***SUPPLEMENTARY MATERIAL***

**Medical History of Disease**

Note the etiology of obesity according to the following categories (1):

* Obesity, iatrogenic
* Obesity arising from or aggravated by drug-induced weight gain
* Obesity arising from or aggravated by other iatrogenic procedures
* Obesity arising from or aggravated by a certain defined disease/condition
	+ Obesity arising from or aggravated by a certain defined endocrine disease
* Obesity arising from or aggravated by a certain defined neoplasm
* Obesity arising from or aggravated by major depressive disorder
* Obesity arising from or aggravated by immobilization/inactivity
* Obesity attributable to more than one certain defined etiological factor
* Obesity due to monogenic disease/genetic syndrome
* Obesity due to other major causes

Use this custom assessment:

|  |  |
| --- | --- |
| Self-report | At what age did you begin to gain significant weight?  |
| In adulthood (age 18 and older), what has been your lowest body weight? What has been your highest body weight? |
| How much weight have you gained or lost in the past 3 months? |
| How many times have you lost 10 kg or more? |

**Basic Background Information**

Use this custom assessment:

|  |  |
| --- | --- |
| Self-report | What is your marital status? Do you have children? How many, and what ages? |
| How many years of formal education have you had? (Include primary, secondary, and post-secondary.) |
| In which country did the majority of your education occur? |

**QoL/handicap**

Measure QoL using the 5Q-5D-5L questionnaire (2), including 6 items: 5 under the EQ-5D-5L and 1 under the EQ VAS.

**Dietary Intake**

Measure dietary intake using the EPIC-Norfolk FFQ (3) or region-specific validated FFQ.

Measure dietary quality using the Dutch Healthy Diet index (4) or region-specific index.

Measure emotional eating using the Emotional Subscale of Dutch Eating Behavior Questionnaire (5)

**Physical activity and sedentary behaviour**

*Physical activity*

Measure vigorous, moderate, and light PA: tri-axial accelerometer (e.g., Actigraph wGT3X) placed on hip; worn continuously for one week (or equivalent 4 days, 3 days week + 1 weekend day); generate raw data (6,7).

Measure context and recent history of vigorous, moderate, and light PA (self-reported): Paffenbarger Physical Activity Questionnaire (8,9).

*Sedentary behaviour*

Measure week day time in SB (less than 100 counts per minute); weekend day time in SB: tri-axial accelerometer (e.g., Actigraph wGT3X) placed on the hip; worn continuously for one week (or equivalent 4 days: 3 weekdays + 1 weekend day); generate raw data (6,10).

In the expanded set, measure sitting time and small-screen recreation time in a habitual week, with week and weekend days using the Lasa Sedentary Behaviour Questionnaire (11).

*Physical fitness*

Measure walking distance using the 6-minute walk test and predict CRF (12).

Measure muscular strength using the Southampton grip-strength measurement (13):

**Sleep**

*Sleep duration*

Assess average sleep duration across the week = (SDwork x week days + SDfree x (7-week days))/7

(Also see questions below in circadian misalignment/ social jetlag section)

*Presence of night work and permanent night work*

Shift work and night work are defined by Directive 2003/88/EC of 4 November 2003 (14):

* "shift work" means any method of organising work in shifts whereby workers succeed each other at the same work stations according to a certain pattern, including a rotating pattern, and which may be continuous or discontinuous, entailing the need for workers to work at different times over a given period of days or weeks
* "night time" is ‘any period of not less than seven hours, as defined by national law, and which must include in any case the period between midnight and 05:00’.
* "night worker" means any worker, who, during night time, works at least three hours of his daily working time as a normal course

Use custom assessment:

|  |  |
| --- | --- |
| Self-report | Do you work in shifts? This includes rotating successive morning/evening or rotating successive morning/evening/night shift or other alternate shifts. (yes / no) |
| Does your work involve working at night (i.e. between 0-5am) even irregularly? (yes / no) |
| Do you work permanently at night (i.e. between 0-5am)? (yes / no) |

*Circadian misalignment/ social jetlag*

Social jetlag is the difference between mid-point of sleep times on free days and on workdays.

Use custom assessment:

|  |  |
| --- | --- |
| Self-report | How many days in a week do you work? |
| On nights before workdays, what time do you fall asleep at?\_ \_:\_ \_ o'clock. |
| On work days, what time do you normally wake up at? \_ \_:\_ \_ o'clock. |
| On nights before free days, what time do you fall asleep at? \_ \_:\_ \_ o'clock. |
| On free days, what time do you normally wake up at? \_ \_:\_ \_ o'clock. |

*Presence of OSA and CPAP*

Use custom assessment:

|  |  |
| --- | --- |
| Self-report | Have you been diagnosed with, and do you currently have, a obstructive sleep apnea syndrome? (yes / no) |
| Do you currently use a mechanical device to help you breathe at night (such as a positive airway pressure ventilator)? (yes / no) |

Use STOP-BANG questionnaire for OSA risk (15)

Diagnosis of OSA with PSG relies on the use of the apnea-hypopnea index (AHI). International Diabetes Federation (IDF) classification: AHI < 5/h = no OSA, AHI 5-14 /h = mild OSA, IAH 15-30 /h = moderate OSA, IAH > 30 /h = severe OSA.

In the expanded set, complement this patient-reported sleep data with objective measures: using overnight PSG for quantifying sleep duration and quality as well as sleep apnea; or wrist-worn actigraphy.

**Stress and other psychological variables**

*Stress*

Measure stress using the Perceived Stress Scale (PSS).

**Anthropometry, body composition, and energy expenditure**

Measure anthropometric parameters as follows:

Body weight (kg) to the nearest 100 g;BMI (kg/m2)

Height (m) to the nearest mm

Waist circumference (cm) Waist-to-hip ratio WHR

Hip circumference (cm) Waist-to-height ratio WHtR

Neck circumference (cm) Neck-to-height ratio NHtR

If the waist circumference exceeds the length of the tape, this fact should be recorded in the data collection form together with the maximum length of the tape.

Hip circumference measurement: Hip circumference should be measured as the maximal circumference over the buttocks.

If the hip circumference exceeds the length of the tape, this fact, together with the maximum length of the tape, should be recorded in the data collection form.

Basal metabolic rate

In the expanded set, use the following energy expenditure equations:

Mifflin Jeor: Men 10 x weight (kg) + 6.25 x height (cm) – 5 x age (y) + 5; Women 10 x weight (kg) + 6.25 x height (cm) – 5 x age (y) – 161

Harris Benedict: Men BMR = 66.5 + ( 13.75 × weight in kg ) + ( 5.003 × height in cm ) – ( 6.755 × age in years ); Women BMR = 655.1 + ( 9.563 × weight in kg ) + ( 1.850 × height in cm ) – ( 4.676 × age in years)

Cunninghams = 500 + 22 (lean body mass [LBM] in kg)

Owen = 655.096 + 1.8496 (height in cm) + 9.5634 (weight in kg) − 4.6759 (age)

Katch-McArdle = 370 + 21.6 (LBM in kg)

Nelson = 25.80 (fat free mass in kg) +4.04 (fat mass in kg).

**Hormonal Status**

*TSH*

Take from serum sample; international standard for analysis. Third generation TSH assays are the most sensitive screening tool for primary hypothyroidism. If TSH levels are above the reference range, then measure free thyroxine (T4).

*Menopausal status in females, current medications*

Use this custom questionnaire:

|  |  |
| --- | --- |
| Self-report | (For females) Have you ceased menstruation? (yes / no) |
| Which medications do you currently take? |

**Diabetes**

*Fasting glycemia*

Conduct two measurements of fasting plasma glucose (mg/dL) via blood sampling and assay. Minimum 8-hour fast.

*HbA1c*

Carry out blood sampling and assay using standardized procedures. HbA1c should be measured using only a standardised method (HPLC-CE or HPLC-MS). Use dried blood spots as an alternative if necessary.

*Fasting insulin (one measurement) and insulin-derived insulin sensitivity indices*

Carry out blood sampling & assay and use the following calculations:

 Glucose x insulin

HOMA-IR = -----------------------

 405

 360 x insulin

HOMA-ß = ------------------- %

 Glucose - 63

Both glucose and insulin should be measured during fasting (at least for 8 hours). Glucose is given in mg/dl, insulin in µU/ml.

|  |  |
| --- | --- |
| Self-report | Does a parent or anyone in your immediate family have type 2 diabetes? (yes / no) |

**Cardiovascular Risk**

*Smoking status*

Use this custom questionnaire:

|  |  |
| --- | --- |
| Self-report | How many cigarettes a day do you smoke, on average? |
| For how long have you had a habit of smoking? |

*Blood pressure & heart rate*

Assess via clinical examination; validated automatic blood pressure device (Omron). Take three repeated measurements in a sitting position; in a comfortable environment.

*Total cholesterol, HDL cholesterol, triglyceride*

Carry out blood sampling using a robust assay. Ensure central lab storage.

*High-sensitivity C-reactive protein (hs-CRP)*

Assess hsCRP using an automated immunonephelometric or immunoturbidimetric or immunoluminometric assay.

*Heart electrical activity*

Carry out 1-5 minute ECG

Measure HR, QTc, and QT dispersion as

QTc diff, normal value -25.8 msec,

95% CI: -28.3/-23.2

N=30-100

*Cardiorespiratory fitness*

Carry out 6-minute walk test using protocols in “ATS Statement,” 2002.

In the expanded set of variables, trials may include markers of endothelial function/ arterial stiffness:

* Flow-mediated dilatation (FMD) provides additional, independent prognostic value beyond the classic CVD risk factors (17)
* Pulse wave velocity (PWV) is related to endothelial function and CVD risk factors (18,19)
* Ankle-brachial index (ABI) is used as a screening tool for peripheral arterial disease but also as a CVD risk marker (20)
* Capillary microscopy measures are associated with relevant physiologic outcomes such as blood pressure and insulin resistance (21)
* Ambulatory blood pressure monitoring (ABPM)

**Liver Disease**

*NFS*

Calculate NAFLD fibrosis score= -1.675

+ 0.037 x age (years)

+ 0.094 x BMI(kg/m2)

+ 1.13 x IFG/diabetes (yes=1, no=0)

+ 0.99 x AST/ALT ratio

- 0.013 x platelet (109/l)

- 0.66 x albumin (g/dl)

See online calculator (22)

*FIB-4 score*

Calculate FIB-4 = (age x AST) / (plateletcount x (ALT)½)

*Alcohol intake*

Use the WHO AUDIT questionnaire:

How often do you have a drink containing alcohol (1 drink contains 12 grams of absolute alcohol = 1 can of beer (33cc), = 1 glass of wine (12 cc), 1 shot of strong alcohol (4 ccs of vodka, gin, whiskey etc)? (0) Never; (1) Monthly or less; (2) 2 to 4 times a month; (3) 2 to 3 times a week; (4) 4 or more times a week

How many drinks containing alcohol do you have on a typical day when you are drinking? (0) 1 or 2; (1) 3 or 4; (2) 5 or 6; (3) 7, 8, or 9; (4) 10 or more

**Osteoarthritis**

*Quality of life*

Use EQ-5D-5L (see above).

**Tissue Phenotyping**

For the expanded set, the group summarized some tissues that are possible to analyse, the type of information gained, and the recommended procedures:

|  |  |  |
| --- | --- | --- |
| **Tissue** | **Type of information** | **Procedures** |
| Blood (Whole blood, plasma/serum and peripheral blood mononuclear cells) | * Blood cells, glucose/insulin/lipid variables, inflammation/immune response
* Hepatic enzymes
* Extraction of genomic DNA for assessing genetic variation
 | Routine procedures |
| Blood (Whole blood, plasma/serum and peripheral blood mononuclear cells) | * Circulating proteins, metabolites, exosomes and noncoding RNAs relevant to energy homeostasis
* Extracted DNA and RNA for assessing gene expression and epigenetic modification
 | Advanced procedures |
| Urine | * Ketone bodies to assess compliance in some dietary studies
* Rapid urine test
 | Routine procedures |
| Urine | * Metabolite and peptide profiling
 | Advanced procedures |
| WAT | * Needle biopsies from abdominal subcutaneous WAT
 | Advanced procedures\* |
| Muscle | * Biopsies from vastus lateralis (Bergström needle)
 | Advanced procedures\*\*  |
| Liver | * Measures of NAFLD
 | Ultrasound |

For BIA or DXA or MRI, use the same equipment for all study samples. Use collagenase treatment for isolation of fat cells (Rodbell method: Rodbell, 1964), measures of fat cell volume by light microscopy (n=100) and corrected for SD in volume (Hirsch method: Hirsch & Gallian, 1968). Glycerol method by bioluminescence.

\*Samples of WAT can be used for various applications:

* Whole tissue: immunohistochemistry; transcriptomic; Western blot; etc.
* Ex vivo explant cultures : secretomics; etc.
* Enzymatic digestion (Rodbell’s and derived methods): fat cells to determine fat cell volume by light microscopy (Hirsch method); metabolic assays on isolated fat cells (e.g. lipolysis through glycerol release measurement); transcriptomic analyses; stromavascular fraction to do differentiated preadipocyte cultures.

\*\*Samples of skeletal muscle can be used for various applications:

* Whole tissue: immunohistochemistry (fiber typing); transcriptomic; Western blot; etc.
* Ex vivo skeletal muscle substrate metabolism
* Enzymatic digestion to prepare myoblasts that can be differentiated into myotubes

The group noted that the definition and diagnosis of sarcopenia were addressed by a recent European consensus (25), and the expert group recommended that, in studies where sarcopenia is of interest (in the expanded set), researchers follow the procedures outlined in the consensus paper: screen for possible sarcopenia using a short questionnaire called the SARC-F (26), assess for sarcopenia using a test of muscle strength (either grip strength or chair stand test), assess muscle mass deficit using BIA, DXA, or CT scan, and finally, assess the severity using muscle performance tests.

**Genetics**

Conduct genetic analysis from blood (not saliva) sample.

**Omics**

The researchers recognized the need to integrate approaches and combine diverse types of data using integrated multi-omics approaches (27), which are the gold standard in the field. The following general guidelines apply:

* Because of the large amount of complex data generated by omics studies, specialized expertise in bioinformatic analysis is essential—not just for analyzing the data after collection (models to apply/build, transformations needed), but also for the initial design of the study (power calculations, planned analysis methods).
* For multi-centre studies, clear standard operating procedures (SOPs) should be in place from sample collection through to final analysis, with appropriate infrastructure in all participating centres. This allows comparability of the data across different sites.
* Work should be centralized as much as possible to minimize variability, lest the interlab variability overshadow any true variability from sample to sample.

*Sample storage recommendations*

SOPs for sample collection and storage are agreed upon and written.

All sites use the exact same tubes, reagent providers, etc.

Conditions for (intermediate) storage are the same across sites: ideally samples should be stored in liquid nitrogen or at -80°C as sampled and should not be aliquoted on-site.

Store as much sample as possible, as cold as possible.

Omics studies should also take sample storage considerations into account, since over time new discoveries will almost certainly prompt re-analysis. In genomics studies, the most conservative storage option should be used to allow future detection of labile molecules (for instance, nucleotide modifications in DNA/RNA).

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