Symposium

SY-01
Muscular expression profiling during unloading and training

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The interdependence of the functional and structural plasticity of skeletal muscle tissue has well been characterized over the last 30 years. Specific protocols such as endurance or strength training paradigms lead to defined adaptations of the musculature. Likewise, nutritional interventions, microgravity and exposition to a hypoxic environment all result in structural modifications of muscle tissue. The phenotypic plasticity of muscle tissue is thus ultimately responsible for observed malleability of muscle function. In order to respond to different external and internal stimuli muscle tissue has several sensory modalities. We can postulate that muscle tissue can respond to mechanical loading or unloading, to metabolic stress, to systemic and local hormonal modifications as well as to nervous input. During exercise training we find a typical pattern of stress whereby we must assume that there is a specific combination of all of these modalities that together result in a "typical" phenotypic adaptation. The use of modern molecular techniques has led to a gradual emergence of a concept of how muscle tissue is responding to the environment. The specific stimuli experienced by the muscle cell are translated into complex molecular signals that intervene dominantly (but not exclusively) on the transcriptional level and thus lead to profound modifications of the transcriptome. It has become apparent that repeated stimulations of signalling pathways lead to transient regulations of early genes and of transcription factors. These transient changes are in turn responsible for the more long-term regulation of structure genes. Using data from studies of the human and the rat transcriptome we will try to demonstrate the potential as well as the limitations of the current approach to study the mechanisms of muscle plasticity.

SY-02
Insulin sensitivity and exercise – an overview

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It is well known that people who regularly perform exercise have a high insulin sensitivity and a reduced risk to develop diabetes type 2. In recent years it has become clear that the ability to use muscle lipids (triglycerides) as a fuel during exercise has a direct link to insulin resistance. Trained subjects have a high capacity to oxidize muscle TG, while obese subjects and patients with type 2 diabetes have low capacities to oxidize muscle TG and develop large muscle TG stores. The size of the muscle TG stores shows a strong positive correlation with insulin resistance. Recent work of the groups of *Shulman and Dohm* is beginning to unravel the molecular link between muscle lipid accumulation and insulin resistance. High muscle lipid concentrations are generally attended by high concentrations of long-chain fatty acylCoA and diacylglycerol in the muscle. This then leads to activation of protein kinase C (q and/or b) and serine phosphorylation of the insulin receptor (IR). As a consequence IR tyrosine kinase activity is downregulated resulting in impaired GLUT-4 translocation and a reduced glucose uptake capacity. In this introductory lecture to the symposium on insulin sensitivity and exercise differences between populations in their ability to oxidize the main fat sources (adipose tissue TG, lipoprotein TG and muscle TG) during exercise will first be summarized, this will be followed by a reflection on the potential nature of impairments in FA mobilization and oxidation pathways that may result in large muscle TG stores, and in the last part a detailed explanation will be given of the molecular link between muscle TG accumulation and insulin resistance.

SY-03
5'-AMP-activated protein kinase as a metabolic master switch

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The AMP-activated protein kinase (AMPK) system is a sensor of cellular energy charge that is activated by ATP depletion. The key activating signal is an increase in AMP, which accompanies falling ATP due to the adenylate kinase reaction. Activation occurs during metabolic stress, including exercise in skeletal and cardiac muscle. Once activated, AMPK acts as a metabolic master switch, switching on catabolic pathways while switching off ATP-requiring processes. The kinase exerts rapid effects due to direct phosphorylation of target proteins involved in the pathway, and more long-term effects on gene expression. Rapid effects in exercising muscle include an increase in glucose uptake due to translocation of GLUT4 to the plasma membrane, and increased uptake and oxidation of fatty acids. Long-term effects include increased expression of GLUT4, hexokinase and mitochondrial proteins, and increased mitochondrial biogenesis. Experiments with transgenic mice expressing a dominant negative AMPK mutant suggest that the system is responsible for the metabolic adaptations to chronic ATP depletion caused by feeding creatine analogues, a treatment which mimics the effects of endurance training.
In well-trained individuals, a similar intensity of exercise produces a much smaller activation of AMPK than in untrained individuals. One potential explanation of this effect lies in the ability of a high cellular glycogen to repress AMPK activation. We have recently found that the beta subunit of AMPK contains a glycogen-binding domain, and it seems likely that this allows the system to act as a sensor of cellular glycogen content as well as ATP content. Recent work on this topic will be discussed.

SY-04
Regulation and expression of hormone-sensitive lipase (HSL) in skeletal muscle

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The enzymatic regulation of intramyocellular triacylglycerol (TG) breakdown has until recently been unknown. The stored muscle TG depot contains more energy than the muscle glycogen depot. Interestingly, this TG depot has recently been related to insulin resistance. We have now shown that it is likely that the enzyme hormone-sensitive lipase (HSL), which controls lipolysis in adipose tissue, may play a key role in the regulation of intramyocellular TG. First HSL was demonstrated in all muscle fibre types by Western blotting of muscle fibres isolated by collagenase treatment or after freeze-drying. The expression of HSL was correlated to fiber type, being higher in oxidative than in glycolytic fibers. Next it was demonstrated that in incubated soleus and extensor digitorum longus muscles stimulation with adrenalin or electrically induced muscle contractions activated HSL by increasing the activity against a triacylglycerol substrate. No measurable activation existed in the presence of an anti-HSL antibody. Furthermore, our studies have shown that it is likely that the effect of adrenaline is mediated by beta-adrenergic activation of protein kinase A (PKA) and the effect of contractions by protein kinase C and the mitogen-activated protein kinase pathway (MAPK). The effect of adrenaline and contractions were partially additive. Training increased adrenaline stimulated HSL activity in rat adipose tissue but not in muscle. Interestingly, training increased contraction-mediated HSL (TG) activity in muscle. In conclusion, HSL is present in skeletal muscle and can be activated by phosphorylation by both adrenaline and muscle contractions. Training increases adrenaline-stimulated HSL activation in adipose tissue and contraction-mediated HSL activation in muscle.

SY-05
Gene polymorphism's and genes coding insulin sensitivity and training

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Genetic factors are playing a role in the regulation of glucose metabolism-related traits such as insulin sensitivity, insulin secretion or insulin resistance. Several genomic scans and candidate gene studies have been performed to localize genes involved in glucose metabolism related traits. Especially clinical relevant phenotypes including diabetes mellitus type 2 and obesity where used as targets for these investigations. However, not much is known about the role these genetic factors play for performance phenotypes (e.g. maximum oxygen uptake) or the response to physical training.
The intention of this talk is to summarize the recent status in the literature dealing with genes involved in glucose and insulin metabolism phenotypes with special respect to training and endurance performance.
A wide range of genes will be reviewed including the alpha-adrenergic receptors, the insulin receptors and IGF family, the PPAR genes, genes coding the Interleukin group as well as the widely investigated ACE and TNF alpha genes. In addition to the comprehensive literature review, a specific summary of these genes investigated in the Genathlete study and the Heritage study will be part of the presentation.
The recent knowledge about the specific topic is quite weak and therefore beside presenting the current status future directions and perspectives will be discussed in the talk.

SY-06
Myofascial forces and the effects on stress on the extracellular matrix

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Movement is caused by force transmission from contracting muscle fibers via aponeurosis/intramuscular connective tissue and tendon to bone. The mechanical characteristics of the human free tendon and aponeurosis - in vivo - remains largely unknown. Development of real-time ultrasound techniques together with MRI, allows for in vivo determination o tendon aponeurosis properties. The free Achilles tendon demonstrates greater strain compared with that of the distal (deep) aponeurosis during voluntary isometric contraction, which suggests that separate functional roles may exist during in vivo force transmission, and that sharing of elongation between myofascial tissue and free tendon is different during passive movement than with active muscle contraction. Tendon tissue adapts to mechanical loading (training) both quantitatively and qualitatively, and trained individuals have thicker tendons than untrained counterparts, a phenomenon that may be beneficial in reducing the amount of stress on the tendon during physical activity, and counteract development of overuse injury.

SY-07
Autrocrine/paracrine growth factor expression in response to stretch and the regulation of muscle mass

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There is a growing appreciation that gene expression in several cell types is influenced by mechanical signals. The growth hormone/IGF-I axis has been regarded as an important main regulator of tissue growth in general. However, locally produced forms of insulin-like growth factor (IGF-I) are important and it is now appreciated that they occur in different forms with different functions. We cloned two different IGF-Is that are expressed by skeletal muscle and both are derived from the IGF-I gene by alternative splicing. One of these is expressed in response to physical activity which has now been called "mechano growth factor" (MGF). The other is similar to the systemic or liver type (IGF-IEa) and is important as the provider of mature IGF-I required for upregulating protein synthesis. MGF differs from systemic IGF-IEa in that it has a different peptide sequence which is responsible for activating muscle satellite (stem) cells. Therefore, it appears these two forms of IGF-I have different actions and that they are important regulators of muscle growth. Growth hormone treatment apparently upregulates the level of IGF-I gene expression and when it is combined with with resistance exercise more is spliced towards MGF. This results in an additional increase in muscle cross sectional area in the elderly subjects who otherwise are less able to produce MGF. There are now new challenges in relation to misuse of these procedures in athletics and professional sports. This topic will also be briefly discussed.

SY-08
Apoptosis and cell signaling in skeletal muscle with aging and caloric restriction

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There is strong evidence that mitochondrial oxidative stress in vivo plays a significant part in the pathogenesis of neurological disorders and sarcopenia (atrophy and loss of skeletal muscle myofibers). Life-long caloric restriction (CR) reduces oxidative damage and has been shown to have neuroprotective effects and prevents the age-associated loss in muscle fibers as well as function, but the mechanisms in vivo are poorly understood. We investigated apoptosis and apoptotic transduction signalling pathways in skeletal muscle and the brain frontal cortex of 12-month old, 26-month old ad libitum fed and 26-month old CR male Fischer-344 rats (CR = 40% of ad lib levels). We found that apoptosis was increased with age in these two post-mitotic tissues and that CR attenuated this age-associated increase significantly. We determined levels of inhibitors, such as ARC (apoptosis repressor with a caspase recruitment domain), which inhibits caspase-2 activity and attenuates cytochrome c release from the mitochondria in addition to levels of XIAP (X-linked inhibitor-of-apoptosis), which inhibits caspase-3 activity. We found a significant age-associated decline in cytosolic ARC levels. CR attenuated the age-associated decline of this anti-apoptotic protein in the cytosol and decreased the levels of ARC in the mitochondria. CR attenuated the increases in cytosolic cytochrome c and caspase-2 activity observed during aging. Moreover, we found that CR suppressed the age-associated rise in cleaved caspase-3 in skeletal muscle and in the cerebral cortices. XIAP protein content increased with age and was reduced by CR. Our studies demonstrate that post-mitotic tissues show significant alterations in apoptotic signalling with aging due to the chronic oxidative stress and that caloric restriction is able to modulate these changes towards cellular survival. Support of the presented work is provided by the NIH-NIA.

SY-09
Adaptive significance of skeletal muscle HSP70 during training

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In response to stress, cells rapidly produce a series of new proteins known as heat shock proteins (HSP). HSPs are considered to be molecular chaperones which play a universal role in maintaining cellular homeostasis. It is known that different HSPs are expressed in skeletal muscle, namely, small HSPs (including ubiquitin, alpha B-crystallin, HSP20 and HSP 27), HSP70, HSP60 and HSP90. It has been proven that exercise is a sufficient physiological stimulus to induce HSPs in blood liver, heart and skeletal muscle. Mechanisms of HSP induction by exercise may involve exercise related contraction cellular stress (i.e. calcium, stress on cytoskeleton), energy depletion, oxidative radicals, metabolic messengers and cytokines, hypoxemia, ischemia and hyperthermia. Moreover, HSP are involved in the antigen-presentation in the inflammatory response to infections or exercise and are involved in the regulation of apoptosis.
Whether HSP70 at the protein level could be induced in human skeletal muscles by exercise had remained unclear until we reported that after a prolonged training programme in well-trained rowers, HSP70 at the protein level increased significantly in response to rowing training. In a study in which two groups of rowers underwent different training strategies the dependence of HSP70 response on exercise amount was mainly atributed to exercise intensity rather than exercise volume. In a third study was found that muscle HSP70 is not induced during low intensity endurance training. The investigation of HSP induction in skeletal muscle (especially in man) by exercise may be used as an indicator of stress. This may be of special interest for monitoring overtraining, and may be useful in the subsequent direction of training. With its protective role, HSP may preserve muscle function in cases of overtraining or muscle injury caused by exercise and may be involved in muscle fiber transition and control of apoptosis. So far we have not been able to show a relationship between HSP expression and physical performance, which would be of great physiological interest.

SY-10
Oxidative Stress

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Exercise has been shown to induce an augmented generation of reactive oxygen species (ROS) via different mechanisms. Resulting actions of ROS affect important mechanisms in the field of exercise physiology. Evidence exists that ROS formation in response to vigorous physical exertion can result in oxidative stress. However, the functional significance of exercise-induced oxidative stress is still discussed controversially. More recent research has revealed the important role of ROS as signaling molecules. In this context, ROS affect a broad array of physiological functions. ROS modulate contractile function in unfatigued and fatigued skeletal muscle. Furthermore, involvement of ROS in modulation of gene expression via redox-sensitive transcription pathways represents an important regulatory mechanism, which has been suggested to be involved in the process of training adaptation. Adaptation of endogenous antioxidative systems in response to regular training may lead to a limitation of oxidative stress and reflects a potential mechanism responsible for augmented tolerance to exercise. This lecture will summarize current knowledge about exercise-related formation of ROS in skeletal muscle by focussing on their generation properties, mechanisms of action, and their involvement in regulatory and adaptational mechanisms.

SY-11
Stress Proteins and Aging

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Cells rapidly synthesize proteins known as heat shock proteins (HSPs), in response to heat or other protein damaging stressors. In the mammalian heart, the over-expression of certain HSPs has been shown to reduce the damage caused by ischemia-reperfusion and thereby confer protection to the heart. The stress-mediated induction of HSPs is regulated by a transcription factor known as the Heat Shock Transcription Factor (HSF1). During unstressed conditions HSF1 exists as an inactive monomer but following stress, HSF1 trimerizes, migrates to the nucleus, binds to the heat shock element and activates HSP genes. This process is known as HSF activation. In mammals, two HSF1 isoforms (HSF1a and HSF1 b) exist. Since the accumulation of abnormal or malfunctional proteins occurs with age and may contribute to the generation of certain diseases, HSPs may play an important role in cells from aged organisms. Comparisons of unstressed cells from aged and adult animals shows HSPs do not accumulate with age. However, when aged cells are stressed and homeostasis perturbed, aged cells show a reduced ability to mount the protective HSP response. Both a decreased HSF1 activation as well as a decreased HSP accumulation has been shown to occur in aged cells. This suggests aged cells lack the ability to translate the biophysical signals of stress into the necessary cellular response. This inability to mount an adequate HSP response may explain, at least in part, why aged organisms are more susceptible to certain stressors.

KV-001
Das kumulierte excess-CO_2 als Belastungskriterium bei hochgradiger Herzinsuffizienz

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Bei Herzinsuffizienten kann der belastungsbedingte Anstieg der Blutlaktatkonzentration durch eine Einschränkung der Perfusion verzögert sein. Die Blutlaktatkonzentration ist bei diesen Patienten daher als Maß für die Leistungsfähigkeit und als Ausbelastungskriterium nicht verwertbar. Das Produkt der Bicarbonatpufferung, CO_2, unterliegt jedoch aufgrund seiner physikalischen Eigenschaften der Perfusion weniger als Laktat. Um zu klären, ob das kumulierte "excess-CO_2" (Int J Sports Med 21 (2000) 419-423) die anaerobe Glykolyse bei Herzinsuffizienten eher anzeigt als die Blutlaktatkonzentration, belasteten wir zwei Patientengruppen bis zur subjektiven Erschöpfung (Fahrradergometrie, Beginn 20 Watt, 10 Watt/min Steigerung). Es wurden 10 Patienten (HI) mit schwerer Herzinsuffizienz (klinisch NYHA III-IV, EF<25%, Alter 58,3±6,8 Jahre) und 10 Patienten (NHI) mit ähnlichen kardialen Grunderkrankungen, jedoch ohne Herzinsuffizienz (EF>45%, Alter 63,5±6,1 Jahre) untersucht. Zum Ende jeder Stufe wurden kapilläre Blutproben aus dem hyperämisierten Ohrläppchen zur Messung der Laktatkonzentration entnommen. VO_2 und VCO_2 als Grundlage für die Berechnung des excess-CO_2, wurden per respiratorischer Massenspektrometrie breath-by-breath gemessen. Koeffizienten für die Anstiegsgeschwindigkeit der Laktatkonzentration (λ(La)) und des kumulierten excess-CO_2 (λ(excessCO_2)) gegen die Leistung wurden aus exponentiellen Kurvenfittings ermittelt. Das Verhältnis von λ(La)/λ(excessCO_2) lag bei HI als Zeichen einer verminderten Laktatverteilung signifikant niedriger als bei NHI. Bei zudem höherer maximaler Leistung von NHI zeigt dies den möglichen Nutzen des excess-CO_2 zur Bewertung 1. einer fraglich erreichten Ausbelastung, 2. der Leistungsfähigkeit in Bezug auf die Beanspruchung der Glykolyse und 3. in der spezifischen Diagnostik von Herzinsuffizienten.