



Insulin resistance since early adulthood and appendicular lean mass in middle-aged adults without diabetes: 20 years of the CARDIA study

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ABSTRACT

Aims: To determine the association between 20-year trajectories in insulin resistance (IR) since young adulthood and appendicular lean mass (ALM) at middle-age in adults without diabetes.

Methods: A prospective cohort study was designed among young and middle-aged US men ($n = 925$) and women ($n = 1193$). Fasting serum glucose and insulin were measured five times in 1985–2005. IR was determined using the homeostasis model assessment (HOMA). ALM was measured in 2005 and ALM adjusted for BMI (ALM/BMI) was the outcome. Sex-specific analyses were performed.

Results: Three HOMA-IR trajectories were identified. Compared to the low-stable group, the adjusted ALM/BMI difference was -0.041 (95% CI: -0.060 to -0.022) and -0.114 (-0.141 to -0.086) in men, and -0.052 (-0.065 to -0.039) and -0.043 (-0.063 to -0.023) in women, respectively, for the medium-increase and high-increase groups. Further adjusting for the treadmill test duration attenuated these estimates to -0.022 (-0.040 to -0.004) and -0.061 (-0.089 to -0.034) in men and -0.026 (-0.038 to -0.014) and -0.007 (-0.026 to 0.012) in women.

Conclusions: Compared to the low-stable insulin resistance trajectory between early and middle adulthood, the high-increase trajectory was associated with lower ALM/BMI in middle-aged men, but not women, without diabetes, after adjusting for cardiorespiratory fitness.

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1. Introduction

Sarcopenia, an age-associated decline in lean mass, muscle strength, and physical performance, is a common health problem in older adults (i.e., 65 years or older).^{1–3} Sarcopenia has been associated with a range of adverse consequences including fall, hospitalization, and mortality.^{4,5} Currently, there is no cure for age- and disease-related lean mass loss.⁶ Lean mass acquired in early life, together with age- and disease-related loss, largely determine lean mass among older adults.^{6–8} Therefore, identifying factors associated with lean mass loss and preservation in early and middle adulthood may play a critical role in improving the quality of life and reducing health and economic burden resulting from sarcopenia in later life.⁹

Elevated insulin resistance and hyperglycemia are associated with accelerated loss of lean mass and/or function in older adults with or without diabetes (mean age of 60 years or older); most of these studies

are cross-sectional.^{10–13} However, our understanding of the association between insulin resistance and lean mass is limited in young and middle-aged adults without diabetes, even though lean mass begins to decline at a rate of roughly 1% annually starting at 30 years of age.¹⁴ Given the aforementioned evidence from older populations, we hypothesize that an increasing trajectory of insulin resistance over time between early and middle adulthood is associated with lower appendicular lean mass, as compared to a relatively stable insulin resistance trajectory, in middle-aged adults without diabetes. To test this hypothesis, we used 20 years of follow-up data from the Coronary Artery Risk Development in Young Adults (CARDIA) Study.

2. Participants and methods

2.1. Study population

The CARDIA study is an ongoing multicenter longitudinal cohort study that enrolled 5115 black and white men and women aged 18–30 years in 1985–86 from four field centers: Birmingham, AL; Oakland, CA; Chicago, IL; and Minneapolis, MN. Participants were followed and received extensive examination 2, 5, 7, 10, 15, 20, 25, and 30 years after enrollment. The details of the CARDIA study design can be found elsewhere.¹⁵ The current study used data from the ancillary CARDIA Fitness Study in which 2704 participants underwent dual-energy X-ray absorptiometry (DXA) for assessment of body composition at year 20. Thus, only data between years 0 and 20 were included for current analyses. Participants with either type 1 or type 2 diabetes mellitus ($n = 236$) at any exam between years 0 and 20 were excluded because i) diabetes is a known strong risk factor for muscle loss⁶; ii) glucose levels of participants with diabetes on glucose-lowering medication do not reflect natural glucose homeostasis and cannot be compared to glucose levels in those with self-management only. Diabetes was defined as having fasting blood glucose ≥ 7 mmol/L (126 mg/dL), or 2-hour glucose ≥ 11.1 mmol/L (200 mg/dL) from the 75-gram glucose tolerance test, or HbA_{1c} $\geq 6.5\%$ (48 mmol/mol), or use of glucose-lowering medications. We further excluded one participant who self-reported transgender status. Glucose and insulin levels at a visit where participants were pregnant or fasted < 8 h before examination were set to missing. Participants with less than three measurements of fasting insulin and glucose ($n = 240$) or missing data for the treadmill test ($n = 109$) were excluded. Participants provided consent at each exam and institutional review boards at each field center approved the study protocols.

2.2. Data collection

All data were collected according to standardized protocols across all exams. For anthropometric assessment, participants were in light clothes and without shoes. For laboratory assays, participants were asked to fast overnight for at least 8 h and not to smoke or perform heavy exercise prior to each examination.

2.3. Anthropometric assessment

Body weight was measured by a balance-beam scale and height was measured using a vertically mounted metal centimeter ruler and carpenter's square. Body Mass Index (BMI) was calculated as weight (kg) divided by height in square meters (m^2). Fat mass, bone mineral content, and non-bone non-fat mass were quantified by DXA (Hologic QDR 4500 W, Delphi 11.2, Discovery XP 12.1, Discovery XP 2002; Hologic, Bedford, MA), and separated into trunk and appendicular components. Quality control and calibration processes for these DXA machines have been previously described.¹⁶ Appendicular lean mass (ALM) was computed as the sum of non-fat, non-bone mass for both arms and legs. Percent total body fat was also determined. We focused on ALM rather than total body lean mass, because ALM is included in the operational criteria

to define sarcopenia by several groups including the Foundation of the National Institutes of Health Sarcopenia Project (FNIHSP),¹ the International Working Group (IWG),² and the European Working Group for Sarcopenia in Older Persons (EWGSOP).³ The correlation between ALM and total body lean mass of our study sample was 0.97. We used ALM adjusted for BMI (ALM/BMI) as the study outcome, as recommended by the FNIHSP.¹ This definition is more conservative than ALM adjusted for height squared, which is the recommendation of the IWG and EWGSOP.¹⁷ Further, in a pooled analysis of six different cohort studies, a consistent association between low lean mass and incident mobility impairment is seen in men and women when ALM/BMI is used as the exposure.¹⁸

2.4. Laboratory measurements, dietary assessment, and questionnaires

Fasting serum blood glucose level and fasting insulin level were measured at years 0, 7, 10, 15, and 20. The insulin resistance index, homeostasis model assessment-estimated insulin resistance (HOMA-IR), was calculated as follows: (fasting insulin in $\mu\text{U/mL} \times$ fasting glucose in mmol/L) divided by 22.5.¹⁹ Estimated glomerular filtration rate (eGFR) was calculated using serum creatinine-based Chronic Kidney Disease Epidemiology Collaboration equation.²⁰ eGFR was available at years 0, 10, 15, and 20. High sensitivity C-reactive protein (hs-CRP) was measured using high-sensitivity nephelometry-based methods (BNII nephelometer, Dade Behring, Eschborn, Germany) at years 7, 15, and 20.

Dietary assessment was performed using the CARDIA 28-day Diet History (approximately 700 items) at years 0, 7, and 20. The reliability and validity of the Diet History has been previously reported.²¹ Dietary intake data were processed with the Nutrition Data Systems for Research software program (Nutrition Coordinating Center, University of Minnesota). Diet quality was quantified according to alternate healthy eating index 2010 (aHEI-2010).

Self-reported age, sex, race, education, smoking, alcohol drinking, and menopause status were collected using standardized questionnaires. Physical activity data were collected using the CARDIA Physical Activity History which queries the frequency, duration, and intensity of 13 different activities during the previous year. A continuous total physical activity intensity score was derived.²²

A graded treadmill exercise testing using a modified Balke protocol was conducted at years 0, 7, and 20 to assess maximal symptom-limited performance for CARDIA participants who completed up to nine 2-min exercise stages of progressively increasing difficulty. The details of the treadmill test protocol have been described elsewhere.²³ We excluded the year 7 test due to a protocol violation at Minneapolis site ($n = 1139$), because Minneapolis participants may be allowed to hold onto the treadmill trailing, leading to longer treadmill test duration.²⁴ Duration of the treadmill test was used to assess cardiorespiratory fitness.

2.5. Statistical analysis

We studied the association between HOMA-IR trajectories over 20 years as the exposure and ALM/BMI measured at year 20 as the outcome. Because of the marked differences in ALM between men and women, all analyses were stratified by sex. Demographic, anthropometric, and clinical characteristics at year 20 were described according to sex-specific quartiles of ALM/BMI.

The primary exposure was determined with latent class models implemented with SAS Proc Traj to identify discrete groups who shared similar underlying trajectories in HOMA-IR.²⁵ The optimal number of trajectory groups was determined by Bayesian Information Criterion²⁶ and ensuring that group size did not fall below 5% of the sample participants. The posterior predicted probability for each participant of being a member in each of the trajectory groups was calculated. Participants were assigned to the group with the highest posterior predicted probability. The names of groups were determined by the baseline HOMA-IR

level (e.g., low, medium, high) and the following 20-year trajectory (e.g., increase, decrease or stable determined by the slope). These terms were selected based on qualitative rather than quantitative assessment, which is in line with the current practice for trajectory analysis.²⁶ Due to the skewed distribution of HOMA-IR, it was natural log-transformed for trajectory analyses. It was then back-transformed to geometric means when plotting the trajectory groups for visualization.

With identified trajectories as the exposure, linear regression was used to determine the association between antecedent distinct HOMA-IR trajectories and current ALM/BMI (in continuous form) at year 20. We tested the hypothesis in the fully adjusted model (i.e., Model 3 below) that an increasing trajectory in insulin resistance, as compared to a relatively stable insulin resistance trajectory, between early and middle adulthood was associated with lower ALM/BMI in middle-aged adults without diabetes, independent of demographic, life-style, clinical factors, and cardiorespiratory fitness. The models were sequentially adjusted for age, race (black and white), field center, highest education completed (Model 1), cumulative smoking pack-years, cumulative number of alcohol drinks, cumulative physical activity score, cumulative total calorie (kcal), cumulative protein intake (gram), cumulative fiber intake (gram), cumulative aHEI score, and cumulative eGFR between year 0 and year 20, and average hs-CRP level measured at years 7, 15 and 20; for women, time-varying menopausal status

(yes/no) was also included (Model 2); however this question was not asked before year 7 due to their young age. eGFR was added as a covariate, because determining insulin resistance trajectories as an independent predictor of ALM/BMI was our primary objective. eGFR can be either a confounder or a mediator in that prevalence of sarcopenia increased in people with early stage chronic kidney disease²⁷ and eGFR might have a bidirectional association with insulin resistance.²⁸ The final Model 3 further adjusted for cardiorespiratory fitness at year 0 and the change in cardiorespiratory fitness between years 0 and 20. Cardiorespiratory fitness has been associated with insulin resistance²⁹ and body composition³⁰ and thus is qualified as a confounder for the association between insulin resistance trajectories and ALM/BMI. We assumed that the value of covariates remained unchanged until the next available value when computing cumulative covariates.

Two secondary analyses were performed. First, we stratified analyses by dichotomizing the study sample at the highest sex-specific quartile of percent total body fat, an approach previously used to categorize body fat in the literature.³¹ Thus in the current study, men with percent total body fat $\geq 26.5\%$ and women with percent total body fat $\geq 40.3\%$ were classified as obese. This stratification analysis by obesity was performed because co-existence of obesity and low muscle mass and function (termed “sarcopenic obesity”) might synergistically influence risk of developing adverse health outcomes.³² Second, we

Table 1
Characteristics by quartiles of ALM/BMI in men and women at year 20.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P
Men					
N	232	230	232	231	
ALM/BMI	0.89 ± 0.08	1.03 ± 0.02	1.11 ± 0.03	1.26 ± 0.08	<0.0001
HOMA-IR ^a	3.3 ± 2.5	2.9 ± 2.1	2.6 ± 1.7	2.4 ± 1.4	<0.0001
BMI, kg/m ²	30.8 ± 8.5	28.0 ± 3.5	27.4 ± 4.0	25.6 ± 3.6	<0.0001
Percent total body fat, %	27.9 ± 5.1	23.4 ± 4.5	21.8 ± 4.5	18.0 ± 4.8	<0.0001
Age, years	45.6 ± 3.5	44.9 ± 3.5	45.2 ± 3.4	44.8 ± 3.4	0.04
Black	18.5	36.1	43.5	48.5	<0.0001
Highest education, years	15.9 ± 2.8	15.5 ± 2.6	15.7 ± 2.6	15.6 ± 2.5	0.3
Current smoker, yes	19.8	18.7	18.1	17.8	0.9
Current alcohol drinker, yes	80.6	81.3	78.0	80.5	0.8
Physical activity score ^a	293 ± 307	357 ± 360	413 ± 458	453 ± 391	<0.0001
Duration of the treadmill test, min	7.8 ± 2.0	8.8 ± 1.9	9.1 ± 2.1	9.9 ± 2.3	<0.0001
eGFR ^a , mL/min per 1.73 m ²	99.9 ± 17.7	93.1 ± 21.1	93.1 ± 23.0	93.1 ± 22.8	0.007
hs-CRP ^a , mg/L	1.14 ± 1.41	0.95 ± 1.30	0.72 ± 1.01	0.52 ± 0.77	<0.0001
Total calories, kcal	2697 ± 1191	2637 ± 1205	2954 ± 1757	3063 ± 1616	0.008
Protein intake, gram	100.4 ± 45.4	100.5 ± 46.0	109.1 ± 56.3	110.6 ± 53.6	0.07
Fiber, gram	22.1 ± 9.9	22.4 ± 11.8	25.1 ± 14.1	25.6 ± 13.2	0.005
Alternate HEI 2010 score ^b	51.4 ± 11.8	52.7 ± 11.8	53.1 ± 12.8	54.3 ± 11.7	0.1
Women					
N	299	298	297	299	
ALM/BMI	0.59 ± 0.05	0.69 ± 0.02	0.77 ± 0.02	0.89 ± 0.07	<0.0001
HOMA-IR ^a	3.2 ± 2.1	2.6 ± 1.8	2.4 ± 1.7	2.0 ± 1.2	<0.0001
BMI, kg/m ²	33.7 ± 7.1	29.2 ± 6.1	26.7 ± 5.3	23.9 ± 4.2	<0.0001
Percent total body fat, %	41.8 ± 5.1	36.6 ± 5.2	32.8 ± 5.7	27.9 ± 5.7	<0.0001
Age, years	45.6 ± 3.6	45.2 ± 3.7	45.1 ± 3.6	44.9 ± 3.6	0.09
Black	45.2	45.3	37.4	41.5	0.2
Highest education, years	15.2 ± 2.4	15.7 ± 2.5	15.9 ± 2.4	16.4 ± 2.4	<0.0001
Current smoker, yes	17.1	16.4	16.5	11.7	0.2
Current alcohol drinker, yes	68.9	74.8	82.5	88.0	<0.0001
Physical activity score ^a	160 ± 212	222 ± 293	281 ± 326	316 ± 381	<0.0001
Duration of the treadmill test, min	4.7 ± 1.6	5.9 ± 1.9	6.7 ± 2.2	8.0 ± 2.4	<0.0001
eGFR ^a , mL/min per 1.73 m ²	101.4 ± 20.2	100.8 ± 23.1	91.9 ± 19.5	89.3 ± 23.5	<0.0001
hs-CRP ^a , mg/L	2.85 ± 5.63	1.59 ± 3.50	0.85 ± 2.18	0.55 ± 0.99	<0.0001
Total calories, kcal	1955 ± 920	2000 ± 844	2136 ± 961	2126 ± 930	0.048
Protein intake, gram	74.3 ± 34.8	75.4 ± 32.4	79.7 ± 35.2	82.7 ± 37.4	0.02
Fiber, gram	18.0 ± 10.1	19.8 ± 9.6	20.9 ± 11.0	23.2 ± 13.0	<0.0001
Alternate HEI 2010 score ^b	52.8 ± 12.3	57.2 ± 13.9	57.3 ± 12.8	61.6 ± 13.0	<0.0001
Menopause	28.4	23.5	17.5	13.7	<0.0001

ALM, appendicular lean mass; hs-CRP, high sensitivity C-reactive protein; eGFR, estimated glomerular filtration rate; HEI, healthy eating index; HOMA-IR, homeostatic model assessment of insulin resistance.

Values were % or mean ± standard deviation or median ± interquartile range when specified by ^a.

P values were obtained from Chi-square test, ANOVA test, or Kruskal-Wallis test for not-normally distributed variables specified by ^a.

^b The range of alternate HEI 2010 score is 0–110. Higher score means higher diet quality.

evaluated the associations of 20-year fasting glucose and insulin trajectories with ALM/BMI to determine the influence of the two component measures of HOMA-IR on ALM/BMI.

A sensitivity analysis with the median imputation for missing data for the treadmill test was conducted to evaluate the robustness of the results. All analyses were performed using SAS version 9.4. Two sided P value <0.05 was considered statistically significant.

3. Results

Of the 925 men and 1193 women included for analyses at year 20, the mean (SD) of ALM/BMI was 0.89 (0.08) in the lowest quartile and 1.26 (0.08) in the highest quartile in men, while women had a mean ALM/BMI of 0.59 (0.05) in the lowest quartile and 0.89 (0.07) in the highest quartile (Table 1). In both men and women, participants with higher ALM/BMI had lower HOMA-IR, lower BMI, and lower percent total body fat, were more physically active, and had longer duration of the treadmill test, lower eGFR, lower hs-CPR, and consumed more fiber and energy ($P < 0.05$). Among men, those with higher ALM/BMI were more likely to be black and slightly younger ($P < 0.05$). Among women, those with higher ALM/BMI had slightly more years of education, were more likely to drink alcohol, consumed more protein and a higher quality diet, and were less likely to have gone through menopause ($P < 0.05$).

Three HOMA-IR trajectories were identified. In men, 31.1% belonged to the low-stable group, 54.8% medium-increase group, 14.1% high-increase group (Fig. 1A). In women, 46.8% belonged to the low-stable group, 40.0% medium-increase group, and 13.2% high-increase group (Fig. 1B).

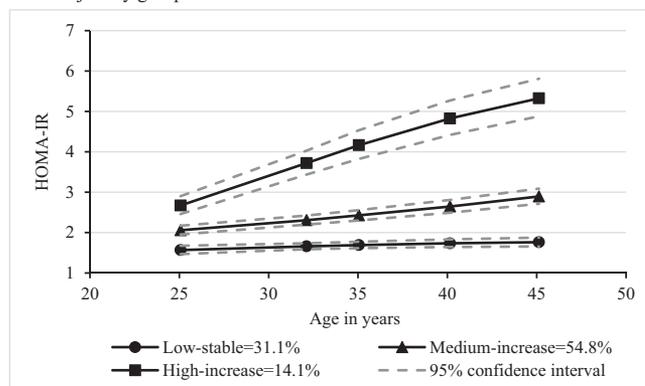
The mean ALM/BMI, mean treadmill test duration at years 0 and 20, and change in the treadmill test duration between years 0 and 20 differed by HOMA-IR trajectory groups similarly in men and women (Supplemental Table 1). In obese men and women (based on percent total body fat), we did not see a difference in the mean ALM/BMI across three trajectory groups ($P \geq 0.2$, Supplemental Table 2). Men or women in the high-increase trajectory had the lowest treadmill test duration at years 0 and 20 ($P \leq 0.003$, Supplemental Table 3). No difference by trajectory groups was found for the change in the treadmill test duration between years 0 and 20.

A graded association was found in men (Fig. 2) but not in women (Fig. 3) between HOMA-IR trajectories and ALM/BMI. In men, compared with the low-stable group in Model 2 where cardiorespiratory fitness was not adjusted, the associated difference in ALM/BMI was -0.041 (95% CI: -0.060 to -0.022) for the medium-increase group and -0.114 (-0.141 to -0.086) for the high-increase group. In women, compared with the low-stable group in Model 2, the associated difference for the medium-increase group (-0.052 [-0.065 to -0.039]) was similar to that for the high-increase group (-0.043 [-0.063 to -0.023]). After adjusting for cardiorespiratory fitness in Model 3, compared with the low-stable group in men, the estimate was -0.022 (-0.040 to -0.004) for the medium-increase group and -0.061 (-0.089 to -0.034) for the high-increase group. In women, the estimate was -0.026 (-0.038 to -0.014) for the medium-increase group and -0.007 (-0.026 to 0.012) for the high-increase group. Although attenuated, the inverse association largely persisted in non-obese men or women.

The sensitivity analyses with the median imputation for the missing data revealed robust results. Compared with the low-stable group in Model 3, in men, the associated difference in ALM/BMI was -0.021 (95% CI: -0.039 to -0.003) for the medium-increase group and -0.057 (-0.084 to -0.030) for the high-increase group. In women, the associated difference in ALM/BMI was -0.029 (95% CI: -0.041 to -0.017) for the medium-increase group and -0.011 (-0.029 to 0.008) for the high-increase group.

The three trajectories were similar between fasting insulin and HOMA-IR (Supplemental Fig. 1A and B). Also, the trajectories of fasting

A. Trajectory groups of insulin resistance in men



B. Trajectory groups of insulin resistance in women

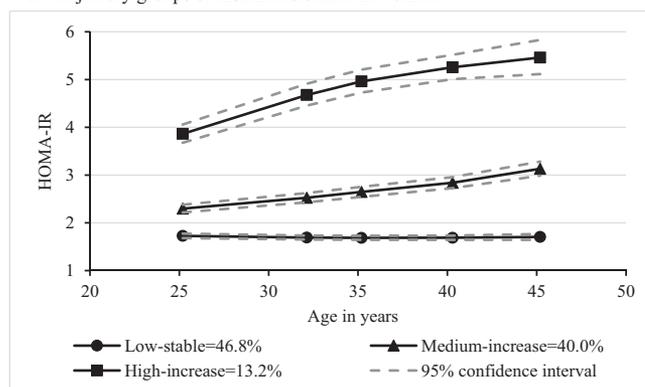


Fig. 1. A. Trajectory groups of insulin resistance in men. B. Trajectory groups of insulin resistance in women. HOMA-IR, homeostatic model assessment of insulin resistance. The percentages in the figures represented proportions of participants in each group.

insulin showed similar associations with ALM/BMI in men (Supplemental Fig. 2) and in women (Supplemental Fig. 3) as the HOMA-IR trajectories. The graded association was also seen in men, but not women. Fasting glucose level in men and women increased over time for each trajectory group (Supplemental Fig. 4A and B). Fully adjusted Model 3 did not reveal a significant association between fasting glucose trajectories and ALM/BMI in men (Supplemental Fig. 5) and in women (Supplemental Fig. 6).

4. Discussion

Among CARDIA participants, a high-increase trajectory in insulin resistance over 20 years between early and middle adulthood was associated with lower ALM/BMI in middle-aged men without diabetes compared with a low-stable trajectory in insulin resistance, even after accounting for cardiorespiratory fitness. In women, this inverse insulin resistance-ALM/BMI association was no longer significant after adjusting for cardiorespiratory fitness. We observed a graded association (i.e., ALM/BMI was lower in each successively higher insulin resistance trajectory group, compared to the low stable group) in men, but not women. The inverse association persisted only in non-obese men and women.

Previous studies investigating the association of diabetes or related biomarkers (blood glucose, insulin, and insulin resistance) with lean mass did not adjust for cardiorespiratory fitness and thus may have overestimated the association.^{10–13} Our study suggests that adjusting for cardiorespiratory fitness considerably attenuated the inverse association between insulin resistance trajectories and ALM/BMI. This attenuation occurred after a number of covariates including physical activity were taken into consideration. Physical activity and cardiorespiratory fitness are two related, but also distinct measures.³³ The former is

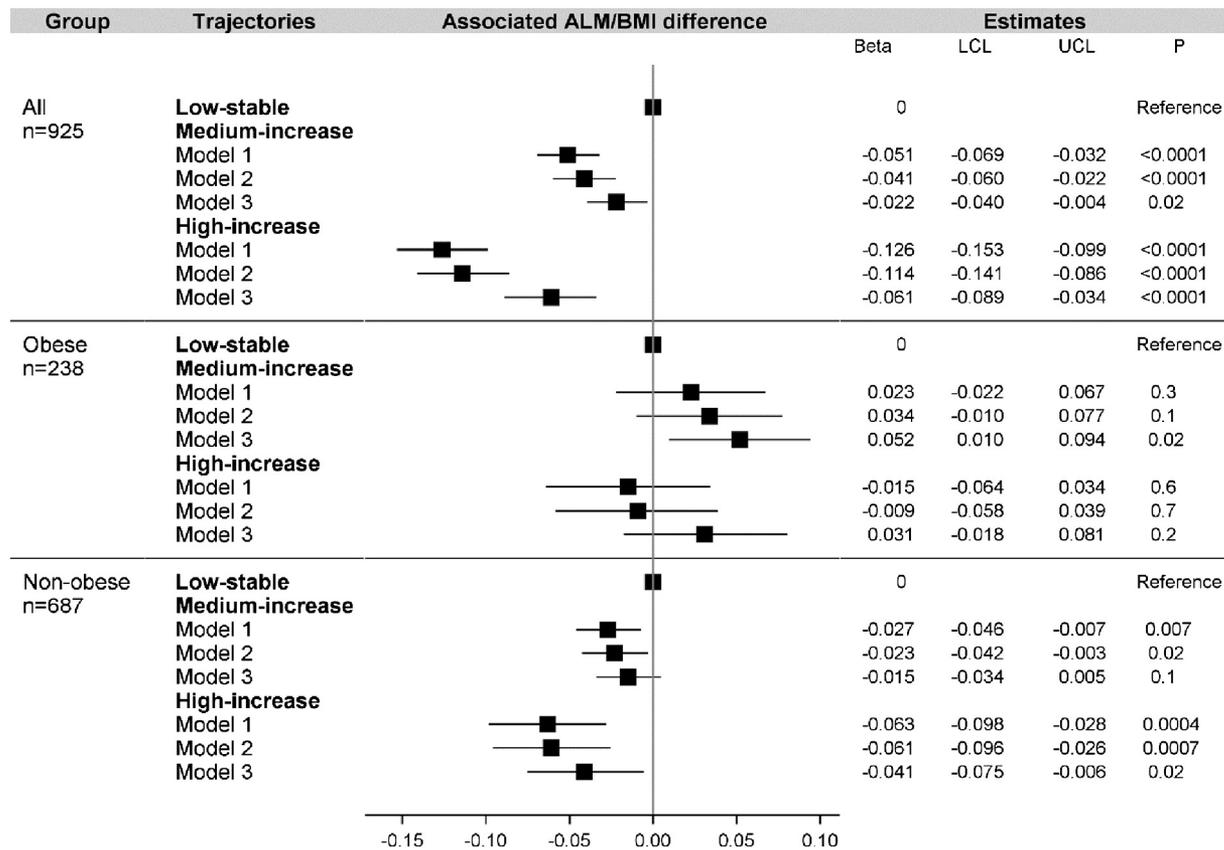


Fig. 2. Association between trajectory groups of insulin resistance and ALM/BMI in men. ALM, appendicular lean mass; LCL, lower confidence limit; UCL, upper confidence limit. “Obese” was defined in men with percent total body fat $\geq 26.5\%$. Model 1 adjusted for age, race, center, education. Model 2 further adjusted for cumulative value of smoking pack years, alcohol consumption units, physical activity score, energy, protein intake, fiber intake, alternate Healthy Eating Index score, and eGFR between year 0 and year 20, and average hs-CRP between years 7 and 20. Model 3 further adjusted for the duration of the treadmill test at year 0 and change in the duration of the treadmill test between year 0 and year 20.

usually self-reported in the literature and also in our study. Therefore, we could not rule out the impact of the imprecise self-reported physical activity data on the obtained association. The latter is an objective reflection of recent physical activity that also has a genetic component.³³ Physical activity and cardiorespiratory fitness may have different implications in the age/disease related loss of lean mass that deserves future investigations, but is beyond the scope of this study.

Our analyses suggest that insulin resistance, rather than glucose levels, is an independent determinant of lean mass in young and middle aged adults without diabetes. In our study, the associations of fasting insulin and insulin resistance trajectories with ALM/BMI were more robust than the association between fasting glucose trajectories and ALM/BMI, particularly in men. It is possible that elevated insulin level and decreased insulin sensitivity in people with high glucose but still within the normal range may have already started to disrupt the balance between muscle protein synthesis and degradation.^{6,34} Insulin resistance is an important underlying mechanism for accelerated muscle loss.³⁴ Elevated insulin resistance breaks the balance between muscle protein synthesis and degradation via various biological pathways including mitochondrial dysfunction, alteration of autophagy pathway, and stimulation of muscle protein degradation via the ubiquitin-proteasome proteolytic pathway.⁶ Decreased muscle mass is also accompanied by reduced mitochondria density and function and less surface area for insulin-mediated glucose uptake, which further exacerbates insulin resistance.^{6,10}

A previous study reported a weaker and less clear graded association between 2-hour glucose and muscle strength in women than men.¹² Similarly, in our study, the graded association between insulin resistance trajectories and ALM/BMI was only seen in men. The difference in body composition, fat distribution (on average, women have more peripheral

fat and men have more visceral fat), sex hormones and adipokines may be relevant to this noted sex difference.³⁵ Also, the sex difference in cardiorespiratory fitness levels has been noted in the published studies using either maximal oxygen uptake or treadmill test duration as the fitness measure^{36,37} and was also confirmed in our study (mean duration of the treadmill test was 2.6 min longer in men than women, $P < 0.0001$). However, it remains unclear how these factors may impact sexual dimorphism in the association between insulin resistance and ALM/BMI. Further, it is unclear if the greater age-related decline in muscle mass over time in men than women,³⁸ which creates greater variation over time in men, may contribute to this sexual dimorphism.

The inverse association between insulin resistance trajectories and ALM/BMI found in non-obese men and women (i.e., those without high percent total body fat) implies that even in individuals without obesity and diabetes, maintaining low insulin resistance over time may be beneficial for preserving lean mass. However, among obese individuals with high percent total body fat, we did not observe an association between insulin resistance trajectories and ALM/BMI. This might be explained by similar ALM/BMI values across the three insulin resistance trajectory groups in obese participants, which was not the case in non-obese participants. Potentially, losing fat mass may be more relevant for maintaining relative muscle mass in obese people than improving insulin sensitivity, although they are not independent from each other. There is evidence that the anabolic action of insulin and physical activity are both less effective at promoting muscle protein synthesis in people with increasing adiposity.^{39,40} Of note, due to the high correlation between body fat and BMI as part of the outcome, stratifying by percent total body fat may be an over-adjustment, because in individuals without high percent total body fat, the association between insulin resistance and ALM/BMI was greatly attenuated.

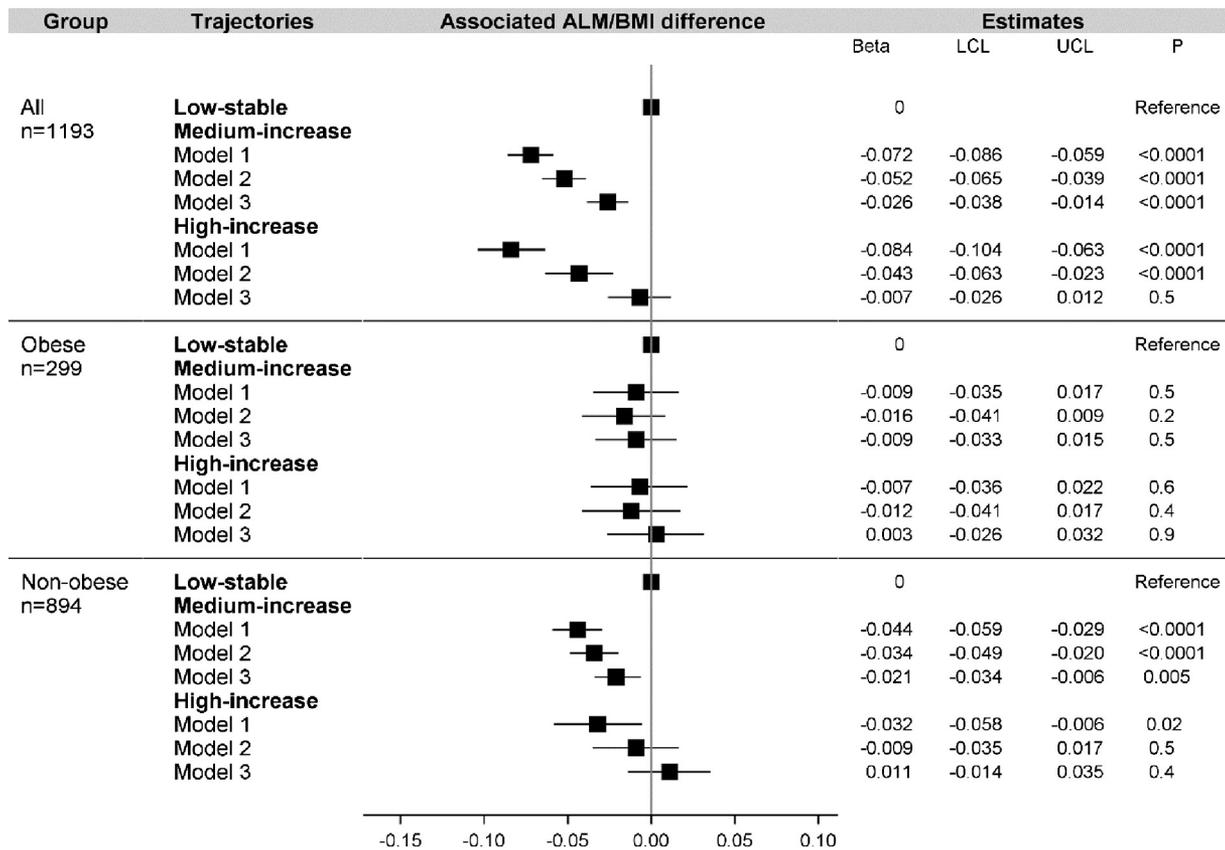


Fig. 3. Association between trajectory groups of insulin resistance and ALM/BMI in women. ALM, appendicular lean mass; LCL, lower confidence limit; UCL, upper confidence limit. "Obese" was defined in women with percent total body fat $\geq 40.3\%$. Model 1 adjusted for age, race, center, education. Model 2 further adjusted for cumulative value of smoking pack years, alcohol consumption units, physical activity score, energy, protein intake, fiber intake, alternate Healthy Eating Index score, and eGFR between year 0 and year 20, average hs-CRP between years 7 and 20, and time-varying menopausal status between year 7 and year 20. Model 3 further adjusted for the duration of the treadmill test at year 0 and change in the duration of the treadmill test between year 0 and year 20.

To the best of our knowledge, ours is the first study investigating the relationship between long-term trajectories in insulin resistance and ALM/BMI in young and middle-aged adults. We have added to the literature by characterizing insulin resistance over a 20-year period between early and middle adulthood where published data are lacking. Our study sample included a large number of black and white men and women within a well characterized cohort (i.e., CARDIA) which allowed for adjustment for a number of potential confounding variables such as cardiorespiratory fitness that has been previously omitted. Some limitations should be noted. First, we excluded participants who developed diabetes during follow-up. Thus, our findings may not be generalized to those who develop diabetes during young and middle adulthood. Second, missing data are generally inevitable in epidemiologic follow-up studies and may bias the estimates upward or downward. Additional analyses found that 9.3% of men and 10.1% of women did not have three or more measurements of fasting glucose and insulin required for trajectory analyses. Those individuals were more likely to be black, but they were not different compared to those with three or more measurements in terms of HOMA-IR, BMI, ALM, and percent total body fat (data not shown in the table). Approximately 5.2% of women and 4.4% of men had missing data for the treadmill test, but the median imputation analysis supported the robustness of the data. Third, although we adjusted for a number of covariates, residual confounding is possible. For example, an important covariate inflammatory marker hs-CRP was only available at years 7, 15, and 20, not in earlier years. Also, we made an arbitrary assumption for calculating cumulative confounders by assuming constant value between last and next available value. Fourth, we had one measure of body composition and thus a cross-sectional analysis of our outcome ALM/BMI. Temporal association and

direction of the association cannot be inferred. Specifically, it was unknown if those participants with low ALM/BMI at year 20 may already have low ALM/BMI at year 0 regardless of their insulin resistance trajectories. Finally, the distribution of BMI within the obese category was constrained due to the table limits of DXA machines.

In conclusion, a high-increase insulin resistance trajectory, as compared to a low-stable insulin resistance trajectory, between early and middle adulthood was associated with lower ALM/BMI in middle-aged men, but not women, without diabetes, after accounting for cardiorespiratory fitness. Future research is needed to understand the differences by sex and obesity status in the insulin resistance and ALM/BMI association and to determine whether insulin resistance can predict change in ALM/BMI over time in young and middle-aged adults.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jdiacomp.2018.09.011>.

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