Ellefsen, S., Vikmoen, O., Slettaløkken, G., Whist, J. E., Nygård, H., Hollan, I. ... Rønnestad, B. (2014). Irisin and FNDC5: effects of 12-week strength training, and relations to muscle phenotype and body mass composition in untrained women. *European Journal of Applied Physiology, 114*, s. 1875-1888.

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Irisin and FNDC5 – effects of 12 wks strength training, and relations to muscle phenotype and body mass composition in untrained women

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Running title: Irisin and strength training in women

Abstract

Purpose

To investigate effects of strength training on abundances of irisin-related biomarkers in skeletal muscle and blood of untrained young women, and their associations with body mass composition, muscle phenotype and levels of thyroid hormones.

Methods

Eighteen untrained women performed 12 weeks of progressive whole body heavy strength training, with measurement of strength, body composition, expression of irisin-related genes (FNDC5 and PGC1α) in two different skeletal muscles, and levels of serum-irisin and -thyroid hormones, before and after the training intervention.

Results

The strength training intervention did not result in changes in serum-irisin or muscle FNDC5 expression, despite considerable effects on strength, lean body mass (LBM) and skeletal muscle phenotype. However, our data indicate that training affects irisin biology in a LBM-dependent manner. No association was found between steady-state serum-irisin or training-associated changes in serum-irisin *and* alterations in body composition. FNDC5 expression was higher in *m.*Biceps brachii than in *m.*Vastus lateralis, with individual expression levels being closely correlated, suggesting a systemic mode of transcriptional regulation. In pre biopsies, FNDC5 expression was correlated with proportions of aerobic muscle fibers, a relationship that disappeared in post biopsies. No association was found between serum-thyroid hormones and FNDC5 expression or serum-irisin.

Conclusion

No evidence was found for an effect of strength training on irisin biology in untrained women, though indications were found for a complex interrelationship between irisin, body mass composition and muscle phenotype. FNDC5 expression was closely associated with muscle fiber composition in untrained muscle.

Abbreviations

18S rRNA – 18S ribosomal RNA

$\beta_2 m$ – β_2-microglobulin

β-a – β-actin

BB – *musculus* Biceps brachii

DXA – Dual-energy X-ray absorptiometry

EIA – enzyme immunoassay

FM – fat mass

FNDC5 – fibronectin type III domain-containing protein 5

FT3 – free triiodothyronine

FT4 – free thyroxine

GAPDH – glyceraldehyde-3-phosphate dehydrogenase

LBM – lean body mass

MyHC – myosin heavy chain

PGC1α – peroxisome proliferator-activated receptor gamma coactivator 1-alpha

PolR2A – polymerase (RNA) II (DNA directed) polypeptide A

PPIA – peptidylprolyl isomerase A (cyclophilin A)

qRT-PCR – quantitative reverse transcriptase-polymerase chain reaction

RM – repetition maximum

RPL32 – ribosomal protein L32 (RPL32)

s-irisin – serum concentrations of irisin

TBP – TATA box binding protein

TSH – Thyroid-stimulating hormone

UBC – ubiquitin C

VL – *musculus* Vastus lateralis

Introduction

The training-associated myokine irisin, which was recently discovered in rodents, has been suggested to play a key role in the somewhat elusive effects of physical activity on health-related parameters in humans. In mice, it leads to browning of adipocytes and reversal of type 2 diabetes (Bostrom et al. 2012), making it a suggestive target for development of therapeutic agents to combat lifestyle-related disease (Enerback 2013). A surge of research activity has thus emerged, attempting to disclose its biological role in human subjects.

In the original paper by Boström et al. (2012), irisin was proposed to play a role in human health and biology, as suggested by expression of the irisin gene fibronectin type III domain-containing protein 5 (FNDC5) in skeletal muscle, abundance of irisin in blood and a two-fold induction of steady-state[1] FNDC5 expression following 10 weeks of endurance training. Despite of this, the true importance of irisin in humans remains disputed and controversial. As an example, Timmons et al (2012) failed to reproduce the training-induced increase in steady-state FNDC5 expression in a larger cohort of individuals, leading to debate concerning inaccuracies of current studies, related to both the choice of human subjects and to the choices of methodology for FNDC5 mRNA assessment (Timmons et al. 2012; Boström et al. 2012). This lack of effect of endurance training and/or strength training on FNDC5 expression (Pekkala et al. 2013; Raschke et al. 2013) or irisin serum levels (s-irisin) (Pekkala et al. 2013; Hecksteden et al. 2013) has been confirmed in recent studies, both on the level of steady-state abundances following prolonged training protocols (Pekkala et al. 2013; Raschke et al. 2013; Hecksteden et al. 2013) and on the level of acutely induced expression following single sessions of training (Pekkala et al. 2013). Raschke et al. (2013) even suggested that the human FNDC5 gene represents a pseudogene, in which a mutation in its Kozak consensus sequence prohibits it from being effectively translated into protein. This was

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[1] Throughout the manuscript, the term «steady-state» refers to gene expression/hormone abundance measured in biological material sampled at rest (in this study: >48h since last training session)

apparent despite near complete conservation of the remainder of the FNDC5 sequence (Raschke et al. 2013), itself suggesting preservation of the biological role.

In contrast to these negative findings, several studies also seem to suggest that irisin is a training-responsive myokine in humans (Huh et al. 2012; Kraemer et al. 2013; Norheim et al. 2014; Lee et al. 2014). These aberrances may of course be caused by *per*-chance statistical errors or technical flaws, perhaps related to dubious quantification of s-irisin (Raschke et al. 2013; Erickson 2013). However, they may also hint towards between-groups or even between-individuals differences in irisin biology, for which observations have been made (Timmons et al. 2012; Boström et al. 2012; Pekkala et al. 2013). Recently, Lee et al. (2014) revitalized the potential significance of irisin in human medicine by demonstrating its natural induction in response to hypothermia and exercise, in turn inducing browning gene expression programs in adipocytes.

In addition to these uncertainties, several aspects of irsin biology remain to be investigated. To our knowledge, no study has investigated the relationship between irsin biomarkers and variables such as differences in muscle phenotypes in human subjects. In fact, an association with traits such as muscle fiber composition seems likely, as its transcriptional regulator PGC-1α has been suggested to show muscle fiber-specific expression, being associated with slow fibers in rodents (Lin et al. 2002) and also in humans (Krämer et al. 2006), though the latter remains controversial (Plomgaard et al. 2006; Norrbom et al. 2004). An association between FNDC5 expression and aerobic fibers has indeed been indicated in rodents (Wrann et al. 2013; Roca-Rivada et al. 2013). Such an association may shed light on the previously observed individual variation in FNDC5/s-irisin abundances (Timmons et al. 2012; Boström et al. 2012; Pekkala et al. 2013; Huh et al. 2012). Moreover, FNDC5 expression may well vary between different groups of muscles, such as those of the upper and lower limbs, which are known to exhibit differences in biology, e.g. they respond differently to strength training (Rønnestad et al. 2007) and exhibit differences in cell biological parameters such as related to androgen signaling (Kadi et al. 2000).

FNDC5 is expressed in a broad range of human tissues (Huh et al. 2012) and its steady-state expression levels in muscle seems to correlate with its levels in adipose tissue (Moreno-Navarrete et al. 2013). Speculatively, this may mean that FNDC5 transcription is regulated in a systemic manner. In this context, the thyroid hormones thyroxine (T4) and triiodothyronine (T3) stand out as intriguing candidates. In addition to being functionally related to irisin, as important regulators of whole-body metabolism, T3 has been shown to lead to increased levels of PGC-1α protein in skeletal muscle tissue (Irrcher et al. 2003), providing a potential molecular link between T3 and FNDC5. Indeed, T_3 supplementation of rats has even been shown to lead to increased levels of FNDC5 expression in brain, constituting one of a mere 149 up-regulated genes (Diez et al. 2008). This connection was partly investigated by Stengel et al. (Stengel et al. 2013), who assessed the correlation between levels of irisin and thyrotropin/thyroid-stimulating hormone (TSH) in human blood, without finding an association. However, it can be argued that it would have been more appropriate to investigate the relationship between s-irisin and T3/T4 hormones, the ultimate messengers of the thyroid axis.

Our aim with the present study was to reveal effects of 12 weeks of progressive whole body heavy strength training on expression of FNDC5 and PGC1α in *musculus* Vastus lateralis (VL) and *musculus* Biceps brachii (BB), and on s-irisin of previously untrained young women. We also aimed to find possible connections between s-irisin and body mass composition, assuming a potential link between training-associated alteration in lean body mass or fat mass and s-irisin. The overall efficacy of the strength training program in altering muscle biology was controlled by measuring muscle strength, lean body mass and muscle fiber composition. Furthermore, we wanted to compare steady-state FNDC5 expression in VL and BB and investigate the potential link between FNDC5 expression and muscle fiber composition. Finally, we aimed to elucidate the potential association between muscular FNDC5 expression *or* s-irisin levels and serum thyroid hormone. To ensure appropriate analysis of mRNA expression, we performed a thorough analysis of the stability

of nine internal reference genes, with their expression patterns being assessed across trained and untrained muscle tissue and across VL and BB biopsies.

Methods

Ethics statement

The study was approved by the Local Ethics Committee at Lillehammer University College and was performed in accordance with the Helsinki Declaration. Written informed consent was obtained from all subjects prior to study participation.

Human material and strength training program

Muscle biopsies. Muscle biopsies were sampled from VL and BB of eighteen previously untrained women (age 26±6 years, see Table 1 for anthropometrics) immediately before and after 12 weeks of progressive strength training, using the Bergstrom procedure as previously described (Hanssen et al. 2012). Biopsies from *one* subject was excluded from analyses due to aberrant quality of RNA. Subjects were instructed to refrain from physical activity for the last 48h leading up to biopsy sampling. In connection with post biopsies, all subjects were set to perform a strength training session in the time window between 48h and 72h of biopsy sampling. These routines were implemented to ensure downstream assessment of standardized steady-state gene expression, i.e. expression in biopsies sampled at rest, resembling those reported in the premier two papers on the role of irsin in humans (Bostrom et al. 2012; Timmons et al. 2012), while at the same time avoiding inactivity-related declines in muscular properties. Adherence to the routines were ascertained through oral communication. Subjects were instructed to refrain from ingestion of anything other than water for the last 2h leading up to biopsy sampling. Pre- and post-biopsies were sampled at the same time of day for each particular subject. In VL, pre-biopsies were sampled from an area situated ~1/3 along the length of femur, as related to its distal end-point in the knee. In BB, biopsies were sampled from a central area. In both VL and BB, post biopsies were sampled ~2 cm proximal of pre biopsies. Biopsies for RNA analyses were immersed immediately in RNA*later*® (Ambion, Life technologies, Carlsbad, CA, USA) and treated according to manufacturer's protocol before storage at -80° C until

RNA extraction. Biopsies for immunohistochemistry were immersed immediately in 10 % buffered formaldehyde solution (Chemi-teknik AS, Oslo, Norway), wherein they were left to fixate for 3-4 days before further preparation. Blood samples for hormonal analyses were sampled before (n=17, i.e. blood samples from one subject was not retrieved) and after (n=12) the strength training period. As for biopsy sampling, subjects were instructed to refrain from physical activity (>48h) and ingestion of anything other than water (>2h) for the time period preceding blood sampling. While irisin analyses were performed on the twelve complete sets of blood samples (pre and post), thyroid hormone analyses were performed on the seventeen pre-samples. Blood samples were drawn from an antecubital vein into serum-separating tubes and were incubated at room temperature for 30 min before centrifugation at 1400g for 15min. Serum were stored at -80° C until analysis.

Insert Table 1 around here

Strength training protocol. In brief, the training protocol consisted of three weekly bouts of bi-lateral exercise, each consisting of three lower body exercises (leg press, leg extension and leg curl) and five upper body exercises (seated chest press, seated rowing, latissimus pull-down, biceps curl and shoulder press), performed at 7-10 repetition maximum (RM), in the order listed. For details, see Rønnestad et al. (2007). Notably, the current study was, in addition to being designed to investigate irisin-related hypotheses, designed to allow elucidation of differences in training adaptations between one and three sets of strength training. Accordingly, prior to upstart of training, subjects were randomly allocated into two groups, one of which was set to perform three sets of the lower body exercises *and* one set of the upper body exercises (3L1U, n=8) throughout the training intervention and one of which was set to perform one set of the lower body exercises *and* three sets of the upper body exercises (1L3U, n=10). For the sake of the current study, we chose to pool 3L1U and 1L3U data, as evaluated from the following information: i) the present protocol is known to ensure similar overall training volumes (Rønnestad et al. 2007); ii) none of the biological adaptations, or lack-thereof, revealed in the current study differed between 3L1U and 1L3U individuals. For example, the decreased MyHC2X mRNA expression observed in both VL and BB after 12 weeks of

strength training (Fig. 1), showed similar magnitudes in 3L1U and 1L3U, amounting to -62±46 % and -67±31 % in VL, and -67±30 % and -72±37 % in BB, respectively, with no statistical differences being found between groups (Fig s3, supplementary); iii) blood samples were only retrieved from a limited number of subjects post training intervention (n=6 in each group), in effect leaving separate 3L1U and 1L3U data sets with low statistical power.

Prior to upstart of the 12 weeks training program, each subject was given a supervised familiarization session to ensure proper lifting technique and testing procedures. Throughout the intervention, each training session was preceded by a 5 minutes general warm-up (cycling or running), followed by two lower body warm-up sets prior to lower body exercises and two upper body warm-up sets prior to upper body exercises, as described in Rønnestad et al. (2007). During each warm-up session, each subject ingested a 50g Squeezy Recovery bar (Squeezy AS, Loddefjord, Norway), containing 15g milk protein, ensuring a muscle growth-stimulating nutritional status (Tipton et al. 2001). Training intensity, measured as numbers of RMs, was increased similarly in 3U1L and 1L3U, as described in Rønnestad et al. (2007): weeks 1-2, 10RM; weeks 3-5, 8RM; weeks 6-12, 7RM. Subjects were encouraged to continuously increase their RM loads throughout the training program and were allowed assistance on the last repetition of each training set. During the first two weeks of training, all training sessions were conducted under supervision from authorized personnel. Thereafter, a minimum of one training session a week was conducted under supervision. To further ensure adequate training efforts, subjects were divided into training groups of three, performing their training together, in effect also improving the safety aspect of the training. Adequate training progression was ensured through continuous monitoring of training diaries and close dialog with subjects. To achieve modified daily undulating periodization, subjects were instructed to reduce training loads by ~10 % in the second training session of each week (Rønnestad et al. 2007).

1RM testing. Effects of the training period on muscle strength were measured as changes in 1RM performance in leg extension and seated chest press. For the sake of the current study, these two

outcome measures were combined into a single measure of overall lower and upper body performance. Prior to the 1RM testing session, subjects were given a familiarization session to ensure proper lifting technique and testing procedures. During this session, the load was gradually increased to allow estimation of a proper starting point for the test session. The 1RM test session was separate from the familiarization session or the last training session (post-testing) by approximately 72 hours. Prior to performing the actual 1RM tests, subjects were given a 10 minute low-intensity warm-up on an ergometer cycle. Subjects were instructed to refrain from food intake for the 2h leading up to the test session, but were allowed to ingest water. In addition, each of the two test procedures were preceded by specific warm-up, consisting of 3 sets with gradually increasing load (40, 75 and 85% of expected 1RM) and decreasing number of repetitions (10→6→3). In both 1RM tests, the first attempt was performed with a load approximately 5% below the expected 1RM. If the lift was successful, the load was increased by approximately 5% or by an amount subjectively decided by the investigator. The test was terminated when the subjects failed to lift the load in 2-3 attempts. Subjects were given three minutes rest between test efforts. The test order was the same pre and post and 1RM testing was overseen by the same investigator for any given subject. Pre- and post-tests were conducted using the same equipment, with identical subject/equipment positioning, and if possible at the same time of day.

Body composition. Effects of training on body composition measured as amounts of lean body mass (LBM, kg) or fat mass (FM, kg) was assessed using Dual-energy X-ray absorptiometry (DXA), using a Prodigy Advance (Lunar, San Francisco, CA, USA), according to manufacturer's protocol. Subjects were instructed to refrain from physical activity for the 48h leading up to DXA scanning and were instructed to refrain from ingesting any food or drinks for the last 2h.

Gene expression

Gene-specific primers for reference genes and target genes were designed as previously described (Ellefsen et al. 2008), using Primer3 Plus (Untergasser et al. 2007). For each gene, a three or four primer pairs were designed, with exception of the MyHC genes, for which a minimum of five

primer pairs were designed. To avoid genomic contamination from affecting gene expression analyses, every primer pair was either located to span an exon-exon boundary containing a genomic intron >~1000 nucleotides *or* to include at least one primer positioned directly across an exon-exon boundary. Primers were ordered from Thermo Scientific (Waltham, MA, USA) (HPLC-purified; in our hands this level of purification provides primers with much more consistent performance than desalted primers). All primer pairs were tested using the below described qRT-PCR protocol, employing a primer concentration of 100 nM and an annealing temperature of 60° C. The primer pair showing the lowest Ct value and at the same time showing distinct melting curves were chosen. Primers sequences are given in the supplementary Table s1.

Total RNA was extracted from muscle biopsies using TRIzol reagent (Invitrogen), as previously described (Ellefsen et al. 2008). Care was taken to remove all remnants of RNA*later* from biopsies. Two-hundred-and-fifty pg external references gene (mw2060) was added *per* mg muscle tissue prior to homogenization. This was done to allow validation of internal reference gene expression on a *per*-mg-tissue basis, a method developed by Ellefsen et al. (2008) and utilized in numerous studies (e.g. Ellefsen et al. 2012). RNA quantities were obtained using Nanodrop (Thermo Scientific, Waltham, MA, USA), whereupon reverse transcription was performed on 500 µg total RNA using Superscript III Reverse Transcriptase (Invitrogen), primed with both random hexamers (Ambion, Life technologies, Carlsbad, CA, USA) and oligo d(T) (Ambion, Life technologies, Carlsbad, CA, USA), according to manufacturer's protocol. For each sample, duplicate cDNA syntheses were performed.

Quantitative real-time PCR (qRT-PCR) was performed on 1/30 dilutions of cDNA using either PerfeCTa® SYBR® Green FastMix® (Quanta Biosciences Inc., Gaithersburg, MD, USA) or SYBR® Select Master Mix (Invitrogen, Life technologies, Carlsbad, CA, USA) and the 7500 Fast Real-Time PCR System (Applied Biosystems, Life technologies, Carlsbad, CA, USA). For PerfeCTa®-based reactions, cycling consisted of an initial denaturation step at 94°C for 30 sec,

followed by 39 repeats of 94°C for 3 s and 60°C for 30 s. For SYBR® Select Master Mix-based reaction, cycling consisted of initial UDG-activation at 50°C for 2 min, followed by denaturation at 95°C for 2 min and 39 repeats of 94°C for 3 s and 60°C for 30 s. For each cDNA synthesis one qRT-PCR reaction was performed for each gene, meaning that two qRT-PCR reactions were performed *per* muscle biopsy *per* gene. For each qRT-PCR reaction, cycle threshold (Ct) was calculated using the 7500 Fast Real-Time PCR System software in an automated manner and priming efficiencies (E) were calculated using the LinRegPCR software (Ramakers et al. 2003; Ruijter et al. 2009). For final calculations of target gene expression average priming efficiencies were utilized, calculated separately for each primer pair. Calculations of target gene expression was performed as described by Ellefsen et al. (2008) and Ellefsen and Stensløkken (2010), depending on whether PPIA-normalization or Gene-family profiling were employed. Average E and Ct values are given for each primer pair in the supplementary Table s1.

In order to identify the most suitable internal reference gene, hence achieving accurate normalization of target gene expression, GeNorm (Vandesompele et al. 2002) was utilized to evaluate the geometric expression stability of nine frequently utilized reference genes: β_2-microglobulin (β_2m), peptidylprolyl isomerase A (PPIA, cyclophilin A), TATA box binding protein (TBP), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), β-actin (β-a), ubiquitin C (UBC), polymerase (RNA) II (DNA directed) polypeptide A (PolR2A), ribosomal protein L32 (RPL32) and 18S ribosomal RNA (18S rRNA). β_2m and PPIA was evaluated to be the two most stable references genes (Fig. s1, supplementary), with an M-value well below the limit set by Vandesompele et al. (Vandesompele et al. 2002), whereupon PPIA was chosen for downstream target gene analyses, performed as previously described (Ellefsen et al. 2008). PPIA's expression stability was confirmed on a *per*-mg-tissue basis across all four experimental groups (untrained *and* trained VL and untrained *and* trained BB), using the external reference gene mw2060 for normalization (Fig. s2, supplementary) (Ellefsen et al. 2008). Furthermore, to provide further insight into how strength

training affected the expression of excluded reference genes, PPIA-normalized expression of GAPDH and TBP are presented in Fig. 1. While GAPDH expression did not display any training-associated alterations, TBP displayed decreased expression in VL (Fig. 1) ($p<0.05$). Notably, these two reference genes were the first and the last, respectively, to be evaluated as unstable and thus excluded during GeNorm analyses (Fig. s1, supplementary). In practice this means that they were evaluated as the least stable and the third most stable reference gene, respectively.

MyHC mRNA composition was calculated using the Gene-family profiling approach developed by Ellefsen and Stensløkken (2010). This approach provides a normalization-free alternative to reference gene-based normalization by treating individual genes as constituting fractions of collective Gene-family expression, thereby increasing the physiological relevance of mRNA data. The approach seems particularly well-suited for MyHC mRNA-based fiber-typing in skeletal muscle tissue (Ellefsen and Stensløkken 2010), for which it was recently validated by our group (Ellefsen et al. 2014). Notably, this validation study was performed on the very same biopsy material that were analysed in the current study. Hence, the MyHC data utilized in Figs. 1, 4 and 5 have been presented elsewhere, though in a different context and, except for those in Fig. 1, in a different format. Here, they are included to shed light on FNDC5 and PGC1α expression patterns.

Immunohistochemistry

Formalin-fixed muscle biopsies were paraffin-embedded and sectioned, whereupon transverse, serial sections were labelled for MyHC1 (*A4.840*), MyHC2A (*EPR5280*) and MyHC2X (*6H1*), as previously described (Ellefsen et al. 2014). For images showing representative labeling, see Fig. 2. Determination of muscle fiber composition was based on analysis of a minimum of 200 fibers, as recommended by Blomstrand & Ekblom (1982), performed using Photoshop CS5 Extended (Adobe, San Jose, CA, USA). Unfortunately, of the eighteen subject for which we had complete sets of biopsies for RNA-analysis from VL and BB (pre and post), we only had sixteen complete sets of biopsies for immunohistochemical analysis. Of these, we were only able to retrieve complete sets of MyHC-labeled serial tissue sections (1+2A+2X) for eleven and twelve subjects in VL and

BB, respectively, as reported in Ellefsen et al. (Ellefsen et al. 2014). This was due to an issue with the performance of the 6H1-antibody, possibly linked to the combined fragility of the antibody and high levels of intramuscular fatty acids, distorting tissue preparation.

Insert Fig. 2 around here

Hormone assessment

S-irisin was measured using an enzyme immunoassay-kit (EIA, catalog #EK 067-29, Phoenix Pharmacauticals Inc., Burlingame, CA, USA), according to protocol supplied by manufacturer. All measurements were performed in duplicates and within one plate. S-irisin was determined by extrapolation to a standard curve using the curve-fitting procedure in GraphPad$^{®}$ Prism6 (Graphpad Software, San Diego, CA, USA).

Serum concentrations of free triiodothyronine (FT3), free thyroxine (FT4) and thyrotropin (s-TSH) were measured on a Cobas e 601 analyzer (Roche Diagnostics GmbH, Mannheim, Germany), using analytical kits from Elecsys (Roche Diagnostics GmbH, Mannheim, Germany). Reference intervals were: s-TSH 0.27-4.2 mIU/L, FT3 3.1-6.8 pmol/L, and FT4 10.0-22.0 pmol/L. Coefficients of variation (%) for analyses were as follows: 1.0 (s-TSH), 2.8 (FT3), and 1.6 (FT4).

Statistics

Prior to statistical testing, gene expression data and hormone abundance data were log2-transformed, performed on factorized raw data (100x). This was done to maximize the likelihood of normal distribution. However, for data representing values ranging from zero to one, such as muscle fiber composition and gene expression measured as VL-BB$_{combined}$ (explained below), square-root arcsine-transformation was performed, representing the recommended mode of transformation for proportional data between -1 and 1 (Dytham 2011).

For correlation analyses performed between serum concentrations of systemic hormones and muscular expression of FNDC5 expression, we developed a systemic indicator of FNDC5 expression, rather than utilizing distinct BB and VL data, following the principle underlying the

performance factor developed by Vegge et al. (2012). First, individual FNDC5 expression data from VL or BB were ranked by dividing them with the individual showing the highest FNDC5 expression in each of the data sets, which thus was set to 1.0. Then, intra-individual VL and BB values were added together and divided by two, providing a combined measure of VL and BB expression, which we termed VL-BB$_{combined}$. This approach resulted in ranking of systemic FNDC expression between individuals in a manner that was not skewed by differences in expression between VL and BB. Prior to statistical testing, VL-BB$_{combined}$ FNDC5 expression data were arcsine-transformed, as previously described.

Effects of training on gene expression in VL and BB and serum s-irisin was tested on log2-transformed data using two-tailed paired samples t-tests. For paired data that did not pass the Shapiro-Wilk test for Normality, a Wilcoxon Signed Rank Test was performed. Comparison of baseline gene expression between VL and BB was performed on log2-transformed data using unpaired Student's t-tests. For unpaired data that did not pass the Shapiro-Wilk test for Normality, a Mann-Whitney Rank Sum Test was performed. Data are presented as log2- or arcsine-transformed in figures or tables, appearing as mean±s.d. or box plots, unless otherwise stated. Data are presented in their original formats in the text, appearing as mean±s.d.. $p < 0.05$ was considered significant; $p < 0.10$ was considered a tendency.

Correlation analyses were performed on log2-transformed, arcsine-transformed or non-transformed raw data using Pearson correlation. For statistically significant correlations ($p < 0.05$), correlation coefficients (r) were interpreted according to Hopkins et al. (2009): $r < 0.1$ = trivial, 0.1-0.3 = small, 0.3-0.5 = moderate, 0.5-0.7 = large, 0.7-0.9 = very large, 0.9 = nearly perfect, and 1.0 = perfect. Correlation coefficients are presented with 95 % confidence intervals (CI) in figures or in the text. Data are presented as individual values. Statistical calculations were performed using Excel (Microsoft, Redmond, WA, USA) or Sigmaplot 12.5 (Systat Software Inc, San Jose, CA, USA).

Results

FNDC5, irisin and responses to strength training. Twelve weeks of progressive whole body heavy strength training did not lead to alterations in steady-state FNDC5 expression (Figs. 1 and s3) or steady-state s-irisin (Fig. 3) in the 3L1U group, the 1L3U group or when the groups were pooled. The lack of training-associated alterations was apparent despite large alterations in muscle phenotype, as evident from a 38±14 % increase in overall upper and lower body muscle strength (P<0.001), a 2.7±4.3 % increase in whole-body LBM (equivalent to 1.1±4.3 kg) (p<0.05) (Table 1), and -65±37% and -70±33% decreases in MyHC2X mRNA in VL and BB, respectively (P<0.001) (Fig. 1). None of these adaptations to strength training differed between the two training protocols. The efficacy of the training program with respect to muscle fiber conversion was comparable to those seen in other studies (Staron et al. 1994; Adams et al. 1993), evident as stable MyHC1 abundances and increased MyHC2A abundances, as discussed in Ellefsen et al. (2014). No effect of training was observed on whole-body FM (Table 1), with individual responses varying from -14 % to +15 %. In VL, steady-state expression of the classic PGC1α-s1 variant tended to decrease by -8±86 % in post-intervention biopsies (p=0.06), while the hypertrophy-associated PGC1α-s4 variant (Ruas et al. 2012) decreased by -22±49 % (p<0.05) (Fig. 1). In BB, no effect was seen of training on expression of PGC1α (Fig. 1).

Insert Figs. 1 and 3 and Table 1 around here

Although no general effect was found of training on s-irisin, effects seemed to be present at the individual level, with responses varying from -36 % to +104 %. These responses correlated negatively with LBM, as measured both pre and post strength training intervention (r = -0.60, CI: -0.87, -0.07; r = -0.69, CI: -0.90, -0.22) (Table 2), posing a role for amounts of muscle mass in determination of training-associated s-irisin responses. Indeed, the 50% of subjects (n = 6) showing lowest LBM, displayed 23±40 % (p<0.05) increases in s-irisin, while the other half of subjects, showing highest LBM (n = 6), displayed -16±10 % (p<0.05) decreases. Notably, these individual

changes in s-irisin were *largely* correlated with changes in muscular FNDC5 expression (r = 0.652, CI: 0.12, 0.89) (Table 3). No association was found between changes in s-irisin and amounts of FM or training-associated changes in amounts of FM.

Steady-state s-irisin was found to correlate with body mass. This was evident as tendencies towards positive correlations between s-irisin and pre LBM and pre FM (r = 0.53, CI: -0.03, 0.84, and r = 0.52, CI: -0.04, 0.83), and a *large* positive correlation with post FM (r = 0.62, CI: 0.10, 0.87) (Table 2).

Insert Tables 2 and 3 around here

FNDC5 expression and its association with muscle phenotype. Steady-state FNDC5 expression was found to be 44±50 % higher in BB than in VL (Fig. 4A; p<0.01), as measured in untrained baseline biopsies. This difference was found despite the evident stability of PPIA expression, as evaluated both on a per-mg-tissue basis and using geNorm (Figs. s1 and s2, supplementary). Also, no differences were found in expression of TBP, GAPDH and MyHC1 and 2X between BB and VL (Fig. 4A), while MyHC2A displayed lowered expression in BB (-32±28 %, p<0.01) (Fig. 4A). Notably, FNDC5 expression levels in BB showed *very large* correlation with FNDC5 expression levels in VL (r=0.75) (Fig. 4B).

Insert Fig. 4 around here

In baseline VL samples, FNDC5 expression showed *very large* correlations with MyHC1 muscle fiber abundances, using both immunohistochemistry- (r=0.78) and GeneFam-determined fiber typing (r=0.75) (Fig. 5A). Conversely, FNDC5 showed a *very large* negative correlation with MyHC2X phenotype (r=-0.78 and r=-0.85, respectively) (Fig. 5A). In VL, no association was found between FNDC5 expression and MyHC2A phenotype (Fig. 5A). In baseline BB samples, FNDC5 expression showed a *large* correlation with MyHC1 muscle fiber composition, determined using GeneFam (r=0.62, p<0.05) (Fig. 5B), and *large* and *very large* correlations with MYHC2A abundances, determined using immunohistochemistry (r=0.62, p<0.05) and Genefam (r=0.70,

p<0.01) (Fig. 5B). Conversely, a *very large* negative correlation was found between FNDC5 expression and MyHC2X composition, determined using GeneFam (r=-0.70, p<0.05) (Fig. 5B), with a tendency towards a negative correlation using immunohistochemistry (r=-0.55, p=0.07). In both VL and BB, the relationship between FNDC5 expression and muscle fiber composition disappeared in post-training biopsies.

Insert Fig. 5 around here

FNDC5, irisin and their association with thyroid hormones. No correlation was found between concentrations of thyroid hormone in serum and muscular FNDC5 expression *or* s-irisin, as investigated using pre-intervention biopsies and blood samples (Table 4). Notably, a tendency towards a negative correlation was observed between FT4 and FNDC5 expression (Table 4).

Insert Table 4 around here

Discussion

In this study we show that 12 weeks of progressive heavy strength training has no effect on muscular expression of FNDC5 in lower or upper body muscle (VL or BB) or on s-irisin in previously untrained young women, despite evidence for extensive alterations in skeletal muscle phenotype, as suggested by increased muscle strength, increased LBM and decreased abundances of MyHC2X mRNA. The latter was recently shown to be accompanied by increased abundances of MyHC2A (Ellefsen et al. 2014). Furthermore, we found FNDC5 expression to be closely correlated with proportions of aerobic muscle fibers in untrained (pre) but not in trained (post) muscle, suggesting that strength training has an intricate effect on irisin biology. Such a lack of a general training-associated FNDC5/irisin response was accompanied by a lack of PGC1α expression, and is in agreement with recent studies showing that neither acute training sessions nor prolonged periods of endurance training and/or strength training has an effect on FNDC5 expression (Timmons et al. 2012; Pekkala et al. 2013; Raschke et al. 2013) or irisin secretion (Hecksteden et al. 2013; Pekkala et al. 2013) in men/mixed gender groups, in turn contrasting the premier data presented by Bostrom

et al. (2012). Importantly, as our data was sampled from subjects in a rested state, the current data does not make feasible analyses of effects of acute training sessions on FNDC5 expression or s-irisin.

Irisin and body mass composition in humans. Studying the role of irisin in human material may not be straight-forward. After all, it seems likely that s-irisin and its biological functionality is regulated through a diversity of signaling pathways, and not only those affected by training. This would make it hard to assess irisin biology using simple descriptive approaches. As such, our data suggests that untrained and trained subjects display different modes of regulation of s-irisin. In untrained subjects (pre-intervention data), a positive relationship was found between amounts of LBM and FM and s-irisin, suggesting that quantities of adipocytes and muscle cells may be an important determinant of irisin secretion, perhaps in a housekeeping-resembling manner. This does not seem unreasonable, as irisin has been shown to act as both a myokine and an adipokine (Roca-Rivada et al. 2013). Indeed, similar correlations have been previously found between s-irisin and measures of muscle mass (Stengel et al. 2013; Huh et al. 2012), FM (Stengel et al. 2013) and BMI (Huh et al. 2012; Stengel et al. 2013). In the trained state on the other hand, the correlation between LBM and s-irisin seemed to disappear, while the positive relationship with FM was retained. Arguably, strength training thus seems to affect regulation of irisin secretion in skeletal muscle fibers but not in adipocytes. This can be argued to have a logical rationale that is likely linked to the complex biological cues imposed on muscle cells by training. This may be further linked to the simultaneous disappearance of an association between FNDC5 expression and muscle fiber composition.

The seemingly complex regulation of irisin biology in humans is further underlined by a strong, negative correlation between training-associated changes in s-irisin and amounts of muscle mass, measured as LBM. In our data set, this was evident as decreased s-irisin with training in individuals with larger amounts of muscle mass and increased s-irisin in those with smaller amounts

of muscle mass. This unexpected relationship is not readily accounted for. However, it may be linked to a training-associated interaction between irisin and muscle fibers, somehow resulting in its removal from the blood stream. With respect to this, irisin has been shown to interact with muscle cells *in vitro* (Vaughan et al. 2014). Alternatively, it may be linked to a higher order of training-associated regulation, perhaps mediated through systemic factors. Irrespective of the underlying mechanism, the relationship suggests that s-irisin is conditionally controlled by training in humans, as was also suggested by Novelle et al. (2013), emphasizing that there is more to irisin biology than can be concluded from population-based studies. Importantly, the relationship between training-associated changes in s-irisin and LBM was insensitive to the type of strength training protocol, as it was equally prominent in combined 3L1U/1L3U data as it was in separate 3L1U and 1L3U data, despite very low statistical powers of these latter analyses (n=6 in each group, data not shown).

The marked individual variation in steady-state s-irisin or training-associated alterations in s-irisin showed no relationship with training-associated changes in amounts of FM. In this regard, it is necessary to note that we did not control for food-intake and hence no conclusive inferences can be made. However, such a tentative lack of an effect of irisin on fat mass is supported by studies such as Raschke et al. (2013) who did not find an effect of irisin on browning of cultured human adipocytes and Norheim et al. (2014) who did not find an association between s-irisin and markers of browning in subcutaneous adipocytes in human subjects. Still, a role for irisin in browning of adipocytes in human subjects cannot be ruled out. Different deposits of human adipocyte have been shown to respond differently to irisin exposure (Lee et al. 2014), which may indicate that the negative results presented by Raschke et al. (2013) and Norheim et al. (2014) was an artefact of adipocyte sampling.

Irisin, FNDC5 expression and muscle phenotype. At baseline, FNDC5 expression was found to be higher in BB than in VL. This difference was apparent despite similar composition of aerobic vs. glycolytic MyHC mRNA, as well as similar expression of all other genes investigated.

This suggests an inherent difference in the capacity to produce irisin between anatomically different muscles. Furthermore, the strong correlation found between FNDC5 expression in VL and BB, in essence implying that individuals with high levels of expression in VL also show high levels of expression in BB, can be taken as an indication for a systemic mode of regulation of FNDC5. This notion is strengthened by a previous observation of an association between FNDC5 expression levels in muscle and adipose tissue (Moreno-Navarrete et al. 2013).

Despite differences in FNDC5 expression between lower body VL and upper body BB at baseline, the two training protocols utilized in the current study, involving either three sets of lower body exercises and one set of upper body exercises *or* one set of lower body exercises and three sets of upper body exercises, were not be associated with differential effects on irisin biology. This was evident as a lack of effects on FNDC5 expression in both VL and BB *and* s-irisin. This may be due to a type II error, as even subtle changes in design of strength training protocols have previously been found to affect training-associated adaptations following upper body strength exercises (Rønnestad et al. 2011). However, this study was performed on men, and its findings may not be relevant to women. Still, it underlines the need for a more thorough assessment of relations between irisin and lower- and upper body strength training. Importantly, in the current study, 3L1U and 1L3U resulted in similar increases in overall gain in strength and LBM and in similar changes in muscle fiber composition in both VL and BB, suggesting that the two training protocols exerted comparable effects also on other aspects of global muscle functionality.

Overall, a positive association was found between FNDC5 expression and proportions of aerobic muscle fibers (MyHC1 and 2A) in untrained VL and BB in human subjects. This notion is supported in the literature by an elevated expression of FNDC5 in oxidative muscles in rodents (Wrann et al. 2013; Roca-Rivada et al. 2013). This may be linked to the fact that low-intensity every-day activities such as walking, which preferentially activate type 1 fibers, may lead to increased nuclear translocation of PGC1α, in turn leading to the observed fiber-specific FNDC5

expression. Indeed, slow-fibre patterned electrical activity has been shown to be capable of resulting in increased PGC1α protein abundance in both rodents and humans (Irrcher et al. 2003; Gundersen 2011; Adams et al. 2011). Alternatively, it may point to a housekeeping-resembling manner of regulation of irisin biology, wherein FNDC5 expression and irisin secretion is a passive consequence of muscle fiber composition. Either way, the correlation disappeared in post-intervention biopsies, pointing towards a complex training-associated manner of transcriptional regulation, which may be related to body mass composition, as previously discussed. Moreover, regulation of FNDC5 expression seems to encompass p38 mitogen-activated protein kinase (MAPK), which in turn is known to be controlled through a range of cellular stressors such as pro-inflammatory cytokines (Nebreda and Porras 2000). This essentially puts FNDC5 expression under control of a range of physiological cues, not only exercise.

FNDC5 expression and thyroid hormones. In light of both the metabolic role ascribed to irisin and the hypothesized systemic mode for regulating its secretion and expression, the thyroid system seemed a particularly interesting candidate. However, in this study, no association was found between FNDC5 expression in skeletal muscle or s-irisin and serum thyroid hormones (FT3, FT4 or s-TSH), despite a range of potential interfaces, including similar roles in regulation of basal energy expenditure (Bostrom et al. 2012; Kim 2008) and the fact that T3 supplementation leads to increased PGC1α protein abundance (Irrcher et al. 2003) and FNDC5 expression (Diez et al. 2008) in rats. This suggests that FNDC5 expression and irisin secretion is not determined by the availability of thyroid hormones. In this context, it should be kept in mind that blood levels of hormones are not necessarily decisive of their biological potency, which is also decided by factors such as hormone transport and metabolism, receptor affinity and quantity, as well as transcriptional and translational aspects (Tjørve et al. 2007). Also, muscular expression of FNDC5 tended to show a negative correlation with $[FT4]_{serum}$, emphasizing on the fact that further study is warranted.

Conclusion. The current study suggests that 12 weeks of whole body heavy strength training does not alter steady-state expression of FNDC5 in skeletal muscle or levels of s-irisin of previously untrained female subjects. No association was found between s-irisin and changes in body mass composition. This lack of an irisin response is in line with previous findings (Pekkala et al. 2013; Raschke et al. 2013; Hecksteden et al. 2013; Timmons et al. 2012) and was seen despite evidence for extensive training-associated alterations in muscle properties, as suggested from increased LBM and a shift towards an aerobic muscle phenotype. The latter could have been expected to be accompanied by increased overall FNDC5 expression, as proportions of MyHC1/2A fibers was found to be positively correlated with FNDC5 expression in untrained pre biopsies. This aberrancy may be linked to a complex relationship between body mass composition and regulation of s-irisin, as observed in our data. Although no general effect was seen of training on s-irisin, we found individual s-irisin responses to correlate with FNDC5 expression responses in skeletal muscle. Together with the complex relationship between s-irisin and body mass composition, this suggests that irisin is a conditionally inducible training-responsive myokine in humans. Such a complex pattern of inter-individual variation may leave studies aiming to investigate mean effects of training on irisin biology in groups of individuals with little or no value, at least in healthy controls. This being said, groups of subjects such as those suffering from lifestyle-related diseases like diabetes type II may display a more uniform group-wise response. No evidence was found for an association between skeletal muscle FNDC5 expression and serum thyroid hormone levels (FT3, FT4 and s-TSH).

Acknowledgements

Conflict of interest

The authors have no conflicts of interest to declare.

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Tables

Table 1. Effects of 12 weeks of progressive heavy strength training on anthropometric measures in 17 previously untrained young women.

ANTHROPOMETRICS	PRE	POST
Height (cm)	169±4.7	*N/A*
Weight (kg)	69.1±12	70.1±12*
BMI (kg/m^2)	24.1±3.8	24.5±3.5***
Whole-body lean body mass (LBM, kg)	41.4±4.3	42.5±4.2*
Whole-body fat mass (FM, kg)	24.7±9.7	24.4±9.7

Effects of training was tested using paired t-tests. * = $p<0.05$, ***=$p<0.001$. Values are mean±s.d.. N/A = not applicable.

Table 2. Relationship between steady-state serum concentrations of irisin (s-irisin) and lean body mass (LBM; kg) or fat mass (FM; kg), measured before (PRE) and after (POST) 12 weeks of progressive heavy strength training in 12 previously untrained young women.

		PRE				POST				Rel. change			
		LBM		FM		LBM		FM		LBM		FM	
		r	p	r	p	r	p	r	p	r	p	r	p
PRE	*s-irisin*	0.53	<0.10	0.52	<0.10	N/A				0.20	NS	0.01	NS
POST	*s-irisin*	N/A				0.35	NS	0,62	<0.05	0.13	NS	0.25	NS
Rel. change	*s-irisin*	-0.60	<0.05	-0.39	NS	-0.69	<0.05	-0.36	NS	-0.22	NS	0.20	NS

Rel. change = relative change in FNDC5 or irisin abundances between PRE and POST samples. Correlation analyses were performed using log2-transformed irisin data and non-transformed anthropometric data using Pearson correlation (r). NS = non-significant. *N/A* = not applicable.

Table 3. Relationship between steady-state FNDC5 expression in VL-BB$_{combined}$ and steady-state serum concentrations of irisin (s-irisin), measured before (PRE) and after (POST) 12 weeks of progressive heavy strength training in 11 previously untrained young women.

		PRE s-irisin		POST s-irisin		Rel. change s-irisin	
		r	p	r	p	r	p
PRE	*FNDC5 BB-VL$_{combined}$*	0.01	NS	*N/A*		-0.25	NS
POST	*FNDC5 BB-VL$_{combined}$*	*N/A*		-0.51	NS	0.46	NS
Rel. change	*FNDC5 BB-VL$_{combined}$*	-0.50	NS	-0.19	NS	0.65	<0.05

VL-BB$_{combined}$ expression was calculated by combining gene expression data from *musculus* Vastus lateralis (VL) and *musculus* Biceps brachii (BB) of each subject (see Methods). Rel. change = relative change in FNDC5 or irisin abundances between PRE and POST samples. Correlation analyses were performed on arcsine-transformed FNDC5 data and log2-transformed irisin data using Pearson correlation (r). NS = non-significant. *N/A* = not applicable.

Table 4. Relationships between steady-state serum concentrations of thyroid hormones and PPIA-normalized FNDC5 expression in VL-BB$_{combined}$ *or* steady-state serum concentrations of irisin (s-irisin) from 17 and 12 untrained young women (sampled before the training intervention), respectively.

		VL-BB$_{combined}$		Serum	
		FNDC5		*s-irisin*	
		r	p	r	p
Serum	*FT3*	-0.36	NS	0.17	NS
	FT4	-0,48	<0.10	-0.09	NS
	s-TSH	0.18	NS	-0.29	NS

VL-BB$_{combined}$ expression was calculated by combining gene expression data from *musculus* Vastus lateralis (VL) and *musculus* Biceps brachii (BB) of each subject (see Methods). Correlation analyses were performed using arcsine-transformed FNDC5 data and log2-transformed irisin/thyroid data using Pearson correlation (r). NS = non-significant. FT3 = serum concentrations of free triiodothyronine; FT4 = serum concentrations of free thyroxine; s-TSH = serum concentrations of thyrotropin

Figure 1. Effects of 12 weeks of progressive heavy strength training on steady-state expression of FNDC5, PGC1α splice variants 1 and 4 (s1 and s4), TBP, GAPDH and MyHC2X in A) *musculus* Vastus lateralis (VL) and B) *musculus* Biceps brachii (BB) of 17 previously untrained young women. Muscle biopsies were sampled before (pre) and after (post) the training period. Data were normalized to PPIA and are presented as gene expression in post-samples relative to pre-samples, measured as log2-fold change. Effects of training on gene expression were tested on log2-transformed data using paired t-tests. However, for PGC1α-s4, a Wilcoxon Signed Rank Test was utilized, as these data sets still did not pass the Shapiro-Wilk Normality Test. MyHC2X data were included to demonstrate the efficacy of the training intervention on muscle phenotype and were retrieved from Ellefsen et al. (2014), wherein data on effects of training on MyHC1 and MyHC2A are also available. *=$p<0.05$; **=$p<0.01$; ***=$p<0.001$. Data are presented as box plots, with the central line marking the data set median.

Figure 2. Representative labeling of myosin heavy chains in serial sections of human skeletal muscle using antibodies towards A) MyHC1 (*A4.840*), B) MyHC2A (*EPR5280*), and C) MyHC2X (*6H1*) (Ellefsen et al. 2014).

Figure 3. Effects of 12 weeks of progressive heavy strength training on steady-state serum concentrations of irisin (s-irisin) in 12 previously untrained young women. Blood samples were sampled in a rested state before (pre) and after (post) the training intervention period. The effect of training on s-irisin was tested using log2-transformed data and a paired t-test. No effect was revealed. Values are mean± s.d..

Figure 4. A) Comparison of steady-state expression of FNDC5, PGC1α splice variants 1 and 4 (s1 and s4), TBP, GAPDH and MyHC1, 2A and 2X between *musculus* Vastus lateralis (VL) and *musculus* Biceps brachii (BB) in 17 untrained young women. Muscle biopsies were sampled before the onset of the training intervention (pre). Data sets were normalized to PPIA, and were then

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referenced to VL values, which were set to 1.0, and log2-transformed. Data are presented as log2-difference in gene expression between VL and BB, with VL being set as the point of reference. Effects of muscle type on gene expression levels were tested using Student's t-tests. However, for GAPDH and MyHC1, 2A and 2X, a Mann-Whitney Rank Sum Test was utilized, as these data sets still did not pass the Shapiro-Wilk Normality Test. *=$p<0.05$; ***=$p<0.001$. Data are presented as box plots, with the central line marking the data set median. B) Relationship between steady-state FNDC5 expression in untrained pre VL and BB. The correlation analysis was performed using log2-transformed data and Pearson correlation. CI denotes the 95 % confidence interval of Pearson r. Values are individual. The solid line illustrates the trend line, while the dotted line indicates a perfect 1:1 slope.

Figure 5. Correlation between steady-state expression of FNDC5 and muscle fiber composition in pre-intervention A) *musculus* Vastus lateralis (VL) and B) *musculus* Biceps brachii (BB) of 10 and 12 untrained young women, respectively. Muscle fiber composition was measured using either immunohistochemistry (IMMUNO) or Gene-family profiling (GeneFam), as described by Ellefsen et al. (2014). Correlation analyses were performed using arcsine-transformed IMMUNO- and GeneFam-determined MyHC data and log2-transformed FNDC5 data using Pearson correlation. CI denotes the 95 % confidence interval of Pearson r. Values are individual. The solid line illustrates the trend line. A separate set of correlation analyses was performed on post intervention biopsies sampled after 12 weeks of progressive strength training (not shown in the figure). In these analyses, no correlation was found between FNDC5 expression and muscle fiber composition, either in VL or BB *or* using IMMUNO or GeneFam, with r values ranging from -0.38 to 0.38.

Figure legends - supplementary figures

Figure s1. Expression stability (M) of nine selected reference genes in A) *musculus* Vastus lateralis (VL) and B) *musculus* Biceps brachii (BB) following 12 wks of strength training in 17 previously untrained women. M was calculated using GeNorm, as described by Vandesompele et al (2002). In both VL and BB, B_2m and PPIA were found to be the most stably expressed reference genes, ranking them as most suitable for normalization of target gene qRT-PCR data.

Figure s2. *Per*-mg-tissue expression of PPIA in untrained (pre, black columns) and trained (post, white columns) *musculus* Vastus lateralis (VL) and *musculus* Biceps brachii (BB). Data were normalized to the external reference gene mw2060, an approach developed by Ellefsen et al. (2008). The effect of training on gene expression was tested using a paired t-test. No differences were found between groups. Values are mean± s.d..

Figure s3. Effects of 12 weeks of progressive heavy strength training on steady-state expression of FNDC5, PGC1α splice variants 1 and 4 (s1 and s4), TBP, GAPDH and MyHC2X in A, C) *musculus* Vastus lateralis (VL) and B, D) *musculus* Biceps brachii (BB) of 17 previously untrained young women. Strength training was performed as either 3L1U (three sets lower body - one set upper body; n = 7; in A-B) or 1L3U (one set lower body - three sets upper body; n = 10; in C-D). Muscle biopsies were sampled before (pre) and after (post) the training period. Data were normalized to PPIA and are presented as post-training expression relative to pre-training expression, measured as log2-fold change. Effects of training on gene expression were tested on log2-transformed data using paired t-tests. *=p<0.05, ***=p<0.001. Data are presented as box plots, with the central line marking the data set median.
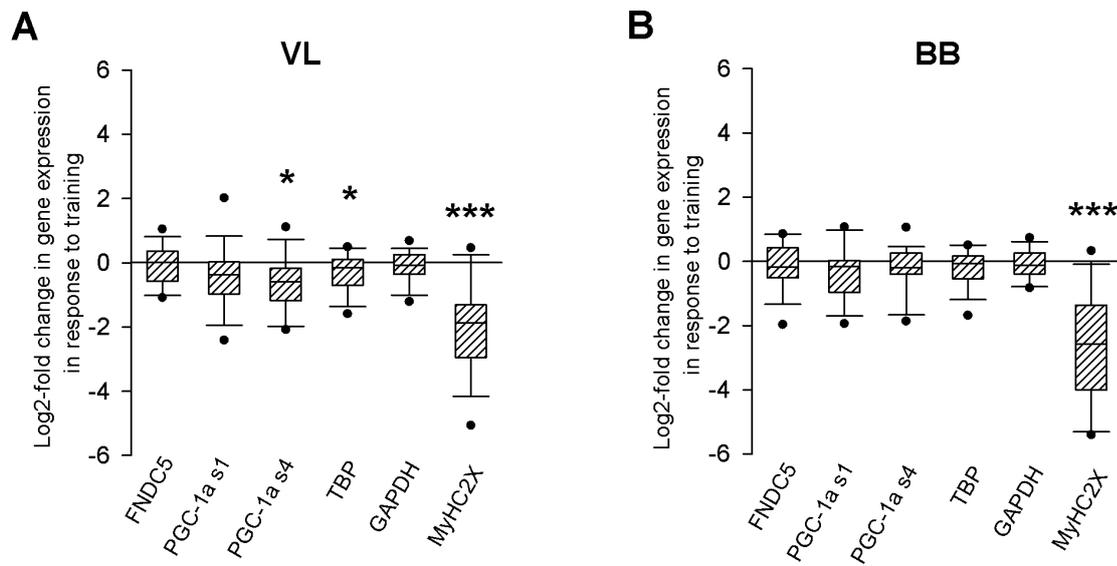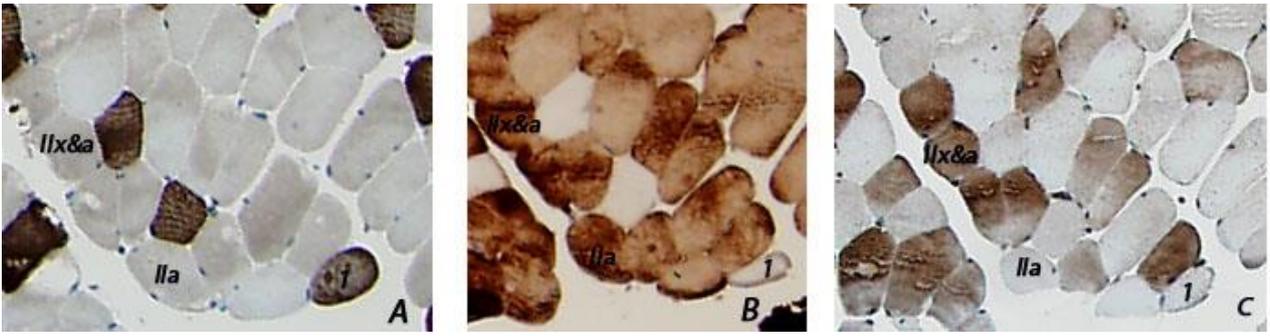
Figure 1

Figure 2
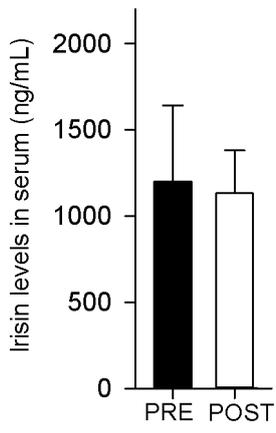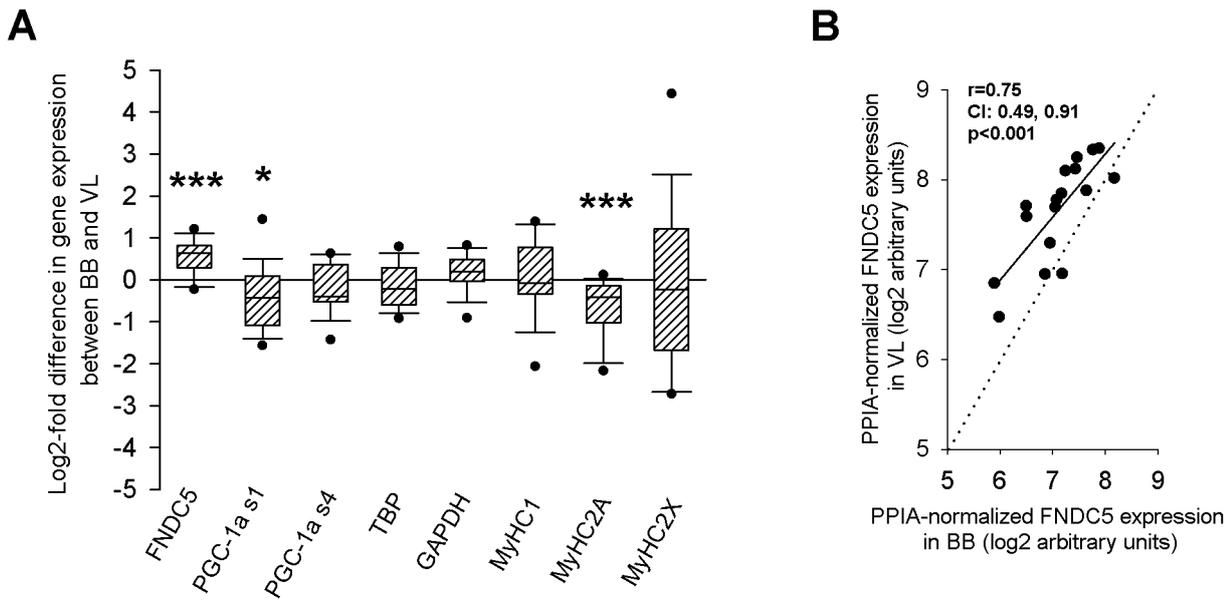
Figure 3

Figure 4

A

VL (baseline)

Type 1
r = 0.78, p<0.01
CI: 0.34, 0.94
p<0.01

Type 2A
r = -0.07
CI: -0.64, 0.55
p = 0.85

Type 2X
r = -0.78
CI: -0.94, -0.34
p<0.01

IMMUNO-determined muscle fibre composition in %

Log2-transformed PPIA-normalized FNDC5 expression

MyHC1
r = 0.75
CI: 0.28, 0.93
p<0.01

MyHC2A
r = 0.48
CI: -0.17, 0.84
p = 0.16

MyHC2X
r = -0.85
CI: -0.96, -0.50
p<0.01

GeneFam-determined muscle fibre composition in %

Log2-transformed PPIA-normalized FNDC5 expression

B

BB (baseline)

Type 1
r = -0.04
CI: -0.46, 0.63
p = 0.89

Type 2A
r = 0.62
CI: 0.13, 0.88
p<0.05

Type 2X
r = -0.55
CI: -0.91, -0.29
p = 0.07

IMMUNO-determined muscle fibre composition in %

Log2-transformed PPIA-normalized FNDC5 expression

MyHC1
r = 0.62
CI: 0.36-0.92
p<0.05

MyHC2A
r = 0.70
CI: 0.20-0.89
p<0.01

MyHC2X
r = -0.69
CI: -0.91, -0.26
p<0.05

GeneFam-determined muscle fibre composition in %

Log2-transformed PPIA-normalized FNDC5 expression

Figure 5

Expression stability of reference genes

Figure s1

Figure s2

Figure s3