draw	Descriptive/ Potential Confounder
Ketones (beta-hydroxybutyrate, lactate, pyruvate)	Days 6, 7, 15, 22	Fasting blood draw	Secondary Outcome / Potential Mediator
Urinary measures (catecholamines and urinary free cortisol)	Days 6, 7, 10, 14, 18, 21	24-h urine collection	Secondary Outcome / Potential Mediator
Food intake measures			
***Ad libitum* food intake**	Days 7-10, 15, 18, 22	Vending machines	Primary Outcome
Appetite sensations	Day 6, 10, 15, 18, 21	Visual analog scale (VAS)	Descriptive / Potential Mediator
Vending machine questionnaire	Day 23	Self-administered	Descriptive

FU: follow-up

* All tasks scheduled prior to the first chamber session can occur in any order, so long as the revised order does not compromise the scientific integrity of those procedures or compromise participant safety. Additional weight-maintaining days or washout days may be inserted before or between chamber sessions throughout the inpatient stay.

REFERENCES

1. Thearle MS, Pannacciulli N, Bonfiglio S, Pacak K, Krakoff J. Extent and determinants of thermogenic responses to 24 hours of fasting, energy balance, and five different overfeeding diets in humans. The Journal of clinical endocrinology and metabolism. 2013;98(7):2791-9. Epub 2013/05/15. doi: 10.1210/jc.2013-1289. PubMed PMID: 23666976; PubMed Central PMCID: PMC3701281.
2. Weise CM, Hohenadel MG, Krakoff J, Votruba SB. Body composition and energy expenditure predict ad-libitum food and macronutrient intake in humans. International journal of obesity (2005). 2013. doi: 10.1038/ijo.2013.85. PubMed PMID: 23736368; PubMed Central PMCID: PMC3909024.
3. Caudwell P, Finlayson G, Gibbons C, Hopkins M, King N, Naslund E, et al. Resting metabolic rate is associated with hunger, self-determined meal size, and daily energy intake and may represent a marker for appetite. The American journal of clinical nutrition. 2013;97(1):7-14. Epub 2012/11/30. doi: 10.3945/ajcn.111.029975. PubMed PMID: 23193010.
4. Blundell JE, Caudwell P, Gibbons C, Hopkins M, Naslund E, King NA, et al. Body composition and appetite: fat-free mass (but not fat mass or BMI) is positively associated with self-determined meal size and daily energy intake in humans. The British journal of nutrition. 2012;107(3):445-9. Epub 2011/07/08. doi: 10.1017/S0007114511003138. PubMed PMID: 21733267.
5. Weise CM, Thiyyagura P, Reiman EM, Chen K, Krakoff J. Fat-free body mass but not fat mass is associated with reduced gray matter volume of cortical brain regions implicated in autonomic and homeostatic regulation. NeuroImage. 2013;64:712-21. doi: 10.1016/j.neuroimage.2012.09.005. PubMed PMID: 22974975; PubMed Central PMCID: PMC4178061.
6. Piaggi P, Thearle MS, Bogardus C, Krakoff J. Lower energy expenditure predicts long-term increases in weight and fat mass. The Journal of clinical endocrinology and metabolism. 2013;98(4):E703-7. Epub 2013/02/19. doi: 10.1210/jc.2012-3529. PubMed PMID: 23418317; PubMed Central PMCID: PMCPMC3615206.
7. Ravussin E, Lillioja S, Knowler WC, Christin L, Freymond D, Abbott WG, et al. Reduced rate of energy expenditure as a risk factor for body-weight gain. The New England journal of medicine. 1988;318(8):467-72. Epub 1988/02/25. doi: 10.1056/NEJM198802253180802. PubMed PMID: 3340128.
8. Luke A, Durazo-Arvizu R, Cao G, Adeyemo A, Tayo B, Cooper R. Positive association between resting energy expenditure and weight gain in a lean adult population. The American journal of clinical nutrition. 2006;83(5):1076-81. Epub 2006/05/11. PubMed PMID: 16685049.
9. Ravussin Y, Xiao C, Gavrilova O, Reitman ML. Effect of intermittent cold exposure on brown fat activation, obesity, and energy homeostasis in mice. PloS one. 2014;9(1):e85876. Epub 2014/01/28. doi: 10.1371/journal.pone.0085876. PubMed PMID: 24465761; PubMed Central PMCID: PMC3895006.
10. Pannacciulli N, Salbe AD, Ortega E, Venti CA, Bogardus C, Krakoff J. The 24-h carbohydrate oxidation rate in a human respiratory chamber predicts ad libitum food intake. The American journal of clinical nutrition. 2007;86(3):625-32. Epub 2007/09/08. PubMed PMID: 17823426; PubMed Central PMCID: PMC2128058.
11. Flatt JP. Use and storage of carbohydrate and fat. The American journal of clinical nutrition. 1995;61(4 Suppl):952S-9S. Epub 1995/04/01. PubMed PMID: 7900694.
12. Flatt JP. Carbohydrate balance and body-weight regulation. The Proceedings of the Nutrition Society. 1996;55(1B):449-65. Epub 1996/03/01. PubMed PMID: 8832812.
13. Piaggi P, Thearle MS, Krakoff J, Votruba SB. Higher Daily Energy Expenditure and Respiratory Quotient, Rather Than Fat-Free Mass, Independently Determine Greater ad Libitum Overeating. The Journal of clinical endocrinology and metabolism. 2015;100(8):3011-20. Epub 2015/06/19. doi: 10.1210/jc.2015-2164. PubMed PMID: 26086330; PubMed Central PMCID: PMCPMC4524995.

14. Celi FS, Brychta RJ, Linderman JD, Butler PW, Alberobello AT, Smith S, et al. Minimal changes in environmental temperature result in a significant increase in energy expenditure and changes in the hormonal homeostasis in healthy adults. European journal of endocrinology / European Federation of Endocrine Societies. 2010;163(6):863-72. Epub 2010/09/10. doi: 10.1530/EJE-10-0627. PubMed PMID: 20826525; PubMed Central PMCID: PMC3113548.
15. Chen KY, Brychta RJ, Linderman JD, Smith S, Courville A, Dieckmann W, et al. Brown fat activation mediates cold-induced thermogenesis in adult humans in response to a mild decrease in ambient temperature. The Journal of clinical endocrinology and metabolism. 2013;98(7):E1218-23. Epub 2013/06/20. doi: 10.1210/jc.2012-4213. PubMed PMID: 23780370; PubMed Central PMCID: PMC3701264.
16. Westerterp-Plantenga MS, van Marken Lichtenbelt WD, Strobbe H, Schrauwen P. Energy metabolism in humans at a lowered ambient temperature. Eur J Clin Nutr. 2002;56(4):288-96. Epub 2002/04/20. doi: 10.1038/sj.ejcn.1601308. PubMed PMID: 11965504.
17. Wijers SL, Saris WH, van Marken Lichtenbelt WD. Cold-induced adaptive thermogenesis in lean and obese. Obesity (Silver Spring). 2010;18(6):1092-9. Epub 2010/04/03. doi: 10.1038/oby.2010.74. PubMed PMID: 20360754.
18. Lee P, Smith S, Linderman J, Courville AB, Brychta RJ, Dieckmann W, et al. Temperature-acclimated brown adipose tissue modulates insulin sensitivity in humans. Diabetes. 2014;63(11):3686-98. doi: 10.2337/db14-0513. PubMed PMID: 24954193; PubMed Central PMCID: PMC4207391.
19. Zhao ZJ, Chi QS, Cao J, Han YD. The energy budget, thermogenic capacity and behavior in Swiss mice exposed to a consecutive decrease in temperatures. The Journal of experimental biology. 2010;213(Pt 23):3988-97. Epub 2010/11/16. doi: 10.1242/jeb.046821. PubMed PMID: 21075940.
20. Henderson D, Fort MM, Rashotte ME, Henderson RP. Ingestive behavior and body temperature of pigeons during long-term cold exposure. Physiology & behavior. 1992;52(3):455-69. Epub 1992/09/01. PubMed PMID: 1409905.
21. van Marken Lichtenbelt WD, Schrauwen P, van De Kerckhove S, Westerterp-Plantenga MS. Individual variation in body temperature and energy expenditure in response to mild cold. American journal of physiology Endocrinology and metabolism. 2002;282(5):E1077-83. Epub 2002/04/06. doi: 10.1152/ajpendo.00020.2001. PubMed PMID: 11934673.
22. Ducreux S, Zorzato F, Muller C, Sewry C, Muntoni F, Quinlivan R, et al. Effect of ryanodine receptor mutations on interleukin-6 release and intracellular calcium homeostasis in human myotubes from malignant hyperthermia-susceptible individuals and patients affected by central core disease. J Biol Chem. 2004;279(42):43838-46. Epub 2004/08/10. doi: 10.1074/jbc.M403612200. PubMed PMID: 15299003.
23. Almada C, Cataldo LR, Smalley SV, Diaz E, Serrano A, Hodgson MI, et al. Plasma levels of interleukin-6 and interleukin-18 after an acute physical exercise: relation with post-exercise energy intake in twins. Journal of physiology and biochemistry. 2013;69(1):85-95. Epub 2012/07/20. doi: 10.1007/s13105-012-0191-x. PubMed PMID: 22810957.
24. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature. 1994;372(6505):425-32.
25. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, et al. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nature medicine. 1995;1(11):1155-61. Epub 1995/11/01. PubMed PMID: 7584987.
26. Rosenbaum M, Leibel RL. Leptin: a molecule integrating somatic energy stores, energy expenditure and fertility. Trends in endocrinology and metabolism: TEM. 1998;9(3):117-24. Epub 2008/04/15. PubMed PMID: 18406252.
27. Ricci MR, Fried SK, Mittleman KD. Acute cold exposure decreases plasma leptin in women. Metabolism: clinical and experimental. 2000;49(4):421-3. PubMed PMID: 10778861.
28. Lee P, Linderman JD, Smith S, Brychta RJ, Wang J, Idelson C, et al. Irisin and FGF21 are cold-induced endocrine activators of brown fat function in humans. Cell metabolism. 2014;19(2):302-9. Epub 2014/02/11. doi: 10.1016/j.cmet.2013.12.017. PubMed PMID: 24506871.

29. Seitz HJ, Krone W, Wilke H, Tarnowski W, Carsten D, Dunkelmann B, et al. Rapid rise in plasma glucagon induced by acute cold exposure in man and rat. Pflügers Archiv European Journal of Physiology. 1981;389(2):115-20.
30. Ortega E, Pannacciulli N, Bogardus C, Krakoff J. Plasma concentrations of free triiodothyronine predict weight change in euthyroid persons. The American journal of clinical nutrition. 2007;85(2):440-5. Epub 2007/02/08. PubMed PMID: 17284741; PubMed Central PMCID: PMC1857361.
31. Rosenbaum M, Hirsch J, Murphy E, Leibel RL. Effects of changes in body weight on carbohydrate metabolism, catecholamine excretion, and thyroid function. The American journal of clinical nutrition. 2000;71(6):1421-32. Epub 2000/06/06. PubMed PMID: 10837281.
32. Ouellet V, Labbe SM, Blondin DP, Phoenix S, Guerin B, Haman F, et al. Brown adipose tissue oxidative metabolism contributes to energy expenditure during acute cold exposure in humans. The Journal of clinical investigation. 2012;122(2):545-52. doi: 10.1172/JCI60433. PubMed PMID: 22269323; PubMed Central PMCID: PMC3266793.
33. van der Lans AA, Hoeks J, Brans B, Vijgen GH, Visser MG, Vosselman MJ, et al. Cold acclimation recruits human brown fat and increases nonshivering thermogenesis. The Journal of clinical investigation. 2013;123(8):3395-403. doi: 10.1172/JCI68993. PubMed PMID: 23867626; PubMed Central PMCID: PMC3726172.
34. Tuschl RJ, Platte P, Laessle RG, Stichler W, Pirke KM. Energy expenditure and everyday eating behavior in healthy young women. The American journal of clinical nutrition. 1990;52(1):81-6. Epub 1990/07/01. PubMed PMID: 2360553.
35. Mellitus ECotDaCoD. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Guideline

Practice Guideline. 2003 Jan. Report No.: 0149-5992 (Print)

0149-5992 (Linking).
36. Geiselman PJ, Anderson AM, Dowdy ML, West DB, Redmann SM, Smith SR. Reliability and validity of a macronutrient self-selection paradigm and a food preference questionnaire. Physiology & behavior. 1998;63(5):919-28. PubMed PMID: 9618017.
37. Hagstromer M, Oja P, Sjostrom M. The International Physical Activity Questionnaire (IPAQ): a study of concurrent and construct validity. Public health nutrition. 2006;9(6):755-62. Epub 2006/08/24. PubMed PMID: 16925881.
38. CoreScan, GE 2015. Available from: http://www3.gehealthcare.com/en/Products/Categories/Bone_Health/CoreScan.
39. Stunkard AJ, Messick S. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. Journal of psychosomatic research. 1985;29(1):71-83. Epub 1985/01/01. PubMed PMID: 3981480.
40. Lowe MR, Butryn ML, Didie ER, Annunziato RA, Thomas JG, Crerand CE, et al. The Power of Food Scale. A new measure of the psychological influence of the food environment. Appetite. 2009;53(1):114-8. Epub 2009/06/09. doi: 10.1016/j.appet.2009.05.016. PubMed PMID: 19500623.
41. Geliebter A, Aversa A. Emotional eating in overweight, normal weight, and underweight individuals. Eating behaviors. 2003;3(4):341-7. PubMed PMID: 15000995.
42. Block G, Coyle LM, Hartman AM, Scoppa SM. Revision of dietary analysis software for the Health Habits and History Questionnaire. Am J Epidemiol. 1994;139(12):1190-6. Epub 1994/06/15. PubMed PMID: 8209877.
43. Block G, Hartman AM, Dresser CM, Carroll MD, Gannon J, Gardner L. A data-based approach to diet questionnaire design and testing. Am J Epidemiol. 1986;124(3):453-69. Epub 1986/09/01. PubMed PMID: 3740045.
44. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. Journal of health and social behavior. 1983:385-96.
45. Blumberg SJ, Bialostosky K, Hamilton WL, Briefel RR. The effectiveness of a short form of the Household Food Security Scale. American journal of public health. 1999;89(8):1231-4. Epub 1999/08/05. PubMed PMID: 10432912; PubMed Central PMCID: PMCPmc1508674.

46. Chapman LJ, Chapman JP, Raulin ML. Scales for physical and social anhedonia. Journal of abnormal psychology. 1976;85(4):374.
47. Barratt ES. Barratt Impulsiveness Scale: Barratt-Psychiatry Medical Branch, University of Texas; 1975.
48. Epel ES, Tomiyama AJ, Mason AE, Laraia BA, Hartman W, Ready K, et al. The reward-based eating drive scale: a self-report index of reward-based eating. PloS one. 2014;9(6):e101350. Epub 2014/07/01. doi: 10.1371/journal.pone.0101350. PubMed PMID: 24979216; PubMed Central PMCID: PMC4076308.
49. Adler NE, Epel ES, Castellazzo G, Ickovics JR. Relationship of subjective and objective social status with psychological and physiological functioning: preliminary data in healthy white women. Health psychology : official journal of the Division of Health Psychology, American Psychological Association. 2000;19(6):586-92. Epub 2000/12/29. PubMed PMID: 11129362.
50. Shewchuk RM, Johnson MO, Elliott TR. Self-appraised social problem solving abilities, emotional reactions and actual problem solving performance. Behaviour research and therapy. 2000;38(7):727-40. Epub 2000/06/30. PubMed PMID: 10875194.
51. Bagby RM, Taylor GJ, Parker JD. The Twenty-item Toronto Alexithymia Scale--II. Convergent, discriminant, and concurrent validity. Journal of psychosomatic research. 1994;38(1):33-40. Epub 1994/01/01. PubMed PMID: 8126688.
52. Hollingshead AB. Four factor index of social status. In: Yale University NH, CT, editor. Unpublished manuscript1975.
53. Bechara A, Damasio H, Tranel D, Damasio AR. Deciding advantageously before knowing the advantageous strategy. Science (New York, NY). 1997;275(5304):1293-5. Epub 1997/02/28. PubMed PMID: 9036851.
54. Stroop JR. Studies of interference in serial verbal reactions. Journal of experimental psychology. 1935;18(6):643.
55. Kongs SK, Thompson LL, Iverson GL, Heaton RK. Wisconsin card sorting test-64 card version (WCST-64). Odessa, FL: Psychological Assessment Resources. 2000.
56. Langenecker SA, Zubieta JK, Young EA, Akil H, Nielson KA. A task to manipulate attentional load, set-shifting, and inhibitory control: convergent validity and test-retest reliability of the Parametric Go/No-Go Test. Journal of clinical and experimental neuropsychology. 2007;29(8):842-53. Epub 2007/09/14. doi: 10.1080/13803390601147611. PubMed PMID: 17852593.
57. Lusk G. Animal calorimetry: analysis of oxidation of mixtures of carbohydrates and fat. J Biol Chem. 1924;59:41-2.
58. Abbott WG, Howard BV, Christin L, Freymond D, Lillioja S, Boyce VL, et al. Short-term energy balance: relationship with protein, carbohydrate, and fat balances. The American journal of physiology. 1988;255(3 Pt 1):E332-7. Epub 1988/09/01. PubMed PMID: 3421330.
59. Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. Int J Obes Relat Metab Disord. 2000;24(1):38-48. Epub 2000/03/07. PubMed PMID: 10702749.
60. Stubbs RJ, Hughes DA, Johnstone AM, Rowley E, Reid C, Elia M, et al. The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. Br J Nutr. 2000;84(4):405-15. Epub 2000/12/05. doi: S0007114500001719 [pii]. PubMed PMID: 11103211.
61. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, et al. Cold-activated brown adipose tissue in healthy men. N Engl J Med. 2009;360(15):1500-8. Epub 2009/04/10. doi: 360/15/1500 [pii]

10.1056/NEJMoa0808718. PubMed PMID: 19357405.
62. Wijers SL, Saris WH, van Marken Lichtenbelt WD. Cold-induced adaptive thermogenesis in lean and obese. Obesity (Silver Spring). 18(6):1092-9. Epub 2010/04/03. doi: oby201074 [pii]

10.1038/oby.2010.74. PubMed PMID: 20360754.

63. Venti CA, Votruba SB, Franks PW, Krakoff J, Salbe AD. Reproducibility of ad libitum energy intake with the use of a computerized vending machine system. The American journal of clinical nutrition. 2010;91(2):343-8. Epub 2009/11/20. doi: 10.3945/ajcn.2009.28315. PubMed PMID: 19923376; PubMed Central PMCID: PMC2806891.
64. O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. Biometrics. 1979:549-56.
65. Pocock SJ. Group sequential methods in the design and analysis of clinical trials. Biometrika. 1977;64(2):191-9.
66. MacKinnon DP. Introduction to statistical mediation analysis. New York: Routledge; 2008.
67. Sobel ME. Asymptotic confidence intervals for indirect effects in structural equation models. Sociological methodology. 1982;13(1982):290-312.