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DXA-derived visceral adipose tissue reference values and cardiometabolic risk factors in an Algerian adult population

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We use this protocol and it's working

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Abstract

This protocol aimed to establish reference values for visceral adipose tissue (VAT) measured by GE Lunar iDXA in a general adult Algerian population. Secondary objectives were to determine cardiometabolic consequences and to propose suggested threshold values for VAT to predict metabolic syndrome.

We conducted a cross-sectional observational study in a general urban adult population in Algeria.

Participants were randomly selected from the electoral list of the municipality of Tlemcen. The required number of subjects for this study was calculated for men and women.

Whole-body scans were conducted with a Lunar iDXATM (GE Healthcare ©, USA) DXA scanner. Body composition parameters were analysed using the software enCORETM (version 18), and VAT was measured with the CoreScanTM (GE Healthcare ©, USA).

The other body composition parameters determined are: Subcutaneous adipose tissue, total and regional fat mass, fat mass index, appendicular lean mass index and dual femur bone mineral density.

Different cardiometabolic risk factors and other diseases associated with obesity that were assessed. Insulin resistance was assessed with different scores particularly HOMA2-IR score.

Physical activity and adherence to the Mediterranean diet were also assessed.

Guidelines

All DXA measurements were performed by a single trained operator who strictly followed the manufacturer's standard protocol, including daily quality control and calibration of the equipment. Participants were also instructed to remove all metal objects and clothing with zippers or underwires before the scan to ensure accurate results.

Materials

DXA measurements

Whole-body scans were conducted with a Lunar iDXA™ (GE Healthcare ©, USA) DXA scanner. Body composition parameters were analysed using the software enCORE™ (version 18), and VAT was measured with the CoreScan™ (GE Healthcare ©, USA).

Clinical measurements

Weight and height were measured using the OMRON HN 289 electronic scale and height chart.

Waist and hip circumference were measured in centimetres with measuring tape at the level of the umbilicus (halfway between the last rib and the upper iliac spine) and the greater trochanters, respectively.

Blood pressure was measured three times, with the average of the last two readings taken using an electronic blood pressure monitor (OMRON model: M3 (HEM-7131-E)).

Laboratory tests

Quantitative measurements of blood glucose, urea, creatinine, total cholesterol, triglycerides, HDL cholesterol, AST, ALT, and uric acid were performed directly from plasma using a SIEMENS ADVIA® 1800 chemistry system.

- **Blood glucose** was measured using the Glucose Hexokinase II (GLUH II) method.

- **Urea** levels were determined by the urease method with glutamate dehydrogenase (GLDH).

- **Creatinine** was measured using the CREA-2 method, which is adapted from the Jaffé method with compensation and intercept correction.

- The **estimated glomerular filtration rate (eGFR)** was calculated using the CKD-Epi (Chronic Kidney Disease Epidemiology collaboration) formula.

- **Total cholesterol** was quantified using the Cholesterol (CHOL) enzymatic method, which involves conversion by cholesterol esterase and cholesterol oxidase, assessed at the endpoint of the Trinder reagent.

- **HDL cholesterol** was measured using the direct HDL cholesterol method. This technique involves the elimination of chylomicrons, VLDL cholesterol, and LDL cholesterol by cholesterol esterase and cholesterol oxidase, followed by the measurement of HDL cholesterol via the Trinder reaction.

- **LDL cholesterol** was calculated using the Friedewald formula: $\text{LDL-Cholesterol (g/L)} = \text{Total Cholesterol (g/L)} - \text{HDL-C (g/L)} - \text{Triglycerides (g/L)}/5$. This formula was not applied if triglyceride levels exceeded 3.40 g/L; in such cases, direct LDL cholesterol was measured.

- **Triglycerides** were measured using the Triglycerides (TRIG) method, based on the three-step enzymatic reaction of Fossati with a Trinder endpoint reaction.

- **Non-HDL cholesterol** was calculated using the following formula: $\text{Non-HDL Cholesterol (g/L)} = \text{Total Cholesterol (g/L)} - \text{HDL Cholesterol (g/L)}$

- The **Triglyceride/HDL cholesterol ratio** was calculated as follows: $\text{Triglyceride/HDL Cholesterol Ratio} = \text{Triglycerides (g/L)}/\text{HDL Cholesterol (g/L)}$

- **Aspartate aminotransferase (AST)** and **alanine aminotransferase (ALT)** were measured using the optimized UV test method according to modified IFCC recommendations.

- **Uric acid** was quantified by the uricase/peroxidase method.

- **Glycated hemoglobin (A1c)** was measured using the D-10® system (Bio-Rad), which employs a high-performance liquid chromatography (HPLC) ion-exchange method.

- **Complete blood count (CBC)** was performed on an ADVIA® 2120i SIEMENS automated hematology analyzer.

- **Quantitative C-peptide** levels were directly measured from plasma using the Maglumi 800 [®] automated immunoassay system via chemiluminescence (CLIA).

Safety warnings

 This study excluded pregnant women as a standard ethical precaution due to the low-dose radiation from the DXA scans.

Ethics statement

The scientific committee of Tlemcen University Hospital, via its Ethics Committee, approved the study protocol (approval number: 12-CSCHUT-2022), and all participants provided informed verbal consent after responding favourably to the invitation.

The study complied with all applicable institutional and governmental regulations regarding the ethical use of human volunteers and with the terms of the Declaration of Helsinki.

Verbal informed consent was chosen for this study due to the specific demographic characteristics of the local population. A portion of participants speak a local dialect, which may hinder their comprehension of both classical Arabic and French. Additionally, some individuals within this population may have low educational attainment, making written consent impractical. The study was considered to be low risk.

The study's procedures, objectives, and all examinations, including DXA scans, were thoroughly explained to participants. Particular emphasis was placed on the minimal radiation risk associated with DXA. These detailed explanations were also supported by a comprehensive invitation and information letter provided to each potential participant. Participants were explicitly informed that their involvement was entirely voluntary and that the study protocol posed no specific risks.

Verbal consent was attested on a dedicated consent form completed by the principal investigator. This form recorded the participant's full name, date of birth, and date of consent approval. This documentation was then securely appended to the participant's data collection form.

Study design

- 1 This is a cross-sectional observational study with an analytical aim over two and a half years, from March 13, 2022 to September 13, 2024, in a general urban adult population in Algeria.

Study Measures

- 2 The study measures included **anthropometric and DXA measurements, laboratory tests**, and an assessment of **cardiometabolic risk factors, physical activity**, and **Mediterranean diet**.

2.1 Anthropometric measurements

Weight and height were measured with light clothing and no shoes in kilograms (kg) and in centimetres (cm).

Waist and hip circumference were measured in centimetres at the level of the umbilicus (halfway between the last rib and the upper iliac spine) and the greater trochanters, respectively.

BMI (weight in kg divided by height in meters (m) squared) was defined as underweight ($<18.5 \text{ kg/m}^2$), normal ($18.5\text{--}24.9 \text{ kg/m}^2$), overweight ($25\text{--}29.9 \text{ kg/m}^2$), or obese ($\geq 30 \text{ kg/m}^2$) with class I obesity ($30\text{--}34.9 \text{ kg/m}^2$), class II obesity ($35\text{--}39.9 \text{ kg/m}^2$) and class III obesity ($\geq 40 \text{ kg/m}^2$). The waist-hip ratio and waist-height ratio were calculated.

2.2 DXA measurements

Whole-body scans were conducted with a Lunar iDXATM (GE Healthcare[©], USA) DXA scanner. Body composition parameters were analysed using the software enCORETM (version 18), and VAT was measured with the CoreScanTM (GE Healthcare[©], USA).

Daily quality control was conducted, and calibration of the model was performed according to the manufacturer's protocol.

Participants were instructed to remove all metal objects, such as jewellery and watches. Shoes, jeans, and all clothes containing zippers or buttons, as well as bras with underwires, had to be removed.

■ **Measurement protocols**

The following measurement protocols were performed for all participants at the following sites:

1. **Total body composition** : The patient's body should be in the center of the table. The table's centerline serves as a reference for patient alignment. Velcro straps are used

to secure the knees and feet. The patient's hands should be turned to the sides with the thumbs facing upwards. The palms should be facing away from the legs, and the arms should be at their sides. The head should be approximately 3 cm below the horizontal line of the table. The patient should neither move nor speak during the scan.

2. **Anteroposterior spine densitometry (L1–L4)** : The "OneScan" tab is not checked for a specific measurement using the foam wedge positioner. The foam wedge was used to elevate the patient's legs. The patient's thighs should form a 60° to 90° angle with the tabletop. This step separates the vertebrae and flattens the lower back. The laser beam was positioned approximately 5 cm below the patient's navel and on the same longitudinal plane as the patient's midline.
3. **Dual femur densitometry** : The patient's arms should be crossed over the chest, away from the side of each hip. The midline of the examination table was used as a reference point to ensure the foot splint was centered. The patient's legs should be facing inward and the feet secured with splints. The laser beam was positioned approximately 7–8 cm below the greater trochanter where the pubic symphysis and the midline of the femur intersect.

All scans were performed and analysed by a single trained operator per a standard protocol provided by the manufacturer.

■ **Parameters measured**

VAT was expressed as a mass in grams (g), volume in cubic centimetres (cm³), and area in square centimetres (cm²). Other body composition parameters determined are:

- Subcutaneous adipose tissue mass (g)
- Total and regional fat mass (%) : android, gynoid, trunk, arms and legs
- Fat mass index: fat mass/height² (kg/m²)
- Appendicular lean mass index: lean mass of four limbs/height² (kg/m²)
- Dual femur and AP spine bone mineral density (g/cm²)

2.3 **Laboratory tests**

Blood samples were collected in the morning after a 12-hour fast. The analyses were performed at the laboratory of the Tlemcen University Hospital including glucose (g/l), urea (g/l), creatinine (mg/l) with the calculation of glomerular filtration rate (GFR) according to CKD-EPI, total cholesterol (g/l), HDL cholesterol (g/l), triglycerides (g/l), calculated LDL cholesterol (g/l), AST (IU/l), ALT (IU/l), uric acid (mg/l), complete blood count, glycated hemoglobin (A1c in %) by high-performance liquid chromatography, and C-peptide (ng/ml) by chemiluminescence.

Insulin resistance was assessed using the HOMA2-IR score with a threshold of 1.80 [2]. HOMA2 estimates pancreatic beta cell function (HOMA_%B), insulin sensitivity (HOMA_%S), and insulin resistance (HOMA2-IR). It was calculated using the HOMA2

calculator v2.2.3 downloaded from the University of Oxford website.

(<https://www.rdm.ox.ac.uk/about/our-clinical-facilities-and-units/DTU/software/homa>).

The other scores calculated were the triglycerides-glucose index, triglycerides HDL cholesterol ratio, FIB-4 [3], and the visceral adipose index [4].

2.4 **Cardiometabolic Risk Factors**

The cardiometabolic risk factors and other diseases associated with obesity that were assessed include: hypertension, type 2 diabetes and prediabetes, dyslipidemia, smoking, metabolic syndrome, chronic kidney disease, family history of cardiovascular events, cardiovascular events (coronary artery disease, stroke, peripheral arterial disease), sleep apnea syndrome, polycystic ovary syndrome, gallstones, osteoarthritis, hypothyroidism, hepatic steatosis, and history of cancers.

- **Hypertension** is defined as :

1. Established and treated hypertension : Individuals currently receiving medication for hypertension.
2. Newly diagnosed during clinical evaluation: Individuals with a systolic blood pressure (SBP) of ≥ 140 mmHg and/or a diastolic blood pressure (DBP) of ≥ 90 mmHg, confirmed by either home blood pressure monitoring (HBPM) or ambulatory blood pressure monitoring (ABPM).

- **Type 2 diabetes** is defined as :

1. Established and treated type 2 diabetes : Individuals with a pre-existing diagnosis of Type 2 Diabetes Mellitus currently receiving medication for diabetes.
2. Newly diagnosed during clinical evaluation: Individuals with fasting blood glucose ≥ 126 mg/dl and/or A1c $\geq 6.5\%$.

- **Prediabetes** is defined as a person with a fasting blood glucose level between 110 mg/dL and 125 mg/dL and/or an A1c between 5.7% and 6.4%.

- **Dyslipidemia** is defined as :

1. Established and treated dyslipidemia : Individuals currently receiving medication for dyslipidemia (For example statins, fibrates).
2. Newly diagnosed during clinical evaluation: Taking into account the parameters of increased LDL cholesterol according to cardiovascular risk assessed by SCORE2, SCORE2OP and SCORE2-Diabetes, hypertriglyceridemia and low HDL cholesterol.

- **Chronic kidney disease** was defined by a eGFR according to CKD-EPI < 60 ml/min.

- **Metabolic syndrome**

The criteria for metabolic syndrome applied were those of the International Diabetes Federation (IDF) harmonized in 2009 [5]: increased waist circumference ≥ 94 cm in men and ≥ 80 cm in women, elevated triglycerides ≥ 1.50 g/l (1.7 mmol/L) (or treatment for dyslipidemia), low HDL cholesterol < 0.40 g/l (1.03 mmol/L) in men and < 0.50 g/l (1.29 mmol/L) in women (or treatment for dyslipidemia), elevated fasting glucose ≥ 1 g/l (or treatment for type 2 diabetes), and elevated blood pressure: systolic ≥ 130 mm Hg and diastolic ≥ 85 mm Hg (or treatment for hypertension). Metabolic syndrome was defined as the presence of three or more of the components including waist circumference.

- **For other cardiometabolic risk factors** we considered the patient's medical history and treatments.

2.5 Physical activity and mediterranean diet

Physical activity and sedentary behaviour were assessed using the version 2 of the GPAQ (Global Physical Activity Questionnaire), which consists of 16 questions and calculates metabolic equivalents [6]. Adherence to the Mediterranean diet was evaluated using the MEDAS (Mediterranean Diet Adherence Screener) [7] and Medi-Lite (Mediterranean diet Literature-based adherence score) [7].

Study population

- 3 Participants were randomly selected from the electoral list with sample size calculation.

3.1 Sampling method

Participants were **randomly** selected from the electoral list of the municipality of Tlemcen, located in the northwest of Algeria.

A random draw of 2,000 people was carried out using **Microsoft Excel** from the electoral roll database of the municipality of Tlemcen. This database, as of October 10, 2021, contained 119,519 voters distributed across 36 centers and 327 polling stations. The following Excel functions were used for the draw: **RAND**, **RANK**, **INDEX**, and **MATCH**. Using the randomly drawn registration numbers, information on each person, including their full name, date of birth, and address, was retrieved. Subsequently, an invitation explaining the details of the examinations was sent by postal mail to each individual, asking them to schedule an appointment by phone.

Authorization to use the electoral roll database for the random draw was granted by the President of the Communal People's Assembly, the Head of the Daïra, and the President of the Independent Authority for Election Monitoring of the Tlemcen Wilaya.

The number of people drawn was **2,000**, based on an estimated response rate of 10% to 20%.

3.2 Sample size calculation

To ensure adequate statistical power, the required sample size for this study was calculated using **MedCalc v22.021** (trial version), specifically addressing the primary objective VAT quantity.

For the alternative hypothesis, we utilized VAT data from the Tromso Study (2021) [1], a reference study. The reported mean VAT mass in that study was 1660.9 ± 876.6 g for men and 936.7 ± 632.5 g for women.

Due to a lack of existing data on the Algerian population for the null hypothesis, we analyzed the first 50 participants from our own study population. This preliminary analysis yielded a mean VAT mass of 1453.36 ± 806.09 g for men and 1154.0 ± 756.82 g for women.

Based on these calculations, the minimum required sample size was determined to be **142 men** and **69 women**, totaling **211 participants**.

3.3 Inclusion and exclusion criteria

We included participants aged 18 to 90 years who responded positively to the invitation. Exclusion criteria included individuals under 18 years and over 90 years, pregnant women, those weighing more than 227 kg (DXA table weight limit), bedridden individuals due to illness, and individuals with decompensated psychiatric disorders.

3.4 Study Procedure

Invitations were sent out periodically in batches of 100, following the order of the random draw.

A total of **1650 invitations were dispatched** until the required sample size for both sexes was reached.

Of those invited, **304 individuals responded**. **Three participants were subsequently excluded** (two pregnant women and one man who was bedridden due to severe illness). This resulted in **301 participants** being included in the study (**147 men and 154 women**).

Participants who responded favorably to the invitation were contacted by phone. The principal investigator provided them with essential information about the examination and its procedure. Two appointments were scheduled for each participant:

The first appointment was set at the Internal Medicine Department of the Tlemcen University Hospital Center for **biological sample collection**. This collection was performed by the paramedical team while the individual was in a fasted state.

The second appointment, for the **DXA examination** and completion of the **data collection form**, was scheduled at the Diabetes Research Laboratory of the Tlemcen Faculty of Medicine. Medical care was also offered if needed.

3.5 Ethical considerations

The scientific committee of Tlemcen University Hospital, via its Ethics Committee, approved the study protocol (approval number: 12-CSCHUT-2022), and all participants provided informed verbal consent after responding favourably to the invitation.

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