Expression of the Irisin Precursor FNDC5 in Skeletal Muscle Correlates with Aerobic Exercise Performance in Patients with Heart Failure

Lecker et al: FNDC5 Correlates with Exercise in HF

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DOI: 10.1161/CIRCHEARTFAILURE.112.969543

Abstract

Background—Exercise induced increase in peroxisome proliferator-activated receptor-γ co-activator-1α (PGC-1α) expression has been shown to increase the expression of the fibronectin type III domain containing 5 (FNDC5) gene and thereby its product, irisin, in mice. Given that exercise intolerance is a hallmark characteristic of heart failure (HF), and since PGC-1α and irisin promote exercise benefits in animals, we hypothesized that expression of these genes relates to aerobic performance in patients with HF.

Methods and Results—Systolic HF (LVEF ≤40%) patients underwent cardiopulmonary exercise testing (CPX) to evaluate aerobic performance. High vs. low aerobic performance was assessed using oxygen consumption [peak VO2 (>14mLO2·kg⁻¹·min⁻¹ vs. ≤14 mLO2·kg⁻¹·min⁻¹)] and ventilatory efficiency [VE/VCO2 slope (<34 vs. ≥34)]. Muscle biopsies of the vastus lateralis and real-time polymerase chain reaction were used to quantify muscle gene expression. Twenty-four patients were studied. FNDC5 (5.7±3.5 vs. 3.1±1.2, p<.05) and PGC-1α (9.9±5.9 vs. 4.5±1.9, p<.01) gene expression was greater in the high peak VO2 group; correlation between FNDC5 and PGC-1α was significant (r=0.56, p<.05) only in the high peak VO2 group. Similarly, FNDC5 and PGC-1α gene expression was greater in the high performance group based on lower VE/VCO2 slopes (5.8±3.6 vs. 3.3±1.4, p<.05 and 9.7±6 vs. 5.3±3.4, p<.05); FNDC5 also correlated with PGC-1α (r=0.55, p<.05) only in the low VE/VCO2 slope group.

Conclusions—This is the first study to show that FNDC5 expression relates to functional capacity in a human HF population. Lower FNDC5 expression may underlie reduced aerobic performance in HF patients.

Key Words: heart failure, genes, physiology, biopsy
Exercise intolerance is a common complaint among heart failure (HF) patients. While cardiac dysfunction contributes to HF symptoms, other mechanisms, including disease-related changes in skeletal muscle are also germane.\textsuperscript{1-3} Skeletal muscle abnormalities including altered metabolism,\textsuperscript{4} altered neurohormonal signaling,\textsuperscript{5} fiber-type shift towards increased anaerobic type II fibers,\textsuperscript{6} and endothelial dysfunction,\textsuperscript{7} are aspects of HF pathophysiology that have been linked to reduced peak oxygen consumption (VO\textsubscript{2})\textsuperscript{8,9} and HF symptoms.\textsuperscript{10,11} Consistently, moderate intensity aerobic exercise training, an intervention which does not substantially improve left ventricular ejection fraction (LVEF), significantly improves the aerobic characteristics of skeletal muscle, along with improvements in peak VO\textsubscript{2} and clinical course.\textsuperscript{12,13,14} Therefore, better understanding of the biological determinants of skeletal muscle dysfunction in HF is of considerable importance as part of broader therapeutic goals to improve functional capacity and the related implications in respect to morbidity, mortality, and quality of life.

Physical activity promotes a more oxidative phenotype in skeletal muscle characterized by increased expression of the peroxisome proliferator-activated receptor $\gamma$ (PPAR-$\gamma$) co-activator-1$\alpha$ (PGC-1$\alpha$). This transcriptional co-activator promotes mitochondrial biogenesis in skeletal muscle that determines physiological and phenotypic changes.\textsuperscript{15} Irisin, a proteolytic hormone derivative (myokine) of the fibronectin type III domain containing 5 (FNDC5) gene, has been demonstrated in a mouse model to be induced by exercise (mediated by PGC-1$\alpha$) and to then directly stimulate phenotypic changes in adipose tissue that mediate changes in systemic metabolism.\textsuperscript{16} As such, this newly identified hormone has the potential to become a therapeutic target that may improve functional performance that is often limited in HF patients.\textsuperscript{2,3}

While the initial description of FNDC5 expression and irisin production by Boström, Spiegelman and co-workers was made in mice,\textsuperscript{16} there is reason to believe that irisin also effects
human physiology since the amino acid sequence of the molecule is identical between the species. It is theorized that FNDC5 may have similar roles in humans as in mice, but it is still not known how FNDC5 expression is controlled in human populations, or how it varies in different physiological or pathophysiological conditions.

In this analysis, we assessed PGC1-α and FNDC5 gene expression in relation to peak VO$_2$ and the VE/VCO$_2$ slope$^{17}$, two well-established cardiopulmonary exercise test (CPX) measures of aerobic exercise performance. This is a potentially important area of research particularly because of the notorious decrements of functional capacity seen among HF patients in association with skeletal muscle abnormalities. Reduced irisin may be a significant component of HF-related skeletal muscle changes that influence functional decline.

**Methods**

Clinically stable male systolic (left ventricular ejection fraction [LVEF] $\leq 40\%$) HF patients aged 50 years and older were enrolled. All were on a standard regimen of evidence-based HF therapy, including a beta-blocker and angiotensin converting enzyme (ACE)-inhibitor or angiotensin receptor blocker (ARB) and were euvoletic at the time of assessment. Additionally, each patient had an echocardiogram within 6 months of enrollment to confirm their LVEFs were $\leq 40\%$ at the time of the study assessments. Mitral valve regurgitation was assessed based on trace, mild, moderate, or severe regurgitation (categorized as 0, 1, 2, or, 3, respectively) and is reported as an average. Patients were excluded if they had severe valvular disease, neurological disorders, pulmonary disease, and/or musculoskeletal problems, which could have confounded functional assessments. This study was approved by the local VA Institutional Review Board and all subjects signed an informed consent prior to participation.
Aerobic Exercise Testing

All patients performed a symptom-limited CPX test on a motorized treadmill (Bari-mill Woodway, Waukesha, WI) using a modified Balke protocol. The ventilatory expired gas analysis system (MedGraphics BreezeSuite St. Paul, MN) was calibrated according to manufacturer’s specifications prior to each test. Continuous gas exchange, telemetry, blood pressure, rating of perceived exertion, and oxygen saturation were assessed for each patient one minute prior to, during, and four minutes following the exercise test in standard clinical fashion.

Both peak VO2 and the VE/VCO2 slope were used to assess aerobic performance. Just as peak VO2 manifests both central (cardiac and pulmonary) and peripheral (skeletal muscle and peripheral vascular) physiologic processes, the VE/VCO2 slope reflects a broad array of physiologic systems, including skeletal muscle chemo- and ergoreflexes and cardiopulmonary coupling.

Aerobic indices including peak VO2 (quantified as the highest 30-second averaged value during the last stage of exercise) and the VE/VCO2 slope were evaluated. VE and VCO2 values, acquired from the initiation of exercise to peak exercise (as 30-second averages), were entered into a spreadsheet software (Microsoft Excel, Microsoft Corp., Bellevue, WA) to calculate the VE/VCO2 slope via least squares linear regression \( y = mx + b \), \( m=\text{slope} \). All subjects achieved a respiratory exchange ratio (RER) of >1.0 at peak exercise, an indication of good exercise effort.

Skeletal Muscle Biopsy

Within one week following the CPX test, a skeletal muscle biopsy of the vastus lateralis muscle in the left leg was performed using a 5 mm Bergstrom muscle biopsy trochar. Biopsy
samples were stored in RNALater (Qiagen, Valencia, CA) at -80°C until gene expression analyses were conducted.

**Gene Expression Analyses**

Total skeletal muscle mRNA was extracted from muscle biopsies using Trizol reagent according to the manufacturer’s instructions. Total RNA was converted to cDNA by reverse transcription using SuperScript reverse transcriptase (Stratagene, Carlsbad, CA) and oligo dT primers in standard reactions according to the manufacturer’s recommendations.

Gene expression levels were determined from diluted cDNA samples (1:100) by real-time polymerase chain reaction (RT-PCR) using the StepOnePlus analyzer (Applied Biosystems, Foster City, CA). Multiplexed amplification reactions were performed using glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) as an endogenous control (GAPDH primers/VIC-labeled probe Applied Biosystems #4310884E) using the TaqMan Universal PCR Master Mix reagents Kit (#4304437, Applied Biosystems). The following amplification settings were used: Stage 1 (denaturation): 95°C for 10 min and Stage 2 (PCR): 95°C for 15 sec and 60°C for 60 sec for 40 cycles. The threshold cycle (Ct) values for each reaction were transferred to a Microsoft Excel spreadsheet and calculation of relative gene expression (to GAPDH) was performed from this data according to published algorithms (TaqMan Cytokine Gene Expression Plate 1 protocol, Applied Biosystems). For each gene studied, all biopsy samples were analyzed on a single 96-well plate. Expression of the following genes were analyzed using FAM-labeled probe/primer sets purchased from Applied Biosystems: PGC-1α [Hs01016724_m1] and FNDC5 [Hs00401006_m1].
Statistical Analysis

Statistical Analysis System (SAS) software version 9.0 (SAS, Cary, NC) was used to analyze the data; values are reported as mean ± the standard deviation (SD) unless otherwise indicated. The overall group was dichotomized into high vs. low aerobic capacity according to clinically established thresholds for peak VO₂ (>14 mlO₂·kg⁻¹·min⁻¹ vs. ≤14 mlO₂·kg⁻¹·min⁻¹)\(^{19}\) and the VE/VCO₂ slope (<34 vs. ≥34).\(^{26}\) Comparisons of high (>14 mlO₂·kg⁻¹·min⁻¹ or VE/VCO₂ slope <34) vs. low (≤14 mlO₂·kg⁻¹·min⁻¹ or VE/VCO₂ slope ≥34) performance among HF patients was determined using non-paired t-tests, while Pearson correlations were used to evaluate the correlation coefficients in the overall group as well as subgroups according to CPX performance. A p-value of <0.05 was used to define statistical significance for all tests.

Results

Twenty-four male systolic HF patients (mean age 67.2±9.2 years, age range 50 to 86) were evaluated. Mean LVEF was 29.5±7.7%, range 15 to 40%.

Table 1 lists the patient characteristics for the entire cohort in the high vs. low aerobic performance groups. Performance stratifications were based on peak VO₂ (> 14 vs. ≤14 mlO₂·kg⁻¹·min⁻¹ [n = 15 and 9, respectively] and VE/VCO₂ <34 vs. ≥34 [n= 14 and 10, respectively]).\(^{19,26}\)

Table 2 lists echocardiography and CPX aerobic indices. In general, patients had depressed systolic function by echocardiography but no differences between groups with respect to morphology or left ventricular function. While pulmonary pressure (pulmonary artery systolic pressure [PASP]) was similar between the high vs. low peak VO₂ groups, PASP was significantly elevated in the low aerobic performance group assessed as VE/VCO₂ slope ≥34.
When defining aerobic performance based on peak VO₂, the high performance group was characterized by a peak VO₂ of 17.0±3.0 mlO₂•kg⁻¹•min⁻¹ vs. 11.4±1.6 mlO₂•kg⁻¹•min⁻¹ in the low performance cohort, p<0.0001 (Table 2). When dichotomized into high and low performance according to the VE/VCO₂ slope, the high functioning cohort demonstrated a significantly better ventilatory efficiency (29±2.8 vs. 42±9.8, p<0.01).

Table 3 shows gene expression in relation to aerobic performance, and demonstrates significant differences between the two functional groups. Subjects with higher aerobic performance indices, defined by either peak VO₂ >14 mlO₂•kg⁻¹•min⁻¹, or by VE/VCO₂ slope <34, had significantly greater expression of both PGC-1α and FNDC5. Within each of these two classifications of high exercise performance, increased expression of FNDC5 correlated with greater expression of PGC-1α (peak VO₂ >14 mlO₂•kg⁻¹•min⁻¹, r= 0.56, p<0.05; VE/VCO₂ slope <34, r=0.55, p<0.05). Similar correlations between PGC-1α and FNDC5 were not present in the lower functional groups (peak VO₂ <14 mlO₂•kg⁻¹•min⁻¹, r= -.22, p=0.58; VE/VCO₂ slope ≥34, r=0.47, p=0.17). As demonstrated in Figure 1, FNDC5 expression correlated with PGC-1α expression in relation to the entire cohort (r=0.61 p=0.001). Correlations between PGC-1α and FNDC5 and peak VO₂ and the VE/VCO₂ slope are illustrated in Figure 2. While all correlations trended in the expected direction, only the relationship between PGC-1α and peak VO₂ reached statistical significance.

Discussion

This is the first study to show that expression of FNDC5, the genetic forerunner to irisin, may impact aerobic performance in HF patients and help to broaden our understanding of the biological determinants of exercise capacity and related cardiovascular health. Traditionally, the
symptoms and progression of HF have been attributed solely to cardiac dysfunction and remodeling. Our findings regarding FNDC5 are part of an expanding literature demonstrating that skeletal muscle and other peripheral tissues mediate HF symptoms and pathophysiology. While most HF patients lose muscle mass leading to frailty and functional decline, the underlying mechanisms remain unclear. We demonstrate that expression in skeletal muscle of PGC-1α and FNDC5 are higher and correlate to one another in a more functional HF cohort, in contrast to the lack of correlation in those who are functionally limited.

The recent identification of irisin, the product of the FNDC5 gene suggests the presence of a hormonal pathway between muscle and adipose tissue that may mediate some of the beneficial effects of exercise. Recent groundbreaking experiments in mice demonstrate that the transcriptional co-activator, PGC-1α, which promotes biogenesis of mitochondria, drives the expression of FNDC5, which in turn leads to increased brown adipose tissue (BAT), increased VO₂, insulin sensitivity and glucose tolerance. Despite the known presence of irisin in human plasma, FNDC5 expression in the skeletal muscle of human systolic HF patients has not been previously reported.

Furthermore, our data are the first to correlate increased PGC-1α and FNDC5 expression in subjects with higher aerobic performance characteristics, as defined by key CPX indices. These findings in human muscle are consistent with the data of Boström, Spiegelman and colleagues in mice linking muscle expression of PGC-1α with FNDC5 expression. It seems likely that higher contractile activity in more aerobically fit HF patients promotes PGC-1α and FNDC5 expression. A sensitive assay for identification of the irisin protein in human plasma is not yet available so we were unable to directly measure irisin levels in this patient cohort.
Peak VO₂ is considered the cornerstone of functional evaluation in HF patients and is assessed for quantification of disease severity and prognosis. However, ventilatory inefficiency (i.e., the VE/VCO₂ slope) is also associated with increased risk for hospitalization and mortality through somewhat overlapping but different physiological mechanisms. While there are conflicting data regarding the optimal parameter for assessing functional capacity in HF patients, we show that regardless of the CPX parameter used for functional stratification, reduced performance is still associated with reduced PGC-1α and FNDC5 expression. Consistently, in the lower functioning study population subgroups, PGC-1α expression did not correlate with FNDC5 expression suggesting that the lack of signaling through these pathways may in part contribute to skeletal muscle abnormalities and functional decline among HF patients whose disease severity is at an advanced stage.

Notably, only PGC-1α linearly correlated significantly with peak VO₂ (Figure 2), and the relationship was only modest. This finding indicates that while dichotomous classification of the cohort according to established peak VO₂ and VE/VCO₂ slope thresholds (as stratifications of disease severity) appear to identify differences in FNDC5 and PGC-1α expression, these same CPX variables do not appear to explain differences in FNDC5 and PGC-1α in a linear, continuous fashion. If confirmed by future investigations in larger cohorts, these findings may lead to recommendations regarding CPX data application in relation to skeletal muscle physiological assessments, i.e., specific CPX thresholds of high vs. low performance may be useful as key categories relative to which skeletal muscle physiology can be better understood and characterized.

Boström et al. also suggest that irisin administration may have therapeutic benefits on glucose tolerance and obesity that mimic and/or amplify effects of exercise. Irisin may have
particular benefits in a systolic HF population in which co-morbidities such as obesity and insulin resistance are often present. It is not yet known whether expression of FNDC5 is particularly low in these populations or if augmenting irisin levels in these patients will lead to clinical improvements. While exercise can improve functional status in HF patients, it is likewise not yet known how FNDC5 expression changes with exercise training in these patients. Studies are currently underway to address these issues.

Limitations

A clear limitation of this study is the small sample size, but since the data reinforce and extend seminal principles published in recent animal studies it still stands out as an important addition to our understanding of irisin biology. Among the implications of a small study population, the degree of correlation between FNDC5 and PGC-1α expression and each of the CPX measurements may have been affected. Thus, while we demonstrated differences of gene expression in relation to categorizations of high aerobic performance based on peak VO₂ and VE/VCO₂ slope, it is not clear whether this reflects a physiological distinction (such as disproportionate elevation of VE/VCO₂ slope despite similar peak VO₂ values in two HF patients based on differences in pulmonary pressures) or variance that is merely attributable to inadequate sampling. Additional research in larger cohorts is needed to further clarify these associations.

A related limitation is that a healthy control population was not included. While we considered comparing our findings with FNDC5 and PGC-1α expression to a cohort without HF, those without HF achieved much more favorable performance measures than HF patients (based on peak VO₂ and the VE/VCO₂ slope). Therefore in contrast to validated prognostic stratifications of peak VO₂ and the VE/VCO₂ slope for HF, it was unclear how to stratify the
much higher performance results of the non-HF cohort and to make meaningful comparisons to gene expression in HF patients.

More fundamentally, we were limited to assessments of FNDC5 expression as assays for irisin are not yet available, but conclusions regarding irisin based on FNDC5 are still physiologically robust. An additional limiting factor is that we have only assessed correlations between PGC-1α and FNDC5 gene expression in functionally stratified HF patients and therefore cannot establish causality.

Influence of gender-specific variables, including hormonal influences, on gene expression and muscle metabolism may also be relevant. While we limited enrollment to male subjects to minimize confounding gender effects, evaluation of FNDC5 expression in females is needed in future investigations. Also, while there were no significant differences among patient demographic characteristics in the high vs. low performance groups, this may be attributed to the small sample size than to true biological equivalence. N-terminal-pro B-type natriuretic peptide levels, for example, were substantially higher in the lower functioning groups (assessed by either peak VO2 or the VE/VCO2 slope). It is uncertain if this peptide is associated with or influences relative differences in PGC-1α and FNDC5 gene expression.

**Conclusion**

The present investigation demonstrates that PGC-1α and FNDC5 gene expression are increased in HF patients who have better aerobic performance, as indicated by the two primary CPX variables assessed in the HF population. Furthermore, PGC-1α correlates with FNDC5 only in the higher functioning groups.
Sources of Funding
This work was supported by the Veterans Affairs Merit Award [F4726R].

Disclosures
None.

References
Table 1. Key Demographic Characteristics

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NYHA Classification

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BMI, body mass index; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; ACE, angiotensin converting enzyme; ARB, angiotensin (II) receptor blocker; VO₂, oxygen consumption; VE/VCO₂, minute ventilation/carbon dioxide production; NS, not significant.
Table 2. Haemodynamic and Cardiopulmonary Indices

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**Echocardiography Parameters**

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**Cardiopulmonary Indices**

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<td>(mlO₂·kg⁻¹·min⁻¹)</td>
<td>14.9±3.3</td>
<td>17.0±2.0</td>
<td>11.4±1.6</td>
<td>&lt;.0001</td>
<td>16±3.1</td>
<td>13.3±3.1</td>
<td>&lt;.05</td>
</tr>
<tr>
<td><strong>VE/VCO₂ slope</strong></td>
<td>34.4±9.2</td>
<td>30.1±3.8</td>
<td>41.5±11.3</td>
<td>&lt;.05</td>
<td>29±2.8</td>
<td>42±9.8</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Peak RER</td>
<td>1.1±0.07</td>
<td>1.1±0.1</td>
<td>1.1±0.1</td>
<td>NS</td>
<td>1.1±0.1</td>
<td>1.1±0.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

LVEF, left ventricular ejection fraction; LVIDd, left ventricular internal dimension at diastole; FS, fractional shortening; PASP, pulmonary artery systolic pressure; MV, mitral valve; VO₂, oxygen consumption; VE/VCO₂, minute ventilation/carbon dioxide production; RER, Respiratory exchange ratio; NS, not significant.
Table 3. Gene Expression

<table>
<thead>
<tr>
<th></th>
<th>Entire Cohort</th>
<th></th>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=24)</td>
<td>(n=15)</td>
<td>(n=9)</td>
<td>(n=24)</td>
<td>(n=14)</td>
<td>(n=10)</td>
<td></td>
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<tr>
<td>Peak VO₂ (mlO₂kg⁻¹min⁻¹)</td>
<td>&gt;14</td>
<td>≤14</td>
<td></td>
<td>P value</td>
<td>&lt;34</td>
<td>≥34</td>
<td>P value</td>
</tr>
<tr>
<td>PGC1-α</td>
<td>7.9±5.4</td>
<td>9.9±5.9</td>
<td>4.5±1.9</td>
<td>&lt;.01</td>
<td>9.7±6</td>
<td>5.3±3.4</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>FNDC5</td>
<td>4.7±3.11</td>
<td>5.7±3.5</td>
<td>3.1±1.2</td>
<td>&lt;.05</td>
<td>5.8±3.6</td>
<td>3.3±1.4</td>
<td>&lt;.05</td>
</tr>
</tbody>
</table>

PGC1- α, PPAR-γ co-activator-1α; FNDC5, fibronectin type III domain containing 5; VO₂, oxygen consumption; VE/VCO₂ slope, minute ventilation/carbon dioxide production. PGC1- α and FNDC5 expression levels are given as mean ± SD relative to the expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) performed in the same sample of cDNA.
Figure Legends

**Figure 1.** Correlation between FNDC5 and PGC-1α in the total study population. PGC1-α, PPAR-γ co-activator-1α; FNDC5, fibronectin type III domain containing 5.

**Figure 2.** Scatter-Plots demonstrating PGC1-α and FNDC5 in relation to Peak VO₂ and the VE/VCO₂ Slope. PGC1-α, PPAR-γ co-activator-1α; FNDC5, fibronectin type III domain containing 5; VO₂, oxygen consumption; VE/VCO₂, minute ventilation/carbon dioxide production.
Expression of the Irisin Precursor FNDC5 in Skeletal Muscle Correlates with Aerobic Exercise Performance in Patients with Heart Failure
Stewart H. Lecker, Alexandra Zavin, Peirang Cao, Ross Arena, Kelly Allsup, Karla M. Daniels, Jacob Joseph, P. Christian Schulze and Daniel E. Forman

Circ Heart Fail. published online September 20, 2012;
Circulation: Heart Failure is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1941-3289. Online ISSN: 1941-3297

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