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Effect of endurance training on skeletal muscle myokine expression in obese men: identification of apelin as a novel myokine

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Abstract

Introduction: It has been suggested that metabolic benefits of physical exercise could be mediated by myokines. We examined here the effect of exercise training on skeletal muscle expression of a panel of myokines in humans. Pathways regulating myokine expression were investigated in human myotubes.

Methods: Eleven obese non diabetic male subjects were enrolled in an 8-week endurance training program. Insulin sensitivity was assessed by oral glucose tolerance test. Subcutaneous adipose tissue and *Vastus Lateralis* muscle biopsies were collected before and after training. RNAs were prepared from adipose tissue and skeletal muscle. Primary culture of myoblasts was established.

Results: As expected, exercise training improved aerobic capacity and decreased fat mass. No significant change in interleukin 6, fibroblast growth factor 21, myostatin or irisin mRNA level was found in muscle after training. A 2-fold increase in apelin mRNA level was found in muscle but not in adipose tissue. No change in circulating myokine and adipokine plasma levels was observed in the resting state in response to training. Interestingly, apelin was significantly expressed and secreted in primary human myotubes. Apelin gene expression was up-regulated by cyclic AMP and calcium unlike the other myokines investigated. Importantly, muscle apelin mRNA levels were positively related to whole-body insulin sensitivity.

Conclusion: Collectively, our data show that exercise training up-regulates muscle apelin expression in obese subjects. Apelin expression is induced by exercise signalling pathways and secreted *in vitro* in human primary myotubes, and may behave as a novel exercise-regulated myokine with autocrine/paracrine action.

Keywords: obesity; exercise; skeletal muscle cells; apelin; myokines

Introduction

Regular physical activity protects against numerous chronic diseases such as obesity, type 2 diabetes and cardiovascular diseases (1). Some of the beneficial effects of regular exercise include lower blood pressure, improved glucose homeostasis and lipid profile, higher resting energy expenditure and reduced fat mass. Several mechanisms underlie such benefits. It is now widely accepted that regular exercise increases nutrient metabolism in various tissues by regulating the expression and activity of key metabolic control genes, leading to enhanced insulin sensitivity and metabolic flexibility (2). Skeletal muscle exhibits remarkable metabolic adaptations to exercise including mitochondrial biogenesis and improved substrate metabolism (3, 4). How exactly contracting muscle mediates the metabolic and physiological adaptations of exercise is still unclear (5). It has long been hypothesized that muscle can produce endocrine signals capable of mediating the health benefits of exercise (6). Since skeletal muscle is the largest organ of the body, the discovery of several factors secreted by contracting muscle has led to a new field of research. These so-called myokines are secreted in response to exercise and can regulate in an autocrine and endocrine fashion the function of muscle and other organs (6). Skeletal muscle has been first considered as an endocrine organ due to its ability to produce interleukin 6 (IL6) as an exercise released factor (7). IL6 induces lipolysis and improves insulin-stimulated glucose uptake (8). Another well documented myokine is myostatin (MSTN). MSTN exhibits anti-hypertrophic effects in skeletal muscle and MSTN null mice are characterized by an excessive muscle mass (9). Irisin originating from the proteolytic cleavage of fibronectin type III domain-containing protein 5 (FNDC5) and fibroblast growth factor 21 (FGF21) were recently identified as novel myokines (10, 11). Irisin improves glucose homeostasis in mice and seems to behave as a thermogenic factor involved in the browning of white subcutaneous adipose tissue in mice (10). FGF21 enhances whole body insulin sensitivity and thermogenesis in brown adipose tissue (12, 13). Although the effect of exercise training on plasma levels of these myokines has been previously studied, inconsistent findings have been reported (14-18).

In the present study, we investigated skeletal muscle myokines (IL6, MSTN, Irisin, FGF21) gene expression and plasma levels in obese individuals in response to an 8-week aerobic exercise training intervention. To extend the knowledge on the adaptation of muscle to training, we performed a comprehensive gene expression profiling of human skeletal muscle. This led to the identification of apelin (APLN) as a novel factor up-regulated by exercise training. Apelin expression, secretion and regulation by exercise-activated signaling pathways were next investigated *in vitro* in human primary myotubes.

Subjects and Methods

Subjects

Eleven sedentary obese male volunteers who had stable weight during the previous 3 months were recruited at the Toulouse Clinical Investigation Centre (Table 1). The subjects were on their usual diet before the study and ate a weight-maintaining diet consisting of 35% fat, 16% protein, and 49% carbohydrates two days before the experiment. None were previously enrolled in endurance activity training. They were asked to maintain their dietary habits during the study and to refrain from vigorous physical activity 48h before each clinical investigation day. Dietary intake was assessed by a dietician from a 3-day weighed food record, including 2 week days and 1 weekend day, the week before the first investigation day. Dietary records were assessed at baseline and during the last week of the program. Nutrient intake was calculated using PRoFIL software v6.7 (Audit Conseil en Informatique Médicale, St Doulchard, BOURGES, France) with CIQUAL french food composition database for diet composition.

Ethics statement

The study was performed according to the latest version of the Declaration of Helsinki and the Current International Conference on Harmonization (ICH) guidelines. Application was

approved by the Ethics Committee of Toulouse University Hospitals and all subjects gave written informed consent. The study is registered in Clinical Trials NCT01083329 and EudraCT 2009-012124-85.

Anthropometric and clinical parameters

Anthropometric parameters, blood samples, adipose tissue and skeletal muscle biopsies were performed during a 2 day investigation, one week apart, before and after the training program as follows. On day 1, after an overnight fast, maximal oxygen consumption (VO_2max) was measured on a braked bicycle ergometer as described in (19). On day 2, after overnight fast, blood samples were drawn and percutaneous biopsies of the V*astus Lateralis* muscle and of abdominal subcutaneous adipose tissue were obtained as previously described (20, 21). Ninety min after the end of biopsy sampling, an oral glucose tolerance test (OGTT) with 75g glucose load was performed at 30 min intervals (times 30, 60, 90, 120). Body composition was assessed by dual-energy X-ray absorptiometry performed with a total body scanner (DPX, Software 3.6, Lunar Radiation Corp., Madison, WI). Blood glucose was assayed using the glucose oxidase technique (Biomérieux, Paris, France). Plasma nonesterified fatty acids were assayed with an enzymatic method (Wako kit, Unipath, Dardilly, France). Serum insulin was measured by using a Bi-insulin IRMA kit (Bertin Pharma, Montigny le Bretonneux, France). Plasma FGF21 and apelin were quantified with Human FGF-21 Quantikine ELISA Kit (R&D Systems Europe, Lille, France) and the human EIA apelin-12 kit (Phoenix Pharmaceuticals, Belmont, CA), respectively. Retinol binding protein 4 (RBP4) was analyzed by immuno-nephelemetry on a BN ProSpec (Siemens HealthCare Diagnostics, Cergy-Pontoise, France). Other parameters were determined using standard clinical biochemistry methods. Because it is a good reflection of the insulin sensitivity measured by euglycemic insulin clamp, the Matsuda insulin sensitivity index (ISI-M) derived from OGTT is widely used in clinical and epidemiological research (22). ISI-M was calculated as : 10 000/square root of [(fasting glucose × fasting insulin) × (mean glucose × mean insulin during OGTT)].

Exercise training program

The exercise training program was performed at the Centre de Ressources d'Expertise et de Performance Sportives (CREPS) of Toulouse. The 45-60 min exercise sessions consisted mainly of cycling and running, 5 times a week for 8 weeks. Subjects exercised 3 times per week under supervision during the first 4 weeks and 2 times per week during the last 4 weeks. They exercised on their own during other sessions. All daily sessions consisted of at least 20 min warm-up at 35% VO_2max followed by progressively increasing exercise intensity (up to 85% VO_2max) and duration (up to 1h) throughout the training program. The subjects exercised at a target heart rate corresponding to 35–85% of their VO_2max. Heart rate was monitored with a Suunto T3 Cardiometer (MSE, Strasbourg, France). Compliance with training was good and the percentage of sessions completed was greater than 85% at the end of the study. Subjects were instructed to keep their usual dietary habits during the study. Adherence to the training program was self-reported. At the end of the 8-week training program they were investigated 48-72h after the last exercise bout.

Indirect calorimetry

For determination of VO_2max, gas exchanges were measured as previously described (23). Breath-by-breath measurements were taken at rest and throughout exercise to assess air flow and O_2 and CO_2 concentrations in expired gases using a computerized ergospirometer (Ultima PFX, Medical Graphics, USA). Oxygen concentration was analysed by a zirconium cell and CO_2 concentration by an infrared analyser. Certified calibration gases were used to calibrate the analysers every day before the beginning of the assay. The VO_2max exercise trial occurred in a ventilated room to ensure a constant room temperature and hygrometry from the calibration just before the trial.

DNA microarray and reverse transcription-quantitative PCR

Total RNA from frozen biopsies was prepared as previously described (20, 24). Based on concentration (Nanodrop ND-1000 Spectrophotometer, Labtech) and quality (Experion, BioRad, Marnes-la-Coquette, France) check, 9 subjects had enough high quality total RNA available for gene expression study. For reverse transcription-quantitative PCR (RT-qPCR), 500ng of total RNA was used for first strand cDNA synthesis using random hexamers and poly(dT) according to the Multiscribe® reverse transcriptase kit (High capacity cDNA reverse transcription kit, Applied Biosystems, Foster City, USA Invitrogen). TaqMan® Assays (Applied Biosystems) were used with 18S RNA (Taqman® Control Assays, Applied Biosystems) for gene expression normalization. Microarray experiments were performed using Agilent 4×44k oligonucleotide arrays as described in (25). Hybridization quality check resulted in the analysis of microarray data from 8 subjects. Microarray data have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE40551.

Cell culture

Satellite cells were isolated from fresh V*astus Lateralis* biopsies obtained before training and cultured as previously described (26). At day 4 of differentiation, the cells were treated as indicated with ionomycin, insulin, GW7647, GW0742 (Sigma-Aldrich, Courtaboeuf, France) or forskolin (Calbiochem Corp. Darmstadt, Germany) as indicated. After 24h, the medium was collected to measure secreted factors and cells were harvested for mRNA extraction. Samples were stored at -80°C.

Statistical analyses

Gaussian distribution and homoscedasticity of data were tested with corrected Kolmogorov-Smirnov and Levene tests, respectively. Microarray data analyses were performed as described in (25). Mann-Whitney or Wilcoxon tests were used in non-parametric simple comparisons. Kruskal-Wallis and Dunn’s post tests were performed in multiple comparisons and non-

parametric data analyses. Spearman correlation analysis was used to assess correlation between variables in non-parametric univariate analysis and p-values adjusted for multiple comparisons using Benjamini-Hochberg procedure. To test for an independent association between insulin resistance and myokines, multiple linear regression models were computed using hierarchical regression in which changes (Δ) in fat mass was entered in a first block, Δ VO_2max, Δ plasma adipokines and myokines, and Δ myokines mRNA levels were introduced in the model using with a stepwise procedure in a second block and Δ *APLN* mRNA level were introduced in subsequent block. Statistical analyses were performed with GraphPad Prism software (GraphPad Software, La Jolla, CA) and SPSS Statistics 17.0 software (SPSS Inc., Chicago, Ill). Threshold for statistical significance was $p < 0.05$.

Results

Anthropometric and clinical characteristics before and after endurance training

The 11 obese male volunteers were aged 35.4 ± 1.5 years. As expected, exercise training increased whole-body aerobic capacity (VO_2max) by about 7%, slightly reduced fat mass (mean fat mass loss 0.8 ± 0.4 kg), and tended to increase fat-free mass (mean fat-free mass gain 1.2 ± 0.6 kg) (Table 1 A). Changes in body composition occurred despite no change in food intake. No significant changes in ISI-M and fasting plasma glucose were observed, while fasting plasma insulin tended to decrease in response to training (Table 1 A).

Effect of endurance training on skeletal muscle myokine gene expression

We investigated the mRNA levels of *IL6*, *FGF21*, *MSTN* and *FNDC5* in human skeletal muscle of obese individuals pre- and post-exercise intervention. Endurance training did not significantly change gene expression of the four candidate myokines (Figure 1).

Apelin mRNA level is increased in skeletal muscle from obese individuals after endurance training

Analysis of human skeletal muscle transcriptome before and after endurance training led to the identification of *APLN* as the most up-regulated transcript encoding a known protein. *APLN* encodes apelin, a peptide that was so far known as an adipokine (27). RT-qPCR confirmed a two-fold increase in *APLN* after endurance training and displayed no change in its receptor *APJ* (Figure 2). A positive correlation between changes of muscle *APLN* mRNA levels and changes in ISI-M was found (r=0.81, p=0.008) in response to exercise training (Figure 3). A significant negative correlation with changes in fasting plasma insulin was also found (r=-0.70, p=0.036). In a stepwise regression analysis, the best predictive model of change in ISI-M included changes in plasma RBP4 (β=-0.723), skeletal muscle *APLN* mRNA (β=0.241) and fat mass (β=-0.203). This model explained 89% of the variability in ISI-M (p=0.008).

Regulation of myokines mRNA level and secretion in human primary myotubes

We next investigated the regulation of *APLN* and other candidate myokines *in vitro* in human primary myotubes established from *Vastus Lateralis* muscle. Myotubes were treated with drugs mimicking the activation of exercise signaling pathways and enhancing calcium and cAMP intracellular levels, ionomycin (a calcium ionophore) and forskolin (an adenylyl cyclase activator), respectively (Figure 4). Ionomycin treatment induced a 1.6-fold increase in *APLN* and 2-fold decrease in *FNDC5* expression. Ionomycin also slightly increased *MSTN* by 30% and decreased *IL6* by 30%. Forskolin treatment decreased *IL6*, *FGF21*, *FNDC5* and *MSTN* by 90, 50, 50 and 35% respectively, while *APLN* increased by 3.3-fold. We also showed that activation of PPAR-α and -δ signaling as well as insulin treatment had no effect of *APLN* gene expression (Supplemental Figure 1). Of interest, FGF21 and apelin were detected in the culture medium at low level when compared to plasma values (Figure 5 and Table 1B). Forskolin and ionomycin respectively decreased by 33% and increased by 2.4-fold FGF21 concentration in the culture

medium (Figure 5 A). Apelin concentration in the culture medium was very low about 50 times less than in plasma and no significant change in apelin concentration was observed compared to control (Figure 5 B).

Effect of endurance training on circulating levels of adipokines and myokines in obese individuals

In agreement with the observed fat mass loss, there was a trend for reduced plasma leptin concentrations, but no significant change in adiponectin or RBP4 (Table 1B). Exercise training did not change significantly resting plasma levels of IL6, FGF21 and apelin (Table 1B).

Discussion

Endurance training is known to improve whole body glucose homeostasis and to reduce the risk of developing type 2 diabetes (2). In this study, we identify apelin as a novel myokine which might contribute to exercise training-mediated improvement of whole-body insulin sensitivity in obese individuals. Interestingly, skeletal muscle gene expression of other myokines with a role evoked in the regulation of insulin sensitivity remained unchanged in response to 8 weeks of exercise training in middle-aged obese male.

Accumulating data suggest that during and following exercise, skeletal muscle synthesizes and releases factors that may act either systemically or locally within muscle tissue to mediate some of the metabolic and physiological adaptations of exercise (6). These secreted factors have been termed myokines. Little is known on the regulation of the few myokines identified so far by acute and chronic exercise. In the present study, skeletal muscle whole transcriptome profiling led us to identify *APLN* as a novel skeletal muscle transcript up-regulated by exercise training. Apelin was so far known as an adipocyte-secreted peptide which modulates skeletal muscle glucose and lipid metabolism and increases insulin sensitivity via its

receptor, APJ (27). No changes in APJ mRNA levels were noted in muscle in response to training. In addition, mRNA levels of other known myokines such as *IL6, FGF21, MSTN* and *FNDC5* remained unaffected by the training intervention in muscle (p values > 0.1). Consistently, exercise training did not significantly change resting plasma concentrations of IL6 and FGF21. A decrease in *MSTN* in skeletal muscle appears to be a hallmark of exercise training. *MSTN* was previously found decreased in muscle of old women after 12 weeks of aerobic training (28). Here, despite non-significant change, a tendency to decrease appears for *MSTN*. *FNDC5* encodes a novel myokine, irisin, with thermogenic potential in white adipose tissue (29). There has been recent controversy on the regulation of skeletal muscle *FNDC5* by training in humans. In agreement with our data, unchanged expression of muscle *FNDC5* was recently reported in response to 6 weeks of endurance cycling (30). In addition, a 12-week endurance exercise training induced no change in serum IL6 despite a 2-fold decrease in its skeletal muscle gene expression (31). Conversely, it was recently shown that a 2-week exercise program increased serum FGF21 in young healthy women (15). However, no report was made on skeletal muscle gene or protein expression. The contribution from each tissue to plasma level is unknown. In summary, part of the discrepancies between our study and other studies may be explained by the age of the subjects, their obesity status and possibly by the difference in exercise training volume and duration.

Despite non-significant change in food intake, the 8-week training program induced a slight but significant fat mass loss indicating that a slight energy deficit was achieved with exercise training. Since apelin was primarily described as an adipokine, a decrease in circulating apelin was expected, as recently described (32). In type 2 diabetic individuals, 12- to 24-month aerobic training programs increased plasma apelin while fat mass was unaffected (33, 34). Here, the absence of changes in plasma apelin level may be the result of compensation due to both the increase in muscle mass and the enhanced gene expression of skeletal muscle *APLN*, while no change in *APLN* expression was found in adipose tissue (data not shown). Also, mice overexpressiong apelin had no increase in plasma apelin content while enhanced metabolic

function was seen in skeletal muscle (35). Alternatively, the up-regulation of muscle *APLN* expression in face of no apparent changes in plasma apelin levels may suggest that apelin is produced to act locally on skeletal muscle fibers in a similar fashion than interleukin 8 (36). In addition, a positive correlation between skeletal muscle *APLN* and ISI-M was observed. Besides change in plasma RBP4, the increase in skeletal muscle *APLN* appeared as a weak independent contributor of insulin sensitivity during exercise training. This is in agreement with at least another study (34).–RBP4 is an adipokine with a controversial role in insulin resistance (37), while apelin has been previously shown to promote glucose uptake in skeletal muscle (38). Collectively, our data indicate that skeletal muscle apelin might be part of the exercise training-induced improvement of insulin sensitivity through autocrine/paracrine effects within skeletal muscle.

To confirm that apelin is a novel myokine, we next investigated its expression, secretion and regulation *in vitro* in human primary myotubes, besides other known myokines (IL6, MSTN, FGF21 and FNDC5). Thus little is known on the regulation of myokines expression *in vitro*. Exercise induces muscle contraction through rise in intracellular cAMP and calcium along with other pathways (5). It was previously shown that activation of cAMP/PKA and calcium signaling pathways in human primary myotubes induces PGC-1α and PPAR-δ gene expression, and promotes mitochondrial biogenesis (39). These pathways also favor lipid oxidation and glycogen storage. In this study, activation of cAMP signaling by forskolin consistently down-regulated *IL6*, *MSTN*, *FGF21* and *FNDC5* expression *in vitro*, while it strongly induced *APLN*. In the same line, activation of calcium signaling by ionomycin significantly induced *APLN* expression while down-regulating *IL6* and *FNDC5*. Since previous studies have shown that both exercise and muscle contraction induce *IL6* mRNA (40), this suggests that the transcriptional regulation of myokines is complex and involves multiple synergistic pathways. Interestingly, we could show that both apelin and FGF21 are secreted in the culture medium of human primary myotubes. Of note, FGF21 secretion paralleled its gene transcription pattern in response to forskolin treatment. In contrast, apelin concentration in the culture medium was very low, about

50 times less than in plasma, and apelin secretion remained unchanged in response to both forskolin and ionomycin treatments. This suggests that apelin may be secreted by skeletal muscle cells, but due to its very short half-life (< 5 min) (41), the peptide may be quickly degraded thus preventing a significant accumulation in the medium overtime. A possible interaction of apelin with circulating proteins (35) and instability in human plasma (42) was also reported. Additionally, skeletal muscle may not contribute significantly to circulating concentrations of plasma apelin. Furthermore, as various apelin isoforms exist in plasma (42), the possibility of a specific skeletal muscle isoform cannot be excluded. It can be hypothesized that apelin is acutely released by skeletal muscle during exercise to act locally on its own receptor as an autocrine/paracrine regulator. In humans, *APLN* and *APJ* mRNA levels are known to be regulated by insulin in adipocytes (27). We found here no direct effect of insulin, PPAR-α and/or PPAR-δ agonists on *APLN* expression in human myotubes (Supplemental Figure 1). These data also indicate a differential regulation of *APLN* expression between fat and muscle cells. The up-regulation of apelin expression in skeletal muscle is in agreement with apelin transgenic and knockout mice data demonstrating a positive role of apelin on skeletal muscle vascular mass and mitochondrial biogenesis (35) and in the maintenance of insulin sensitivity (43). Considering the potential role of apelin in the regulation of lipid metabolism and insulin sensitivity (27), future studies should investigate the functional and metabolic role of apelin in human skeletal muscle.

In summary, these data highlight apelin as a novel exercise-regulated myokine in humans. Apelin is expressed, secreted, and responsive to exercise-activated signalling pathways in cultured human primary myotubes. Skeletal muscle *APLN* expression is up-regulated by 8 weeks of endurance exercise training in obese male subjects and might contribute to exercise training-mediated improvement of whole-body insulin sensitivity. Collectively, these data suggest that apelin may be locally produced by skeletal muscle fibers in response to exercise and acts locally to improve muscle metabolism and function. Future studies should investigate

the influence of acute exercise on skeletal muscle apelin expression as well as its metabolic role in human skeletal muscle.

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Supplementary information

Supplementary information is available at the journal's website.

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Tables

Table 1. Changes in bio-clinical characteristics before and after 8weeks of exercise training

A. Anthropometric and clinical parameters.

	Before training	After training	P-value
BMI (kg/m²)	32.6 ± 2.3	32.7 ± 2.5	0.722
Body weight (kg)	102.3 ± 6.5	102.6 ± 7.4	0.722
Fat free mass (kg)	62.1 ± 6.3	63.3 ± 7.4	0.075
Fat mass (kg)	36.3 ± 6.3	35.5 ± 6.8	0.033
Food intake (kcal/day)	2462 ± 774	2226 ± 519	0.183
Fasting insulin (mUI/L)	17.6 ± 7.9	13.7 ± 5.9	0.093
Fasting glucose (mM)	5.0 ± 0.5	4.9 ± 0.3	0.424
AUC glucose	167.8 ± 17.7	170.0± 20.7	0.214
AUC insulin	9208 ± 4633	11361 ± 5705	0.169
ISI-Matsuda	3.08 ± 1.51	3.63 ± 2.43	0.646
VO_2 max/fat free mass (mL/min/kg)	44.8 ± 3.8	47.9 ± 6.0	0.022

B . Circulating adipokines and myokines.

	Before training	After training	P-value
Adiponectin (µg/ml)	13.1 ± 3.7	12.4 ± 2.4	0.722
Leptin (ng/ml)	37.6 ± 8.4	29.6 ± 7.1	0.077
RBP4 (µg/ml)	43.9 ± 7.2	41.4 ± 7.1	0.155
IL6 (pg/ml)	0.26 ± 0.07	0.24 ± 0.06	1.000
Apelin (pg/ml)	514 ± 76	490 ± 89	0.328

FGF21 (pg/ml)	123.1± 21.5	143.8 ± 33.0	0.354

BMI, body mass index; ISI-Matsuda, insulin sensitivity Matsuda index.

Data are mean ± SD (n=11). P-value is extracted from Wilcoxon test

Figure legends

Figure 1: Effect of endurance training on myokines mRNA level in human skeletal muscle. mRNA levels were measured before and after 8 weeks of endurance training using RT-qPCR normalized to 18S. Data are mean ± SEM (n=9).

Figure 2: Effect of endurance training on apelin and APJ mRNA level in human skeletal muscle. Apelin (APLN) and apelin receptor (APJ) mRNA levels were measured before and after 8 weeks of endurance training using RT-qPCR normalized to 18S. Data are mean ± SEM (n=9). ** $p < 0.01$ in a Wilcoxon test.

Figure 3: Correlation between changes in skeletal muscle apelin mRNA levels and insulin sensitivity index during endurance training. Spearman correlation between changes in skeletal muscle apelin (Δ APLN) mRNA levels and insulin sensitivity index (Δ ISI Matsuda) during a 8-week training of male volunteers (n=9).

Figure 4: Effect of exercise mimetic signaling compounds on myokines mRNA level in human myotubes. Cells were treated with forskolin (4 µM), ionomycin (0.5µM) or vehicle (control) for 24h. Myokines mRNA level was measured using RT-qPCR normalized to 18S. Data are presented as base-2 log of mean fold change ± SEM relative to control cells (n=6-10). *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$ versus control cells in a one way Kruskal-Wallis and Dunn's post hoc tests.

Figure 5: Effect of exercise mimetic signaling compounds on myokine concentrations in the culture medium of human myotubes. Cells were treated with forskolin (4 µM), ionomycin (0.5µM) or vehicle (control) for 24h. Culture media was tested for FGF21 (A) and apelin (B).

Data are presented mean ± SEM (n=6). ** $p < 0.01$ versus control cells in a one way Kruskal-Wallis and Dunn's post hoc tests.

Figure 1

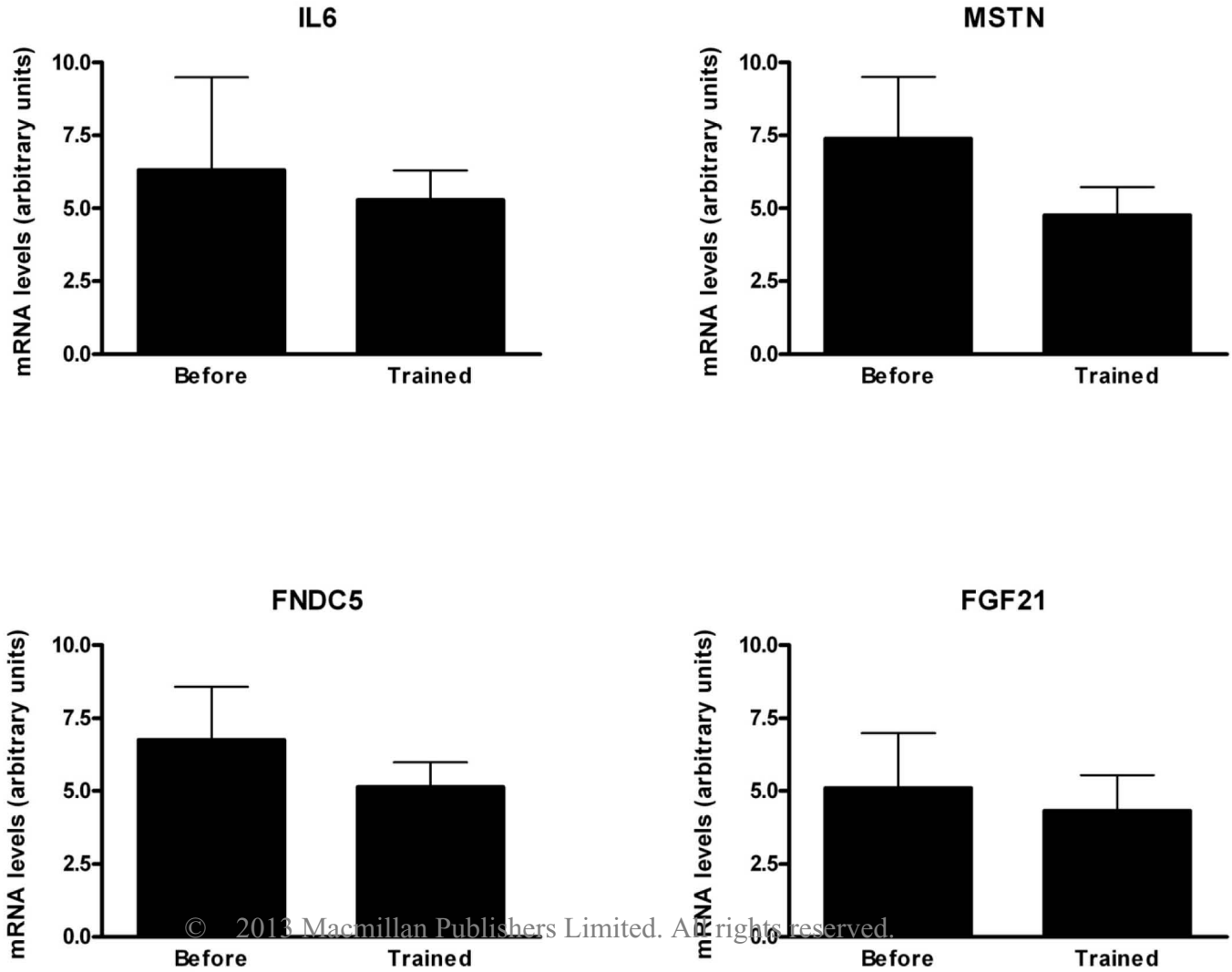

Figure 2

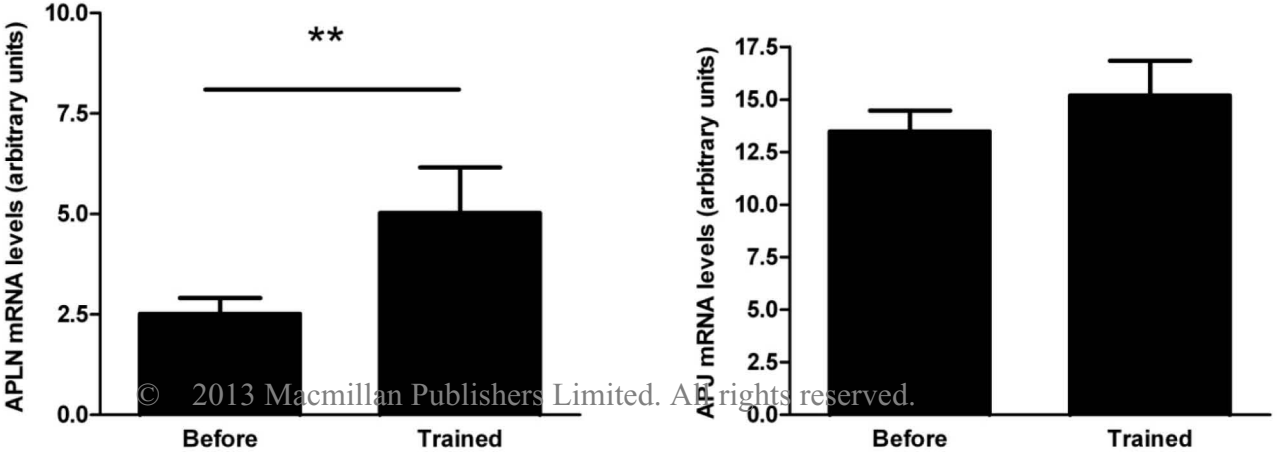

Figure 3

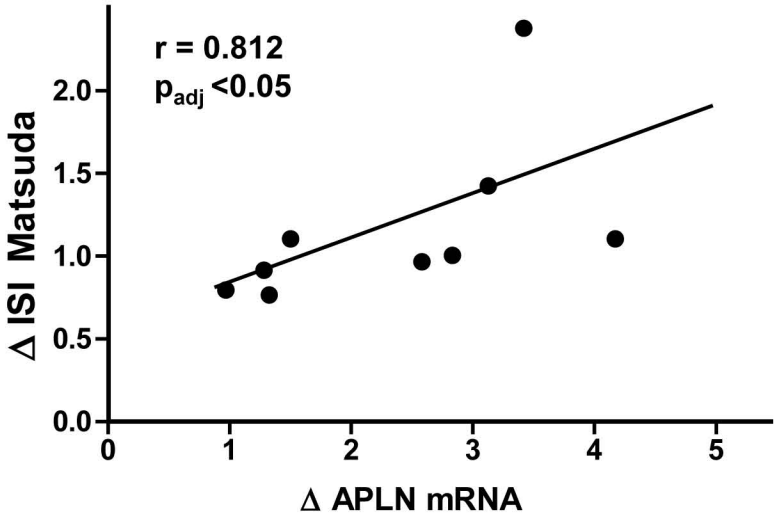

Figure 4

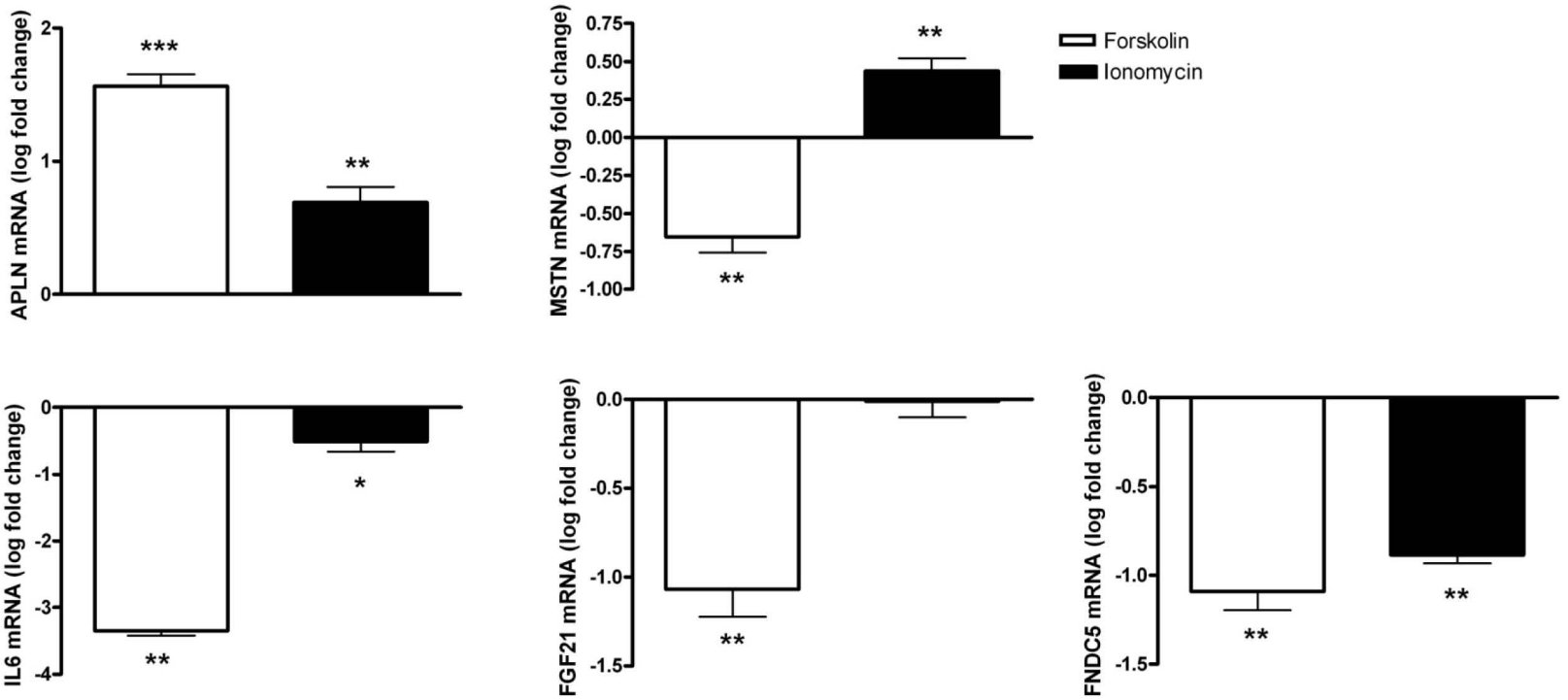

Figure 5