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Cross-sectional and longitudinal study protocols of the 'ADIposity and BOne metabolism: effects of eXercise-induced weight loss in obese adolescents' (ADIBOX) project

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ABSTRACT

Introduction: A need exists for sustainable and clinically effective weight management interventions, suitable for preventing well-linked chronic disease such as diabetes and cardiovascular disease and some less investigated secondary conditions such as bone alteration. The ADIposity and BOne metabolism: effects of eXercise-induced weight loss in obese adolescents (ADIBOX) protocol was designed to provide a better understanding of the interaction between adipokines and bone hormones in adolescents with obesity and how a 10-month physical activity programme may affect these interactions.

Methods and analysis: The ADIBOX protocol combines 2 studies. The first study involves a total of 68 adolescents aged 12–16 years. This cross-sectional study will include both males and females (1:1 ratio), either living with obesity/overweight (n=34; body mass index (BMI) ≤97th centile and ≥85th centile) or normal weight (n=34; BMI<85th centile). The second study is a longitudinal study that will include 50 obese adolescent girls and track them over a period of 42 weeks. Weight loss programme will consist of a combination of physical activity and a normocaloric diet. Bone and adiposity-related measurements will be performed every 14 weeks. Both studies will assess participants' anthropometric profile, nutrition and physical activity, body composition, bone densitometry and blood markers of bone, growth and adiposity.

Ethics and dissemination: The ADIBOX protocol complies with the ethics guidelines for clinical research and has been approved by their respective ethics committee (Australian Catholic University Committee Ethic, Australia and Hospital Sud Est 1 committee, France). Findings from this protocol are expected to clarify the possible interactions between adiposity and bone in childhood obesity and will be disseminated at several research conferences and published articles in peer-reviewed journals.

Trial registration number: NCT02626273; Pre-results.

Strengths and limitations of this study

- Advancing the understanding of the bone adipocyte cross-talk in obese adolescents.
- Investigating the effects of physical activity-induced weight loss on bone, growth and adiposity markers.
- Longitudinal study only assessing female adolescents.
- Unable to accurately account for the different phases of the female menstrual cycle.

INTRODUCTION

The complex consequences of childhood obesity represent major concerns in most developed countries,[1] largely contributing to metabolic complications with costly repercussions for the burden of disease.[2 3] This burden is exemplified by high prevalence rates of overweight or obesity. For instance, in Australia the prevalence of overweight and obesity is 24% in boys and 23% in girls, while in France the prevalence is 20% of boys and 16% of girls.[4]

Understanding the effects of obesity-induced fat mass accumulation on bone during growth is of particular interest given that obesity is a risk factor for fracture during the peripubertal period.[5] Childhood and adolescence are characterised by significant bone accrual. The achievement of optimal skeletal gains throughout the maturation process is crucial in order to optimise bone mass before adulthood. However, the levels of some circulating hormones, either dependent or independent of fat mass, vary widely from childhood to puberty and adulthood and may subsequently strongly affect bone density.

Owing to their common origin, bone cells and adipocytes are intimately associated, suggesting a cross-talk between adipose tissue and bone tissue. Adipose tissue has long been considered an inert tissue dedicated for energy storage. Recent advances have established that both adipose tissue and bone tissue are endocrine organs. Adipose tissue is involved in satiety, energy balance and pubertal development,[6] while bone tissue acts on energy expenditure and glucose homoeostasis.[7]

The impact of obesity on bone metabolism is gaining the attention of researchers. The skeletal system is stressed from mechanical loading and also through the metabolic effect of some of the adipokines secreted by the adipose tissue. Indeed, obesity leads to hormonal alterations associated with increasing proinflammatory cytokines and oxidative stress. These events favour the accumulation of fat mass and loss of bone mass. Moreover, weight loss induced by dietary restriction can lead to weakening bones.[8]

Decreased mechanical loading on the skeleton,[9] altered hormonal secretion involved in bone regulation[10] or decrease of caloric intake[9] may contribute to the bone breakdown generated by weight loss strategies during childhood. However, weight bearing physical activity may be anabolic for bone, even during periods of weight loss.

A better understanding of the effects of weight loss programmes on the bone adipocyte cross-talk in adolescents is required in order to prescribe effective and safe weight loss programmes. As previously shown, the effectiveness of weight loss programmes for improving the overall health in obese youth may be compromised by potential side effects (ie, loss of bone mass).

Our team recently justified the need of further investigations exploring the bone adiposity cross-talk in adolescents with obesity.[11] Fat and bone are linked by multiple possible interactions but the low volume and inconsistent methodologies of existing studies prevent strong conclusions about the presence of definitive relationship. A specific project is required to advance the understanding of the complex relationship between fat mass and bone mass in adolescents with obesity.

The overall purpose of this work is to investigate the effects of body mass and its variations (taking into account body composition) on the bone adipocyte cross-talk in adolescents with obesity. To do so, a cross-sectional and a longitudinal study will be conducted. The ADIposity and BOne metabolism: effects of eXercise-induced weight loss in obese adolescents (ADIBOX) protocol will contribute to the evidence required to promote the holistic significance of sustainable weight management in obese youth.

METHODS AND ANALYSIS

Cross-sectional study—study I

Protocol design

This study will investigate whether the interaction between adipokines and bone hormones differ between obese and their normal weight peers (figure 1).

Selection criteria

Adolescents aged between 12 and 16 years, over Tanner stage 3, free of any recent history of hospitalisation (past 2 years) or of systemic illness lasting more than 2 weeks in the past 12 months will be recruited. In compliance with Human Ethics guidelines, adolescents and their legal representatives will sign assent and consent forms, respectively. Also, the recruited adolescents will not have any known history of metabolic bone or muscle disease, nor metabolic diseases such as diabetes, insulin resistance, or hypothyroid or hyperthyroid activity.

Additional inclusion criteria relate to being free from a diagnosis of congenital cardiovascular disease, not regularly consuming alcohol, being a non-smoker and not taking medication known to alter bone metabolism, nor hormones or calcium preparations (vitamin D, calcium, protein). Owing to the low exposure to radiation, women will be excluded if they are pregnant and will need to have a regular menstrual cycle. Obese men and women recruited for this study will have a body mass index (BMI) ≥95th centile.[12] Furthermore, adolescents who are overweight or with obesity will be ineligible if they were enrolled in a weight management programme during thepast 2 years.

Age-matched and gender-matched normal weight participants (BMI<85th centile)[12] will also be recruited. If necessary, in order to include a sufficient number of participants, we will consider recruiting both obese and overweight (BMI≥85th centile) adolescents. Normal weight, overweight and obese adolescents will be excluded if they participate in more than 250 min of physical activity outside of school per week. The 250 min was derived by excluding the physical education and school sport from guidelines recommending 60 min per day or 420 min per week.[13] This ensured that none of the participants exceeded the recommended guidelines for physical activity. Minutes of weekly physical activity will be monitored during the screening visit using the International Physical Activity Questionnaire (IPAQ).

Power analysis

Owing to a lack of data and reported discrepancies in the literature it seemed difficult to propose an a priori accurate sample size estimation. The number of participants to be included in the study has been extrapolated from the data obtained on previous works.[14] For a two-side type I error at 5% and a statistical power equivalent to 80%, 17 participants of each sex per group of obese and lean body composition status (a total of 68 participants) will allow us to highlight a clinical and realistic effects size of 1 SD between groups in key obesity outcomes.[15]

Participants

As previously stated participants engaged in this protocol will be adolescents aged between 12 and 16 years, with a self-reported pubertal development status equal to or exceeds Tanner stage 3. Differences in race and

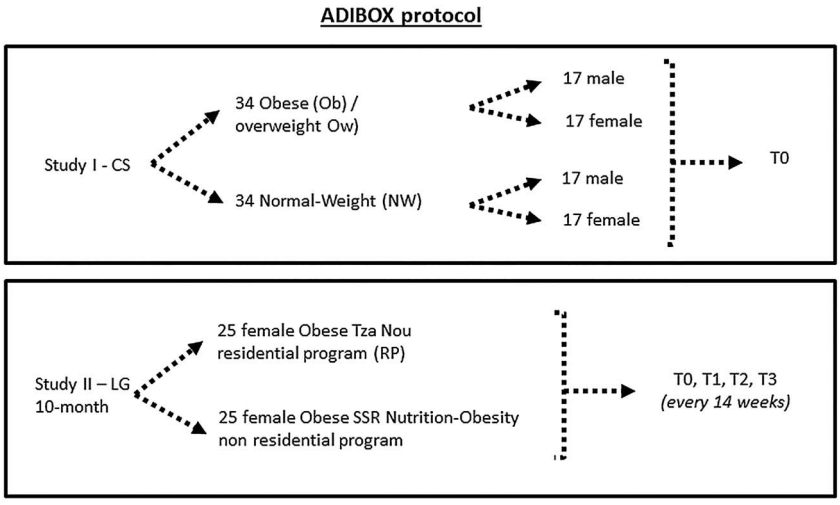

Figure 1 ADIBOX protocol. ADIBOX, ADIposity and BOne metabolism: effects of eXercise-induced weight loss in obese adolescents; CS, cross-sectional study; LG, longitudinal study.

ethnicity of participants will be accepted. The adolescent stage of development was selected to advance the understanding of maturation processes, growth changes and the possible exploratory aspects of weight changes. For this study, we will recruit both male and female adolescents who are obese or overweight (1:1 ratio) as well as their age-matched and sex-matched lean peers. The non-obese group is necessary for a better understanding of the hormonal changes induced by obesity.

Participant recruitment

Following approval from ethic committees, and based on our calculation, a total of 68 adolescents, men and women will be enrolled in the first study of the ADIBOX protocol. All participants as well as their legal representatives will be given written information regarding the project and be asked to sign consent and assent forms. Adolescents will be recruited from medical clinics in Melbourne and Sydney, Australia, and from the Australian Catholic University (Victoria or New South Wales—Australia) using the 'snowballing' principle. The 68 adolescents will be split into two groups of 34 each; a non-obese group and an obese/overweight group. Groups will comprise 17 men and 17 women, in order to understand more about sex-based comparisons.

Measurements

After a screening visit to ensure the suitability of adolescents to complete the study, each adolescent will perform a battery of tests. Data collection will be performed only once (figure 2).

Ability to complete the study

The researcher will make a phone call prior to the beginning of the study to ensure the suitability of adolescents to complete it. If the participant is willing to participate, general information such as medical history of the family, early childhood development and history of obesity will be gathered during this phone call.

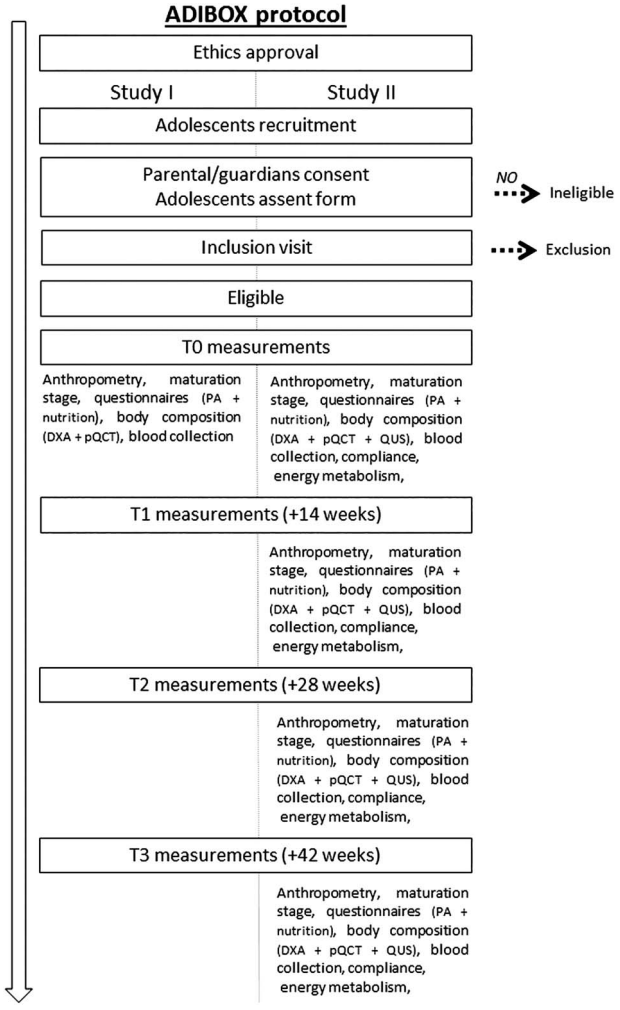

Figure 2 ADIBOX protocol overview. ADIBOX, ADIposity and BOne metabolism: effects of eXercise-induced weight loss in obese adolescents; DXA, dual energy X ray absorptiometry; PA, physical activity; pQCT, peripheral quantitative CT; QUS, quantitative ultrasound.

Maturation

Pubertal status will be assessed using the Tanner's Stage of Pubertal Development model for biological

maturation. An experienced researcher will be provided with a series of pictures showing the five stages of puberty for breast or genitalia and pubic hair development.[16] Despite the fact that self-reported maturation stages is less precise than a paediatric assessment it still has a good validity and reliability.[17]

Anthropometry

Anthropometric measurements will be taken accordingly to the anthropometrics recommendations of the International Society for the Advancement of Kinanthropometry for standing height (m) and body mass (kg), waist circumference (cm), and lower limb bone lengths/breadths (cm).[18]

Physical activity and nutrition questionnaires

Among participants, validated sedentary activity[19] and physical activity questionnaires (IPAQ)[20] will be distributed to assess physical activity. In addition, a food frequency questionnaire,[21] current eating habits questionnaire[22] and a food preferences questionnaire[23] will be distributed.

Body composition

Body composition will be measured by dual energy X ray absorptiometry (DXA) (DXA, iDXA, GE healthcare, Lunar Corporation, Madison, Wisconsin, USA). Bone mineral density (BMD, g/cm^2), bone mineral content (BMC, g), bone area (cm^2), and lean and fat mass (subcutaneous and visceral) will be determined for each adolescent. The DXA measurements will be taken for the whole body, lumbar spine (L2–L4) and the non-dominant hip (including the femoral neck, trochanteric and intertrochanteric regions). All DXA scans will be conducted by the same technician and quality assurance checks will be performed routinely. This scanning protocol and offline analyses have been validated among comparable population.[24]

Peripheral Quantitative CT

Musculoskeletal parameters for bone geometry including bone strength will be obtained using a peripheral quantitative CT (pQCT) XCT 2000 in study I (XCT 2000, Stratec Medizintechnik, Pforzheim, Germany). BMC (g/cm), volumetric cortical and trabecular BMC (mg/cm^3), total area (mm^2), cortical and trabecular area (mm^2) and density (g/cm^2), bone strength (mm^3) will be assessed at the distal (4%), proximal (66%) site of the non-dominant tibia and radius. A planar scout scan was first conducted to determine the anatomical reference line for the radius and tibia. Tomographic slices of 1 mm thickness were obtained at the 4% and 66% sites measured distally. Scan speed and voxel size were 30 mm/s and 0.5 mm, respectively. To assure quality of measurement, calibration checks will be performed by scanning a standard phantom with known densities, prior to each scan.

In order to calculate BMC, volumetric cortical BMC, volumetric trabecular BMC, cortical area (CoA), cortical density (CoD), trabecular area (TrA), trabecular density (TrD) and stress–strain index (SSI) will be analysed with Stratec pQCT software. Contour mode 1 with a threshold of 180 mg/cm^3 was used to separate soft tissue and bone in order to analyse trabecular bone. Cortical bone was identified and removed using a constant default threshold of 710 mg/cm^3. A contour mode 3 with peel mode 1 at a threshold of 40 mg/cm^3 was used to assess muscle and fat cross-sectional area. Radial distribution and polar density will be estimated with an open source bone image analysis tool, known as Image J (rsbweb.nih.gov/ij). To differentiate the cortical bone a threshold of 710 mg/cm^3 and a 3×3 median filtering of the image was used. For mean cortical polar bone mass distribution and polar distribution the cortex is divided into equal sectors, while the 66% slice radial distribution will be estimated by subdividing the cortex into concentric rings. For more information, this open source software was validated and described in two studies of Rantalainen *et al*.[25 26]

Endocrine assays

Blood samples will be collected by a qualified paediatric nurse after the participants have fasted overnight. Blood will be collected by a venipuncture at the brachial vein. After collection, blood will be centrifuged and aliquots will be stored (-80°) for subsequent analysis.

Basic biology (triglycerides (TG), cholesterol, low-density lipoprotein (LDL), high-density lipoprotein cholesterol (HDLC), glycaemia, insulin, ultrasensible C reactive protein (CRP)) will be assessed in the biochemistry laboratory of the Australian Catholic University (Sydney, Australia). Bone markers will be assayed in the biochemistry laboratory of the Sydney University (Sydney, Australia): serum osteoprotegerin (OPG), receptor activator of nuclear κB ligand (RANKL), sclerostin, bone alkaline phosphatase and undercarboxylated osteocalcin (unOc) will be assayed using ELISA kits while type I collagen C-telopeptides (CTX), PINP, osteocalcin total and vitamin D by Cobas 6000 (Roche Diagnostics).

All other biochemical determination (leptin, adiponectin, tumour necrosis factor (TNF)-α, interleukin (IL)-6, growth hormone (GH), insulin-like growth factor-1 (IGF1), insulin-like growth factor-binding protein 3 (IGFBP-3), oestradiol, follicle stimulating hormone/luteinizing hormone (FSH/LH) and parathyroïd hormone (PTH)/calcitonin) will be made using commercial kits, following manufacturers' recommendations, including sampling steps, allowing the best performances of coefficient of variation and sensitivity. All analyses will be conducted in duplicate by the same technician.

Statistical analysis

Data will be analysed using Stata (StataCorp, College Station, Texas, USA) and significance will be accepted at

a two-sided α level of p<0.05. After testing for normal distribution (Shapiro-Wilk test), data will be treated either by parametric or non-parametric analyses according to statistical assumptions. Means and SDs will be reported for descriptive statistics and used to summarise the data. In case of non-Gaussian distribution, median and IQR will be reported. For general linear model (GLM) or multilevel model, the data will be log transformed or Box-Cox transformed, where appropriate. Group comparisons will be assessed using analysis of covariance (ANCOVA) to compare quantitative parameters between obese and lean adolescents, as well as for gender comparison after controlling for pubertal status.

Ethical considerations and dissemination

The cross-sectional study has been approved by the Australian Catholic University Ethic Committee (2014 320N). In accordance with ethical considerations, the principal investigator is responsible of ensuring that participants understand the potential risks and benefits of taking part in the study. Moreover, the principal investigator is responsible for obtaining a written consent from adolescents and their legal guardians/parents.

Ten-month longitudinal study—study II

Protocol design

This 42-week longitudinal study with repeated measures on four occasions (baseline and thereafter every 14 weeks) will allow us to understand the effect of physical activity-induced weight loss on the bone adipocyte cross-talk in obese adolescent females (figure 1). For this study, we will compare two groups of obese adolescents: one following a residential weight loss programme and compare their results to a group not receiving any intervention or advice for weight loss.

Selection criteria

Similarly to the cross-sectional study, obese females (BMI>95th centile)[12] who are invited to take part in this study will be aged between 12 and 16 years, with a self-reported pubertal status equal to or exceeding Tanner stage 3, free of any recent history of hospitalisation (past 2 years) or of systemic illness lasting more than 2 weeks in the past 12 months. In compliance with Human Ethics guidelines, adolescents and their legal representatives will sign assent and consent forms, respectively, and will have to be covered by social health insurance.

Also, the recruited adolescents will need to have no limitations to being physically active and will not have a known history of metabolic bone or muscle disease, nor metabolic diseases such as diabetes, insulin resistance or hypothyroid or hyperthyroid activity. Additional inclusion criteria relate to being free from a diagnosis of congenital cardiovascular disease, not regularly consuming alcohol, being a non-smoker and not taking medication known to alter bone metabolism, nor hormones or calcium preparations (vitamin D, calcium, protein). Owing to the low exposure to radiation, women will be excluded if they are pregnant and will need to have a regular menstrual cycle.

Exclusion criteria

Adolescents will be excluded if major treatment and/or protocol deviations are observed by the obesity centre team. In addition, they will be excluded and not considered in the analysis if their compliance to the programme is <80%. This threshold has been chosen based on our previous experience[27] and the fact that adolescents are integrated as a specific institution.

Power analysis

Sample size estimation has been set within the context of the variation index of body fat relative to the variation of a marker of bone mass measured at the lumbar spine. Currently, few articles provide information about the combined effects of body fat and lumbar spine parameters in the literature. However, based on limited previous research[28] the index difference of the variation index of body fat, relative to the variation of a marker of bone mass at lumbar spine between groups, of 1.2–1.5 (SDs) can be expected. It seemed reasonable to set the variation index at 1.3 (SDs). Based on this, a sample size of 21 female participants was predicted to highlight statistically significant differences with a statistical power of 90% and a two-sided type I error less of 5%. Anticipating a potential 20% drop-out among participants, a minimum of 25 volunteers per body composition group will be invited to take part in the study.

Participants

As previously stated participants in this second study will be adolescents aged between 12 and 16 years, with a paediatrician-assessed maturation stage equal to or exceeding Tanner stage 3. The adolescent stage of development was selected to advance the understanding of maturation processes, growth changes and the possible exploratory aspects of weight changes. Race and ethnicity of participant is mixed.

As the obesity centre that our study group is associated with is hosting only women, we will restrict recruitment to adolescent women. The community dwelling control group with obesity is necessary in this study to provide useful information on setting the effects of exercise-inducing weight loss on the bone adipocyte cross-talk.

Participant recruitment

Following approval from ethic committees, and based on our calculations, a total of 50 adolescents' girls will be enrolled for the second study of the ADIBOX protocol. All participants as well as their legal representatives will be given written information regarding the project. Both the adolescents and their legal guardians will have to sign consent forms before enrolment of the adolescent. Adolescents will be recruited from the 'Tza Nou' Medical Center for Children and the SSR (ambulatory care and rehabilitation department) Nutrition-Obesity

in Auvergne (France). The participants will be divided into two groups on a convenient basis: an intervention group (25 obese women from 'Tza Nou'—France) who will undertake the residential programme and an obese control group (25 obese women from the SSR Nutrition-Obesity—France) who will remain with their family and in a community setting.

Institution programme

Adolescents from the intervention group will be enrolled at the obesity centre for the whole school year (ie, 10 months). The obesity centre employs a multidisciplinary team to provide the best weight management care to adolescents during their stay. The weight loss programme is an integral part of the obesity centre programme and fundamentally combines physical activity with a normocaloric diet monitored by a dietician. The physical activity programme consists of two training sessions (aerobic and resistance training) per week. Moreover, adolescents will be engaged in two additional sessions per week, consisting in recreational activities such as ball and racquet games, trekking, snowshoeing or swimming.

Measurements

After a screening visit to ensure the suitability of adolescents to complete the group in the study to which they have been assigned, each adolescent will perform a battery of tests (described below). Data collection will be performed four times for the 42-week longitudinal study. Adolescents will be screened at baseline (T0) and thereafter every 14 weeks. This period of testing coincides with school holidays and includes the bone remodelling cycle period of 3 months (figure 2).

Ability to complete the study

A paediatrician will meet participants assigned to the weight management group prior to the beginning of the study to ensure the suitability of adolescent to complete the weight loss protocol. If the participant is willing to participate, general information such as medical history of the family, early childhood development and history of obesity will be gathered by the paediatrician.

Maturation

Pubertal status will be assessed by a paediatrician using Tanner's Stage of Pubertal Development model for biological maturation[16] as well as laboratory blood analyses (oestradiol, testosterone, FSH/LH). Information about the age at menarche and regularity of menstruation will be gathered by the paediatrician.

Anthropometry

Anthropometric measurements will be taken according to the recommendations of the International Society for the Advancement of Kinanthropometry for the following: standing height (m) and body mass (kg), waist circumference (cm), and lower limb bone length/breadth (cm).[18]

Physical activity and nutrition questionnaires

Among participants, validated sedentary activity[19] and physical activity questionnaires (IPAQ)[20] will be distributed to assess physical activity. In addition, a food frequency questionnaire,[21] current eating habits[22] and food preferences questionnaire[23] will be distributed.

Body composition

Body composition will be measured by DXA (DXA, QDR-4500A, Hologic, Waltham, Massachusetts, USA). BMD (g/cm^2), BMC (g), bone area (cm^2), and lean and fat mass (subcutaneous and visceral) will be determined for each adolescent. The DXA measurements will be taken for whole the body, lumbar spine (L2–L4) and the non-dominant hip (including the femoral neck, trochanteric and intertrochanteric regions). All DXA scans will be conducted by the same technician and quality assurance checks will be performed routinely.

Peripheral quantitative CT

Similarly to the cross-sectional study, musculoskeletal parameters for bone geometry including bone strength will be obtained using the pQCT XCT 3000L (XCT 3000L, Stratec Medizintechnik, Pforzheim, Germany). BMC (g/cm), volumetric cortical and trabecular BMC (mg/cm^3), total area (mm^2), cortical and trabecular area (mm^2) and density (g/cm^2), and bone strength (mm^3), will be assessed at the distal (4%) and the proximal (66%) site of the non-dominant tibia and radius. Pictures will be analysed with Stratec pQCT software and Image J, an open source bone image analysis tool (rsbweb.nih.gov/ij).[25 26]

Quantitative ultrasound

This method may provide additional information on bone quality and architecture. To predict fracture risk, quantitative ultrasound (QUS) measurements will be made with Achilles Insight+ (Achilles Insight, GE, Lunar Corporation, Madison, Wisconsin, USA) on the non-dominant calcaneus. The QUS results will be expressed in terms of broadband ultrasound attenuation (BUA, dB/MHz) which is postulated to reflect bone mass and architecture, and speed of sound (SOS, m/s) which is estimated to reflect the mass and elasticity of bone. This technique has been shown to be useful in child and adolescent bone investigations.[29] The in vivo coefficient of variation assessed in our laboratory for paediatric use is 1.4% for BUA and 0.16% for SOS measurements. All analyses will be conducted in duplicate by the same observer.

Endocrine assays

Similarly to the cross-sectional study, blood samples will be collected by a qualified paediatric nurse after the participants have fasted overnight. Blood will be collected

by a venipuncture at the brachial vein. After collection, blood will be centrifuged and aliquots will be stored (-80°) for subsequent analysis.

Basic biology (TG, cholesterol, LDL, HDLC, glycaemia, insulin, ultrasensible CRP) will be assessed in the biochemistry laboratory of Clermont-Ferrand University Hospital, while bone markers will be assayed in the biochemistry laboratory of Montpellier University Hospital (OPG, RANKL, sclerostin, bone alkaline phosphatase, unOc, CTX, PINP, osteocalcin total and vitamin D).

All other biochemical determination (leptin, adiponectin, TNF-a, IL-6, GH, IGF1, IGFBP-3, oestradiol, FSH/LH and PTH/calcitonin) will be made using commercial kits, following manufacturers' recommendations, including sampling steps, allowing the best performances of coefficient of variation and sensitivity. All analyses will be conducted in duplicate by the same technician.

Energy metabolism

Routinely performed in our laboratory, resting metabolism rate (RMR) is a reliable measure that reflects muscle mass. RMR will be measured in the morning using indirect calorimetry (K4b^2, Cosmed, Rome, Italy). Before each test, gas analysis is calibrated in accordance with the manufacturer's recommendations. Participants will be asked to lie in a supine position in a thermoneutral environment (22–25°C room temperature) for 45 min before starting the measurements. After achieving a steady state, O_2 consumption and CO_2 production standardised for temperature, barometric pressure and humidity will be recorded at 1 min intervals for 20–45 min and averaged over the whole measurement period.

Compliance

Volunteers engaged in the residential arm of the longitudinal study will be monitored by educators working at the obesity centre in order to control for their daily engagement and adherence to the weight loss lifestyle intervention combining physical activity and nutrition (normocaloric diet). Educators will complete an individualised daily journal on the compliance of each participant.

Statistical analysis

Data will be analysed using Stata and IBM Statistics SPSS V.22 (IBM Corp, 2013, Chicago, Illinois, USA) and significance will be accepted at a two-sided α level of $p<0.05$. After testing for normal distribution (Shapiro-Wilk test), data will be treated either by parametric or non-parametric analyses according to statistical assumptions. Means and SDs will be reported for descriptive statistics and used to summarise the data. In case of non-Gaussian distribution, median and IQR will be reported. For a GLM or a multilevel model, the data will be either log transformed or Box-Cox transformed, where appropriate.

Student's t-tests or Mann-Whitney U test (when assumptions of t-test are not met: normal distribution and homoscedasticity will be studied using the Fisher-Snedecor test) will be performed to compare adipose tissue (total, subcutaneous, visceral) variation reported to bone mass variation at the lumbar spine between groups at baseline. χ^2 tests or the Fisher's exact tests will be used for categorical variables. Group comparisons will be assessed using ANCOVA to compare quantitative parameters between obese and non-obese adolescents, as well as for gender comparisons after controlling for pubertal status. Moreover, Pearson's (or Spearman's when appropriate) correlation coefficients will be used and compared with the Fisher test (command corcor Stata) to measure the potential links between exercise-induced weight loss and changes in adipose tissue and bone mass. Longitudinal data will be treated using a mixed model analyses in order to treat fixed-effects group, time and group×time interactions taking into account between and within participant variability. Moreover, the impact of covariates (ie, compliance, BMI, hormonal status) will be explored. A sensitivity analysis of missing data will be performed to ensure the relevance of the longitudinal data (missing at random or MCAR missing completely at random). In order to assess the problem caused by missing longitudinal data, estimation methods developed by Verbeke and colleagues will be proposed.

Ethical considerations and dissemination

The longitudinal study has been registered with a clinical trial number (NCT02626273). Ethics approval has been obtained from the Hospital Sud Est 1 committee (2015-33).

In accordance with ethical considerations, the principal investigator of each study is responsible for ensuring that the participants understand the potential risks and benefits of taking part in the study. Moreover, the principal investigator is responsible for obtaining written consent from the adolescents and their legal guardians/parents.

Link between studies

The ADIBOX protocol combines two studies: a cross-sectional study addressing gender and weight effects of the bone adipocyte cross-talk while the longitudinal study will focus on the effects of weight loss induced by physical activity and nutrition in obese adolescents. Since the actual literature remains unclear regarding the interactions between adipocyte and osteocyte, both studies appear necessary to advance the understanding of these interactions in obesity and how they change over time. As previously stated by our team[11] only limited and heterogeneous literature (ie, large heterogeneity: including gender, age, pubertal status, maturations stage) is available to date for explaining the inability to draw clear conclusions. Using the same

methodology, our studies will provide complementary approaches with transversal and longitudinal results.

Radiation

Both DXA and pQCT provide measures of body composition and bone properties by exposing participants to low-level radiation: 0.0056 mSv from DXA scans (whole body, lumbar and hip) and 0.0014 mSv from the pQCT scans (tibia and radius measures).[30] Over the duration of each study, the effective dose of 0.007 and 0.03 mSv will be administered, respectively, for both the cross-sectional study and the 10-month study.

Confidentiality

For both studies, data will be stored in the principal investigator's office in password-protected computer only accessible to members of our research team. Within the electronic database, participants' names will be replaced with numeric identity codes. Blood samples will also be labelled with the numeric identity codes and samples will be stored in our laboratories. Only aggregate results will be reported, ensuring participants' anonymity.

Dissemination

The results of both studies will be disseminated at several research conferences and published articles in peer-reviewed journals.

DISCUSSION

The ADIBOX protocol has been developed to advance the understanding of the bone adipocyte cross-talk in adolescents and to extend what is currently known about growth-related and body mass changes that may or may not influence obesity. The specific effects of weight loss on bone tissue are uncertain, particularly in young people. Growth responses cannot be overlooked. Targeting puberty stages should help to highlight growth-stimulated responses which are often masked by assessing young participants across puberty ranges. Adolescence is a very sensitive period, determined by positive and/or negative influences that may contribute or adversely alter adolescents' health. Despite the well-described regular exercise-induced benefits in terms of health improvement and maintenance, adolescence is characterised by a marked decline of physical activity level in men and women.

The ADIBOX protocol will attempt to clarify the impact of the bone adipocyte interaction in obese adolescents and should provide a better understanding about the bone strength indices and adiposity cross-talk in adolescent men and women. In addition, this will be the first protocol investigating the effects of physical activity-induced weight loss on bone, growth and adiposity markers. Findings from this protocol are expected to clarify the possible interactions between adiposity and bone in childhood obesity.

Current study status

The ADIBOX protocol began recruiting participants in September 2015. Data collection will be completed in January 2017. Regarding the longitudinal study, data collection started in September 2015 and will be running for 10 months.

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Contributors ADIBOX principal investigators DC, GN, DT and DG are responsible along with EC (PhD student) for identifying the research question, the design of the protocol. GN, DG and EC were responsible for obtaining ethics committee approval and recruiting participants for study I, while DC, DT, FD and EC were responsible for obtaining ethics committee approval and recruiting participants for study II. BP was responsible for all the statistical part of this protocol. All authors were responsible for the draft of this manuscript and have read and approved the final version.

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REFERENCES

1. WHO. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organ Tech Rep Ser* 2000;894:i–xii, 1–253.
2. Daniels S. Complications of obesity in children and adolescents. *Int J Obes (Lond)* 2009;33:S60–5.
3. Ebbeling CB, Pawlak DB, Ludwig DS. Childhood obesity: public-health crisis, common sense cure. *Lancet* 2002;360:473–82.
4. Ng M, Fleming T, Robinson M, *et al*. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2014;384:766–81.
5. Dimitri P, Bishop N, Walsh JS, *et al*. Obesity is a risk factor for fracture in children but is protective against fracture in adults: a paradox. *Bone* 2012;50:457–66.
6. Karsenty G. Convergence between bone and energy homeostasis: leptin regulation of bone mass. *Cell Metab* 2006;4:341–8.
7. Lee NK, Sowa H, Hinoi E, *et al*. Endocrine regulation of energy metabolism by the skeleton. *Cell* 2007;130:456–69.
8. Ensrud KE, Ewing SK, Stone KL, *et al*. Intentional and unintentional weight loss increase bone loss and hip fracture risk in older women. *J Am Geriatr Soc* 2003;51:1740–7.
9. Shapses SA, Sukumar D. Bone metabolism in obesity and weight loss. *Annu Rev Nutr* 2012;32:287–309.
10. Ricci TA, Heymsfield SB, Pierson RN, *et al*. Moderate energy restriction increases bone resorption in obese postmenopausal women. *Am J Clin Nutr* 2001;73:347–52.
11. Chaplais E, Thivel D, Greene D, *et al*. Bone-adiposity cross-talk: implications for pediatric obesity. A narrative review of literature. *J Bone Miner Metab* 2015;33:592–602.
12. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, *et al*. CDC growth charts: United States. *Adv Data* 2000:1–27.

13. Medicine ACoS. *ACSM's guidelines for exercise testing and prescription*. Lippincott: Williams & Wilkins, 2013.
14. Campos RM, Lazaretti-Castro M, Mello MT, *et al*. Influence of visceral and subcutaneous fat in bone mineral density of obese adolescents. *Arq Bras Endocrinol Metabol* 2012;56:12–18.
15. Cohen J. *Statistical power analysis for the behavioral sciences*. Academic Press, 2013.
16. Tanner JM. Growth at adolescence. Growth at adolescence, 2nd ed. Thomas: Springfield, Ill. 1962.
17. Duke PM, Litt IF, Gross RT. Adolescents' self-assessment of sexual maturation. *Pediatrics* 1980;66:918–20.
18. Marfell-Jones MJ, Stewart AD, de Ridder JH. International standards for anthropometric assessment. 2012.
19. Hardy LL, Booth ML, Okely AD. The reliability of the adolescent sedentary activity questionnaire (ASAQ). *Prev Med* 2007;45:71–4.
20. Hagströmer M, Bergman P, De Bourdeaudhuij I, *et al*. Concurrent validity of a modified version of the International Physical Activity Questionnaire (IPAQ-A) in European adolescents: the HELENA Study. *Int J Obes (Lond)* 2008;32:S42–8.
21. Hodge A, Patterson AJ, Brown WJ, *et al*. The Anti-Cancer Council of Victoria FFQ: relative validity of nutrient intakes compared with weighed food records in young to middle-aged women in a study of iron supplementation. *Aust N Z J Public Health* 2000;24:576–83.
22. Isacco L, Lazaar N, Ratel S, *et al*. The impact of eating habits on anthropometric characteristics in French primary school children. *Child Care Health Dev* 2010;36:835–42.
23. Chaput JP, Jomphe-Tremblay S, Lafreniere J, *et al*. Reliability of a food menu to measure energy and macronutrient intake in adolescents. *Eur J Clin Nutr* 2016;70:104–8.
24. Aucouturier J, Meyer M, Thivel D, *et al*. Effect of android to gynoid fat ratio on insulin resistance in obese youth. *Arch Pediatr Adolesc Med* 2009;163:826–31.
25. Rantalainen T, Nikander R, Daly R, *et al*. Exercise loading and cortical bone distribution at the tibial shaft. *Bone* 2011;48:786–91.
26. Rantalainen T, Nikander R, Heinonen A, *et al*. An open source approach for regional cortical bone mineral density analysis. *J Musculoskelet Neuronal Interact* 2011;11:243–8.
27. Vinet A, Obert P, Dutheil F, *et al*. Impact of a lifestyle program on vascular insulin resistance in metabolic syndrome subjects: the RESOLVE study. *J Clin Endocrinol Metab* 2014;100:442–50.
28. Campos RM, de Mello MT, Tock L, *et al*. Aerobic plus resistance training improves bone metabolism and inflammation in adolescents who are obese. *J Strength Cond Res* 2014;28:758–66.
29. Van den Bergh J, Noordam C, Özyilmaz A, *et al*. Calcaneal ultrasound imaging in healthy children and adolescents: relation of the ultrasound parameters BUA and SOS to age, body weight, height, foot dimensions and pubertal stage. *Osteoporos Int* 2000;11:967–76.
30. Damilakis J, Adams JE, Guglielmi G, *et al*. Radiation exposure in X-ray-based imaging techniques used in osteoporosis. *Eur Radiol* 2010;20:2707–14.

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