Comparative Biochemistry and Physiology, Part A 153 (2009) S71–S76

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Society for Experimental Biology Annual Main Meeting
28th June — 1st July 2009, Glasgow, UK

A2 - Molecular and Cellular aspects of tissue adaptation and repair: *A symposium in honour of Professor Geoffrey Goldspink on the occasion of his retirement*

A2.1
09:15 Wednesday 1st July 2009
Myogenesis and the regulation of muscle hypertrophy in teleost fish

Ian A. Johnston, Neil I. Bower (University of St. Andrews)

Muscle growth requires a population(s) of myogenic progenitor cells (MPCs) or myoblasts that remain capable of proliferation and are regulated by signaling pathways responsive to both nutritional status and environmental conditions. Myotube formation involves the recognition and adhesion of myoblasts, the breakdown of muscle membranes and the remodeling of the actin cytoskeleton. Myotube production in fast myotomal muscle typically continues until ~40% of the maximum adult body length, and subsequent growth only involves fibre hypertrophy. Microarray experiments will be described to identify genes associated with the transition from hyperplastic to hypertrophic growth phenotypes in zebrafish. Insulin-like growth factor I (IGF-I) together with associated binding proteins and receptors plays a key role in regulating myogenesis and muscle fibre hypertrophy. The signals that regulate atrophy and hypertrophy are linked through the PI3K/AKT/mTOR pathway. Activation of the PI3k/AKT/mTOR pathway by IGF-I activates a phosphorylation cascade that leads to an increase in translation and protein synthesis, resulting in fibre hypertrophy. Phosphorylation of Akt also inhibits regulators of fibre atrophy such as the muscle specific ubiquitin ligases MAFbx and MuRF1, through phosphorylation of FOXO transcription factors. The myotomal muscles act as a reservoir of amino acids during seasonal fasting and for the elaboration of gonads prior to reproduction. The use of fasting-refeeding paradigms to challenge the genetic pathways regulating muscle fibre hypertrophy in Atlantic salmon will be described. *In vitro* cell culture systems have also been developed which enable manipulative experiments and analysis of the function of individual genes.

Supported by BBSRC.

Email Address for correspondence: iaj@st-and.ac.uk

doi:10.1016/j.cbpa.2009.04.026

A2.2
09:45 Wednesday 1st July 2009
Larry Rome (Univ. of Pennsylvania)

To be confirmed

doi:10.1016/j.cbpa.2009.04.027

A2.3
10:40 Wednesday 1st July 2009
Myosin heavy chains responsible for temperature plasticity of fish muscle

Shugo Watabe (The University of Tokyo)

Freshwater temperate fish such as goldfish and carp alter myofibrillar ATPase activity in response to changes in environmental temperature. This phenomenon was discovered by G. Goldspink et al. [1]. Since then, various biochemical and molecular biological investigations related to this alteration have been conducted and our group also carried out extensive research mainly focusing on myosin, a major element of myofibrils. Muscle tissues contain the most classical myosin of sarcomeric type called myosin II which consists of two heavy chains (MYHs) and four light chains. Various types of sarcomeric MYHs have been found in fish muscles as the cases of mammals. Interestingly, some of their encoded MYH isoforms have been found to exhibit different ATPase and motor activities and to be differentially expressed following temperature acclimation Three types of MYH encoding these isoforms were isolated from fast muscle of common carp acclimated to either 10 °C or 30aC. The 10 °C-type MYH (MYHF10) and the 30aC-type MYH (MYHF30) are the genes predominantly expressed in 10 °C- and 30aC-acclimated fish, respectively. The third gene is expressed over a relatively broad temperature range and named the intermediate-type (MYHFint). Such changes were observed not only with evolutionarily tetraploid common carp, but also with diploid fish such as grass carp and medaka. This talk will summarize these

findings, further deals with the results recently obtained and discuss on their physiological significance.

1) Johnston, I. A., Davison, W. and Goldspink, G. (1975). Adaptations in Mg2+-activated myofibrillar ATPase activity induced by temperature acclimation. FEBS Lett. 50, 293–295.

Email Address for correspondence: awatabe@mail.ecc.u-tokyo.ac.jp

doi:10.1016/j.cbpa.2009.04.028

A2.4

11:10 Wednesday 1st July 2009

Natural variations in myosin heavy chain genotype in gammarid amphipods

Nia M. Whiteley (Bangor University), Jenny Rock (Bangor University), Julia L. Magnay (Keele University), Alicia El Haj (Keele University) David Lunt (Hull University), Geoff Goldspink (University College London)

Myosin heavy chain (MyHC) sequences were examined between and within conspecifics of the amphipod genus Gammaridae distributed along the coastal fringes of the north Atlantic and Arctic Oceans. The purpose was to determine whether functional sequence variation in the surface loop regions of the subfragment 1 domain of MyHC occur in response to natural thermal gradients. To this end, hypervariable MyHC loop 1 and loop 2 regions were characterised in the abdominal muscles of 7 gammarids species collected at various latitudes from North Wales to Svalbard in the Arctic. PCR amplification of MyHC cDNAs and subsequent sequencing of multiple clones from individual amphipods made it possible to make comparisons between individuals, latitudinal populations and species.

Loop 2 MyHC sequences proved to be more interesting than loop 1 with 4 potential isoforms identified. Each isoform showed amino acid divergence in the loop 2 site resulting in changes in loop length and distribution of the charge suggesting significant biochemical changes. Even though there were some species-specific differences in the expression of loop 2 MyHC isoforms, differences were not related to phylogeny. Latitudinal differences, however, did occur and certain species showed greater MyHC isoform diversity with increasing latitude, while others relied on a general cold-water isoform. It appears that thermal histories are important, with the most tolerant high intertidal species showing greater MyHC isoform diversity in order to maintain muscle performance across wide and rapid variations in temperature.

Email Address for correspondence: n.m.whiteley@bangor.ac.uk

doi:10.1016/j.cbpa.2009.04.029

A2.5

11:40 Wednesday 1st July 2009

Developmental programming of muscle and consequences for later growth — From fish to rats

Neil Stickland (Royal Veterinary College)

It is now widely accepted that conditions *in utero* (e.g. levels of maternal nutrition) can often be related to problems in later adult life such as cardiovascular disease. This is often referred to as the Fetal programming or Developmental origins hypothesis.

For muscle tissue, we have shown that maternal nutrition can have a significant and permanent influence on secondary muscle fibre formation in the porcine fetus. There also appears to be a shift (probably regulated by IGFBP5) in the proportion of non muscle tissue (fat and connective tissue) as well as a shift in oxidative metabolism within the offspring's muscles. These effects correlate with postnatal consequences for muscle growth and adiposity. In fish also the development of muscle tissue can be influenced by egg incubation temperature. In some species raised incubation temperature can lead to faster muscle development relative to bone and may be the underlying reason for the increasing problem of musculoskeletal deformities seen in many farmed fish species. And although the muscle development is faster, the muscle produced has fewer muscle fibres and nuclei to fuel posthatch muscle growth. Overfeeding pregnant rats also leads to muscle fibre hypoplasia and weaker muscles with increased adiposity in the offspring.

Taken as a whole these results demonstrate the significant plasticity of muscle in early stages of development with many of the changes being permanent and with important consequences for later growth and function.

Email Address for correspondence: nstickland@rvc.ac.uk

doi:10.1016/j.cbpa.2009.04.030

A2.6

12:10 Wednesday 1st July 2009

Expression patterns of multiple myosin heavy chain genes identify tissue-specific fibre types in adult skeletal muscles of torafugu *Takifugu rubripes*

Dadasaheb B. Akolkar, Shigeharu Kinoshita, Yousuke Ono, Shugo Watabe (The University of Tokyo)

Fish skeletal muscles are highly specialized to adapt themselves to a wide range of body movement both during sustained swimming as well as in high-velocity burst. Unlike other tetrapod, teleost contains fast-glycolytic and slow-oxidative muscles in distinct anatomical position, with the former being located deeply in myotomes and the later lying beneath the lateral line. In addition to slow muscle of lateralis superficialis, torafugu *Takifugu rubripes* has erectors and depressors linked to dorsal and anal fin. However, little is known regarding fibre types in adult fish including torafugu. Here, histochemical analysis of myofibrillar ATPase demonstrated high diversity in fibres in both fast and slow muscles that reacted differently at low pH preincubation. Fast muscle fibres were glycolytic and slow muscle fibres oxidative as revealed by staining for NADH-tetrazolium reductase. Myosin is a principle component of thick filaments in sarcomere and possesses two heavy chains (MYHs) and four light chains. To examine fibre-type specific expression of MYH genes (*MYHs*), we cloned *MYHs* from adult fast and slow muscles of torafugu. $MYH_{M86\text{-}1}$ and MYH_{M8248} were expressed exclusively in fast and slow muscles, respectively, whereas $MYH_{M2528\text{-}1}$ and MYH_{M1034} were found in both fast and slow muscles. $MYH_{M2126\text{-}2}$ and MYH_{M5} were expressed in slow and cardiac muscles. Fibre-type specific expression of these *MYHs* was revealed in fast and slow muscles by *in situ* hybridization. Our comprehensive molecular and biochemical investigation provided a direct evidence of distinct fibre types in adult skeletal muscles of torafugu.

Email Address for correspondence: akolkardada@gmail.com

doi:10.1016/j.cbpa.2009.04.031

A2.7

13:30 Wednesday 1st July 2009

The E-domain region of MGF preserves cardiac function and mobilizes resident stem cell populations following myocardial infarction

Tamara Los, Paul H. Goldspink, Krystyna M. Shioura, Evangelos Mavrommatis (University of Illinois at Chicago)

IGF-I exists as two splice variants in the heart, a predominant isoform IGF-IEa, and IGF-IEb, also known as mechano-growth factor (MGF). We have found a significant increase in both MGF transcript and protein expression 24 h post-infarction in mice. Since MGF differs in the E-domain region of the pro-hormone, we are interested in determining whether this region has biological function. A synthetic peptide corresponding to the E-domain of MGF was delivered at the time of myocardial infarction (MI) in mice. At 14-days post-MI, cardiac function was assessed using pressure-volume analysis *in situ*. Several hemodynamic parameters and cardiac contractility were preserved in the MI + E-domain group, and were significantly improved compared to both MI and MI + scrambled peptide groups. Pathologic hypertrophy was apparent in the MI group, but inhibited in the MI + E-domain group. Immunohistochemical analysis of sectioned hearts revealed numerous small troponin I (TnI) positive cells that co-express both Nkx2.5 and Islet-1 in the E-domain treated mice. Gene expression analysis of these small TnI^+ cells grown *in vitro*, revealed expression of MEF2C, GATA4, Flk-1, the slow skeletal isoform of TnI, cardiac TnT and L-type Ca^{2+} channel. Furthermore, an increase in the number of c-Kit^+ cells and co-expression of c-Kit^+ and ssTnI suggests that resident stem cells may contribute to the appearance of the small TnI positive cells in the E-domain treated hearts. These data suggest that administration of the E-domain peptide derived from the pro-hormone form of IGF-I produced during injury, maybe facilitate the actions of IGF-I to improve cardiac function and mobilize resident stem cell populations.

Email Address for correspondence: pgolds@uic.edu

doi:10.1016/j.cbpa.2009.04.032

A2.8

14:00 Wednesday 1st July 2009

Calcineurin and the slow-oxidative skeletal muscle fibre type

Kin-Chow Chang (University of Nottingham), Joachim Meissner (Hannover Medical School), Joanne Mallinson (University of Nottingham)

In skeletal muscle, signalling by calcineurin, a widely distributed calcium-dependent serine-threonine phosphatase, is crucial for myocyte differentiation, muscle regeneration and in fibre conversion to the slow-oxidative phenotype. Such processes are vital to muscle performance, metabolic health and even to meat animal production. Downstream effector genes of calcineurin activation are potential targets for pharmaceutical intervention to elicit desirable oxidative changes. Recent advances in our molecular understanding of calcineurin signalling in fast-to-slow fibre conversion will be examined.

Email Address for correspondence: kin-chow.chang@nottingham.ac.uk

doi:10.1016/j.cbpa.2009.04.033

A2.9

14:30 Wednesday 1st July 2009

A little bit of this and a little bit of that! 3D tissue engineered skeletal muscle—Generating basic mechanisms in maintenance and turnover

Mark P. Lewis (UCL Eastman Dental Institute), Alec Smith (UCL Eastman Dental Institute), Rishma Shah (UCL Eastman Dental Institute), Khalid Al-Qahtani (UCL Eastman Dental Institute), Karin Carlqvist (UCL Eastman Dental Institute), Andrea C. Sinanan (UCL Eastman Dental Institute), Vivek Mudera (UCL Institute of Orthopaedics and Musculoskeletal Sciences)

The new field of tissue engineering is starting to offer insights into the basic structural and functional biology of skeletal muscle. Our work in developing 3D biomimetic constructs has shown that the principle stimulus in this system is mechanical force and we now seek to dissect the molecular pathways that such stimulation activates. One such critical pathway is activation of proteases/integrins. The ability to apply the knowledge generated in basic cell biology research to the formation of "tissue engineered" constructs and progenitor cells in these systems are critical to success. Our work on precursor cells from skeletal muscle will enable us to ultimately "engineer" a 3D construct with muscle correctly integrated with other musculoskeletal systems (bone, tendon, ligament) and the neural system to mimic the in vivo structures. The translational aspect of this work aims to take the knowledge regarding muscle response to physical forces through to applications that could range from study of physiological response e.g. exercise through to surgical therapies to improve outcomes in terms of stability and success and also to develop characterised biomimetic models that will help reduce in vivo experiments.

Email Address for correspondence: mark.lewis@ucl.ac.uk

doi:10.1016/j.cbpa.2009.04.034

A2.10

15:30 Wednesday 1st July 2009

Developmental and adaptive growth of skeletal and cardiac muscle

David F. Goldspink (Liverpool John Moores)

Muscle growth (i.e. protein accretion) and atrophy (i.e.net protein loss) are controlled by the relative rates of protein synthesis and protein breakdown; both processes are under physiological control and require ATP [1]. In all muscle types during development the average rates of protein synthesis and breakdown decline and converge to yield steady-state conditions [2], whilst remaining responsive to anabolic (e.g. stretch) and catabolic (e.g. weightlessness, calorie restriction) stimuli [3]. Although skeletal muscle fibres change their phenotype, their number remains fairly constant throughout most of postnatal life [4]. In contrast, large numbers of cardiomyocytes are lost with age, especially from male rodent and human hearts [5]. In both muscle types cell death occurs, via apoptosis and necrosis, during natural ageing or in heart failure [6,7]. Most injured skeletal fibres are replaced via satellite cells, and some cardiomyocytes by resident stem cells [7]. Exercise stimulates myocyte renewal in both muscle types, leading to skeletal fibre hypertrophy and elongation [8], and increases in cardiomyocyte number and size. However, the intensity of the exercise is critical.

1. DF Goldspink, Ergonomics 48; 1334–1351, 2005.
2. SEM Lewis et al. Biochemical Journal 217; 517–526,1984.
3. DF Goldspink et al. Cardiovascular Research 9; 672–678,1986.
4. CAG Boreham et al. Journal of Anatomy 157; 111–125,1988.
5. G Olivetti et al. Journal of American College of Cardiology. 26;1068–1079,1995.
6. DF Goldspink et al. Experimental Physiology 89; 407–416, 2004.
7. GM Ellison et al. Journal of Biological Chemistry. 282;11397–11409, 2007.
8. RS James et al. J Applied Physiology 83; 398–406,1997.

Email Address for correspondence: d.goldspink@ljmu.ac.uk

doi:10.1016/j.cbpa.2009.04.035

A2.11

16:00 Wednesday 1st July 2009

Differential effects of high strain and insulin-like growth factor 1 on adaptation of muscle fiber size and force

Richard Jaspers (Research Institute MOVE VU University Amsterdam), Willem J. Van der Laarse (Department of Physiology Institute for Cardiovascular Research VU University Medical Center), Ramaswamy Krishnan (Program in Molecular and Integrative Physiological Sciences, Harvard School of Public Health), Carla Offringa (Research Institute MOVE VU University Amsterdam),; Christophe P. Bagowski (Institute of Biology, Department of Integrative Zoology Leiden University), Janwillem Testerink (Research Institute MOVE VU University Amsterdam)

In vivo, immobilization of muscle at extended length causes hypertrophy and an increase in serial sarcomere numbers. The aim of this study was to investigate the separate and possible synergetic effects of high muscle fibre strain and IGF-1 on adaptation of fibre cross-sectional area (CSA) and number of sarcomeres in series. Mature, single muscle fibers of *Xenopus laevis* were cultured at slack length (sarcomere length 2.3 μm, "$l_{2.3\ \mu m}$") or at extended length (12% over slack, "high strain") for 10 to 24 days in serum-free medium with or without human IGF-1. During culture, tetanic force and CSA of fibers cultured at $l_{2.3\ mm}$ without IGF-1 remained unchanged. Fibers cultured at high strain without IGF-1 reduced tetanic force by 1.4 ± 0.2% (mean ± SEM) per day, whereas fiber CSA was constant. In contrast, tetanic force of fibers cultured at $l_{2.3\ mm}$ with IGF-1 increased by 1.0 ± 0.1% per day whereas CSA increased by 33.4 ± 3.8% after 16.6 ± 0.6 days. CSA of high strain cultured fibers with IGF-1 increased to 28.8 ± 3.7% after 16.6 ± 1.4 days. The IGF-1 induced increase in tetanic force at high stain (0.6 ± 0.2% per day) was lower than at $l_{2.3\ mm}$. For all conditions, numbers of sarcomeres in series and myonuclei were unchanged. mRNA levels of actin and IGF-1 were increased by IGF-1 and not by high strain. We conclude that high strain imposed on an isolated muscle fiber does not stimulate hypertrophy or increase the serial sarcomere number, whereas IGF-1 may stimulate hypertrophic signaling via increasing IGF-1 mRNA and induce hypertrophy by increasing actin mRNA.

Email Address for correspondence: r.jaspers@fbw.vu.nl

doi:10.1016/j.cbpa.2009.04.036

A2.12

16:30 Wednesday 1st July 2009

Genes associated with athletic performance

Barbara Wessner, Harald Tschan, Norbert Bachl (University of Vienna)

Human physical performance is determined by a variety of factors including training history, nutritional status, technical aids, psychological strength, social environment but also genetic factors. Several studies have revealed repeatedly that heritability is a strong component of key endurance (maximal oxygen uptake, lactate/ventilatory threshold, economy of movement, ...) and strength phenotypes (muscle strength, sprint performance, ...). To date more than 200 gene entries and quantitative trait loci have shown some associations or linkages with exercise-related phenotypes (Bray et al. 2009). Many of these associations seem to be rather week or need to be proven in larger populations, but the impact of the R577X single nucleotide polymorphism of the a-actinin 3 (ACTN3) gene on elite athletic performance and trainability has been confirmed in a series of studies (MacArthur and North 2007). This holds true for a couple of other polymorphisms such as the insertion/deletion polymorphism of the angiotensin I-converting enzyme (ACE), the Ser49Gly polymorphism of the beta2-adrenergic receptor (ADRB2), the 34C>T transition in exon 2 of the adenosine monophosphate deaminase 1 (AMPD1) or the microsatellite repeat in the promoter region of the insulin-like growth factor-1 (IGF-1). However, it is very likely that more than one genetic variant will be responsible for a complex trait such as athletic performance. Therefore, a combinatory polymorphic approach would be necessary to predict human elite status or response to a certain type of exercise (Williams and Folland 2008).

Bray, M. S., J. M. Hagberg, et al. (2009). "The human gene map for performance and health-related fitness phenotypes: the 2006–2007 update." Med Sci Sports Exerc 41(1): 35–73.

MacArthur, D. G. and K. N. North (2007). "ACTN3: A genetic influence on muscle function and athletic performance." Exerc Sport Sci Rev 35(1): 30–4.

Williams, A. G. and J. P. Folland (2008). "Similarity of polygenic profiles limits the potential for elite human physical performance." J Physiol 586(1): 113–21.

Email Address for correspondence: Barbara.Wessner@univie.ac.at

doi:10.1016/j.cbpa.2009.04.037

A2.13

16:55 Wednesday 1st July 2009

Mechano Growth Factor, that is produced by the splicing of the IGF-1 gene increases muscle size and strength

Geoffrey Goldspink (Royal Free and University College Medical School London University)

Muscles in humans are genetically programmed to respond to mechanically signals and carry out repair but selection has only been up to the age of reproduction. Here attention is focused on the role of the GH/IGF-1 axis and the discovery of mechano growth factor (MGF). The latter is derived from the IGF-I gene by alternative splicing and in the young is associated with increasing contractile strength in response to exercise. This involves activating the muscle satellite (progenitor) cells that kick start local muscle repair and induce hypertrophy. Following a bout of exercise, the IGF-I gene is initially spliced to MGF which due to a reading frame shift, has a unique C-terminal peptide for which the natural version has a short half life. Recent studies using primary muscle cell cultures have shown that this MGF C-peptide activates muscle satellite (stem/progenitor) cells in normal human muscle. Interestingly, although the initial yield of these cells was less from dystrophy and ALS patients the numbers were increased by MGF C-peptide within 48 h During the second phase following the exercise the IGF-1 gene is spliced to IGF-1Ea. which is the main source of anabolic agent although the other function of IGF-I is to induces the progenitor cells to enter the myogenic pathway and to fuse with the muscle fibres. With the increased number of nuclei and gene copies, IGF-I which is a major metabolic agent increases protein synthesis for the second stage of muscle repair and hypertrophy. During ageing growth hormone levels decline markedly and administration of hGH appears to upregulate the number of primary transcripts of the IGF-I gene so more MGF and IGF-1Ea can be produced in older people by exercise. In the young, MGF offers the explanation of why muscle tissue can adapt to mechanical stress and it offer the prospect of treating muscle wasting during the ageing process and muscle cachexia that is associated with many diseases.

Email Address for correspondence: gb.goldspink@btinternet.com

doi:10.1016/j.cbpa.2009.04.038

A2.14

Poster Session — Tuesday 30th June 2009

Differential gene expression in South African Abalone (*Haliotis midae*) in response to oxidative and thermal stress.

Dalene Vosloo, Andre Vosloo, Paula Sommer, Anel Laas, Jessika Samuels (University of KwaZulu-Natal, Durban, South Africa)

In land-based abalone holding systems, animals may be exposed to temperature and oxygen levels outside the ranges of what they might experience in their natural habitat. Juvenile abalone however must have some way of mediating the effect of the large range of environmental oxygen levels of the algal forests where they live. We found at least 39 genes that are differentially expressed in response to changing environmental oxygen levels and temperature, when analysed by FDD-RT-PCR. These include malate dehydrogenase, cytochrome C oxidase, ubiquitin and arginine-*N*-methyltransferase. Western blot immunodetection of heat shock protein 70 protein levels showed significantly higher levels at high environmental oxygen levels, but not to elevated temperature. In the mammalian model all these proteins are implicated in functions that include protein repair, DNA repair, protein trafficking and cell proliferation, all of which may be impacted by changes in temperature and environmental oxygen levels. These gene responses will be used to facilitate our understanding of the seemingly differential susceptibility of juvenile and adult abalone to heat and oxidative stress.

Email Address for correspondence: voslood@ukzn.ac.za

doi:10.1016/j.cbpa.2009.04.039

A2.15

Poster Session — Tuesday 30th June 2009

Thermal imprinting in pearlfish: How embryonic temperature modulates postembryonic muscle growth

Julia Marschallinger, Astrid Obermayer, Walter Stoiber, Alexandra M. Saenger, Peter Steinbacher (University of Salzburg, Austria)

Temperature is a most important external factor to influence muscle cellularity in teleost fish. Related effects are heterogeneous and include the phenomenon that the thermal experience during embryonic life is likely to become 'imprinted' and to have a lasting influence on muscle growth later in ontogeny. However, our understanding of the imprinting process as a whole, including cellularity change over a long time range, is rather incomplete.The present work uses digital morphometry to examine the long-term effects of embryonic temperature on larval and juvenile muscle growth in the cyprinid pearlfish *Rutilus meidingeri*. To perform imprinting, fish were reared at three different temperatures (8.5°, 13°, 16°) until hatching and subsequently all kept at 16 °C. Samples from each thermal regime were taken from end of somitogenesis to 7 months post hatching. To precisely assess spatio-temporal variation of intramyotomal growth dynamics, trunk quadrants were subdivided into three zones representing the distinct modes of teleost muscle growth (lateral and apical zones: stratified growth; central zone: mosaic growth). Results to date demonstrate that at hatching body lengths, fast muscle cross sectional areas and fast fibre numbers are lower at 8.5° and 13° than at 16°. During the larval period, this situation becomes inverted in the 13° fish, mainly due to enhanced hyperplasia, so that these fish finally outgrow those of the two other temperature groups. These results confirm the existence of long-term thermal history effects and are discussed in relation to their relevance for patterns of myogenic stem cell activation and aquaculture practice.

Email Address for correspondence: julia.marschallinger@sbg.ac.at

doi:10.1016/j.cbpa.2009.04.040

A2.16

Poster Session — Tuesday 30th June 2009

Keeping cool makes big fish — The temperature dependence of myotomal growth dynamics in the zebrafish *Danio rerio*

Astrid Obermayer, Julia Marschallinger, Peter Steinbacher, Alexandra M Saenger, Walter Stoiber (University of Salzburg, Austria)

The zebrafish *Danio rerio*, a small, fast developing cyprinid, has become a most important model for vertebrate development within the last decade, and most of the present knowledge on early teleost muscle formation and its genetic/molecular control comes from this species. However, muscle cellularity and growth dynamics have almost exclusively been examined in large-growing species which are of interest to aquaculture, while data from zebrafish are entirely missing. In order to close this gap and to enquire about the temperature dependence of the myotomal growth dynamics, we use digital morphometry to analyse muscle cellularity of zebrafish reared under different thermal conditions (25°, 28.5° and 31 °C). Developmental stages from hatching to adult life are examined. Total body length as well as fast muscle fibre numbers and cross sectional areas are assessed. To trace intramyotomal differences of the hypertrophy/hyperplasia balance, fibre measurements are done separately for three zones of the myotome (lateral, apical, central). Preliminary results from comparison of two thermal regimes (25° and 28.5°) indicate that a lower rearing temperature promotes growth to a larger body size in later life stages. The implications of this for the hypertrophy/hyperplasia balance as reflected in cellularity change are discussed. The present work is intended to open new directions for investigation of the regulatory mechanisms that govern muscle growth and its temperature dependence at the genetic/molecular level.

Email Address for correspondence: astrid.obermayer@sbg.ac.at

doi:10.1016/j.cbpa.2009.04.041

A2.17

Poster Session — Tuesday 30th June 2009

Effects of different training interventions on the expression and synthesis of important factors of skeletal muscle plasticity regulation

Lydia Mueller, Klaus Richter, Herbert Wimmer, Peter Steinbacher, Walter Stoiber, Susanne Ring-Dimitriou, Alexandra M. Saenger (University of Salzburg, Austria)

A common feature along with senescence is the deterioration of the muscular system. This process of body composition changes and related functional decline is called sarcopenia which generally starts around the fourth decade of life. Especially women, having a smaller muscle mass than men *per se*, are afflicted with this age-related loss of muscle strength. It is well known that gene expression changes with aging affecting among others skeletal muscle protein synthesis, oxidative defence and mitochondrial enzyme activity. Based on previously performed light microscopical findings the purpose of the study was to test the effects of two modes of endurance training (intermittent *vs.* consecutive method) as well as two modes of resistance training (hypertrophy *vs.* SuperSlow method) on skeletal

muscle of perimenopausal women (45–55 yrs) with respect to the expression and synthesis of important factors of both the regulation of skeletal muscle plasticity and skeletal muscle damage. Muscle biopsy samples of the *M.vastus lateralis* of the non dominant leg were proceeded for RNA isolation and gene array. In particular, gene expression profiles included in adaptational processes, inflammatory responses and genes encoding metabolic enzymes were investigated. The data will underpin previous findings of our group regarding the advantage of one or the other training mode such as the SuperSlow one.

Email Address for correspondence: lydia.mueller@sbg.ac.at

doi:10.1016/j.cbpa.2009.04.042

A2.18

Poster Session — Tuesday 30th June 2009

Molecular characterization of lipase family in pufferfish *Takifugu rubripes*

Gen Kaneko (The University of Tokyo), Toshihiro Yamada (The University of Tokyo), Reiko Nagasaka (Tokyo University of Marine Science and Technology), Hideki Ushio (Tokyo University of Marine Science and Technology), Shugo Watabe (The University of Tokyo)

Fish has two types of lipid accumulation pattern. While pufferfish and flounder accumulate lipid predominantly in liver, red seabream and eel do both in liver and muscle. Lipase family members, which hydrolyze triacylglycerols in plasma lipoproteins to provide various tissues with free fatty acids, play a critical role in lipid metabolism. Here we characterized several genes encoding lipases in pufferfish *Takifugu rubripes* for better understanding of the mechanisms underlying its liver-specific lipid accumulation. Four lipase genes were found on the JGI fugu genome database. Two genes were monophyletic with lipoprotein lipase (LPL) gene, and each one of another two with endothelial lipase (EL) and hepatic lipase (HL) gene from other animals in the neighbor-joining tree, and thus termed *trLPL1*, *trLPL2*, *trEL* and *trHL*, respectively. Subsequently, the transcripts of these genes were analyzed in fast muscle, gill, skin, liver and intestine from adult fish reared at 25 °C by reverse transcription-PCR. The transcripts of both *trLPL1* and *trLPL2* were predominant in liver and intestine, whereas those of *trEL* and *trHL* showed ubiquitous and liver-specific distribution, respectively. Starvation during 10 days led to the decrease in the mRNA levels of *trLPL1* and *trLPL2* in liver with a concomitant increase in fast muscle and skin, but no apparent changes in the case of *trEL* and *trHL*. Meanwhile, insulin response elements were found within a 1 kb 5′-flanking region in *trLPL1* and *trLPL2*, but not in *trEL* and *trHL*, suggesting the possible involvement of *trLPL1* and *trLPL2* in lipid accumulation.

Email Address for correspondence: gkaneko1@me.com

doi:10.1016/j.cbpa.2009.04.043