Methods

Study Participants

Participants were derived from the Framingham Heart Study, a cohort study initiated in 1948 with 5209 participants.[1] The offspring of the original participants as well as offspring spouses were recruited to the study in 1971[2] and the third generation and their spouses were recruited in 2002.[3] Participants undergo regular examinations and health history updates.

This study was comprised of Framingham Heart Study offspring and third generation participants who had undergone multidetector computed tomography (MDCT) from 2002-2005. Men were required to be over 35 years of age and women over 40 years. Participants weighing >160 kg did not undergo MDCT due to weight restrictions. Of the 3529 participants who underwent CT scanning, 3127 had muscle attenuation (MA) measurements. Of the 3127, 2945 had a complete set of covariates and were included in the final analysis (1479 women, 1466 men). Of those final analysis participants without diabetes (1398 women, 1365 men), 1280 women and 1277 men had insulin data available and were included in analyses of HOMA-IR and insulin resistance.

Measurement of Muscle Attenuation and Visceral and Subcutaneous Fat

Participants underwent abdominal CT imaging while in a supine position. As previously described, an eight-slice MDCT (LightSpeed Ultra, General Electric, Milwaukee, WI, USA) was used to image 25 contiguous 5-mm thick slices (120 kVp, 400 mA, 500 ms gantry rotation time, 3:1 table feed).[4] One hundred and twenty-five millimeters above S1 level were covered by the scan.

A ~1 cm region of interest was placed on both the left and right paraspinous muscles at mid-abdomen level. The MA of these two areas were averaged in order to calculate mean MA; the correlation between the two reads is 0.86. The inter-reader correlation was 0.88. We

focused on paraspinous muscles due to their slow-twitch characteristics, which tend to have more intramuscular fat than fast-twitch muscles.[5;6] Skeletal MA on MDCT scans to evaluate intramuscular fat has been previously validated by muscle biopsy study.[7] In vivo vastus lateralis MA via MDCT had a Pearson correlation coefficient of -0.43 ($P<0.01$, $n=45$) with vastus lateralis muscle fiber lipid content (% area) determined with histochemical staining of lipid after biopsy.[7]

Visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) volumes were determined via manual tracing of the abdominal muscular wall on 25 continuous 5 mm slices from an eight-slice MDCT scan of the abdomen. The window for fat was -195 to -45 Hounsfield units with a window center of -120 Hounsfield units. All fat outside of the manual tracing was defined as SAT while that inside of the tracing was defined as VAT. The inter-reader correlation for SAT was 0.997 and for VAT was 0.992.[8]

Cardiovascular Disease Risk Factor Assessment

Risk factors for this study were determined at the seventh offspring examination (1998-2001) and the first third generation examination (2002-2005). Fasting glucose and lipids were measured after an overnight fast. Impaired fasting glucose (IFG) was defined as 100-125 mg/dL fasting plasma glucose for participants not on diabetes treatment. Diabetes (DM) was classified as fasting plasma glucose of ≥ 126 mg/dL or current use of insulin or a hypoglycemic treatment. The modified Adult Treatment Panel III criteria was used to classify metabolic syndrome.[9] Insulin was calculated via radioimmunoassay (Offspring Cohort) and ELISA (Third Generation cohort). Third Generation cohort insulin values were standardized to the values of the Offspring Cohort in order to account for the different insulin calculation methods.[10] HOMA-IR was determined by fasting glucose multiplied by fasting insulin divided by 22.5.[11] Insulin resistance (IR) was classified as the top quarter of HOMA-IR in participants without diabetes.[12] High TG was classified as ≥150 mg/dL or if the participant was on lipid-lowering medication. Low HDL was defined as HDL < 50 mg/dL for women and <40 mg/dL for men. Blood pressure was

determined by averaging two reads taken at rest with a mercury column sphygmomanometer. Hypertension (HTN) was determined by systolic blood pressure (SBP) ≥ 140 mmHg, diastolic blood pressure (DBP) ≥ 90 mmHg or current use of hypertension treatment medication.

Measurement of Covariates

Participants were designated as current smokers if they had smoked ≥ 1 cigarette per day in the year prior to examination. Alcohol use was determined via a physician-administered questionnaire. Participants were split into two alcohol use groups based on whether they had > 7 drinks/week for women or > 14 drinks/week for men. Physical activity was calculated on the physical activity index (PAI) after a physician-administered questionnaire concerning the average number of hours of sleep and sedentary, slight, moderate and heavy activity the participant took part in per day. Women without menstrual bleeding for ≥ 1 year were considered postmenopausal. BMI was determined by participant weight in kilograms divided by the square of participant height in meters. Waist circumference was calculated at the level of the umbilicus.

Statistical Analysis

MA approximated the normal distribution for both women and men. Logs of triglycerides and HOMA-IR were performed to normalize their distributions. Sex-specific, age-adjusted correlations between MA and metabolic risk factors were calculated.

For continuous risk factors, multivariable-adjusted linear regression models were used to model the relative change in the outcome per one standard deviation decrease in MA (i.e., more intra-muscular fat). For dichotomous risk factors, a multivariable-adjusted logistic model was used. Three sex-specific models were fit separately for each of the following outcomes: plasma glucose, log HOMA-IR, insulin resistance, impaired fasting glucose, diabetes, metabolic syndrome, log triglycerides, high triglycerides, HDL, low HDL (<40 mg/dl in men and <50 mg/dl

in women), SBP, DBP, and hypertension. In the first model, covariates included age, current smoking status, alcohol use, physical activity, treatment for hypertension (for SBP and DBP models), dyslipidemia (for triglyceride and HDL models), and diabetes (for glucose models) as well as menopausal status and hormone replacement therapy in models in women. The second model additionally adjusted for BMI. The third model additionally adjusted for VAT (but not for BMI).

The Framingham Risk score was calculated as previously described[13] and examined in association with muscle HU among participants free of clinical CVD.

Given previously observed interactions with sex, we tested the interactions between MA and sex; we also examined the interaction with age and metabolic risk factors. We also stratified our results by cohort to test whether the lack of temporality between the offspring clinical risk factor data and the computed tomography scans might impact the results.

Analyses were calculated with SAS version 9.2. P-values less than 0.05 were considered statistically significant for primary analyses. Because of the many interactions that we tested, we used a significance level of 0.01 to assess the significance of interaction terms.

Reference List

1. DAWBER TR, KANNEL WB, LYELL LP. An approach to longitudinal studies in a community: the Framingham Study. *Ann N Y Acad Sci*. 1963;107:539-556.
2. KANNEL WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families. The Framingham offspring study. *Am J Epidemiol*. 1979;110:281-290.
3. Splansky GL, Corey D, Yang Q, Atwood LD, Cupples LA, Benjamin EJ, D'Agostino RB, Sr., Fox CS, Larson MG, Murabito JM, O'Donnell CJ, Vasan RS, Wolf PA, Levy D. The Third Generation Cohort of the National Heart, Lung, and Blood Institute's Framingham Heart Study: design, recruitment, and initial examination. *Am J Epidemiol*. 2007;165:1328-1335.

4. Maurovich-Horvat P, Massaro J, Fox CS, Moselewski F, O'Donnell CJ, Hoffmann U. Comparison of anthropometric, area- and volume-based assessment of abdominal subcutaneous and visceral adipose tissue volumes using multi-detector computed tomography. *Int J Obes (Lond)*. 2007;31:500-506.

5. Komiya H, Mori Y, Yokose T, Kurokawa N, Horie N, Tajima N. Effect of intramuscular fat difference on glucose and insulin reaction in oral glucose tolerance test. *J Atheroscler Thromb*. 2006;13:136-142.

6. Johnson MA, Polgar J, Weightman D, Appleton D. Data on the distribution of fibre types in thirty-six human muscles. An autopsy study. *J Neurol Sci*. 1973;18:111-129.

7. Goodpaster BH, Kelley DE, Thaete FL, He J, Ross R. Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content. *J Appl Physiol*. 2000;89:104-110.

8. Fox CS, Massaro JM, Hoffmann U, Pou KM, Maurovich-Horvat P, Liu CY, Vasan RS, Murabito JM, Meigs JB, Cupples LA, D'Agostino RB, Sr., O'Donnell CJ. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. *Circulation*. 2007;116:39-48.

9. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA*. 2001;285:2486-2497.

10. Preis SR, Massaro JM, Robins SJ, Hoffmann U, Vasan RS, Irlbeck T, Meigs JB, Sutherland P, D'Agostino RB, Sr., O'Donnell CJ, Fox CS. Abdominal subcutaneous and visceral adipose tissue and insulin resistance in the Framingham heart study. *Obesity (Silver Spring)*. 2010;18:2191-2198.

11. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28:412-419.

12. Balkau B, Charles MA. Comment on the provisional report from the WHO consultation. European Group for the Study of Insulin Resistance (EGIR). *Diabet Med*. 1999;16:442-443.

13. D'Agostino RB, Sr., Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, Kannel WB. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation*. 2008;117:743-753.