Research Project Plan & Protocol

Research involving human subjects with the exception of clinical trials

Project Title

Phenotyping for thrifty metabolic traits in young adults born small: a risk factor for obesity and cardiometabolic diseases

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Research legislation:	Ordinance on human research with the exception of Clinical trials
Type of Research Project:	Research project involving human subjects

Project Leader: Prof. Abdul Dulloo
Professeur titulaire
Department of Medicine/Physiology
University of Fribourg
Chemin du Musée 5
1700 Fribourg
SWITZERLAND
abdul.dulloo@alumni.unifr.ch

The project leader has approved the protocol version ***(dated 20.12.2017)***, and confirms hereby to conduct the project according to the protocol, the Swiss legal requirements, current version of the World Medical Association Declaration of Helsinki and the principles of Good Clinical Practice.

TABLE OF CONTENTS

ABBREVATIONS

EE	Energy Expenditure
RQ	Respiratory Quotient
LBW	Low birth weight
NBW	Normal birth weight
RPM	Revolutions per minute
W	Watts

1. BACKGROUND, PROJECT RATIONALE & SIGNIFICANCE

Background

Obesity has reached epidemic proportions worldwide (1) and is the driving force behind an equally alarming explosion of type 2 diabetes and cardiovascular diseases (2). While our 'obesogenic' environment - with abundant energy-dense foods and sedentary lifestyle – plays a central role in the pathogenesis of obesity and cardiometabolic diseases, it is also recognized that factors operating during fetal life - and resulting in low birth weight (LBW) - may program the individual for greater susceptibility towards the development of excess adiposity and chronic diseases later in life. There is indeed compelling evidence - from large population and clinical studies - that adults who had LBW (which reflects fetal growth constraint) have higher susceptibility for obesity, type 2 diabetes or cardiovascular diseases, particularly when accelerated or catch-up growth occurs early in postnatal life (3). These risks for cardiometabolic disease have also been reported in people born appropriate-for-gestational-age but who were nonetheless relatively light at birth, possibly because of more subtle intrauterine growth constraint (4). Thus, the relationships between birth size and chronic metabolic diseases in adulthood are graded and operate across a range of birth weight which would generally be considered normal, and are not simply a feature of the extreme of growth retardation (4).

The mechanisms by which such early growth patterns (relatively slow growth followed by catch-up growth) confer predisposition to obesity and insulin-related diseases remain unclear, amid theories of fetal or neonatal programming for a thrifty phenotype. According to the Thrifty Phenotype Hypothesis (5), a consequence of early growth constraint (due to inadequate nutrition, small womb size, maternal smoking or other insults) can be that adaptations occur to reduce the detrimental effects of growth retardation on vital tissues/organ development and functions. If these adaptations take place during critical windows of development, they may become permanent, and hence may persist during improved nutrition later in life, thereby predisposing the individual to adverse metabolic and cardiovascular consequences.

This hypothesis is strongly supported by studies in animal models showing that interventions (e.g. nutritional, hormonal, other insults) that slow growth in utero followed by accelerated or catch-up growth during postnatal life can have permanent effects on mechanisms that regulate blood glucose homeostasis and on control systems that regulate body weight, body composition (6). These include changes in structure and function of neuronal pathways in the brain which lead to deregulation of pathways mediating insulin release/action, energy intake and/or energy expenditure (EE), as well as changes in peripheral physiological systems that participate in glucose homeostasis and underlie thrifty (energy conservation) metabolism directed at an increased metabolic efficiency for fat storage associated with impaired glucose homeostasis, thereby making the animal more prone to develop insulin-related disorders and obesity.

Project Rationale

In humans, however, the existence of thrifty (energy conservation) metabolism has yet to be demonstrated in adults born small, and hence forms the basis of this research. Our working hypothesis is that the mechanisms that lead to both thrifty metabolism (via suppressed thermogenesis) and diminished insulin sensitivity in skeletal muscle - and which constitute the thrifty energy metabolism for catch-up fat – are 'hard-wired' or 'imprinted' during early growth perturbations, and can therefore operate well beyond the phase of catch-up growth and into adulthood to confer increased risks for obesity and cardiometabolic diseases (3). Such thrifty metabolism operating to conserve energy could theoretically operate in EE at rest in response to specific dietary challenges, in non-resting compartments of EE, and/or in the resetting of core body temperature to a lower level. By merging techniques of indirect calorimetry, novel approaches for EE phenotyping during low-level physical activity and continuous core body temperature monitoring by ingestible capsule telemetric sensors, we plan to test aspects of the above-mentioned working hypotheses about the occurrence of thrifty metabolic traits in humans with LBW.

Significance of Project

This project will provide valuable knowledge about the metabolism of a population sub-group with a high predisposition to obesity and cardiometabolic diseases. Indeed, the application of classic and novel approaches to phenotype potential thrifty metabolic traits is an essential step towards defining the mechanisms by which humans born small may express an increased metabolic susceptibility to obesity and cardiometabolic disease. Such research will advance knowledge and contribute towards the development of more refined approaches and diagnostic tools for identifying individuals most prone to obesity and associated cardiometabolic risks, and in the design of intervention strategies that would confer advantages for long-term health while preventing adverse health outcomes.

2 PROJECT OBJECTIVES AND DESIGN

2.1 Hypothesis and objectives

Hypothesis

In young adults with LBW, thrifty metabolic traits may be expressed through one or more compartments of energy expenditure (EE) namely (i) in diminished glucose-induced thermogenesis, (ii) diminished dietary protein-induced thermogenesis, (iii) diminished energy cost of performing low-intensity physical activity, and (iv) in a lower core body temperature.

Primary objective

Metabolic phenotyping of young adults distinguished on the basis of birth weight, LBW vs normal birth weight (NBW) for potential differences in EE in response to dietary challenges (glucose or a moderately-high protein meal), to low-intensity physical activity, as well as to potential differences in core body temperature in the post-absorptive state and in response to an acute cold water drink challenge.

Secondary objective

To assess the extent to which potential differences in EE and core temperature according to birthweight can be associated with differences in:

- Anthropometry and body composition
- Cardiovascular health and cardiovascular functions

2.2 Primary and secondary endpoints

Primary endpoints

- Measurements of EE in the pre- and post-prandial states in response to glucose or a mixed meal (moderately high in protein), assessed at rest in a comfortably seated position by gas exchange using indirect calorimetry.
- Measurements of EE in response to standardized low-intensity exercises by indirect calorimetry. Methodological approaches have been developed and validated in our laboratory for assessing the energy cost posture maintenance during sitting and standing (7, 8), the energy cost of dynamic (cycling) exercise at low power outputs (9), as well as during intermittent isometric (leg press) exercise of low-intensity (10), i.e. at levels that are energetically comparable to low-intensity physical activities of daily life.
- Measurement of core temperature continuously over 24h by telemetry using ingested pill-sized sensors (11). This will allow core temperature to be monitored under standardized conditions of overnight fasted post-absorptive state at rest, in response to standardized movements that are primarily dynamic or isometric, and in response to a cold water drink challenge.

Secondary end-points

- Respiratory Quotient (RQ), assessed concomitant to EE by indirect calorimetry
- Anthropometry and body composition (fat mass, lean mass)
- Cardiovascular health assessed by assay of glycemic profile and lipid profile
- Cardiovascular functions (blood pressure, cardiac output, vascular resistance) in fasted state and in response to glucose during an oral glucose tolerance test.
- Comparison of the responses between male and female subjects to test for potential sex differences in the overall response.

2.3 Design & Protocols

General study design

The study will be observational and monocentric. The metabolic phenotyping is planned to be performed through three experimental protocols (I, II and III) on mornings, and separated by at least 2 days for subjects participating in more than one of the protocols. In women, the protocols will be performed during the follicular phase of the menstrual cycle. Participants will be requested to avoid any strenuous physical activity, caffeine and dietary supplements in the 24 h before metabolic testing. On the day of testing,

subjects will arrive at the laboratory in the morning (between 08:00 and 08:30) in the overnight (~12h) fasted state. On arrival at the laboratory, subjects will be asked to empty their bladder, and after resting in a comfortable seat for 15 min, the subjects are then connected to equipment for metabolic and cardiovascular monitoring. After a baseline recording of 30 min, the specific experimental protocol I, II or III will then be undertaken. All tests are performed in an air-conditioned (22°C) quiet laboratory.

Protocol I: Glucose-induced thermogenesis

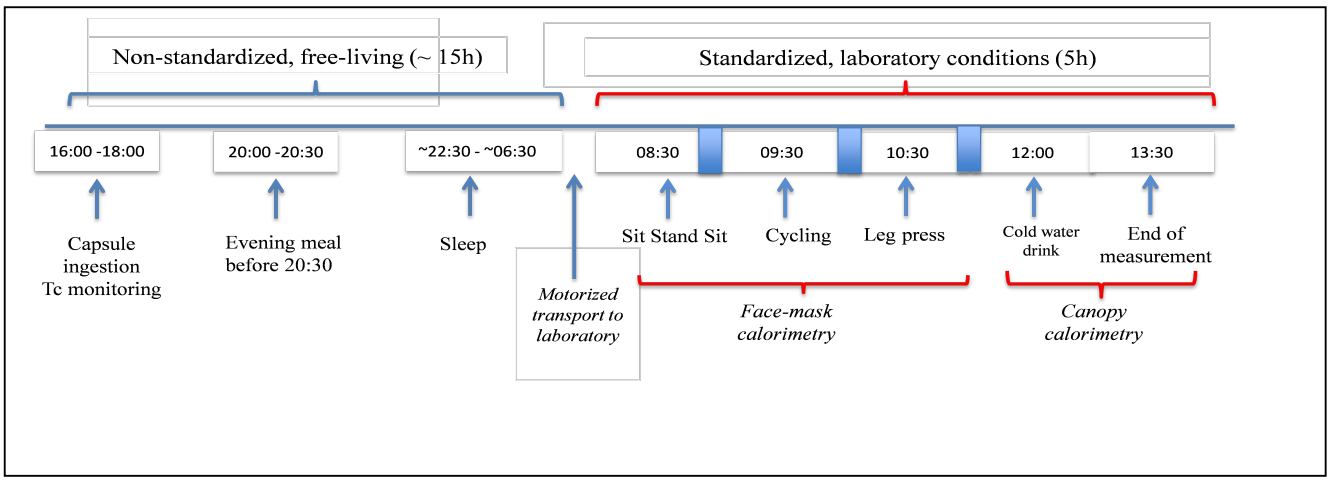
Following the 30 min baseline measurement of resting EE by ventilated hood (canopy) indirect calorimetry, cardiovascular parameters (blood pressure, heart rate, stroke volume) and a first blood sampling (time-point 0), the glucose drink (75g in 300ml water at 22°C) is ingested over a period of 5 min. Postprandial EE and cardiovascular parameters at rest are monitored for the next 120 min, with blood sampling made at 30 min intervals over 2 hours post-drink.

Protocol II: Protein-induced thermogenesis

Following the 30 min baseline measurement of resting EE (by ventilated hood indirect calorimetry), the moderately-high protein meal is ingested over a period of 10 min. Post-prandial resting EE are then monitored for the next 180 min after ingestion of a 590 kcal test meal moderately-high in protein of the following composition (by energy): protein 24%, carbohydrate 44% and fat 32%; with 24% protein being 2-2.5 times more than the average daily protein intake (expressed by energy) of the adult population in many countries (11-13%). The test meal will consist of a simple breakfast meal base (two slices of toast and a 200 mL milk-based drink (Nestlé Resource, Nestlé Health Science, Florham Park, New Jersey) to which protein powder (Protifar, Nutricia, Schiphol, the Netherlands), butter, and jam are added to adjust the protein content as necessary to 24% of total meal energy intake; the ratio of carbohydrate to fat being 1.41.

Protocol III: Energy cost of physical activity and body temperature (Tc)

In the early afternoon (13:30-14:30) of the day preceding protocol III, the capsule telemetric sensor to be ingested by the subject is first calibrated ex-vivo against a mercury thermometer in a water bath across the range of 35-40 °C, as we have recently detailed (11). Following ingestion of the capsule later in the afternoon (15:00–18:00), the subject is fitted with the temperature-recording device (logger) worn on a belt around the waist. Subjects will be provided with an activity log and asked to record the timing of their movements, meal consumption, as well as sleep and awakening times, advised to be at around 22:30 and 06:30, respectively. They will be instructed to consume a standardized evening meal no later than 20:30. The standardized EE measurements of protocol III will be undertaken the following morning (after an overnight fast of ~ 12 h) using face-mask indirect calorimetry to measure (i) the energy cost of standing posture maintenance, (ii) the energy cost of performing low power cycling (dynamic) exercise, (iii) the energy cost of performing the intermittent low-intensity leg press (isometric) exercise. After a 15 min period of rest, the subject is then connected to the ventilated hood (canopy) calorimetry system for resting EE monitoring for 30 min before and 90 min after drinking 500 ml of cold water at 3°C ingested over a 10 min period. Finger skin temperature will be measured by an infra-red thermography camera prior to the cold water drink, and then every 15 min during 90 min post-drink period



2.4 Methods

Questionnaire

Medical history and lifestyle (dietary and exercise habits) will be assessed by a standardized questionnaire. As well as being used to assess whether or not the participant meets the inclusion/exclusion criteria, this information (along with anthropometry and body composition) will be included in co-variate analyses of our primary and secondary variables.

Anthropometry and body composition

At the onset of the study, after voiding the bladder, body weight, height and sitting height will be measured using a scale with integrated stadiometer (Seca model 709, Hamburg, Germany), while limb length and waist circumference using a tape. In addition, anthropometric measures of fluctuating asymmetry (i.e. differences between right and left) will be assessed at several sites including measurements of calf and mid-thigh circumferences, length of tibia and femur, as well as length of fingers, ear height, and foot length and width by use of calipers, as described by Wells et al. (12). Body composition (fat mass and fat-free mass) will be assessed using a multi-frequency bioelectrical impedance analysis (Inbody 720, Biospace Co., Ltd, Seoul, Korea), Trunk (abdominal) fat by bioelectrical impedance analysis using ViScan (Tanita Corporation, Japan).

Indirect calorimetry: Energy expenditure (EE) and respiratory quotient (RQ):

Respiratory gas exchange will be measured non-invasively by indirect calorimetery using an open-circuit indirect calorimeter (Quark CPET Cosmed, Rome, Italy) using either the ventilated hood (canopy) system (protocol I, II, IIII) for EE measurements at rest or using a Hans Rudolph silicon facemask system for non-resting EE, i.e. for EE phenotyping in response to standardized physical activities during protocol III involving assessing energy cost of standing posture maintenance and during the exercise tests). EE is calculated according to Weir equation (10).

- *Assessment of energy cost of posture maintenance during standing*

Following a period for metabolic stability (usually 10-20 minutes), a baseline recording will be made over 30 minutes whilst the participant is seated comfortably. Following this baseline measurement, participants will be asked to stand relaxed and avoiding movement for 12 minutes, as we have previously described (40, 41). A second sitting measurement of 30 minutes (or until stability in the metabolic and heart rate measurements for 20-30 minutes) will then be made.

- *Assessment of energy cost of dynamic (cycling) exercise:*

This is performed by measuring oxygen consumption and carbon dioxide by face-mask indirect calorimeter for 10 min while seated at rest on a bicycle ergometer (Cosmed E100 P) and during the subsequent graded cycling exercise; EE was calculated according to the Weir equation. Values of EE are averaged over the last 5 min of the resting period (no cycling) and over the last 2 min of cycling at each power output. Each subject performs the graded exercise protocol by pedaling at 60 RPM for 5 min at each of the following incremental (low) power outputs: 10W, 20W, 30W, 40W and 50W. The steady state EE during the last 2 min cycling at each power load is then plotted against the various power outputs. The slope of regression line of the EE-power relationship provides the energy cost of the exercise (kcal/min/watt), the reciprocal of the slope can be calculated to provide the delta efficiency of the subject in performing this cycling exercise. The linearity of the EE-power relationship across such low power cycling exercise (10-50W) has been validated in our laboratory (9).

- *Assessment of energy cost of isometric (intermittent leg press) exercise:*

The isometric exercise test is performed by exerting low-intensity isometric work by intermittently pressing both legs simultaneously against a press-platform incorporating a weighing scale; a procedure developed and validated in our laboratory (10). The intermittent leg press exercise is performed at 4 different isometric loads, namely +10, +15, +25 kg force. At each isometric load, the exercise consists of a succession of 5 cycles of press/rest, with the periods for press (contraction) and rest (relaxation) lasting 30 seconds each. Each 5-min period of intermittent leg press exercise is followed by 10 min of continuous rest. The overall data of EE in response to each press load level - comprising the 5 press/rest cycles - is analyzed as the integrated mean EE of the 5 min EE data points corresponding to the 5 press/rest cycles; this is analogous

to the method for calculating the energy cost (and delta efficiency) of dynamic work. For each experiment, a plot of EE vs isometric loads yields a strong linear relationship, with the correlation coefficient r > 0.9. The slope of this linear regression therefore provides the energy cost of performing the standardized isometric exercise per kg force applied half of the time (i.e. per kg force t1/2); this is referred to as the "delta energy cost" of the exercise. Validation studies in our laboratory (10) indicate that the slope of the EE-Load regression (i.e. the energy cost of the standardized exercise) show good repeatability.

- *Core body temperature, physical activity and finger temperature assessment*

Core body temperature will be measured following ingestion of a small, radio transmitting capsule measuring 17.7 mm long x 8.9 mm diameter and weighing 1.7 g (e-Celsius, BodyCap, France) with temperature data transmitted from the capsule to the monitor every 15 seconds; the monitor is worn on a belt around the waist, and weighs only 75g. Ingested pill-sized capsules have often been used in various research settings including human obesity research (13-15), and meet all relevant ISO standards. The capsules will be ingested under our supervision in the afternoon (3-6 pm) of the day preceding the morning for the laboratory tests. Typical transit time, as noted by loss of the telemetric signal following a bowel movement, is 48 h (range: 12 to 200 h). The timing of capsule ingestion will enable us to measure body temperature throughout the evening prior to the standardized laboratory testing, and during test itself, thus allowing us to investigate both resting and sleeping core body temperature, and to determine effect of each standardized low-intensity physical activity on core body temperature.

Finger skin temperature will be assessed, using infrared camera (FLIR, Sweden Model E63900); at the third fingernail bed of the right hand. The hand will be placed on a grid (1mm2; made of thin nylon line fixed on a solid ring) suspended from, but anchored to, a small table. This grid allows the hand to be supported while avoiding heat transmission to/from the table surface. A series of three consecutive thermographic images will be taken immediately at each time-point before and after the cold water drink, with the camera positioned at a distance of 50 cm from the hand, and perpendicular to the region of interest. The infrared images will be analysed using ENVI software (ITT Visual Information Solutions, Boulder, CO) and the two regions of interest processed across sequential images with a circular cursor (1 cm diameter). The infrared camera has a resolution of 160 x 120 and a thermal sensitivity below 0.06°C.

Oral glucose tolerance test and Blood sampling

Blood sampling is performed during the oral glucose tolerance test in protocol I by the medical doctor collaborating on this project. Every test starts whilst the subject is sitting in a comfortable armchair, with a resting period of at least 10 min, after venous cannulation. After taking a first blood sample (10 ml) in the overnight fasted state, the subject ingests of 75g glucose dissolved in 300 mL water at room temperature (22°C), within five minutes, and blood glucose samples (10 ml) will be drawn every 30 minutes thereafter for up to 120 minutes post-drink. The blood samples are collected into tubes containing the appropriate anticoagulants and stored on ice. Samples will be centrifuged as soon as possible (<2h after collection), with the extracted plasma/serum and stored at -20°C for later analysis. Blood assays will be performed, for glucose and insulin. Furthermore, triglycerides and cholesterols will also be assessed on fasting blood samples..

Cardiovascular functions

These will be monitored continuously and non-invasively in protocol I using the Task Force Monitor (CNSystems, Medizintechnik, Graz, Austria), for beat-to-beat recording of heart rate, blood pressure and cardiac output, namely: (i) Heart rate will be recorded by standard four-lead electrocardiogram at a sampling rate of 1000 Hz; (ii) Blood pressure will be recorded continuously from the middle finger using the non-invasive vascular unloading technique; (iii) Stroke volume and cardiac output will be assessed on a non-invasive and continuous basis by impedance cardiography. Values of cardiac output and total peripheral resistance are determined by calculation from the stroke volume, heart rate and blood pressure signals.

3 PROJECT POPULATION AND STUDY PROCEDURES

3.1 Project population, inclusion and exclusion criteria

Project population

Healthy young adults (n=60) will be recruited for each protocol, with birth weight in the range of 1.5 and 4 kg; the latter range being within the 7th and 90th percentiles for birth weight in Switzerland.. Weight at birth will be validated by presentation of birth record or other document from hospitals or pediatric clinics.

Inclusion criteria

Both parents of Caucasian ancestry; Young men and women (18-35 years old); Healthy (as determined from medical history); Non-smokers; Non-obese, body mass index (BMI) in the range of 18-30 kg/m^2; birth weight in the range of 1.5–4 kg

Exclusion criteria

Subjects born very premature defined as less than 28 weeks of gestation; Subjects who were treated for short stature (e.g. with growth hormone therapy); Subjects taking medications that may alter body temperature, and any other condition that might impair the subject's ability to participate in the study; Competition athletes, smokers, and those have eating disorders; Subjects with a history of alcohol or any other drug abuse ; Weight loss > 5% during 6 weeks prior to inclusion in the study; Pregnant and lactating women or females who desire to become pregnant over the study period (a pregnancy test is performed prior to each experimental protocol); Subjects with gastro-intestinal complications, and fulfilling the official contraindications for the ingestion of the telemetry pill such as those diagnosed for diverticulitis, inflammatory bowel disease, gag reflex disorders or impairments, previous gastrointestinal surgery, hypo-motility of the gastrointestinal tract; Any person who might undergo magnetic resonance imaging (MRI) scanning while the telemetry pill is within the body, or having a cardiac pacemaker or another implanted electro-medical device.

3.2 Recruitment, screening and informed consent procedure

The subjects will be recruited by advertisements (placed at different signposts of buildings) to the general public and to the student and staff population of our university and other higher educational institutes in Fribourg, including Ecole d'ingénieurs et d'Architecture, Haute école de la Santé, Ecole des métiers, etc. All the participants will be informed about the research project and consent form will be sought from each participant.The timeline of the screening and informed consent processes are provided in the section below on 'Study procedures'.

3.3 Study procedures

Interested subjects will participate in one, two or all three of protocols of the proposed project, with at least two days in between any two protocols. The duration of each protocol is ~ 4 hours per morning. All experiments will take place in a quiet, temperature-controlled (~22 °C) room dedicated for human physiological measurements. The subjects will be instructed to refrain from heavy exercise and from taking caffeinated drinks and alcohol on the day before each experiment, and to take a normal meal on the evening before the experiment. Women will only be tested during the follicular phase of their menstrual cycle.

In addition to the day(s) scheduled for test experiment(s), participants will have come previously to the lab for two short preliminary visits. On the 1st visit (20-30 min), participant will (**a**) have all the information about the study, (**b**) be able to ask all the questions about the protocol or measuring devices and (**c**) visit the lab to become familiar with devices used during testing. On the 2nd visit (~40 min), participants will (**d**) give their written consent for the study (after appropriate reflection time and prior to any procedure or collection of data relating to the study), (**e**) complete a questionnaire (medical, nutrition, physical activity, lifestyle) and (**f**) complete initial anthropometric and body composition measurements.

For this project, only one **pregnancy test** will be carried out before the inclusion of women in the study during **[visit 2]**. If a woman has any doubts about her pregnancy, she will have to inform the investigator

and we will provide her a quick pregnancy test before the experiment. The pregnancy tests used (EVIAL Schwangerschaftstest, Inopharm, GmbH Switzerland) are CE approved and registered with Swissmedic.

On arrival at the laboratory around 8AM, subjects will be asked to empty their bladders if necessary, and to sit in a comfortable armchair. The metabolic and cardiovascular monitoring equipment will then be connected. Throughout the procedures when at rest, subjects will be permitted to watch neutral documentaries on a flat TV screen set at eye level. Following a variable period for reaching metabolic and cardiovascular stability (usually between 10-15 min), and after a stable baseline recording of at least 30 minutes, the subjects will then perform the test specific to the experimental protocol.

3.4 Withdrawal and discontinuation

Participants are free to discontinue the project at any stage, without reason. In addition, participants may be asked to withdraw from the project by the investigator in the event of failure to respect the instructions (non-compliance), in case of illness or discomfort to any experimental monitoring. In these cases, the participants will be excluded from the study population, and new participants will be recruited to replace them. In each instance, the biological samples and personal data related to health will be anonymized after analysis.

4. STATISTICAL ANALYSIS PLAN

The data will be analysed by two approaches: (i) by applying regression analyses to investigate the extent to which variability in the various measured parameters is determined by birth weight, and (ii) through category analysis comparing those with (LBW and NBW, using cut-offs for LBW of below 2.5 kg (WHO cut-off) as well as below 3 kg (corresponding to below the 25th percentile for birth weight in Switzerland), and which is often used as cut-off in studies on European Caucasians; the upper cut-off value for 'normal' birth weight (NBW) being 4 kg (90th percentile for birth weight in Switzerland).
Power analysis, with Type I error (α) of 0.05 and a desired power (1 - β) of 0.90 suggests that, for the primary outcome parameters (EE and core temperature), a total number of 25 subjects per group would be required for between-group comparisons (LBW group vs NBW group) with the following assumptions: (i) for EE: a standard deviation (σ) of 0.05 kcal/min across subjects, and a between-group difference (δ) of 0.05 kcal/min, and (ii) for core temperature: a standard deviation (σ) across subjects of ~ 0.15°C and a between-group difference (δ) of 0.15 °C. However, in order to take into account potential drop-outs, we plan to study 30 subjects per group. In addition to data analysis '*by category*' (LBW vs NBW), we will also perform the analysis of data *'by continuum'* using regression analysis of outcomes (y-axis) against birth weight (x-axis).

5. RISK-BENEFIT ASSESSMENT

- In terms of risk of the procedure, this study involves non-invasive techniques for metabolic and cardiovascular phenotyping – except for blood taking which may cause a small amount of pain, but which will be minimised by using well-trained staff (our medical doctor collaborator) to collect the samples.

- Our laboratory has a long-standing experience in similar studies analyzing the acute metabolic and cardiovascular experiments in daily-life situations. In the last 15 years, we have reported the acute metabolic and cardiovascular responses to low-intensity physical activities (7-11), to the ingestion of cold water (16, 17), various sugars such as fructose, glucose and sucrose (18, 19) and high-protein meals (20) – all of which are well-tolerated. We also have first-hand experience using ingestible pill telemetry for 24 hour continuous monitoring of core body temperature (11). It is important that the body temperature capsules will not be given to individuals who have gastrointestinal tract problems (an exclusion criterion). Also, because an individual should not undergo magnetic resonance imaging (MRI) while the capsule is in the body, participants will be given durable wrist bands to wear, from ingestion of the capsule until it is eliminated from the body, to alert medical personnel to the capsules presence in the event of an accident or serious illness.

- Risk of unauthorised data access and/or unwanted identification of project participants will be minimised through appropriate data coding and storage procedures.

- Although this observational study is of no potential personal benefit to the participants, it will provide a better understanding of the mechanisms underlying the increased risks for disease in this population subgroup, and provide important information regarding diagnosis of humans with predisposition to obesity and cardiometabolic diseases (*see above sub-section 1.4 on 'Significance of research project'*).

6. FUNDING / PUBLICATION / DECLARATION OF INTEREST

Publication policy
The results of this study will be published in peer-reviewed journals and presented at national and international conferences.

Data sharing
If the situation arises, data (excluding health related personal data) obtained during this project may be shared with interested third parties (e.g., for publishing requirements allowing for replication or further collaborative analysis) and upon reasonable request to the project leader.

Funding Sources
This research project is mainly funded by the SNF (Swiss National Science Foundation). Furthermore, it also receives intramural funding of our department which covers expenses related to maintenance of equipment involved in this research.

Conflict of Interest
The investigators involved in this research project have no conflict of interest.

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