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Original Article

Circulating MicroRNA Are Predictive of Aging and Acute Adaptive Response to Resistance Exercise in Men

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Abstract

Circulating microRNA (c-miRNA) have the potential to function as novel noninvasive markers of the underlying physiological state of skeletal muscle. This investigation sought to determine the influence of aging on c-miRNA expression at rest and following resistance exercise in male volunteers (Young: $n = 9$; Older: $n = 9$). Primary findings were that fasting c-miRNA expression profiles were significantly predictive of aging, with miR-19b-3p, miR-206, and miR-486 distinguishing between age groups. Following resistance exercise, principal component analysis revealed a divergent response in expression of 10 c-miRNA, where expression profiles were upregulated in younger and downregulated in older participants. Using Ingenuity Pathway Analysis to test c-miRNA-to-mRNA interactions in skeletal muscle, it was found that response of c-miRNA to exercise was indicative of an anabolic response in younger but not older participants. These findings were corroborated with a positive association observed with the phosphorylation status of p-Akt^{Ser473} and p-S6K1^{Thr389} and expression of miR-19a-3p, miR-19b-3p, miR-20a-5p, miR-26b-5p, miR-143-3p, and miR-195-5p. These important findings provide compelling evidence that dysregulation of c-miRNA expression with aging may not only serve as a predictive marker, but also reflect underlying molecular mechanisms resulting in age-associated declines in skeletal muscle mass, increased fat mass, and "anabolic resistance."

Keywords: Anabolic resistance—miR-19b-3p—miR-206—miR-486

Deleterious changes in body composition, where there is a concomitant loss of skeletal muscle mass and increase in fat mass, are a consistent pathophysiological condition that occurs with aging (1,2). Without intervention, these detrimental modifications in body composition increase the risk of developing metabolic syndrome and diminished mobility and physical function, ultimately decreasing health span and elevating health care costs (3,4). Age-associated alterations in body composition are not only attributed to reduced physical activity, but also a blunting of cellular mechanisms involved in the regulation of muscle hypertrophy in response to an acute stimulus, termed "anabolic resistance" (5,6). Recently, our laboratory (7) identified altered expression of microRNA (miRNA; small noncoding RNA that negatively regulate gene expression (8)) in skeletal muscle as a mechanism for impairment of exercise-induced adaptations in older individuals. Specifically, discordant response of miRNA expression to a bout of resistance exercise with aging inhibited the acute adaptive response of insulin-like growth factor 1 (IGF-1) signaling (7). Similarly, altered expression of miRNA with aging has been observed to predict the anabolic response of the downstream target of IGF-1, mechanistic target of rapamycin complex 1 (mTORC1), which is the central regulator of hypertrophy and muscle mass (9).

Though assessment of miRNA expression in skeletal muscle has yielded valuable insight into their functional role with age-induced changes to body composition, this type of data collection requires invasive muscle biopsies, which may cause discomfort and limit tissue available for analysis. Determination of noninvasive markers is thus warranted to capture larger data sets to improve our understanding of mechanisms altering body composition and metabolic health with aging. Recently, miRNA have been reported to be present in circulation (c-miRNA), with alterations in c-miRNA profile

reflective of the underlying physiological state that negatively affect skeletal muscle mass (10–12). The ability for c-miRNA expression to provide insight into changes of pathogenic processes in skeletal muscle makes them a promising noninvasive marker. While aging has been demonstrated to alter c-miRNA profiles (13–16), whether these differences relate to age-associated alterations in body composition and metabolic health have not been assessed. Furthermore, it remains undetermined if the expression of c-miRNA following an acute anabolic stimulus can distinguish "anabolic resistance" in older individuals.

The objective of the present investigation was to assess the influence of aging on c-miRNA expression under resting fasted conditions and determine their relationship to body composition and circulating metabolic markers of health. Furthermore, this investigation sought to determine the influence of an acute bout of resistance exercise on changes in c-miRNA expression and establish if c-miRNA are predictive of exercise-induced adaptions. We hypothesized that aging would result in altered c-miRNA profiles associated with differences in body composition and circulating metabolic markers of health. Additionally, we hypothesized that following resistance exercise a divergent response in c-miRNA expression with aging will be predictive of "anabolic resistance." Overall, findings from this investigation will demonstrate the predictive and potentially mechanistic role of c-miRNA in age-associated decrements in body composition.

Methods

Participants and Study Design

Data for this analysis were collected as part of a previous investigation aimed to assess markers of anabolism and catabolism in younger and older individuals in response to an acute bout of exercise (7,17). For the present investigation, data were analyzed on a total of 18 younger (22 ± 1 years, $n = 9$) and older (74 ± 2 years, $n = 9$) male participants (Supplementary Table 1). As previously described, all participants were in good health as determined by medical screening and did not regularly engage in resistance or endurance exercise. Data collection was performed at the U.S. Department of Agriculture (USDA) Human Nutrition Research Center on Aging (HNRCA). This investigation was approved by the Tufts University Health Sciences Campus Institutional Review Board, with informed written consent obtained from all participants.

One week prior to data collection, one repetition maximum was determined for all participants to prescribe exercise intensity for the acute resistance exercise bout. Participants were admitted to the HNRCA 24 hours prior to the acute resistance exercise bout. Baseline blood samples were collected at this time. The following morning participants performed three sets of bilateral knee extension exercise and three sets of bilateral leg press exercise for 10 repetitions at 80% of their one repetition maximum. Blood sampling was conducted immediately (0-hour) post exercise and again at 6-hour post exercise (recovery). Participants were fasted throughout the entire 6-hour data collection period.

Anthropometrics and Body Composition

Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Body mass and composition were determined using dual-energy x-ray absorptiometry (DXA; Hologic, Bedford, MA) as previously described (17).

Blood Sampling

Blood samples were obtained at baseline, post exercise, and recovery. All samples were obtained following an overnight fast. To minimize hemolysis and potential contamination of serum by red blood cells, venipuncture was performed by a highly experienced registered nurse trained in phlebotomy at each time point of the trial. Blood were allowed to clot at room temperature and then centrifuged at 2,135*g* for 10 minutes at 4°C. Derived sera were stored in 500 µL aliquots at −80°C, prior to analysis. No serum sample that was visually hemolyzed (red in color) was used in analysis.

Substrate and Hormone Analysis

Serum glucose and triglyceride concentrations were assessed using Beckman Coulter AU400e Chemistry analyzer (Beckman Coulter, Inc., Brea, CA).

c-miRNA Extraction and Expression

c-miRNA were extracted from 200 µL serum using miRNeasy Serum/Plasma Kit, which allows for extraction and purification of small (<200 nt) cell-free RNA (217184; Qiagen, Valencia, CA). To avoid introduction of potentially contaminating material, prior to RNA extraction, serum samples were centrifuged for 10 minutes at 4°C to remove cellular debris. Supernatant was removed and transferred to a new tube without disturbing the pellet. Due to the small amount of RNA in the serum, 3.5 µL of a Spike-In Control (*Caenorhabditis elegans* miR-39; 219610; Qiagen) was added to all samples prior to extraction of RNA to determine the yield of template recovered. Reverse transcription (RT) using a fixed amount (1.5 µL) of RNA was performed using miScript II RT Kit (218161; Qiagen). Quantitative polymerase chain reaction (RT-qPCR) amplifications were conducted following manufacturer's instructions using a QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems, Foster City, CA) to assess cDNA template in a 384-well miScript miRNA PCR Array (MIHS-3106Z; Qiagen). Arrays were run using a miScript SYBR Green PCR Kit, quantifying 84 miRNA.

Additional miRNA of interest (miR-34a, miR-181, miR-206, miR-208b, miR-324, miR-486) were analyzed using TaqMan MicroRNA Assays (4427975; Applied Biosystems) following previously described multiplex RT and preamplification protocol (18). Briefly, miRNA were reverse-transcribed using the TaqMan MicroRNA RT Kit (4366596; Applied Biosystems) with the eight miRNA-specific stem-loop RT primers pooled in 1× Tris–EDTA (TE) buffer for a final dilution of 0.05× for each miRNA RT primer. The RT primer pool (6 µL) was added to the RT reaction mix (0.3 µL 100 mM dNTP, 3 µL enzyme, 1.5 µL 10× RT buffer, 0.19 µL RNase inhibitor) and 3 µL of serum RNA. A preamplification step was performed to increase cDNA template using a primer pool of 20× TaqMan Small RNA Assay for the eight miRNA of interest at 0.05× concentration in 1× TE buffer. Preamplification reaction mix was constituted of 3.75 µL primer pool, 2.5 µL cDNA, 12.5 µL TaqMan Universal PCR Master Mix (2×), no UNG (4440040; Applied Biosystems), and 6.25 µL nuclease-free H_2O. RT and preamplification were conducted following manufacturer's instructions in a T100 Thermal Cycler (Bio-Rad, Hercules, CA). Following preamplification, RT-qPCR amplifications were conducted following manufacturer's instructions using CFX96 Touch Real-Time PCR Detection System (Bio-Rad).

Older participants may be more prone to hemolysis during blood draws, which may lead to miRNA contamination of serum fractions, compared to younger participants. To ensure that differences

in miRNA expression with aging and resistance exercise were not due to difference in serum contaminated by hemolyzed red blood cells, we examined the cycle threshold (C_t) of miR-16-5p, a miRNA that is highly enriched in erythrocytes and has been shown to cross the C_t earlier with hemolysis (19). Comparison of C_t values for younger (baseline: 23.91, post exercise: 23.40, recover: 23.60) and older (baseline: 25.72, post exercise: 24.96, recover: 26.03) participants suggests age-induced hemolysis was not a factor in differences in miRNA expression with aging and resistance. Similarly, assessing the difference in delta C_t values for miR-16-5p to miR-23a-3p, a reference miRNA that expression is unaffected by hemolysis (19), no differences were observed between younger (baseline: 2.73, post exercise: 2.44, recover: 3.21) and older (baseline: 2.30, post exercise: 2.05, recover: 2.66) participants, indicating hemolysis did not factor into differences observed with aging or resistance exercise in c-miRNA expression profiles in the current investigation.

If a miRNA did not cross the C_t for any time point or if the average miRNA expression was ≥35 C_t (20), it was not included in analysis (Supplementary Table 2). All miRNA were normalized to the geometric mean of Spike-In Control miR-39 (external control) and SNORD95 and U6 (internal controls). A total of six internal controls (SNORD61, SNORD68, SNORD72, SNORD95, SNORD96A, and U6) were assessed for this analysis. SNORD95 and U6 were determined to be the most stable and least variable internal controls in the present investigation. Combination of external and internal controls allows for both technical and interindividual normalization (21). Geometric mean of controls was used to correct for possible outlying values and abundance differences between the different controls (22). Furthermore, geometric mean of Spike-In Control miR-39, SNORD95, and U6 was determined to be a homogenously and stably expressed housekeeper with a coefficient of variation of 4.5% (23). Fold changes were calculated using the ΔΔ cycle threshold ($\Delta\Delta C_t$) method as described below in Statistical Analysis section.

Discriminate Analysis

To determine a potential grouping of c-miRNA that distinguishes between age groups, stepwise discriminant analysis with forward selection based on Wilk's lambda was conducted. A nonsignificant result for Box's test of equality of covariance matrices confirmed model appropriate for discriminant analysis. Cross-validation method was performed to assess the reliability and generalizability for results from discriminant analysis. Based on outcomes from discriminant analysis, receiver operating characteristic curve and area under curve were used to assess the potential for miR-19b-3p, miR-206, miR-221-3p, and miR-486 as predictive markers for aging.

Clustering and Assessment of miRNA-Associated Pathways

Principal component analysis (PCA) was performed for grouping of relevant miRNA, while controlling for type 1 error. The data set was verified suitable for factor analysis by a Kaiser–Meyer–Olkin of sampling adequacy > 0.6 with a significant < 0.05 Bartlett's test of sphericity. Related clusters of miRNA were then uploaded to DNA Intelligent Analysis (DIANA)-miRPath 3.0 (Alexander Fleming Biological Sciences Research Center [BSRC], Athens, Greece; http://diana.cslab.ece.ntua.gr) to determine potential molecular pathways that these miRNA have previously been reported to regulate. Relevant Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/) pathways were identified using experimentally verified targets from TarBase 7.0 (Alexander Fleming BSRC).

Ingenuity Pathway Analysis

Ingenuity miRNA target filter analysis was conducted for all possible c-miRNA-to-mRNA interactions from the top 10 c-miRNA identified in Component 1 of PCA results to gene array data set of altered mRNA expression in skeletal muscle 6-hour post resistance exercise in young and older participants (7). Data of differentially expressed c-miRNA and mRNA were integrated using the expression pairing function to obtain c-miRNA-to-mRNA relationships. The interacting networks were further explored for relevance to biological pathways and upstream regulators. Experimentally validated interactions and predictions characterized as "high" confidence were considered based on TargetScan and Ingenuity Knowledgebase. Based on top canonical pathways observed in younger and older participants, the association of the top 10 c-miRNA identified in Component 1 of PCA results to phosphorylation status of p-Akt^{Ser473} and p-S6K1^{Thr389} was determined from data obtained from a previous investigation of these participants (17). Phosphorylation of these protein targets was determined with Western blotting as previously published (17). Muscle samples used for Western blotting were obtained using percutaneous muscle biopsy taken from the vastus lateralis.

Statistical Analysis

Normality of data was assessed using Shapiro–Wilk tests for dependent variables. Data were not normally distributed ($p < .05$) and fold change data for c-miRNA were log transformed ($\log_2$) for statistical analysis, but presented as original values (mean ± *SEM*). Baseline data (younger and older) were set as control to calculate fold change ($2^{-\Delta\Delta Ct}$) of c-miRNA expression at baseline, post exercise, and recovery. Mixed-model repeated measures ANOVA was performed to determine main effects of age (younger and older), time (baseline and post exercise and recovery), and age-by-time interaction. Based on Akaike's information criterion, unstructured covariance was determined as the appropriate model for this analysis. For c-miRNA with a significant main effect of time or age-by-time interaction, Bonferroni correction was used for post hoc pairwise comparisons. Backwards linear regression analysis was conducted to determine the relationship of miR-19b-3p, miR-206, and miR-486 to age, fat-free mass, fat mass, and glucose and triglyceride concentrations. Spearman's rho rank correlation coefficient was utilized to determine associations. The α level for significance was set at $p \leq .05$. Data were analyzed using IBM SPSS Statistics for Windows Version 22.0 (IBM Corp., Armonk, NY).

Results

Expression of miR-19b-3p, miR-206, and miR-486 Discriminant of Aging

Of the 90 miRNA assess, 65 miRNA crossed the cycle threshold (C_t) before 35 cycles in both younger and older participants at each time point (Supplementary Table 2). Stepwise discriminant analysis revealed four c-miRNA (miR-19b-3p, miR-206, miR-221-3p, miR-486) that correctly classified 93% of participants by age, with cross-validated group cases correctly classified with 89% accuracy (Figure 1A). Receiver operating characteristic curve analysis was then conducted, measuring the sensitivity (true positive) and specificity (false positive) of these four c-miRNA. miR-19b-3p differentiated aging with an area under curve of 0.75, sensitivity 89%, and specificity 67% (Figure 1B). miR-206 distinguish aging with an area under curve of 0.70, sensitivity 89%, and specificity 78%, whereas miR-486 had an area under curve of 0.77, sensitivity 89%, and specificity

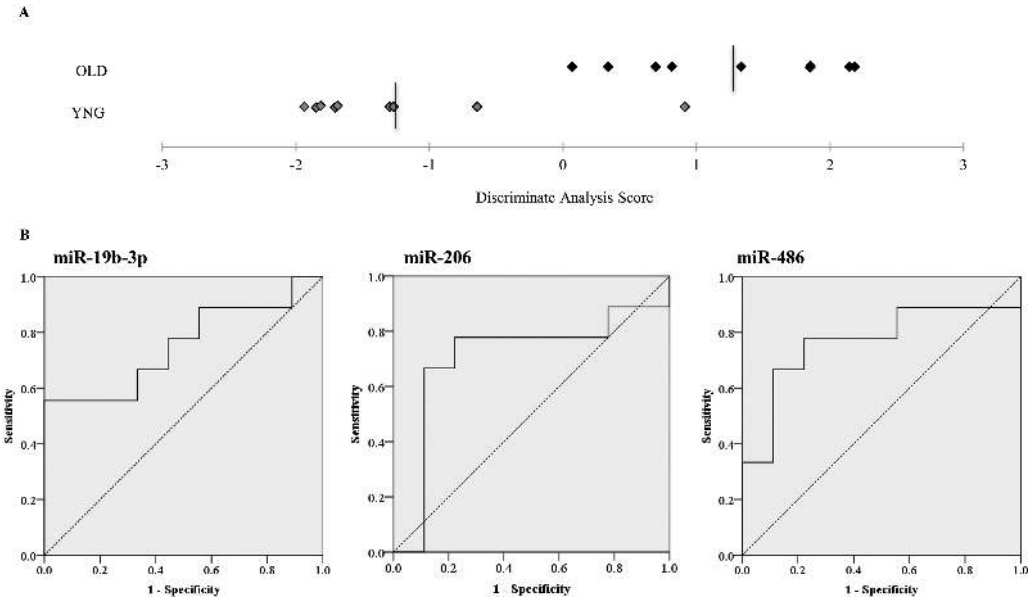

Figure 1. Discriminant analysis using miR-19b-3p, miR-206, miR-221-3p, and miR-486 for Younger (♦) and Older (♦) participants under fasted resting conditions (**A**). Receiver operating characteristic curve analysis to determine sensitivity and specificity of miR-19b-3p, miR-206, and miR-486 (**B**).

89%. Though miR-221-3p was identified in the stepwise discriminant analysis, it was not sensitive or specific to age using receiver operating characteristic curve analysis.

Accordingly, backwards linear regression was conducted to test the association of miR-19b-3p, miR-206, and miR-486 expression to descriptive characteristics, which included age, fat-free mass, fat mass, and glucose and triglyceride concentrations (Supplementary Table 1). Positive and negative associations of fat-free mass and glucose concentrations, respectively, accounted for 36% of the variance in miR-19b-3p expression (r = .60, r^2 = .36; p = .04), triglyceride concentrations accounting for 47% of the variance (r = .68, r^2 = .47; p < .01) in miR-206 expression, and fat mass and glucose concentration explained 52% of the variance (r = .72, r^2 = .52; p < .01) for miR-486 expression. Furthermore, when fat-free mass and fat mass were expressed as a percentage of total body weight to account for difference in body weight between age groups, percent fat-free mass was negatively associated with miR-206 (r = –.51, r^2 = –.16; p = .03) and miR-486 (r = –.65, r^2 = –.39; p < .01), whereas percent fat mass was positively associated with miR-206 (r = .56, r^2 = .22; p = .02) and miR-486 (r = .68, r^2 = .39; p < .01).

To determine if common regulatory function could explain the association of discriminate miRNA to body composition and markers of metabolic health, KEGG pathways were identified using predicted targets of miR-19b-3p, miR-206, and miR-486 using DIANA-miRPath 3.0. Mutual relevant pathways were identified as FoxO signaling, p53 signaling, cell cycling, PI3K-Akt signaling, apoptosis, and AMPK signaling, revealing that these three distinguishing miRNA serve as regulators of skeletal muscle anabolism and atrophy, metabolism, immune-regulation, and oxidative stress. All KEGG pathways common to miR-19b-3p, miR-206, and miR-486 are presented in Supplementary Table 3.

Altogether, these comprehensive analysis not only identified miR-19b-3p, miR-206, and miR-486 as predictive of aging, but common molecular pathways and associations with body composition and markers of metabolic health plausibly illustrate their critical role as regulators in aging processes.

Circulating miRNA Predictive of "Anabolic Resistance" With Aging

Impairment of the acute adaptive response to exercise in skeletal muscle with aging (eg, anabolic resistance) plays an important role in age-associated alterations in body composition (6). Though determination of molecular adaptions in muscle typically requires invasive muscle biopsies procedures, assessment of c-miRNA expression may provide insight into the underlying physiological state of tissue while minimizing discomfort to the participant. In the current investigation, aging and/or response to acute resistance exercise altered (p < .05) the expression of 25 c-miRNA (Supplementary Table 4; Figure 2A). PCA was performed to group c-miRNA based on fold change in response to aging and exercise. Five c-miRNA components were extracted from PCA with eigenvalues > 1. Of these five c-miRNA components, Component 1 explained the majority of the variance (60%) in the data set (Supplementary Table 5), while the remaining four components combined only explained 24% of the variance in the data set. Additional analysis was performed on the top 10 c-miRNA in Component 1 to assess between-group differences and associated pathways. A main effect of age (p < .05) was observed for 9 of the 10 c-miRNA, with c-miRNA expression being greater in younger compared to older participants (Figure 2B). To determine potential regulatory function, these 10 c-miRNA were uploaded into DIANA-miRPath 3.0, with KEGG pathways identified using predicted targets. Relevant pathways related to exercise-induced adaptations containing at least 8 of the 10 c-miRNA were reported (Figure 3A). Disease-related targets, such as cancers, were not reported. Results of this analysis revealed that these 10 c-miRNA predicted hypertrophic, proteolytic, inflammatory, and metabolic pathways whose activity have characterized to be augmented with exercise.

To further characterize the potential use of c-miRNA as a marker of aging and acute adaptive response to exercise, Ingenuity Pathway Analysis (IPA) was performed on c-miRNA in Component 1 of PCA to skeletal muscle mRNA at 6 hours into recovery of resistance exercise (7). Results of IPA showed that top-regulated canonical pathways

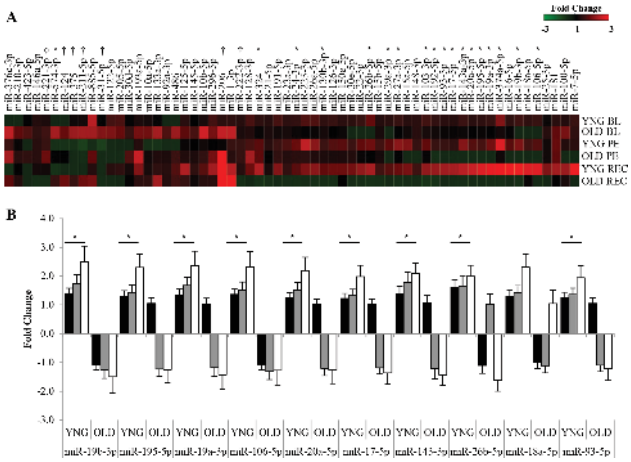

Figure 2. Fold change c-miRNA expression baseline (BL), 0-hour post exercise (PE), and 6-hour post exercise (REC) in Younger (YNG) and Older (OLD; **A**). Fold change of c-miRNA from Component 1 from PCA at baseline (■), 0-hour post exercise (■), and 6-hour post exercise (□) in Younger (YNG) and Older (OLD; **B**). Fold change calculated using average baseline data for both younger and older participants as controls. Mixed-model repeated measures ANOVA with Bonferroni adjustment used to determine main effect of age, time, and age-by-time interaction. Data presented as mean ± *SEM*. *Main effect of age, older significantly different from younger participants; $p < .05$. †Main effect of time; $p < .05$. c-miRNA = circulating microRNA; PCA = principal component analysis.

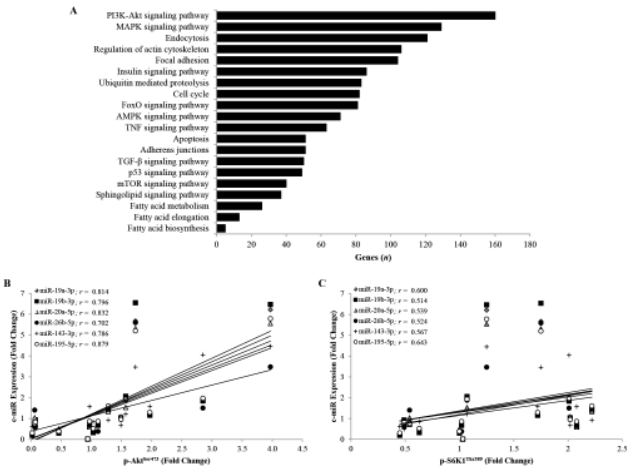

Figure 3. Identification of significant ($p < .05$) and relevant KEGG pathways associated with exercise-induced adaptations and number of genes regulated by top 10 c-miRNA in Component 1 (**A**). Determined with PCA and identified with DIANA-miRPath 3.0 using experimentally verified targets from TarBase 7. Correlation of c-miRNA expression to phosphorylation status of Akt^{Ser473} (**B**) and S6K1^{Thr389} (**C**) from Western blot analysis conducted in a previous investigation (17). Associations determined using Spearman's rho rank correlation coefficient. All correlations significant; $p < .05$. c-miRNA = circulating microRNA; DIANA = DNA Intelligent Analysis; KEGG = Kyoto Encyclopedia of Genes and Genomes; PCA = principal component analysis.

differed with aging, as anabolic pathways, IGF-1, and mTOR signaling, were present in younger, but not older participants (Table 1). Furthermore, the top physiology system development and function category produced by IPA for younger participants was skeletal and muscular system development, which again was not present in the results for older participants. Absence of anabolic signaling in the IPA results with aging suggests that a downregulation of the 10 c-miRNA in Component 1 is indicative of "anabolic resistance." Strengthening the IPA results, positive correlations were observed between the expression of c-miRNA in Component 1 compared to phosphorylation status of upstream and downstream targets of mTORC1, p-Akt^{Ser473}, and p-S6K1^{Thr389}. Of the 10 c-miRNA in Component 1, miR-19a-3p, miR-19b-3p, miR-20a-5p, miR-26b-5p, miR-143-3p, and miR-195-5p were significantly associated with both p-Akt^{Ser473} and p-S6K1^{Thr389} (Figure 3B and C; Supplementary Table 6). These current findings highlight that not only is the expression of c-miRNA altered following exercise, but that this change may be reflective of the underlying molecular processes being activated in skeletal muscle.

Discussion

The main findings from the present investigation provide initial evidence that c-miRNA have the potential to function as noninvasive markers of physiological and metabolic adaptations with aging and acute adaptive response to resistance exercise. Using advanced statistical methodologies and integrative bioinformatics analysis, this investigation is the first to report that alterations in c-miRNA expression profiles with aging are predictive of age-associated modifications in body composition and metabolic health. Furthermore, combining measurements of c-miRNA expression with skeletal muscle mRNA and signaling proteins, findings from this investigation not only show that aging results in a divergent c-miRNA profile following resistance exercise, but that this discordant response may be reflective of underlying acute adaptive responses in skeletal muscle.

Paralleled reductions in skeletal muscle mass and increased fat mass with aging result in impaired whole-body metabolism, over time propagating a feed-forward loop leading to further reductions in muscle and elevated fat mass (24), a process termed "sarcopenic obesity." Due to this concomitant shifts in body composition metabolically compromised older individuals may maintain a normal BMI (<25 kg/m^2), masking their risk for developing weight-related comorbidities such as cardiovascular disease and diabetes (25). In the present investigation despite having a BMI only slightly above normal (25.6 kg/m^2), we observed that c-miRNA (miR-19b-3p, miR-206, and miR-486) which distinguished aging were associated with body composition and markers of metabolic health (eg, serum glucose and triglyceride concentrations). The ability for these c-miRNA to discriminate aging, despite older participants being of good health, suggests c-miRNA expression may be a highly sensitive predictive tool for development of more severe conditions, such as "sarcopenic obesity" and metabolic syndrome. Previous investigations have provided promising evidence that c-miRNA expression is reflective of metabolic health. Alteration of c-miRNA expression profiles have been reported in morbidly obese (BMI > 40 kg/m2) and diabetic individuals (12,26). When treated (eg, bariatric surgery and Metformin), modulation in c-miRNA expression is corrected, with profiles being comparable to healthy control participants (12,26). Additionally, c-miRNA have been shown to be predictive of improvements in insulin resistance, with circulating miR-486 expression indicative of responders and nonresponders to Thiazolidinedione therapy (27). Given that potential functions of discriminant c-miRNA in the present investigation may regulate pathways involved in anabolism and atrophy, metabolism, immune-regulation, and oxidative stress (28–31), dysregulation of their expression in circulation may be attributed to the aging process. While findings from the present investigation cannot definitely show that dysregulation in

Table 1. c-miRNA Component 1 Comparison Analysis to Skeletal Muscle Gene Expression

Younger	*P*	Older	*P*
		Canonical pathways	
AMPK signaling	7.28E-05	AMPK signaling	6.21E-04
Coagulation system	2.64E-04	Xenobiotic metabolism signaling	1.63E-0.3
IGF-1 signaling	4.39E-04	Glucocorticoid receptor signaling	1.75E-03
ILK signaling	5.70E-04	IL-10 signaling	1.77E-03
mTOR signaling	6.57E-04	Pyridoxal 5′-phosphate salvage pathway	1.87E-03
		Physiology system development and function	
Skeletal and muscular system development	1.96E-03–7.27E-08	Connective tissue development and function	2.93E-03–4.29E-07
Tissue development	1.96E-03–9.23E-08	Cardiovascular development and function	3.57E-03–2.09E-06
Connective tissue development and function	1.96E-03–1.42E-07	Organismal development	3.74E-03–2.09E-06
Tissue morphology	2.07E-03–1.42E-07	Tissue development	3.48E-03–2.09E-06
Organismal survival	1.55E-04–3.40E-07	Tissue morphology	3.48E-03–1.07E-05

Note: c-miRNA = circulating microRNA; IGF-1 = insulin-like growth factor 1; mTOR = mechanistic target of rapamycin. Functional analysis of c-miRNA-to-mRNA data interactions using Ingenuity Pathway Analysis with the miRNA target filter. Italics denote relevant pathways.

these discriminate miRNA alters skeletal muscle physiology with aging, these results do provide compelling preliminary evidence to warrant further in-depth investigation.

Assessment of the acute response to an anabolic stimulus in aging is critical when assessing mechanism related to age-associated alterations in body composition, as a blunted response (eg, "anabolic resistance") to such events potentially results in declines in skeletal muscle mass and increased fat mass (32,33). While several studies have investigated altered expression of c-miRNA in response to an acute bout of aerobic exercise (34–36), the response of c-miRNA expression to an acute bout of resistance exercise with aging remains undefined. Findings from the present investigation are the first to report that not only is there a divergent response in the expression of specific c-miRNA with aging, but also that upregulation or downregulation of these c-miRNA may be indicative of underlying physiological processes in skeletal muscle following resistance exercise. Functional analysis of c-miRNA–mRNA interactions using IPA elucidated an absence of an anabolic response to exercise with aging, as the well-characterized hypertrophic signaling cascades, IGF-1 and mTOR, were identified as top canonical pathways in young, but not aged males. This finding is consistent with the hypothesis that aging results in "anabolic resistance" (5,6), where the synthetic response to an acute anabolic stimulus such as resistance exercise is blunted (37–39). Further strengthening the bioinformatics data of our current investigation, a positive association was observed between the expression of c-miRNA in Component 1 of PCA to the phosphorylation status (eg, activity) of p-Akt^{Ser473} and p-S6K1^{Thr389}. This finding shows that an upregulation in the expression of these c-miRNA, in particular miR-19a-3p, miR-19b-3p, miR-20a-5p, miR-26b-5p, miR-143-3p, and miR-195-5p, is indicative of a hypertrophic response within skeletal muscle. Coupling state-of-the-art integrative analytic techniques with findings from traditional bench-top techniques assists in validating that our IPA findings are accurate, and that c-miRNA can be used as noninvasive markers to predict adaptations reflective of molecular processes in skeletal muscle to acute resistance exercise with aging.

Interestingly, many of the c-miRNA (miR-17-5p, miR-18a, miR-19a, miR-19b, and miR-20a) observed in Component 1 of PCA results are members of the miR-17~92 cluster (40). Additionally, this cluster has been extended to miRNA families which contain miR-93 and miR-106b (41). These clusters of miRNA are generated from a primary transcript and have a large overlap in their function (41). Though little is known regarding the influence of acute resistance exercise on this cluster of miRNA, disease models have shown convergence of these miRNA on Akt-mTOR signaling within tissue (42). A main target of these miRNA is PTEN, an inhibitor of the PI3K-Akt pathway. Inhibition of PTEN can promote cellular survival and proliferation through increased activation of Akt-mTOR signaling (40). Results from these previous investigations corroborate findings from the present study that c-miRNA in Component 1 target anabolic pathways (39–41). Furthermore, identification of divergent c-miRNA profiles following resistance exercise being members of the same family of miRNA, sharing similar target genes, further strengthens the potential use of c-miRNA as potential predictive markers of resistance exercise-induced adaptions.

Use of c-miRNA expression profiles as a predictive biomarker for the pathophysiological state of tissue has gained favor in recent years, as c-miRNA have been shown to be stable, reproducible, and predictive of clinical outcomes in disease states (43). The biogenesis and maturation of miRNA occur in the nucleus and cytoplasm of the cell, respectively (44,45). Mature miRNA can be released into the circulation in membrane-derived vesicles (exosomes), complexed with proteins, lipoproteins (cholesterol), or apoptotic bodies (plasma membrane fragments) (46). As miRNA serve to regulate anabolic and metabolic molecular pathways, with transport of miRNA in the circulation potentially involved in cell-to-cell communication (46,47), dysregulation of c-miRNA expression with aging may not only serve as predictive marker, but may also provide insight into underlying mechanisms resulting in age-associated declines in skeletal muscle mass, increased fat mass, and "anabolic resistance." While presently it can be observed how c-miRNA expression profiles are altered under various conditions, and potential function can be inferred using bioinformatics analysis, causation may not be determined. Advancement of methodologies to isolate exosomes and microvesicles, as well as determination of tissue source in vitro, is required to fully realize the functional potential of c-miRNA to determine causal effects (48). Future investigations should focus on attempting to isolate skeletal muscle–derived miRNA in serum to gain further insight into their use as noninvasive markers of altered skeletal muscle physiology with aging.

To the best of our knowledge, results from this work are the first of their kind to identify c-miRNA as predictive markers for aging and "anabolic resistance." Although this investigation was limited in

sample size and conducted only in male participants, here we demonstrated that aging results in altered c-miRNA expression profiles with distinguishing miR-19b-3p, miR-206, and miR-486 being associated with body composition and metabolic health. Furthermore, using advanced statistical and bioinformatics analysis, we were able show that divergent responses in c-miRNA expression were predictive of acute adaptations to resistance exercise, where c-miRNA expression was associated with altered phosphorylation status of upstream and downstream targets of mTORC1. Enhanced understanding of alterations in c-miRNA expression with aging may result in clinically relevant diagnostic tools and further substantiating the use of "liquid biopsies" in aging diseases. Unlike traditional markers of health, c-miRNA are not end products of altered molecular pathways, but rather serve in a functional role. As such, deregulation of c-miRNA with aging and association to body composition and metabolic health may not only be used to predict risk of developing conditions such as sarcopenic obesity and metabolic syndrome, but also provide an understanding into mechanisms involved in the aging process.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biomedical Sciences and Medical Sciences* online.

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Conflict of Interest

The authors report no conflicts of interest.

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