Federazione Italiana Fitness
1st International Conference

Exercise and muscle hypertrophy
From cell biology to training

Riccione, March 4, 2000
Palazzo del Turismo, Piazzale Ceccarini 11,
Riccione (Rimini), Italy

PROGRAM

9.30 R Dall'Aglio, Parma, Italy: Muscle Cell: How to achieve muscular hypertrophy

10.00 M Sandri, Padova, Italy: The role of apoptosis or programmed cell death in exercise-induced muscle damage

10.30 S Salmons, Liverpool, UK: Pre-electrostimulation improves tissue resistance to exercise-induced muscle damage

11.10 Discussion

11.50 U Carraro, Padova, Italy: *The protocols of LD wrap stimulation: a Key Issue in the Italian Trial of Demand Dynamic Cardiomyoplasty*

12.05 C Reggiani, Padova, Italy: *Muscular hyperplasia and exercise*

12.35 H Kern, Vienna, Austria: *Muscular recovery and hypertrophy*

13.15 Discussion

Lunch

15.00 U Carraro, Padova, Italy: *Muscle damage and regeneration*

15.30 P Hespel, Leuven, Belgium: *Creatine and muscle hypertrophy*

16.10 R Manno, Roma, Italy: *Strength and hypertrophy*

16.40 Discussion

17.20 OM Rutherford, London, UK: *Hormones as stimuli for muscle growth*

18.00 R Maughan, Aberdeen, Scotland: *Nutrition and hypertrophy*

18.40 A Paoli and M Neri, Padova and Ravenna, Italy*: Exercise and muscular development: from molecular basis to training*

19.10 Discussion

ABSTRACTS

MOLECULAR MARKERS OF DAMAGE AND HEALING IN SKELETAL MUSCLE

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Full dependence from function of nerve and vessels, and myofibre plasticity heavily influence ethiology, and damage/healing processes of skeletal muscle. Given as known relation of muscle tropism to nutrients and blood perfusion, main effects of peripheral and central denervation, and secondary pathogenesis of genetic muscle disorders, three models of muscle damage spot actual knowledge of the complex processes of skeletal muscle healing.

The restitutio ad integrum (full healing) by muscle regeneration of a massive death of muscle tissue due to myotoxic agents (bupivacaine and snake venom) displays the potentials of satellite cells, major if not unique source of myoblasts of regenerative myogenesis, and of the co-ordinate interactions of phagocyte and myoblast in successful muscle regeneration.

Exercise-induced damage of normal muscle occurs during and after hours of physical activity performed at submaximal force, if abrupty higher than mean daily levels. This is a good model to study myofibre damage and the processes that exacerbate or moderate the primary lesions due to a kind of exercise usually performed with some pleasure.

Induction of progressive dystrophic changes by chronic electrical stimulation of transposed or mobilised muscles, anyhow activated at suboptimal resting length and tension, rise interesting questions, though give a few of the answers that are essential to applications of muscle plasticity to the health or the rehabilitation of severe handicap.

During the past fifty years electron microscopy provided the foundations of muscle functions, and of tissue damage and healing at cellular and subcellular levels.

During the last twenty years molecular approaches exponentially accumulated the knowledge on ethiology and pathogenesis of muscle lesions and of the healing processes by resolution of focal subcellular damage, and/or regeneration/repair of tissue insults providing fine markers of quasi all the steps of the processes. One can take advantage of surface markers to identify in complex mixtures of cells and of their debris phagocytes, fibroblasts, endothelia, and myoblasts. Monoclonals combined with structural approaches recognise in tissue extracts or sections the isoforms of the sarcomere, cytoskeleton and sarcolemma proteins, and those of the connective machinery that distribute or concentrate contraction force. Cytokines, their receptors and the intracellular or intranuclear network of mediators talk the language of intercellular communications and effects. Among the lasts, the transcription factors of the myogenic program in activated satellite cells tells us that muscle is more than meat, it is a living population

of myofibers which either mourn deaths or wear Sunday clothes to give new babies a hearty welcome. In vitro and in situ analyses of the mRNA of all these and other proteins add valuable information on successful or abortive changes of gene expression in myofibers confronting heavy functional demands. All these are robust analyses, surprisingly cost-effective.

Notwithstanding all that, we are still awaiting the answers to trivial-looking queries. The mains are related to the mechanisms of the contractile apparatus turnover and of the healing of spotted lesions of sarcolemma and sarcomeres. We need more human and financials resources, since knowledge of the limits of muscle plasticity under extreme stresses will pay the high dividends of the innovative applications to prevent diseases and restore decaying or lost functions.

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CREATINE AND MUSCLE HYPERTROPHY

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In the 1990's creatine monohydrate has become a very popular ergogenic supplement in athletic populations. Meanwhile it has been well established that creatine supplementation can increase muscle total creatine store, which is accompanied with enhanced muscle power output during exercise modes involving rapid muscle contractions and relaxations. This ergogenic action of creatine is probably at least partly due to facilitation of muscle relaxation. However, over the last 5 years substantial evidence has also been provided that creatine supplementation, in conjunction with heavy-resistance training, stimulates muscle hypertrophy and maximal muscle strength. In fact the first evidence that creatine exerts an anabolic action on muscle indeed, comes from a study in patients afflicted by atrophy of the choroid and retina because of deficient creatine biosynthesis. Creatine intake in these patients (1.5g/day, 1 year) consistently induced type II muscle fibre hypertrophy as a side-effect[2]. However, the effect of creatine supplementation on muscle hypertrophy was not further investigated until recently. In a study in young female volunteers, we found 10 weeks of heavy-resistance training (3h/week) in conjunction with creatine intake (4x5g/day for 1 week, and 1 x5g/day thereafter), to cause a greater increment of fat-free mass than the same training load without creatine supplementation. Meanwhile, two other studies have indicated that oral creatine supplementation can enhance hypertrophy of both type I and type II muscle fibers during heavy-resistance training. Accordingly, the responses of maximal muscle force and power to resistance training were found to be significantly stimulated by creatine intake [4]. Most recently we also investigated the impact of oral creatine supplementation on the cross-sectional area and functional capacity of m. quadriceps during leg immobilisation (2 weeks) and rehabilitation (10 weeks) in healthy volunteers (Hespel et al., unpublished observations). The magnitude of the muscle atrophy caused by the immobilisation was not reduced by creatine supplementation (4x4g per day). However, in line with the above-mentioned observations with heavy-resistance training, creatine intake was demonstrated to markedly facilitate the recovery of muscle mass and muscle functional capacity following the disuse atrophy. It is also important to emphasise that in none of the above double-blind placebo-controlled studies, any significant side effects occurred during or after the period of creatine supplementation (10 to 20 weeks). It is certainly worthwhile to further evaluate the potential of long-term creatine supplementation with regard to the improvement of muscle functional capacity in the context of fitness training, athletic training and rehabilitation training, for individuals at risk or afflicted by muscle atrophy.

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MUSCULAR RECOVERY AND HYPERTROPHY

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The known effects of Neuromuscular Electrical Stimulation (NMES) in Sports and Physical Medicine and Rehabilitation are presented in a review of current literature. Influences of NMES on strength endurance, neural adaptations, therapy of edema and pain as well as functional improvements will be

focused on. Basics of physiology and histology in human muscle tissues are discussed.
The central topic is the influence of NMES on human skeletal muscle regarding histological changes, fibre transformation as well as strength and endurance of muscles involved.
We optimised NMES characteristics and then conducted a series of investigations with 9 volunteers that had 30 minutes of NMES applied twice daily for 7weeks.
Muscle biopsies were taken from the vastus lateralis portion of quadriceps femoris muscle on both the stimulated and non-stimulated leg before and after 7 week NMES period. Additionally participants performed isometric strength measurements of quadriceps femoris muscle at different knee joint angles before and after the 7 week stimulation period. Biopsies from the nonstimulated side showed no significant changes and may therefore serve as proof for the quality of the biopsies and as a standard for comparison.
The increment of type IIa fibre volume was12.3% and of type IIa fibre number 16%, respectively. Volume density of interfibrillar mitochondria increased by 22%. Subsarcolemmal mitochondria remained unchanged which contrasts the effects seen in voluntary non-NMES muscle training. Capillary density was augmented by 14.58%.
NMES is an appropriate means of adjusting longstanding muscle dysbalance regarding strength, endurance and improving oxidative metabolism in human skeletal muscle. Daily muscle fibre workload (i.e. number of stimuli per day) was found to be the main determinant of fibre transformation.

MUSCLE STRENGTH AND HYPERTROPHY

R. Manno

Muscular force is deeply influenced by muscle transverse section and by neuromuscular functionality. Both motor and sport demands can have different importance. In subject with different sex and during different periods of life hypertrophy answer to changing demands could be more or less emphasised with a relation that sometimes can be considered competitive to neuromuscular functionality

Training care doses muscular tension induced by external load (weights, springs, gravity, inertia, and so forth ...) in order to modulate both metabolic and neuromuscular function according to practicised sports or fitness aims.
Really stressing weights (85 to 100%), but few repeats seem to train neuromuscular function, while middle and low loads (30 to 40%) increase the energetic metabolism, but does not seem to induced hypertrophy. Loads at intermediate intensity (70 to 85%) seem to be able to induce hypertrophy.
In different sports hypertrophy with its relative increase of body weight is sometimes desirable, while in sports (like jumps and sprints) that reside in explosive movements and impose body propulsion it is desirable to contain muscle hypertrophy.
Muscle hypertrophy induced by workload varies at different ages. It is low in children, and maximised in adolescents and adults. It reduces in elderly. In women it is usually lower than in men.

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NUTRITION AND MUSCULAR HYPERTROPHY

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In sports that required strength and power a highly lean body mass, and especially a high muscle mass, confers a definite advantage. Supplement use is widespread among athletes in strength sports, and a wide variety of supplements are used. A few of the supplements that are more commonly used by athletes are described, but the list is by no means comprehensive.

EXERCISE AND HYPERTROPHY FROM MOLECULAR BASES TO TRAINING PROGRAMMES

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Scientific research on hypertrophy training has been always neglected if compared to training force, speed, etc. This because of an intrinsic difficulty on hypertrophy measurements and of ghettoizing of those who are looking for hypertrophy *per se*. On the other hand, studying the body builders' methods we found physiological bases for high intensity training. In spite of the classical 8 repeats suggested by trainers and their books, athletes obtained better results with more repeats or peculiar techniques. High intensity methods of Body Builders seem to stimulate cellular and biochemical mechanisms, which only now scientific research is describing.
These strategies are different an directed to different goals:
Mechanical Stimuli
- Emphasis on eccentric phase of movements
- Differential loads to involve different fiber types and muscular masses
- Different forms and speeds of exercising different fiber types

- exercise movements at very low speed

Metabolic Stimuli

- Maximal loss of phosphate and alteration of ATP/ADP ratio
- lactic acidhemia

Hypertrophy special techniques using one or more strategies, like forced, negative, stripping, 21 technique, super set, isometric and peak contractions, etc., are just some of the methods used to obtain hypertrophy.

Mechanical Stimuli. One of the factors that seems more involved in muscle growth is stretch, that is forced extension of muscle fibers. Indeed it is well known that passive stretch induce muscle growth even in denervated muscles, and in the absence of Growth Ormone, insulin and adequate nutrition [1]. Vanderburgh [2] recognized two class of second messengers, which translate mechanical force into muscle growth. First class involves molecules of the extracellular matrix [3]. Second class of messengers is related to sarcolemma-associated proteins [2, 4].

Satellite Cells. Muscle-specific growth factors (IGF-1 and FGF) are released in muscle tissue when satellite cells loose their contact to myofibers [5, 6]. Satellite cells enter the cell cycle and proliferate, as part of muscle hypertrophy. Increased content of muscle DNA support this hypothesis [7]

Mitochondria. In muscle hypertrophy beside contractile proteins mitochondria add to muscle mass if endurance training is performed. Molecular mechanisms are related to increased cAMP [8], to ADP/ATP ratio and to decreased creatin phosphate content.

Myofibres. Increased volume of myofibres is due to increased protein synthesis consequent to biochemical (ADP/ATP ratio and p70 phosphorilation) [9, 10], paracrine (IGF1 and FGF) [5, 6] and mechanical events. In these last changes are based the tropic effects induced by negative repeats and low speed performance applied by body builders. Eccentric contractions are known to be responsible of exercise-induced muscle damage [11, 12], while long contractions seem to prolong the mechanical stress to which the nuclei respond (Booth, personal communication). If this a major factor, we could understand the difference between elite sportsmen which use short contractions and body builders. It is well known that overloading increases expression of proto-oncogenes, like c-myc and c-fos [13]. Since free radicals also induce expression of proto-oncogenes, they also could be important to induce muscle hypertrophy.

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HYPERPLASIA IN EXERCISE-INDUCED MUSCLE GROWTH?

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Compensatory growth in response to exercise is an important adaptation mechanism of skeletal muscles, as they become in this way able to move heavier mechanical loads. The increase of muscle mass is primarily the result of the increase of muscle fibre size (fibre hypertrophy). Whether increase of fibre number (hyperplasia) also contributes to compensatory growth is still matter of debate. The aim is to analyse some data in favour or against this possibility.

HORMONES AS STIMULI FOR MUSCLE GROWTH

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Skeletal muscle is affected by many hormones and growth factors, both catabolic and anabolic in nature. Knowledge of

these effects has increased with the growth of recombinant technology leading to more studies on replacement therapies such as growth hormone (GH). Our understanding of the synthesis, release, transport and tissue sensitivities of these hormones has also increased greatly in recent years. Despite
This knowledge we still do not know the link between hormonal/growth factor release, training and muscle hypertrophy. Studying the effects of strength training on the endocrine system is complicated by a variety of factors related to both the exercise regime itself and the accurate measurements of the relevant hormones and growth factor.
As the latter are often synthesised in muscle itself, measurements mode from blood samples may be irrelevant. Many of the hormones that affect protein synthesis within muscle exhibit circadian and seasonal variations which further complicates accurate measurement.
From the work that has been carried out it would appear, that if sufficient high resistance exercise is carried out, the hormonal response is not qualitatively different to that following a bout of endurance exercise. This involves an acute increase in cortisol, GH, testosterone, and catecholamines and a decrease or no change in insulin and thyroid status. In the longer term there may also be changes in muscle sensitivity, perhaps through a changing receptor density.
About 20 years ago much attention was focused on the anabolic effects of androgens with the advent of steroid abuse amongst athletes.
The evidence that exogenous derivatives of testosterone are anabolic in eugonadal men is still controversial. In supraphysiological doses, and combined with training there is some evidence for their anabolic effects. This needs to be weighed against their potentially harmful side-effects. Much excitement was generated by studies on GH replacement in GH-deficiency which strongly indicated an important role for GH in the maintenance of lean tissue mass. Despite its role in clinical situations, the controlled trials have failed to demonstrate a clear anabolic role for GH in either the healthy younger person or elderly.
Considering all the evidence that is available, a likely candidate for causing muscle hypertrophy as a result of resistance training, is IGF-1. This would appear to be independent of GH release and act in an autocrine/paracrine fashion. A splice variant if IGF-1 has been identified and is expressed locally in muscle during repair and overload. This has been termed Mechano Growth Factor (MGF) and may be an important factor linking a mechanical stimulus and activation of gene expression and growth.
More recently there has been renewed interest in searching far an “anabolic hormone” due to the increasing numbers of elderly people within populations and the associated frailty common in this group. After the age of 75-80, frailty is one of the major health concerns and the major contributor to this is muscle weakness. Reduced physical activity levels and a shifting anabolic/catabolic ratio all contribute to this problem. The focus of attention is shifting to addressing this issue and identifying countermeasures.

PRE-ELECTROSTIMULATION IMPROVES TISSUE RESISTANCE TO EXERCISE INDUCED MUSCLE DAMAGE

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Deterioration of the latissimus dorsi (LD) muscle wrap after cardiomyoplasty has been demonstrated in both animals and man. It is probably the consequence of several factors, of which the most important is the combination of ischaemia with the increased metabolic demands imposed by electrical stimulation [1]. Ischaemia is most severe in the distal part of the muscle. Delaying stimulation for 2-3 weeks after reconfiguring the muscle is not entirely effective and also delays the benefit that the patient could otherwise derive from the operation.
We have confirmed the presence in the LD muscle of anastomotic channels connecting le vascular trees of le thoracodorsal artery and the perforating arteries[2]. The perforating arteries have to be divided during mobilization of the graft, but the distal region of the muscle is still perfuse by the thoracodorsal artery via the arterial anastomoses. Stimulation of the muscle prior to mobilization (‘prestimulation’) appeared to enhance flow through these anostomotic channels, since it abolished the characteristic proximodistal gradients in flow. Associated with these changes, there was an increased resistance to surgical intervention. When untreated muscles were lifted, handled, cooled and replaced at reduced tension, the usual signs of distal ischaemia were observed, and these had not recovered to a significant extent 5 days later. When prestimulated muscles were subjected to the same manipulations there was a smaller reduction in blood flow, the distal region was no longer selectively affected, and any initial ischaemia was completely reversed by 5 days [3, 4].
To determine whether these changes in blood flow would be reflected in the viability of the graft, we recently examined LD muscle grafts in rats, using Nitroblue Tetrazolium staining to distinguish living from necrotic muscle. The area of viable tissue was significantly greater in grafts that had received prestimulation. We also compared, in pigs, the effects of prestimulation with those of the more invasive true vascular delay procedure, in which the collateral vessels are divided but the LD muscle is left in situ for 10 d before elevating it as a graft. In the distal part of le muscle a significantly greater proportion of baseline blood was maintained after prestimulation than after vascular delay.
These findings make a substantial case for stimulating the LD muscle before raising it as a graft. Such a procedure will both improve the viability of the muscle and enable cardiac assis-

tance to be delivered to the patient at an earlier postoperative stage.

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ROLE OF APOPTOSIS IN MUSCLE DISORDERS

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Mononucleated cells from multicellular organisms self-destroy when no longer needed in organogenesis or when damaged. They do this by activating genetically controlled machinery that lead to apoptosis. Apoptosis has been described in developing and in adult human skeletal muscle. Alterations in the pathways that regulate myoblasts proliferation/differentiation processes lead to the induction of apoptosis during ontogenetic and regenerative myogenesis. Fully differentiated syncytial cells make skeletal muscle tissue in adults. In this case, apoptosis seems to start from segmental area of myofibre often producing loss of a single myonucleus. Tough apoptosis has been shown to occur in the skeletal muscle, the role played in neuromuscular disorders and the pattern followed in developing and adult muscle cells are far from being clear. The bcl2/bax system is active in muscle when apoptosis occurs, but conflicting results are reported on the role played by Fas/FasL and caspases systems. Some of the caspase cascades seem to be inhibited in adult myofibers, but others are activated in disease.

The role of apoptosis in such diverse pathological processes as tumour growth, immune response and neurodegeneration suggests that its regulation by drugs will become important to the medical community. A number of compounds have been used to inhibit or enhance some of the fundamental mechanisms of apoptosis. This knowledge is paving the path toward applications to treat muscular disorders.
