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Molecular basis of skeletal muscle plasticity-from gene to form and function

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Abstract Skeletal muscle shows an enormous plasticity to adapt to stimuli such as contractile activity (endurance exercise, electrical stimulation, denervation), loading conditions (resistance training, microgravity), substrate supply (nutritional interventions) or environmental factors (hypoxia). The presented data show that adaptive structural events occur in both muscle fibres (myofibrils, mitochondria) and associated structures (motoneurons and capillaries). Functional adaptations appear to involve alterations in regulatory mechanisms (neuronal, endocrine and intracellular signalling), contractile properties and metabolic capacities. With the appropriate molecular techniques it has been demonstrated over the past 10 years that rapid changes in skeletal muscle mRNA expression occur with exercise in human and rodent species. Recently, gene expression profiling analysis has demonstrated that transcriptional adaptations in skeletal muscle due to changes in loading involve a broad range of genes and that mRNA changes often run parallel for genes in the same functional categories. These changes can be matched to the structural/functional adaptations known to occur with corresponding stimuli. Several signalling pathways involving cytoplasmic protein kinases and nuclear-encoded transcription factors are recognized as potential master regulators that transduce physiological stress into transcriptional adaptations of batteries of metabolic and contractile genes. Nuclear reprogramming is recognized as an important event in muscle plasticity and may be related to the adaptations in the myosin type, protein turnover, and the cytoplasma-to-myonucleus ratio. The accessibility of muscle tissue to biopsies in conjunction with the advent of high-throughput gene expression analysis technology points to skeletal muscle plasticity as a particularly useful paradigm for studying gene regulatory phenomena in humans.

Abbreviations
Stimuli: *CLFS* Chronic low-frequency electric stimulation · *CR* Caloric restriction · *DE* Detraining · *DV* Denervation · *EE* Endurance exercise · *ER* Endurance runners · *I* Immobilization · *IN* Inactivity · *MG* Real or simulated microgravity · *RT* Resistance training · *WL* Weightlifters

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Structure, function: *βOX* β-Oxidation · *DEL* Deltoidus · *EC coupling* Excitation-contraction coupling · *EDL* Extensor digitorum longus · *Gls* Glycolysis · *H+* Reducing equivalents · *IMF mitochondria* Interfibrillar mitochondria · *IMCL* Intra-myocellular lipid · *KC* Krebs cycle · *M* Muscle · *NMJ* Neuromuscular junction · *SR* Sarcoplasmic reticulum · *S mitochondria* Subsarcolemmal mitochondria · *Tn* Troponin · *VL* Vastus lateralis · *VO2max* Maximal oxygen consumption

Signals, sensors and transducers: *ACTH* Corticotropin · *AMPK* 5'-AMP-activated protein kinase · *ATP* Adenosine 5'-triphosphate · *Ca2+* Intracellular calcium · *CaMKII* Ca^{2+}/CaM kinase II · *Cor* Cortisol · *EN* Epinephrine · *ERK* Extracellular signal-regulated kinase · *GH* Growth hormone · *IGFBP-3* Insulin-like growth factor binding protein 3 · *IGF-I* Insulin-like growth factor I · *JNK* c-jun N-terminal kinase · *c-jun* cellular counterpart of retroviral insert from avian sarcoma virus 17 · *Ins* Insulin · *lep* Leptin · *MAPK* Mitogen-activated (microtubule-associated) protein kinase · *NRF-1 and -2* Nuclear respiratory factor 1 and 2 · *p38* p38 MAPK · *RE* Renin · *ROS* Reactive oxygen species · *T3* Triiodothyronine · *T4* Tetraiodothyronine · *Tes* Testosterone · *TFAM* Mitochondrial transcription factor · *TSH* Thyroid stimulating hormone

Genes: *3'UTR* 3' Untranslated region · *5'UTR* 5' Untranslated region · *ACAA2* 3-ketoacyl-CoA thiolase · *ACC* Acetyl-CoA carboxylase · *AIF1* Allograft inflammatory factor 1 · *AOX1* Aldehyde oxidase · *ATP5A* ATP synthase alpha chain · *ATP5C* ATP synthase gamma chain · *ATP5G3* ATP synthase lipid-binding protein · *ATP5J* ATP synthase coupling factor 6 · *ATPB* ATP synthase beta chain · *Cat H* Cathepsin H · *CDC16Hs* Cell division cycle 16 · *c-fos* c-fos Proto-oncogene · *c-jun* c-jun Proto-oncogene · *CK* Creatine kinase · *Col* Collagen type · *CPT I and II* Carnitine *O*-palmitoyltransferases I and II · *COX* Cytochrome C oxidase subunits · *CSF-1* Colony stimulating factor · *CXCL5* Small inducible cytokine B5 · *CYP2A6* Cytochrome P450 2A6 · *CYP2B6* Cytochrome P450 2B6 · *CYP2C8* Cytochrome P450 2C8 · *CYPdb1* Cytochrome P450 db1 · *DDO* D-aspartate oxidase · *DIA1* NADH-cytochrome b5 reductase · *DNA* Deoxyribonucleic acid · *DP5* Neuronal death protein · *EGFR* Epidermal growth factor receptor · *ERF* Ets2 repressor factor · *FAP* Fibroblast activation protein · *FAT/CD36* Fatty acid translocase · *FBP2* D-fructose-1,6-bisphosphate 1-phospho-hydrolase · *FN* Fibronectin · *Fum* Fumarase · *gamma 1* Interferon gamma treatment inducible mRNA · *glut-1* Glucose transporter 1 · *HIAP1* Inhibitor of apoptosis protein 1 · *HIF-1α* Hypoxia-inducible factor 1 alpha · *HPARG* Poly(ADP-ribose) glycohydrolase · *HPXEL* Peroxisomal enoyl-CoA hydratase-like protein · *HSC* Heat shock cognate · *HSP* Heat shock protein · *HSP27 and 70* Heat shock protein 27 and 70 · *HSP2* Heparan sulfate proteoglycan 2 · *IL-1, -12 and -18* Interleukin 1, 12 and 18 · *IMPDH1* Inosine-5'-monophosphate dehydrogenase 1 · *LDH* lactate dehydrogenase · *LIF* Leukaemia inhibitory factor precursor · *LN* Laminin · *LPL* Lipoprotein lipase · *LRP1* Low-density lipoprotein receptor-related protein 1 · *LTC4S* Human leucotriene C4 synthase · *Mac-2* Macrophage subpopulation-specific antigen 2 · *MARK3* MAP/microtubule affinity-regulating kinase 3 long · *MAT1* CDK-activating kinase assembly factor · *MCAD* Medium chain-specific acyl-CoA dehydrogenase · *MCT1, 2 and 4* Monocarboxylate transporters 1, 2 and 4 · *MHC* Myosin heavy chain · *MHC10* Nonmuscle type B myosin heavy chain (MYH10) · *MHC9* Nonmuscle type A myosin heavy chain (MYH9) · *mRNA* Messenger ribonucleic acid · *MSH3* DNA mismatch repair protein · *MT1B* Metallothionein-IB · *MT1F* Metallothionein I F · *MEF2* Myocyte enhancer factor 2 · *myoD* Myoblast determination protein · *MRF4* Muscle regulatory factor 4 · *myf 5 and 6* Myogenic factors 5

and 6 · *nAChR* Nicotinic acetyl choline receptor · *NADH6* Mitochondrially-encoded NADH dehydrogenase subunit · *NF-kappa B* Nuclear factor kappa B · *NCAM1* Neural cell adhesion molecule 1 · *NDUFV1* NADH-ubiquinone oxidoreductase 51 kDa subunit · *NDUFV2* NADH-ubiquinone oxidoreductase 24 kDa subunit complex core protein 2 · *NF-kB p65* Nuclear factor kappa B p65 subunit · *NF-Y* Nuclear factor Y protein subunit A · *NNT* Mitochondrial NAD(P) transhydrogenase · *NRG-1* Neuregulin · *ORP150* 150 kDa Oxygen-regulated protein · *p21* Cyclin-dependent kinase inhibitor 1 · *PON2* Paraoxonase 2 · *PDK4* Pyruvate dehydrogenase kinase 4 · *PP2Ag1* Serine/threonine protein phosphatase 2A · *PPAR* Peroxisome proliferator-activated receptor · *RANTES* Regulated upon activation, normal T cell expressed and secreted · *RECQ2* RecQ-like type 2 DNA helicase · *RNA* Ribonucleic acid · *RYK* Related to receptor tyrosine kinase · *SCHAD* Short chain 3-hydroxyacyl-CoA dehydrogenase · *SDH* Succinate dehydrogenase · *SH3GL3* SH3-containing GRB2-like protein 3 · *SOD2* Manganese superoxide dismutase · *SVCT2* Sodium-dependent vitamin C transporter · *tie 2* Angiopoietin 1 receptor · *TRAP230* Thyroid hormone receptor-associated protein complex component · *TSP4* Thrombospondin 4 · *UCP* Mitochondrial uncoupling protein · *UQCRB* Ubiquinol-cytochrome C reductase · *UQCRC2* Ubiquinol-cytochrome C reductase · *UQCRH* Ubiquinol-cytochrome C reductase complex 11 kDa protein · *VEGF* Vascular endothelial growth factor

Introduction

Skeletal muscle structure and function can be changed as a consequence of stimuli that modify skeletal muscle contractile activity (endurance exercise, electrical stimulation, denervation), load on the muscle (resistance training, microgravity), substrate supply (nutritional interventions) or environmental factors such as hypoxia and thermal stress. Moreover, skeletal muscle tissue undergoes gradual changes with ageing. This malleability is an important feature of skeletal muscle tissue and allows for an economic design of muscle tissue sufficient for given conditions at a certain time but adaptable to changes imposed by muscle use and the environment (Hoppeler and Flück 2002). The phenomenon of plasticity is observed in all vertebrate species; however, a large variability in the responsiveness and degree of muscle adaptations is observed among species, individuals of a species and among different functional systems of muscle tissue such as the contractile apparatus, energy conversion and excitation-contraction coupling.

In the context of muscle malleability, the fibre and its environment appear to conspire. Recent data from rodent models indicate that skeletal muscle plasticity involves an integrated response of fibre (i.e. cytoarchitecture and composition; Chopard et al. 2001; Frenette and Tidball 1998) and fibre-associated structures (i.e. nerves, capillaries; Tyml and Mathieu-Costello 2001; Deschenes et al. 2001). Transcriptional reprogramming of nuclei is recognized to be a major event early on in this process (Wittwer et al. 2002a; Carson et al. 2002). The involvement of signalling in the sensation and transduction of stimuli into activation of specific gene expression events is widely recognized (Sakamoto and Goodyear 2002; Martineau and Gardiner 2002; Nader and Esser 2001). The present review gives an overview of the molecular basis of structure-function adaptations of skeletal muscle in rodents and humans. The current state of knowledge is summarized in a model describing how physiological stimuli are sensed at the molecular level and translated into the changes that modify skeletal muscle phenotype. Due to the rapid progress in this field, this

article cannot be exhaustive but reflects the bias of the authors of what they perceive to be major factors involved in skeletal muscle plasticity. The reader is also referred to previous articles with a similar focus (Booth and Thomason 1991; Hood 2001; Baldwin and Haddad 2001; Booth and Baldwin 1995; Howald 1982).

Concepts

Muscle function

Contraction of mammalian skeletal muscle is under neuronal control. Triggering of an action potential at the neuromuscular junctions through the activity of an efferent α-motor neuron causes the synchronous contraction of the muscle fibres constituting the motor unit. The contraction process in the muscle fibre is then initiated by the release of Ca^{2+} from intracellular stores and provoking ATP-dependent interaction changes of myosin and actin in the contractile apparatus (Huxley 1988). In addition to the α-neurons responsible for the control of contractile activity, muscle contains thin myelinated (A delta or group III) and unmyelinated (C or group IV) afferents receptive to chemical, mechanical, and thermal stimuli, playing a role in the circulatory and respiratory adjustments during exercise (Kniffeki et al. 1981; Christensen and Galbo 1983).

Specific molecular diversities of the main proteins in the Ca^{2+}-dependent proteins involved in the EC coupling process largely determine the contractile properties of muscle fibres (Berchtold et al. 2000). Changes in the expression of slow (type I) and fast (type IIa, IIx and IIb) myosin heavy- and light-chain components of the sarcomere, and changes in the firing frequency of the innervating nerve are both known to contribute to differences in contraction speed of single fibres (Pette and Staron 2000; Schiaffino and Reggiani 1994, 1996).

Energy demand of skeletal muscle can be increased by two orders of magnitude by contractile activity (Hargreaves 2000; Booth and Thomason 1991). At the onset of exercise, the creatine and arginine ATP stores are self-sufficient for a few seconds. Subsequently, ATP production by glycolysis takes over. Glycolysis yields ATP at high rates but delivers the by-product lactate, which is exported into the blood stream and into adjacent muscle fibres and may hinder muscle performance (Brooks and Mercier 1994; Brooks 2000). The ATP for continued muscle work is regenerated from ADP through oxidative phosphorylation of reduction equivalents arising from the combustion of carbohydrates and fatty acids (Fig. 1). Glycolysis is an order of magnitude less efficient in terms of ATP yield per mole of glucose than the mitochondrial oxidation of carbohydrates or fatty acids (Hargreaves 2000; Weibel 2002b). During aerobic work, the balance between carbohydrate and fat metabolism is controlled by the intensity of exercise and the supply of nutrients from intra- (IMCL, glycogen) and extracellular stores (in liver and adipose tissue). Extracellular substrates need to be delivered through the capillary system whereby it is recognized that the design of the capillary network is determined by the conditions for oxygen transfer to muscle cells, as there are practically no stores of oxygen in muscle cells (Hoppeler and Weibel 2000 see Fig. 1). In general terms, combustion of carbohydrates is favoured at high-intensity exercise (over 50% of VO_2max), whereas the proportion of energy production through β-oxidation is larger during prolonged exercise and at moderate intensities (below 50% of VO_2max; Brooks and Mercier 1994).

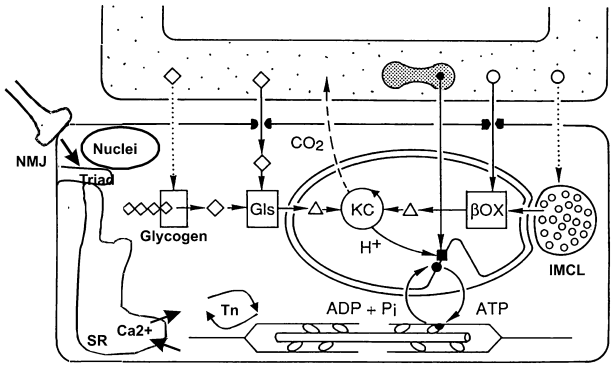

Fig. 1 Substrate pathways and muscle contraction. Model for structure-function relationships of oxygen and intracellular substrate supply to the mitochondria of skeletal muscle cells and control of muscle contraction by nerve activity. The generation of an action potential at the NMJ causes release of Ca^{2+} from the sarcoplasmic reticulum into the cytoplasmic space. Through binding to troponin, this provokes the modulation of the actin-myosin interaction, thereby shortening the sarcomere. This so-called cycle of excitation-contraction coupling is reversible and ATP-dependent. The ATP equivalents necessary for muscle contraction are mainly derived from metabolic conversion of carbohydrates and fatty acids. *Open circles* indicate fatty acids, *diamonds* indicate glucose; a *row of diamonds* indicates polymerized glycogen and *triangles* indicate acetyl-CoA. *Arrows* show the pathways of intracellular substrate breakdown from the intracellular stores to the terminal oxidase in the mitochondrial inner membrane (*black square*). Other *arrows* indicate the supply routes of oxygen (*black dot*) and substrates from the capillaries, with *dotted arrows* for the supply route to intracellular stores, temporally split from the phase of oxidation. Paired *semicircles* (*in black*) indicate transport systems facilitating glucose and fatty acid uptake. *Dashed arrow* indicates CO_2 discharge to the blood. For full-length names, the reader is referred to the list of abbreviations

The molecular composition of the sarcomere, its metabolic make-up, capillary supply and the mode of motor unit recruitment all have an influence on the functional muscle phenotype by determining the key parameters contractility and resistance to fatigue. In addition, we must recognize that muscle tissue, by virtue of its size, making up as much as 50% of body mass in some athletic species, is a key metabolic organ importantly involved in conserving the energy balance of the individual (Booth and Thomason 1991; Hoppeler and Flück 2002).

Molecular mechanisms of muscle malleability

During development, skeletal muscles are formed by fusion of mesodermal precursor cells to primary myotubes. A population of muscle precursor cells stays residual under the basal lamina (satellite cells) and can respond to various stimuli. Satellite cells may divide and proliferate, thus forming new muscle fibres (Schultz and McCormick 1994). Alternatively, they can fuse with existing muscle fibres, thereby adding to the pool of nuclei when increased need for transcriptional activity arises (Schiaffino and Reggiani 1994). The nuclear domain limitation theory predicts that nuclei can provide mRNA for only a limited myocellular volume (the nuclear domain; Allen et al. 1999). As skeletal muscle nuclei provide the necessary mRNA coding for ribosomal, structural and metabolic proteins, it was suggested that the cytoplasma-to-myonucleus ratio determines the capacity of a muscle to support protein turnover rates as well as increased synthesis of perturbed protein classes (reviewed in Booth and Baldwin 1995). The number of myonuclei and thus the size of the

nuclear domains appear to play a critical role in establishing the window of adaptive potential of skeletal muscle to alter its phenotype. In these events, the recruitable satellite cell population seems to be of importance. Last but not least, selective apoptotic death of myonuclei has been proposed to occur during muscle atrophy as a mechanism for removing parts of myofibres without affecting their viability (Sandri 2002).

On a structural level, skeletal muscle plasticity involves modifications of cellular (mitochondria, myofibrils, etc.) and extracellular compartments (capillaries, nerves, connective tissue). Modulation of the proteic and to a lesser degree also of the lipidic make-up of muscle tissue has been shown to be the molecular components of muscle malleability (Booth and Baldwin 1995; Hoppeler and Weibel 1998; Bruhn et al. 1991). In particular, changes in expression of myofibrillar and metabolic proteins have often been demonstrated to be involved in skeletal muscle plasticity (Schiaffino and Reggiani 1996; Booth and Thomason 1991).

The change of protein content and of protein isoforms towards a new steady-state during an adaptive event can potentially be controlled by modifications in many steps from DNA to the assembled translation products (Fig. 2). Altered pretranslational, translational and post-translational events are all involved in the molecular regulation of the skeletal muscle phenotype. Three decades ago, solid evidence for the essential role of de novo RNA synthesis (transcription) in loading induced skeletal muscle hypertrophy was presented (Goldberg and Goodman 1969; Sobel and Kaufman 1970). These investigators showed the activity of RNA polymerase to be increased with hypertrophy. Treatment with actinomycin D, an inhibitor of DNA-dependent RNA synthesis was shown to prevent skeletal muscle hypertrophy. More recently, rapid transcriptional regulation of gene expression in human skeletal muscle during recovery from exercise has been demonstrated by nuclear run-ons (Pilegaard et al. 2000). Endurance exercise training has been shown to increase the steady-state levels of certain metabolic mRNAs in human skeletal muscle (Vogt et al. 2001; Pilegaard et al. 2000; Puntschart et al. 1995a).

The level of mRNA is determined by the rate of mRNA synthesis and the rate of mRNA decay (i.e. its chemical half-life). It is well established that the half-life time of many mRNAs can fluctuate in response to environmental stimuli such as nutrient levels, cytokines, hormones, temperature shifts and viral infections (Day and Tuite 1998). So far, only indirect evidence has been provided for control of RNA stability in muscle with exercise, by demonstrating that contractile activity modulates the level of factors interacting with a region of the 3'UTR of the cytochrome C transcript known to determine mRNA degradation (Yan et al. 1996). The observations indicate that enhanced RNA stabilization may contribute to the enhanced mRNA level and thus ultimately to the increased mitochondrial densities seen in human skeletal muscle with endurance exercise.

As expected, in general we find that cellular and molecular adaptations in human and animal models for muscle plasticity are congruent when physiologically equivalent stimuli are compared. For example, a single bout of resistance training causes similar increases in protein synthesis in humans and in rats (Phillips et al. 1999; Hernandez et al. 2000). Likewise an acute bout of endurance training causes a similar increase in VEGF expression, with a similar time course in humans and rats (Richardson et al., 1999; Breen et al. 1996). However, interspecies congruency of adaptive events is by no means absolute. Involvement of the same biological processes, but to different extents, is noted in some skeletal muscle adaptations. Differences between human and rat models for load-induced muscle hypertrophy include the extent as well as the kinetics of cellular events such as the change

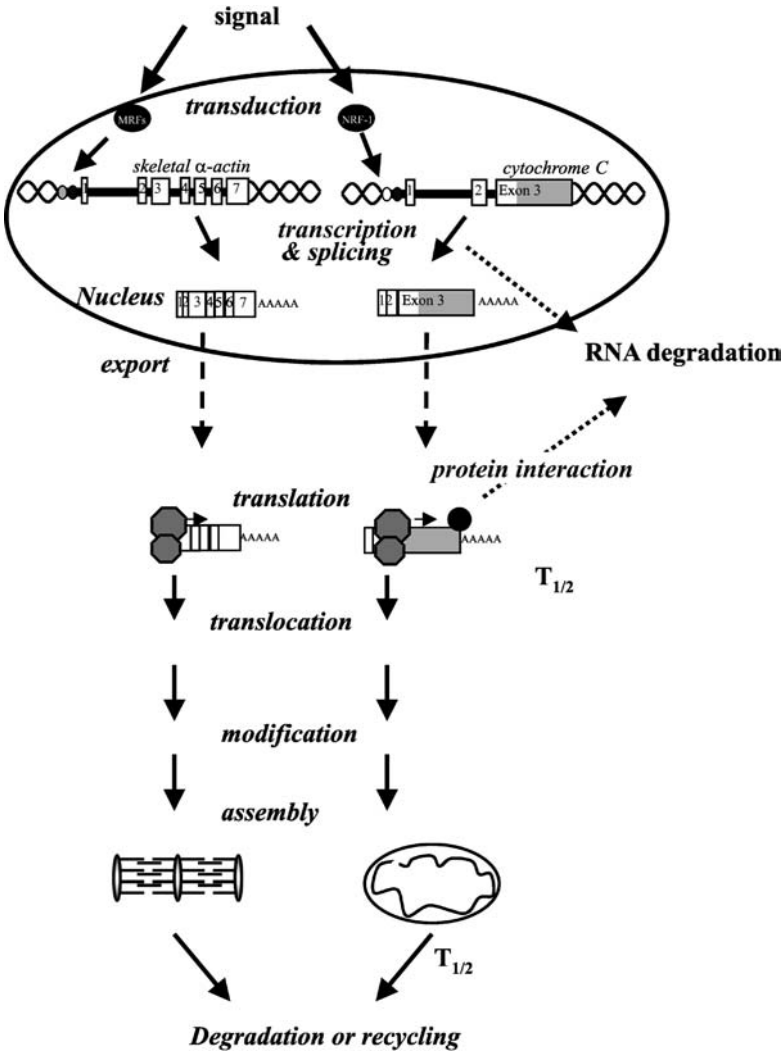

Fig. 2 Control of gene expression. Various extracellular signals initiate transcriptional, post-transcriptional, translational and post-translational responses. This influences the rate of synthesis and degradation and ultimately determines the steady-state of each expressed gene and protein. Expression steps indicated in *italics* are demonstrated to be involved in the molecular mechanism of muscle plasticity

in fibre diameter and the activation of satellite cells (Kadi and Thornell 2000; Snow 1990). These differences in response were tentatively explained by the higher relative severity of the stimuli in the rat versus the human model (Booth and Thomason 1991). Alternatively, we have to consider that observed differences in malleability and the time course of adaptations may simply reflect allometric scaling, i.e. be related to the 3/4 power relationship between basal metabolic rate and body mass (Weibel 2002a).

A survey of the molecular responses of skeletal muscle to changes in contractile activity demonstrates that as a general rule the directional change of a given mRNA is in the

same direction as the directional change of the protein during its adaptation (Booth and Baldwin 1995). When RNA and translation products do not change in the same direction, in some specific cases this may be explained by high turnover or high basal concentrations of the protein (Andersen and Schiaffino 1997). An increased level of mRNA is assumed to be translated into protein and to cause a micro-adaptation in protein concentration (Day and Tuite 1998). In the absence of a proportional increase in degradation, this would increase the total amount of protein. However, for highly abundant proteins or in the presence of increased protein degradation, increases in mRNA content may not be detected as net changes in protein concentration (Fig. 2). The scenario for increased protein turnover is supported by the fact that 2 weeks of daily wheel running augmented skeletal alpha actin mRNA in fast- but not slow-twitch hindlimb muscle and increased fractional protein synthesis in slow- and fast-twitch hindlimb muscle in rats (Munoz et al. 1994; Morrison et al. 1989).

Post-translational modifications of proteins are a universal and efficient way to modulate the functional properties of enzymes and of pathways. Modifications of enzymes involved in metabolism and signalling have been reported as acute responses to several physiological stressors of skeletal muscle (Booth and Baldwin 1995). Moreover, modulation of translation efficiency has been noted to occur with muscle loading and increased contractile activity (reviewed in Booth and Baldwin 1995). Several of these adaptations are short-lived and are down-regulated by degradation or recycling of the modified protein or by removal of the post-translational modification (Bergamini 1992; Wilkinson 2000; Sorkin and Waters 1993).

The focus of this review will be on changes in muscle mRNA steady-state levels, as these can be taken as an index of changes in gene expression and currently appear to be the best described and understood molecular events that underlie muscle plasticity.

Adaptations of skeletal muscle to different stimuli

Contractile activity

The importance of contractile activity in determining the muscle phenotype is widely recognized. An important role in modulating phenotype has been assigned to the motoneuron (Gundersen 1998). It has been demonstrated that the extent and modality of neuronal activity is crucial for regulating contractile properties such as speed, strength and endurance. The signals that determine muscle phenotype are to a large extent coded in the pattern and frequency of electrical activity (Pette and Staron 2000). Large amounts of continuous low-frequency activity lead to a slowing of shortening velocity by influencing, among other things, myosin heavy-chain expression. Short bursts of high-frequency activity lead to a fast muscle phenotype (Pette 1998; Booth and Baldwin 1995).

Endurance exercise

Endurance exercise training has been the intervention of choice to explore the malleability of mitochondrial structure and function in humans. In a typical training experiment during which initially untrained subjects were trained intensely for 6 weeks (5 times/week for 30 min on a bicycle ergometer); total mitochondrial volume density was increased by 40% in

m. vastus lateralis (Hoppeler et al. 1985). The data are compatible with the contention that it is a relatively small fraction of the total muscle volume which is specifically trained with an exercise intervention and it is the mitochondria in these muscles that adapt to the stimulus and can be held responsible for the increase in VO_2max (Saltin 1986; Hoppeler et al. 1985).

Endurance exercise training increases the smaller volume fraction of subsarcolemmal mitochondria (SS mitochondria) more than the larger fraction of interfibrillar mitochondria (IMF mitochondria; Hoppeler et al. 1985). This was noted as early as 1973 (Hoppeler et al. 1973) and has since been confirmed in many studies using different separation techniques for mitochondria (Chilibeck et al. 1998; Bizeau et al. 1998; Roussel et al. 2000). Differences in mitochondrial functions such as protein import (Takahashi and Hood 1996) or protein synthesis and degradation (Connor et al. 2000) point to possible mechanisms by which the two mitochondrial subpopulations are capable of reacting differently to stress. In some studies, IMF mitochondria were shown to have higher respiration rates and higher respiratory control ratios than SS mitochondria (Lombardi et al. 2000). However, a consensus on the functional identity of SS and IMF mitochondria and the significance of the differential response to a variety of stimuli has not been reached.

Using a morphometric approach on individual histochemically typed muscle fibres Howald et al. (Howald et al. 1985) were able to demonstrate that bicycle endurance exercise training of 6 weeks duration (same study as Hoppeler et al. 1985) leads to an increase of the volume density of mitochondria in all three fibre types, the increase being larger in type IIa than in type I and type IIb/x fibres. This finding is surprising, as one would not expect type IIb/x fibres to be recruited in an endurance-type exercise such as bicycling at low intensity (approx. 20% of maximal short-term power output). Howald et al. (1985) also noted that there was a broad overlap of mitochondrial volume densities among all three fibre types before and after training. This begs the question as to the diversity of molecular phenomena, which might occur in different fibre types as a consequence of a functionally uniform intervention such as a classic endurance-type training intervention. The complex response of the individual muscle fibre types also calls for circumspection in the interpretation of molecular responses obtained on bulk extracts of muscle biopsy material. Moreover, there is evidence that aerobic exercise (4 h of exercise at 40% of VO_2max) stimulates protein turnover by increasing protein breakdown and protein synthesis in the recovery phase in human skeletal muscle (Carraro et al. 1990b). Moreover, the same type of exercise increased the fractional synthesis rate of fibronectin but not of albumin (Carraro et al. 1990b). This indicates that in response to exercise, there is also a stimulation of the synthesis of some acute-phase (extracellular matrix) proteins.

Endurance exercise training leads to a shift in skeletal muscle mitochondria towards an increased use of lipids as a substrate source (Holloszy and Booth 1976; Holloszy and Coyle 1984). We noted early (Hoppeler et al. 1973) that the intramyocellular lipid content (IMCL) in trained subjects was significantly larger than in controls. Likewise, 6 weeks of endurance exercise training approximately doubled IMCL (0.47 vs. 0.92%) of muscle fibre volume (Hoppeler et al. 1985; Howald et al. 1985; Table 1). It is generally accepted now that endurance exercise training leads to an increased use of lipids as a substrate both with regard to relative and absolute exercise intensities (van Loon et al. 2001) and augments IMCL content of muscle fibres. However, the mechanisms by which these phenomena are controlled remain to be established. Last but not least, recent observations demonstrating an increase in reduced glutathione after recurrent episodes of high-intensity exercise ses-

Table 1 Structural/functional adaptations of primate skeletal muscle to physiological stimuli (for the full-length names, the reader is referred to the list of abbreviations; blank field, to the best of our knowledge not reported; *s* seconds, *m* minutes, *h* hours, *d* days, *w* weeks, *mo* months, *y* years)

	Stimuli														
	High contractile activity-low loading					Low contractile activity-high loading			Metabolic intervention			Hypoxia		Ageing	
	EE	CLFS	DV	IN/DT	References	RT	MG	References	FA supp.	CR	References		References		References
STRUCTURAL															
Myofibrils															
Cross-sectional area															
Total muscle	–	↑mo-y	–	–	–	↑w	↓w-mo (VL)	1-7	–	–	–	↓mo	8	↓y	7
Total fibre	→y	↑mo-y	↓mo	↓w ↑w in ER	9-12	→w↑mo	↓w-mo (VL)	5, 13, 14	–	–	–	↓mo	8	↓y	7
Type I	→y	→w-mo	↓y	↑w in ER	9, 11, 12, 15-17	→w↑mo	↓mo (VL) →w-mo (DEL)	1, 5, 18-20	–	–	–	–	–	→y	16
Type IIa	→y	→w-mo	↓y	↓w in WL ↑w↓mo in ER	9, 11, 12, 15-17	→w↑mo	↓mo (VL) →w-mo (DEL)	1, 5, 18-20	–	–	–	–	–	↓y	16
Type IIx	→y	→w-mo	↓y	↓w	9, 11	→w↑mo	↓mo (VL) →w-mo (DEL)	1, 5, 18-20	–	–	–	–	–	↓y	16
Volume density															
Total fibre	–	–	–	–	–	→w↑mo	→w	2, 5, 21	–	–	22	↑mo	23	–	–
% I Fibres	↑mo-y	→mo-y		→d-w↓y	9, 12, 21, 24-30	→↓w-mo	→w-mo	1, 2, 25, 31	–	–	–	–	–	–	–
% IIa Fibres	↓y	↑mo-y	↑mo-y	→d-w	9, 10 24	↑w-y(↓mo)	→w-mo	1, 2, 19, 25, 31-33	–	–	–	–	–	↓y	34
% IIx Fibres	↓d	↓mo-y	↑mo-y	→d-w↑mo	9-12, 21, 24, 27	→↓w	→w-mo	1, 2, 19, 25, 31, 35	–	–	–	–	–	↓y	34
% Hybrid I/IIa fibres	↑y	–	–	↑mo-y	36	↓ mo	–	37	–	–	–	–	–	↑s	38
% Hybrid IIa/IIx fibres	↑y	–	–	↑mo-y	36	↓mo	–	25, 31, 37	–	–	–	–	–	↑s	38
Mitochondria (total)															
[Volume density]	↑w	–	–	–	39	↓w-y	↓mo (VL) →w-mo (DEL)	2, 13, 19	→w	–	40	↓mo	8	–	–
[Absolute volume]	–	–	–	–	–	→w	↓mo(VL)	2, 13, 19	–	–	–	–	–	–	–

Table 1 (continued)

	Stimuli														
	High contractile activity-low loading					Low contractile activity-high loading			Metabolic intervention			Hypoxia		Ageing	
	EE	CLFS	DV	IN/DT	References	RT	MG	References	FA supp.	CR	References		References		References
Muscle nuclei															
Myonuclei/ fibre	–	–	–	–	–	↑mo-y	↓mo	33, 42, 43	–	–	–	–	–	–	–
Satellite cells	–	–	–	–	–	↑mo	–	33, 42	–	–	–	–	–	–	–
Capillarity															
Capillary-to-fibre ratio	↑w	→w-mo	↓y	↓w / type I fibre	11, 15, 16	→w	→w-mo	2, 5, 18	–	–	–	↓mo	8	↓y / IIa and IIx fibre	16
[Volume density]	↑w	–	–	–	11, 15, 43	→w	→w-mo	2, 5, 18	–	–	–	↑mo	8	–	–
Fuel stores															
Glycogen	↓m	–	–	–	44	–	–		–	–	–	–	–	–	–
IMCL (volume density)	↓h↑w-mo	–	–	–	27, 39	→w	→↑w	2, 5, 13, 18	↑w	–	40	→mo	8	–	–
Oxidative damage (myofibrils/ lipids)	–	–	–	–	–	–	–	–	–	↓y	45	–	–	↑y	45
Motoneurons and -units	–	–	–	–	–	–	–	–	–	–	–	–	–	↓y	34, 46
FUNCTIONAL															
Regulation															
Neuronal activity	↑s-m	–	–	↓d-mo in WL	11, 47, 48	↓d↑w	↑d-w	7, 30, 49-51	–	–		–		–	
Endocrine	↑m GH, RE, EN ↓m Ins ↓h T3/T4, TSH, Ins	–	↓h Tes, GH, IGF-I	↑w GH, Tes ↓w cor in WL	11, 52-54	↑m GH, Tes, IGFBP-3 ↑w Tes ↓m lep ↓w cor	–	35, 55-58	–	–		(+EE) ↑m ACTH (+EE)↓– m Ins	54	↓y Tes, IGF-I, IGFBP-3	57
Mechano	↑m-h JNK, ERK, p38 activity	–	–	–	59-63	–	–	–	–	–	–	–	–	–	–

Table 1 (continued)

	Stimuli														
	High contractile activity-low loading					Low contractile activity-high loading			Metabolic intervention			Hypoxia		Ageing	
	EE	CLFS	DV	IN/DT	References	RT	MG	References	FA supp.	CR	References		References		References
Metabolic	↑m AMPKα2 activity	–	–	–	44, 64, 65	–	–	–	–	–	–	–	–	–	–
Contraction															
Maximal shortening velocity	–	↑w-mo	–	–	66	↑w	→↑w	3, 14, 67-71	–	–		-	–	↓y	7, 72
Muscle strength/ power	↓h→y	↑mo-y	–	→d-w↓mo in WL	10, 11, 73-75	↑w	↓w-mo	1, 3-6, 20, 35, 49, 76	–	–		–	–	↓y	7
Metabolism															
Myoglobin	→w	–	–	→w-mo	11, 77	–	–	–	–	–	↑y	78	–	–	
CHO metabolism	↑w	–	–	↑↓w	11, 79	↑w	→w	1, 2, 18	↓d	–	80, 81	(+EE)↑m	82-84	↓y	85
FA oxidation (mito ox. capacity)	↑h-d-w	↑mo-y	–	↓w-mo	11, 15, 16, 65, 86, 87	–	↓w-mo (VL) →w-mo (DEL)	1-3, 18	→↑d	–	80, 81	(+EE)↓m	83, 84	–	–
Krebs cycle (respiration)	↑w	↑w-mo	↓y	↓w-mo	11, 15, 88	↑w	→w	1-3, 18	–	–		–	–	↓y	85, 89
Energy transfer	–	–	–	→mo	11	↑w	–	1, 6	–	–	–	–	↓y	85	
Fatty acid synthesis	↑m phospho ACC	–	–	–	44, 90	–	–	–	–	–		–	–		–
Resistance to fatigue	↑w	↑mo-y	–	–	10, 24, 66, 91	↑w	–	76	↑w	–	92	–	–	↓y	9
VO_2max	↑w	↑mo-y	–	↓d-mo	9-11, 24, 39	→w	↓w-mo (VL)	2, 69	→w	–	92	–	–	–	
Synthesis rate															
Protein synthesis	↑h (mixed and FN)	–	–	–	93, 94	↑h-w (mixed and MHC)	–	7, 28, 95-99	–	–		–	–	↓y (mixed + MHC) →y (actin)	28
Protein degradation	–	–	–	–	–	↑h (mixed)	–	96, 98	–	–		–	–	–	–
RNA activity	–	–	–	–	–	↑h	–	97	–	–	–	–	–	–	

Table 1 (continued)

[1] Costill et al. 1979, [2] Ferretti et al. 1997, [3] Fitts et al. 2001, [4] Hespel et al. 2001, [5] Luethi et al. 1986, [6] VandenBorne et al. 1998, [7]Vandervoort 2002, [8] Hoppeler et al. 1990, [9] Ingjer 1979, [10] Mohr et al. 1997, [11] Mujika and Padilla 2001, [12] Round et al. 1993, [13] MacDougall et al. 1979, [14] Widrick et al. 1999, [15] Martin et al. 1992, [16] Proctor et al. 1995, [17] Schaub et al. 1989, [18] Desplanches et al. 1998, [19] Hikida et al. 2000, [20] Hortobagyi et al. 2000, [21] Lotta et al. 1991, [22] Hoppeler and Weibel 2000, [23] Hoppeler and Weibel 1998, [24] Andersen and Schiaffino 1997, [25] Andersen et al. 2000, [26] Burnham et al. 1997, [27] Howald et al. 1985, [28] Hasten et al. 2000, [29] Ricoy et al. 1998, [30] Sale 1988, [31] Andersen et al. 1994b, [32] Andersen et al. 1994a, [33] Kadi et al. 1999, [34] Carmeli and Reznick 1994, [35] Staron et al. 1994, [36] Klitgaard et al. 1990c, [37] Williamson et al. 2001, [38] Klitgaard et al. 1990a, [39] Hoppeler et al. 1985, [40] Hoppeler et al. 1999, [41] Kadi and Thornell 2000, [42] Ohira et al. 1999, [43] Zumstein et al. 1983, [44] Stephens et al. 2002, [45] Zainal et al. 2000, [46] Panenic and Gardiner 1998, [47] Ray and Gracey 1997, [48] Seals and Enoka 1989, [49] Connelly and Vandervoort 2000, [50] Ertl et al. 2002, [51] Hakkinen and Kauhanen 1989, [52] Bauman and Spungen 2000, [53] Hesse et al. 1989, [54] Kjaer et al. 1999, [55] Craig et al. 1989, [56] Hansen et al. 2001, [57] Kraemer et al. 1999, [58] Tuominen et al. 1997, [59] Aronson et al. 1997, [60] Aronson et al. 1998, [61] Boppart et al. 1999, [62] Wong and Booth 1990, [63] Yu et al. 2001, [64] Chen et al. 2000, [65] Tunstall et al. 2002b, [66] Hjeltnes et al. 1998, [67] Degens et al. 1999, [68] Larsson et al. 1996, [69] Paavolainen et al. 1999, [70] Trappe et al. 2000, [71] Widrick et al. 1997, [72] Larsson et al. 1997, [73] Bentley et al. 1998, [74] Harridge 1996, [75] Leveritt et al. 2000, [76] Lesmes et al. 1978, [77] Masuda et al. 2001, [78] Reynafarje 1962, [79] Linossier et al. 1997, [80] Carey et al. 2001, [81] Goedecke et al. 1999, [82] Green et al. 1992, [83] Parolin et al. 2000a, [84] Parolin et al. 2000b, [85] Pastoris et al. 2000, [86] Brooks and Mercier 1994, [87] Martin 1996, [88] Bengtsson et al. 2001, [89] Trounce et al. 1989, [90] Chen et al. 2000, [91] Harridge et al. 2002, [92] Hoppeler and Vogt 2001a, [93] Carraro et al. 1990a, [94] Carraro et al. 1990b, [95] Balagopal et al. 2001, [96] Biolo et al. 1995, [97] Chesley et al. 1992, [98] Phillips et al. 1997, [99] Yarasheki et al. 1993b

sions indicate that redox potential could be increased with endurance training (Rabinovich et al. 2001) and argues for expressional changes in redox metabolism.

The effects of different exercise training paradigms on myosin heavy-chain expression have recently been reviewed by Baldwin and Haddad (2001). World-class marathon runners and extreme endurance athletes have been shown to have a strong bias towards the expression of the slow type I MHC. As much as 80-90% of the MHC pool is composed of the slow type I MHC, with the remainder being type IIa MHC (Andersen et al. 2000; Table 1). In the context of these observations, it has been shown in humans that a short period of high-intensity endurance training induces a shift from fast MHC isoforms toward the slow variety within histochemically typed fibres of the m. vastus lateralis (Schaub et al. 1989). Furthermore, a report by O'Neill et al. (1999) demonstrated that 7 days of cycling exercise induces a significant down-regulation of fast IIx MHC mRNA (Table 2). Thus, although gifted athletes may have a genetic predisposition to excel in certain athletic events due to their inherent leg muscle MHC profiles, it appears that the MHC gene profile of an individual can be altered via chronic increases in contractile activity such as physical training. As indicated above, world-class marathon runners and ultra-endurance athletes are reported to have remarkably high type I fibre numbers in their trained muscle groups (Andersen et al. 2000; Ricoy et al. 1998), whereas muscles of sprinters and weightlifters predominantly consist of IIa/IIx fibres (Andersen et al. 1994b, 2000). It further appears that extreme usage (and disuse) induced alterations of the muscle MHC isoform profile result in hybrid fibres that express a combination of MHC isoforms such as type I/IIa and IIx/IIa (Klitgaard et al. 1990a). How much of these patterns of MHC gene expression is due to genetic predisposition and how much to the specificity of training (or some combination of these) is unresolved (reviewed in Baldwin and Haddad 2001).

Table 2 Expressional adaptations of primate skeletal muscle to physiological stimuli (for the full-length names, the reader is referred to the list of abbreviations; blank field, to the best of our knowledge not reported)

	Stimuli														
	High contractile activity-low loading					Low contractile activity-high loading			Metabolic intervention			Hypoxia		Ageing	
	EE	CLFS	DV	IN/DV	References	RT	MG/I	References	FA supp.	CR	References		References		References
FUNCTIONAL UNIT															
Contraction-structure	–	↑w MHCI	–	–	[1]	↑↓w-m MHCI	↓w MHCI	[2-4]	–	–	–	–	–	→y MHCI	[2]
Myosin heavy Chains	–	–	–	–	–	→↑w-m MHCIIa ↓m (aged)	→w MHCIIa	[1, 3-5]	–	–	–	–	–	↓y MHCIIa	[2]
	→m ↓d-w-mo MHCIIx	↓w MHCIIx	↑w-mo MHCIIx	↑w-mo MHCIIx	[1, 4, 6-8]	↓→↑w-m MHCIIx	↑w MHCIIx	[1, 3, 4, 6]	–	–	–	–	–	↓y MHCIIa	[2]
ECM and cytoskeleton	–	–	–	–	–	–	–		–	↑y Col I and III and VI and VIII, FN, HSP2, LN α2, desmin, tubulin, actin β, MHC9 and 10	[9]	–	–	↓y TSP4	[9]
Metabolism															
Carbohydrates															
Glycolysis	↑m-h HKII	–	–	–	[10]	–	–	–	–	–	–	–	–	–	–
Glucose stores	↑m-h glut-4, GYS ↑d GYS	–	–	–	[10, 11]	–	–	–	–	–	–	–	–	↓y glycogenin, glut-3, FBP2	[9]
Oxygen															
Myoglobin	–	–	–	–	–	–	–	–	–	–		↑y +EE↑w	[12, 13]	–	–
Fatty acids															
Import and β-oxidation	↑m-h CPTI, LPL, FAT ↑wCPTI, MCAD, VLCAD	–	–	–	[10, 13-15]	–	–	–	–	↓y ACAA2, HPXEL ↑yLRP1	[9]	–	–	↓y SCHAD	[9]

Table 2 (continued)

	Stimuli														
	High contractile activity-low loading					Low contractile activity-high loading			Metabolic intervention			Hypoxia		Ageing	
	EE	CLFS	DV	IN/DV	References	RT	MG/I	References	FA supp.	CR	References		References		References
ATP production and transfer															
Krebs cycle	↑m-hPDK4 ↑w SDH, Fum	–	–	–	10, 16, 17	–	–	–	↑dPDK4	↑yPDK4 ↓y MDH1	9, 29	–	–	↓y SDH, MDH1, Fum	9
El chain and ox. phos.	↑wNADH6, COXI and IV	–	–	–	17, 18	–	–	–	–	↑h-d UCP-3↓y UQCRC2 UQCRB, NDUFV2, CytC, COX IV and VIII, ATPB, NNT ↑y NDUFV1, DIA1	9, 19	–	–	↓y UQCRH, NDUFV2, ATP5A, ATP5C, ATP5G3, ATP5J, COX IV and VIII and VIa	9
Proton uncoupling	↑m-h UCP-3	–	–	–	10	–	–	–	↑w UCP-2 and 3	–	20, 29	–	–	–	–
Energy transfer	–	–	–	–	–	–	–	–	–	–	–	–	–	↓y CK	9
Protein turnover															
Turnover	–	–	–	–	–	↑d HSC/HSP70, HSP27	–	21	–	–	–	–	–	↓y Cat H	9
Folding	↑m-h HO-1, HSP-70	–	–	–	10, 22		–	–	–	–	–	–	–	↑y HSP27 and 70	9, 23
Regulation															
Signal transduction															
Mechano	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Metabolic	↑mo PPARα	–	–	–	14	–	–	–	–	–	–	(+EE) ↑w HIF-1α	13	–	–
Endocrine response	–	–	–	–	–	–	–	–	–	–	–	–	–	↓y TRAP230	9

Table 2 (continued)

	Stimuli														
	High contractile activity-low loading					Low contractile activity-high loading			Metabolic intervention			Hypoxia		Ageing	
	EE	CLFS	DV	IN/DV	References	RT	MG/I	References	FA supp.	CR	References		References		References
Transcription	↑m-h c-fos, fosB,fra-1, c-jun, junB and D ↑wTFAM	–	–	–	17, 18, 24	↑h-d c-jun, ↑w myogenin →w MRF4, MyoD, Myf5, ↓↑h-d EGR-1	→w myogenin, MRF4, MyoD, Myf5	23, 25	–	–	–	–	–	↓y ERF	9
Cell cycle															
Angiogenesis	↑m-hVEGF	–	–	–	26, 27	↑h-d VEGF	–	23	–	–	–	+EE ↑w VEGF	13	–	–
Satellite cell proliferation	–	–	–	–	–	↑w-m Leu19	–	4	–	–	–	–	–	–	–
Myonuclei incorporation	–	–	–	–	–	↑y dMHC	–	4	–	–	–	–	–	–	–
Muscle cell growth arrest	–	–	–	–	–	–	–	–	–	–	–	–	–	↑y p21	9
Cell proliferation	–	–	–	–	–	–	–	–	–	↑y RYK, FAP	9	–	–	↓y IGF-BP 5 and 7, MAT1, IMPDH1, EGFR, Mac-2, PP2Ag1, MARK3	9
(Neuronal) cell death	–	–	–	–	–	–	–	–	–	–	–	–	–	↑y SH3GL3, DP5, granzyme A	9
Neuronal remodelling	–	–	–	–	28	–	–	–	–	–	–	–	–	↑y NRG-1, NCAM1, reelin, phogrin	9
Repair															
(Oxidative) Stress/ damage	–	–	–	–	–	–	–	–	–	–	–	–	–	↑y MT1F, ORP150, NF-kB p65	9, 23
DNA repair	–	–	–	–	–	–	–	–	–	–	–	-	–	↓y XRCC1, HPARG, MSH3, RECQ2	9

Table 2 (continued)

	Stimuli														
	High contractile activity-low loading					Low contractile activity-high loading			Metabolic intervention			Hypoxia		Ageing	
	EE	CLFS	DV	IN/DV	References	RT	MG/I	References	FA supp.	CR	References		References		References
Detoxification	–	–	–	–	–	–	–	–	–	–	[9]	–	–	↓y SOD2, PON2 ↑y AOX1, CYP2B6, CYP2A6, CYP2C8	[9]
Inflammation	–	–	–	–	–	↑h-d RANTES, IL-1β	–	[23]	–	↓y RING6 OTF-2	[9]	–	–	↑y granulin, gamma 1, JAK1, LTC4S, CSF-1, chitotriosidase, IL-18, CXCL5	[9]

[1] Harridge et al. 2002, [2] Balagopal et al. 2001, [3] Hortobagyi et al. 2000, [4] Kadi and Thornell 1999, [5] Andersen et al. 1994a, [6] Andersen and Aagaard 2000, [7] Andersen et al. 2000, [8] O'Neill et al. 1999, [9] Kayo et al. 2001, [10] Pilegaard et al. 2000, [11] Kraniou et al. 2000, [12] Reynafarje 1962, [13] Vogt et al. 2001, [14] Horowitz et al. 2000, [15] Tunstall et al. 2002b, [16] Martin et al. 1992, [17] Puntschart et al. 1995a, [18] Bengtsson et al. 2001, [19] Tunstall et al. 2002a, [20] Schrauwen et al. 2001a, [21] Thompson et al. 2001, [22] Puntschart et al. 1996, [23] Jozsi et al. 2000, [24] Puntschart et al. 1998, [25] Hespel et al. 2001, [26] Gustafsson et al. 1999, [27] Richardson et al. 1999, [28] Panenic and Gardiner 1998, [29] Peters et al. 2001

The effects of endurance exercise such as running on the MHC profile appear to be both muscle-specific and dose-dependent. For example, in rodents, when animals are trained to run at moderate to high intensity (~30 m/min; ~20% incline at ~75% VO_2max) for several weeks, the running effects on the MHC profile of the soleus are manifested only when animals run for longer durations (60 and 90 min/day; Demirel et al. 1999). In mixed fast muscles (red vastus and gastrocnemius), which have a composition bias to type IIx and IIa MHC expression, both the type IIa and IIx MHCs are up-regulated relative to the sedentary state, whereas the IIb MHC is significantly down-regulated under these training conditions (Demirel et al. 1999). If running is extended for longer durations, it is even possible to induce increased expression of the type I MHC in fast muscles (Demirel et al. 1999; Green et al. 1984).

It has been shown that systematic endurance exercise training increases the steady-state level of a number of mRNAs encoding mitochondrial proteins in proportion to the increase in mitochondrial volume density (Puntschart et al. 1995a). Moreover, acute bouts of endurance exercise cause a significant rapid (0-4 h after exercise) transient increase in the mRNA level of several proteins involved in regulating mitochondrial functions (Pilegaard et al. 2000). Additionally, exercise seems to rapidly affect cellular trafficking, i.e. glut-4 and possibly small G proteins (Kraniou et al. 2000) (reviewed in Booth and Baldwin 1995). Endurance exercise training further causes a fine-tuning of the mRNA level of MCAD involved in beta oxidation of medium chain fatty acids (Vogt et al. 2001). This observation is compatible with the functional observation that endurance exercise shifts the functional capacity of mitochondria towards an increased use of lipids as a substrate (Holloszy and Booth 1976; Holloszy and Coyle 1984). Bicycling exercise also affects expression of genes involved in the modulation of muscle substrate supply, i.e. to an induction of key components of fatty acid transport (FAT/CD36, FABPpm, LPL) and β-oxidation (CPTI) in human m. vastus lateralis during the recovery phase (Pilegaard et al. 2000; Tunstall et al. 2002b). Data from animal experiments support that LPL expression is at least in part controlled by local contractile activity (Hamilton et al. 1998). Activation of gene expression therefore appears to be the main mechanism for the accumulation of post-transcriptional micro-adaptations responsible for the subsequent structural and biochemical adaptations of the mitochondrial compartment in exercised skeletal muscle.

Looking at mitochondria in more detail, we find that the mitochondrial organelle incorporates numerous proteins involved in beta oxidation, the Krebs cycle and the mitochondrial respiratory chain, in the inner and outer membrane and its matrix. Only a few of these proteins are coded on the mitochondrial genome while most are coded on the nuclear genome. Therefore, proteins from nuclear-encoded genes, as well as phospholipids, must be synthesized in the cytosol, imported into mitochondria and incorporated into their appropriate locations for mitochondrial biogenesis to be successful (Hood 2001). The steady-state level of mitochondrially, as well as nuclear-encoded mRNAs of the mitochondrial respiratory chain and the mitochondrial DNA concentration is increased in m. vastus lateralis of athletes versus untrained subjects. By contrast, genomic DNA was not increased in athletes while mitochondrial DNA was increased (Puntschart et al. 1995a). These observations indicate a specific way in which the expression of mitochondrial proteins from the nuclear and from the mitochondrial genomes is coordinated. For nuclear encoded genes, we find an increase in transcription leading the observed increase in mRNA of downstream genes. For mitochondrially encoded species, the rate of transcription is unchanged and the total quantity of mitochondrial DNA is up-regulated. Thus, the proportion of mito-

chondrial RNA to mitochondrial DNA remains constant. This is achieved because endurance exercise causes an increase in the level of the mitochondrial transcription factor (TFAM) in proportion to the increased abundance of mitochondrial- and nuclear-encoded mitochondrial proteins. A transient increase in TFAM and nuclear respiratory factor 1 and 2 (NRF-1 and NRF-2) mRNA has also been documented for rat muscle (Bengtsson et al. 2001; Gordon et al. 2001a; Murakami et al. 1998). Therefore, post-exercise transient expression of NRF-1 through controlling TFAM expression may be a key factor for coordinating nuclear and mitochondrial gene transcription for mitochondrial biogenesis in exercised skeletal muscle.

The increase in oxidative capacity of skeletal muscle with endurance training in human (and rats) is accompanied by a similar increase in muscle capillarity. Using morphometric techniques, the capillary-to-fibre ratio is significantly higher in orienteers (2.70) than in untrained men (2.07) and in untrained women (1.37) (Zumstein et al. 1983). To our knowledge, the molecular events leading to concerted capillary growth in human exercise training have yet to be studied in detail. In human skeletal muscle, exercise affects expression of the *VEGF* gene, which is involved in formation and permeability of blood vessels (Richardson et al. 1999; Gustafsson et al. 1999).

Data from animal studies further indicate the involvement of other molecules such as angiotensin II, basic fibroblast growth factor and transforming growth factor β1, but not of nitric oxide, in capillary remodelling induced by exercise (Amaral et al. 2001b; Breen et al. 1996; Lloyd et al. 2001; Richardson et al. 1999). Blocking of angiotensin II-signalling by angiotensin-converting enzyme inhibitors and angiotensin II type I receptor blockers completely blocked VEGF-dependent short-term (3 days) capillary remodelling in rat skeletal hindlimb muscle induced by intense running exercise or 7 days of electrical stimulation (Amaral et al. 2001a, 2001b). The involvement of the renin-angiotensin system in control of VEGF expression and vessel density support for an important role of microcirculation in capillary remodelling of skeletal muscle through eventual modulation of vascular resistance and shear stress (Hudlicka 1998).

Using microarray analysis (Atlas array no. 7740-1) we recently analysed the level of distinct mRNAs involved in regulatory processes in human m. vastus lateralis and the difference in their expression between untrained subjects (mean VO_2max=39 ml/min/kg; n=7) and professional cyclists (mean VO_2max=72 ml/min/kg; n=7). High expression of genes involved in inflammation (*IL-1*, *IL-13*) and stress response (*HSP-27*, *c-jun*, UV excision repair protein RAD23A) was detected pointing to an eventual role in skeletal muscle homeostasis while others with well-established muscle regulatory function were often less expressed (Fig. 3). Using the Mann-Whitney U-test, we identified only 15 out of 408 expressed genes to be differently expressed between untrained subjects and professional cyclists. At the 0.05% significance level, this number of transcripts is within the range of randomly expected events in a population of 408. We have therefore refrained from a biological interpretation of these results. It is assumed that a highly fluctuating gene expression between individuals could mask adaptations in regulatory gene expression (Wittwer et al. unpublished observations).

Inactivity/detraining

The adaptability of skeletal muscle to variable levels of functional demands is well underlined by the observation that muscular detraining occurs during periods of insufficient

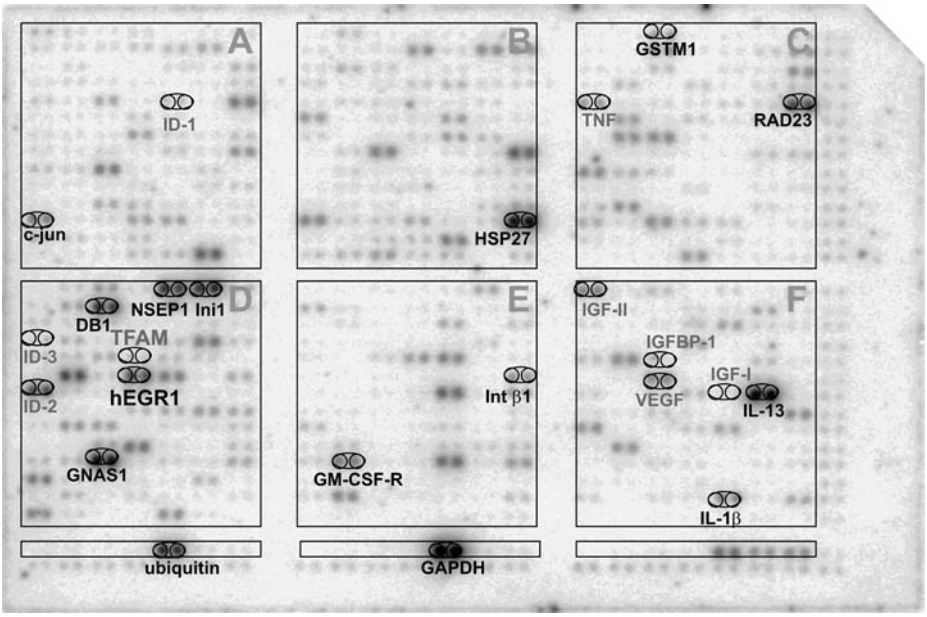

Fig. 3A-F Regulatory gene profile in human m. vastus lateralis. Autoradiogram of a microarray that was hybridized with radiolabelled target cDNA synthesized from RNA isolated from untrained human m. vastus lateralis. The *boxes* denote sectors that were doubly spotted with probes of genes belonging to related functional categories: **A** cell cycle regulation; **B** signal transduction; **C** cell death and DNA interaction; **D** transcription; **E** cell surface receptors; **F** cell-cell communication. The spots assigned with *black and grey text* correspond to the ten genes which were on average most highly expressed and to genes with established muscle regulatory function

training stimulus. The underlying structural-functional changes to detraining have been recently reviewed by Mujika and Padilla (2001). Fibre distribution remains unchanged during the initial weeks of inactivity, but oxidative fibres may decrease in endurance athletes and increase in strength-trained athletes within 8 weeks after training was stopped. Muscle fibre cross-sectional area declines rapidly in strength and sprint athletes, and in recently endurance-trained subjects, whereas it may increase slightly in endurance athletes. Force production declines slowly and in relation to decreased EMG activity. Strength performance in general is readily maintained for up to 4 weeks of inactivity, but highly trained athletes' eccentric force and sport-specific power, and recently acquired isokinetic strength, may decline significantly. Detraining may be characterized by a decreased capillary density, which could take place within 2-3 weeks of inactivity. Arterial-venous oxygen difference declines if training arrest continues beyond 3-8 weeks. Moreover, rapid and progressive reductions were noted within 3-46 weeks of detraining in oxidative and Krebs cycle enzyme activities (β-hydroxyacyl-CoA dehydrogenase, citrate synthase, succinate dehydrogenase, malate dehydrogenase) to bring about a reduced mitochondrial ATP production. The above changes are related to the reduction in VO_2max observed during long-term training cessation. In contrast, myoglobin concentration was found unchanged in the m. gastrocnemius of endurance-trained runners and cyclists after 3 and 12 weeks of training cessation. Moreover, glycolytic enzyme activities (phosphofructokinase, lactate dehydrogenase, phosphorylase) in muscles of trained and untrained individuals show nonsys-

tematic changes during periods of training cessation (Table 1). These muscular characteristics remain above sedentary values in the detrained athlete but usually return to baseline values in recently trained individuals. Interestingly, increases were observed the concentration of growth hormone and testosterone while cortisol was decreased in the plasma concentrations of weightlifters after 12 weeks of detraining. The complete pattern of adaptations indicates that the qualitative changes in fibre types may depend on the training status of the individuals. To the best of our knowledge, no data are available on the expressional events that underlie the phenomenon of muscular detraining in humans.

Cross-reinnervation

Cross-reinnervation (cross-union) experiments performed by Buller, Eccles and Eccles on cats (Buller et al. 1960) were at the origin of the paradigm of skeletal muscle phenotype. This kind of experiment demonstrated that the type of motor neuron innervation is determining many muscle characteristics. When a denervated muscle exhibiting fast-twitch properties is ectopically reinnervated by a slow nerve, the muscle adopts most slow-twitch properties, and vice versa (Buller et al. 1960).

The early cross-reinnervation experiments are supplemented now with studies leading to the identification of molecular events involved in muscle reprogramming. A particular focus has been the study of the myogenic transcription factors myogenin, myoD, MRF4/herculin/myf-6 and myf5. Together, these factors play an important role in myogenesis through binding to consensus Ebox recognition sites, which exist in numerous muscle-specific genes (Olson 1990). Cross-reinnervation of the slow rat soleus muscle with a fast nerve decreased myogenin mRNA expression in regions usually expressing fast myosin heavy chain (Hughes et al. 1993). Moreover, thyroid hormone treatment resulted in activation of the myoD and fast myosin heavy-chain gene expression in the slow soleus while no significant change in either slow MHC, MRF4 or myogenin mRNA accumulation was detected. Based on the observations that the levels of myogenin are higher in slow-twitch muscles, while myoD is associated with fast-type fibres, it was proposed that myogenin and myoD may function as intermediaries to selectively regulate fibre-type-dependent contractile gene expression. Specifically, because myogenin controls expression of a battery of muscle-specific genes, including slow MHC expression (Olson 1990), it was suggested that myoD is involved in controlling fast glycolytic and that myogenin would control slow *MHC* gene expression (Hughes et al. 1993). Consequently, an increase in the myogenin:myoD ratio would preferentially drive expression of slow-type contractile proteins in skeletal muscle (Fig. 4).

Electric stimulation

Chronic low-frequency stimulation (CLFS) of mammalian skeletal muscle causes specific changes in muscle properties (Tables 1, 2). Much of the early work on the effect of chronic stimulation was done in animal models (Pette and Vrbova 1992). These experiments have led to the conclusion that the firing frequency and activity pattern of motor-neurons are the key determinants of the contractile characteristics of muscle fibres.

CLFS activates all motor units of the target muscle synchronously and with the same impulse pattern. It is thus possible to attain much higher levels of muscle activity over

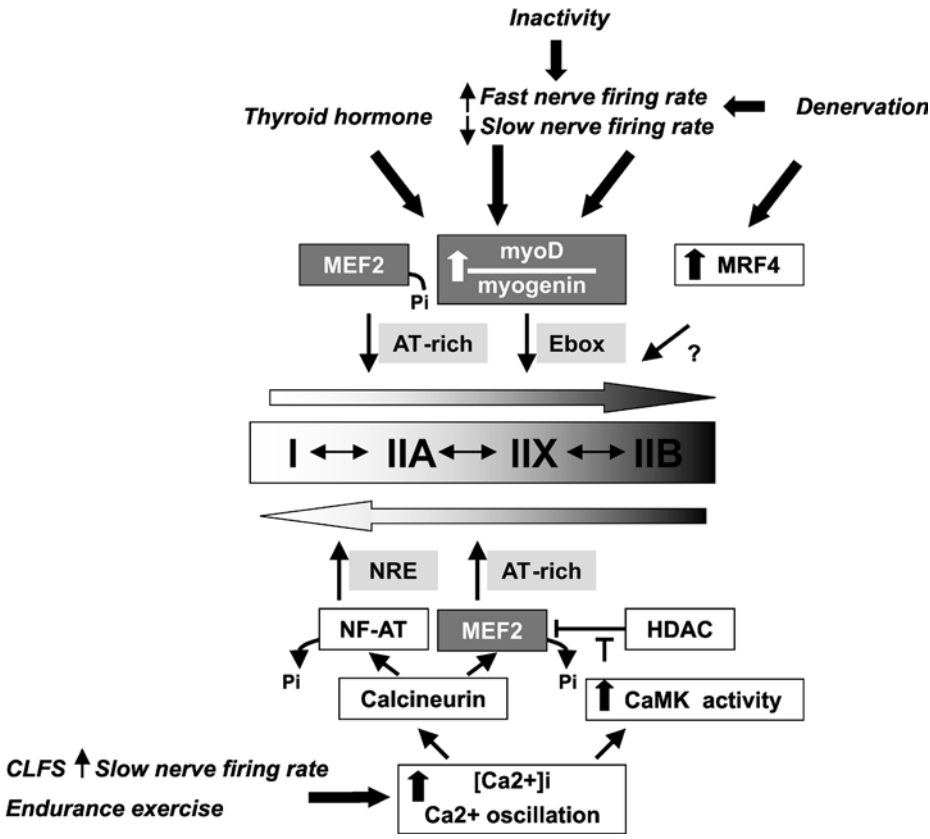

Fig. 4 Molecular control of fibre-type-specific MHC expression. Model indicating the molecular factors that have been implied in control of myosin heavy-chain expression as a consequence of different stimuli in (rodent) skeletal muscle myonuclei. Reduced rate of slow nerve firing, denervation, thyroid hormone and eventually inactivity cause an increase in the myoD/myogenin ratio of myogenic transcription factors and phosphorylated MEF2 drives expression of fast type myosin II (Hughes et al. 1993). Conversely, through increases in intracellular calcium $[Ca^{2+}]i$, chronic low-frequency stimulation (*CLFS*) and endurance exercise cause the activation of the phosphatase calcineurin and the protein kinase CaMKII. Calcineurin causes dephosphorylation of transcription factor NF-AT and MEF2 and initiates transcription of myogenin. CaMKII provokes the liberation of MEF2 from the complex with the transcriptional repressor histone deacetylase (*HDAC*) (Olson and Williams 2000). These events promote the expression of slow type myosins (Chin et al. 1998; Wu et al. 2001, 2002)

time than with any voluntary exercise regimen. CLFS thus challenges muscle to its full adaptive potential (Pette 2001). The major adaptations observed with CLFS affect the contractile apparatus and its energy supply. Chronic stimulation of fast-type animal muscle increases time to peak, twitch tension and half relaxation time while the maximal rate of tetanic tension development is decreased (Pette and Vrbova 1992). Moreover, increased resistance to fatigue and increased aerobic-oxidative capacity are consistent findings observed with CLFS of animal skeletal muscle (Pette and Vrbova 1992; Reichmann et al. 1985; Table 1). The observed functional changes are explained by a drop in muscle fibre diameter, changes in the quality of the myofibrillar apparatus and the Ca^{2+} regulatory system towards the slow muscle type as well as an increase in capillarity (reviewed in Pette

and Vrbova 1992). The increase in activities of enzymes of the aerobic-oxidative metabolism is held responsible for the increased fatigue resistance. A linear correlation is shown to exist between the increase in citrate synthase activity, total mitochondrial volume and aerobic-oxidative capacity (Reichmann et al. 1985). Recent data indicate that the functional changes provoked by increasing the contractile activity of skeletal muscle of spinal cord-injured humans by chronic low-frequency stimulation are qualitatively similar to those seen with endurance exercise (Tables 1, 2). Exercise training of spinal cord-injured individuals (30 min/day, two to three times per week for 1 year) on an ergometer under electrical stimulation reverses inactivity-associated performance changes in skeletal muscle (Mohr et al. 1997). Resistance to fatigue, work output, oxygen uptake, muscle cross-sectional area were all increased, while the number of MHC-type IIa fibres was increased and the type IIb (IIx) fibre number was reduced. Similar changes in MHC expression with an augmentation of MHC type IIa and a drop in MHC type IIa fibres were seen after 6 and 12 months of functional electric stimulation (Andersen et al. 1996). Intriguingly, in both studies, MHC type I fibres did not increase.

In the clinical setting, electrical stimulation therapy either of individual muscles or in a coordinated functional manner is occasionally used to counteract atrophy, pressure sores, bone loss, and glucose intolerance (Mohr et al. 1997). Improvements in muscle metabolic properties, insulin sensitivity, and oxidation capacity may be closely related to the transition of fibre types within the fast phenotypes (i.e. from MHC IIx to MHC IIa) (Andersen et al. 1996; Burnham et al. 1997; Mohr et al. 1997).

It has been demonstrated early on that low-frequency (10 Hz, 12 h/day) electric stimulation of fast-twitch rabbit muscles induces the tissue yield of the total polyadenylated RNA, polyadenylated RNAs specifically translatable in vitro, total ribosomes whereby an increase in monosomes precedes the increase in the polysomes (Seedorf et al. 1986). The increase in lactate dehydrogenase isoenzymes and citrate synthase was found to be due to altered transcription levels as well as post-transcriptional control mechanisms. The latter are held responsible for the early rise in CS concentration. Later, data from human and animal models indicated that numerous pretranslational changes contribute to adaptations of skeletal muscle to chronic electrical stimulation. For example, continuous indirect electrical stimulation of tibialis anterior muscle increases CPT II mRNA (Yan et al. 1995). Recent data from paraplegic humans indicate that the up-regulation of mRNA for MHC-I isoform and down-regulation of the MHC-IIx isoform both contribute to changes in the contractile apparatus and hence to the reduction in speed of contraction with chronic electrical stimulation (Harridge et al. 2002). Last but not least, expression of the myogenic factors myogenin and MyoD were found to increase as a consequence of electric stimulation while myogenin is implicated in the control of the increase in nAChR expression with CLFS (reviewed in Buonanno et al. 1998).

Denervation

Denervation due to spinal cord injury leads to drastic adaptations of human skeletal muscle. Paralyzed muscles of individuals with chronic traumatic spinal cord injury (SCI) are characterized by a massive reduction of the size of all muscle fibres, by a high incidence of fast muscle fibres and in some instances even by a complete loss of slow fibres in what would normally be mixed fibre-type muscles (Burnham et al. 1997; Lotta et al. 1991; Martin et al. 1992; Round et al. 1993) (Fig. 5; Tables 1, 2). For both type I and type II fibres,

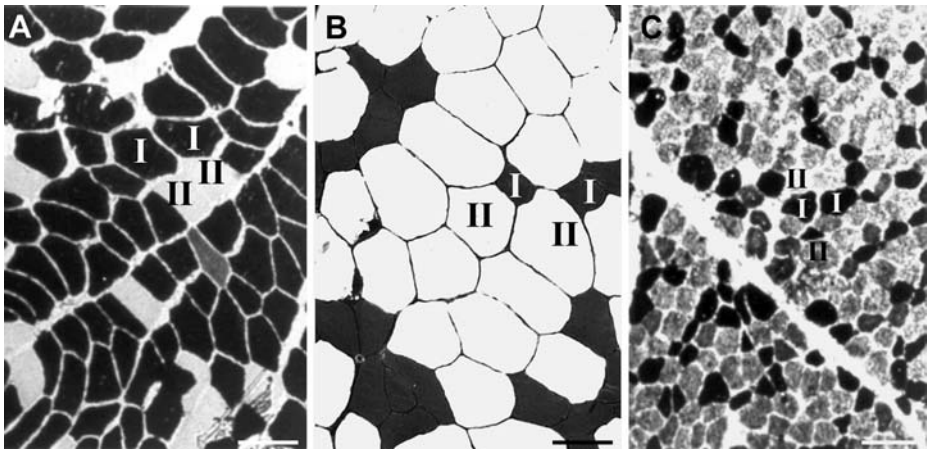

Fig. 5A-C . Plasticity of human skeletal muscle fibre types. Histochemical pictures showing adaptations of fibre types in human m. vastus lateralis with endurance exercise (**A**), resistance training (**B**) and denervation subsequent to spinal cord injury (**C**). Slow- (*I*) and fast-type (*II*) fibres are indicated. Bar, 100 μM

the cross-sectional area, activities of succinate dehydrogenase, and the capillary-to-fibre ratio are significantly reduced in paralyzed muscles (Martin et al. 1992). Moreover, the activity of the primary enzyme for conversion of circulating lipoprotein triglyceride into (membrane-permeable) free fatty acids, lipoprotein lipase (LPL), is decreased in denervated rat soleus muscle, supporting the concept that contractile activity is importantly involved in the regulation of the metabolic muscle phenotype (Smol et al. 2001).

The myosin heavy-chain (MHC) isoform expression appears to be altered towards an increase in MHC II. Furthermore, SCI leads not only to a general relative increase in MHC II expression, but also to a relative increase in the expression of the fastest of the human MHC isoforms, MHC IIx (Burnham et al. 1997; Lotta et al. 1991; Round et al. 1993). These changes are thus opposite to the adaptations seen with increased motoneuron activity such as during CLFS (Tables 1, 2). This observation supports the suggestion that human MHC IIx (formerly called IIb) is the default *MHC* gene (Goldspink et al. 1991) and that the high expression of this isoform in paraplegic muscle is an adaptive response to a lack of usage, specifically of the loss of neural input and mechanical loading (Andersen et al. 1996; Harridge 1996; Talmadge 2000).

In animal models, the myogenic factors myogenin, myoD, MRF4/herculin/myf-6 and myf5 have been demonstrated to undergo rapid nuclear accumulation in mature myofibres after denervation (Weis et al. 2000; Eftimie et al. 1991; Walters et al. 2000). There are data indicating that the expression of the myogenic regulatory factors in denervated muscles depend on the muscle phenotype (Walters et al. 2000). Fast muscles respond very quickly to denervation by increasing the level of MRF transcripts MyoD and myogenin while slow muscles did not show significant increases in expression up to 48 h after denervation (Walters et al. 2000; Fig. 4). MRF4 has been suggested to regulate genes encoding adult contractile proteins and acetylcholine receptor subunits, indicating that MRF4 may have important roles in the gene programs activated after denervation and during muscle regeneration (Weis et al. 2000). Overall, the data indicate that muscle-specific transcription factors of the helix-loop-helix family such as myoD and myogenin are important for

regulating genes related to metabolic profile, fibre size and possibly myosin heavy-chain expression in response to changes in nerve-dependent contractile activity (Gundersen 1998; Buonanno et al. 1998).

Experiments with rats show that denervation-induced transient increases in myogenin and sustained up-regulation of levels of myoD cause an increase in MHC IIx expression in soleus and EDL but no atrophy of these muscles (Dupont-Versteegden et al. 1998). These findings indicate that the mechanisms underlying maintenance of muscle mass are distinct from those controlling myosin heavy-chain expression. The results support the contention that altered myogenin and myoD levels are involved in control of MHC expression. It was shown that MyoD was preferentially expressed in satellite cells in soleus muscle but was more readily detectable in myofibre nuclei in EDL. By contrast, myogenin was readily detected in both myofibre and satellite cell nuclei, suggesting varied functions of these myogenic factors in different muscles (Dupont-Versteegden et al. 1998). These findings are compatible with a scenario whereby new satellite cells have to be recruited and to fuse with type I fibres of slow-twitch solei to express type II MHC in these former type I fibres. This suggests that the recruited myonuclei substitute during this fibre transformation for a lack of myonuclei which switch back to or re-express the default-type type II MHC. In the fast-twitch EDL muscle, however, myonuclei can be induced to express Myo D and type II MHC isoforms (Fig. 4).

However, there are a number of negative correlations between the activity-dependent regulation of MyoD and myogenin and genes encoding contractile proteins that challenge the model of Hughes et al. (1993), which predicts that the myogenin:myoD ratio determines expression of slow versus fast-type contractile proteins in skeletal muscle. An alternative model was proposed that postulates that motor neuron innervation alone induces transcription regulatory mechanism(s) that activate the expression of numerous genes encoding contractile proteins and metabolic enzymes (Buonanno et al. 1998; Olson and Williams 2000; Fig. 4). Future experiments are now necessary to identify to what extent regulatory factors in addition to the myogenin:myoD ratio are needed to explain the observed plasticity of MHC expression, contractile activity and metabolic pathways observed as a consequence of denervation in rodent and human skeletal muscle (Talmadge 2000).

Muscle loading

Several manipulations which change the mechanical load to which muscle is exposed have been shown to have pronounced effects on muscle structure and function. In the following sections, we discuss the structural and functional changes as well as the molecular events associated with various modalities for increasing or decreasing muscle loading.

Resistance training

Using high-intensity, low-repetitive (strength-type) exercise, human skeletal muscles show marked gains in strength that are due both to neuronal adaptations and to an increase in muscle cross-sectional area (see Sale1988). There is consensus that the gain in muscle cross-sectional area is mainly due to an increase in myofibrillar volume (i.e. contractile protein; MacDougall et al. 1979; Luethi et al. 1986). The cross-sectional area of all fibre types is increased following resistance training, with a tendency for larger increases in

type II than type I fibres (Hortobagyi et al. 2000; Table 1). Volume densities of mitochondria are found to be reduced and may be as low as 2% in some elite athletes. This decrease is thought to be due to a dilution of a constant mitochondrial volume in larger muscle fibres (MacDougall et al. 1979; Luethi et al. 1986). Marked structural abnormalities such as central nuclei and atrophied fibres were noted in elite power lifters and body builders (MacDougall et al. 1982). These fibre abnormalities have been assumed to be related to the use of anabolic steroids in elite strength athletes. For further structural and functional characteristics of strength-trained human muscle, see also Alway et al. (1988) and Prince et al. (1981). The fact that strength training seems to leave the mitochondrial compartment relatively unaffected is supported by an unchanged distribution of subsarcolemmal versus interfibrillar mitochondria demonstrated by quantitative SDH stain of mitochondria (Chilibeck et al. 1999). Neuronal adaptations within the earlier weeks of resistance training have been suggested (Hakkinen and Kauhanen 1989; see also Sale 1988). However, some studies have not been able to demonstrate changes in the control properties of the nervous system after 8 weeks of resistance training (Rich and Cafarelli 2000). Conversely, the pronounced effect of resistance training in elderly subjects (strength gain up to 100%) has been shown to reside more in neuronal adaptations of the motor control than in the moderate increase in muscle cross-sectional area of 5-10% (Vandervoort 2002; Connelly and Vandervoort 2000).

Numerous experiments point to the importance of changes in protein turnover for the hypertrophic process (Wong and Booth 1990; Table 1). Resistance training is known to cause a (maximal) increase in fractional synthesis rate and fractional breakdown rate within 3 h after exercise that are maintained up to 48 h after the training in humans (Phillips et al. 1999; Biolo et al. 1995). Mixed and MHC synthesis rates remain enhanced as a consequence of continued resistance training of young and old subjects (Yarasheski et al. 1993b; Balagopal et al. 2001; Hasten et al. 2000). Due the lack of pertinent expression data, it is currently not known whether the absence of mitochondrial adaptations with strength training is due to transcriptional regulatory events (Chesley et al. 1992) or to enhanced mitochondrial turnover.

In humans, resistance training has been shown to up-regulate fast IIa MHC and eventually the slow type MHCI isoforms while expression of the fast type IIx MHC may be down-regulated (Adams et al. 1993; Andersen et al. 1994b; Klitgaard et al. 1990b). An increased mRNA level probably contributes to the increased size of type I and IIa fibre types. Recent data indicate that the increase in pure type MHC IIa-expressing fibres can be attributed to a decrease in the number of fibres that are hybrid with regard to the expression of slow and fast MHC isoforms (Williamson et al. 2001). The eventual drop in total MHC IIx expression with resistance training is probably due to a reduction in MHC IIx expression as a consequence of differentiation of IIx-expressing hybrid fibres towards pure IIa-expressing fibres. The increase in size of pure MHC IIx-expressing fibres also observed seems to be of lesser importance.

If subjects are detrained for several weeks, it appears that this process is reversed and the re-expression of the fast type IIx in (hybrid) fibres appears to be greater than in the pretraining period (Andersen and Aagaard 2000). However, even though resistance training increases type IIa MHC expression, it is uncertain how these molecular changes relate to the functional changes seen with strength training, in particular in the initial stages of strength training (Carroll et al. 1998).

In animal models, it has been clearly established that satellite cell activation is involved and may be a prerequisite for fibre hypertrophy (Schultz and McCormick 1994). Using a

marker for satellite cells it has been documented that expression of early markers of myogenesis is activated in satellite cells and muscle fibres in response to resistance training in humans (Kadi and Thornell 1999). Satellite cells are believed to proliferate and fuse with existing fibres, thereby contributing to an increase in myonuclei per muscle fibre. Another study indicated that the number of myonuclei was increased with fibre hypertrophy and positively correlated with the increased number of satellite cells (Kadi and Thornell 2000). This suggests that the acquisition of additional myonuclei is required to support the enlargement of muscle cells during strength training. Moreover, hypertrophy of skeletal muscle of untrained elderly men induced by high-intensity resistance training did not result in significant changes in the cytoplasma-to-myonucleus ratio despite the increased cross-sectional size of all fibre types and transition of type IIx towards IIa fibres (Hikida et al. 2000). The cellular changes occurring during hypertrophic adaptations thus essentially confirm the nuclear domain theory, suggesting that the cytoplasma-to-myonucleus ratio is a function of the myosin type and the amount of protein turnover (see Booth and Baldwin 1995). Taken together, the available evidence suggests that satellite cells are recruited during hypertrophy of muscle fibres in order to maintain the cytoplasma-to-myonucleus ratio.

Rodent models have provided a great deal of evidence on the molecular events responsible for muscle plasticity with changes in the loading conditions. Goldberg and Goodman (1969) used actinomycin D (a transcription inhibitor) to block load-induced muscle hypertrophy, demonstrating the importance of mRNA changes for muscle plasticity. The advent of microarray techniques now allows for studying the regulation of hundreds of genes simultaneously and therefore provides the opportunity for the detection of concerted regulation of gene clusters (synexpression groups; Niehrs and Pollet 1999). Recent gene profiling data document that work-induced muscle hypertrophy is an integrated transcriptional response whereby genes related to carbohydrate and protein metabolism, autocrine/paracrine factors, extracellular matrix proteins, transcription factors and cell regulatory factors change together (Carson et al. 2002). It has been proposed that the pronounced increase in the muscle regulatory factor myogenin in overloaded muscle supports its involvement as a major controller of the complex fast-to-slow transformation process (Carson et al. 2002). Another important player in muscle hypertrophy seems to be the *ski* gene (Sutrave et al. 1990). It has been demonstrated that type II fast fibres undergo selective hypertrophy in muscles of transgenic mice that overexpress the *ski* gene. Supporting evidence for the role of the *ski* gene in muscle hypertrophy comes from experiments indicating a threefold increase in *ski* mRNA after injury (Soeta et al. 2001). MRF4 has been identified as an additional factor of potential importance for muscle hypertrophy (Hespel et al. 2001). This role is suggested by an increase in MRF4 expression during recovery of muscle fibre diameter from disuse atrophy after 10 weeks of knee-extension exercise under oral creatine monohydrate supplementation in humans (Hespel et al. 2001). Recently it has been demonstrated that a single bout of resistance exercise in young male subjects has significant effects on the transcriptome (Jozsi et al. 2000). The expression of mRNAs involved in the stress/damage (HSP27) and inflammatory response, the angiogenic factor VEGF and several transcription factors were found changed in biopsies of m. vastus lateralis 24 h after a single bout of resistance exercise. Similarly, large increases in the level of the heat-shock proteins (HSPs) HSP27 and HSC/HSP70 were identified 48 h after a single bout of resistance exercise in human m. biceps brachii (Thompson et al. 2001). HSPs fulfil diverse and distinct functions in protein folding and stabilization (Liu and Steinacker 2001), indicating

that their increase after exercise may be related to the longer-lasting changes in protein turnover observed after resistance exercise (see Table 1).

Real and simulated microgravity (immobilization)

The adaptations of skeletal muscle tissue to microgravity have recently been reviewed (Fitts et al. 2001). Adaptations to microgravity conditions have been studied in biopsies from astronauts before and after space flights of various durations as well as in experiments involving prolonged bedrest (simulated microgravity). As far as skeletal muscle tissue is concerned, both interventions lead to similar structural and functional adaptations. After 42 days of a head-down tilt bedrest (with no countermeasures), we found a 17% reduction in muscle cross-sectional area and a similar reduction in volume density of mitochondria in biopsies of m. vastus lateralis (Ferretti et al. 1997). The total loss of oxidative capacity in this muscle thus amounted to 28.5%, with a concomitant loss of 22% of total capillary length. Biopsies obtained in the same subject from m. deltoideus showed no loss of muscle cross-sectional area or fibre size, no difference in muscle mitochondrial volume and no decrease in capillarity (Desplanches et al. 1998). Space flight of 6 months duration leads to a decrease in maximal voluntary contraction of the plantar flexors of the foot of 20-48% (see review by Fitts et al. 2001). This loss of muscle strength is due both to muscle atrophy as well as to selective loss of contractile proteins, leading to a decrease in force per cross-sectional area. Already after 17 days of space flight, type I fibres in m. soleus of astronauts were found to manifest less average peak Ca^{2+}-activated force. This was attributed to a reduction in fibre diameter and/or force per cross-sectional area (Widrick et al. 1999). Conversely, mean velocity during unloaded contractions was greater but could not be explained by alterations in myosin heavy- or light-chain composition. After microgravity exposure there is an increase in the muscle fatigability and a shift away from lipid towards glucose metabolism in rats (reviewed in Fitts et al. 2001). However, the results of muscle inactivity after real space flights must be interpreted with caution, as the observed changes occur despite vigorous counter measures designed to minimize loss of muscle structure and function in orbit.

Comparing real with simulated microgravity, the results in m. vastus lateralis are found to be similar under both conditions. However, the results of m. deltoideus remain puzzling. It is unclear whether upper and lower limb muscles react differentially to inactivation or whether m. deltoideus escaped atrophy in the bedrest experiment because of an increased circumstantial use of this muscle under these particular circumstances. This could also be the case in real space flight where an increased use of upper body muscles for stabilization is likely.

The molecular mechanisms responsible for microgravity adaptations have mainly been studied using earth-bound animal models. Hindlimb suspension of rats and immobilization are two experimental interventions that reproduce many of the gross functional and structural adaptations in skeletal muscle observed with space flight. Hindlimb suspension leads to fibre atrophy in m. soleus with a selective loss of myofibrils, a shift towards higher speeds of contraction, a reduced resistance to fatigue and a reduced oxidative capacity (Fitts et al. 2001; Thomason and Booth 1990). The hindlimb suspension model has produced evidence for a reduced protein synthesis combined with increased protein degradation, a shift towards expression of fast myosin heavy-chain isoforms and a shift towards a preferred utilization of carbohydrates over fatty acids as substrates (Fitts et al. 2001; Ste-

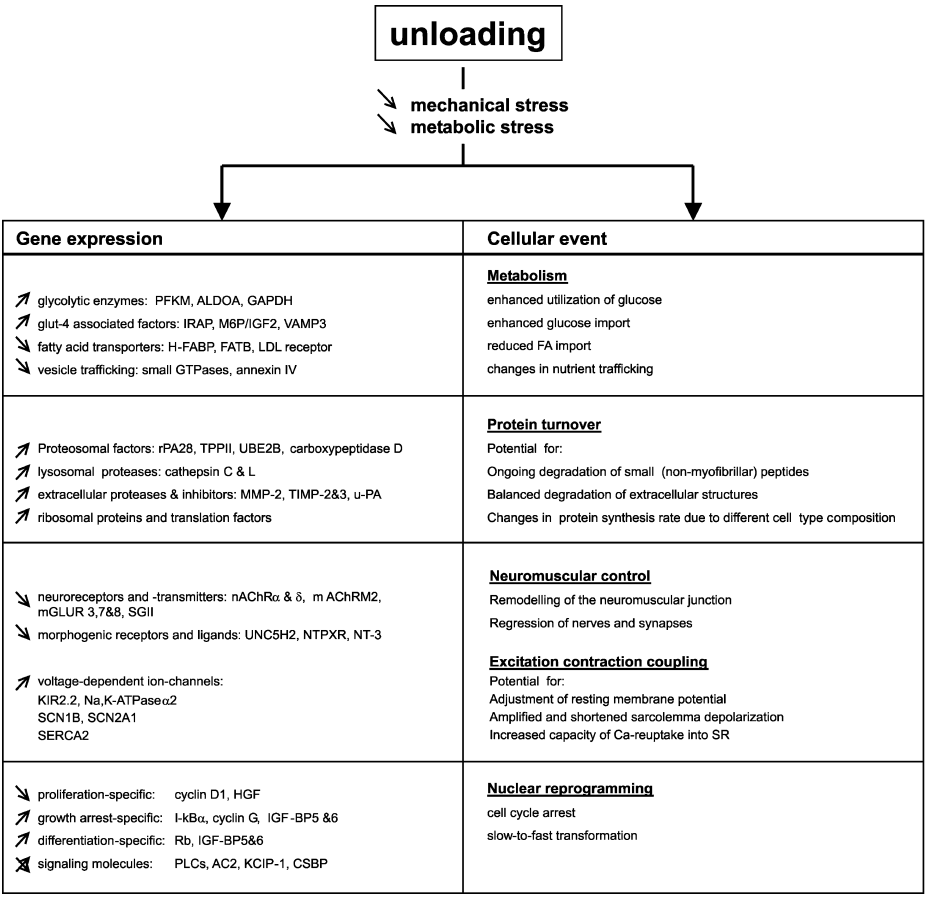

Fig. 6 Effect of simulated microgravity on the rat M. soleus gene profile. Schematic diagram summarizing the expressional changes in functional categories in hindlimb suspended rat m. soleus and their interpretation in terms of affected cellular event (Wittwer et al. 2002a). Unloading is expected to cause these expressional adaptations through a reduction in mechanical as well as metabolic stress

vens et al. 1999; Baldwin and Haddad 2001; Booth and Baldwin 1995). The latter is due to post-transcriptional (enzymatic) events. Moreover, it has been demonstrated that activation of the ubiquitin-proteasome pathway, the NF-kappa B pathway and apoptosis is involved in muscle loss with rodent m. soleus atrophy (Hunter et al. 2002; Ikemoto et al. 2001; Allen et al. 1997; Sandri 2002). Recently, we have demonstrated that prolonged unloading of rat soleus muscle causes massive adaptations of the gene profile (Wittwer et al. 2002). Expression levels of many genes involved in fibre transformation, metabolism, nutrient trafficking, protein turnover and cell regulation were changed. Additionally, expressional changes of extracellular proteases, of genes involved in nerve-muscle interaction and of EC coupling were identified to change with hindlimb suspension-induced soleus atrophy (Fig. 6). The results support a modulation of nutrient trafficking by glut-4 associated factors and small G proteins. Many of the changes of the transcriptome observed in this study were described for the first time. The data on molecular markers indicating changes

at the neuromuscular junction with microgravity are supported by structural evidence demonstrating that space flight modifies the morphology of the neuromuscular junction (Deschenes et al. 2001). We would assume that the widespread changes in the transcriptome elicited by hindlimb suspension in rats is typical for disuse atrophy and we suspect that prolonged microgravity exposition would lead to similar changes in human muscles.

Nutritional interventions

High-fat diet

It has long been known that adequate glycogen stores are necessary for optimal muscle performance (Bergstroem et al. 1967). However, it has only recently been recognized that nutritional interventions manipulating the macronutrient composition of the diet can have a significant impact on muscle structure and function (Helge 1996; Helge et al. 1998; Hoppeler et al. 1999). As indicated above, endurance exercise training leads to an increased use of fat as a substrate. This adaptational feature has been identified as an important means of sparing glycogen during long-term exercise (Holloszy and Coyle 1984). Experiments in rats have suggested that high-fat diets alone can enhance endurance performance by increasing muscle tissue capacity for oxidative metabolism, in particular the ability to oxidize fat (Miller et al. 1984). It was also found that these changes were additive to endurance exercise training (Simi et al. 1991). Likewise, in humans a sizable number of studies report a decrease in the respiratory exchange ratio as a consequence of a high-fat diet (Carey et al. 2001; Jansson and Kaijser 1982; Phinney et al. 1983; Lambert et al. 1994), indicating an increased reliance of muscle tissue on fat as a substrate. High-fat diets gradually increase fat oxidation both at rest and during exercise, while there is no change in rate of appearance of fatty acids and plasma-derived fatty acid oxidation (Schrauwen et al. 2000). However, unlike in animals, a high-fat diet in humans has no effect on muscle oxidative capacity (Goedecke et al. 1999; Hoppeler et al. 1999). Of the enzymes involved in beta-oxidation, only carnitine acyl transferase activity was increased, while citrate synthase and 3-hydroxyl-acyl-CoA dehydrogenase was not changed, in human m. vastus lateralis after 10 days of high-fat diet (Goedecke et al. 1999; Peters et al. 2001; Table 1). Likewise, muscle mitochondrial content seems to be unaffected by a high-fat diet in humans, while intramyocellular lipid (IMCL) content of muscle cells appears to be elevated by this diet intervention (Hoppeler 1999; Vogt et al., unpublished observations). Interestingly, the reports on performance gains in human athletes after high-fat diets remain controversial (Vogt et al., unpublished observations). An increase in endurance capacity seems to be most likely for ultraendurance events when care is taken that the muscle glycogen pool is fully loaded (Lambert et al. 2001).

Little is known on the molecular events underlying these metabolic adaptations of the muscle cell. The available data support the notion that the expression of enzymes involved in regulation of mitochondrial function, i.e. proton uncoupling (UCP-2 and -3) and the entry of carbohydrate-derived pyruvate into the Krebs cycle (PDK4), is affected (Schrauwen et al. 2001a; Peters et al. 2001). The induction of PDK4 mRNA and its activity 1 day after onset with the high-fat diet indicates enhanced entry of carbohydrate-derived pyruvate into the Krebs cycle (Peters et al. 2001). The up-regulation of UCP-2 and 3 mRNA in m. vastus lateralis of subjects after 10 weeks of high-fat diet was more pronounced in humans with high proportions of type IIa fibres, suggesting a role for UCPs in lipid utilization (Schrau-

wen et al. 2001a). This eventually indicates protection of mitochondria against accumulation of non-esterified fatty acids inside the mitochondria (Schrauwen et al. 2001b). An important adaptive mechanism is the activation of the key regulator fat metabolizing enzyme expression, peroxisome proliferator-activated receptor (PPAR), through lipid products. Activation of PPARα and PPARγ may be related to the induction of UCP-2 and -3 mRNA expression (Vidal-Puig et al. 1997; Pedraza et al. 2000).

Recently, it has been demonstrated that metabolic interventions affect the regulation of metabolism via transcriptional and post-translational events. It has been realized that amino acids are not only substrates of chemical reactions but also act as signalling molecules in control of protein synthesis in skeletal muscle (Kimball 2002). The branched-chain amino acid leucine mediates signalling, leading to increased initiation of mRNA translation as well as to an increase in the phosphorylation status of the translational repression protein 4E-BP1 and the ribosomal protein S6 kinase (S6K1) (Greiwe et al. 2001). Conjugated linoleic acid isoforms have been demonstrated to improve glucose transport and glycogen synthase activity and to increase UCP-2 mRNA in skeletal muscle of obese rats (Ryder et al. 2001). A challenge for future studies is to identify whether the adaptations identified in rodents play a role in explaining the metabolic changes following diet interventions in humans.

Caloric restriction

Caloric restriction (CR) has been recommended to control weight and the blood lipid profile, particularly in the elderly (Schlienger et al. 1995). Animal experimentation shows that caloric restriction reduces fibre loss and mitochondrial abnormalities in aged rat muscle (Aspnes et al. 1997). To our knowledge, no data have been published on the effect of caloric restriction on structural or functional adaptations in human skeletal muscle. Recent data from experiments with rodents indicate a significant modulation of gene expression under caloric restriction (Weindruch et al. 2002). Caloric restriction increased expression of genes involved in carbohydrate and fatty acid metabolism and decreased expression of genes involved in stress-response/DNA repair and detoxification processes. Notably, these data suggest that caloric restriction could reverse age-related changes in gene expression.

It is obvious that nutritional preferences and requirements vary widely between humans and mice. Nevertheless, there are some indications of similar transcriptional adaptations occurring in both species as a consequence of caloric restriction. An important role of caloric restriction in regulation of muscle plasticity in humans is indicated by the recent observation that caloric restriction results in an up-regulation of cytoskeletal protein-encoding genes and a decrease in the expression of genes involved in mitochondrial bioenergetics in m. vastus lateralis of rhesus monkeys (Kayo et al. 2001). Moreover, caloric restriction in primates is found to reduce oxidative damage (Zainal et al. 2000). These results indicate that the induction of an oxidative stress-induced transcriptional response may be a common feature of ageing in skeletal muscle of rodents and primates. However, the extent to which caloric restriction modifies these responses may be species-specific.

Hypoxia

Intermittent and continuous exposure to hypoxia causes many adaptive events in human skeletal muscle tissue (see Hoppeler and Vogt 2001a). A significant 10% loss in thigh muscle cross-sectional area in 14 subjects after return from mountaineering expeditions to the Himalayas has been reported (Hoppeler et al. 1990). Post-expedition volume density of mitochondria was reduced by 23%, such that the total loss of oxidative capacity of m. vastus lateralis was close to 30%. The muscle capillary bed was not much affected by high-altitude exposure, with the result that a practically unchanged capillary network supplied a smaller muscle oxidative capacity after return from expedition. The results of a substantial loss of muscle oxidative capacity as a consequence of permanent exposure to hypoxia is substantiated by biochemical results obtained from the same subjects (Howald et al. 1990) but also from results of Operation Everest II, a simulation of an ascent to Mt. Everest in the pressure chamber (MacDougall et al. 1991; Green et al. 1989). The combined structural and functional evidence suggests that lowlanders do not tolerate long-term severe hypoxia well. This view is supported by the observation that the quantity of lipofuscin present in muscle fibres is increased by over threefold after returning from an expedition (Martinelli et al. 1990). Lipofuscin is a degradation product formed by lipid peroxidation and characterizes cytological damage possibly incurred by radical formation in muscle cells in hypoxia (Radak et al. 1994). Hypoxia training also initiates a shift in muscle metabolism towards more carbohydrate oxidation (Vogt et al. 2001).

Changes in gene expression in mammalian skeletal muscles as a consequence of hypoxia exposure have not yet received much attention. Training in intermittent hypoxia has been shown to improve oxygen transfer to muscle mitochondria by increasing muscle capillarity and muscle myoglobin content (Vogt et al. 2001). These changes are believed to be related to hypoxia-inducible factor 1 alpha (HIF-1α), which is a transcription factor controlling the expression of many genes making up the hypoxia response (Semenza 1999). HIF-1α is constitutively expressed but degraded rapidly under normoxic conditions. Hypoxia has been demonstrated to stabilize HIF-1α and thus make it available for transcription regulation (Jewell et al. 2001) (see also "Metabolic factors"). We were also able to show recently that HIF-1α mRNA is induced in human skeletal muscle after endurance exercise training in hypoxia (Vogt et al. 2001). Recent experiments with rats hint at the molecular changes possibly responsible for the functional adaptations in skeletal muscle to chronic hypoxia. Protein expression of the mitochondria-associated Bcl-2 was inversely related to the oxidative character of muscles and markedly induced in rat muscles after 3 weeks' exposure to chronic hypoxia. This change paralleled the oxidative character of the muscles (heart > soleus > gastrocnemius). No sign of apoptosis was detected in these experiments (Riva et al. 2001). It was suggested that the adaptations were an anti-apoptotic mechanism allowing protection against the lack of oxygen in oxidative muscles. It has recently been shown that protein expression of the enzymes and transporters of lactate, lactate dehydrogenase (LDH) and monocarboxylate transporters (MCT1, 2 and 4) is affected in a tissue-specific manner by long-term exposure to hypobaric hypoxia (McClelland and Brooks 2002).

It is currently not known whether the adaptability of the lactate shuttle (LDH and MCTs) contributes to the changes in the control of lactate metabolism that relate to the paradoxical observation that maximal lactate concentration at VO_2max is reduced after adaptation to altitude (Hochachka et al. 2002).

The involvement of transcriptional mechanisms in skeletal muscle adaptations to hypoxia have been thoroughly studied in fish (see Gracey et al. 2001). These gene expression profiling data document a very rapid shut-down of energy-requiring processes such as protein synthesis and locomotion in skeletal muscle and a concurrent suppression of cell growth and proliferation involved in hypoxia survival mechanisms. Future studies will have to provide the evidence on whether similar expressional changes may be responsible for the observed reduction in fibre size and shift in energy metabolism observed in human skeletal muscle with stays at high altitudes.

Ageing

Age-related changes in muscle mass and functional properties are the result of a complex hierarchical system of regulation of cell ageing processes and corresponding cell adaptive responses (Navarro et al. 2001; Tables 1, 2). It is generally accepted that there is a decrease in muscle mass and a decrease in muscle oxidative capacity, in particular after the age of 70 in humans. It is not clear, however, how much of the observed changes are due to inactivity and how much are due to the ageing process *per se*. It has been demonstrated that old muscle retains its ability to enlarge during resistance training (Booth and Baldwin 1995; Balagopal et al. 2001; Hikida et al. 2000) and to increase oxidative capacity after endurance training (Kiessling et al. 1973). Numerous aspects of skeletal muscle plasticity thus do not seem to be compromised by the ageing process. Ageing muscle has been shown to be different in motor-unit innervation, post-synaptic properties (sarcolemmal channel alterations and depolarization properties), EC coupling and calcium homeostasis (Weindruch1995; Navarro et al. 2001; Klitgaard et al., 1990c). Old muscle also responds differently to growth hormone and testosterone release as a consequence of an acute bout of resistance exercise than young muscle (Craig et al. 1989). Old muscle has a higher proportion of type I/IIa and IIa/IIx hybrid fibres (Klitgaard et al. 1990a), exhibits reduced state III mitochondrial respiration rates (Trounce et al. 1989) and a decrease in citrate synthase activity (Pastoris et al. 2000). Data from rhesus monkeys demonstrate age-dependent accretion of signs of oxidative damage in myofibrils (Zainal et al. 2000). These changes may be related to impaired mitochondrial respiration rates, as the O_2 that is incompletely reduced by mitochondria yields reactive oxygen species (Chandel and Schumacker 2000). The fractional synthesis rate of MHC and mixed protein is reduced in aged muscle (Hasten et al. 2000). As contributing factors to muscle ageing hormonal factors, modulation by caloric restriction and decreased exercise activities have been recognized. Gene profiling data imply that specific expressional changes are associated with the ageing process in mouse skeletal muscle (Weindruch et al. 2002). In particular, it was noted that expression of genes involved in stress response and neuronal growth were increased while expression of genes responsible for protein turnover, calcium handling and energy metabolism were reduced. The observations of differences in the expression of factors involved in nerve morphogenesis are supportive evidence for a process of denervation and reinnervation in ageing human muscle (Vandervoort 2002).

In rhesus monkeys, experiments taking advantage of high-density oligonucleotide arrays indicate a selective up-regulation of transcripts involved in inflammation and oxidative stress and a down-regulation of genes involved in mitochondrial electron transport and oxidative phosphorylation with ageing (Kayo et al. 2001). These data are broadly compatible with the changes of the transcriptome described in human skeletal muscle with

ageing. A recent gene profiling study comparing skeletal muscle tissue from healthy elderly (62-75 years old) to young (20-34 years old) men demonstrated elevated expressions of genes typically involved in stress and damage response and a decrease in expression of a gene encoding a DNA repair/cell cycle checkpoint protein (Jozsi et al. 2000; Table 2). Several genes with important roles in the adaptation of muscle to exercise, specifically the processes of angiogenesis and cell proliferation, showed a similar response to exercise in older and younger subjects. Other responses, however, such as those seen after resistance exercise were different between the two age groups. Jozsi et al. (2000) concluded from these results that there may be a molecular basis for the difference in the response of skeletal muscle tissue to resistance training in elderly people.

Thermal stress

Very little is known concerning the adaptations of skeletal muscle to extreme thermal conditions. During exercise, multiple afferent signals including body temperature feed back on sympathetic centres and in turn influence cardiovascular, hormonal, and metabolic responses, electrolyte homeostasis and muscular performance (Christensen and Galbo 1983). Using a combination of indirect calorimetry and stable isotope methodology, it has also been demonstrated recently that exposure for 2 h at 10°C stimulates heat production by involuntary muscle contraction (cold shivering) and increased the oxidation of plasma glucose, muscle glycogen and of lipids by more than twofold (Haman et al. 2002). These data indicate that lipids and muscle glycogen provide most of the energy for heat production. Reversible ultrastructural modifications possibly related to muscle temperature have been described in horses exercising at higher intensities (McCutcheon et al. 1992). Heat-stress is known to partially prevent hindlimb suspension-induced atrophy through a process involving heat-shock proteins (Naito et al. 2000). Moreover, it has been shown for rat gastrocnemius muscle that 6 weeks of uphill running (5 days/week, 60 min/day) on a treadmill at cold temperature (4° or 8°C), concomitant with a drop in exercising core temperature, causes a greater increase in cytochrome-c oxidase activity than running at ambient temperature of 23°C (Mitchell et al. 2002). The data indicate that decreasing exercising core temperature may enhance mitochondrial biogenesis. However, there seems to be practically no further data in the literature on mammalian skeletal muscle response to a temperature challenge. Muscle tissue in certain fish species exposed to water temperature changes with season exhibits a marked and well-described molecular plasticity (Johnston and Temple 2002). However, the changes occurring in fish muscle as a consequence of temperature adaptations are outside the scope of this article.

Signals, sensors and transducers

What could be the sensing mechanisms responsible for the specific adaptational events induced in skeletal muscle by exercise and changes in the environment? For example, strength training causes a selective increase in the myofibrillar compartment, thus increasing the capacity for force production. By contrast, endurance exercise predominantly increases the mitochondrial and capillary compartment, giving rise to an improvement of the capacity for aerobic energy turnover. The unique and specific muscle cellular responses to strength- and endurance-type exercise make it likely that different sensing systems

are involved with high-intensity low-repetitive (strength) training versus low-intensity high-repetitive (endurance) exercise training. The massive and selective perturbations of cellular homeostasis with exercises of different intensity and duration suggests that both metabolic *and* mechanical factors can be sensed separately and need to be integrated into complex transcriptional responses. The consequence of these transcriptional regulations is seen as the malleability of cellular structure. The modified structures then enable the transformed muscle to better withstand an imposed physiological stress (Booth and Thomason 1991; Booth and Baldwin 1995). Apart from metabolic and mechanical signals, there is ample evidence for the involvement of hormonal (growth hormone, IGFs, ACTH, thyroid state and testosterone) as well as neuronal factors (via intracellular Ca^{2+}) playing important roles in the plastic response of muscle tissue.

For the sake of simplicity, this article will focus on the concept that mechanical, metabolic, hormonal and neuronal factors are the key signals distinctly sensed by skeletal muscle tissue (Fig. 7). These signals will be discussed separately. Finally we will focus on the integration of these signals into the transcriptional adaptations that are seen in skeletal muscle.

Mechanical factors

The role of mechanical factors is best supported by animal model studies demonstrating that mechanical factors stretch or modulate transcriptional induction of the *c-jun* and *c-fos* genes within 1 h (Dawes et al. 1996). In situ hybridization experiments of human m. vastus lateralis show that an increase in c-fos and c-jun mRNA after running occurs in a patchy expression pattern not corresponding to fibre type distribution. This has been taken to support the notion that factors other than metabolic products related to fibre recruitment contribute to the observed massive up-regulation of *c-fos* gene transcription in exercised human skeletal muscle (Puntschart et al. 1998).

The contention that mechanical factors are involved in regulation of the mRNA concentration with exercise training is further supported by the observations in mechanically stressed skeletal muscle fibres that mechanisms known to contribute to pre-transcriptional control are affected concomitantly with the transcription of downstream target genes. In particular, exercise can induce all three limbs of the MAP-kinase pathways (ERK1/2, JNK and p38) in human m. vastus lateralis (Aronson et al. 1997, 1998; Yu et al. 2001; Boppart et al. 1999; Widegren et al. 2001; Table 1). Activation of these kinases is known to affect transcription factors of the jun/fos and ATF/CREB family (Hunter and Karin 1992; Table 2). The latter are known to bind to the promoter of many genes, e.g. *c-jun*, *c-fos* and cytochrome C, that are affected simultaneously by exercise (Hood 2001). In situ studies delineated that mechanical factors, i.e. the degree of tension in rat muscle fibres, control JNK and ERK1/2 differently and directly (Martineau and Gardiner 2001). In contrast, the p38 pathway in skeletal muscle is only responsive when mechanical stress (stretch, exercise) is applied in vivo (Boppart et al. 2001). This can be taken as an indication that p38 is indirectly controlled by mechanical factors, possibly through endocrine mechanisms. Furthermore, the activation of the p70S6 kinase has also been linked to mechanical or metabolic stimuli, which may induce muscle hypertrophy by influencing translation (factors) (Nader and Esser 2001). The mechanically induced release and production of growth factors (IGF-I, the IGF-I splice form MGF, IL-6) has been recognized as another signalling route in skeletal muscle exercise, potentially representing an important link between contracting

Mechanical factors
Metabolic factors
Neuronal factors
Hormonal factors
Integrins
JNK MAPK
AP-1
c-jun
?
c-fos
HSP70
Signal transduction
Gene transcription
Translation protein assembly
Stress response
↓O2
ROS
HIF-1α
?
Glut-1, GAPDH
VEGF
AMP/ATP
AMPK
FFA
PPARα/γ
NRF-1
TFAM
Cyt C
COXVIc
δALS
CPT I
MCAD
UCP-3
Ca2+/CaM
CaMK
PGC-1
Calcineurin
MEF2
NF-AT
Troponin
MHCI
MyoD
MHCII
GH
IGF-1
T3
Tes
S6K
HSP70
HSP27
?
AAA
acyl-CoA import
β-oxid.
krebs cycle
respiratory chain
TFAM
mtDNA
transcription & translation
Mitochondrial biogenesis
metabolism
Sarcomerogenesis
Protein turnover

skeletal muscles and exercise-related metabolic changes (Goldspink 1999; Pedersen et al. 2001; Adams et al. 1999). Current thinking suggests that the activation of all these mechano-transduction events is ultimately linked to mechano-sensation via integrins and associated kinases (Gordon et al. 2001b; Carson and Wei 2000; Chiquet and Flück 2001).

Metabolic factors

Several metabolic perturbations that occur in severely exercised skeletal muscle, such as a drop in pH and oxygen tension and an increase in AMP concentration and free radical production (to name only a few), may be sensed and could serve to control changes in gene expression. The acute chemical perturbations of muscle cells are likely linked in multiple ways to signal transduction events that ultimately influence gene expression. A drop in the ATP/AMP ratio, activating 5'-AMP-activated protein kinase (AMPK) (Bergeron et al. 2001) is believed to be an important sensing mechanisms that links metabolic changes to gene expression (Aronson et al. 1998; Hood 2001). The sensing of cellular energy charge by the AMPK cascade supports the energy charge hypothesis first proposed in 1960 by Daniel Atkinson (in Hardie and Hawley 2001). Unsaturated long-chain fatty acids are released from adipose tissue during exercise and can directly stimulate PPARα-activated gene transcription (Escher and Wahli 2000; Kliewer et al. 1997; Huss et al. 2001). Unsaturated long-chain fatty acids must thus be considered to be paracrine factors modulating the capacity of muscle cells to transport and metabolize fatty acids (Fig. 7).

Ever since Reynafarje (1962), local tissue hypoxia has been assumed to be an important stimulus for muscle tissue adaptations. Using ^{1}H nuclear magnetic resonance spectroscopy, it was shown that myoglobin desaturation occurs within 20 s of onset of exercise in human quadriceps muscle (Richardson et al. 1995). The finding of a low muscle oxygen tension during exercise is supported by the earlier studies of Gayeski and Honig (1978). Using the same technique, Richardson et al. (2001) have shown recently that oxygen saturation in human skeletal muscle, calculated from myoglobin desaturation, is reduced with onset of exercise, to a plateau. This has been taken to indicate that maximal muscle local hypoxic conditions may prevail even during low-intensity exercise in normoxia. This reasoning is based on the assumption that oxygen is homogenously distributed within and among muscle fibres. This may not be the case. Mitochondria, the oxygen sink, are more prevalent in oxidative fibres by a factor of at least three and even within fibres they are clustered in the fibre periphery (Howald et al. 1985). As a consequence, we would assume that the average ^{1}H nuclear magnetic resonance deoxy signal does not account for local hypoxia conditions within individual fibres. However, it is these local conditions that are likely to be relevant for the molecular response of muscle fibres (Lindstedt and Wells 1988; Gayeski and Honig 1978).

The observation of a decrease in muscle oxidative capacity with long-term hypoxia exposure came as a surprise (MacDougall et al. 1991; Kayser and Hoppeler 1991). A number

Fig. 7 Integration of signals into changes of skeletal muscle gene expression. Mechanical, metabolic, neuronal and hormonal factors are transduced via complex pathways into adaptations in gene expression. A prominent increase in activity of several kinases and downstream transcription factors (for details see text) is observed within minutes after application of stress to muscle tissue. This may link to induced transcription of downstream genes and functional adaptations. Recent data indicate that convergence between multiple pathways exists

of studies have been designed to maximize hypoxic stress on muscle (and to minimize muscle deterioration) by exposing working subjects to normobaric or hypobaric hypoxia for the period of the exercise training sessions only (short-term hypoxia; Terrados et al. 1988, 1990; Desplanches et al. 1993; Emonson et al. 1997; Melissa et al. 1997; Vogt et al. 2001). The consensus seems to be that training in short-term hypoxia produces training effects similar to, but not identical with the effects seen after training in normoxia (see Hoppeler and Vogt 2001b). The reason why long-term hypoxia is damaging for muscle cells is not yet fully understood (Hoppeler and Vogt 2001b).

Hypoxia has been demonstrated to instantaneously stabilize the transcription factor HIF-1α, which appears to be a master gene involved in the hypoxia response of most mammalian cells (Semenza 1999). In HeLaS3 cell culture, 0.5% O_2 provokes an increase in HIF-1α from undetectable levels within 2 min (Jewell et al. 2001). Continuous hypoxia induces HIF-1α with maximal levels after 1 h and gradually decreasing thereafter in various organs of mice (Stroka et al. 2001). The increase in HIF-1α is mediated by reducing hydroxylation of a proline and in the oxygen-dependent degradation domain of HIF-1α. This is achieved by interference with a hypoxia-inducible proline hydroxylase; as in normoxia, HIF-1α is targeted for destruction via the ubiquitin pathway (Wenger and Bauer 2001; Zhu and Bunn 1999; Kietzmann et al. 2000). Recently, hydroxylation of an asparagine residue within the HIF-1α C-terminal transactivation domain has been noted to block its association with co-activators (Lando et al. 2002; Mahon et al. 2001).

The increase in HIF-1α and its dimer formation with HIF-1β (ARNT) subsequently induces expression of a number of hypoxia-activated genes (Semenza 1999). The heterodimeric complex (HIF-1) drives transcription of a variety of hypoxia-inducible genes including angiogenic factors, glucose transporters and glycolytic enzymes through binding to cis-acting hypoxia-response elements (HREs) in the promoters (Semenza 1999; Pages et al. 2000). Recently, direct evidence was provided that in rat skeletal muscle, oxidant production is increased during prolonged exercise, with as potential sources for oxidants both the mitochondrial respiratory chain and the NADPH oxidase (Bejma and Ji 1999). Radicals (reactive oxygen species, ROS) produced in this process are known to activate multiple pathways that influence gene expression and have been shown to be linked to the hydroxylation of HIF-1α (Kietzmann et al. 2000). In this regard it is interesting to note that mitochondria-derived ROS are required to initiate HIF-1α stabilization during hypoxia (Chandel et al. 2000; Chandel and Schumacker 2000) and that enzymic and non-enzymic oxygen free radical-generating systems have been implicated to control hydroxylation of prolyl and arginyl residues (Stadtman 1990). These lines of evidence indicate that the ubiquitous transcription factor, HIF-1α, could be a major molecular component that integrates the events that translate increased muscle activity into plastic remodelling of muscle with local tissue hypoxia as a signal.

Neuronal factors

It is well known that transient increases in intracellular Ca^{2+} are involved in fibre transformation. This is believed to occur through modifying Ca^{2+}-calmodulin-dependent processes (Sreter et al. 1987). Recent data provide indirect evidence for neuronal modulation of muscle function by Ca^{2+}-dependent processes and indicate that calmodulin may in fact function as a sensor (Talmadge 2000). Ca^{2+} is periodically released from the SR during con-

tractions, thereby increasing cytoplasmic Ca^{2+} concentration by nearly two magnitudes before Ca^{2+} is pumped back into the SR via Ca-ATPAse(s) (Berchtold et al. 2000). Such periodic increases in intracellular Ca^{2+} might trigger the activation of Ca^{2+}/calmodulin-dependent enzymes acting as calcium sensors (Talmadge 2000). This scenario is supported by the observation that Ca^{2+}/calmodulin kinase II is a decoder for Ca^{2+} oscillations (Dupont and Goldbeter 1998) and that Ca^{2+}-independent CaMK(II) activity is increased in exercised rodent muscle (Flück et al. 2000). The latter observation could eventually be related to an elevation of intracellular free calcium to levels high enough to activate CaMK. This would then be similar to the situation observed in chronically stimulated muscles (Chin and Allen 1996, reviewed in Talmadge 2000). An increase in autonomous CaMK activity may in turn favour mitochondrial biogenesis, as CaMK seems to be involved in mitochondrial biogenesis by causing up-regulation of a master regulator of mitochondrial biogenesis, PGC-1 which resides upstream of PPARs (Wu et al. 2002). Much attention has also been drawn to results that implicate the Ca^{2+}/calmodulin-dependent calcineurin:NF-AT and CaMK pathways in the regulation of muscle-specific gene expression (Talmadge 2000; Wu et al. 2002). Calcineurin:NF-AT has also been found to influence the expression of slow muscle fibre specific contractile proteins (i.e. troponin) as well as to help to control transformation from type II towards type I fibres (Chin et al. 1998; Figs. 4,7).

Recently, molecular correlates for neuronal reprogramming as an important mechanism in muscle plasticity have been identified by the demonstration that synapses (Deschenes et al. 2001), elements of the neuromuscular junction (Wittwer et al. 2002a) and motor units (Leterme and Falempin 1996) are modified in animal models of microgravity. The observations that serotonin receptors adapt to hindlimb unloading (Wittwer et al. 2002a) suggest that afferent metabolic receptors in afferent nerves are also modulated by changes in muscle loading conditions. Taken together, the available evidence suggests that neuronal factors are involved in muscle plasticity either by interfering with contractile activity or by modulating mechanical and metabolic signals, on several levels of hierarchical organization.

Hormonal factors

It is well documented that hormones have a pronounced effect on muscle phenotype (Frisch 1999; Everts 1996; Florini et al. 1991). There is considerable evidence that serum levels of many hormones are influenced by exercise, muscle loading and ageing. For example, endurance exercise acutely increases serum growth hormone, endorphin and renin levels while insulin levels are reduced (Kjaer et al. 1999). Also, a single resistance exercise session (45-60 min) involving all major muscle groups increases serum GH by more than fourfold but had no effect on testosterone levels (trained or untrained people) (Craig et al. 1989). By contrast, an acute high-intensity weightlifting protocol increased the levels of serum testosterone, cortisol and growth hormone in junior elite male weightlifters (Kraemer et al. 1992). Total serum testosterone levels were increased in both men and women after 6 and 8 weeks of heavy resistance training while the increase in serum growth hormone levels seen after exercise did not result in a change in basal GH concentration (Kraemer et al. 1998). Resistance training was found to cause alterations in the acid-labile subunit of the circulating IGF system in humans (Nindl et al. 2001). Such an increase in protein could also be seen with overloading of rat muscle potentially preceding increases in IGF mRNA (Adams et al. 1993). Space flight lowers thyroxine and triiodothy-

ronine levels in human, causes a loss of insulin sensitivity, increases the activity of the sympathetic system but leaves growth hormone levels unaffected (Strollo 1999). The level of 13 hormones including thyroid stimulating hormone (TSH) and growth hormone were dramatically increased after a triathlon event where dehydration did not significantly contribute to the observed endocrine changes (Malarkey et al. 1993). In well-trained young subjects, long-distance runs (45-75 km) were shown to selectively increase T4 levels, whereas in older and less high-performance athletes, the same stimulus provoked a significant decrease of the levels of T4, T3 and TSH (Hesse et al. 1989; Table 1). Differences in the response of GH levels with lower-body resistance training involving a combination of concentric and eccentric muscle work indicate that serum GH levels are sensitive to the type of muscle action (Kraemer et al. 2001). Subjects with a spinal cord injury were shown to have a depression of the anabolic hormones, testosterone and growth hormone/IGF-I and decreased serum T3 levels (Bauman and Spungen 2000). In this context, it may also be noted that hypothyroidism is characterized by exercise intolerance (McAllister et al. 1997).

Evidence for involvement of hormones in muscle plasticity is indicated by observations on the influence of the thyroid state on MHC expression in rodents (Booth and Baldwin 1995). Skeletal muscle is one of the major target organs for thyroid hormones. Although thyroid hormones are essential during growth, both their excess and their deficiency cause muscle wasting by yet unknown mechanisms. The muscles most commonly affected are those used during prolonged effort (slow-twitch muscles). Most of the peripheral effects of the thyroid hormones can be ascribed to the action of triiodothyronine (T3), which is produced by de-iodination of thyroxine (T4) in liver and kidney. From the plasma, T3 is actively transported into skeletal muscle (Everts 1996). Thyroid hormone (T3) plays an important role in the regulation of muscle plasticity. Hyperthyroidism and doses of the thyroid hormones T3, cause an up-regulation of IIa and possibly IIx fibres in 30-40% of rat soleus fibres (Baldwin and Haddad 2001). In contrast, hypothyroidism is known to cause IIa fibres to shift to express only type I MHC. The thyroid state and particularly a single T3 injection affects Ca^{2+} ATPase and Na+, K+ ATPase as early as after 24 h. In humans, a linear correlation between the Na+, K+ ATPase concentration of skeletal muscle and the free T4 index was established (Everts 1996). Current evidence indicates that T3 largely exerts its effect by first interacting with receptor complexes in the nucleus. The subcellular action of the thyroid hormone-thyroid receptor interaction is thought to be mediated via their combined interaction with DNA sequences located in the promoter (regulatory) sequence of thyroid-responsive genes designated as TRE elements (reviewed in Booth and Baldwin 1995).

Excess doses of the thyroid hormone triiodothyronine have been shown in young subjects to increase β-adrenergic receptor density in all types of skeletal muscle fibres (Martin 1993). Thyroid exposure results in alterations of the myogenin/myoD mRNA expression pattern and MHC (Hughes et al. 1993). This latter observation is in general agreement with the finding that triiodothyronine up-regulates expression of genes involved in cellular functions including transcriptional control, mRNA maturation, protein turnover, signal transduction, cellular trafficking, and energy metabolism in skeletal muscle of healthy men (Clement et al. 2002). Non-genomic actions of thyroid hormone independent of nuclear receptors for the hormone have been described. These actions include alterations in solute transport (Ca^{2+}, Na+, glucose), changes in activities of several kinases, including protein kinase C, cAMP-dependent protein kinase and pyruvate kinase M2, effects on efficiency of specific mRNA translation and mRNA t1/2, modulation of mitochondrial respi-

ration, and regulation of actin polymerization (Davis and Davis 1996). All these effects could influence muscle phenotype.

A link has been established between β-adrenergic receptor level density and oxidative capacity in skeletal muscle. The administration of β-adrenergic agonist to humans during long-term bedrest experiments has been shown to maintain or increase the activity of CS and SDH in skeletal muscle (reviewed in Booth and Baldwin 1995).

Hormones with an anabolic action (growth hormone, insulin-like growth factors, and testosterone) stimulate human muscle growth mainly by increasing protein synthesis or by decreasing protein breakdown (insulin) (Rooyackers and Nair 1997). The stress hormones (glucagon, glucocorticoids, and catecholamines) cause muscle catabolism when up-regulated together; however, the effects of the individual hormones on human muscle and their mechanisms of action remain to be defined more clearly. Moreover, it has been demonstrated that the adipocyte-derived hormone leptin increases glucose and fatty acid metabolism in skeletal muscle (Ceddia et al. 2001). Despite the evidence that heavy, glycogen-depleting exercise lowers serum leptin concentrations in correlation with serum triglyceride concentration (Tuominen et al. 1997), the involvement of increased secretion of this adipocyte-derived factor in control of exercise-induced muscle plasticity and fuel homeostasis in humans remains to be established (Bradley et al. 2001).

Testosterone has pronounced effects on muscle size and strength in normal men, especially when combined with strength training (Bhasin et al. 1996). This implies that the increased serum testosterone levels seen after resistance exercise may contribute to increased protein synthesis (Table 1). Similarly, doses of GH administration increase fasting serum IGF-I but may increase the rate of muscle protein synthesis in normal adults (Gamrin et al. 2000; Fryburg et al. 1992; Yarasheski et al. 1993a, 1993b) but not in experienced weightlifters (Yarasheski et al. 1993a). Growth hormone stimulates growth of skeletal muscle and connective tissue, increases rate of protein synthesis, and decreases rate of glucose use, thereby counteracting insulin action on lipid and glucose metabolism (Dominici and Turyn 2002; Howrie 1987). Recently, GH administration alone was demonstrated to decrease fat mass, increase fat-free mass, and induce MHC IIx in healthy elderly men whereas resistance training did overrule the changes in MHC (the MHC IIx isoform was decreased) composition induced by GH administration alone (Lange et al. 2002). This parallels the observation that supplementation of resistance training with GH dose not further enhance muscle anabolism and function (Yarasheski et al. 1992). Secretion of most of the hormones mentioned above is under the control of the central nervous system. This points to the important role of the brain for muscle plasticity. Growth hormone (somatotropin) is secreted by the anterior pituitary gland in response to various stimuli, including exercise, hypoglycaemia, and arginine.

Taken together, many hormones have been shown to modulate specific aspects of muscle phenotype. An integrated view of muscle plasticity therefore needs to account for the hormonal milieu experienced by the muscle during any adaptive event.

Signal integration

All factors discussed in the preceding paragraph are likely to interact with each other and to affect skeletal muscle gene expression at multiple levels. It is likely that many sig-

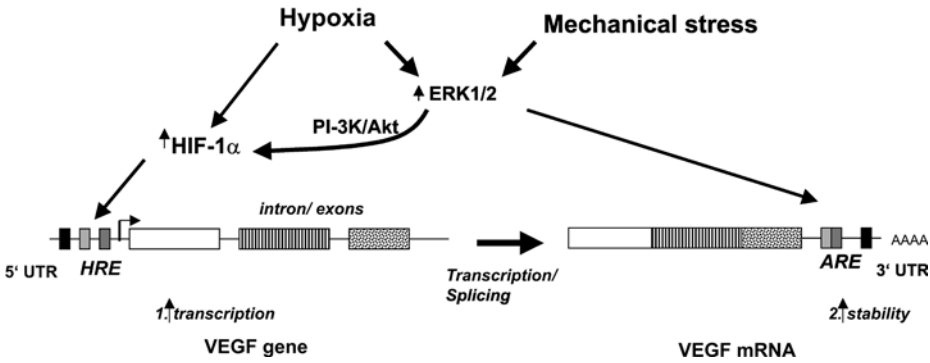

Fig. 8 Possible cooperation of oxygen and mechanical signal into changed VEGF mRNA level. The level of VEGF mRNA synthesis and degradation is controlled through cis-elements. In normoxia, VEGF mRNA is rapidly degraded through a mechanism involving two instability elements in its 3'UTR (adenylate-uridylate-rich elements, *ARE*). A drop in oxygen tension (pO_2) stabilizes the transcription factor HIF-1α that in turn interacts through hypoxia response elements (*HRE*) with the VEGF 5'UTR, which induces VEGF transcription. Moreover, a drop in oxygen tension and mechanical stress induce the MAPK pathway which increases in the half-life time of VEGF mRNA through modulating the binding of a hypoxia-inducible protein to a stability element in the VEGF 3'UTR. Finally, phosphorylation of HIF-1α by ERK1/2 has been shown to enhances HIF-1-dependent transcriptional activation of VEGF. Thus mechanical factors and metabolic factors (oxygen tension) can possibly interact at the transcriptional and post-transcriptional level to control VEGF mRNA in skeletal muscle through HIF-1α and MAPK

nalling pathways exert different and superimposed effects on promoter activity as well as on RNA stabilization, degradation and translation.

So far, no study reports on the complex task of monitoring the effect of exercise on multiple levels of expression control for one single gene. However, how signal integration could work is illustrated by the regulation of VEGF transcription and mRNA stability by hypoxia (Fig. 8). The VEGF mRNA falls into a class of labile messengers which codes for transiently expressed proteins, induced by acute stress signals. The prime mechanism for hypoxia-mediated regulation of VEGF mRNA synthesis acts through cis-elements (Hypoxia response elements, HRE) within its 5'UTR (Pages et al. 2000). The decay of VEGF mRNA is determined through two instability elements (adenylate-uridylate-rich elements, ARE) in the 3'UTR, which promote VEGF mRNA degradation in normoxia, and a stability element, which increases the VEGF mRNA half-life time in hypoxia. In culture, it has been shown that hypoxia increases the steady-state levels of VEGF mRNA through inducing the VEGF promoter activity via binding of the transcription factor HIF-1 (hypoxia-inducible factor 1) to HREs (Forsythe et al. 1996). HIF-1 is a heterodimer of the HIF-1α and HIF-1β proteins and its activity in acutely regulated on the post-translational level by stabilization of HIF-1α in hypoxia (Semenza 1999). Moreover, under low oxygen tension HIF-1α is directly phosphorylated via the mechano-sensitive and hypoxia-sensitive ERK1/2 MAPK pathway and enhances HIF-1-dependent transcriptional activation of VEGF (Berra et al. 2000; Seko et al. 1997; Fig. 8). Hypoxia also enhances the stability of the VEGF mRNA through binding of a hypoxia-inducible protein to the 3'UTR (Levy et al. 1996). Furthermore, the tyrosine kinase inhibitor genistein, at doses known to inhibit ERK phosphorylation (Tang et al. 1998), inhibits hypoxia-induced protein binding to the VEGF 3'UTR and blocks the hypoxia-induced stabilization of VEGF via the 3'UTR (Levy et al. 1996). Finally, dominant-negative mutant forms of c-Src or of Raf-1, both of which can

activate the MAPK pathway, blocked the hypoxia-induced stabilization of VEGF 3'UTR transcripts (Mukhopadhyay et al. 1995). Thus mechanical factors and metabolic factors (oxygen tension) can interact through HIF-1α and MAPK at transcriptional and post-transcriptional levels to control VEGF mRNA in skeletal muscle.

Concerning the changes in mitochondrial mass or volume in human skeletal muscle, it is apparent that the factors that orchestrate mitochondrial biosynthesis with endurance training are manifold. Factors that are involved in the coordination of mitochondrial protein expression from the nuclear and mitochondrial genome include the nuclear respiratory factors 1 and 2 (NRF-1 and NRF-2) and the mitochondrial transcription factor A (TFAM). Expression of TFAM, and several nuclear encoded proteins (cytochrome C), is promoted by the nuclear-encoded transcription factors NRF-1 and NRF-2 (Virbasius and Scarpulla 1994; Evans and Scarpulla 1990). TFAM is essentially involved in general mitochondrial biogenesis (Larsson et al. 1998). It translocates into the mitochondria where it induces transcription and replication of mitochondrial DNA (Shadel and Clayton 1997; Fig. 7).

The involvement of factors other than TFAM and NRF-1 in exercise induced mitochondrial adaptations is suggested by the observations that selective transcriptional up-regulation of enzymes involved in beta oxidation of fatty acids with exercise is not explained by NRF-1 or NRF-2 alone (Lehman et al. 2000 and references therein).

Candidate signalling molecules that emerge as additional transducers of exercise stimuli to increased expression of mitochondrial proteins include the transcription factor complex activator protein-1 (AP-1), 5'AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor-alpha and gamma (PPARα/γ). Treadmill running and ergometer training, for 30 min above the aerobic threshold, induce expression of the jun/fos family of transcription factors in human m. vastus lateralis (Puntschart et al. 1998; Aronson et al. 1998). Dimers of the jun/fos family bind as AP-1 complex to the promoter, and/or influence the promoter activity of several nuclear encoded mitochondrial proteins, i.e. cytochrome C, carnitine palmitoyltransferase (CPT) and mitochondrial malic enzyme, some of which are affected acutely by exercise (Hood 2001; Hunter and Karin 1992; Butta et al. 2001; Brady et al. 1992; Pilegaard et al. 2000). AMPK activation is involved in the regulation of skeletal muscle metabolism during exercise (Winder et al. 2000; Zhou et al. 2000) and the α2 isoform of AMPK has been demonstrated to be activated by ergometer exercise in an intensity-dependent manner in human m. vastus lateralis (Wojtaszewski et al. 2000; Fig. 7). AMPK activation in rat muscle was held responsible for increasing cytochrome C and delta-aminolevulinic acid synthase protein levels, the activity of mitochondrial enzymes citrate synthase, succinate dehydrogenase, and malate dehydrogenase, and the skeletal muscle mitochondria-specific uncoupling protein-3 mRNA (Winder et al. 2000; Zhou et al. 2000). Moreover, Ca^{2+}-calodulin-dependent kinases recently have been implicated in regulation of mitochondrial biogenesis of skeletal muscle by a process that involves induced expression of the master regulator of mitochondrial biogenesis, the peroxisome proliferator-activated receptor γ co-activator PGC-1 (Wu et al. 2002). PGC-1 leads to a powerful induction of *NRF-1* and *NRF-2* gene expression and co-activates the transcriptional function of NRF-1 on the promoter of the mitochondrial transcription factor TFAM (Wu et al. 1999). A role for Ca^{2+} and CaMK in control of mitochondrial biogenesis during contractile activity is further supported by the finding that electric stimulation of cardiac cell cultures induces the mRNA of the transcription factor NRF-1 (Xia et al. 1997).

The inverse relationship between the cellular oxygen concentration and transcription factor HIF-1α protein levels indicates that HIF-1α is a main molecular sensor integrating increased muscle activity and oxygen availability into muscular remodelling. Recent evidence indicates that also phosphorylation of HIF-1α, through hypoxia-activated mitogen-activated protein kinases (MAPK), contributes to induced HIF-1α activity and promotes expression of its target VEGF (Pages et al. 2000; Minet et al. 2000; Semenza1999). There is increasing evidence that phosphorylation of HIF-1α activity, by a MAPK-dependent mechanism, is also important in gene regulation under normoxic conditions in response to growth factors in culture (Richard et al. 2000; Zelzer et al. 1998). On the other hand, it is found that exercise, presumably due to increased mechanical stress during contractile activity (Zou et al. 1998), also induces MAPK activity (Sherwood et al. 1999). Moreover, functional cooperation of HIF-1α with the MAPK-dependent transcription factor c-jun in hypoxia-induced gene transcription has been demonstrated (Alfranca et al. 2002). Last but not least, HIF-1α accumulates in response to mechanical stress (haemodynamic overload) in the nuclei of cardiac myocytes in a manner dependent on the concomitantly activated phosphatidylinositol 3-kinase (PI-3K)-Akt pathway and links to induced VEGF mRNA expression (Kim et al. 2002; Sodhi et al. 2001). A picture is therefore suggested whereby integration of exercise signals into transcription of angiogenic factors in skeletal muscle could be achieved by a cooperation and/or competition of mechano- and (local) hypoxia-sensitive pathways at the level of HIF-1α (Fig. 8).

The results on the expressional co-regulation of myofibrillar proteins MHC and the myogenic factors (foremost myoD and myogenin) suggest that myogenic factors integrate nerve activity and thyroid state into remodelling of the contractile apparatus. However, these factors do not appear involved in the mechanisms underlying maintenance of muscle mass (Dupont-Versteegden et al. 1998).

Conclusions

The present data suggest that exercise and environmental stress acting on skeletal muscle cause adaptive events in muscle fibres and associated structures and contribute to muscle plasticity. Changes in the gene profile are recognized as an interface that integrates the physiological perturbations by providing the necessary instructive information for the remodelling process of muscle structure and function.

The enormous and well-described plasticity of muscle tissue and its accessibility to biopsies makes muscle the organ of choice to study gene regulatory phenomena in humans. With the advent of technology to monitor thousands of genes at once, the skeletal muscle plasticity is a paradigm whose exploration is of greatest potential to understand the adaptations of human biological systems to physiological stimuli in the tissue context of the living. With the appropriate molecular techniques it has been demonstrated in the past 10 years that rapid changes in mRNA occur with exercise in human and rodent species (Pilegaard et al. 2000; Neufer and Dohm 1993; Puntschart et al. 1998). Recent expression profile data demonstrate that transcriptional adaptations in muscle due to changes in loading involve co-incident adaptations of genes in several functional categories. The co-directional expressional changes of genes belonging to functional categories (synexpression groups) indicates that expression of a battery of genes is controlled by a master transcriptional regulators. Signalling pathways involving the nuclear-encoded transcription factors

NRF-1, TFAM, AP-1, PPAR and AMPK, HIF-1α, and myogenic regulatory factors are implicated in transducing and integrating physiological stress into transcriptional adaptations of metabolic and contractile genes. These changes are matched to structural/functional adaptations and enzyme activity known to occur with corresponding stimuli. Nuclear reprogramming is recognized as an important event in muscle plasticity and may be related to the adaptations in the myosin type, protein turnover, and the cytoplasma-to-myonucleus ratio, as predicted by nuclear domain theory.

Future experiments will have to aim at understanding the acute regulatory phenomena occurring as a consequence of a single exercise intervention. Acute regulatory phenomena will have to be linked to the steady state changes in mRNA levels of structural genes ultimately responsible for the gross and subtle structural and functional modifications described as skeletal muscle plasticity. To this end, future approaches will make use of high-throughput technology such as protein and DNA arrays to identify the characteristic molecular adaptations in animal and human models.

Moderate exercise has a beneficial effect of body weight, metabolism and insulin resistance and energy expenditure and can counteract the syndrome of physical frailty (Evans 1995; Booth and Baldwin 1995). Understanding the molecular key to muscle plasticity therefore may be of importance for understanding the development of diseases such as obesity, NIDDM, hypertension and hyperlipidaemia and will give insight into these important clinical processes (Booth et al. 2002).

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