Materials and Methods

Study population

The Coronary Artery Risk Development in Young Adults (CARDIA) Study began in 1985 with recruitment of 5,115 participants aged 18 to 30 years at field centers located in Birmingham, AL, Chicago, IL, Minneapolis, MN, and Oakland, CA[1]. Recruitment was balanced on race (black and white), sex, age (18-24, 25-30 years) and education (≤12 years, >12 years), and participants have been followed longitudinally. The current cross-sectional study includes data from participants who agreed to undergo computed tomography (CT) scans at the year 25 CARDIA examination (3,189 of 3,498 year 25 participants; 91%). Of these 3,189 participants with CT scans, 3,160 participants had measures of both CAC and abdominal muscle composition. Among the 3,160 participants with complete CT measures, 109 participants were missing key year 25 risk factor data leaving 3,051 participants in the present cross-sectional study. All participants provided written informed consent, and institutional review boards from each field center and the coordinating center approved the study annually.

Clinical measures

Clinic visit procedures were standardized for all field centers at the year 25 visit and have been consistent across examinations as previously published in detail[1]. Briefly, blood pressure was measured in triplicate after a 5-minute rest using an automated blood pressure monitor (Omron model HEM907XL; Omron Healthcare Inc., Lake Forest, IL) with the average of the second and third measurements used in analyses. Fasting plasma lipids and lipoproteins were measured using enzymatic methods at Northwest Lipids Research Laboratory (Seattle, WA). HDL cholesterol was measured after dextran-magnesium precipitation. Serum glucose was measured using the Roche Modular P hexokinase method(Roche Diagnostics, Rotkreuz, Switzerland), insulin was measured using Elecsys sandwich immunoassay (Roche Diagnostics, Rotkreuz, Switzerland), and percent Hemoglobin A1c was measured using Tosoh G7 HPLC (Tosoh, San Francisco, CA, USA). Plasma C-reactive protein (CRP) was measured using high-sensitivity nephelometry-based method (BNII nephelometer, Dade Behring, Eschborn, Germany). Diabetes was defined as fasting glucose ≥7.0 mmol/l (≥126 mg/dl), self-report of oral hypoglycemic medications or insulin, 2-hour postload glucose ≥11.1 mmol/l (≥200 mg/dl), or HbA1c ≥6.5% at the year 25 visit. The homeostasis model assessment (HOMA-IR) was calculated as [fasting glucose (mg/dl) × fasting insulin (μU/ml)]/405 to estimate insulin resistance[2].

The CARDIA Physical Activity History questionnaire was used to estimate weekly leisure, occupational, and household physical exertion over the past 12 months[3]. Weight and height were measured with participants wearing light clothing and no shoes. Body weight was measured to the nearest 0.2 kg on a calibrated scale whereas height was measured to the nearest 0.5 cm using a fixed vertical ruler. BMI was calculated as weight in kilograms divided by height in meters squared. Cigarette smoking status was classified as never, ever or current; education was reported in years; and information on use of antihypertensive and lipid lowering medication was collected through interviewer-administered questionnaires.

CT measures of arterial calcification and adipose deposition

Participants underwent a multi-detector CT exam without additional contrast media of the chest and abdomen using a standardized chest scan protocol for CAC measurement described previously[4–7]. Women who were pregnant or potentially pregnant were excluded from the CT exam. The scans were performed at the four CARDIA field centers using 64 channel multi-detector GE CT scanners (GE Healthcare Milwaukee, WI) at the Birmingham, AL and Oakland, CA centers and Siemens CT scanners (Siemens, Erlangen, Germany) at

the Chicago, IL and Minneapolis, MN centers. Technical parameters included: KVp of 120-130, tube current increased by 25% for individuals weighing greater than 100 kg, and for CAC measures, ECG-gating of image acquisition in late diastole, and slice thickness of 2.5-3.0 mm. A quality control phantom with known quantities of calcium hydroxyapatite was positioned beneath the participant and included in each scan (INTableTM Calibration Pad, Image Analysis, Inc., Columbia, KY). CT images were electronically transmitted to the central CT reading center located at Wake Forest University School of Medicine, Winston-Salem, NC. Experienced image analysts (masked to participant information) measured calcified plaque in the epicardial coronary arteries producing total calcium scores based on a modified Agatston method that accounts for slice thickness[8]. Analyses were performed using a dedicated FDA-approved workstation (Aquarius Workstation, TeraRecon, Foster City, CA). Minimum lesion size measured was four adjacent pixels (an area of at least 1.87 mm^2) at calcium density >130 Hounsfield units.

The standard-format 2.5-3.0 mm thick CT images of the chest used to measure CAC were also used to measure pericardial adipose tissue (PAT) as described previously[9–11]. CT slices within 15 mm above and 30 mm below the superior extent of the left main coronary artery were analyzed for PAT using an image processing workstation (OsiriX, Pixmeo, Geneva, Switzerland). This 45 mm block of images (18 slices of 2.5 mm or 15 slices of 3.0 mm thickness) was selected for analysis as it covers pericardial fat located around the proximal coronary arteries (left main coronary, left anterior descending, right coronary, and circumflex arteries). The anterior boundary of the volume is the chest wall and the posterior boundary is the aorta and the bronchus. Experienced analysts manually segmented the interface between the lungs and the paracardial space and then applied a threshold of -190 to -30 Hounsfield units to isolate adipose tissue. The volume (cm^3) of PAT was determined by summing the adipose tissue containing pixels and accounting for the slice thickness. These methods quantify the combined epicardial (adipose tissue within the pericardium in direct contact with the coronary arteries) plus paracardial (adipose superficial to the pericardium) adipose tissues providing total PAT volume within the 45 mm thick block of images analyzed. We have previously shown that epicardial adipose is highly correlated with PAT ($r=0.92$, $p<.0001$, $n=159$)[12].

Abdominal CT scans were analyzed using the 50 cm display field-of-view to include the whole abdomen. Adipose tissue depots were measured volumetrically within a 10 mm block of 10 x 1 mm or 8 x 1.25 mm contiguous slices based on the nominal slice thickness produced by the specific scanner centered at the level of the disk between the 4th and 5th lumbar vertebrae as previously described[13,14]. Tissues with attenuation of -190 through -30 Hounsfield units were defined as adipose tissue. Medical Image Processing, Analysis, and Visualization (MIPAV--http://mipav.cit.nih.gov/index.php) software was used to quantify subcutaneous (SAT) and visceral adipose tissue (VAT) volume.

Abdominal muscle composition (fat, lean and total) was measured volumetrically from a 10 mm block of contiguous slices centered between the 3rd and 4th lumbar disks. The abdominal muscles were measured at the L3-L4 level to avoid changes in muscle orientation related to the pelvic bones and specifically the iliac wings encountered in some individuals at the L4-L5 level. Muscle volumes at L3-L4 and L4-L5 were highly correlated ranging from 0.87 (rectus) to 0.98 (psoas and paraspinous). Pixels within muscle with attenuation of -190 and -30 Hounsfield units (HU) were defined as adipose tissue, and -31 to 160 HU as lean tissue. Fat, lean, and total muscle volumes were quantified for the psoas, paraspinous, lateral oblique, and rectus muscles

(shown in Figure 1) using a custom MIPAV plug-in developed by study investigators. The left and right side measures for each muscle group were highly correlated, so mean adipose volume and total volume of the left and right sides were calculated and analyzed for each muscle group separately and overall for all abdominal muscles.

Analysis reliability of CT measures was assessed through intra- and inter-reader re-reads of 158 scan pairs (~5%). Participant scans randomly selected for quality control were relabeled with new identifiers and dates, and sent through the reading queue as new scans, so that scan analysts would remain blinded to the fact that these were quality control scans and, other than the programmer, no one at the reading center knew the matching participant scans. Overall (intra- and inter-reader) technical error in re-analysis of 158 pairs of scans was 6.6% for CAC, 4.2% for PAT, 6.0% for VAT, and 7.7% for psoas muscle total volume with correlations for re-reads >0.95 in each measure.

Statistics

Mean abdominal IMAT volume across all muscles (overall IMAT) was the primary independent variable. Mean overall IMAT volume and mean IMAT/total muscle volume (IMAT normalized to total muscle size) were analyzed as continuous data and were also broken into quartiles to facilitate interpretation and evaluate potential threshold effects. The association of IMAT with CAC was consistent across sex and race strata (IMAT by sex, IMAT by race, and three-way interactions including IMAT all nonsignificant, $p>0.25$). Nevertheless, the race by sex interaction was significantly associated with CAC and thus included in all models. Associations of presence/absence of prevalent CAC (Agatston score >0 versus 0) and Agatston score categories in quartiles (0, 1-19.9, 20-99.9, and ≥100) with IMAT volume were tested in multivariable binomial and multinomial logistic regression models, respectively. Multivariable model covariables were chosen *a priori.* Model 1 included the demographic/socioeconomic variables age, sex, race, sex*race, and education and the potential confounders study center and height. Model 2 included model 1 covariables plus traditional CVD risk factors thought to potentially lie within the causal pathway between IMAT and CAC including diabetes or HOMA-IR, systolic blood pressure, CRP, HDL-cholesterol and triglycerides or potential modifiers of associations between IMAT and CAC such as physical activity, smoking history (never/ever/current), alcohol consumed per day, and current use of anti-hypertensive or cholesterol medicines. BMI, VAT, and PAT were separately added to model 2 to test IMAT and CAC associations after adjustment for generalized and ectopic adipose tissues. We present odds ratios (OR) and 95% confidence intervals (CI) for the association of IMAT with CAC in higher quartiles of IMAT versus the lowest quartile. In the multinomial case, we back-transformed the multinomial logistic regression solutions to adjusted probabilities and presented the probability distributions for the CAC score categories 1-19.9, 20-99.9, and ≥100 AU graphically. All analyses were performed using SAS JMP Pro 12 and SAS V9.4.

References

1. Friedman GD, Cutter GR, Donahue RP, Hughes GH, Hulley SB, Jacobs DRJ, Liu K, Savage PJ. CARDIA: study design, recruitment, and some characteristics of the examined subjects. *J Clin Epidemiol*. 1988;41(11):1105-1116.
2. Matthews DR, Hosker JR, Rudenski AS, Naylor BA, Treacher DF, Turner RC, Infirmary R. Diabetologia. 1985:412-419.
3. Jacobs DR, Hahn LP, Haskell WL, Pirie P, Sidney S. Validity and Reliability of Short Physical Activity History: Cardia and the Minnesota Heart Health Program. *J Cardiopulm Rehabil Prev*. 1989;9(11):448-459. doi:10.1097/00008483-198911000-00003.
4. Carr JJ, Nelson JC, Wong ND, McNitt-Gray M, Arad Y, David R. Jacobs J, Sidney S,

Bild DE, Williams OD, Detrano RC. Calcified Coronary Artery Plaque Measurement with Cardiac CT in Population-based Studies: Standardized Protocol of Multi-Ethnic Study of Atherosclerosis (MESA) and Coronary Artery Risk Development in Young Adults (CARDIA) Study. *Radiology*. 2005;234(1):35-43. doi:10.1148/radiol.2341040439.

5. Loria CM, Liu K, Lewis CE, Hulley SB, Sidney S, Schreiner PJ, Williams OD, Bild DE, Detrano R. Early Adult Risk Factor Levels and Subsequent Coronary Artery Calcification: The {CARDIA} Study. *J Am Coll Cardiol*. 2007;49(20):2013-2020. doi:http://dx.doi.org/10.1016/j.jacc.2007.03.009.
6. Reis JP, Loria CM, Steffen LM, Zhou X, van Horn L, Siscovick DS, Jacobs DRJ, Carr JJ. Coffee, decaffeinated coffee, caffeine, and tea consumption in young adulthood and atherosclerosis later in life: the CARDIA study. *Arterioscler Thromb Vasc Biol*. 2010;30(10):2059-2066. doi:10.1161/ATVBAHA.110.208280.
7. Carr JJ, Jacobs DR Jr., Terry JG, Shay CM, Sidney S, Liu K, Schreiner PJ, Lewis CE, Shikany JM, Reis JP GDJ. Coronary artery calcium in adults 32-46 years and incident coronary heart disease and death: The CARDIA Study. *JAMA Cardiol*. 2017;2(4):391-399. http://dx.doi.org/10.1001/jamacardio.2016.5493
8. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol*. 1990;15(4):827-832. doi:10.1016/0735-1097(90)90282-T.
9. Alman AC, Jacobs DRJ, Lewis CE, Snell-Bergeon JK, Carnethon MR, Terry JG, Goff DCJ, Ding J, Carr JJ. Higher pericardial adiposity is associated with prevalent diabetes: The Coronary Artery Risk Development in Young Adults study. *Nutr Metab Cardiovasc Dis*. 2016;26(4):326-332. doi:10.1016/j.numecd.2015.12.011.
10. Ding J, Kritchevsky SB, Hsu F-C, Harris TB, Burke GL, Detrano RC, Szklo M, Criqui MH, Allison M, Ouyang P, Brown ER, Carr JJ. Association between non-subcutaneous adiposity and calcified coronary plaque: a substudy of the Multi-Ethnic Study of Atherosclerosis. *Am J Clin Nutr*. 2008;88(3):645-650. http://ajcn.nutrition.org/cgi/content/long/88/3/645. Accessed August 8, 2014.
11. Ding J, Hsu F-C, Harris TB, Liu Y, Kritchevsky SB, Szklo M, Ouyang P, Espeland MA, Lohman KK, Criqui MH, Allison M, Bluemke DA, Carr JJ. The association of pericardial fat with incident coronary heart disease: the Multi-Ethnic Study of Atherosclerosis (MESA). *Am J Clin Nutr*. 2009;90(3):499-504. doi:10.3945/ajcn.2008.27358.
12. Carr JJ, Ding J. Response to “Epicardial and Pericardial Fat: Close, but Very Different.” *Obesity*. 2009;17(4):626-627. doi:10.1038/oby.2008.622.
13. VanWagner LB, Ning H, Lewis CE, Shay CM, Wilkins J, Carr JJ, Terry JG, Lloyd-Jones DM, Jacobs Jr. DR, Carnethon MR. Associations between nonalcoholic fatty liver disease and subclinical atherosclerosis in middle-aged adults: The Coronary Artery Risk Development in Young Adults Study. *Atherosclerosis*. 2014;235(2):599-605. doi:http://dx.doi.org/10.1016/j.atherosclerosis.2014.05.962.
14. VanWagner LB, Wilcox JE, Colangelo LA, Lloyd-Jones DM, Carr JJ, Lima JA, Lewis CE, Rinella ME, Shah SJ. Association of nonalcoholic fatty liver disease with subclinical myocardial remodeling and dysfunction: A population-based study. *Hepatology*. 2015;62(3):773-783. doi:10.1002/hep.27869.