Clinical Research Protocol

National Institute of Diabetes, Digestive and Kidney Diseases

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TITLE: Thyroid hormones homeostasis and energy metabolism changes during stimulation of endogenously secreted bile acids (BAs)

SHORT TITLE: Thyroid, energy metabolism, and thermoregulation

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ABOUT THE PROTOCOL:
PROJECT **DOES NOT** USE IONIZING RADIATION.
PROJECT **DOES NOT** INVOLVE AN IND/IDE.
PROJECT **DOES NOT** USE "DURABLE POWER OF ATTORNEY".
STUDY LOCATION IS THE CLINICAL CENTER, NIH.
THIS IS NOT A MULTI-INSTITUTIONAL PROJECT.

Précis

Postprandial thermogenesis, or "thermic effect of food" are terms that describe the increase in utilization of energy by the human body following a meal. The mechanisms involved in this process are believed to differ according to the type of food consumed, whether fat, protein or carbohydrate.
The bile acids (BAs), unique substances secreted by the gall bladder into the gut after a meal, play an important role in the absorption of fat and the management of cholesterol stores in the body. Recent studies suggest that BAs may also serve as regulators of energy expenditure (consumption) in the cells of our body by increasing the production of T3, an active form of thyroid hormone. T3 in turn is believed to increase the efficiency with which our bodies burn calories thereby generating heat. Although this process has been shown to be effective in rodents who demonstrated weight loss after treatment, the role of BAs in humans is poorly understood. Thus we do not know whether endogenous (produced by the body) or exogenous (taken as medication) BAs play a significant role in the maintenance of body weight. We hypothesize that, similarly to rodents, humans will respond to BAs by increasing energy expenditure via the production of the active form of thyroid hormone.

This randomized, cross-over study will look at changes in thyroid hormones and energy consumption in response to stimuli of endogenous BA secretion including dietary content, and to the intake of pharmacological doses of bile acids.

Following a two-day period of equilibration diet, 30 healthy volunteers will be randomly assigned to receive either a high-fat or high-carbohydrate isocaloric meal followed by a 6-hour metabolic chamber stay; the next day they will be crossed-over to the alternate intervention. During the following three days, the study subjects will again be randomized to receive either an intravenous injection of sincalide (the C-terminal octapeptide fragment of cholecystokinin) 0.04 mcg/kg or placebo and P.O. placebo, or I.V. placebo and 15 mg/kg of BA (ursodiol) with similar metabolic chamber stays and cross-over design.
The following parameters will be recorded and compared to placebo:

Energy expenditure
Substrate utilization
Spontaneous movements
Skin and core temperature
Serial changes in circulating thyroid hormones
Serial changes in bile acid serum concentrations

The data gathered from this study will provide greater insight into the physiological and molecular mechanism(s) regulating the relation between endogenous bile acid secretion and energy metabolism in response to meals, as well as the role of BAs *per se* on energy metabolism.

Introduction

The overall energy balance is maintained by multiple redundant mechanisms regulating both energy intake and expenditure (EE) (1, 2). While a considerable amount of experimental and clinical data on the mechanisms regulating food intake is currently available, comparatively little is known about the regulation of postprandial thermogenesis (PPT). Classical studies by Rubner (3) and Lusk (4) have revealed the unique role of protein in PPT and termed it "specific dynamic action". However, the roles of fat and carbohydrate, the major sources of energy intake, on PPT are less clear. Recently, Blaak et al. (5) have reported that the level of increase in 3-hr postprandial EE on a high-fat (95%) meal was lower in obese versus individuals. On the other hand, it has been shown that the metabolism of ^{14}C labeled dietary oleic acid to $^{14}CO_2$ began after 6 hours and end around 15 hours. Thus, the cause for this acute rise in PPT (<6 hours) cannot be attributed directly to the oxidation *per se* of the dietary fat.

BAs are steroid acids found predominantly in the bile of mammals. In humans, the most important BAs are cholic acid, chenodeoxycholic acid, and their conjugates with taurine and glycine (glycocholate and taurocholate). These molecules are derived from cholesterol and synthesized endogenously by the liver. Storage of excess bile acid takes place within the gall bladder from where it is available for secretion upon stimulation by such factors as cholecystokinin (CCK) and high-fat diet. CCK, a gut-peptide hormone, naturally secreted by the duodenum in response to fat and protein rich meals causes increased production of hepatic bile, stimulation of gallbladder contraction and the relaxation of the Sphincter of Oddi, resulting in the delivery of bile into the duodenum. CCK and high-fat meals induce, together with an increase in the secretion of bile in the duodenum, a rapid rise in the plasma concentration of BAs(6).

BAs have long been known to play a central role in lipid, fat soluble vitamin absorption and cholesterol catabolism, however in recent years an important role for BAs as signaling molecules has emerged. BA binding to FXR nuclear receptor can reduce expression of 7-alpha hydroxlase a rate-limiting enzyme in the synthesis of BAs in hepatic cells. Interestingly, BAs also act through an alternate pathway by binding a cell surface receptor TGR5. TGR5 is a G protein coupled receptor (GPCR), which upon binding with its ligand, leads to an increase in intracellular cAMP production. Recent evidence indicates that the cAMP-driven Type-2 Deiodinase gene (D2) is the naturally occurring target of the TGR5 signal pathway, ultimately resulting in an increase in the intracellular concentration of T3(7, 8) the active form of thyroid hormone.

This phenomenon has been demonstrated in BAT of mice and human skeletal muscle cells, tissues that express both TGR5 and D2 (8). In rodents fed a high fat diet, cholic acid prevented the development of diet-induced obesity by increasing energy expenditure (EE) and oxygen consumption in BAT through increased D2-mediated T4 to T3 conversion (8-11) This effect was not observed in D2 knockout mice or those rodents fed a regular diet. These data can thus be interpreted as an endocrine action of the bile acids whose postprandial serum levels increase would lead to an increase in D2 transcription and activity ultimately generating an increase in thermogenesis, thus responding in a homeostatic fashion to the caloric load. It is postulated that elevated intracellular cAMP and T3 concentrations induce the expression of uncoupling proteins (UCPs)(12). The UCPs, by reducing the "efficiency" of mitochondrial oxidative

phosphorylation, generate heat and thereby increase EE. In rodents this is achieved by the activation of the UCP-1 in the brown adipose tissue (BAT). Indeed, the UCP-1 gene is a thyroid-hormone dependent gene, and mice devoid of D2 are unable to produce non-shivering thermogenesis in response to cold exposure.

In humans, the pattern of expression of D2 is strikingly different from the rodent(13, 14). Specifically, while adult individuals have no "anatomically defined" BAT, D2 and UCP-3 have been demonstrated in skeletal muscle, raising the possibility that this tissue, which is approximately 40-80% of the individual's weight, could represent a "physiological equivalent" of BAT(10, 15). UCP-3 induction in skeletal muscle, similar to UCP-1 in rodents, may mediate an increase in EE after BA treatment thereby promoting weight loss and insulin sensitivity. Indirect evidence suggests that the D2-mediated T4 to T3 conversion plays a major role in the production of the circulating T3(16). Interestingly, two common genetic variants of the human D2 gene, Thr92Ala and -258 A/G SNP, have been associated both in cross sectional and in *in vitro* studies with changes in the enzymatic activity (17-20).

At the present, the role of BAs in the regulation of metabolism and energy expenditure in human BAT or "BAT-physiological equivalent" is unclear. *In vitro* evidence from cultured human skeletal muscle cells suggests that BAs increase the metabolic rate in this tissue. If this is true, one could hypothesize that the manipulation of circulating bile acid levels, by pharmacologic or dietary means, could result in an overall increase in EE, ultimately facilitating weight loss. Data available in the gastroenterology literature clearly demonstrate that either a high-fat meal or a single injection of CCK is able to generate respectively a 1.5 to 3-fold and 2-fold rises in circulating BAs (6, 21, 22) without any significant residual effect to the following day(23). Interestingly, the chronic use of bile acid salts for the treatment of primary biliary cirrhosis resulted in a mild increase in body weight(24). This finding was attributed to an overall improvement of the general (nutritional?) conditions of the patients (Dr K. Lindor, Mayo Clinic, *personal communication*). No data is currently available relative to the synchronous evaluation of energy expenditure and endocrine metabolism during manipulation of endogenous secretion or circulating levels of BAs.

The recently opened Metabolic Unit, a state-of-the-art metabolic phenotype characterization laboratory, has the capability, through the temperature-controlled and fast response metabolic chambers, to analyze changes in the human physiology in controlled environmental conditions. This hypothesis-driven natural history protocol/physiology is thus aimed, by taking full advantage of the capabilities of the Metabolic Unit, to the detailed characterization of the physiological response to the stimulation of endogenous BA secretion and to characterize the role of BAs in the postprandial thermogenesis.

Study Hypotheses

1) If BAs have an endocrine/paracrine action on BAT-equivalent tissue in humans, the acute pharmacological stimulation of the endogenous BAs secretion by CCK and/or the oral administration of BAs will result in an increase in thermogenesis similar to the one

observed in the postprandial period of a high-fat meal, and greater than the one observed in a high-carbohydrate one.

2) If the raise in circulating BAs is coincidental but not causative of the increase in thermogenesis observed in the postprandial period, we do not expect to observe any change in EE after an oral dose of BAs. Consequently, if the increase in thermogenesis observed in the postprandial period is secondary to endocrine effects of CCK, but not to BAs *per se*, we will observe an increase in EE after CCK infusion but not after an oral dose of BAs.

3) Conversely, if the postprandial thermogenesis is secondary to calorie intake, irrespective of macronutrient composition, while no thermogenic effect will be observed after acute administration of BAs (or the pharmacological stimulation of the endogenous BAs secretion), a comparable increase in thermogenesis will be observed after a high carbohydrate or a high-fat meal.

4) If the postprandial thermogenesis is dependent to the secondary macronutrient composition, a differential increase in the thermogenesis will be observed after a high carbohydrate as compared to a high-fat meal.

Aim of the study:

1) To characterize the metabolic and hormonal responses to changes in endogenous BAs secretion following CCK stimulation or pharmacological administration of BAs.

2) To characterize the role of BAs and macronutrient composition in postprandial thermogenesis.

Secondary aims

1) To characterize the role of genetic variants of the type-2 deiodinase gene (Thr92Ala and -258 A/G) in the modulation of the hormonal and metabolic response to changes in circulating BAs.

2) To analyze the changes in glucose metabolism and substrate utilization in response to changes in circulating BAs.

Study Protocol

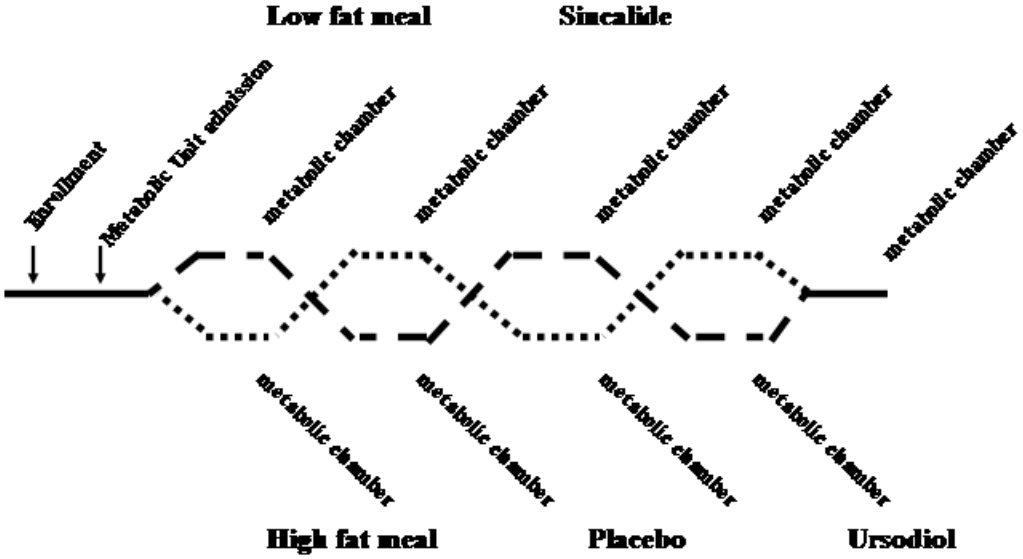
Subjects and Recruitment

Subjects will be healthy non-obese (BMI<27) male and female adult volunteers.

Study design and methods

This natural history research protocol is designed as a double blind-cross over study with both pharmacological and nutritional interventions. During a seven-day stay at the NIH, volunteers will undergo five 6-hour clinical and experimental observations in the metabolic chamber. The timing of the experiments have been based on the observations of Hoffman and colleagues who have shown that the peak of BA's occurs at ~2 hours postprandially in a physiological pharmacokinetic model in man (25) and of Foberg et al. who showed that CCK induced a rise in serum bile acids (3α-HBA) peaked at ~30 minutes and returned to baseline within 120 min (6). The overall outline of the study is reported in Figure 1. Briefly, eligible volunteers will be invited for a seven-day stay at the Metabolic Unit. After a two-day isocaloric equilibration diet, study volunteers will be randomly assigned to receive either a very low or high-fat isocaloric standardized liquid meal during a 6-hour stay in the metabolic chamber. After an 18-hour resting period the volunteers will then be crossed-over to the second type of meal. After an 18-hour resting period the volunteers will again be randomly assigned to receive an IV injection of sincalide (*Kinevac*, a sterile lyophilized powder of the C-terminal octapeptide fragment of cholecystokinin) 0.04 mcg/kg or placebo during a 6-hour stay in the metabolic chamber. After an 18-hour post infusion resting period, the volunteers will be crossed-over to the alternate study arm; during these two interventions the volunteers will also receive a P.O. placebo. The next day, after an 18-hour post infusion resting period from the previous metabolic chamber stay, the volunteers will receive an IV infusion of placebo and 15 mg/kg of ursodiol (as 300 mg capsules rounded up to the nearest 300 mg), and will undergo the same recording. The volunteers will be blinded relative to the composition of the IV and PO compounds. This study design is necessary to avoid carry-over effects of the PO BAs.

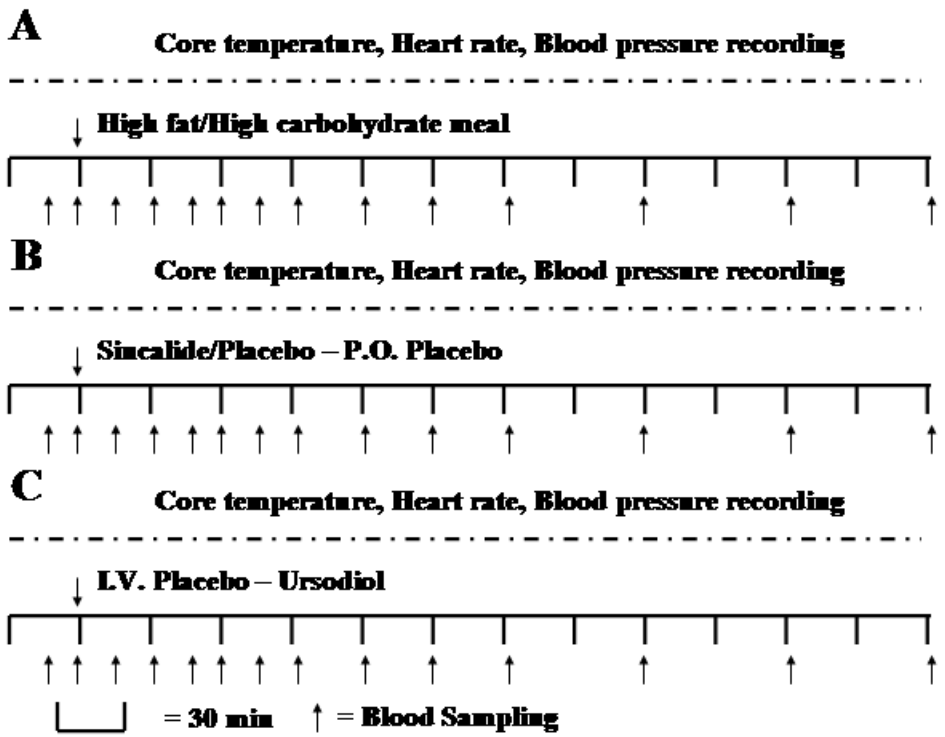
Fig. 1 Study protocol, overview



Equilibration diet: 2 days; Metabolic chamber stay: 6-hours

During each 6-hour stay in the metabolic chamber we will record the following parameters and will perform these procedures (Fig. 2, **A**: High fat/High carbohydrate meal, **B**: Sincalide/IV Placebo-P.O. Placebo, **C**: IV Placebo-ursodiol):

Fig. 2 6-hour Metabolic Chamber stays, procedures and blood drawing protocol



1) Real-time energy expenditure, RQ
2) Spontaneous movements
4) Real-time core and skin temperature
5) Frequent blood samples for the following parameters:
    Free fatty acids
    TSH
    T4
    T3
    Free T4
    Free T3
    rT3
    Glucose
    Insulin
    Bile acid levels
    Ghrelin
    CCK
    GLP-1
    β-hydroxybutirrate
    Interleukin-6,
    Adiponectin.

Inclusion Criteria:

- Age ≥18 years, male or female.
- Written informed consent.

Exclusion Criteria:

- Hypo- or hyperthyroidism (history or serum TSH > 5.0 or < 0.4 miU/L)
- Blood pressure greater than 140/90 mmHg (26) or receiving antihypertensive therapy
- History of cardiovascular disease
- BMI ≤ 20 or ≥ 27 Kg/m2.
- Diabetes mellitus (fasting serum glucose ≥ 126 mg/dL).
- Hyperlipidemia (serum total cholesterol ≥ 240 mg/dL, triglycerides ≥ 220 mg/dL, and/or use of antilipemic therapy).
- Liver disease or ALT serum concentrations greater than 1.5 times the upper laboratory reference limit.
- Hyperbilirubinemia (serum total bilirubin > 1.5 mg/dL)
- Renal insufficiency or estimated creatinine clearance ≤ 50 mL/min (MDRD equation).
- Anemia (Hemoglobin concentration ≤ 11.1 g/dL females , and 12.7 g/dL males)
- History of cholecystectomy or cholelithiasis (by ultrasound at screening).
- History of malabsorption, or food allergies/intolerances that would preclude participant from consuming foods required for study
- Claustrophobia.
- History of illicit drug or alcohol abuse within the last 5 years; current use of illicit drugs (by history) or alcohol (CAGE > 3).
- Psychiatric conditions or behavior that would be incompatible with safe and successful participation in this study
- Current use of medications/dietary supplements/alternative therapies known to alter thyroid function, energy expenditure or bile acid secretion.
- History of weight loss or weight gain of >3% body weight over the past 2 months (self-reported)
- Pregnancy/breastfeeding/hormonal contraceptive use and childbirth within the last 6 months.
- Perimenopausal (as self-described within two years from onset of amenorrhea or current complaints of hot flashes)
- Current smoker

All subjects will be fully informed of the aim, nature, and risks of the study prior to giving written informed consent.

Methods:

Screening visit
Subjects will be screened and counseled at the initial screening visit. After obtaining informed consent, the study volunteers will undergo a history and physical examination, and the following screening tests will be performed, including: TSH, glucose, creatinine, AST, ALT, CBC, cholesterol, EKG and gall bladder ultrasound. Subjects are required to fast for at least 12-hour prior to the laboratory testing, but will be allowed to drink water during the fasting period. During the screening visit, the study participants will be interviewed by a dietitian in order assess the caloric intake and food preferences of each study participant.

Hospital stay
Study volunteers will be housed in the Metabolic Unit of the NIH Clinical Center. Non-menopausal female volunteers will be admitted during the early follicular phase (day 2-5) of the menstrual cycle and a pregnancy test will be performed at admission. During the hospital stay, the volunteers will be asked not to perform strenuous physical activity and to abstain from tobacco use. After two days of equilibration diet the subjects will be randomly assigned to receive low and high fat meal during the metabolic chamber stay. The next day the volunteers will receive the alternate meal. The following day the volunteers will be again randomized to sincalide or placebo and P.O. placebo; the next day volunteers will receive the alternate treatment. In the last day of hospital stay the volunteers will again receive IV placebo and 15 mg/kg of ursodiol.

Metabolic Diet
Throughout the stay at the Metabolic Unit study volunteers will receive an isocaloric, caffeine-free diet with the following macronutrient distribution: 50% carbohydrate, 20% protein, 30% fat. A research dietitian will meet with participants during the screening visit to plan the isocaloric equilibration diet and to assess food allergies and intolerances. Approximate total energy needs for weight maintenance will be calculated using the Mifflin-St.Jeor equation (27). During two of the metabolic chamber stays study volunteers will be randomized to receive an isocaloric high fat or fat free meal. After an 18 hour resting period the subjects will be crossed over to the second type of meal. Both of the meals will consist of a shake with approximately 600 calories. The high fat meal will contain 50 grams of fat and will have an approximate macronutrient distribution of 72% fat, 8% protein, and 20% carbohydrate. The fat free meal will contain 0 grams of fat and will be 100% carbohydrate. The shakes to be consumed in ≤15 min, will be similar in volume and will be served at room temperature.

Body composition
A DEXA scan and an air-displacement plethysmography body composition analysis (BodPod) will be performed at either day 1 or 2 of the hospital stay.

Metabolic chamber
Study volunteers will enter the metabolic chamber at 08:00 AM after a 10-hour fast, voiding, and ingestion of the core temperature probe. Superficial skin temperature probes will also be placed (see below). Study volunteers will wear a standard suit (hospital scrub) and will be allowed to perform regular physical activity while in the metabolic chamber.

The respiratory chamber is a specially constructed room to assess the metabolism of subjects for a period of 6 hours in this study under constant thermoneutral condition (73.5±0.5° F). Designed as a walk-in "pull" calorimeter, it is an open circuit unit that draws conditioned room air into the chamber at the same flow rate as it is extracted into the gas analysis system.(28) The room is equipped with toilet and sink with privacy screen, treadmill, bed, desk, and computer with access to television and other forms of entertainment. Physical activity level is measured continuously through a wall mounted monitoring device (microwave sensor). Food and fresh water is passed through an air-lock drawer system. Using this rapid response chamber, we will first measure the resting EE after the patient enters the room. While still fasted, the subject will lie in supine posture and with minimum movement for 30 minutes. After each meal, the subject will be instructed to remain sedentary for 2 hours where the postprandial thermogenesis (EE above the resting level) will be determined. The energy cost of spontaneous physical activity (29) will also be assessed. The respiratory quotient (RQ), as determined by the ratio of carbon dioxide excretion and oxygen consumption, during resting, post-prandial, and overall 6-hour periods will be used to estimate whole-body carbohydrate and fat oxidation rates.

The following parameters will be recorded:

Spontaneous movements
Spontaneous physical movements will be quantified with minute-to-minute resolutions during the study periods using small, portable pager-type and watch accelerometers at the subjects' hip and wrist (30).

Real-time core and skin temperature
The VitalSense® monitor (Mini Mitter/Respironics, Bend OR) is used to receive transmissions from multiple miniature, wireless, temperature sensors. Core temperature is sensed by the ingestible Jonah™ capsule. Dermal temperatures are recorded from hypoallergenic, non-irritating, adhesive dermal patches. Both sensor types are disposable, but designed for multi-day use under demanding physical and environmental conditions. Both types of sensors use low-power radio frequency transmissions to communicate with the Monitor. The core temperature capsules transmit at various frequencies, therefore, it is possible to track more than one capsule at a time.

Blood sampling, urine collection
All blood samples will be drawn under sterile technique through an indwelling catheter using the Vacutainer® system (Becton Dickson and Co.). Serum and plasma aliquots will be stored at -80 C° until analysis. Blood samples will be drawn according to the

following scheme during each metabolic chamber stay: -15',0',15',30', 45', 60', 75', 90', 120', 150', 180', 240', 300',360'.
The following laboratory parameters will be recorded: T4, T3, free T4, free T3, TSH, glucose, insulin, BAs, Ghrelin, CCK, GLP-1, and free fatty acids.

Genetic testing
DNA samples will be obtained from leukocytes obtained during the screening visit. Samples will be analyzed for the Thr92Ala and -258 A/G polymorphisms, and for subsequent screening of known mutations of genes involved in the energy, carbohydrate, thyroid hormone and glucose metabolism, as well as scanning for novel mutations in genes involved in these same metabolic pathways. Since these data do not currently have clinical relevance, study volunteers will not be notified of the results.

Laboratory testing
Screening tests (Chem 20, lipid panel, CBC with differential, TSH, HCG) will be performed in the Department of Laboratory Medicine (DLM). Genomic DNA will be isolated from peripheral mononuclear cells using the QIAmp® system (QIAGEN) in Dr. Celi's laboratory. The following tests will be performed by the Department of Laboratory Medicine (DLM): Chem 20, insulin, cholesterol, HDL, triglycerides, free fatty acids, TSH, total and free T3, and total and free T4. Aliquots of serum and plasma will be also collected and stored at -80 C° for further analysis. GLP-1, CCK, and Ghrelin will be measured by immunometric assay in the clinical research core laboratory of NIDDK. Serum BAs (Cholic acid, Deoxycholic acid, Chenodeoxycholic acid, Total bile acids) will be measured by radio-immunoassay technique (Mayo Clinic).

Radiology studies
Right upper quadrant ultrasound to screen for the presence of gallstones will be performed at the screening visit. DEXA scan for body composition will be performed during hospital day #1 or #2 using the Metabolic Unit GE iDXA.

Statistical analysis

The primary endpoint of this mechanistic study is the analysis in changes in energy expenditure in response to changes (pharmacological or diet-induced) in serum bile acids concentration. The data will be analyzed using Analysis of Variance (ANOVA) and, should the data suggest an interaction, we will explore statistical models including delta in bile acid concentration, thyroid hormone concentration, and macronutrient diet (as a categorical data). Power analysis: Although this is a pilot study, we use previously published data comparing the effect on PPT (31) to help power the study, The PPT was 7.6-10.1% of the total energy intake, with a standard deviation of 2.6%. If we detect a desired difference of 2.0% in PPT with CCK or high fat diets, 28 study subjects will be needed to provide an 80% statistical power (α error of 0.05, using a two-tailed independent t-test design). We plan to enroll 30 volunteers considering the possibility of missing values.

Research use, storage, and disposition of human subject's samples and data

Serum, plasma, and nucleic acid samples will be coded and stored in a -80°C refrigerator located in the PI's laboratory. Tissue samples will be coded and stored in a liquid nitrogen tank located in the PI's laboratory. A registry of the samples location will be kept in the PI's office. This will allow the PI and the AIs to analyze research data in relation to clinical data stored in the Medical Record Department. Data and samples will be retained for the foreseeable future in order to allow secondary analyses after the completion of the protocol. Such analyses include association studies for known mutations of genes involved in the energy, carbohydrate, thyroid hormone and glucose metabolism, as well as gene scanning for novel mutations. Serum, plasma and nucleic acids samples will be similarly utilized for future association studies of known and novel markers of energy, carbohydrate, thyroid hormone and glucose metabolism. The PI will inform the IRB in case of loss of data/samples secondary to intrusion in the laboratory or database.

Possible Risks and Hazards:

This study, aimed to analyze the role of BAs in postprandial thermogenesis, is not a therapeutic trial. Research-related risks in this study include those associated with study procedures, namely blood drawing. We do not expect serious study medication-related risks except nausea or mild and transient diarrhea (see below) due to the single injection of sincalide or to a single dose of ursodiol, nor do we expect any significant risk connected to the respiratory chamber stay.

Metabolic Chamber.
Besides inconveniences that can reasonably be expected as a result of spending 6 hours in the live-in room calorimeter, the serious risk to subjects' health is minimum.

Blood drawing.
The placement of intravenous needles may cause transient pain, and occasional infection or bruising at the insertion site. Study subjects will undergo 5 venipunctures (14 blood draws during each metabolic chamber stay) during the study. Each blood drawing will be for 5 mL of blood. However, in compliance with NIH guidelines, no more than 6 mL/Kg will be drawn from any study participants during a six-week period.

Core-body and surface temperature measurements.
A small temperature "pill" and wireless surface patches (VitalSense, Mini Mitter, Bend OR) will be used to measure the diurnal variation of body temperature, and a small receiver will need to remain close to the body. The "temp pill" is a capsule (8.7 mm x 23 mm) which is swallowed with water and will be stay in the digestive tract for 2-3 days until being eliminated in the feces. Patients are advised not to undergo MRI procedure until the capsule is passed. No other known side-effect has been reported.

Frequent blood-pressure monitoring.

An ambulatory blood pressure monitor (Oscar 2, SunTech Medical, Morrisville NC), using a cuff on the upper arm, will be programmed to measure BP very 15 minutes. During the measurement, temporary pressure/restriction to the arm is typical. Minor pain may be experienced if the cuff is too tight. Adjustment of the cuff is allowed. No other known side-effect has been reported.

DEXA Scan.
The use of DEXA scan apparatus may cause some minimal discomfort in claustrophobic subjects and may cause some minimal back pain in a small minority of the individuals.

Radiation Safety.
The radiation exposure of a whole-body DEXA Scan is less than 1 mrem. A typical radiation dosage from a chest X-ray is 20 mrem. A normal individual is exposed to 300 mrem/year from natural sources.

Study drugs:

Sincalide (*Kinevac*)
Reactions to sincailde are generally mild and of short duration. Common adverse effects (reported in 20% of patients) associated with sincalide include abdominal pain, cramps, and nausea. Less frequent side effects, such as dizziness, diaphoresis, diarrhea, dyspnea, fecal urgency, flushing, headache, hyper-/hypotension, rash, sneezing, numbness, and/or vomiting occur in ≤ 2% of patients.

Ursodeoxycholic Acid
Possible side effects occurring in more than 1% of patients receiving ursodiol for cholecystitis or primary biliary cirrhosis have included: headache (up to 25%), dizziness (up to 17%), constipation (up to 26%), rash (<1%-3%), hair loss (<1% to 5%), diarrhea (1%), nausea, vomiting (felt to be related to cholecystitis), musculoskeletal pain (6-8%), cough or respiratory infections (5-15%), leukopenia (3%), and allergy (5%). The incidence of many of these side effects was similar to those reported in the placebo group, and, according to product labeling, doses of ursodiol in the range of 16-20 mg/kg/day have been tolerated in some patients for 6-37 months without symptoms.

Acetaminophen (Tylenol)
This analgesic will be prescribed for headache and minor pain. For the dose and duration of use in this study, serious adverse effects would not be expected.

Event Characterization and Reporting to the IRB and Clinical Director (CD)

Adverse events, protocol deviations, unanticipated problems (UP), Unanticipated Adverse Device Effects (UADEs), serious adverse events, sponsor and serious, are defined as

described in NIH HRPP SOP 16 ("Reporting Requirements for Unanticipated Problems, Adverse Events and Protocol Deviations."). All adverse events occurring during the study, including those observed by or reported to the research team, will be recorded. Serious unanticipated problems and serious protocol deviations will be reported to the IRB and CD as soon as possible but not more than 7 days after the PI first learns of the event. Not serious unanticipated problems will be reported to the IRB and CD as soon as possible but not more than 14 days after the PI first learns of the event. Not serious protocol deviations will be reported to the IRB as soon as possible but not more than 14 days after the PI first learns of the event.

Deaths are not expected to occur since no subjects are being recruited. In the unlikely event that a death is reported to the Investigator, it will be reported to the Clinical Director and IRB within 7 days after learning of the event.

Non-Serious Protocol Deviations

Non-serious protocol deviations will only be reported to the IRB (within 14 days after the PI first learns of the event) if they represent a departure from NIH policies for the conduct of human subjects research, adversely affect the health care of the subject(s) or compromise the interpretation or integrity of the research. Non-serious protocol deviations that result from technical issues associated with sampling that does not impact the health of the subject or the interpretation of the study data will not be reported.

Adverse Events

The PI is responsible for summarizing all serious adverse events and adverse events at least possibly related to the research procedure and interventions at the time of Continuing Review.

Data and Safety Monitoring Plan

Due to the modest risk of the study procedures, no DSMB will be instituted to evaluate adverse events. For this protocol the PI will serve as study monitor.

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 visit results will be reported to the Principal Investigator for further reporting as appropriate. Study documents and pertinent hospital or clinical records will be reviewed to verify that the conduct of the study is consistent with the protocol plan.

Recruitment of Women, children and minority individuals

We will actively encourage the participation of women and minorities. On the other hand, the study design precludes the recruitment of children, since the differences in the

biomarkers between adults and children would impede the interpretation of the data collected. Moreover, this is not a therapeutic trial and children will not be prevented from receiving potentially beneficial therapy by the exclusion from this study.

Benefits

Study subjects will receive no direct benefit from participation in this study. However, a thorough medical examination, and a series of diagnostic tests will be provided as part of the evaluation. Abnormal values will be discussed with the study volunteers and forwarded to their primary care physicians.

Remuneration

Patients will receive payments for time and discomfort connected with the visits/procedures according to the following scheme:

1. Daily compensation for impatient stay/venipunctures	$250
2. Inconvenience for the study medications administration	$50
3. Compensation for metabolic chamber stay ($100 each)	$500
4. Compensation for completion of the study	$100
Maximum compensation	**$900**

In case of premature termination of the study the volunteers will be remunerated only for the actual procedures, and the bonus compensation for the completion of the study, as well as the study medications administration will be forgone.

Confidentiality

Study data will be kept in locked files, and subjects will be identified by codes.

Genetic testing

DNA samples will be obtained from leukocytes obtained during the screening visit. For subsequent screening of known mutations of genes involved in the energy, carbohydrate, thyroid hormone and glucose metabolism, as well as scanning for novel mutations in genes involved in the energy, carbohydrate, thyroid hormone and glucose metabolism. Since these data do not currently have clinical relevance, study volunteers will not be notified of the results.

Consent process and documents

Written informed consent will be obtained at the time of the screening visit and confirmed before the procedure.

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