

Heat shock proteins in exercised human skeletal muscle



*Örebro Studies in Sport Sciences 28*



MATTIAS FOLKESSON

**Heat shock proteins in exercised human skeletal  
muscle**

© Mattias Folkesson, 2018

*Title:* Heat shock proteins in exercised human skeletal muscle

*Publisher:* Örebro University 2018  
[www.oru.se/publikationer-avhandlingar](http://www.oru.se/publikationer-avhandlingar)

*Print:* Örebro University, Repro 09/2018

ISSN 1654-7535  
ISBN 978-91-7529-260-1

## Abstract

Mattias Folkesson (2018): Heat shock proteins in exercised human skeletal muscle. Örebro Studies in Sport Sciences 28.

Exercise is considered as an important stressor accompanied by concerted molecular and cellular changes leading to adaptations at the level of skeletal muscle size and function. An important protein family produced by cells in response to stressful conditions is the heat shock proteins (HSPs). It is suggested that the different HSPs play specific roles in acute and long-term responses to exercise-induced stress. The overall aim of this thesis was to explore the expression of four different HSPs ( $\alpha$ B-crystallin, HSP27, HSP60 and HSP70) in human skeletal muscle exposed to exercise, with a special emphasis on the role played by HSP27 in the hypertrophy of human skeletal muscle.

One of the major findings was the fibre type-specific expression of HSPs in resting human skeletal muscle, including the preferential expression of HSP27 in fast type II muscle fibres. Another finding was the occurrence of training background-related differences in the expression of HSPs. Also, a cytoplasmic relocation of HSP27, occurring specifically in type II muscle fibres, was shown in response to a single bout of resistance exercise. Interestingly, there were no corresponding changes in response to an endurance exercise bout, suggesting that HSP27 may be specifically involved in the adaptations to resistance exercise. In order to test this hypothesis, an *in-vitro* exercise model based on the electrical pulse stimulation (EPS) of muscle cells was developed. The EPS protocol, including an 8 h restitution period, induced a significant hypertrophy of muscle cells together with molecular changes similar to those previously described in response to exercise in humans. The role of HSP27 in the hypertrophy of human muscle cells was examined through the downregulation of HSP27. Based on data from morphological and micro-array analyses, findings indicate that HSP27 is not mandatory for the hypertrophy of human muscle cells. Overall, the present thesis clarified the expression of different HSPs in human skeletal muscle and provided an *in-vitro*-based approach for the elucidation of the exact role played by HSPs in the adaptations of human skeletal muscle to exercise.

**Keywords:** Endurance training, Resistance training, Muscle Fibre Type, Electrical Pulse Stimulation, Muscle Hypertrophy

Mattias Folkesson, School of Health Sciences  
Örebro University, SE-701 82 Örebro, Sweden, mattias.folkesson@oru.se



## List of publications

- I. Folkesson M, Mackey A L, Langberg H, Oskarsson E, Piehl-Aulin K, Henriksson J, Kadi F (2013). The expression of heat shock protein in human skeletal muscle: effect of muscle fibre phenotype and training background. *Acta Physiol* 209, 26-33
- II. Folkesson M, Mackey A L, Holm L, Kjaer M, Paulsen G, Raastad T, Henriksson J, Kadi F (2008). Immunohistochemical changes in the expression of HSP27 in exercised human vastus lateralis muscle. *Acta Physiol* 194, 215-222
- III. Tarum J, Folkesson M, Atherton P J, Kadi F (2017). Electrical pulse stimulation: an in-vitro exercise model for the induction of human skeletal muscle cell hypertrophy. A proof of concept study. *Exp Physiol*. Nov 1;102(11):1405-1413
- IV. Folkesson M, Kruse R, Kadi F. HSP27 is not mandatory for the hypertrophy of human skeletal muscle cells. In manuscript.

*Published papers have been reprinted with permission from the publisher.*

## List of abbreviations

|                     |                                                                                        |
|---------------------|----------------------------------------------------------------------------------------|
| 1RM                 | One repetition maximum                                                                 |
| 4E-BP1              | 4E-binding protein 1                                                                   |
| ACT                 | Healthy active subjects (study I)                                                      |
| EC                  | Endurance ergometer cycling group (study II)                                           |
| ELISA               | Enzyme-linked immunosorbent assay                                                      |
| END                 | Endurance trained athletes (study I)                                                   |
| EPS                 | Electrical Pulse Stimulation                                                           |
| FBXO32              | F-box only protein 32 (commonly also called Muscle atrophy f-box (MAFbx) or atrogin-1) |
| FC                  | Fold change                                                                            |
| FOXO                | Forkhead box                                                                           |
| GO                  | Gene ontology                                                                          |
| HSE                 | Heat shock element                                                                     |
| HSF                 | Heat shock factor                                                                      |
| HSP                 | Heat Shock Protein                                                                     |
| IGF-1               | Insulin-like growth factor 1                                                           |
| IL-6                | Interleukin 6                                                                          |
| MFI                 | Myogenic fusion index                                                                  |
| mRNA                | Messenger RNA                                                                          |
| mTOR                | Mechanistic target of rapamycin                                                        |
| MPD                 | Muscle protein degradation                                                             |
| MPS                 | Muscle protein synthesis                                                               |
| MyHC                | Myosin heavy chain                                                                     |
| NF- $\kappa$ B      | Nuclear factor kappa-light-chain-enhancer of activated B cells                         |
| RE                  | Resistance exercise group (study II)                                                   |
| RES                 | Resistance trained athletes (study I)                                                  |
| RNA                 | Ribonucleic acid                                                                       |
| ROS                 | Reactive oxygen species                                                                |
| RT-qPCR             | Real-time quantitative polymerase chain reaction                                       |
| S6K1                | Ribosomal protein S6 kinase                                                            |
| siRNA               | Short interfering RNA                                                                  |
| TRIM63              | Tripartite Motif Containing 63 (commonly also called Muscle RING finger-1 (MuRF-1))    |
| VO <sub>2</sub> max | Maximal oxygen consumption                                                             |



# Table of Contents

|                                                                     |    |
|---------------------------------------------------------------------|----|
| INTRODUCTION .....                                                  | 11 |
| Skeletal muscle: an overview .....                                  | 11 |
| Skeletal muscle adaptations to exercise .....                       | 13 |
| Muscle fibre hypertrophy .....                                      | 13 |
| <i>In-vitro</i> exercise models .....                               | 15 |
| Heat shock proteins: An overview .....                              | 15 |
| AlphaB-crystallin .....                                             | 16 |
| HSP27 .....                                                         | 17 |
| HSP60 .....                                                         | 18 |
| HSP70 .....                                                         | 18 |
| Heat shock proteins in exercised human skeletal muscle .....        | 18 |
| Acute and chronic effects of resistance exercise .....              | 18 |
| Acute and chronic effects of endurance exercise .....               | 19 |
| Research gaps .....                                                 | 20 |
| AIMS OF THE THESIS .....                                            | 21 |
| MATERIAL AND METHODS .....                                          | 22 |
| Subjects and study design .....                                     | 22 |
| Muscle biopsy sampling .....                                        | 23 |
| <i>In-vitro</i> studies (study III and IV) .....                    | 24 |
| Purification of muscle cells .....                                  | 24 |
| Electrical pulse stimulation (EPS) .....                            | 24 |
| Down-regulation of HSP27 using siRNA .....                          | 24 |
| Immunohistochemistry and immunofluorescence .....                   | 25 |
| Staining procedures (study I, II, III and IV) .....                 | 25 |
| Muscle fibre type composition and fibre area (study I and II) ..... | 26 |
| Myotube diameter and myogenic fusion index (study III and IV) ..... | 27 |
| Western blot analyses (study III and IV) .....                      | 28 |
| Enzyme-linked Immunosorbent Assay (ELISA) (Study III) .....         | 29 |
| Gene expression analyses (study III and IV) .....                   | 29 |
| Real-time quantitative polymerase chain reaction (RT-qPCR)          |    |
| (Study III) .....                                                   | 29 |
| Microarray analysis (study IV) .....                                | 30 |
| Statistical analyses .....                                          | 30 |
| Ethical considerations .....                                        | 31 |

|                                                                                                                                                |    |
|------------------------------------------------------------------------------------------------------------------------------------------------|----|
| MAIN RESULTS AND DISCUSSION .....                                                                                                              | 32 |
| Expression of $\alpha$ B-crystallin, HSP27, HSP60 and HSP70 in resting skeletal muscle from athletes with different training backgrounds ..... | 32 |
| HSP27 .....                                                                                                                                    | 32 |
| $\alpha$ B-crystallin .....                                                                                                                    | 33 |
| HSP60 .....                                                                                                                                    | 33 |
| HSP70 .....                                                                                                                                    | 34 |
| Acute changes in the expression of HSP27 in human skeletal muscle following resistance and endurance exercise .....                            | 34 |
| Development of an <i>in-vitro</i> exercise model promoting the hypertrophy of human muscle cells.....                                          | 36 |
| EPS and muscle cell size .....                                                                                                                 | 36 |
| Molecular response to EPS .....                                                                                                                | 38 |
| The role of HSP27 in the growth of cultured human skeletal muscle cells.....                                                                   | 39 |
| Methodological considerations.....                                                                                                             | 41 |
| FUTURE PERSPECTIVES.....                                                                                                                       | 43 |
| CONCLUSIONS .....                                                                                                                              | 44 |
| ACKNOWLEDGEMENTS .....                                                                                                                         | 45 |
| SVENSK SAMMANFATTNING .....                                                                                                                    | 46 |
| REFERENCES .....                                                                                                                               | 48 |

# Introduction

Human skeletal muscle exhibits a great capacity to undergo extensive adaptations in response to the stress imposed by changes in the mechanical and metabolic demands. Being a highly plastic tissue, skeletal muscle can adapt its size and function to varying physiological requirements. In this respect, physical exercise is considered as an important stressor as it is accompanied by several mechanical and metabolic disturbances. An exercise that requires the generation of high muscle force during a short period of time will impose a different challenge than an exercise requiring prolonged muscle activity.

Each time skeletal muscle is exposed to a new physiological stress, concerted cellular mechanisms will take place to adapt to changes in usage. An important protein family generated by cells in response to exposure to stressful conditions is the heat shock proteins (HSPs). Although it is suggested that HSPs are involved in the exercise-induced changes in skeletal muscle, knowledge on the specific function played by these proteins during the adaptation to specific exercise modalities is limited. The present thesis explores the expression of four different HSPs ( $\alpha$ B-crystallin, HSP27, HSP60 and HSP70) in human skeletal muscle exposed to acute and chronic exercise, with a special emphasis on the role played by one member of this family (HSP27) in the exercise-induced muscle growth.

## Skeletal muscle: an overview

The entire skeletal muscle is surrounded by a thick layer of connective tissue, the epimysium, and fascicles of muscle fibres are enveloped by another layer of connective tissue, called the perimysium. Finally, a third layer of connective tissue, the endomysium, surrounds each separate muscle fibre (Martini & Timmons, 1997). Connective tissue layers converge to form a tendon, which main function is to anchor muscles onto bones. Skeletal muscle fibres are also surrounded by a vast capillary bed facilitating substrate and gas exchange processes. Finally, the contraction of muscle fibres occurs when motor nerve impulses reach a specific part of the sarcolemma, the inner layer of the endomysium, called the motor endplate.

A specificity of muscle fibres, compared to other cell types, is that they are multinucleated cells containing thousands of myonuclei that are located beneath the sarcolemma. Indeed, during muscle development, thousands of mononucleated myoblasts (committed muscle cell precursors) fuse together

and differentiate into large fusiform multinucleated myotubes that will ultimately become muscle fibres. It is hypothesized that each myonucleus controls a limited cytoplasmic area, a concept called the myonuclear domain (Cheek, 1985).

Each muscle fibre contains thousands of cylindrical myofibrils and each myofibril is made of repetitive units called the sarcomeres, which represent the smallest functional units of a muscle fibre. The myofibrils represent the contractile elements of muscle fibres and the main contractile proteins are actin, myosin, troponin and tropomyosin. Another family of proteins called the cytoskeletal proteins, including titin, desmin and  $\alpha$ -actinin are involved in the maintenance of muscle fibre architecture (Fig 1).

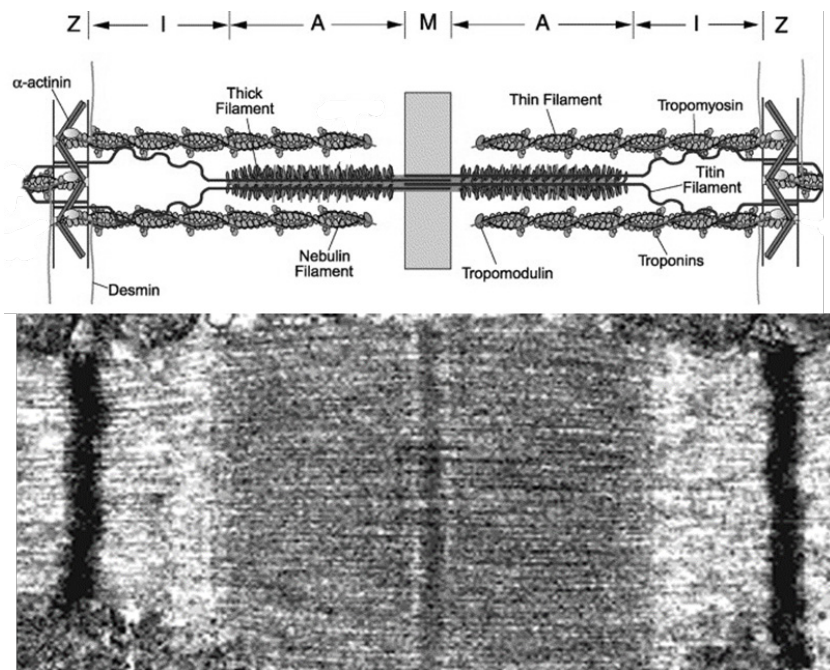


Figure 1. Top: Model of sarcomere structure. Bottom: Electron microscopy photograph of the ultrastructural organization of a sarcomere.

Human skeletal muscle consists of different muscle fibre types. Based on myosin heavy chain (MyHC) content, human muscle fibres are commonly classified into three main types (I, IIA, IIX) and two hybrid types (I/IIA –

IIA/IX) (Pette & Staron, 2000; Schiaffino, 2010). The three main fibre types display differences regarding morphological, contractile and metabolic properties. Compared to type IIX fibres, type I fibres show higher capillary and mitochondrial density, higher oxidative and lower glycolytic potential and longer time to peak tension (Schiaffino & Reggiani, 2011). Type I fibres are recruited during sustained muscle contractions with relative low force generation whereas type IIX fibres are recruited during rapid muscle contractions with relative high force generation. Type IIA fibres display intermediate morphological, contractile and metabolic properties between type I and type IIX fibres (Schiaffino & Reggiani, 2011). Type I and type IIA muscle fibres are the predominant muscle fibre types in human limb muscles (Schiaffino, 2010).

### **Skeletal muscle adaptations to exercise**

Human skeletal muscle is characterized by a high degree of plasticity, enabling adaptations to a variety of physiological demands. Exercise-related skeletal muscle adaptations are specific to the exercise modality. Although a continuum of different exercise modalities exists, two broad categories can be distinguished: resistance and endurance exercises. These two main exercise modalities are associated with distinct morphological, metabolic and functional adaptations in human skeletal muscle. The adaptations that occur in skeletal muscle following a period of endurance exercise include improved oxidative metabolism, increased mitochondrial function and capillary density (Holloszy & Coyle, 1984). In response to resistance exercise, increases in the force generating capacity and the size of muscle fibres (mainly that of type II muscle fibres) are two main adaptations in skeletal muscle (Fry 2004, Folland & Williams 2007). Additionally, a type IIX to type IIA fibre transition is also reported following prolonged periods of resistance exercise (Fry, 2004), whereas a shift from type IIA to type I has been reported following prolonged periods of endurance exercise (Wilson et al., 2012).

### **Muscle fibre hypertrophy**

One of the major adaptations to resistance training is the occurrence of muscle fibre hypertrophy. The exercise-induced increase in the size of muscle fibres, with concomitant improved muscle strength, has important implications both in term of athletic performance and optimal physical function in the general population. A key factor determining the magnitude of increased or decrease muscle mass is the net balance between muscle protein

synthesis (MPS) and muscle protein degradation (MPD). Several signalling pathways including the mechanistic target of rapamycin (mTOR) and myostatin pathways have been linked to the occurrence of fibre hypertrophy. Moreover, the involvement of satellite cells and myonuclei in the hypertrophic process has also been documented.

### 1. Mechanistic target of rapamycin (mTOR) and myostatin pathways

In order to increase protein synthesis, muscle cells need to increase the mRNA-governed translation of amino acids into proteins by the ribosome. Activation of the growth-regulating kinase mTOR has been shown to increase protein synthesis via activation of the downstream targets ribosomal protein S6 kinase (S6K1) and 4E-binding protein 1 (4E-BP1) (Bodine, 2006; Bodine et al., 2001). Upstream activators of mTOR include hormonal (for example insulin-like growth factor 1, IGF-1), mechanical (due to the tension in the muscle fibre) or nutritional (amino acids) stimuli whereas factors that can inhibit mTOR signalling include glucocorticoids and myostatin (McCarthy & Esser, 2010; Schiaffino et al., 2013; Yoon, 2017). In addition to inhibition of mTOR activation, an increased level of myostatin has been shown to enhance muscle protein degradation via activation of Forkhead box (FOXO) transcription factors, including F-box only protein 32 (FBXO32, in literature also commonly called MAFbx or atrogin-1) and Tripartite Motif Containing 63 (TRIM63, also commonly called MuRF-1) (McCarthy & Esser, 2010; Rodriguez et al., 2014). Although not reported in all studies, several reports have shown that engagement in resistance exercise is associated to activation of the mTOR pathway (Walker et al., 2011) and repression of myostatin (Roth et al., 2003), which may be a prerequisite for increased muscle mass.

### 2. Satellite cells and myonuclei

Satellite cells are the stem cells of skeletal muscle and are essential for the generation of new muscle fibres and reparation of exercise-induced muscle fibre damage (Relaix & Zammit, 2012). Satellite cells may also generate new myonuclei to enable the hypertrophy of muscle fibres (Kadi & Thornell, 2000; Montarras et al., 2013). Given that satellite cells are donors of new myonuclei in adult muscle fibres, several reports have highlighted their role in the hypertrophy of muscle fibres in response to resistance exer-

cise (Snijders et al., 2015). It is currently suggested that myonuclear accretion occurs in human skeletal muscle when a ceiling size is reached (Fry et al., 2014; Kadi et al., 2005). Moreover, using *in-vivo* imaging techniques in rodents, it was clearly shown that a significant enlargement of single fibres requires addition of new myonuclei (Egner et al., 2016).

### ***In-vitro* exercise models**

Current knowledge on the mechanisms underlying the adaptations to exercise remains limited due to the complexity of the molecular and cellular processes occurring in skeletal muscle and other organs. In this respect, it is currently suggested that the use of *in-vitro* models with cultured muscle cells would allow progress in understanding the mechanisms underlying changes at the level of skeletal muscle. Although these models do not reflect the complexity of an *in-vivo* context, they do offer a number of possibilities to modulate the cellular environment in order to facilitate our understanding of specific physiological adaptations. Additionally, the use of human muscle cells may prove useful given that myogenic cells isolated from a muscle biopsy may retain the *in-vivo* phenotype of that muscle (for example impaired glucose metabolism) (Aas et al., 2013; Gaster et al., 2002; Henry et al., 1996).

Electrical pulse stimulation (EPS) of cultured human muscle cells has been used in order to mimic *in-vivo* muscle adaptations to exercise. In such an approach, motor neuron activation of muscle fibres would be replicated by EPS. Indeed, it has been shown that EPS of muscle cells may reproduce some adaptations similar to those commonly observed in response to exercise in humans, including changes at the mRNA and protein levels of key exercise-regulated factors (Nikolic et al., 2017). Despite inherent limitations including the absence of innervation and interaction with other cells and tissues, the use of EPS and cultured human muscle cells is currently regarded as a valuable tool for the exploration of the mechanisms underlying the adaptations of skeletal muscle to exercise (Nikolic et al., 2017). However, further research is needed to refine and adapt these models in order to better mimic the main *in-vivo* adaptations to exercise in humans.

### **Heat shock proteins: An overview**

Different triggers including hypoxia, reduced glucose availability, oxygen-derived free radicals and mechanical stress can initiate the adaptations of human skeletal muscle to exercise through specific down-stream molecules.

Among these molecules, the heat shock proteins (HSPs) have been shown to be involved in the exercise-related adaptations of skeletal muscle (Morton et al., 2009b). The HSPs were discovered in the early 1960's (Ritossa, 1962) and in the 1990's it was reported that the stress initiated by physical exercise can elicit an HSP response in mammalian skeletal muscle (Locke et al., 1990). HSPs is a highly conserved family of proteins present in almost all eukaryotic cells, including skeletal muscle cells (Liu & Steinacker, 2001). Although these proteins are induced in response to several stressors they are still called heat shock proteins in reference to the early studies examining cellular thermotolerance. The primary roles of HSPs include (1) the maintenance of cellular function by stabilizing and refolding denatured proteins, (2) the targeting of irreversible denatured proteins for degradation and (3) the participation in cellular signalling (Locke & Noble, 2002; Stice & Knowlton, 2008).

As reviewed by Liu and Steinacker (2001), the regulation of HSP level is suggested to occur via a negatively self-regulated loop involving a heat shock factor (HSF). During non-stress conditions, HSF is bound to an HSP, whereas upon stress application the HSP is released in order to assist damaged or impaired proteins. The unbound HSF can translocate into the nucleus and interact with a region on the HSP gene called the heat shock element (HSE) leading to the up-regulation of HSP gene expression. The newly produced HSP will assist in cellular protection whereas excess HSP will re-bind free HSF, thereby decreasing the HSF-HSE interaction, which leads to down-regulation of HSP generation.

Members of the HSP family are frequently named according to their molecule weight (e.g. the HSP with a molecule weight of 27 kDa is called HSP27). More recently, a recommended nomenclature has been issued by the HUGO Gene Nomenclature Committee (Kampinga et al., 2009). In this thesis, the following HSPs have been investigated: HSP27,  $\alpha$ B-crystallin, HSP60 and HSP70, corresponding respectively to HSPB1, HSPB5, HSPD1 and HSPA1A according to the HUGO-nomenclature. These four HSPs are the most frequently studied HSPs in the context of skeletal muscle adaptation to exercise (Morton et al., 2009b).

### **AlphaB-crystallin**

AlphaB-crystallin is present in several tissues, including heart, lung, brain, kidney and skeletal muscle (Horwitz, 2003). AlphaB-crystallin is classified as a small heat shock protein (Klemenz et al. 1991) and it is shown to facilitate protein synthesis, folding and assembly (Horwitz, 1992; Jakob et al.,



1993). In rodent skeletal muscle,  $\alpha$ B-crystallin is found abundantly in fibres with high oxidative capacity i.e. type I fibres, whereas it is rarely detected in glycolytic type II fibres (Neufer & Benjamin, 1996). In human skeletal muscle,  $\alpha$ B-crystallin has been reported to bind to myofibrillar molecules like actin and desmin to increase myofilament stability e.g. during high-force muscular contractions, especially eccentric contractions (Paulsen et al., 2009; Paulsen et al., 2007). In addition to the putative role of  $\alpha$ B-crystallin in muscle cell protection during exercise-induced stress, it is suggested that this HSP is involved in skeletal muscle differentiation and muscle growth (Dimauro et al., 2017).

## **HSP27**

HSP27 shows several structural similarities with  $\alpha$ B-crystallin (de Jong et al., 1993) and has also been shown to act as a molecular chaperone (Jakob et al., 1993). HSP27 is suggested to stabilize stress-altered proteins until other molecular chaperones like HSP70 either refold the damaged proteins or recognize and target them for degradation (Arrigo, 2012; Mymrikov et al., 2011). In rodent skeletal muscle, HSP27 is expressed abundantly in type I fibres and to a smaller extent in type II fibres (Neufer & Benjamin, 1996). During non-stress conditions HSP27 is localized in muscle cytoplasm, whereas upon stress application it can be translocated to the nucleus and, similar to  $\alpha$ B-crystallin, HSP27 can also translocate to cytoskeletal/myofibrillar proteins immediately following forceful eccentric contractions (Morton et al., 2009b).

It is hypothesized that HSP27 is involved in the regulation of skeletal muscle mass. While some animal studies suggested that changes in HSP27 expression may play an important role in the regulation of muscle mass (Huey, 2006; Kawano et al., 2007; Zhang et al., 2014); (Middleton & Shelden, 2013), no significant difference in fibre size were found between mice genetically manipulated to lack HSP27 (HSP27<sup>-/-</sup> mice) and control mice (Kammoun et al., 2016). It is further hypothesized that HSP27 may be involved in the regulation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) signalling pathway (Dodd et al., 2009) and that it may promote net protein synthesis in rodent muscles by the attenuation of muscle protein degradation through down-regulation of FOXO transcription factors TRIM63 and FBXO32 (McCarthy & Esser, 2010). Currently, the involvement of HSP27 in the hypertrophy of human skeletal muscle is unknown.

## **HSP60**

In human skeletal muscle, HSP60 is primarily located within the mitochondria and is suggested to assist in the proper folding and assembly of proteins translocating from the cytosol into the mitochondria (Liu & Steinacker, 2001). In rodent skeletal muscle, HSP60 expression was found highly correlated to mitochondrial content (Ornatsky, Connor, & Hood, 1995). HSP60 in rodents is present at a higher extent in slow-twitch muscles with high oxidative capacity compared to fast-twitch muscles (Mattson et al., 2000). In human vastus lateralis, a higher level of HSP60 is found in endurance trained men compared to untrained men (Morton et al., 2008), possibly due to a higher proportion of oxidative type I fibres in endurance trained men.

## **HSP70**

The term HSP70 relates to a subfamily of HSPs rather than one specific HSP member. Several different isoforms exist in skeletal muscle and among these, HSP72 is the best characterized in response to exercise (Locke & Noble, 2002) and is frequently referred to as HSP70 in the literature. HSP70 is not abundantly expressed in non-stress conditions and can be rapidly synthesized in response to different forms of stress (Henstridge et al., 2016; Liu & Steinacker, 2001). HSP70 acts to maintain correct protein folding, prevent protein aggregation and assist in degradation of unstable proteins (Locke & Noble, 2002). In rodents, muscles consisting of primarily type I fibres show higher expression of HSP70 than muscles consisting of primarily type II fibres (Locke et al., 1991). In mice genetically manipulated to overexpress HSP70 in skeletal muscle, a better preservation of muscle function after immobilization was reported (Miyabara et al., 2012).

## **Heat shock proteins in exercised human skeletal muscle**

### **Acute and chronic effects of resistance exercise**

Increases in  $\alpha$ B-crystallin, HSP27 and HSP70 content, both at mRNA and protein levels, have been reported in the hours and days following a single bout of exercise including forceful contractions (Paulsen et al., 2009; Paulsen et al., 2007; Thompson et al., 2002; Thompson et al., 2001). In addition to the upregulation of HSP levels, an intracellular relocation of HSP27 and  $\alpha$ B-crystallin from a cytosolic location to sarcomeric structures has been suggested to occur (Koskinen et al., 2017; Paulsen et al., 2009; Paulsen et al., 2007). It is hypothesized that this relocation allows these

small HSPs to act as so called “holdases” in order to stabilize stress-altered proteins following the high mechanical stress induced by high-force muscular contractions (Arrigo, 2012). Indeed, as indicated by increased levels of circulating creatine kinase and decreased post-exercise voluntary force production, the exercise performed in the studies above induced pronounced muscle damage. Interestingly, similar findings including an immediate relocation of HSP27 and  $\alpha$ B-crystallin to cytoskeletal structures were reported in response to a non-damaging resistance exercise protocol based on low-load blood-flow-restricted exercise (Cumming et al., 2014).

A longer period of strength training (5-11 weeks) is shown to up-regulate baseline cytosolic protein levels of  $\alpha$ B-crystallin, HSP27 and HSP70 (Gjovaag & Dahl, 2006; Paulsen et al., 2012). It is currently suggested that HSP levels increase mostly during the first period of training when muscles are still unaccustomed to the exercise modality (Gjovaag & Dahl, 2006). This is supported by studies showing a reduced HSP response in the second exercise session compared to the first one (Morton et al., 2009b; Paulsen et al., 2009; Thompson et al., 2002; Vissing et al., 2009).

### **Acute and chronic effects of endurance exercise**

Two to six days following a single session of endurance exercise (cycling or running at an intensity corresponding to approximately 70% of  $\text{VO}_2\text{max}$ ), increases in protein expression of HSP60 and HSP70 but not  $\alpha$ B-crystallin or HSP27 were reported in exercised skeletal muscle (Khassaf et al., 2001; Morton et al., 2006). Morton et al. (2006) suggested that the exercise protocol used in their study does not elicit a high mechanical stress in skeletal muscle and thus, was not accompanied by elevated  $\alpha$ B-crystallin or HSP27 levels. In another study involving 30 min of downhill treadmill running, the expression of  $\alpha$ B-crystallin and HSP27 increased in human skeletal muscle at 1 and 14 days after the exercise (Feasson et al., 2002). These increases were suggested to be related to the occurrence of exercise-induced muscle damage given the high mechanical stress generated during the eccentric muscle contractions due to the negative incline of the treadmill. Following a non-damaging endurance exercise, the HSP response is suggested to be related to the metabolic stress, including increased levels of reactive oxidative species (ROS) (Khassaf et al., 2001; Morton et al., 2006).

An investigation of the chronic effects of endurance exercise on HSP levels in human skeletal muscle showed higher levels of  $\alpha$ B-crystallin and HSP60 and similar levels of HSP27 and HSP70 in trained compared to untrained subjects (Morton et al., 2008). In contrast, significant increases

in the expression of HSP70 in skeletal muscle were reported in response to chronic endurance training (Liu et al., 2000; Liu et al., 1999). It was also suggested that the chronic HSP response is related to training intensity as levels of HSP70 were higher following a training phase with increased intensity compared to a training phase with reduced training intensity (Liu et al., 2000).

## **Research gaps**

Overall, several evidences suggest that HSPs are involved in the remodelling process of skeletal muscle in response to different exercise modalities. There are, however, several aspects that need to be clarified in order to improve our knowledge on the exact involvement of HSPs in the adaptive processes of skeletal muscle. Indeed, knowledge on the expression of different HSPs in skeletal muscle of athletes with a background in either endurance or resistance training remains scarce. There is also paucity of studies investigating the fibre type specific expression of different HSPs in human skeletal muscle of athletes with different training backgrounds. Moreover, the exact role of HSPs in the adaptation of skeletal muscle to exercise remains unclear. For example, although HSP27 is suggested to be involved in the adaptation to resistance exercise (Huey, 2006; Kawano et al., 2007), whether this HSP is instrumental for muscle hypertrophy needs to be clarified.

## Aims of the thesis

The overall aim of the studies performed within the frame of the present thesis was to examine the expression of four heat shock proteins (HSPs) in exercised human skeletal muscle and to determine the role played by HSP27 in the hypertrophy of human muscle cells exposed to an *in-vitro* exercise model.

The specific aims were:

- To examine the fibre type-specific expression of  $\alpha$ B-crystallin, HSP27, HSP60 and HSP70 in resting skeletal muscle from athletes with different training backgrounds (study I)
- To investigate the fibre type-specific acute changes in the expression of  $\alpha$ B-crystallin, HSP27, HSP60 and HSP70 in human skeletal muscle following resistance and endurance exercise (study II)
- To develop an *in-vitro* exercise model based on electrical pulse stimulation and promoting the hypertrophy of human muscle cells (study III)
- To examine the role of HSP27 in the hypertrophy of human muscle cells during muscle differentiation and in response to *in-vitro* electrical pulse stimulation (study IV)

# Material and methods

## Subjects and study design

In study I, three groups of subjects with different training backgrounds were recruited (Table 1). One group consisted of physically active healthy subjects not engaged in any specific training (ACT). The other two groups included well-trained athletes, either endurance trained athletes (END) or athletes with a long history of resistance training (RES). Subjects in the END group were experienced runners or adventure racers with a  $\text{VO}_{2\text{max}}$  of  $4.7 \pm 0.7 \text{ l}\cdot\text{min}^{-1}$  (equivalent to  $60.4 \pm 6.8 \text{ ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ ) and athletes in the RES group were power lifters competing at a national level, with personal records in squat lift, bench press and dead lift corresponding to  $281 \pm 47 \text{ kg}$ ,  $180 \pm 32 \text{ kg}$  and  $284 \pm 45 \text{ kg}$  respectively. All muscle biopsies were taken at rest.

Table 1. Characteristics of subjects included in study I. ACT = Healthy active subjects, END = Endurance trained athletes, RES = Resistance trained athletes.

|                                       | ACT            | END                       | RES                  |
|---------------------------------------|----------------|---------------------------|----------------------|
| N (female / male)                     | 12 (1/11)      | 8 (0/8)                   | 6 (0/6)              |
| Age (year)                            | $22 \pm 4$     | $24 \pm 3$                | $25 \pm 6$           |
| Height (cm)                           | $179 \pm 5$    | $182 \pm 7$               | $175 \pm 6$          |
| Weight (kg)                           | $78 \pm 12$    | $78 \pm 11$               | $92 \pm 10$          |
| BMI ( $\text{kg}\cdot\text{m}^{-2}$ ) | $24.3 \pm 2.8$ | $23.4 \pm 1.8$            | $29.9 \pm 1.4^*$     |
| <i>Muscle fibre type composition</i>  |                |                           |                      |
| Type I (%)                            | $46.9 \pm 2.5$ | $67.4 \pm 14.0^{\dagger}$ | $41.7 \pm 4.3$       |
| Type IIA (%)                          | $44.5 \pm 3.1$ | $32.6 \pm 14.0^{\#}$      | $58.3 \pm 4.3^*$     |
| Type IIX (%)                          | $8.5 \pm 2.4$  | $0 \pm 0$                 | $0 \pm 0$            |
| <i>Muscle fibre area</i>              |                |                           |                      |
| Type I ( $\mu\text{m}^2$ )            | $3655 \pm 551$ | $6415 \pm 885^{\#}$       | $6180 \pm 1091^{\#}$ |
| Type II ( $\mu\text{m}^2$ )           | $3945 \pm 617$ | $7281 \pm 939^{\#}$       | $9941 \pm 2440^*$    |

\* Significantly different from ACT and END

$\dagger$  Significantly different from ACT and RES

$\#$  Significantly different from ACT

In study II, six participants ( $26 \pm 5$  years,  $74 \pm 9$  kg and  $172 \pm 3$  cm) performed endurance ergometer cycling (EC) and nine participants ( $24 \pm 2$  years,  $80 \pm 16$  kg and  $183 \pm 5$  cm) resistance exercise (RE). All subjects were healthy and physically active male students, not engaged in any specific exercise training programme. The EC group performed one-legged ergometer cycling for 30 minutes at two different exercise intensities, corresponding to 40% and 75% of one-legged peak  $\text{VO}_2$ . Pedalling cadence was determined to 60 revolutions per minute. The two bouts of biking were separated 6-9 days apart and at both sessions exercise was performed using the right leg while the left leg served as a non-exercised control leg. The RE group performed a resistance exercise consisting of one-legged knee extensions at an intensity corresponding to 70% of 1 RM. This exercise session included ten sets of eight repetitions and was performed at a self-selected pace, typically less than three seconds to complete each repetition, including both the concentric and the eccentric phase. Non-exercised leg served as a control.

In study III, myoblasts were obtained from muscle biopsies taken from five healthy active subjects (1 female and 4 male, age  $36 \pm 5$  years). Muscle cells were purified and cultured. Cultured muscle cells underwent electrical pulse stimulation (EPS). Several EPS protocols were evaluated in order to develop a protocol able to promote the hypertrophy of cultured human muscle cells.

In study IV, myoblasts were obtained from muscle biopsies taken from ten healthy and physically active subjects (6 female and 4 male, age  $49 \pm 18$  years). The expression of HSP27 was down-regulated using short interfering RNA (siRNA) in order to examine its role in the hypertrophy of muscle cells during the differentiation of muscle cells and in response to EPS.

## **Muscle biopsy sampling**

All muscle biopsies were taken by trained medical doctors from the mid-portion of the vastus lateralis muscle. After local anaesthesia was performed, a small incision was made through the skin and muscle fascia before a muscle sample was taken. Biopsies were rinsed from visible fat and connective tissue using sterile scalpels. For study I and II, muscle samples were immediately embedded in an embedding medium, frozen in isopentane cooled in liquid nitrogen and stored at  $-80^\circ\text{C}$  before they were assessed using immunohistochemistry. In study III and IV, muscle samples were minced into smaller pieces and placed in freezing media (90% fetal bovine serum (HyClone, France) and 10% dimethyl sulfoxide (Sigma Aldrich,

USA)) and then stored in liquid nitrogen before they were processed for cell culture experiments.

## ***In-vitro* studies (study III and IV)**

### **Purification of muscle cells**

Minced pieces were cultured for 6-15 days embedded in a layer of Matrigel (6mg/ml, Matrigel Matrix, BD Biosciences, Le Pont de Claix, France) with growth media (containing 20% fetal bovine serum) replaced every 48 h. Following migration, cells were harvested and subcultured for 24-48 h in growth media. Myoblasts were then immunomagnetically purified and proliferated in growth media for 48-72 h. At purification myoblasts were considered to be at passage 0. All experiments were performed at passage 4 or 5. For each experiment, myoblasts were seeded and proliferated for approximately 48 h to reach 80% of full confluence.

### **Electrical pulse stimulation (EPS)**

Cultured myoblasts were fully differentiated into multinucleated myotubes following 48 h incubation in differentiation media (containing 2% fetal bovine serum). Myotubes were then exposed to electrical pulse stimulation using C-Pace EP culture pacer (IonOptix, Dublin, Ireland). EPS protocols with different durations (1.5 to 24 h), voltages (5 to 30 V), frequencies (0.5 or 1 Hz) and length of pulse trains (2 or 5 ms) were tested. Different restitution times (0, 4 or 8 h) were applied following the EPS protocol in order to let stimulated myotubes recover from “training”. The final EPS protocol, able to promote hypertrophy of myotubes, consisted of 8 hours stimulation with 12 V and 2 ms long pulse trains at 1 Hz followed by 8 hours of restitution. All experiments were run in parallel with myotubes either stimulated (EPS) or with non-stimulated myotubes acting as controls.

### **Down-regulation of HSP27 using siRNA**

During the differentiation of myoblasts into myotubes, cells were transfected with short interfering RNA (siRNA) specifically targeting HSP27 (HSP27 siRNA II, catalogue number 6526, Cell signalling technology), using lipofectamine (Lipofectamine 2000, Invitrogene) as a transfection agent. Transfecting cells with HSP27 siRNA degraded HSP27 mRNA and subsequently also reduced HSP27 at protein level. A non-specific scrambled siRNA (catalogue number 6201, Cell signalling technology) was used as a control to validate the specificity of the HSP27 siRNA. In this procedure,



several different concentrations of HSP27 siRNA and lipofectamine were combined in order to determine the optimal concentrations able to down-regulate levels of HSP27 mRNA using the HSP27 siRNA, but keeping levels of HSP27 mRNA almost unaffected using the scrambled siRNA.

## **Immunohistochemistry and immunofluorescence**

### **Staining procedures (study I, II, III and IV)**

To prepare samples for immunohistochemical analyses, muscle biopsies (study I and II) were cut at -20°C into 5 µm-thin cross-sections using a cryotome (Leica CM1850, Leica Microsystems, Germany) and mounted on glass slides. In study III and IV, cultured myotubes were fixed using 2% paraformaldehyde and permeabilized with 0.25% Triton X-100 (Sigma Aldrich). Primary antibodies used are described in table 2. For study I and II, visualization of primary antibody binding sites was made according to the Avidin-Biotin-Complex (ABC) method. This method included binding of a biotinylated secondary antibody to the specific primary antibody, thereafter an avidin-biotin-peroxidase complex was bound to the biotinylated secondary antibody. Finally, DAB (3,3'-diaminobenzidine) substrate kit for peroxidase was used, giving the targeted antigen a brown colour with an intensity proportional to the presence of the actual antigen in the sample. Images were acquired using a light microscope (Nikon Eclipse E400, Nikon Instruments, the Netherlands) connected to a digital camera (SPOT Insight, Diagnostic Instruments, Sterling Heights, MI, USA). Analyses of images were performed using Sigma Scan Pro (Image Analysis, Version 5.0.0). Densitometric analyses of staining intensity in muscle fibre cytoplasm were performed by software quantification of pixel intensity on a 0-255 scale, where 0 corresponds to black and 255 to white.

In study III and IV fluorescent labelled secondary antibodies were used, yielding either green (Alexa 488, goat anti-mouse, Thermo Fisher Scientific) or red (Alexa 568, goat anti-rabbit, Thermo Fisher Scientific) fluorescent labelling when visualized using a fluorescent microscope (Zeiss Axiovert, Carl Zeiss AG, Oberkochen, Germany). DAPI (4',6-diamidino-2-phenylindole) was used to stain nuclei and yield a blue fluorescent colour. Image acquisition was made using ZEN Microscope software (Carl Zeiss AG) and images were analysed with ImageJ Software (U.S. National Institutes of Health, Maryland, USA).











Table 2. Primary antibodies used for immunohistochemical staining in study I-IV.

| Study        | Primary anti-body     | Type       | Dilution |
|--------------|-----------------------|------------|----------|
| I and II     | A4.951                | Monoclonal | 1:50     |
| I and II     | N2.261                | Monoclonal | 1:100    |
| I, II and IV | HSP27                 | Monoclonal | 1:100    |
| I and II     | HSP60                 | Monoclonal | 1:300    |
| I and II     | HSP70 (HSP72)         | Monoclonal | 1:300    |
| I and II     | $\alpha$ B-crystallin | Monoclonal | 1:400    |
| III and IV   | Myogenin              | Polyclonal | 1:200    |
| III and IV   | Troponin-T            | Monoclonal | 1:100    |

### Muscle fibre type composition and fibre area (study I and II)

Determination of muscle fibre type was performed according to Kadi et al. (1998) using two monoclonal antibodies against myosin heavy chains (MyHC); A4.951 against MyHC type I and N2.261 against MyHC I and IIA. By combining staining patterns from these two monoclonal antibodies five fibre types were identified as described in table 3 and figure 2.

Table 3. Immunohistochemical determination of fibre type composition based on myosin heavy chain content.

| Antibody | I                                                                                   | I/IIA                                                                               | IIA                                                                                 | IIA/IIIX                                                                            | IIIX                                                                                |
|----------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| A4.951   |  |  |  |  |  |
| N2.261   |  |  |  |  |  |

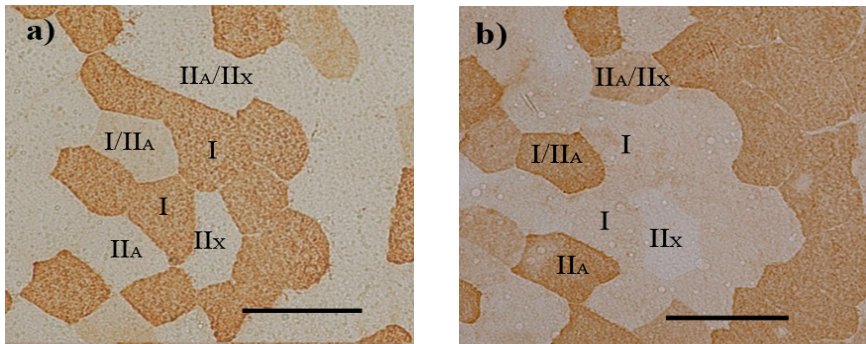


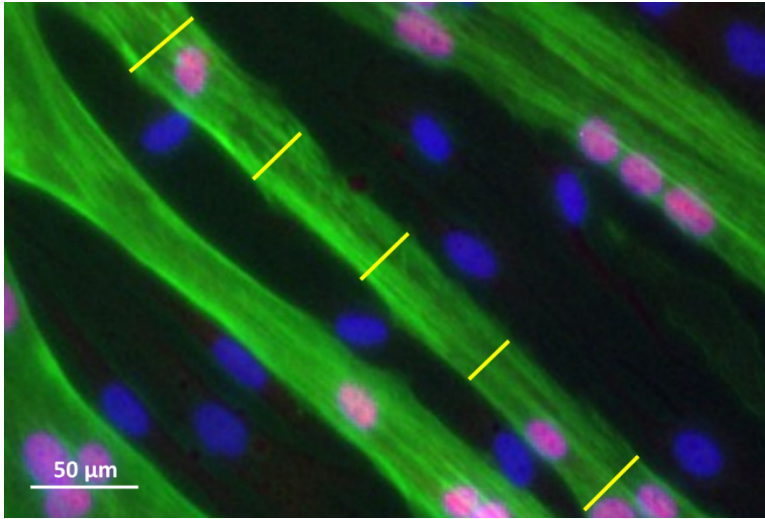
Figure 2. Classification of muscle fibre types using two consecutive muscle cross-sections stained with primary antibodies A4.951 (a) and N2.261 (b). (Scale bar = 200  $\mu$ m)

Fibre area was measured using images taken at magnification x20. As type I/II<sub>A</sub>, type II<sub>A</sub>/II<sub>X</sub> and type II<sub>X</sub> fibres were rare, measurement of fibre area was not performed for these fibre types.

### Myotube diameter and myogenic fusion index (study III and IV)

For assessment of myotube diameter the mean of five measurements along the length of a myotube was used (Fig. 3). Measurements from at least five randomly selected areas in each well, including in total at least 100 myotubes, were used for determination of tube diameter in each cell culture. During measurements, the operator was blinded to the culture conditions being analysed.

Myogenic fusion index (MFI) was defined as the ratio between the number of nuclei inside the myotubes and expressing myogenin and the total number of nuclei in a given microscopic field. For calculation of MFI at least five randomly selected fields were used and an average of 1500 myonuclei per well were counted in each culture.



*Figure 3. Myotubes immunohistochemically stained for assessment of tube diameter and myogenic fusion index (MFI). An antibody against troponin T was used to stain myotubes in green. DAPI stains all nuclei in blue and myogenin-positive nuclei are stained in red. In order to calculate the average diameter of the selected myotube, five measurements along the length of that myotube were used (yellow lines).*

### **Western blot analyses (study III and IV)**

Wells were rinsed with PBS before adding 50  $\mu$ l of lysing buffer containing Tris-HCL 50 mM, EDTA 1 mM, EGTA 1 mM,  $\beta$ -Glycerophosphate 10 mM, NaF 50 mM, Sodium Orthovanadate 0.5 mM and protease inhibitor tablet (Roche Applied Science). Cell lysates were then sonicated, vortexed, incubated on ice for 30 min and centrifuged at 12000  $\times$  g for 10 min at +4° before supernatant was collected. Protein concentration was determined using Pierce<sup>TM</sup> BCA protein assay kit (Thermo Scientific) with a microplate reader. Equal amounts of protein from cell lysates prepared in Laemmli buffer were separated on 8-12% SDS-polyacrylamide gels at 200 V for 65-90 min and subsequently transferred to nitrocellulose membranes at 100 V for 60 min (Mini Trans-Blot Electrophoretic Transfer Cell, Bio-Rad). A blocking step for 1 h at room temperature with 5% non-fat dry milk was followed by 2 h incubation with primary antibodies against HSP27 (1:1000,

NCL-HSP27, Novocastra), phosphorylated HSP27 (1:2000, phospho S15, ab76313, AbCam), phospho-mTOR (1:1000, Ser2448, cat no #5536, Cell Signalling Technology), phospho-4E-BP1 (1:1000, Thr37/46, cat no #9459, Cell Signalling Technology), phospho-AMPK (1:1000, Thr172, cat no #4188, Cell Signalling Technology) and phospho-P70S6K1 (1:1000, Thr389, cat no #9234, Cell Signalling Technology). All incubations were performed at room temperature and blots were normalized to  $\beta$ -actin (cat no #4967, Cell Signaling Technology). Membranes were treated with chemiluminescent HRP-substrate (LI-COR Biosciences, Nebraska, USA) to visualize bands and Image Studio<sup>TM</sup>Software was used for quantification of band intensities.

### **Enzyme-linked Immunosorbent Assay (ELISA) (Study III)**

For assessment of myotube secretion of interleukin-6 (IL-6) into cell culture media, a human IL-6 ELISA-kit (cat no #900-T16, PeproTech) was used together with a TMB ELISA buffer kit (cat no #900-T00, PeproTech). All procedures followed the instructions given by the manufacturer and all incubations were performed at room temperature. In this sandwich ELISA an IL-6 specific capture antibody (100 $\mu$ g/ml) was coated to the wells and after blocking (1% BSA in PBS) to prevent unspecific binding to the well, samples and standards were added. For detection, a biotinylated detection antibody against IL-6 (100  $\mu$ g/ml) was used. As secondary antibody streptavidin horseradish peroxidase (S-HRP; 0.05  $\mu$ g/ml) was used and Tetramethylbenzidine (TMB) was used as a visualizing agent together with 1 M hydrochloric acid (HCl) as a stop solution. Recombinant human IL-6 was used as standards (ranging from 3.9 pg/ml to 2000 pg/ml). Cell culture media was diluted 1:1 in supplied diluent buffer (0.05% Tween-20, 0.1% BSA in PBS) and analysed in triplicates. A microplate reader at 450 nm was used to monitor colour development, and from the generated standard curve the amount of IL-6 in the samples could be calculated.

### **Gene expression analyses (study III and IV)**

#### **Real-time quantitative polymerase chain reaction (RT-qPCR) (Study III)**

For analysis of gene expression using RT-qPCR, total RNA was extracted from cultured myotubes using NucleoSpin<sup>®</sup> RNA Isolation kit (Macherey-Nagel, Düren, Germany). Quantity and purity were determined using a NanoDrop 2000 (Thermo Fisher Scientific). Following extraction, total RNA was reversely transcribed into complementary DNA (cDNA) using a

High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Carlsbad, CA, USA). Real-time qPCR was performed using TaqMan® Fast Universal PCR Master Mix (2X; Applied Biosystems) with gene-specific primers to quantify myostatin (Hs00976237\_m1, Applied Biosystems) mRNA levels in relation to reference gene GAPDH (glyceraldehyde 3-phosphate dehydrogenase) (Hs03929097\_g1, Applied Biosystems). The  $\Delta\Delta C_t$  method was used to analyse normalized levels of myostatin mRNA in stimulated myotubes compared to non-stimulated controls.

#### **Microarray analysis (study IV)**

Total RNA was extracted using NucleoSpin® RNA Isolation Kit (Macherey-Nagel, Düren, Germany). RNA concentration and purity were checked by spectrophotometric measurement using a NanoDrop 2000 (Thermo Fisher Scientific). RNA quality was further assessed using the Agilent 2100 Bioanalyzer with RNA 6000 nano kit (Agilent Technologies, Waldbronn, Germany). All samples showed RNA integrity number above 9.7 on a scale where 10 is the maximum value. One-color Microarray-Based Gene Expression Analysis (Agilent Technologies) was performed according to protocol “One-color Microarray-based Gene expression Analysis” (Agilent Technologies, version 6.9.1). An input of 50 ng total RNA was used to prepare labelled cRNA according to manufacturer instructions, using One-color Low Input Quick Amp Labelling Kit (Agilent). Samples were hybridized onto SurePrint G3 Human Gene Expression 8x60k glass slides (Agilent) in a G2545A hybridization oven (Agilent). Slides were scanned using a G2565A array laser scanner (Agilent) and image analysis and data extraction were performed with Feature Extraction Software (version 10.7.3.1, Agilent Technologies).

#### **Statistical analyses**

In study I, between-group differences in subject and muscle characteristics were analysed using one-way ANOVA followed by Bonferroni post-hoc procedure. Comparison of staining intensities between type I and type II fibres was performed using Wilcoxon’s signed-rank test.

In study II, Wilcoxon’s signed-rank test was used to analyse differences in protein expression between pre and post exercise conditions and the Spearman rank correlation was used for evaluating the association between two variables.

In study III, Wilcoxon's signed-rank test was used to determine differences in several cell culture parameters between the control and EPS conditions. Time-course analysis of IL-6 was assessed using the Friedman test.

In study IV, microarray data was analysed using Gene Spring GX version 14.0 (Agilent). Raw data was normalized with per chip and gene 75<sup>th</sup> percentile shift normalization prior to statistical testing with paired t-tests followed by Benjamini-Hochberg multiple testing correction. Statistical significance was set at a corrected  $p < 0.05$  and biological relevance was set at a fold change (FC)  $\geq 2$ . Significance for GO term enrichment, single experiment pathway analysis and GSEA enrichment were set at a  $p$ -value  $< 0.05$ . Paired t-tests were used to determine differences in normally distributed variables (myotube diameter, myogenic fusion index, HSP27 mRNA expression and level of HSP27 phosphorylation) between two experimental conditions.

Statistical analyses were performed using SPSS version 18.0 or later (SPSS, Chicago, IL, USA) (studies I, II, III and IV) and Gene Spring GX version 14.0 (study IV). Significance level was set at  $p < 0.05$ .

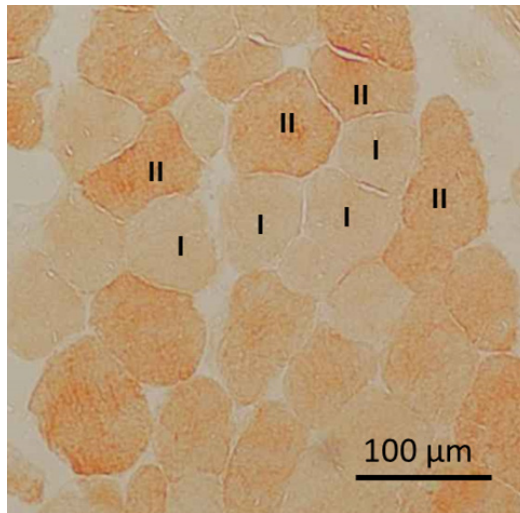
## **Ethical considerations**

All studies have been conducted in accordance to the principles set by the declaration of Helsinki and have been approved by regional ethical boards of Uppsala (DNR 2005:239 and DNR 2015:489), Karolinska institutet (DNR 96-362) and Copenhagen/Fredriksberg (DNR 01-171/04). Participants consented to the study after being informed about the study procedures and related discomfort and potential risks. Personal data are treated confidentially and only authorized persons have access to collected data.

## Main Results and discussion

### Expression of $\alpha$ B-crystallin, HSP27, HSP60 and HSP70 in resting skeletal muscle from athletes with different training backgrounds

Immunohistochemical analysis of muscle biopsies revealed different levels of constitutive expression of HSPs in human vastus lateralis. Generally, there was no detectable staining at the level of capillaries or connective tissue. A first important finding in this study was the occurrence of a fibre type-specific expression of some HSPs (Fig. 4). A second main finding was the occurrence of training background-associated differences in the fibre type-specific expression pattern of HSPs in skeletal muscle.



*Figure 4. Muscle fibre cross section stained with the antibody against HSP27 showing a mosaic staining pattern with higher intensity in type II fibres compared to type I fibres.*

### HSP27

HSP27 staining intensity was higher in type II compared to type I muscle fibres in half of the subjects from ACT and RES. In contrast, there were no differences in staining intensity between the two fibre types in END. The preferential expression of HSP27 in type II muscle fibres is supported by



one study in humans (Paulsen et al., 2009), but is in contrast to data reported in rodents (Golenhofen et al., 2004; Inaguma et al., 1993; Larkins et al., 2012; Neufer & Benjamin, 1996), where HSP27 expression was stronger in type I fibres compared to type II fibres. HSP27 is suggested to be involved in the acute changes related to the production of high-force muscle contractions (Koh, 2002; Paulsen et al., 2009). Thus, the higher expression of HSP27 in type II muscle fibres in humans is suggested to be related to the predominant recruitment of fast fibres in activities requiring the generation of high levels of muscle force. A notable finding was the lack of a fibre type-specific HSP27 staining in skeletal muscle of athletes from END. This can be interpreted as a long-term adaptation to endurance exercises, where muscle fibres are put under a high metabolic stress rather than a mechanical one.

### **$\alpha$ B-crystallin**

A stronger  $\alpha$ B-crystallin staining intensity was detected in type I compared to type II fibres in all subjects from ACT and RES. In contrast, similar intensity in  $\alpha$ B-crystallin staining was found in type I and II fibres in all subjects from END. In rodent and human skeletal muscle, higher levels of  $\alpha$ B-crystallin were found in slow oxidative fibres compared to fast glycolytic fibres (Atomi et al., 2000; Cumming et al., 2014; Larkins et al., 2012; Neufer & Benjamin, 1996; Paulsen et al., 2009). Since  $\alpha$ B-crystallin is predominantly expressed in type I muscle fibres, our findings suggest that this HSP can be involved in the long-term adaptations to prolonged low-force muscle activities. Our data also suggest that an adaptation to long-term endurance training include an upregulation of  $\alpha$ B-crystallin in type II fibres in order to improve the ability of this specific fibre type to sustain an elevated oxidative workload during endurance training. In support of this hypothesis, a higher total level of  $\alpha$ B-crystallin has been shown in endurance-trained athletes compared to untrained subjects (Morton et al., 2008) and in rodents, continuous low-frequency motor nerve stimulation has been shown to alter the fibre type specific pattern of  $\alpha$ B-crystallin from being expressed only in type I fibres to become expressed in all fibre types (Neufer & Benjamin, 1996).

### **HSP60**

No fibre type-specific expression of HSP60 was detected in any subject, regardless of training background. However, in rodents slow oxidative muscle fibres have been shown to express higher levels of HSP60 (Barone et al.,

2016; Ornatsky et al., 1995). Furthermore, a period of endurance training increased the level of HSP60 specifically in type I fibres in trained mice (Barone et al., 2016). Discrepancies between these and our findings may be related to biological differences between different species or to methodological issues such as antibody specificity. Nevertheless, to our knowledge, a fibre type-specific expression of HSP60 has never been described in human skeletal muscle. Given that HSP60 is suggested to act specifically within the mitochondria to facilitate the correct folding of proteins (Martinus et al., 1995) and given the differences in mitochondrial content between type I and type II fibres, the lack of a fibre type-specific HSP60 staining was not expected. Nevertheless, our study does not support the occurrence of changes at the level of HSP60 expression in human skeletal muscle following long-term training.

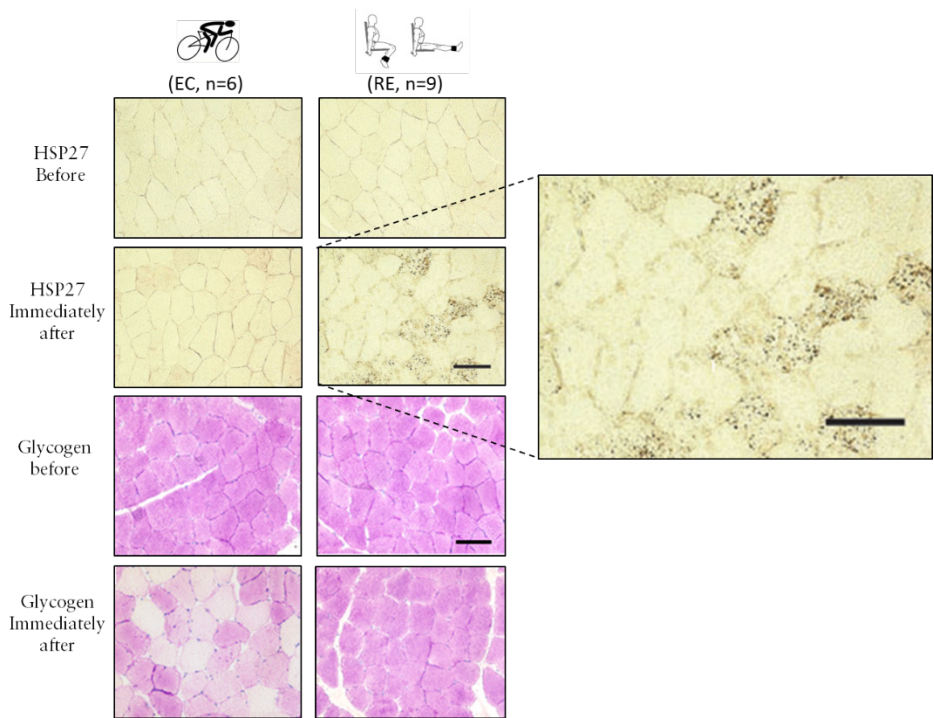
### **HSP70**

Regarding HSP70, no differences in staining intensity level between type I and type II fibres were observed in END and RES. In muscles from ACT, four out of twelve subjects exhibited a lower staining intensity in type II compared to type I fibres. This result may indicate that an elevation of HSP70 expression level can occur in type II fibres in response to long-term training in humans regardless of the training modality. This hypothesis is supported by data showing an up-regulation of HSP70 in rodent fast twitch fibres following long-term training (Gonzalez et al., 2000; Neufer et al., 1996).

### **Acute changes in the expression of HSP27 in human skeletal muscle following resistance and endurance exercise**

In order to evaluate acute changes in the expression of  $\alpha$ B-crystallin, HSP27, HSP60 and HSP70 in exercised human skeletal muscle, two different exercise modalities were used: one session of endurance ergometer cycling (EC) and one session of resistance exercise (RE). In response to RE but not to EC, granular accumulations of HSP27 were seen in muscle fibre cytoplasm (Fig. 5). The granular accumulations of HSP27 were seen in biopsies from five of the nine subjects in RE. In average  $6.3 \pm 10.3\%$  of muscle fibres in biopsies from RE displayed these granular accumulations. Further, these granular accumulations were observed exclusively in type II muscle fibres. Analysis of muscle glycogen content in muscle fibres indicated that there was no glycogen depletion in fibres displaying the HSP27 accumulations

(Fig. 5). With respect to  $\alpha$ B-crystallin, HSP60 and HSP70, no granular accumulations were detected following both exercise modalities.



*Figure 5. Muscle cross-sections stained with the antibody against HSP27 and stained for glycogen content using the PAS method. Biopsies are taken at rest and immediately after endurance ergometer cycling (EC) and resistance exercise (RE). Granular accumulations of HSP27 are seen in response to RE only. Glycogen depletion occurred in muscle fibres following EC but not RE (scale bar = 100  $\mu$ m)*

The finding of granular accumulations of HSP27 in muscle fibres in response to RE is supported by similar observations made following exercise protocols involving high-force eccentric contractions inducing myofibrillar disruptions (Cumming et al., 2014; Koh & Escobedo, 2004; Paulsen et al., 2009; Paulsen et al., 2007). On the basis of our findings, it is suggested that HSP27 protein relocation is an early event in the adaptive process to re-

sistance exercise, including the protection of myofibrillar organization during exercise inducing high mechanical stress. The resistance exercise performed in our study is unlikely to induce fibre damage to the same extent as in studies showing relocation of HSP27 following exercises inducing overt myofibrillar disruption. Moreover, we found that HSP27 relocation is not related to exercises relying on high glycogen utilization. Thus, HSP27 response is likely related to a given level of mechanical stress rather than a metabolic one. Another important finding was the preferential HSP27 accumulation in type II fibres. As HSP27 is suggested to play an important role in the adaptation to forceful contractions, this preferential expression may be related to the involvement of type II fibres in movements requiring the production of high levels of muscle force.

### **Development of an *in-vitro* exercise model promoting the hypertrophy of human muscle cells**

Given evidences pointing at the involvement of HSP27 in skeletal muscle adaptation to exercise resistance, we sought to study the role of this HSP in the occurrence of muscle hypertrophy, which is a major adaptation to resistance exercise. For this purpose, we aimed to develop an *in-vitro* physiological model based on the electrical pulse stimulation (EPS) of differentiated human muscle cells that mimics the hypertrophy of skeletal muscle fibres following resistance exercise.

Several EPS parameters including pulse duration and amplitude were tested before the final setting was defined. The final EPS protocol consisted of 2 ms pulses at 12 V, with a frequency of 1 Hz during 8 h. After completion of EPS, a restitution period of 8 h was applied. By 48 h of differentiation preceding EPS, multinucleated myotubes were formed, and no further fusion contributing to diameter increase was observed. The EPS protocol did not induce any visible cell detachment, and assessment of the cytotoxic effect of EPS based on the release of lactate dehydrogenase (a marker of cell integrity) in culture media using a colorimetric cytotoxicity assay (CytoTox 96; Promega, Finnboða, Nacka, Sweden) showed that lactate dehydrogenase activity was unchanged in medium from stimulated compared with unstimulated myotubes.

### **EPS and muscle cell size**

Compared to non-stimulated myotubes, a significant hypertrophy of stimulated myotubes occurred in response to the EPS protocol including 8 h of

restitution. Interestingly, we tested whether the hypertrophy occurred immediately after EPS or after 4 h of restitution and found no significant difference between the diameter of stimulated and non-stimulated myotubes (Fig. 6).

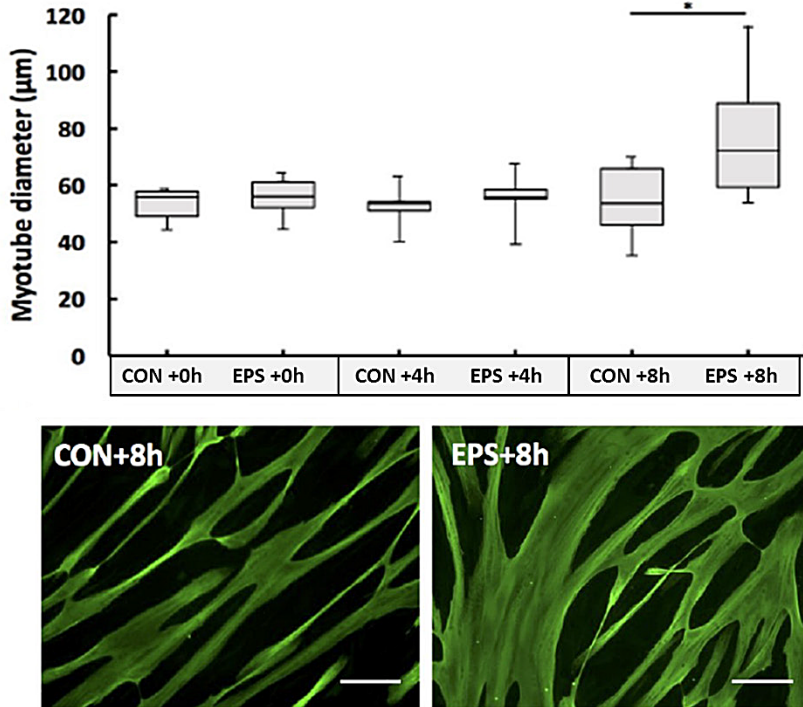


Figure 6. Size of human myotubes ( $n = 5$ ) exposed to electrical pulse stimulation (EPS) assessed without (+0h) and with (+4h and +8h) time for restitution following EPS. CON indicates non-stimulated myotubes (CON). An antibody against troponin T was used to visualize myotubes. \* $p < 0.05$

*In-vivo*, skeletal muscle hypertrophy does not occur during or immediately after an exercise session (Atherton et al., 2017; Dreyer et al., 2006; Kumar et al., 2009). Similarly, the EPS-induced hypertrophy of *in-vitro* cultured myotubes required a period of restitution following the stimuli before the

hypertrophy occurred. Interestingly, there were large variations in the degree of hypertrophy between cultures from different donors. This indicates that our model also mimics the occurrence of inter-individual variability in the response to a similar physiological stimulus in humans. Thus, the use of muscle cells obtained from human donors may represent a biologically relevant tool when exploring mechanisms involved in human skeletal adaptations to exercise (Aas et al., 2013).

### **Molecular response to EPS**

In addition to the study of myotube size, which is the major physiological endpoint, the expression and phosphorylation of proteins commonly involved in the exercise-related hypertrophy of muscle fibres was explored. First, a down-regulation of myostatin, a negative regulator of skeletal muscle mass (Latres et al., 2015; Trendelenburg et al., 2009), occurred in stimulated myotubes. Second, there was an increase in the amount of phosphorylated mTOR and 4E-BP1 in stimulated myotubes. These two factors have previously been shown to be involved in the stimulation of muscle protein synthesis (Qin et al., 2016; Schiaffino et al., 2013). Third, the level of 5'-AMP-activated protein (AMPK) remained unchanged in response to EPS. AMPK is a key cellular energy-sensor suggested to be mainly responsible for adaptations to endurance exercise (Jager et al., 2007) and also suggested to negatively regulate mTOR and protein synthesis (Hawley et al., 2014). Another biological event which further supports the use of this model is the EPS-mediated increased interleukin-6 (IL-6) release. Indeed, the contraction-regulated IL-6 release is considered as an important physiological change in response to exercise in humans (Pedersen & Febbraio, 2008). Altogether, these changes indicate that EPS induced molecular changes at the level of several mechanisms previously described in the context of exercise-induced muscle hypertrophy.

Together with the EPS-mediated hypertrophy, an increase in the amount of phosphorylated HSP27 occurred in stimulated compared to non-stimulated myotubes ( $+20 \pm 8\%$ ;  $p < 0.05$ ), indicating an up-regulation of HSP27 activity. Increased phosphorylation of HSP27 has previously been shown in human skeletal muscle following a single bout of exercise including forceful contractions (Frankenberg et al., 2014; Gonzalez et al., 2016). In rodents, increased phosphorylation of HSP27 is suggested to be related to the modulation of structural proteins and the regulation of muscle mass (Huey, 2006; Kawano et al., 2012; Kawano et al., 2007). Thus, increased level of

phosphorylated HSP27 in response to the EPS model promoting muscle hypertrophy indicates that this model leads to changes similar to those reported following *in-vivo* exercise. Similar to findings following exercise in humans, HSP27 is suggested to be involved in mechanisms related to EPS-induced muscle hypertrophy.

### **The role of HSP27 in the growth of cultured human skeletal muscle cells**

In order to examine the role of HSP27 in the hypertrophy of human muscle cells, the level of this HSP was down-regulated using short interfering RNA (siRNA) designed to specifically target HSP27. Using HSP27 siRNA transfection, the HSP27 mRNA levels in myotubes was reduced by  $53 \pm 6\%$ , but above all, Western blot analysis revealed no detectable HSP27 at the protein level. This was further confirmed by the lack of HSP27 staining using immunohistochemistry (Fig. 7 C and D). Transfected myotubes were able to proliferate and fuse into multinucleated myotubes and no differences in myotube diameter or myogenic fusion index were observed between cells transfected with HSP27 siRNA compared to cells transfected with non-specific scrambled siRNA (Fig. 7 A and B). This important finding indicates that reduced HSP27 does not impair the growth of human muscle cells in our cell culture system. This is supported by the result of a study indicating that inactivation of HSP27 in mice does not induce apparent morphological or anatomical alterations in skeletal muscle (Kammoun et al., 2016). In contrast to this, reduced cytoskeletal protein levels and decreased formation of myotubes were reported in bovine muscle cells with reduced HSP27 levels (Zhang et al., 2014).

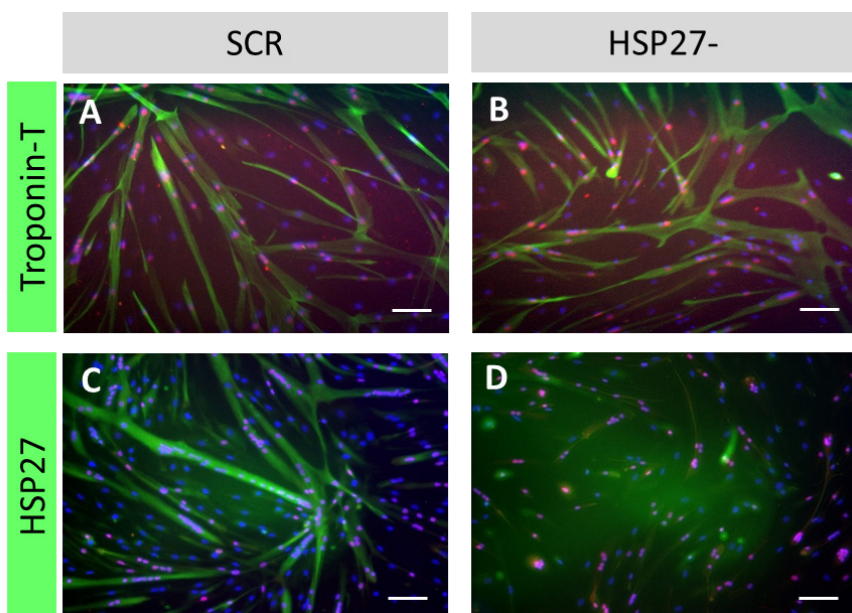


Figure 7. Immunohistochemical staining of differentiated (non-stimulated) myotubes transfected with scramble siRNA (SCR, A and C) or with HSP27 siRNA (HSP27-, B and D). Green fluorescent myotubes are visualized using Troponin-T antibody (A and B). DAPI was used to label myonuclei in blue and myogenin-labelled myonuclei are stained red. The expression of HSP27 was visualized using green fluorescence (C and D). Note the absence of HSP27 staining in D.

In order to further explore the role of HSP27 in the hypertrophy of human muscle cells, we evaluated the expression of genes and sets of genes related to the hypertrophy of human skeletal muscle using micro-array analysis. Compared to controls, a total of 794 genes were statistically ( $p < 0.05$ ) and biologically (fold change (FC)  $\geq 2$ ) differentially expressed (338 genes were up-regulated and 456 genes were down-regulated) in HSP27 transfected muscle cells. In accordance to our results from PCR, western blot and immunohistochemistry analyses, the micro-array analysis also revealed a significant down-regulation of HSP27 (FC = -4.7,  $p < 0.05$ ). Out of the 794 differentially expressed genes, six (IGFB2, MSTN, FBXO32, MTPN, MYF5 and TRIM63) have previously been shown to be involved in the regulation



of muscle fibre size. To assess the enrichments of gene ontologies, a gene ontology (GO) enrichment analysis was performed. There were 200 significantly enriched GO terms in muscle cells with down-regulated HSP27 levels. Importantly, none of these were related to skeletal muscle hypertrophy. Similarly, a geneset enrichment analysis (GSEA), using a set of genes derived from genesets previously found associated to muscle hypertrophy together with a literature derived set of genes, did not reveal a significant enrichment of genesets related to muscle hypertrophy. Together, findings from the micro-array analysis support data on muscle fibre morphology and indicate that HSP27 is not mandatory for the growth of human skeletal cells.

Finally, the evaluation of the role of HSP27 during the hypertrophy of muscle cells in response to EPS was also conducted. Here again, no significant differences in myotube diameter ( $33 \pm 5 \mu\text{m}$  vs  $32 \pm 6 \mu\text{m}$ ;  $p > 0.05$ ) or myogenic fusion index ( $72 \pm 3\%$  vs  $70 \pm 2\%$ ;  $p > 0.05$ ) were found between stimulated HSP27 transfected cells and stimulated control cells. This further confirms our previous finding, indicating that the occurrence of hypertrophy is not controlled by HSP27.

## Methodological considerations

One of the main findings in this work was the relocation of HSP27 within the muscle fibre, which occurred in type II fibres after resistance but not endurance exercise. The use of immunohistochemistry on muscle cross-sections was essential to detect this phenomenon. In general, quantitative analysis methods such as western blot rather than immunohistochemistry provide a reliable tool to evaluate the amount of a specific marker in skeletal muscle. However, protein relocation within a specific fibre type cannot be assessed using the quantitative analysis of homogenized human samples consisting of both slow and fast muscle fibres.

Comparison of HSP expression pattern between participants engaged in long-term training revealed significant training background-related and fibre type-specific differences in the expression of HSPs. It is important to note that due to the cross-sectional design, the contribution of factors other than the training background, including genetic or lifestyle factors cannot be excluded.

In order to evaluate the role of HSP27 in the exercise-induced hypertrophy of human skeletal muscle, an *in-vitro* exercise model using EPS of cultured human muscle cells was developed. Common to all *in-vitro* experiments, extrapolation of findings to an *in-vivo* context is challenging. It is

currently suggested that the use of cultured human muscle cells can be considered a valid model to explore adaptations of human skeletal muscle (Aas et al., 2013). Indeed, the use of muscle cells isolated from human biopsies instead of commercially available cell-lines, may be more appropriate as it introduces an inter-individual variability in genetic background. For example, cells isolated from patients with metabolic disorders exhibit altered metabolic properties (Corpeleijn et al., 2010; Gaster et al., 2002). It is also interesting to note that the use of isolated muscle cells might prove useful in order to describe whether a given factor can be produced by the muscle cell itself or surrounding tissues. *In-vivo*, skeletal muscles are surrounded by other tissues such as connective tissue and muscle contractions are generated after motor neuron firing. Although it is difficult to mimic exercised human skeletal muscle *in-vivo*, several changes seen after electric pulse stimulation of cultured human muscle cells are similar to those described in human skeletal muscle following exercise (Nikolic et al., 2017).

Down-regulation of HSP27 using siRNA with lipofectamine as a transfection agent was performed. In this respect, transfection experiments may lead to increased cell death. In our study, although cell viability was not quantitatively evaluated, transfected myoblasts did not detach and were able to fuse into multinucleated myotubes. Additionally, myotubes were able to increase in size and there were no apparent alterations in the shape of myotubes.

## Future perspectives

Our data on basal expression of HSP27,  $\alpha$ B-crystallin, HSP60 and HSP70 as well as early changes in HSP27 expression following resistance and endurance exercise were collected in subjects aged between 20 and 30 years. Interestingly, an attenuated HSP response has been shown in skeletal muscle of old compared to young rats following contractile activity (Vasilaki et al., 2002) and reduced production of HSP following heat stress has been shown in aged animals and humans (Morton et al., 2009b). Therefore, further investigations are warranted in order to clarify the role and expression of HSP in aged skeletal muscle.

With one exception, all participants included in the first and second studies were male. In rodents, it has been shown that females displayed a lower HSP response compared to males in several tissues, including heart, liver, lung and skeletal muscle (Paroo et al., 1999). Similarly, a lower HSP response to exercise in women compared to men has previously been reported (Morton et al., 2009a). Thus, the occurrence of sex differences in the involvement of HSPs in the response of skeletal muscle to exercise deserves further attention.

Our data also suggested that HSP27 is not mandatory for the hypertrophy of cultured human skeletal muscle cells. Nevertheless, future investigations should clarify whether this HSP may play a role in other processes than muscle hypertrophy during exercise.

## Conclusions

The present thesis provides new information about the expression of specific HSPs in exercised skeletal muscle using classical exercise models in humans. Additionally, an *in-vitro* model of physiologically-mediated muscle growth has been developed in order to determine the exact function of a specific HSP. The major conclusions were:

- In resting human skeletal muscle, a fibre type-specific expression of some, but not all, HSPs was demonstrated. This fibre type-specific expression can be influenced by the exercise training modality (endurance and resistance training).
- In exercised human skeletal muscle, a relocation of HSP27 in the cytoplasm of type II muscle fibres occurs in response to an acute resistance but not endurance exercise bout.
- EPS of human muscle cells followed by a restitution period may be used as an *in-vitro* exercise model promoting the hypertrophy of human muscle cells, recapitulating a major physiological endpoint to resistance exercise in human skeletal muscle
- The level of phosphorylated HSP27 in muscle cells increases during the EPS-mediated cell hypertrophy. However, reducing HSP27 content in muscle cells does not impair the regulation of cell size, indicating that HSP27 is not mandatory for the EPS-induced muscle hypertrophy.

## Acknowledgements

The scientific work presented in this thesis was performed at the School of Health Sciences in the research group Metabolism, Inflammation and Physical Activity (MIPA), Örebro University, Sweden. Collaborating centres included Department of Sports Medicine, Norwegian School of Sports Sciences, Oslo, Norway; Institute of Sports Medicine, Bispebjerg Hospital and Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark; Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden; School of Medicine, Royal Derby Hospital, University of Nottingham, Derby, UK

I wish to express my sincere gratitude to all of you who one way or another contributed to the studies and made this thesis possible, especially:

**Fawzi Kadi**, my supervisor, for giving me this opportunity. I am truly grateful for all your support and encouragement during these years. I will never stop being impressed and inspired by your knowledge and work capacity.

**Britta Wåhlin-Larsson** and **Robert Kruse**, my co-supervisors, for guiding me on the winding roads of cell culture and microarray analysis.

Members of the past “**Muscle group**” from my early years as a PhD student and Members of the present group **Research in Sport and Physical Activity (RISPA)** for fruitful discussions, constructive feedback on texts and presentations and all the hours of company in the lab.

Past and present **Colleagues and Students at the Department of Sport Sciences** for making this the best place to work.

**My Family** for making this the best life to live.

## Svensk sammanfattning

Fysisk träning utlöser en rad olika molekylära och cellulära förändringar som leder till antingen förbättrad uthållighetsförmåga eller ökad muskelmassa och styrka. Heat shock proteiner (HSP) är en proteinfamilj som produceras när muskler utsätts för fysiologiska belastningar. Tidigare studier har föreslagit att dessa proteiner uppfyller viktiga funktioner vid muskelns anpassning till träning.

I denna avhandling studerades förekomsten av fyra medlemmar i HSP familjen ( $\alpha$ B-crystallin, HSP27, HSP60 och HSP70) i human muskel i samband med ett akut träningspass och även till följd av flerårig styrke- och konditionsträning. Vidare studerades även betydelsen av HSP27 vid muskelhypertrofi.

Ett av huvudresultaten var det fibertypspecifika uttrycket av HSP i muskulaturen där ett högre uttryck av  $\alpha$ B-crystallin och HSP70 sågs i långsamma muskelfiber (typ I) jämfört med snabba muskelfibrer (typ II). Däremot sågs det motsatta, dvs. högre uttryck i typ II än typ I, avseende HSP27. Dessutom kunde skillnader i detta fibertypspecifika uttryck av olika HSP relateras till individernas träningsbakgrund där det fibertypspecifika uttrycket av  $\alpha$ B-crystallin och HSP27 framträdde hos individer med bakgrund inom styrkelyft men inte hos uthållighetsträde. Omedelbart efter ett styrketräningspass men inte ett uthållighetspass sågs en intracellulär omfördelning av HSP27 i muskelfibrer. Ingen av de andra undersökta HSP uppvisade en liknande förändring efter någon form av träning. Resultaten tyder alltså på att HSP27 kan vara specifikt involverat i mekanismer kopplat till anpassning vid styrketräning.

För att testa hypotesen att HSP27 är involverad i muskelhypertrofi, en av de viktigaste anpassningarna till styrketräning, utvecklades en *in-vitro* cellmodell som efterliknar styrketräning i detta avseende. Den utvecklade modellen baserades på pulserande elektrisk stimulering av humana muskelceller. Ett protokoll med 8 timmar stimulering följt av 8 timmar vila inducerade en signifikant hypertrofi hos stimulerade jämfört med icke-stimulerade muskelceller. Parallellt med hypertrofiprocessen observerades flera molekylära förändringar liknande de som tidigare beskrivits som en följd av styrketräning. Den specifika rollen av HSP27 vid muskelcellhypertrofi undersöktes genom att nedreglera nivåerna av HSP27. Våra resultat visar inga signifikanta skillnader i muskeltillväxt mellan celler med nedreglerade nivåer av HSP27 och kontrollceller. Nedregleringen av HSP27 medförde förändringar i uttrycket av flera enskilda gener, men resulterade inte i någon

signifikant påverkan på de cellulära signaleringsvägar som reglerar muskel-tillväxt. Med hjälp av den framtagna träningsmodellen på cellnivå påvisades att HSP27 inte är nödvändig för utveckling av muskelhypertrofi. Denna modell kan med fördel användas i framtida studier som syftar till att undersöka mekanismer bakom anpassning av skelettmuskler till fysiskt arbete.

# References

- Aas, V., Bakke, S. S., Feng, Y. Z., Kase, E. T., Jensen, J., Bajpeyi, S., Thoresen, G. H., & Rustan, A. C. (2013). Are cultured human myotubes far from home? *Cell Tissue Res*, 354(3), 671-682. doi:10.1007/s00441-013-1655-1
- Arrigo, A. P. (2012). Pathology-dependent effects linked to small heat shock proteins expression: an update. *Scientifica (Cairo)*, 2012, 185641. doi:10.6064/2012/185641
- Atherton, P. J., Kumar, V., Selby, A. L., Rankin, D., Hildebrandt, W., Phillips, B. E., Williams, J. P., Hiscock, N., & Smith, K. (2017). Enriching a protein drink with leucine augments muscle protein synthesis after resistance exercise in young and older men. *Clin Nutr*, 36(3), 888-895. doi:10.1016/j.clnu.2016.04.025
- Atomi, Y., Toro, K., Masuda, T., & Hatta, H. (2000). Fiber-type-specific alphaB-crystallin distribution and its shifts with T(3) and PTU treatments in rat hindlimb muscles. *J Appl Physiol*, 88(4), 1355-1364.
- Barone, R., Macaluso, F., Sangiorgi, C., Campanella, C., Marino Gammazza, A., Moresi, V., Coletti, D., Conway de Macario, E., Macario, A. J., Cappello, F., Adamo, S., Farina, F., Zummo, G., & Di Felice, V. (2016). Skeletal muscle Heat shock protein 60 increases after endurance training and induces peroxisome proliferator-activated receptor gamma coactivator 1 alpha1 expression. *Sci Rep*, 6, 19781. doi:10.1038/srep19781
- Bodine, S. C. (2006). mTOR signaling and the molecular adaptation to resistance exercise. *Med Sci Sports Exerc*, 38(11), 1950-1957. doi:10.1249/01.mss.0000233797.24035.35
- Bodine, S. C., Stitt, T. N., Gonzalez, M., Kline, W. O., Stover, G. L., Bauerlein, R., Zlotchenko, E., Scrimgeour, A., Lawrence, J. C., Glass, D. J., & Yancopoulos, G. D. (2001). Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nat Cell Biol*, 3(11), 1014-1019. doi:10.1038/ncb1101-1014
- Cheek, D. B. (1985). The control of cell mass and replication. The DNA unit--a personal 20-year study. *Early Hum Dev*, 12(3), 211-239.
- Corpeleijn, E., Hessvik, N. P., Bakke, S. S., Levin, K., Blaak, E. E., Thoresen, G. H., Gaster, M., & Rustan, A. C. (2010). Oxidation of intramyocellular lipids is dependent on mitochondrial function and the availability of extracellular fatty acids. *Am J Physiol Endocrinol Metab*, 299(1), E14-22. doi:10.1152/ajpendo.00187.2010



- Cumming, K. T., Paulsen, G., Wernbom, M., Ugelstad, I., & Raastad, T. (2014). Acute response and subcellular movement of HSP27, alphaB-crystallin and HSP70 in human skeletal muscle after blood-flow-restricted low-load resistance exercise. *Acta Physiol (Oxf)*, 211(4), 634-646. doi:10.1111/apha.12305
- de Jong, W. W., Leunissen, J. A., & Voorter, C. E. (1993). Evolution of the alpha-crystallin/small heat-shock protein family. *Mol Biol Evol*, 10(1), 103-126. doi:10.1093/oxfordjournals.molbev.a039992
- Dimauro, I., Antonioni, A., Mercatelli, N., & Caporossi, D. (2017). The role of alphaB-crystallin in skeletal and cardiac muscle tissues. *Cell Stress Chaperones*. doi:10.1007/s12192-017-0866-x
- Dodd, S. L., Hain, B., Senf, S. M., & Judge, A. R. (2009). Hsp27 inhibits IKKbeta-induced NF-kappaB activity and skeletal muscle atrophy. *FASEB J*, 23(10), 3415-3423. doi:10.1096/fj.08-124602
- Dreyer, H. C., Fujita, S., Cadenas, J. G., Chinkes, D. L., Volpi, E., & Rasmussen, B. B. (2006). Resistance exercise increases AMPK activity and reduces 4E-BP1 phosphorylation and protein synthesis in human skeletal muscle. *J Physiol*, 576(Pt 2), 613-624. doi:10.1113/jphysiol.2006.113175
- Egner, I. M., Bruusgaard, J. C., & Gundersen, K. (2016). Satellite cell depletion prevents fiber hypertrophy in skeletal muscle. *Development*, 143(16), 2898-2906. doi:10.1242/dev.134411
- Frankenberg, N. T., Lamb, G. D., Overgaard, K., Murphy, R. M., & Vissing, K. (2014). Small heat shock proteins translocate to the cytoskeleton in human skeletal muscle following eccentric exercise independently of phosphorylation. *J Appl Physiol* (1985), 116(11), 1463-1472. doi:10.1152/jappphysiol.01026.2013
- Fry, A. C. (2004). The role of resistance exercise intensity on muscle fibre adaptations. *Sports Med*, 34(10), 663-679.
- Fry, C. S., Lee, J. D., Jackson, J. R., Kirby, T. J., Stasko, S. A., Liu, H., Dupont-Versteegden, E. E., McCarthy, J. J., & Peterson, C. A. (2014). Regulation of the muscle fiber microenvironment by activated satellite cells during hypertrophy. *FASEB J*, 28(4), 1654-1665. doi:10.1096/fj.13-239426
- Gaster, M., Petersen, I., Hojlund, K., Poulsen, P., & Beck-Nielsen, H. (2002). The diabetic phenotype is conserved in myotubes established from diabetic subjects: evidence for primary defects in glucose transport and glycogen synthase activity. *Diabetes*, 51(4), 921-927.
- Gjovaag, T. F., & Dahl, H. A. (2006). Effect of training and detraining on the expression of heat shock proteins in m. triceps brachii of untrained males and females. *Eur J Appl Physiol*, 98(3), 310-322. doi:10.1007/s00421-006-0281-y

- Golenhofen, N., Perng, M. D., Quinlan, R. A., & Drenckhahn, D. (2004). Comparison of the small heat shock proteins alphaB-crystallin, MKBP, HSP25, HSP20, and cvHSP in heart and skeletal muscle. *Histochem Cell Biol*, 122(5), 415-425. Epub 2004 Oct 2012.
- Gonzalez, A. M., Hoffman, J. R., Townsend, J. R., Jajtner, A. R., Boone, C. H., Beyer, K. S., Baker, K. M., Wells, A. J., Mangine, G. T., Robinson, E. H. t., Church, D. D., Oliveira, L. P., Fukuda, D. H., & Stout, J. R. (2016). Intramuscular MAPK signaling following high volume and high intensity resistance exercise protocols in trained men. *Eur J Appl Physiol*, 116(9), 1663-1670. doi:10.1007/s00421-016-3417-8
- Gonzalez, B., Hernando, R., & Manso, R. (2000). Stress proteins of 70 kDa in chronically exercised skeletal muscle. *Pflugers Arch*, 440(1), 42-49.
- Hawley, J. A., Hargreaves, M., Joyner, M. J., & Zierath, J. R. (2014). Integrative biology of exercise. *Cell*, 159(4), 738-749. doi:10.1016/j.cell.2014.10.029
- Henry, R. R., Ciaraldi, T. P., Abrams-Carter, L., Mudaliar, S., Park, K. S., & Nikoulina, S. E. (1996). Glycogen synthase activity is reduced in cultured skeletal muscle cells of non-insulin-dependent diabetes mellitus subjects. Biochemical and molecular mechanisms. *J Clin Invest*, 98(5), 1231-1236. doi:10.1172/jci118906
- Henstridge, D. C., Febbraio, M. A., & Hargreaves, M. (2016). Heat shock proteins and exercise adaptations. Our knowledge thus far and the road still ahead. *J Appl Physiol* (1985), 120(6), 683-691. doi:10.1152/jappphysiol.00811.2015
- Holloszy, J. O., & Coyle, E. F. (1984). Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J Appl Physiol Respir Environ Exerc Physiol*, 56(4), 831-838.
- Horwitz, J. (1992). Alpha-crystallin can function as a molecular chaperone. *Proc Natl Acad Sci U S A*, 89(21), 10449-10453.
- Horwitz, J. (2003). Alpha-crystallin. *Exp Eye Res.*, 76(2), 145-153.
- Huey, K. A. (2006). Regulation of HSP25 expression and phosphorylation in functionally overloaded rat plantaris and soleus muscles. *J Appl Physiol* (1985), 100(2), 451-456. doi:10.1152/jappphysiol.01022.2005
- Inaguma, Y., Goto, S., Shinohara, H., Hasegawa, K., Ohshima, K., & Kato, K. (1993). Physiological and pathological changes in levels of the two small stress proteins, HSP27 and alpha B crystallin, in rat hindlimb muscles. *J Biochem (Tokyo)*. 114(3), 378-384.
- Jager, S., Handschin, C., St-Pierre, J., & Spiegelman, B. M. (2007). AMP-activated protein kinase (AMPK) action in skeletal muscle via direct

- phosphorylation of PGC-1 $\alpha$ . *Proc Natl Acad Sci U S A*, 104(29), 12017-12022. doi:10.1073/pnas.0705070104
- Jakob, U., Gaestel, M., Engel, K., & Buchner, J. (1993). Small heat shock proteins are molecular chaperones. *J Biol Chem.*, 268(3), 1517-1520.
- Kadi, F., Charifi, N., Denis, C., Lexell, J., Andersen, J. L., Schjerling, P., Olsen, S., & Kjaer, M. (2005). The behaviour of satellite cells in response to exercise: what have we learned from human studies? *Pflugers Arch*, 451(2), 319-327. doi:10.1007/s00424-005-1406-6
- Kadi, F., & Thornell, L. E. (2000). Concomitant increases in myonuclear and satellite cell content in female trapezius muscle following strength training. *Histochem Cell Biol*, 113(2), 99-103.
- Kammoun, M., Picard, B., Astruc, T., Gagaoua, M., Aubert, D., Bonnet, M., Blanquet, V., & Cassar-Malek, I. (2016). The Invalidation of HspB1 Gene in Mouse Alters the Ultrastructural Phenotype of Muscles. *PLoS One*, 11(8), e0158644. doi:10.1371/journal.pone.0158644
- Kampinga, H. H., Hageman, J., Vos, M. J., Kubota, H., Tanguay, R. M., Bruford, E. A., Cheetham, M. E., Chen, B., & Hightower, L. E. (2009). Guidelines for the nomenclature of the human heat shock proteins. *Cell Stress Chaperones*, 14(1), 105-111. doi:10.1007/s12192-008-0068-7
- Kawano, F., Fujita, R., Nakai, N., Terada, M., Ohira, T., & Ohira, Y. (2012). HSP25 can modulate myofibrillar desmin cytoskeleton following the phosphorylation at Ser15 in rat soleus muscle. *J Appl Physiol* (1985), 112(1), 176-186. doi:10.1152/jappphysiol.00783.2011
- Kawano, F., Matsuoka, Y., Oke, Y., Higo, Y., Terada, M., Wang, X. D., Nakai, N., Fukuda, H., Imajoh-Ohmi, S., & Ohira, Y. (2007). Role(s) of nucleoli and phosphorylation of ribosomal protein S6 and/or HSP27 in the regulation of muscle mass. *Am J Physiol Cell Physiol*, 293(1), C35-44. doi:10.1152/ajpcell.00297.2006
- Khassaf, M., Child, R. B., McArdle, A., Brodie, D. A., Esanu, C., & Jackson, M. J. (2001). Time course of responses of human skeletal muscle to oxidative stress induced by nondamaging exercise. *J Appl Physiol*, 90(3), 1031-1035.
- Koh, T. J. (2002). Do small heat shock proteins protect skeletal muscle from injury? *Exerc Sport Sci Rev*, 30(3), 117-121.
- Koh, T. J., & Escobedo, J. (2004). Cytoskeletal disruption and small heat shock protein translocation immediately after lengthening contractions. *Am J Physiol Cell Physiol*, 286(3), C713-722. Epub 2003 Nov 2019.

- Kumar, V., Atherton, P., Smith, K., & Rennie, M. J. (2009). Human muscle protein synthesis and breakdown during and after exercise. *J Appl Physiol*, (1985), 106(6), 2026-2039. doi:10.1152/japplphysiol.91481.2008
- Larkins, N. T., Murphy, R. M., & Lamb, G. D. (2012). Absolute amounts and diffusibility of HSP72, HSP25, and alphaB-crystallin in fast- and slow-twitch skeletal muscle fibers of rat. *Am J Physiol Cell Physiol*, 302(1), C228-239. doi:10.1152/ajpcell.00266.2011
- Latres, E., Pangilinan, J., Miloscio, L., Bauerlein, R., Na, E., Potocky, T. B., Huang, Y., Eckersdorff, M., Rafique, A., Mastaitis, J., Lin, C., Murphy, A. J., Yancopoulos, G. D., Gromada, J., & Stitt, T. (2015). Myostatin blockade with a fully human monoclonal antibody induces muscle hypertrophy and reverses muscle atrophy in young and aged mice. *Skelet Muscle*, 5, 34. doi:10.1186/s13395-015-0060-8
- Liu, Y., Lormes, W., Baur, C., Opitz-Gress, A., Altenburg, D., Lehmann, M., & Steinacker, J. M. (2000). Human skeletal muscle HSP70 response to physical training depends on exercise intensity. *Int J Sports Med.*, 21(5), 351-355.
- Liu, Y., Mayr, S., Opitz-Gress, A., Zeller, C., Lormes, W., Baur, S., Lehmann, M., & Steinacker, J. M. (1999). Human skeletal muscle HSP70 response to training in highly trained rowers. *J Appl Physiol*, 86(1), 101-104.
- Liu, Y., & Steinacker, J. M. (2001). Changes in skeletal muscle heat shock proteins: pathological significance. *Front Biosci*, 6, D12-25.
- Locke, M., & Noble, E. G. (2002). *Exercise and the stress response; the role of stress proteins*.: CRC Press LLC.
- Locke, M., Noble, E. G., & Atkinson, B. G. (1990). Exercising mammals synthesize stress proteins. *Am J Physiol.*, 258(4 Pt 1), C723-729.
- Locke, M., Noble, E. G., & Atkinson, B. G. (1991). Inducible isoform of HSP70 is constitutively expressed in a muscle fiber type specific pattern. *Am J Physiol.*, 261(5 Pt 1), C774-779.
- Martini, F., & Timmons, M. (1997). *Human Anatomy* (2 ed.): Prentice Hall PTR.
- Martinius, R. D., Ryan, M. T., Naylor, D. J., Herd, S. M., Hoogenraad, N. J., & Hoj, P. B. (1995). Role of chaperones in the biogenesis and maintenance of the mitochondrion. *FASEB J*, 9(5), 371-378.
- Mattson, J. P., Ross, C. R., Kilgore, J. L., & Musch, T. I. (2000). Induction of mitochondrial stress proteins following treadmill running. *Med Sci Sports Exerc.*, 32(2), 365-369.
- McCarthy, J. J., & Esser, K. A. (2010). Anabolic and catabolic pathways regulating skeletal muscle mass. *Curr Opin Clin Nutr Metab Care*, 13(3), 230-235. doi:10.1097/MCO.0b013e32833781b5

- Middleton, R. C., & Shelden, E. A. (2013). Small heat shock protein HSPB1 regulates growth of embryonic zebrafish craniofacial muscles. *Exp Cell Res*, 319(6), 860-874. doi:10.1016/j.yexcr.2013.01.002
- Miyabara, E. H., Nascimento, T. L., Rodrigues, D. C., Moriscot, A. S., Davila, W. F., AitMou, Y., deTombe, P. P., & Mestril, R. (2012). Overexpression of inducible 70-kDa heat shock protein in mouse improves structural and functional recovery of skeletal muscles from atrophy. *Pflugers Arch*, 463(5), 733-741. doi:10.1007/s00424-012-1087-x
- Montarras, D., L'Honore, A., & Buckingham, M. (2013). Lying low but ready for action: the quiescent muscle satellite cell. *Febs j*, 280(17), 4036-4050. doi:10.1111/febs.12372
- Morton, J. P., Holloway, K., Woods, P., Cable, N. T., Burniston, J., Evans, L., Kayani, A. C., & McArdle, A. (2009a). Exercise training-induced gender-specific heat shock protein adaptations in human skeletal muscle. *Muscle Nerve*, 39(2), 230-233. doi:10.1002/mus.21182
- Morton, J. P., Kayani, A. C., McArdle, A., & Drust, B. (2009b). The exercise-induced stress response of skeletal muscle, with specific emphasis on humans. *Sports Med*, 39(8), 643-662. doi:10.2165/00007256-200939080-00003 [pii]
- Morton, J. P., MacLaren, D. P., Cable, N. T., Bongers, T., Griffiths, R. D., Campbell, I. T., Evans, L., Kayani, A., McArdle, A., & Drust, B. (2006). Time course and differential responses of the major heat shock protein families in human skeletal muscle following acute nondamaging treadmill exercise. *J Appl Physiol*, 101(1), 176-182. Epub 2006 Mar 2024.
- Morton, J. P., Maclaren, D. P., Cable, N. T., Campbell, I. T., Evans, L., Kayani, A. C., McArdle, A., & Drust, B. (2008). Trained men display increased Basal heat shock protein content of skeletal muscle. *Med Sci Sports Exerc*, 40(7), 1255-1262.
- Mymrikov, E. V., Seit-Nebi, A. S., & Gusev, N. B. (2011). Large potentials of small heat shock proteins. *Physiol Rev*, 91(4), 1123-1159. doi:10.1152/physrev.00023.2010
- Neufer, P. D., & Benjamin, I. J. (1996). Differential expression of B-crystallin and Hsp27 in skeletal muscle during continuous contractile activity. Relationship to myogenic regulatory factors. *J Biol Chem*, 271(39), 24089-24095.
- Neufer, P. D., Ordway, G. A., Hand, G. A., Shelton, J. M., Richardson, J. A., Benjamin, I. J., & Williams, R. S. (1996). Continuous contractile activity induces fiber type specific expression of HSP70 in skeletal muscle. *Am J Physiol*, 271(6 Pt 1), C1828-1837.

- Nikolic, N., Gorgens, S. W., Thoresen, G. H., Aas, V., Eckel, J., & Eckardt, K. (2017). Electrical pulse stimulation of cultured skeletal muscle cells as a model for in vitro exercise - possibilities and limitations. *Acta Physiol (Oxf)*, 220(3), 310-331. doi:10.1111/apha.12830
- Ornatsky, O. I., Connor, M. K., & Hood, D. A. (1995). Expression of stress proteins and mitochondrial chaperonins in chronically stimulated skeletal muscle. *Biochem J*, 311 ( Pt 1), 119-123.
- Paroo, Z., Tiidus, P. M., & Noble, E. G. (1999). Estrogen attenuates HSP 72 expression in acutely exercised male rodents. *Eur J Appl Physiol Occup Physiol*, 80(3), 180-184. doi:10.1007/s004210050579
- Paulsen, G., Hanssen, K. E., Ronnestad, B. R., Kvamme, N. H., Ugelstad, I., Kadi, F., & Raastad, T. (2012). Strength training elevates HSP27, HSP70 and alphaB-crystallin levels in muscoli vastus lateralis and trapezius. *Eur J Appl Physiol*, 112(5), 1773-1782. doi:10.1007/s00421-011-2132-8
- Paulsen, G., Lauritzen, F., Bayer, M. L., Kalhovde, J. M., Ugelstad, I., Owe, S. G., Hallen, J., Bergersen, L. H., & Raastad, T. (2009). Subcellular movement and expression of HSP27, alphaB-crystallin, and HSP70 after two bouts of eccentric exercise in humans. *J Appl Physiol*, 107(2), 570-582. doi:00209.2009 [pii] 10.1152/japplphysiol.00209.2009
- Paulsen, G., Vissing, K., Kalhovde, J. M., Ugelstad, I., Bayer, M. L., Kadi, F., Schjerling, P., Hallen, J., & Raastad, T. (2007). Maximal eccentric exercise induces a rapid accumulation of small heat shock proteins on myofibrils and a delayed HSP70 response in humans. *Am J Physiol Regul Integr Comp Physiol*, 293(2), R844-853. Epub 2007 May 2023.
- Pedersen, B. K., & Febbraio, M. A. (2008). Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiol Rev*, 88(4), 1379-1406. doi:10.1152/physrev.90100.2007
- Pette, D., & Staron, R. S. (2000). Myosin isoforms, muscle fiber types, and transitions. *Microsc Res Tech*, 50(6), 500-509. doi:10.1002/1097-0029(20000915)50:6<500::aid-jemt7>3.0.co;2-7
- Qin, X., Jiang, B., & Zhang, Y. (2016). 4E-BP1, a multifactor regulated multifunctional protein. *Cell Cycle*, 15(6), 781-786. doi:10.1080/15384101.2016.1151581
- Relaix, F., & Zammit, P. S. (2012). Satellite cells are essential for skeletal muscle regeneration: the cell on the edge returns centre stage. *Development*, 139(16), 2845-2856. doi:10.1242/dev.069088
- Ritossa, F. (1962). A new puffing pattern induced by temperature shock and DNP in drosophila. *Experientia*, 18(12), 571-573. doi:10.1007/BF02172188

- Rodriguez, J., Vernus, B., Chelh, I., Cassar-Malek, I., Gabillard, J. C., Hadj Sassi, A., Seiliez, I., Picard, B., & Bonniieu, A. (2014). Myostatin and the skeletal muscle atrophy and hypertrophy signaling pathways. *Cell Mol Life Sci*, 71(22), 4361-4371. doi:10.1007/s00018-014-1689-x
- Roth, S. M., Martel, G. F., Ferrell, R. E., Metter, E. J., Hurley, B. F., & Rogers, M. A. (2003). Myostatin gene expression is reduced in humans with heavy-resistance strength training: a brief communication. *Exp Biol Med (Maywood)*, 228(6), 706-709.
- Schiaffino, S. (2010). Fibre types in skeletal muscle: a personal account. *Acta Physiol (Oxf)*, 199(4), 451-463. doi:10.1111/j.1748-1716.2010.02130.x
- Schiaffino, S., Dyar, K. A., Ciciliot, S., Blaauw, B., & Sandri, M. (2013). Mechanisms regulating skeletal muscle growth and atrophy. *Febs j*, 280(17), 4294-4314. doi:10.1111/febs.12253
- Schiaffino, S., & Reggiani, C. (2011). Fiber types in mammalian skeletal muscles. *Physiol Rev*, 91(4), 1447-1531. doi:10.1152/physrev.00031.2010
- Snijders, T., Nederveen, J. P., McKay, B. R., Joannis, S., Verdijk, L. B., van Loon, L. J., & Parise, G. (2015). Satellite cells in human skeletal muscle plasticity. *Front Physiol*, 6, 283. doi:10.3389/fphys.2015.00283
- Stice, J. P., & Knowlton, A. A. (2008). Estrogen, NFkappaB, and the heat shock response. *Mol Med*, 14(7-8), 517-527. doi:10.2119/2008-00026.Stice
- Thompson, H. S., Clarkson, P. M., & Scordilis, S. P. (2002). The repeated bout effect and heat shock proteins: intramuscular HSP27 and HSP70 expression following two bouts of eccentric exercise in humans. *Acta Physiol Scand*, 174(1), 47-56.
- Trendelenburg, A. U., Meyer, A., Rohner, D., Boyle, J., Hatakeyama, S., & Glass, D. J. (2009). Myostatin reduces Akt/TORC1/p70S6K signaling, inhibiting myoblast differentiation and myotube size. *Am J Physiol Cell Physiol*, 296(6), C1258-1270. doi:10.1152/ajpcell.00105.2009
- Walker, D. K., Dickinson, J. M., Timmerman, K. L., Drummond, M. J., Reidy, P. T., Fry, C. S., Gundermann, D. M., & Rasmussen, B. B. (2011). Exercise, amino acids, and aging in the control of human muscle protein synthesis. *Med Sci Sports Exerc*, 43(12), 2249-2258. doi:10.1249/MSS.0b013e318223b037
- Vasilaki, A., Jackson, M. J., & McArdle, A. (2002). Attenuated HSP70 response in skeletal muscle of aged rats following contractile activity. *Muscle Nerve*, 25(6), 902-905. doi:10.1002/mus.10094

- Wilson, J. M., Loenneke, J. P., Jo, E., Wilson, G. J., Zourdos, M. C., & Kim, J. S. (2012). The effects of endurance, strength, and power training on muscle fiber type shifting. *J Strength Cond Res*, 26(6), 1724-1729. doi:10.1519/JSC.0b013e318234eb6f
- Vissing, K., Bayer, M. L., Overgaard, K., Schjerling, P., & Raastad, T. (2009). Heat shock protein translocation and expression response is attenuated in response to repeated eccentric exercise. *Acta Physiol*, 196, 283-293.
- Yoon, M. S. (2017). mTOR as a Key Regulator in Maintaining Skeletal Muscle Mass. *Front Physiol*, 8, 788. doi:10.3389/fphys.2017.00788
- Zhang, Q., Lee, H. G., Kang, S. K., Baik, M., & Choi, Y. J. (2014). Heat-shock protein beta 1 regulates androgen-mediated bovine myogenesis. *Biotechnol Lett*, 36(6), 1225-1231. doi:10.1007/s10529-014-1489-2



PUBLICATIONS *in the series*  
ÖREBRO STUDIES IN SPORT SCIENCES

1. Gustafsson, Henrik (2007). *Burnout in competitive and elite athletes.*
2. Jouper, John (2009). *Qigong in Daily Life. Motivation and Intention to Mindful Exercise.*
3. Wåhlin Larsson, Britta (2009). *Skeletal Muscle in Restless Legs Syndrome (RLS) and Obstructive Sleep Apnoea Syndrome (OSAS).*
4. Johansson, Mattias (2009). *Qigong: Acute affective responses in a group of regular exercisers.*
5. Cardell, David (2009). *Barndomens oas. En tvärvetenskaplig studie av kulturindustriella intressen och organisering av evenemang genom exemplet Stadium Sports Camp.* Licentiat.
6. Isberg, Jenny (2009). *Viljan till fysisk aktivitet. En intervention avsedd att stimulera ungdomar att bli fysiskt aktiva.*
7. Oskarsson, Eva (2010). *Lateral epicondylitis. Intramuscular blood flow, pressure and metabolism in the ECRB muscle.*
8. Andersson, Helena (2010). *The physiological impact of soccer on elite female players and the effects of active recovery training.*
9. Åkesson, Joakim (2010). *Idrottens akademisering. Kunskapsproduktion och kunskapsförmedling inom idrottsforskning och högre idrottsutbildning.* Licentiat.
10. Eliason, Gabriella (2010). *Skeletal muscle characteristics and physical activity patterns in COPD.*
12. Stigell, Erik (2011). *Assessment of active commuting behaviour – walking and bicycling in Greater Stockholm.*
13. Wahlgren, Lina (2011). *Studies on Bikeability in a Metropolitan Area Using the Active Commuting Route Environment Scale (ACRES).*
14. Thiborg, Jesper (2011). *ATT GÖRA (E)SPORT: Om datorspel och sportifiering.* Licentiat.
15. Maivorsdotter, Ninitha (2012). *Idrottsutövandets estetik: En narrativ studie av meningsskapande och lärande.*
16. Hellström, John (2012). *Expert Performance in Golf.*
17. Hjältn, Sören (2014). *Utbrändhet och återhämtning bland elittränare i fotboll.*

18. Brolin, Magnus (2014). *Hälsoarbete i skolan- mer än motion, morötter och moral?* Licentiat.
19. Tolgfors, Björn (2014). *Bedömning för lärande i ämnet idrott och hälsa, didaktiska konsekvenser av styrning.* Licentiat.
20. Andreas Jacobsson (2014). *Träffar vi alltid rätt? - Lärares tal om resurser för elevers lärande i ämnet Idrott och hälsa.* Licentiat.
21. Seger, Izabela (2014). *Betygsättningsprocess i ämet idrott och hälsa. En studie om betygsättningsdilemman på högstadiet.* Licentiat.
22. Huitfeldt, Åke (2015). *Passar jag in? Nyanlända ungdomars möte med idrottsundervisning.* Licentiat.
23. Hosford-Donovan, Adrian (2015). *C-reactive protein, skeletal muscle mass and blood pressure in elderly women: observational and mechanistic links.* Licentiat.
24. Li, Jing (2016). *The Culturally Significant Key Component of Qigong, 'Heart adjustment', is Lost in Translation.* Licentiat.
25. Tolgfors, Björn (2017). *Bedömning för vilket lärande? En studie av vad bedömning för lärande blir och gör i ämnet idrott och hälsa.*
26. Strandberg, Emelie (2017). *Skeletal Muscle Mass & Function in Older Women: Health-Enhancing Influences of Combined Resistance Exercise & Diet.*
27. Ericson, Helena (2018). *A Salutogenic perspective on resistance training – a study on healthy old adult women.* Licentiat.
28. Folkesson, Mattias (2018). *Heat shock proteins in exercised human skeletal muscle.*