2-5 Intermittent Caloric Restriction for Weight Loss and Insulin Resistance in HIV-infected Adults with Features of the Metabolic Syndrome

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Sponsored by:
National Institute of Allergy and Infectious Diseases (NIAID)

Principal Investigator (PI): Colleen Hadigan, MD, MPH
Laboratory of Immunoregulation (LIR)
NIAID
National Institutes of Health (NIH)
Building 10, Room 11C103
(301) 594-5754
hadiganc@niaid.nih.gov

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List of Abbreviations

2-5 ICR	2 days intermittent caloric restriction/week with 5 days of ad lib eating
AE	Adverse Event
AHA	American Heart Association
AIDS	acquired immunodeficiency syndrome
ART	antiretroviral therapy
BDNF	brain-derived neurotrophic factor
BMI	body mass index
CAP	controlled attenuation parameter
CCR	continuous caloric restriction
CD4	cluster of differentiation 4
CRIMSON	Clinical Research Information Management System of the NIAID
CRP	C-reactive protein
DXA	dual-energy X-ray absorptiometry
GCP	Good Clinical Practice
HIV	human immunodeficiency virus
HOMA-IR	homeostatic model assessment of insulin resistance
ICR	intermittent caloric restriction
IRB	Institutional Review Board
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
OGTT	oral glucose tolerance test
PANAS	Positive and Negative Affect Schedule
PI	Principal Investigator
PYY	peptide YY
REE	resting energy expenditure
SAE	Serious Adverse Event
TFEQ	Three-factor Eating Questionnaire
VAS	Sisual analog scale

Protocol Summary

Full Title:	2-5 Intermittent Caloric Restriction for Weight Loss and Insulin Resistance in HIV-infected Adults with Features of the Metabolic Syndrome
Short Title:	2-5 to WIN
Conducted by:	National Institute of Allergy and Infectious Diseases and National Institutes of Health Clinical Center
Principal Investigator:	Colleen Hadigan, MD, MPH
Sample Size:	N = 50; 25 subjects in intermittent caloric restriction arm, 25 subjects in lifestyle modification standard of care arm
Accrual Ceiling:	100
Study Population:	Human immunodeficiency virus (HIV)-infected adults aged 18 to 65 with a body mass index ≥30 kg/m^2 (obese) and at least one feature of the metabolic syndrome (increased waist circumference, systolic hypertension, dyslipidemia)
Accrual Period:	3 years
Study Design:	Randomized study with 2 parallel arms
Study Duration:	5 years (estimated start date of February 2018; end date January 2023
Intervention Description:	Prospective randomized 12-week diet intervention study; 2 days of intermittent caloric restriction (fasting) per week vs standard of care.
Primary Objective:	To evaluate the effects of 12 weeks of intermittent caloric restriction 2 days per week on weight and insulin sensitivity in obese HIV-infected individuals with features of the metabolic syndrome
Primary Endpoint:	Changes in weight and insulin sensitivity as measured by homeostatic model assessment of insulin resistance (HOMA-IR) between baseline and week 12

Précis

The high prevalence of obesity coupled with chronic inflammation and immune activation places human immunodeficiency virus (HIV)-infected individuals at increased risk for metabolic complications, emphasizing the need for more aggressive management of obesity and related comorbidities in the aging HIV-infected population. The most effective treatment for obesity and metabolic syndrome is lifestyle modification, usually with a combination of caloric restriction and increased exercise. Intermittent caloric restriction (ICR) or intermittent fasting simplifies caloric restriction by severely limiting calories only a few days per week and allowing ad lib diet on the other days. Weight loss benefits are similar to those seen with conventional diets; however, data suggest possible added health benefits from intermittent fasting.

We propose to study the benefits of a 2-5 ICR strategy on weight, insulin resistance, and cardiovascular disease markers in obese HIV-infected adults with features of the metabolic syndrome. In a prospective pilot study, 50 HIV-infected adults will be randomized 1:1 to ICR or standard-of-care instruction of healthy diet and lifestyle for a 12-week intervention period. We hypothesize that ICR (2 days per week) will be an effective and acceptable diet strategy that will result in significant weight reduction, improvements in insulin sensitivity, and related metabolic parameters.

1 Background Information and Scientific Rationale

1.1 Background Information

Since the introduction of antiretroviral therapy (ART), human immunodeficiency virus (HIV)-infected individuals have lower morbidity from opportunistic infections and increased life expectancy. However, as HIV-infected individuals live longer, the prevalence of comorbid conditions, including obesity and metabolic syndrome, is increasing.

In HIV-negative populations, obesity, alone or as part of the metabolic syndrome, is associated with increased risk of diabetes, hypertension, cardiovascular disease, and dementia. HIV infection and ART are independent risk factors for comorbidities, including central obesity, insulin resistance, diabetes, atherosclerosis, and dementia. Coupled with HIV-related inflammation and immune activation, the high prevalence of obesity places HIV-infected individuals at increased risk for metabolic complications and highlights the need for more aggressive management of obesity and related comorbidities in the aging HIV-infected population.

Both treated HIV infection and excess adipose tissue promote similar changes in immune activation that are implicated in the development of a range of chronic diseases; however, at present, there are little data on whether the effects of treated HIV and excess adiposity are synergistic or additive.

1.1.1 Weight Gain and Obesity in HIV

Many HIV-infected individuals starting ART experience a significant gain in weight, regardless of weight at the time of antiretroviral initiation [1-4]. With the move towards earlier start of ART, an increasing number of HIV-infected individuals will initiate ART with normal or excess body weight.

In a cohort of 175 HIV-infected ART-naïve adults with advanced HIV/acquired immunodeficiency syndrome (AIDS) (cluster of differentiation 4 [CD4] < 100 cell/mm^3) followed in the NIAID/Clinical Center intramural HIV/AIDS outpatient clinic from 2010 to 2012, 32% were overweight or obese at the time of ART initiation; rates nearly doubled after one year of therapy, with 63% of individuals overweight or obese. We have recently shown that this weight gain is accompanied by metabolic complications that include a high prevalence of non-alcoholic steatohepatitis and cardiac dysfunction [5, 6].

Our observations are mirrored in other cohorts. In one large US outpatient cohort, nearly half of HIV-infected individuals were overweight or obese at the time of ART initiation and weight increased to a higher body mass index (BMI) category within 2 years of initiation in 20% [4]. In a Veterans' Affairs cohort, HIV-positive veterans gained significantly more weight one year after initiation of ART compared to age and sex-matched HIV-negative controls [7].

Not surprisingly, these weight changes translate to increased risk of metabolic complications. In the Veterans' Affairs cohort, weight gain was more strongly associated with increased risk of incident diabetes mellitus in HIV-positive individuals (10% more risk per 5-lb gain) compared with HIV-negative individuals (6% more risk per 5-lb gain).

In the multi-cohort Data Collection on Adverse Events (AEs) of Anti-HIV Drugs (D:A:D) study, weight gain in the first year after initiation of ART is associated with an increased risk of cardiovascular disease and diabetes [8]. Taken together, these data suggest that weight gain in HIV-infected adults has significant implications for long-term health outcomes.

Treatment

For both HIV-infected and non-HIV-infected individuals, the most effective treatment for obesity and metabolic syndrome is lifestyle modification, usually a combination of caloric restriction and increased exercise [9-11]. A few small studies looking at lifestyle modification in HIV have shown improvement in dyslipidemia, waist circumference, systolic blood pressure, and hemoglobin (Hgb) A1C levels with dietary or exercise interventions [12-14].

Beyond lifestyle modification, subsequent approaches include drug therapy and, in obese individuals with serious medical comorbidities, bariatric surgery. In HIV-infected adults with lipodystrophy, a number of potential therapies have been studied unsuccessfully, including changes in ART, use of insulin-sensitizing agents, hormonal therapies such as growth hormone, growth hormone–releasing hormone, testosterone, and HMG-CoA reductase inhibitors (statins) [15].

1.1.2 Caloric Restriction and Intermittent Fasting

Caloric restriction without malnutrition slows aging and increases lifespan in a variety of organisms, including worms, spiders, fish, and rodents. In rhesus monkeys, caloric restriction reduces the incidence of type 2 diabetes mellitus, cardiovascular disease, and cancer, and may increase longevity [16, 17]. Caloric restriction also protects monkeys against age-related muscle loss and grey matter volume shrinkage in several key subcortical regions [18].

Studies in humans have confirmed the benefits of sustained calorie restriction; however, controversy exists related to subject selection, diet composition, and the duration of restriction required for beneficial effects to emerge. In one trial, adults following a calorie-restricted diet for 6 years (n=18) had improved body composition, lipid profile, blood pressure, insulin sensitivity, C-reactive protein (CRP), and carotid artery intima-media thickness compared to adults on an unrestricted diet (n=18) [19]. In a 6-month, randomized study of calorie restriction with or without exercise in overweight, non-obese adults (n=48), caloric restriction reduced metabolic rate independently of weight change and improved insulin sensitivity [20].

One hypothesis to explain the beneficial, anti-aging effects of calorie restriction is reduced energy expenditure with a consequent reduction in the production of reactive oxygen species (ROS) [21]. However, other metabolic effects associated with calorie restriction, including alterations in insulin sensitivity and signaling, neuroendocrine function, stress response, or a combination of these, may also contribute to the benefits [22].

ICR or intermittent fasting simplifies caloric restriction by severely limiting calories only a few days per week and allowing ad lib diet on the other days. Benefits are similar to those seen with caloric restriction (where daily caloric intake is reduced but meal

frequency is maintained). However, data from animal models and a few human studies suggest possible added benefits from intermittent fasting [23-25]. For example, in mouse studies performed by Dr. Mattson and his colleagues, intermittent fasting is superior to continuous calorie restriction for improving insulin sensitivity, cardiovascular function, and for protecting neurons in animal models of Alzheimer's and Parkinson's diseases [25-28].

In a cohort of overweight adults with asthma, Dr. Mattson's group has shown that 8 weeks of alternate day caloric restriction decreased weight (~ -8% of baseline body weight), reduced peripheral insulin resistance, reduced plasma inflammatory markers, including decreased tumor necrosis factor-α (TNF-α, Figure 1), and decreased levels of ROS-induced 8-isoprostane, protein carbonyl, and nitrotyrosine (Figure 2) [29].

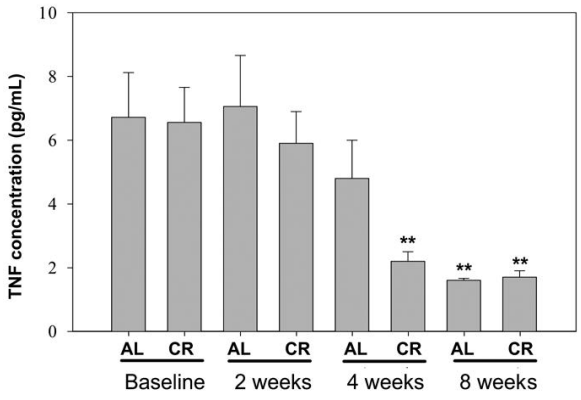

Figure 1.

The effect of alternate day calorie restriction (CR) on serum TNF-α. By the 8-week time point, concentrations were greatly reduced regardless of assessment on ad libitum (AL) or restricted (CR) days.

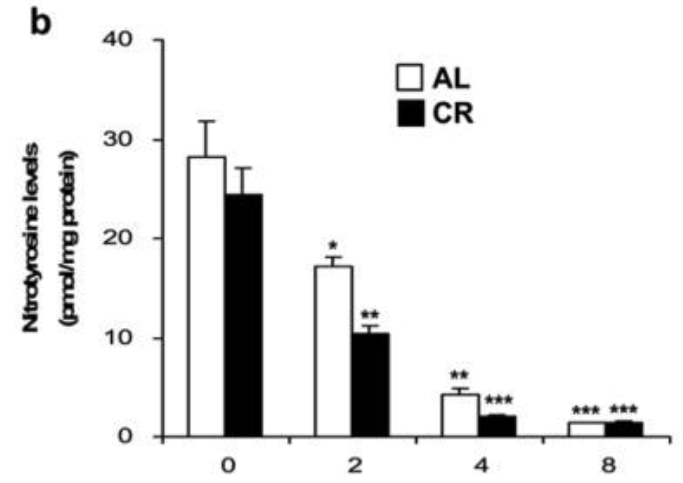

Figure 2.

The effect of alternate day calorie restriction (CR) on serum levels of nitrotyrosine. By the 8-week time point, levels decreased regardless of assessment on ad libitum (AL) or restricted (CR) days.

In a 6-month study comparing intermittent with continuous caloric restriction (CCR) in overweight women, both regimens produced comparable weight loss (average loss of 6 kg) and reductions in total and low-density lipoprotein cholesterol, triglycerides, blood pressure, and CRP [30]. ICR resulted in significantly greater reduction in visceral fat and improvement in insulin sensitivity relative to continuous restriction and was easier for subjects to maintain. Several short-term studies have shown similar benefits and confirmed the safety of the approach, with no evidence of overconsumption on unrestricted diet days [31-35].

The mechanisms underlying the benefits of intermittent fasting are beginning to be established. Fasting mice have higher levels of brain-derived neurotrophic factor (BDNF), a protein that regulates synaptic plasticity, neurogenesis, and neuronal survival in the brain. Recent work by Dr. Mattson and others has identified a widespread role for BDNF in regulation of energy homeostasis. BDNF influences multiple cell types in the body that are involved in glucose metabolism, including pancreatic β cells (increased insulin production), hepatocytes (decreased glucose production), and skeletal muscle (increased insulin sensitivity) [36-38]. Fasting also appears to increase autophagy, a mechanism by which cells can eliminate damaged molecules, including ones that have been previously associated with Alzheimer's, Parkinson's, and other neurological diseases. Moreover, in contrast to caloric restriction without fasting, intermittent fasting results in depletion of liver glycogen stores, release of fatty acids from adipose cells, and metabolism of the fatty acids to generate ketone bodies. Ketone bodies may mediate some of the beneficial effects of intermittent fasting on the brain and cardiovascular systems [39], and recent findings suggest that ketones can protect neurons in experimental models relevant to HIV-associated neurocognitive disorders [40].

1.2 Rationale

With the rising prevalence of overweight and obesity in HIV-infected individuals, strategies to prevent and reverse weight-related comorbidities are needed. Caloric restriction can reduce weight and improve insulin resistance; however, daily caloric restriction is difficult to maintain long-term. Limited data suggest ICR may be easier to achieve and is superior to CCR for improving insulin sensitivity, cardiovascular function, and neuroprotection.

This study seeks to evaluate the tolerability and effects of ICR on body weight, insulin sensitivity, cardiovascular disease risk, and cognitive function. Additionally, we aim to characterize the effects on body composition, hepatic steatosis, resting energy expenditure (REE), plasma biomarkers of inflammation, immune activation, and metabolism. The results of this study may provide valuable information on the interactions between obesity and HIV-related inflammation and associated risk of non-AIDS disorders, and educate the design of future studies of obesity and associated comorbidities in HIV-infected populations.

2 Study Objectives

2.1 Primary Objectives

- To evaluate the effects of 12 weeks of ICR 2 days per week on weight and insulin sensitivity in obese HIV-infected individuals with features of the metabolic syndrome.

2.2 Secondary Objectives

- To evaluate the effects of ICR on body composition, including visceral adiposity and hepatic steatosis, lipids levels, metabolic biomarkers, biomarkers of inflammation and immune activation, and mood.
- To assess the tolerability of ICR.

3 Study Design

3.1 Description of the Study Design

After completing screening procedures and establishing eligibility, all subjects will receive standard-of-care recommendations for healthy diet and lifestyle and will be randomized 1:1 to either 2 days of ICR per week with 5 days of ad lib eating (2-5 ICR) or no additional intervention (see below).

Standard of Care (n=25)	**2-5 ICR (n=25)**
• AHA dietary counselling • Lifestyle and physical activity counselling • Pedometer and instructions for use	• AHA dietary counselling • Lifestyle and physical activity counselling • Pedometer and instructions for use
-----	• ICR guidelines and instructions • Supplemental beverages for ICR days

All subjects will receive dietary counseling consistent with the American Heart Association (AHA) diet recommendations. Like the standard-of-care arm, the 2-5 ICR subjects will implement the AHA diet 5 days per week; however, on 2 days per week will consume approximately 25% of their estimated daily caloric needs in the form of supplemental beverages or small meals. Assessments will occur prior to randomization and at 1, 4, 8, and 12 weeks. All subjects will then complete a follow-up visit to assess changes in weight and metabolic parameters 12 weeks after the intervention period is complete.

3.1.1 Study Endpoints

3.1.1.1 Primary Endpoint

Change in weight and insulin sensitivity as measured by homeostatic model assessment of insulin resistance (HOMA-IR) between baseline and week 12.

3.1.1.2 Secondary Endpoints

1. Tolerance and adherence to diet assignment
2. Change in the following after 12 weeks of caloric restriction:
    - Visceral adiposity as measured by dual x-ray absorptiometry (DXA) and waist circumference
    - Fat and lean mass as measured by DXA
    - Hepatic steatosis and liver stiffness as measured by transient elastography
    - Lipid profile
    - REE as measured by indirect calorimetry
    - Serum biomarkers of inflammation (eg, CRP, TNF-alpha) and immune activation

4 Study Population

4.1 Recruitment Plan

Subjects will be recruited from existing National Institutes of Health (NIH) cohorts as well as HIV care clinics in the greater Washington, DC area. In addition, we will use the OP8 Clinic recruitment strategies that are in place and include a full-time subject recruiter and community-based outreach within local area clinics specializing in HIV care.

4.2 Subject Inclusion Criteria

1. Aged 18 to 65 years
2. HIV RNA level ≤200 copies/mL for ≥1 year (1 measure ≥200 allowed if also <500 and preceded and followed by one or more undetectable values)
3. CD4 >200 cells/mL and no active opportunistic infection or malignancy
4. BMI ≥30 kg/m^2
5. One or more components of the metabolic syndrome as defined in the table below.

Table 1. *ATPIII Clinical Identification of the Metabolic Syndrome [41]*

Risk Factor	Defining Level
Waist circumference	
Men	>102 cm
Women	>88 cm
Triglycerides	≥150 mg/dL
High density lipoprotein (HDL) cholesterol	
Men	<40 mg/dL
Women	<50 mg/dL
Blood pressure	≥130/≥85 mmHg
Fasting glucose	≥110 mg/dL

6. Fasting blood glucose >60 mg/dL at screening
7. Willingness to allow sample storage for future research
8. Able to provide informed consent

4.3 Subject Exclusion Criteria

1. Established diagnosis of diabetes mellitus, use of anti-diabetes medications, or a Hgb A1C of >7.0%
2. History of eating disorder, uncontrolled mood or thought disorder, significant gastrointestinal disorder or malabsorption, or significant hepatic or renal impairment
3. Current use of medical therapy for overweight/obesity including phentermine, orlistat, lorcaserin, naltrexone/bupropion, and liraglutide or history of weight loss surgery. Concomitant use of medications with side effects known to potentially influence appetite are allowed if on a stable dose for at least 12 months
4. History of symptomatic hypoglycemia.
5. Use of systemic glucocorticoids (stable dose daily inhaled corticosteroid allowed)
6. Chronic viral hepatitis C; subjects with a history of hepatitis C successfully treated can enroll >12 months after sustained virologic response
7. Alcohol or substance use disorder in the past year as defined by DSM-V or positive urine drug screen
8. Current pregnancy, actively seeking to become pregnant or breastfeeding

9. Any serious health or other condition which, in the opinion of the PI or their designee, could potentially interfere with the ability of a subject to comply with the procedures and assessments of the protocol or to safely participate and complete the study.

Co-enrollment Guidelines: Co-enrollment in other trials is restricted, other than enrollment on observational studies. Study staff should be notified of co-enrollment as it may require the approval of the PI.

4.4 Justification for Exclusion of Special Populations

Exclusion of Pregnant and/or Breastfeeding Women:

- **Pregnancy:** Pregnant women are excluded from this study because the effects of ICR on the developing human fetus are unknown. Additionally, the metabolic changes associated with pregnancy could would confound assessment of the effects of diet intervention.
- **Breastfeeding:** Nursing mothers are excluded from study participation because the nutritional needs of nursing mothers require stable caloric intake and the potential risk of maternal intermittent fasting nursing is unknown.

Exclusion of Subjects <18 years of age: Persons age <18 years are excluded from this study because there are insufficient data regarding the risk of ICR in adults to judge the potential risk in growing children and young adults.

Exclusion of Subjects >65 years of age: Persons age >65 are excluded from this study because there are insufficient data regarding the risk of ICR in adults <65 years of age to judge the potential risk in aging adults. Additionally, changes in body composition and resting metabolic rate independent of weight change, occur with advanced age and could potentially confound study measures [42].

Exclusion of Subjects Who Cannot Provide Informed Consent: To ensure compliance with study-mandated diet and lifestyle, subjects must be able to provide initial and ongoing informed consent to participate in this study.

5 Study Interventions

5.1 Assessment of Subject Compliance with Study Intervention(s)

Subjects will be asked to keep a daily diary, which will record how well they complied with their assigned diet as well as their daily step count. Subjects in the ICR arm will be asked to indicate which days they restrict calories. These diaries will be reviewed with the study team at all study visits.

5.2 Concomitant Medications and Procedures

All concomitant medications, including prescription, over-the-counter, and non-prescription medications will be recorded.

5.3 Prohibited Medications and Procedures

During study participation, subjects may not initiate medical therapy or undergo surgical intervention intended to promote weight loss. Any change in medications, including supplement and vitamin use, or proposed surgical intervention should be discussed with the study team.

6 Study Schedule and Procedures

6.1 Screening

The following procedures will be performed at the NIH Clinical Center to determine if a subject is eligible for study participation and are expected to be completed over 2 visits:

1. History and physical exam and vital signs including measurement of blood pressure, height, and weight
2. Blood collection for laboratory assessments including acute care, hepatic, and mineral panels, Hgb A1c, fasting glucose and insulin, fasting lipid profile, complete blood counts, CRP, HIV viral load, CD4+ T-cell count, and hepatitis serologies
3. Urine studies including urinalysis, urine protein/creatinine ratio and albumin/creatinine ratio, and urine drug screen
4. Blood or urine pregnancy testing for women of childbearing potential
5. Nutrition consultation for body composition measurements including weight, and waist and hip circumference. Subjects will also receive instructions on completion of 3-day food diary.
6. Receive a pedometer and instruction on its use to assess daily activity level
7. 1-day trial of the very low-calorie diet (approximately 25% of estimated needs) to assess ability and motivation to lose weight through ICR

6.2 Baseline Assessments

Prior to randomization, subjects will undergo baseline assessments to include:

1. Additional laboratory studies including fasting glucose and insulin (for HOMA-IR), lipid panel, CRP, leptin, ghrelin, peptide YY (PYY), BDNF, urine ketones, and inflammatory biomarkers
2. Oral glucose tolerance test (OGTT)
3. Indirect calorimetry (REE)
4. Nutrition consultation for review of 3-day food diary and counseling on the AHA diet and caloric restriction. Subjects will also receive information on recommended physical activity and ways to increase daily steps.
5. Questionnaires:
    a. Three-factor eating questionnaire (TFEQ) [43]

b. Symptoms Review Questionnaire
c. Beck Depression Inventory II
d. RAND Medical Outcomes Study 36-Item (SF-36; quality of life)
e. Positive and Negative Affect Schedule (PANAS)
f. Modified visual analog scales (eg, mood, hunger)

6. DXA
7. Blood or urine pregnancy testing for women of childbearing potential
8. Transient elastography with controlled attenuation parameter (CAP) (Fibroscan®, Echosens, Paris, France)

Baseline assessments may be completed over multiple visits within 4 weeks of randomization.

6.3 Randomization (Day 0)

Study subjects will be randomized sequentially to the ICR arm or the observation arm.

All subjects will receive dietary counseling consistent with the AHA diet recommendations, physical activity recommendations for healthy lifestyle, and a pedometer. Subjects randomized to the 2-5 ICR group will implement the AHA diet 5 days per week, and on 2 non-consecutive days per week, will consume approximately 25% of estimated caloric need in the form of supplemental beverages or small meals. Subjects randomized to the standard-of-care group will receive the AHA dietary guidelines, which will include healthy food selection, portion size control, education on caloric goals for healthy weight management, physical activity recommendations, and pedometers, but not instruction on intermittent fasting.

6.4 Study Phase (Weeks 1-12)

Interim safety visits will be scheduled for weeks 1, 4, 8, and 12. Subjects will visit the NIH Clinical Center as outpatients. The following assessments and procedures will occur:

1. Vital signs including weight and blood pressure
2. Physical exam and interval medical history at weeks 4, 8, and 12
3. Fasting blood collection for acute care, hepatic, and mineral panels, insulin, glucose and lipids, and CRP, as well as serum, plasma, and PBMCs for storage at weeks 4, 8 and 12. Hgb A1C at week 12. Leptin, ghrelin, PYY, BDNF, inflammatory markers, urine ketones and HIV viral load will be repeated at weeks 4, 8, and 12.
4. Nutrition consultation at weeks 4 and 12: Completion of 3-day food diary and waist and hip measurements at weeks 4 and 12
5. Review of subject diary documenting compliance and daily steps at weeks 1, 4, 8, and 12

6. Symptoms Review Questionnaire at weeks 1, 4, 8 and 12
7. TFEQ at weeks 4, 8, and 12
8. Modified visual analog scales at week 12
9. At week 12, baseline assessments will be repeated. Assessments include:
    - Beck Depression Inventory, RAND SF-36, and PANAS questionnaires
    - REE
    - OGTT
    - DXA
    - Blood or urine pregnancy testing for women of childbearing potential
    - Transient elastography with CAP (Fibroscan®, Echosens, Paris, France)

Study visit windows are as follows: Week 1: +/-3 days, Weeks 4, 8, and 12: +/- 7 days.

6.5 Final Follow-up Visit (Week 24)

After completing the 12-week randomized portion of the study, subjects will be followed for an additional 12 weeks during which time they will not be instructed to follow any specific dietary pattern. Both groups' visits will occur at week 24. (+/- 4 weeks)

The following procedures will be performed:

1. Vital signs including weight and blood pressure
2. Physical exam and interval medical history
3. Fasting blood collection for acute care, hepatic, and mineral panels, Hgb A1C, insulin, glucose and lipids, as well as serum, plasma, PBMCs for storage
4. Symptoms Review Questionnaire
5. Modified visual analogue scale
6. Transient elastography with CAP
7. 3-day food diary and anthropometric measurements
8. REE
9. DXA scan
10. Blood or urine pregnancy testing for women of childbearing potential

6.6 Early Termination Visit

If a subject's participation is terminated before completing the study, then they will be asked to return to the NIH Clinical Center for a Termination Visit. Laboratory tests and study evaluations to be conducted at that visit will be determined on the basis of the subject's health, their willingness, and the appropriateness of the test at that time point.

6.7 Pregnancy and Follow-up Visit

If a subject becomes pregnant during the course of participation, then her participation in the study will be stopped and she will be counseled to discuss diet and nutrition with her obstetrician. The study team will request that the subject notify the team of the pregnancy outcome.

7 Study Procedures/Evaluations

History and Physical Examination

The **initial history**, performed at the screening visit, will consist of a comprehensive review of the subject's medical history, including any previous surgical procedures and any previous significant medical conditions. A comprehensive review of systems will also be performed. A detailed medication history will be taken. Allergies and all current medications will be recorded. **Interval history**, performed at all subsequent visits, will consist of questioning subjects about any new medical issues or any noted changes in health. The most recent medication list will be reviewed with subjects to assess for any changes in dosing, new medications, or medication discontinuation. **Physical examination** will consist of a review of vital signs as well as examination of general appearance (visualization), heart (auscultation), lungs (auscultation), abdomen (palpation and auscultation), and extremities (visualization and palpation). Additional elements of the physical examination may be performed at the investigator's discretion.

Blood Draw

To assess clinical safety parameters, as well as research measures, blood will be drawn at time points listed above.

Table 2. Venipuncture Volumes for Subjects

	Screen	***Baseline***	**Week 1**	**Week 4**	**Week 8**	**Week 12**	**Week 24**
Day +/- Window	**−1 to 1**	**1 ± 1**	**3 ± 1**	**5 ±1**	**8 ± 1**	**11 ± 1**	**29 + 6**
Safety hematology, chemistry and OGTT tests	X 26mL	X 22mL	--	X 19mL	X 19mL	X 32mL[2]	X[3] 22mL[2]
Blood for Serum	X 8mL	X 16mL	--	X 16mL	X 16mL	X 16mL	X 16mL
Blood for Plasma	X 12mL	--	--	X 12mL	X 12mL	X 12mL	X 12mL
Blood for PBL Storage	--	20mL	--	20mL	20mL	20mL	20mL
Total volume	46mL	58mL	--	67mL	67mL	80mL	70mL
Total for all study visits							388mL

Pedometer

To assess activity at baseline and the impact of the diet intervention on activity, subjects will use a pedometer to record daily steps. This will be recorded on a patient diary and reviewed by the study staff at clinic visits.

Transient Elastography and CAP

Liver stiffness will be determined using transient elastography [44] and hepatic fat content estimated by CAP. At least ten measurements will be made using the M-probe according to the manufacturer's recommendations and the median will be expressed in kilopascal (kPa) units. If measurements from the M-probe are unsuccessful at the baseline assessment, the XL probe may be used at the operator's discretion. Use of the XL probe at baseline will be noted, and the XL probe will also be used for subsequent assessment of that subject.

Whole Body DXA will be performed to determine total body and regional percent fat, lean body mass, and bone density. The technique has a precision error (1 SD) of 3% for fat and 1.5% for lean body mass [45]. Trunk, extremity, and trunk-to-extremity ratio will also be assessed [46, 47].

REE

REE is measured by indirect calorimetry. Following at least a 4-hour fast, a large plastic cover or "bubble" is placed over the subject's head while a plastic sheet covers the subject's upper body to prevent external air from entering the bubble. Oxygen flows into the bubble through a valve at the top. The calorimeter measures the amount of oxygen (O_2) consumed and the amount of carbon dioxide (CO_2) produced while at rest by comparing the concentrations of O_2 and CO_2 in the air inspired by the subject with the concentration in the air expired by the subject. The modified Weir equation is used to convert the volume of O_2 consumed and the volume of CO_2 produced per minute into a value for REE expressed in calories [48].

Genetic Analysis

PBMCs will be stored and analyzed for single nucleotide polymorphisms that may affect metabolism and immunology.

OGTT

75-g OGTT will be performed with sampling for insulin and glucose at 0, 30, 60, and 120 min.

Nutritional Analysis

A 3-day food record, completed at baseline and weeks 4 and 12, will be analyzed for protein, carbohydrate, fat, micronutrient, and alcohol intake (Nutrition Data Systems).

Anthropometric Measurements

Measurements of waist-to-hip ratio will be performed using a standardized technique [49].

TFEQ and Modified Visual Analog Scale

The TFEQ will be administered to measure three aspects of eating attitudes: cognitive restraint of eating, disinhibition, and hunger [50]. Modified visual analog scales (VAS) will be used to assess subjective hunger and satiety three times a day the days prior to week 0 and week 12 assessments [51, 52]. VAS assesses 'desire to eat,' 'hunger', 'fullness,' and 'prospective consumption.'.

PANAS

Subjects will report on Likert scales how they feel emotionally. The PANAS is a common index of emotional state [53].

Symptoms Review Questionnaire

Subjects will report on Likert scales their mood, level of energy, difficulty concentrating, headaches, feeling cold, constipation, negative emotions (depression, anxiety, irritability), hunger, and preoccupation with food for the week prior to the visit.

RAND SF-36 scale

This questionnaire gauges physical and psychosocial effects of the dietary interventions. It has been used as a quality of life assessment in previous studies of ICR [30].

7.1 Return of Research Results

Any clinically relevant test results will be shared with the subject throughout the study. Results of research procedures or evaluations (including incidental findings) will be shared with participants if they are medically actionable. Such results will be discussed with the participant along with guidance for appropriate follow-up with their healthcare provider. Any findings discovered beyond the completion of the primary research will not be returned.

8 Potential Risks and Benefits

8.1 Potential Risks

Blood Draw. Collection of blood may be associated with discomfort, bruising, local hematoma formation and, on rare occasions, infections, lightheadedness, and fainting. The amount of blood drawn for research purposes will be within the limits allowed for adult subjects by the NIH CC (Medical Administrative Policy 95-9: Guidelines for Limits of Blood Drawn for Research Purposes in the Clinical Center: http://cc-internal.cc.nih.gov/policies/PDF/M95-9.pdf.

Pedometer. No risks are associated with pedometer use and it is associated with minimal inconvenience.

OGTT. No serious risks are associated with the OGTT. Some subjects experience temporary abdominal discomfort after drinking the sugar solution. If the results of an OGTT demonstrate glucose intolerance or insulin resistance, recommendations will be made for appropriate follow-up monitoring and management.

REE. Subjects may experience claustrophobia during the measurements.

Transient elastography. There are no foreseeable risks associated with this non-invasive procedure.

DXA. Subjects will be exposed to radiation from three DXA scans, for total estimated effective dose in 1 year of 0.00007 rem. This is considered a low exposure. The risk of this exposure is too low to be reliably measured.

Caloric restriction. [29, 30, 57, 58] In previous studies of ICR, no serious adverse events (SAEs) occurred. In the study by Harvie et al., a 6-month trial, 8% of subjects on the 2-5 ICR regimen reported physical symptoms including lack of energy, headaches, feeling cold, and constipation. Minor psychological symptoms were reported in 15% of the 2-5 ICR group, including lack of concentration, bad temper, and a preoccupation with food. These adverse effects abated after caloric restriction was stopped [29, 30]. Conversely, 6% of 2-5 ICR subjects reported better health and increased energy [30]. Furthermore, 32% of 2-5 ICR had increased self-confidence and positive mood. Overall, a minority of subjects (9-15%) would be expected to develop minor physical and psychological AEs related to 2-5 ICR. Such AEs include lethargy, feeling cold, difficulty concentrating, and irritability. Study subjects will be counseled on potential side effects and will record symptoms in the diet and symptom diary.

8.2 Potential Benefits

Subjects may not receive any benefit from this study. However, there is the potential that the nutrition counseling and diet support received may result in reductions in weight and/or improvements in insulin sensitivity, visceral adipose tissue, hepatitis steatosis, and adipose-tissue associated inflammation, potentially reducing cardiovascular disease risk.

9 Research Use of Stored Human Samples, Specimens or Data

- **Intended Use:** Samples and data collected under this protocol may be used to study HIV, immune disorders, metabolic disorders, and inflammation. Limited genetic testing will be performed.
- **Storage:** Access to stored samples will be limited using a locked room or locked freezer. Samples and data will be stored using codes assigned by the investigators. Data will be kept in password-protected computers. Only investigators will have access to the samples and data.
- **Tracking:** Samples and data acquired as part of this protocol will be tracked using the Clinical Research Information Management System of the NIAID (CRIMSON) database.
- **Disposition at the Completion of the Protocol:** In the future, other investigators (both at NIH and outside) may wish to use these samples and/or data for research purposes. If the planned research falls within the category of “human subjects research” on the part of the NIH researchers, IRB review and approval will be obtained. This includes the NIH researchers sending out coded

and linked samples or data and getting results that they can link back to their subjects.

- **Reporting the Loss or Destruction of Samples/Specimens/Data to the IRB:**

  Any loss or unanticipated destruction of samples (for example, due to freezer malfunction) or data (for example, misplacing a printout of data with identifiers) that meets the definition of a reportable event will be reported to the NIH IRB according to Human Research Protections Program (HRPP) Policy 801. Additionally, subjects may decide to withdraw from research participation at any point and not have their samples stored. In this case, the PI will destroy all known remaining samples and report what was done to both the subject and to the IRB. This decision will not affect the subject's participation in other protocols at NIH.

10 Remuneration Plan for Subjects

Eligible subjects will receive partial remuneration from the NIH for immediate costs associated with study-related expenses (eg, transportation and lodging) according to established NIH/NIAID guidelines. Subjects will also be compensated for the time and inconvenience of study participation according to the attached schedule (see Appendix A). Thus, remuneration up to $880 for completion of the entire study, commensurate with the time and inconvenience of study participation including blood draws, imaging, inpatient assessments, and optional adipose tissue biopsies.

11 Assessment of Safety

11.1 Toxicity Scale

The investigator will assess all AEs with respect to **Seriousness** (criteria referenced in Section 11.3 Definitions), **Severity** (intensity or grade) and **Causality** (relationship to study interventions according to the following guidelines

Severity: The Investigator will grade the severity of each AE according to the "Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events" Version 2.1, July 2017, which can be found at:

https://rsc.tech-res.com/docs/default-source/safety/division-of-aids-(daids)-table-for-grading-the-severity-of-adult-and-pediatric-adverse-events-corrected-v-2-1.pdf

Some grade 1 lab parameters on the DAIDS Toxicity Table (fibrinogen, potassium [low], uric acid [males only, elevated]) fall within the NIH lab reference range for normal values. These normal values will not be recorded as grade 1 AEs. The grade 1 values for these tests will be recorded as follows:

- Fibrinogen: 100-176 mg/dL
- Potassium (low): 3.0-3.3 mmol/L
- Uric acid (males): 8.7-10.0 mg/dL
- Magnesium (low): 0.60-0.65 mmol/L

Causality: Causality (likelihood that the event is caused by the study intervention will be assessed considering the factors listed under the following categories:

Definitely Related:

- reasonable temporal relationship
- follows a known response pattern
- clear evidence to suggest a causal relationship
- there is no alternative etiology

Probably Related:

- reasonable temporal relationship
- follows a suspected response pattern (based on similar agents)
- no evidence of a more likely alternative etiology

Possibly Related

- reasonable temporal relationship
- little evidence for a more likely alternative etiology

Unlikely Related

- does not have a reasonable temporal relationship

  or

- good evidence for a more likely alternative etiology

Not related

- does not have a temporal relationship

  or

- definitely due to an alternative etiology

Other factors will also be considered for each causality category when appropriate. Causality assessment is based on available information at the time of the assessment of the AE. The investigator may revise the causality assessment as additional information becomes available.

11.2 Recording/Documentation

At each contact with the subject, information regarding AEs will be elicited by appropriate questioning and examinations. All events, both expected/unexpected and related/unrelated will be recorded on a source document. Source documents will include progress notes, laboratory reports, consult notes, phone call summaries, survey tools and data collection tools. Source documents will be reviewed in a timely manner by the research team. All reportable AEs that are identified will be recorded in CRIMSON. The start date, the stop date, the severity of each reportable event, and investigator judgment of the relationship and expectedness to the study intervention(s) will also be recorded in CRIMSON.

11.3 Definitions

Reportable events and adverse events are defined in NIH HRPP Policy 801.

11.4 Reporting Procedures

Unanticipated problems (UPs), non-compliance, and other reportable events will be reported to the NIH IRB according to Policy 801.

The principal investigator will report UPs, major protocol deviations, and deaths to the NIAID clinical director according to institutional timelines.

11.5 Reporting of Pregnancy

In the event of pregnancy:

- The subject will discontinue the study intervention (caloric restriction) and procedures but continue to follow-up in person and/or over the phone for safety and outcome
- Pregnancy will be reported to the IRB
- Research subject will be advised to notify the obstetrician of her study participation

11.6 Type and Duration of the Follow-up of Subjects after AEs

AEs that occur from the time of enrollment of the subject are followed until the final outcome is known or until the end of the study follow-up period. SAEs that have not resolved by the end of the follow-up period are followed until final outcome is known. If it is not possible to obtain a final outcome for an SAE (eg, the subject is lost to follow-up), then the reason a final outcome could not be obtained will be recorded by the investigator.

11.7 Withdrawal of an Individual Subject

An individual subject will be withdrawn for any of the following:

- An individual subject's decision (the investigator will attempt to determine the reason for the subject's decision.)
- Non-compliance with study procedures to the extent that it is potentially harmful to the subject or to the integrity of the study data.
- Any clinical AE, laboratory abnormality, intercurrent illness, other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject.
- The subject loses the capacity to provide ongoing informed consent.
- Development of an exclusion criteria may be cause for discontinuation.

11.8 Replacement of a Subject Who Discontinues Study Treatment

If a subject withdraws after fewer than 12 weeks on study diet assignment, then they will be replaced. If a subject is replaced, their data will still be included for the safety assessments.

11.9 Safety Monitoring Plan

The data gathered during this study will be monitored by the PI for safety and compliance with protocol-specified requirements. Subjects will be closely monitored for weight loss. If a subject loses ≥5% of their body weight within a 2-week period, additional safety visits will be scheduled to monitor body weight more closely. If weight loss in that range or greater continues to occur, subjects will be asked to discontinue caloric restriction and monitored.

12 Statistical Considerations

12.1 Overview and Design

This is a randomized controlled, study of 12 weeks of ICR versus standard of care. Changes from baseline to 12 weeks in measures of the primary endpoints (weight and insulin sensitivity) and secondary endpoints (eg, hepatic fat content, inflammatory biomarkers) will be compared between the 2 study arms. The 12-week follow-up period after the randomized portion of the study is designed to monitor changes in weight and metabolic parameters after completion of the 2-5 ICR diet arm and the standard-of-care arm.

12.2 Study Hypotheses

Subjects in the 2-5 ICR group will experience greater weight loss and greater improvements in insulin sensitivity and related metabolic parameters, including decreased visceral adipose tissue and hepatic fat content, compared to subjects in the standard of care arm.

12.3 Sample Size Justification

Using an alternate day fasting strategy for weight loss in obese adults (n=13), Catenacci et al [57] observed that subjects lost 8.2±0.9 kg (mean ± standard error of the mean) after 8 weeks. For sample size justification, we will use a conservative estimate of 8-kg weight loss after 12 weeks with ICR, and assume 4-kg weight loss among the observation group due to monitoring and counselling through study visits. Therefore, using the standard deviation estimate from Catenacci et al. of 3.24, we will have 96.7% power to detect a 4-kg difference in weight loss between treatment arms with 25 subjects in each arm. With 17 subjects per arm (ie, approximately a 30% dropout rate), the power will be 93.7% to detect a 4-kg difference in weight loss.

In previous studies of ICR, HOMA-IR improvements ranged from 9% to 30%, depending on a number of factors, including the duration of the study, extent and timing of caloric restriction, and whether or not meals were provided [59]. Assuming a baseline HOMA-IR of 1.5, a standard deviation of change in HOMA-IR of 0.25, a study size of

25 subjects per arm, we will have >80% power to detect the difference between a 25% improvement in insulin sensitivity with ICR versus a 10% improvement in the observation arm as measured by HOMA-IR. The power to detect this difference will be 72.1% with 17 subjects per arm.

12.4 Description of the Analyses

For the primary endpoint, the between group effect on weight will be tested using a two-sided t-test (p-value <0.05) of the calculated change in weight from baseline to 12 weeks. This will be conducted using on-treatment data, ie, data from all randomized subjects who completed baseline and 12 weeks evaluations regardless of reported compliance. Similarly, between group differences in insulin resistance measured by HOMA-IR will be determined. A secondary analysis will use analysis of covariance to adjust for the baseline value of the given endpoint.

In the analyses of the secondary endpoints (eg, visceral adiposity, serum lipids, markers of inflammation, hepatic fat content results), two-sided t-test comparing change from baseline to 12 weeks will be calculated between groups. In addition, within group paired t-tests may be performed to determine if, during the 12 week follow up if any of the changes during the randomized intervention portion of the study are sustained after 12 weeks of no intervention.

13 Ethics/Protection of Human Subjects

13.1 Informed Consent Process

Informed consent is a process where information is presented to enable persons to voluntarily decide whether or not to participate as a research subject. It is an on-going conversation between the human research subject and the researchers which begins before consent is given and continues until the end of the subject's involvement in the research. Coercion and undue influence will be minimized by informing participants that their decision to join the study will not affect any medical care they are currently receiving or their eligibility to participate in other research studies at the NIH. Participants will be given as much time as they need to read the consent form and ask questions of the investigators. Participants will also be given time to discuss their participation with family members, friends, and other healthcare providers.

Informed consent will be obtained in person in a private setting by a study team member authorized to obtain consent. The subjects will sign the informed consent document prior to undergoing any research procedures. The subjects may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subjects for their records. The researcher will document the signing of the informed consent form in the subject’s medical record. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

13.1.1 Considerations for Consent of NIH Staff

Even though NIH staff members are not targeted, if they are incidentally enrolled, then informed consent will be obtained as detailed above with following additional protections:

Consent from staff members will be obtained by an individual independent of the staff member's team whenever possible. Otherwise, the consent procedure will be independently monitored by the Clinical Center Department of Bioethics Consultation Service in order to minimize the risk of undue pressure on the staff member.

13.2 Subject Confidentiality

All records will be kept confidential to the extent provided by federal, state and local law. The study monitors and other authorized representatives of the Sponsor may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records. Records will be kept locked and all computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB or Office for Human Research Protections.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the NIH. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

14 Data Handling and Record Keeping

14.1 Data Capture and Management

Study data will be maintained in CRIMSON and collected directly from subjects during study visits and telephone calls, or will be abstracted from subjects' medical records. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary to confirm the data abstracted for this study. Data entry into CRIMSON will be performed by authorized individuals. The Investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner.

14.2 Record Retention

The investigator is responsible for retaining all essential documents listed in the International Conference on Harmonisation Good Clinical Practice Guideline. Study records will be maintained by the PI for a minimum of 7 years and in compliance with institutional, IRB, state, and federal medical records retention requirements, whichever

is longest. All stored records will be kept confidential to the extent required by federal, state, and local law.

Should the investigator wish to assign the study records to another party and/or move them to another location, the investigator will provide written notification of such intent to Office of Clinical Research Policy and Regulatory Operations (OCRPRO)/NIAID with the name of the person who will accept responsibility for the transferred records and/or their new location. NIAID will be notified in writing and written OCRPRO/NIAID permission shall be obtained by the site prior to destruction or relocation of research records.

15 Data Sharing

What data will be shared?

The study team will share human data generated in this study for future research as follows

- De-identified data in an NIH-funded repository.
- De-identified or identified data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?

Data will be shared through:

- An NIH-funded repository. After the study is completed data and samples will be transferred and maintained under the following protocol: Protocol for the Management and Use of Stored Human Specimens NIAID Protocol Number: 09-I-N066.
- Approved outside collaborators under appropriate individual agreements.
- Publication and/or public presentations.

When will the data be shared?

At the time of publication or shortly thereafter.

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APPENDICES

Appendix A: Remuneration Schedule

Visit/Procedure	Hours (remuneration)	Inconvenience Units (remuneration)	Total Remuneration Per Visit
Screening visit(s): Consent, history and physical, anthropometrics, laboratory studies, Nutrition consult	4 ($50)	3 ($30)	$80
Baseline visit(s): 1 day Diet Trial, Nutrition consult, REE, Fibroscan and CAP, OGTT, DEXA	6 ($80)	12 ($120)	$200
Randomization	2 ($30) per visit	2 ($20) per visit	$50
Weeks 1, 4, 8, 12: history and physical, education, laboratory assessments	2 ($30) per visit	2 ($20) per visit	$200
Week 12: History and physical, anthropometrics, laboratory assessments, Fibroscan and CAP; REE, DXA, OGTT	6 ($80)	12 ($120)	$200
Week 24: History and physical, anthropometrics, laboratory assessments, Fibroscan and CAP, REE, DEXA	4($50)	10 ($100)	$150
TOTAL COMPENSATION FOR COMPLETE STUDY PARTICIPATION			**$880**
CAP controlled attenuation parameter; REE resting energy expenditure; DXA dual-energy x-ray absorptiometry; OGTT iOral glucose tolerance testing			
Note: Screening, baseline, week 12 and week 24 visits may be scheduled over multiple days as necessary for participant convenience.			