

# Futsal and Continuous Exercise Induce Similar Changes in Specific Skeletal Muscle Signalling Proteins

## Authors

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## Key words

- intermittent exercise
- protein signalling
- ATF2
- football

## Abstract

Exercise elicits skeletal-muscle adaptations which are important for improved health outcomes. We compared the effects of a futsal game (FUT) and moderate-intensity continuous exercise (MOD), on the skeletal-muscle protein signalling responses in young, healthy individuals. 16 men undertook an incremental exercise test and a resting muscle biopsy performed >48 h apart. They were then randomly allocated to either FUT (n=12) consisting of 2 x 20 min halves, or MOD (n=8) consisting of a work-matched running bout performed at an intensity corresponding to the individual ventilatory threshold 1. Work matching was achieved by means of tri-axial accelerometers. Immediately after FUT and

MOD, participants underwent a second biopsy to assess exercise-induced changes in protein signalling. Total and phosphorylated protein abundance was assessed via western blotting. Both FUT and MOD altered signalling responses in skeletal muscle. FUT increased total ATF2 protein abundance (p=0.048) and phosphorylation (p=0.029), while no changes occurred with MOD. Both exercise regimes increased ACC phosphorylation (p=0.01) and returned a trend for increased p38MAPK phosphorylation. Futsal may be employed as an alternative to continuous exercise to elicit muscle adaptations which may be associated with improved health outcomes. As only FUT increased ATF2 activation, this protein might be a target of future investigation on exercise-induced signalling.

## Introduction

Exercise is a significant lifestyle determinant to chronic disease risk [39,42]. Exercise elicits numerous skeletal muscle adaptations, such as metabolic changes and glycaemic control, which contribute to improved health outcomes [15,26]. Particular attention has been directed towards understanding the molecular mechanisms that are believed to be fundamental in obtaining such adaptations. In particular, the signalling pathways associated with mitochondrial biogenesis and exercise- or insulin-mediated glucose transport have received considerable interest [11]. One pivotal pathway in metabolic responses to exercise is mediated by the activation of the 5'AMP-activated protein kinase (AMPK), which in turn induces an up-regulation of mitochondrial transcription factors and an increased mRNA transcription of the glucose transporter 4 (GLUT4) [20,21,31]. Within the AMPK signalling pathway, a putative role has been proposed for acetyl-CoA carboxylase (ACC) in the regulation of cellular lipid metabolism [22]. Similarly, the acti-

vation of a calcium-related protein, the Ca<sup>2+</sup>/calmodulin-dependent protein kinase II (CaMKII) induces an increase in the expression of mitochondrial transcription factors and GLUT4 via a mechanism involving the repressor histone deacetylase 5 (HDAC5) [33,46]. Additionally, the p38 mitogen-activated protein kinase (p38 MAPK) is involved in liver gluconeogenesis and glucose transport in the skeletal muscle cell partly via the action of the activating transcription factor 2 (ATF2) [28,32].

These signalling proteins and their associated pathways are highly sensitive to both acute and chronic exercise [11,38,40]. Traditionally, research investigating skeletal-muscle molecular responses to exercise has employed continuous aerobic exercise as the experimental model, typically of 30–90 min in duration and with an intensity of 60–80% of  $\dot{V}O_{2peak}$ . These protocols increased the phosphorylation of AMPK [24,35,44], p38 MAPK [30] and CaMKII [36]. More recently, focus has been directed toward low-volume, high-intensity protocols to establish whether an increased exercise intensity, together

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## Bibliography

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with the intermittent nature of exercise, play an important role in skeletal-muscle molecular adaptations [5,27,38]. There is evidence that low-volume, high-intensity intermittent exercise is at least as efficacious as continuous exercise in increasing the activation of several signalling proteins [4,16]. We have also previously demonstrated that as little as 1 min of repeated-sprint exercise increases the phosphorylation of ACC, CaMKII and HDAC5 [38].

However, outcomes originating from laboratory-based exercise research may have limited external validity. Some of these laboratory exercise protocols are not always applicable to “real-life” conditions, as they may be poorly motivating [43] or too exhausting [12] for the general population. Team sports may be an appropriate alternative for studying the effect of physical activity on skeletal-muscle molecular adaptations. They generally comprise bouts of high-intensity intermittent exercise and provide higher motivation for the participants, due to the enhanced social context [23]. Futsal (5 a-side indoor football) is a high-intensity intermittent activity, with approximately 70–85% of a game being performed above 85% of an individual’s maximal heart rate [3,9,34]. Approximately 50 % of the total distance covered during a game is achieved through moderate-intensity running, high-intensity running and sprinting [3]. Given the physiological characteristics of the game [9], futsal might induce greater acute signalling responses compared to low-intensity continuous physical activity. Secondly, futsal is one of the indoor sports with the highest participation rate worldwide, especially from a recreational perspective. In 2006, 1.1 million individuals

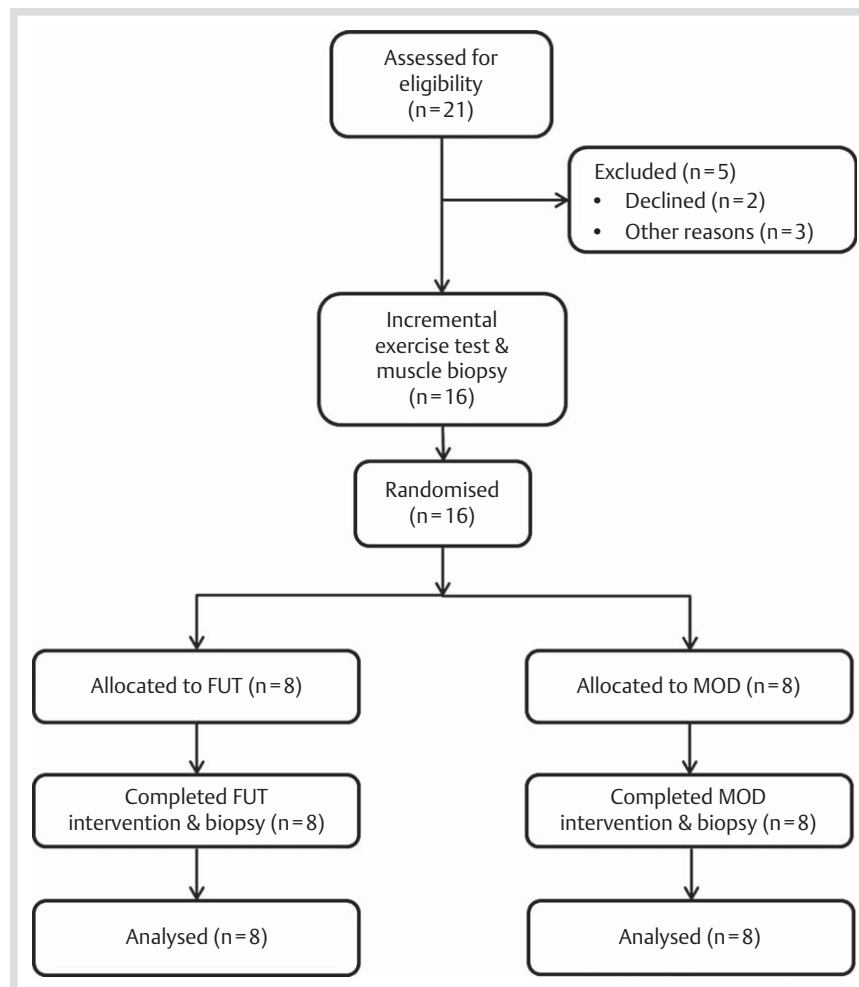
played futsal as registered players worldwide and it was estimated that 12 million individuals played futsal at a recreational level [41]. Therefore, research involving futsal as the experimental model can provide a high external validity, as the outcomes can be applied to real-life physical activity contexts on a large scale.

The aim of this study was to compare the effects of a futsal game and work-matched continuous running exercise on skeletal-muscle signalling responses in young, healthy men. We hypothesised that i) futsal would alter protein signalling in the skeletal muscle, and ii) futsal would induce greater signalling responses compared to continuous running exercise.

## Methods

### Participants

Twenty-one young, healthy men, were initially assessed as eligible to participate according to the following inclusion criteria: i) age 18–35 years; ii) absence of major cardiovascular pathologies; iii) absence of major musculoskeletal injuries; iv) previous experience in futsal and running exercise. Following preliminary screening, 5 participants decided not to commence the testing phase for personal reasons and 16 individuals took part in the study, which was conducted following a randomised parallel-group, pre-post design (◉ Fig. 1). The study was approved by the Ethics Committee of the University of Verona and was performed in accordance with recognised ethical standards [17].



**Fig. 1** Participant assessment and group allocation flow chart.

Participants' physical characteristics were (Mean±SD): age 21.4±1.7 years, height 179.2±6.0 cm, body mass 74.8±5.9 kg, and  $\dot{V}O_{2peak}$  52.0±6.3 mL kg<sup>-1</sup> min<sup>-1</sup>.

### Experimental overview

For the baseline testing, participants visited the laboratory on 2 occasions. During the first visit, participants performed an incremental test to exhaustion. At least 48 h after the incremental test participants underwent a resting muscle biopsy. One week after the completion of baseline testing, participants were randomly assigned (randomly-permuted blocks, <http://www.randomization.com>) to either a futsal (FUT) or a moderate-intensity running group (MOD). The FUT group performed a single futsal game, while MOD performed a work-matched running exercise bout on a treadmill. Immediately after the acute exercise, another muscle biopsy was taken. Participants were asked to refrain from alcohol, caffeine intake and physical exercise for the 48 h preceding all visits. Participants were also asked to accurately report the 3 meals preceding the resting biopsy and to exactly replicate the same diet before the main exercise trial.

### Incremental exercise test

The test was performed on a motorised treadmill (RUNRACE™, Technogym, Italy) and consisted of an initial 3-min stage at 8 km h<sup>-1</sup>, with the intensity thereafter increased by 1 km h<sup>-1</sup> every minute until exhaustion, defined as the subject's inability to maintain the required intensity. During the test, expired gases were analysed breath-by-breath using a metabolic cart (Quark PFT, COSMED srl, Italy) with the data averaged to obtain 10-s periods. Peak O<sub>2</sub> uptake was calculated as the average of the 2 highest values in 2 consecutive 10-s periods. Ventilatory threshold 1 (VT1) was calculated as an increase in  $\dot{V}E/\dot{V}O_2$  without a concurrent increase in  $\dot{V}E/\dot{V}CO_2$  [8]. During the test, participants wore a triaxial accelerometer (X6-2; 160 Hz, ±6 g, 16-bit; Gulf Coast Data Concepts, USA) placed in a dedicated vest between the scapulae. The changes in acceleration on the 3 anatomical axes were used to calculate a scaled vector magnitude (hereafter defined as 'work') corresponding to each stage of the incremental test, according to the following equation [7]:

$$work(AU) = \frac{\sqrt{((x1 - x0)^2 + (y1 - y0)^2 + (z1 - z0)^2)}}{100}$$

Where *x* represents vertical accelerations, *y* represents lateral accelerations, *z* represents frontal accelerations and the value '100' represents a scaling factor.

### Exercise trials

One week after the completion of baseline testing, participants performed the main exercise trial. The FUT group performed a game consisting of a standardised 5-min warm-up, followed by two 20-min (uninterrupted time) halves with 5 min of rest. To standardise the time between the end of the game and the biopsy, and due to the inability to perform multiple post-exercise biopsies at the same time, participants commenced and finished the game with a 10-min delay between one another. To achieve this, the missing players were temporarily substituted by players who did not participate in the study.

The MOD group performed a continuous exercise on a treadmill (RUNRACE™, Technogym, Italy) at an intensity corresponding to the speed at VT1 measured during the incremental test. The duration of exercise for each participant was calculated based on

the total work performed by FUT and matched according to their individual work performed at VT1. In summary, participants wore an accelerometer during the futsal game, and the total work for the game (including warm-up) was recorded. The duration of exercise required for MOD to match FUT was then calculated by dividing the average FUT work by the work corresponding to 1 min of exercise at VT1. Assessments of physical activity loads with triaxial accelerometers are widely used in research and an acceptable validity has been found on the comparison of work measured during treadmill- and ground-based physical activity [18].

### Muscle biopsy

A muscle biopsy was performed at rest and immediately after exercise on the *vastus lateralis* muscle of the participants' dominant leg. In short, following injection of a local anaesthetic (lidocaine hydrochloride 2 %, Monico SpA Italy) a muscle sample was collected using a semi-automatic biopsy needle (Vantage 13G, ZAMAR srl, Italy). Following collection, the samples (~60 mg) were immediately blotted on a filter paper to remove excess blood and quickly frozen in liquid N<sub>2</sub> before being stored at -80 °C for subsequent analysis. The time elapsed between the end of exercise and the post-exercise biopsy was between 4 and 6 min.

### Immunoblotting

Approximately 50 mg of frozen muscle samples were analysed as previously published [29]. Membranes were incubated with the following primary antibodies (all from Cell Signalling Technology unless otherwise reported): ATF2 (#9226), phospho-ATF2 Thr<sup>71</sup> (#5112), phospho-ACC Ser<sup>79</sup> (#3662), AMPKα (#2603), phospho-AMPKα Thr<sup>172</sup> (#2535), CaMKII (Santa Cruz Biotechnology, #SC-13082), phospho-CaMK II Thr<sup>286</sup> (#3361), p38 MAPK (#9212), phospho-p38 MAPK Thr<sup>180</sup>/Tyr<sup>182</sup> (#9211), phospho-HDAC4/5/7 (#3443). Individual blots were normalised for loading with GAPDH (Abcam, #ab8245). A dilution of 1:1 000 was used for all antibodies, with the exception of CaMKII (1:200) and GAPDH (1:10 000). Representative western blotting images are presented in **Fig. 2**.

### Statistical analysis

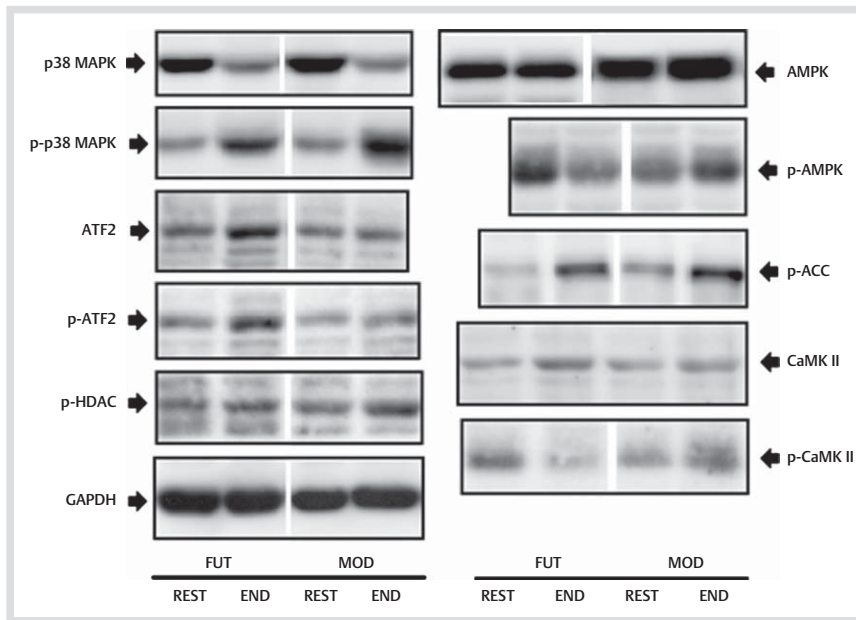
Data are presented as mean±SD. Scores were tested for normal distribution using a Shapiro-Wilk W test and, when the assumption of normality was not met, data were natural log-transformed. An analysis of covariance (ANCOVA) was used to assess group differences post-exercise, using the baseline values as a covariate for each variable. The magnitude of the changes was assessed using effect size (ES) statistic with 90 % confidence intervals, adjusted for the baseline values for each variable. ES were defined as follows: <0.2=trivial, 0.2–0.6=small, 0.6–1.2=moderate, 1.2–2.0=large, >2.0=very large [19].

## Results



### Baseline characteristic

At baseline, there was a strong trend towards a difference between FUT and MOD for  $\dot{V}O_{2peak}$  (49.1±6.2 and 55.0±5.2 mL kg<sup>-1</sup> min<sup>-1</sup>, respectively; *p*=0.057, ES 0.95±0.83), and  $\dot{V}O_2$  at VT1 (37.5±5.0 and 42.9±5.6 mL kg<sup>-1</sup> min<sup>-1</sup>, respectively; *p*=0.061, ES 0.92±0.84). However, when expressed as relative to  $\dot{V}O_{2peak}$ , there were no differences in VT1 between FUT



**Fig. 2** Representative western blot images from one individual of each experimental group. FUT, futsal game; MOD, moderate-intensity continuous exercise.

( $76.7 \pm 7.8$  %) and MOD ( $78.1 \pm 6.1$  %). There was a significant difference between FUT and MOD for the running velocity at VT1 ( $10.0 \pm 1.1$  and  $12.1 \pm 1.0$  km h<sup>-1</sup>, respectively;  $p=0.001$ , ES  $1.88 \pm 0.84$ ), and height ( $175.1 \pm 4.8$  and  $183.3 \pm 4.1$  cm, respectively;  $p=0.003$ , ES  $1.71 \pm 0.83$ ). There was no difference between FUT and MOD for age ( $22.0 \pm 2.2$  and  $20.9 \pm 0.8$  years, respectively;  $p=0.198$ , ES  $0.61 \pm 0.84$ ) and body mass ( $74.4 \pm 6.5$  and  $75.1 \pm 5.7$  kg, respectively;  $p=0.809$ , ES  $0.13 \pm 0.84$ ).

### Skeletal muscle protein signalling

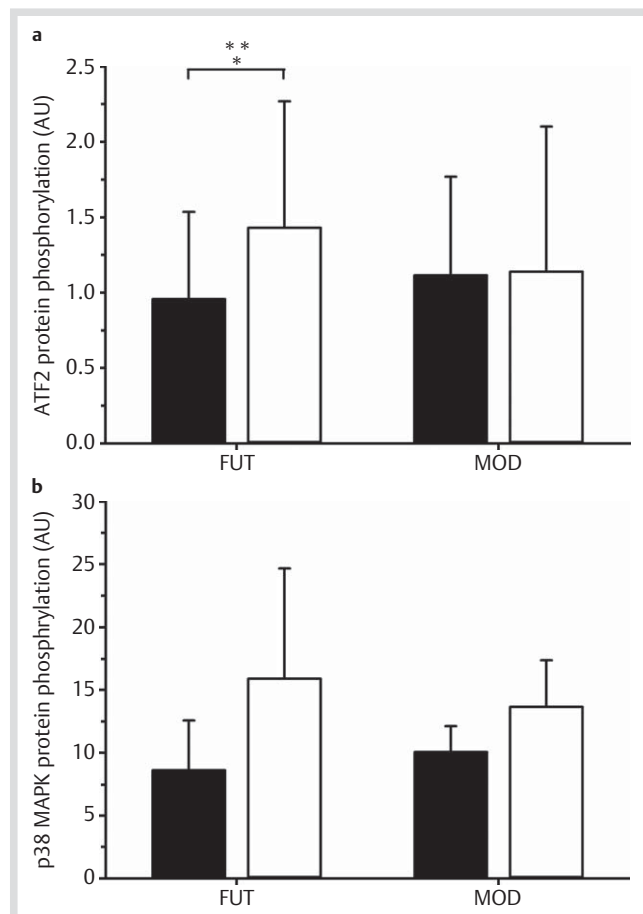
An acute futsal game was associated with a significant, 50 % increase in ATF2 phosphorylation ( $p=0.029$ , **Fig. 3**), while no change was detected in response to MOD. Similarly, FUT increased total ATF2 protein abundance, while no change was obtained with MOD ( $p=0.048$ ). Both FUT and MOD significantly increased ACC phosphorylation by 119 and 75 %, respectively ( $p=0.01$  for time factor, **Fig. 4**). There was also a trend towards an increased p38 MAPK in both groups after exercise. A summary of the changes in protein abundance and phosphorylation in response to FUT and MOD is presented in **Table 1**.

### Workloads and MOD exercise duration

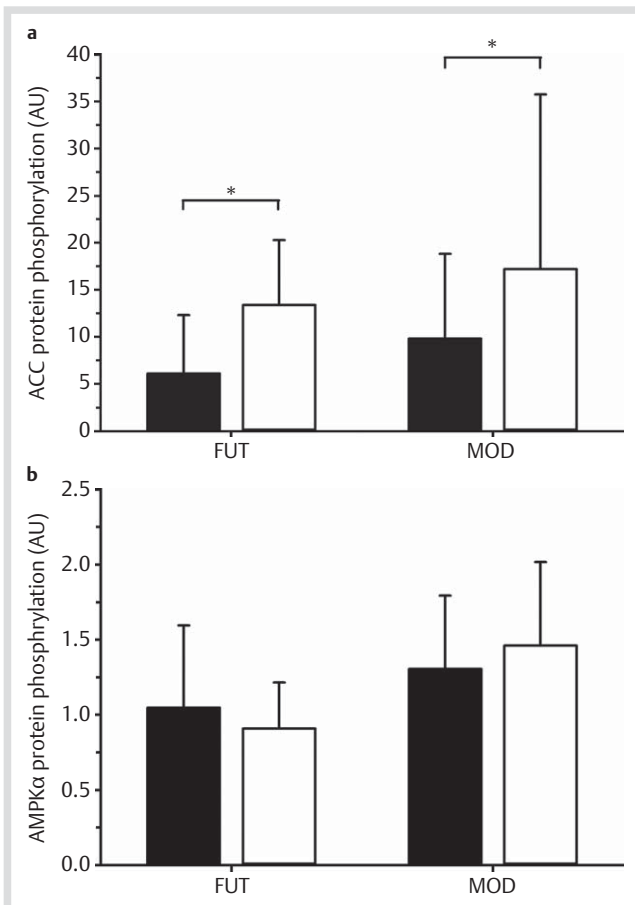
The average work during the FUT game was  $621 \pm 69$  arbitrary units. The average work for 1 min of running exercise at a speed corresponding to VT1 for the MOD group was  $27.7 \pm 2.4$  arbitrary units. The average duration of the experimental exercise for the participants in the MOD group was  $23.1 \pm 1.9$  min. This is similar to the average percentage of effective playing time (55%, ~22 min) compared to uninterrupted time in futsal games.

### Discussion

There were 3 main findings in this study. Firstly, ATF2 protein phosphorylation was increased only after FUT, despite no differences between groups in the regulation of its upstream p38 MAPK. Secondly, both FUT and MOD increased the phosphorylation of ACC. Finally, there were no differences between groups in the regulation of CaMKII and HDAC.



**Fig. 3** Skeletal muscle ATF2 **a** and p38 MAPK **b** protein phosphorylation at rest (filled bars) and immediately after (open bars) an acute game of futsal (FUT) and a work-matched running exercise performed at a moderate intensity (MOD). \*, significantly different from pre-exercise, main factor for both groups combined ( $p < 0.05$ ); \*\*, significantly different between FUT and MOD at post-exercise compared to pre ( $p < 0.05$ ). Data are presented as Mean  $\pm$  SD;  $n=8$  for both groups.



**Fig. 4** Skeletal muscle ACC **a** and AMPK $\alpha$  **b** protein phosphorylation at rest (filled bars) and immediately after (open bars) an acute game of futsal (FUT) and a work-matched running exercise performed at a moderate intensity (MOD). \*, significantly different from pre-exercise, main factor for both groups combined ( $p < 0.05$ ). Data are presented as Mean  $\pm$  SD;  $n = 8$  for both groups.

### ATF2 phosphorylation may depend on exercise intensity

We found that ATF2 phosphorylation was increased in response to FUT, while no change was detected with MOD. The increase in ATF2 phosphorylation appears to be the result of an increase in total ATF2 abundance. This transcription factor has received recent attention as an important downstream signalling protein of p38 MAPK [14]. Thorough investigations in rodent and in vitro models have revealed a double role for ATF2, as an important transcription factor of genes such as PGC-1 $\alpha$ , TNF- $\alpha$  and IL-6, but also as a DNA-damage response protein [6]. The differential increase of ATF2 in response to FUT and MOD is of interest, considering that phosphorylation of p38 MAPK, which is the direct upstream of ATF2, was not different between the two exercise regimes. The role of ATF2 in the regulation of gene expression in response to exercise has received little attention. It has been demonstrated that exercise-induced PGC-1 $\alpha$  mRNA expression was increased via a mechanism involving p38 MAPK, ATF2, and the cAMP response element (CRE) [1], which is the main transcription factor implicated in PGC-1 $\alpha$  gene expression. In this proposed mechanism, upon contraction-induced phosphorylation of ATF2 by p38 MAPK, ATF2 binds to CRE, which in turn increases PGC-1 $\alpha$  mRNA expression. However, the results of our study suggest a differential response to work-matched intermittent or continuous exercise for ATF2 and p38 MAPK. This is consistent with previous research showing that ATF2 phosphorylation was increased only in response to exercise performed at 80%  $\dot{V}O_{2peak}$  but not to an isocaloric exercise at 40%  $\dot{V}O_{2peak}$ , despite an almost identical increase in p38 MAPK phosphorylation [14]. There is thus emerging evidence pointing to the differential response of ATF2 and its upstream regulator p38 MAPK in skeletal muscle. This suggests that ATF2 might be regulated by kinases other than p38 MAPK. Despite mechanistic research on this topic lacking in human skeletal muscle experiments, it has been shown that ATF2 can be activated by signalling pathways involving the extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) in human cancer cells

Protein	FUT		MOD		FUT-MOD $\Delta$
	Pre	Post	Pre	Post	ES $\pm$ CI
AMPK $\alpha$	1.2 $\pm$ 0.5	1.0 $\pm$ 0.4	1.0 $\pm$ 0.4	0.8 $\pm$ 0.3	0.34 $\pm$ 0.73
p-AMPK $\alpha$	1.0 $\pm$ 0.5	0.9 $\pm$ 0.3	1.3 $\pm$ 0.5	1.5 $\pm$ 0.6	-0.72 $\pm$ 0.71
p-AMPK $\alpha$ /AMPK $\alpha$	1.1 $\pm$ 0.7	1.1 $\pm$ 0.5	1.1 $\pm$ 0.2	2.2 $\pm$ 1.4	-0.57 $\pm$ 1.02
p-ACC	6.1 $\pm$ 6.2	13.4 $\pm$ 6.9*	9.8 $\pm$ 9.0	17.2 $\pm$ 18.6*	0.19 $\pm$ 0.79
CaMKII	1.7 $\pm$ 1.2	1.6 $\pm$ 0.9	1.1 $\pm$ 0.4	1.3 $\pm$ 0.8	0.08 $\pm$ 0.57
p-CaMKII	2.5 $\pm$ 2.7	1.9 $\pm$ 1.3*	2.5 $\pm$ 1.7	2.9 $\pm$ 3.0*	-0.22 $\pm$ 0.61
p-CaMKII/CaMKII	1.7 $\pm$ 2.0	1.3 $\pm$ 0.7*	2.8 $\pm$ 2.0	3.6 $\pm$ 3.8*	-0.18 $\pm$ 0.81
p38 MAPK	1.8 $\pm$ 0.5	1.4 $\pm$ 0.6	1.9 $\pm$ 0.7	1.4 $\pm$ 0.6	-0.03 $\pm$ 0.94
p-p38 MAPK	8.6 $\pm$ 4.0	15.9 $\pm$ 8.8	10.1 $\pm$ 2.1	13.7 $\pm$ 2.7	0.10 $\pm$ 0.77
p-p38/p38 MAPK	0.45 $\pm$ 0.21	1.44 $\pm$ 1.30	0.64 $\pm$ 0.34	1.22 $\pm$ 0.62	-0.20 $\pm$ 0.93
ATF2	6.0 $\pm$ 2.6	11.1 $\pm$ 4.4**	8.0 $\pm$ 2.5	8.2 $\pm$ 2.9	1.09 $\pm$ 0.78
p-ATF2	1.0 $\pm$ 0.6	1.4 $\pm$ 0.8**	1.1 $\pm$ 0.7	1.1 $\pm$ 1.0	0.62 $\pm$ 0.48
p-ATF2/ATF2	0.17 $\pm$ 0.08	0.15 $\pm$ 0.09	0.15 $\pm$ 0.09	0.14 $\pm$ 0.08	-0.17 $\pm$ 0.45
p-HDAC4/5/7	1.0 $\pm$ 0.5	1.2 $\pm$ 0.5	1.3 $\pm$ 0.7	1.3 $\pm$ 0.3	-0.09 $\pm$ 0.61
GAPDH	1.1 $\pm$ 0.1	1.1 $\pm$ 0.1	1.0 $\pm$ 0.2	1.0 $\pm$ 0.1	0.28 $\pm$ 0.65

Data are presented as mean  $\pm$  SD and expressed as arbitrary units normalised for GAPDH;  $n = 8$  for each group

FUT, futsal group; MOD, moderate-intensity continuous exercise group; FUT-MOD  $\Delta$ , outcome of the difference between groups post-exercise, adjusted for baseline values; ES, effect size; CI, confidence interval; \*, significantly different from pre-exercise, main factor for both groups combined ( $p < 0.05$ ); \*\*, significantly different between FUT-MOD at post-exercise compared to pre ( $p < 0.05$ )

AMPK $\alpha$ , 5'AMP-activated protein kinase  $\alpha$ ; ACC, Acetyl-CoA carboxylase; CaMKII, Ca<sup>2+</sup>/CaM-dependent protein kinase II; p38 MAPK, p38 mitogen-activated protein kinase; ATF2, Activating transcription factor 2, HDAC, Histone deacetylase; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase. FUT, futsal group; MOD, moderate-intensity continuous exercise group

**Table 1** Skeletal muscle protein abundance and phosphorylation following an acute futsal game and work-matched continuous exercise.

[25]. Therefore, ATF2 may be identified as a more suitable target within the MAPK pathway to be measured when comparing different exercise characteristics on molecular responses in skeletal muscle, as ATF2 appears to be more sensitive to high-intensity intermittent exercise than p38 MAPK. However, mechanistic research in a more controlled environment must be conducted to support this hypothesis. Additionally, measurements of p38 MAPK and ATF2 should be integrated with a wider array of upstream and downstream measures to better understand the importance of MAPKs in exercise-induced signalling.

### Differential responses of ACC and AMPK to acute exercise

In the present study we found a significant increase in ACC phosphorylation in response to both FUT (119%) and MOD (75%). As the biochemical function of ACC is to catalyse the formation of malonyl CoA from acetyl CoA, and considering that one of the mechanisms of activation of ACC is via direct phosphorylation by AMPK, considerable focus has been directed towards the effects of contraction on ACC responses [22]. The magnitude of the increases in ACC phosphorylation observed in our study was consistent with those measured in response to a single bout of repeated-sprint exercise [38]. On one hand, this is a confirmation of the intense nature of futsal. On the other hand, the increase in ACC phosphorylation in response to FUT was similar to the increase with MOD. This is in contrast to the hypothesis that the AMPK pathway is sensitive to exercise intensity [10]. The discrepancy is more evident when ACC phosphorylation is compared to the activation of its direct upstream kinase, AMPK (◉ Fig. 4). Neither FUT nor MOD increased AMPK phosphorylation immediately after exercise. However, when considering the ratio between the abundance of the phosphorylated and total AMPK protein, there was a small effect for the difference between the two groups (ES  $0.57 \pm 1.02$ ), with the ratio being increased by approximately 100% in MOD and no change in FUT (◉ Table 1). This may signify that continuous, moderate-intensity running exercise is more effective than futsal in inducing AMPK phosphorylation, though the 2 protocols are similar in promoting ACC phosphorylation. It has been previously demonstrated that the activation of ACC might be independent of AMPK [13], this also being supported by the altered chronological sequence in the phosphorylation of the two proteins [38]. It is evident that further research is required to define the role of AMPK and ACC in skeletal-muscle signalling, and that these two proteins might not be optimal markers for differentiating between high- and moderate-intensity exercises, or between continuous and intermittent exercises, when these are matched by total volume. It must also be acknowledged that the delay between the end of exercise and the muscle sampling in this study may have not allowed detection of the peak of exercise-induced AMPK phosphorylation.

### Neither FUT nor MOD increases CaMKII phosphorylation

An interesting finding was that CaMKII total protein abundance and phosphorylation were not changed in response to either FUT or MOD. One of the elements behind the hypothesis that FUT may induce large adaptations in the skeletal muscle was that the activation of CaMKII plays a major role in the molecular adaptations induced by high-intensity intermittent exercise. This is supported by evidence that higher intracellular  $Ca^{2+}$  concentrations are recorded in response to muscle fibre stimula-

tions at high frequencies compared to low frequencies [2,45]. It was demonstrated that the phosphorylation of both CaMKII and its downstream target phospholamban were increased after exercise performed at a higher, but not a lower intensity [14,37]. We have also previously demonstrated that an acute exercise comprising only 60 s of sprinting was capable of increasing CaMKII phosphorylation by approximately 70% [38]. Therefore, the results of the present study are surprising. A possible explanation might be found in the observation that both CaMKII autonomous activity and phosphorylation at Thr<sup>287</sup> were initially increased after 1 min of continuous exercise at 67%  $\dot{V}O_{2peak}$ , then reduced by 100–200% during the following 90 min [37]. This might suggest a very short-term response of CaMKII to exercise. However, this is in contrast to the results presented above showing a detectable increase in CaMKII phosphorylation 1 h after the conclusion of repeated sprint-exercise [38]. Therefore, it is not possible to conclude that the activation of CaMKII is dependent on exercise intensity or exercise characteristics when two different exercise protocols are matched by total work performed. The lack of a clear difference in CaMKII phosphorylation between groups, together with the unclear results about AMPK, may also explain the absence of an increased HDAC phosphorylation after exercise in FUT and MOD. We have previously shown that as little as 1 min of high-intensity intermittent exercise increased HDAC5 phosphorylation by approximately 90% [38]. Additionally, 36 min of cycling at 80%  $\dot{V}O_{2peak}$  induced a phosphorylation of HDAC that was almost double that following a 70-min isocaloric exercise performed at 40%  $\dot{V}O_{2peak}$  [14]. However, the effectiveness of low-volume, high-intensity intermittent exercise to increase HDAC phosphorylation could not be replicated in the present study. We acknowledge that a limitation of the present study was that the randomisation process resulted in a difference between groups for  $\dot{V}O_{2peak}$  and VT1, though not statistically significant. As this physiological difference between FUT and MOD may play an important role in the skeletal muscle signalling responses to acute exercise, we determined whether  $\dot{V}O_{2peak}$ , and  $\dot{V}O_2$  and running speed at VT1 showed patterns of covariation with our main outcomes. There was no correlation between any of the physiological variables and the resting abundance/phosphorylation of the main signalling protein variables, or with the pre-post changes. Similarly, the inclusion of the physiological variables in the ANCOVA model did not produce any alteration of the statistical outcomes.

### Conclusions



This study was the first ever to examine the effects of an acute game of futsal on skeletal muscle protein signalling in young healthy adults. The effects of futsal were compared to a work-matched exercise consisting of continuous running performed at an intensity corresponding to VT1. The results indicate that FUT was comparable to MOD with respect to inducing protein-signalling in skeletal muscle. As these molecular responses have been linked to skeletal muscle adaptations important for the reduction of risk factors for chronic diseases, futsal might be employed as an alternative to traditional aerobic exercise as a lifestyle modification intervention. This is of particular interest, considering that team sports can be more motivating to some individuals than individual exercise [23]. From a more mechanistic perspective, this study also suggests that the activating transcription factor 2 (ATF2) might represent an appropriate tar-

get for investigating the effects of acute exercise of different characteristics (e.g., intensity) on protein-signalling in skeletal muscle.

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**Conflict of interest:** The Authors declare that they have no conflict of interest.

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