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The 12th Conference of the International Research Group on the Biochemistry of Exercise was held at Maastricht University, Maastricht, The Netherlands on 13–16 July 2003

Conference on 'The biochemical basis of the health effects of exercise'

Physical activity and health, novel concepts and new targets: report from the 12th Conference of the International Research Group on the Biochemistry of Exercise

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The present paper is the introductory paper to a series of brief reviews representing the proceedings of a recent conference on 'The biochemical basis for the health effects of exercise' organized by the International Research Group on the Biochemistry of Exercise in conjunction with the Nutrition Society. Here the aim is to briefly review and highlight the main innovations presented during this meeting. The following topics were covered during the meeting: exercise signalling pathways controlling fuel oxidation during and after exercise; the fatty acid transporters of skeletal muscle; mechanisms involved in exercise-induced mitochondrial biogenesis in skeletal muscle; new methodologies and insights in the regulation of fat metabolism during exercise; muscle hypertrophy: the signals of insulin, amino acids and exercise; adipose tissue–liver–muscle interactions leading to insulin resistance. In these symposia state-of-the-art knowledge on how physical exercise exerts its effects on health was presented. The fast-growing number of identified pathways and processes involved in the health effects of physical exercise, which were discussed during the meeting, will help to develop tailored physical-activity regimens in the prevention of inactivity-induced deterioration of health.

Physical activity: Health

The lack of physical activity has now been recognized by the World Health Organization (2002) as a major underlying cause of death, disease and disability. Preliminary data from a WHO study on risk factors suggest that inactivity, or sedentarism, is one of the ten leading global causes of death and disability. These data imply that each year $>2\times10^6$ deaths can be attributed to the lack of physical activity. For example, a sedentary lifestyle doubles the risk of CVD, diabetes and obesity.

Not only does physical activity help to prevent certain diseases, but favourable effects of regular physical activity have also been reported in individuals already suffering from chronic diseases such as type 2 diabetes mellitus, obesity and CVD. Hence, advocating regular physical activity to promote health will be one of the most prominent recommendations in the upcoming WHO report *Global Strategy on Diet, Physical Activity and Health* (for draft report, see World Health Organization, 2003). This report will be presented to the World Health Assembly at about the same time as the proceedings of the 12th Conference of the International Research Group on the Biochemistry of Exercise (IRGBE) will be published (May 2004). During this conference, which was held in July 2003 in Maastricht, The Netherlands, the biochemical basis for the health effects of exercise was discussed by recognized authorities in the field. In order to explore the

Abbreviations: AMPK, AMP-activated protein kinases; CaMK, Ca^{2+}/calmodulin-dependent protein kinase; FABPpm, plasma membrane fatty acid-binding protein; FAT, fatty acid translocase; GS, glycogen synthase; HSL, hormone-sensitive lipase; IGF-1, insulin-like growth factor 1; IMCL, intramyocellular lipids; IRGBE, International Research Group on the Biochemistry of Exercise; PDK4, pyruvate dehydrogenase kinase 4; PGC1α, PPARγ co-activator α; PI3K, phosphatidylinositol 3-kinase; PKB, PKC, protein kinase B and C respectively; UCP3, uncoupling protein 3.

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full potential of physical activity in the prevention and management of disease, the mechanisms that are triggered by physical activity, their regulation and the way they lead to health effects need to be investigated. In the oral presentations during the meeting the major focus was on the metabolic health effects of exercise. The conference programme comprised six symposia in which speakers presented not only the state-of-the-art of their field, but also presented exciting new unpublished data.

The topics of the symposia were: exercise signalling pathways controlling fuel oxidation during and after exercise (chair: A Bonen, Guelph, Ont., Canada); the fatty acid transporters of skeletal muscle (chair: J Glatz, Maastricht, The Netherlands); mechanisms involved in exercise-induced mitochondrial biogenesis in skeletal muscle (chair: J Holloszy, St Louis, MO, USA); new methodologies and insights in the regulation of fat metabolism during exercise (chair: A Wagenmakers, Birmingham, UK); muscle hypertrophy: the signals of insulin, amino acids and exercise (chair: M Hesselink, Maastricht, The Netherlands); adipose tissue–liver–muscle interactions leading to insulin resistance (chair: K Frayn, Oxford, UK).

The present issue of the *Proceedings of the Nutrition Society* will comprise brief reports from all invited speakers for this meeting. These reports have been peer reviewed by the chair of each session and at least one additional expert referee. In this introductory paper the aim is to briefly review and highlight the main innovations presented during this symposium.

Also, the organisers would like to take the opportunity to express sincere gratitude to the conference sponsors, Numico Research, DSM, Pfizer, Masterfoods, AstraZeneca, Medical Laboratories Stein, Hutchinson, Diabetes Fonds, The Netherlands Ministry of Welfare Health and Sport and the Nutrition and Toxicology Research Institute Maastricht, who all played a pivotal role in facilitating the invitation of so many distinguished experts in the field of the biochemistry of exercise. In addition, the organisers would like to thank the IRGBE for giving them the opportunity to organize this meeting in conjunction with the Nutrition Society. Finally, the organisers are very grateful to the Nutrition Society for publishing the papers from this conference in the *Proceedings of the Nutrition Society*.

‘Thrifty’ genes

In his opening lecture Dr Booth (Booth & Shanely, 2004) discussed the current excessive increase in obesity and type 2 diabetes from an evolutionary perspective. As a framework for his presentation he used the thrifty genotype hypothesis defined by Neel (1962). In this hypothesis diabetes is envisioned as an untoward aspect of a ‘thrifty’ genotype. This genotype originates from times when food availability depended on hunting and gathering; hence, food was available in cycles of feast–famine (Neel, 1962). Gene selection occurred 50 000–10 000 years ago while physical activity in the population was high. In the last 10 000 years the human genome has not changed markedly, and it is only since the industrial age that physical activity has become drastically reduced. Thus, the pandemic increase in the prevalence of type 2 diabetes over the last 40 years has occurred without a change in the human genome, but rather is the consequence of lifestyle changes. Dr Booth introduced four concepts to understand the selection of thrifty genes and to exemplify how physical (in)activity links to overt clinical symptoms of chronic diseases. The first concept states that cycles of feast–famine and physical activity–recovery trigger the cycling of metabolic processes, hence affecting the selection of thrifty genes and genotypes. A parallel between hunter–gatherer cycles and exercise–recovery cycles can be drawn in terms of cycling of muscle glycogen depletion–repletion. The latter is considered ‘normal’ physiology, thus oscillations in metabolites can be considered homeostatic cycling. This notion leads to the definition of the second concept that exercise–recovery cycles trigger glycogen depletion–repletion cycles that are critical in the initiation of other metabolic processes and ensure appropriate metabolic fluxes to maintain health. Importantly, cycles of depletion–repletion, which are not only restricted to glycogen, can be stalled by physical inactivity. Thus, a third concept is defined, stating that inadequate physical activity precipitates the stalling of metabolic cycling leading to misexpression of some thrifty genes so that a biological threshold to overt clinical symptoms is crossed. Unlimited food supply without exercise-induced metabolic cycling results in thrifty storage of metabolites and the loss of cycling of metabolic processes. Stalling of metabolic processes leads to the manifestation of chronic diseases and an increased mortality rate. The fourth concept postulates that by re-introduction of physical activity the stalling of metabolic cycling can be broken and the critical gene–environment interaction to prevent the development of chronic diseases can be restored. As stalling of metabolic cycling is considered abnormal physiology and is obviously disadvantageous from a health perspective, homeostasis should be redefined in terms of metabolic cycling rather than a constant level of muscle glycogen and intramyocellular lipids. In line with this viewpoint, Dr Booth finished with the provocative and intriguing call to be careful when referring to the sedentary group as the control group, since in terms of feast–famine and metabolic cycling the sedentary group can just as well be considered the chronically-diseased group. After this lecture, the challenge for the other speakers was to identify the lines along which stalled metabolic cycles could best be reactivated.

Exercise signalling pathways controlling fuel oxidation during and after exercise

This symposium focused on the role of various signalling pathways in the regulation of the fuel mix that is oxidized during and after exercise.

In the lecture given by Dr Fujii (Fujii *et al*. 2004) the role of AMP-activated protein kinases (AMPK) as a myocellular energy sensor was discussed. In past years AMPK has been considered to be a cAMP-independent member of the metabolite-sensing protein kinase family.

Generation of AMPKα2-dominant negative mice by over-expressing the AMPKα2 inactive form has resulted in important novel information on the role of AMPK as a fuel sensor. These mice, which are indeed characterized by complete inhibition of AMPKα2 activity, have normal AMPKα1 expression and activity levels, which can be increased by dinitrophenol and 5-aminoimidazole-4-carboxamide ribonucleoside. Phenotypically, the AMPK-α2-dominant negative mice are characterized by higher blood glucose levels but lower basal glycogen levels and decreased exercise capacity. Despite complete absence of contraction-induced AMPKα2 activity, contraction-induced glucose uptake is unaffected in the AMPKα2-dominant negative mice. This finding implies that another, as yet unidentified AMPKα-independent, signal plays a pivotal role in contraction-mediated glucose uptake.

These findings in the AMPKα2-dominant negative mice were confirmed and extended in the next lecture by Dr Richter (Richter *et al*. 2004), who has developed AMPKα2 knock-out mice. In fact, in the AMPKα2 knock-out mice contraction-induced glucose uptake is increased compared with wild-type mice whereas 5-aminoimidazole-4-carboxamide ribonucleoside-induced glucose uptake is completely blunted. Despite the lack of AMPKα2 activity in the knock-out mice, the signalling effect of AMPK *in vivo*, as indicated by contraction-induced phosphorylation of acetyl-CoA carboxylase β at serine residue 227, is largely unaffected, possibly explaining the normal contraction-induced glucose transport in the α2AMPK knock-out mice. In his lecture Dr Richter also stressed the possible importance of Ca-stimulated kinases such as protein kinase C (PKC) isoforms and Ca^{2+}/calmodulin-dependent protein kinase II (CaMkII). Novel data for these kinases indicate that atypical PKC can be activated during exercise in a duration- and intensity-independent manner. There are also data supporting PKC involvement in contraction-induced glucose transport. The participating isoforms still need to be identified but atypical PKC, rather than conventional PKC, appear to be likely candidates. Preliminary data on CaMkII during exercise show increased CaMKII activity during exercise at 100% $V_{O_2\,max}$ and a gradual increase during exercise for 40 min at 77% $V_{O_2\,max}$. The exact role of these and possibly other kinases, however, requires further investigation before solid conclusions can be drawn.

In his lecture on muscle glycogen and metabolic regulation Dr Hargreaves (Hargreaves, 2004) elegantly outlined the role of glycogen as a signalling molecule. The potential regulatory mechanisms of glycogen can be via direct effects of glycogen binding, via enzyme activities and signalling and via hormonal effects. However, delineation of the effects of glycogen *per se* from the secondary effects of the diet or exercise regimens used to manipulate glycogen levels remains complex. One of the genes apparently affected by the level of glycogen is pyruvate dehydrogenase kinase 4 (PDK4).

The involvement of PDK4 was covered by Dr Pilegaard (Pilegaard & Neufer, 2004) in her lecture on its regulation during and after exercise. PDK4 transcriptional rate and mRNA levels are already increased during prolonged exercise at low intensity and also during 45 min of exhaustive exercise. The transcriptional rate and also the mRNA levels of PDK4 are affected by the level of glycogen and the substrate availability after exercise. Infusion of Intralipid to raise plasma NEFA levels indicates that the regulation of PDK4 is (at least partly) under the control of fatty acids. Also, incubation of primary muscle cells with the glucocorticoid dexamethasone increases PDK4 mRNA, suggesting that the transcription factors PPARα and/or forkhead homologue in rhabdomyosarcoma (which belongs to the FOXO subfamily of forkhead type transcription factors and is also termed FOXO1) may be involved. In the high-fat condition, while recovering from exercise, forkhead homologue in rhabdomyosarcoma is indeed increased whereas PPARα is not, suggesting that forkhead homologue in rhabdomyosarcoma may play a role in the transcriptional regulation of PDK4.

Other molecular mechanisms involved in the regulation of gene expression and glucose metabolism by exercise were highlighted by Dr Zierath (Long *et al*. 2004). The observation of a mutation in the gene encoding a muscle-specific isoform of the regulatory subunit of AMPKγ3 has led to the suggestion that AMPKγ3-containing complexes are important in the regulation of glucose homeostasis. The putative role of AMPKγ3 in glucose homeostasis has been examined in mice that overexpress AMPKγ3 and AMPKγ3 knock-out mice. Overexpression of the *AMPKγ3* gene in wild-type and *ob/ob* mice results in increased glycogen stores in the fasted and the fed state in both strains. The mice that overexpress AMPKγ3 are characterized by normal glucose and insulin tolerance and are resistant to exercise-induced muscle fatigue; in the AMPKγ3 knock-out mice, however, glycogen content and insulin- and contraction-mediated glucose uptake are normal. These findings challenge the hypothesis that contraction-induced glucose uptake occurs via AMPKγ3-containing complexes.

In the last lecture of this session Dr Wojtaszewski (Nielsen & Wojtaszewski, 2004) discussed the seemingly paradoxical activation of glycogen synthase (GS) during exercise. Regulation of GS activity is closely controlled by (de)phosphorylation at both N- and C-terminal serine residues. Exercise-induced phosphorylation of the N-terminal residues (probably mediated by systemic and local factors) appears to be the most important mechanism by which GS activity is unaltered or decreased during exercise, while dephosphorylation at the C-terminal residues increases GS activity during and after exercise (via local factors such as GS kinase 3). AMPKα1 and AMPKα2 knock-out mice from the laboratory of Dr Richter have been used to show that AMPK is a GS kinase, hence affecting GS activity. Whilst more research is needed to elucidate the precise role of AMPK in the regulation of GS activity, the AMPKα2 isoform appears to be the main candidate for this regulatory role.

Fatty acid transporters of skeletal muscle

In this symposium the transport of fatty acids in skeletal muscle and the role of fatty acid binding and transporting proteins were discussed. The first lecture was given by Dr Kiens (Roepstorff *et al*. 2004), who reported data from human intervention studies designed to manipulate protein

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expression of plasma membrane fatty acid-binding protein (FABPpm) and fatty acid translocase (FAT)/CD36 *in vivo*. Immunohistochemistry reveals that FABPpm is present at the sarcolemma as well as in the sarcoplasm. The latter signal may represent mitochondrial localization of FABPpm, although double staining of FABPpm with a mitochondrial protein was not shown at the meeting. For FAT/CD36 the immunohistochemical data seem to indicate that in human skeletal muscle FAT/CD36 is associated with the sarcolemma and is also expressed in capillaries, while intracellularly the staining for FAT/CD36 is absent. Both FABPpm and FAT/CD36 increase following a high-fat diet; FAT/CD36 is already increased after only 5 d on a high-fat diet. Exercise training is associated with an increase in protein expression of FABPpm, but not of FAT/CD36. Intriguingly, expression of the putative lipid-binding protein caveolin 1 (expressed in endothelial cells, adipocytes and fibroblasts) is increased 10 h after glycogen-depleting exercise in combination with a low-carbohydrate diet, while caveolin 3 (expressed almost exclusively at the sarcolemma) is unaffected. The glycogen-depletion studies indicate that transport across the sarcolemma is not always the rate-limiting step, but that mitochondrial fatty acid uptake in some cases is the determining step in fatty acid oxidation.

This observation links nicely to the lecture by Dr Bonen (Bonen *et al*. 2004) who focused on the role of FAT/CD36 in fatty acid transport. In his lecture Dr Bonen showed that fatty acid uptake across the sarcolemma can be regulated within minutes by insulin and contraction as a result of intracellular redistribution of FAT/CD36. Transmembrane transport of fatty acids, determined in giant vesicles from lean, overweight and obese individuals and in individuals with type 2 diabetes, is drastically increased without changes in total FAT/CD36 expression levels but with increases in sarcolemmal levels of FAT/CD36, suggesting a link between FAT/CD36 fatty acid transport and intramyocellular accumulation of triacylglycerols. Regulation of *FAT/CD36* gene expression is under the control of insulin rather than PPARα or PPARγ, hence alterations in FAT/CD36 can be observed in human and animal models of insulin resistance and insulin insufficiency. In addition to transmembrane transport of fatty acids, FAT/CD36 also appears to have a role in mitochondrial fatty acid handling. The expression of FAT/CD36 is more abundant in subsarcolemmal mitochondria than in intramyofibrillar mitochondria. This finding deserves more in-depth investigation.

The routes for regulation of cellular redistribution of fatty acid transporters were discussed by Dr Luiken (Luiken *et al*. 2004). In a model for cardiac fatty acid uptake it has been shown that FAT/CD36 can be recruited from an insulin- and a contraction-sensitive pool and the effects of insulin and contraction are additive. The original hypothesis that cAMP-activated PKA would be involved in contraction-inducible FAT/CD36 translocation could not be confirmed. An elegant series of physiological and pharmacological interventions used to study the putative involvement of AMPK and PKC in contraction-induced translocation of FAT/CD36 have revealed that AMPK and one or more of the PKC isoforms play an important role.

The last lecture in the fatty acid transport session, which was given by Dr Pohl (Pohl *et al*. 2004), outlined the role of the fatty acid transport protein and caveolae in fatty acid uptake. Caveolin 1 is putatively involved in the uptake of long-chain fatty acids from the blood across the endothelial cell layer and across the membrane of adipocytes. The latter is substantiated by the observation that pharmacological inhibition of caveolae in 3T3-L1 adipocytes drastically reduces the uptake of oleate. Interestingly, caveolin 1-deficient mice have a low body mass, reduced adipocyte diameter, increased fatty acid levels and are resistant to diet-induced obesity. Together with the observation that FAT/CD36 is expressed in caveolae and that long-chain fatty acids indeed bind to caveolae, it is obvious that caveolae play an important role in uptake of fatty acids.

The metabolic role of IL6 produced by muscle during exercise

In her lecture on the role of IL6 in human substrate metabolism Dr Pedersen (Pedersen *et al*. 2004) clearly outlined the multiple roles of IL6 that have recently been identified. Exercising muscles produce and release IL6 in a fibre type-independent fashion. The induction of the *IL6* gene during exercise is more pronounced in untrained subjects with low pre-exercise glycogen levels than it is in subjects with high pre-exercise glycogen levels after exercise training. Increased circulatory levels of IL6 increase lipolysis and fat oxidation and inhibit TNF-production. Given that TNF may be involved in mediating insulin resistance, these data provide one mechanism whereby IL6 may enhance insulin sensitivity. The putative role of IL6 in insulin sensitivity is currently under investigation.

Mechanisms involved in exercise-induced mitochondrial biogenesis in muscle

In this session the biogenesis of the cellular powerhouse and the role of exercise in this process was discussed. In the first lecture Dr Baar (Baar, 2004) focused on transcriptional regulation of mitochondrial biogenesis. Mitochondrial biogenesis is the result of coordination of nuclear- and mitochondrial-encoded genes. The nuclear respiratory factors 1 and 2 are transcription factors central to this process. Many of the respiratory-chain genes, including the mitochondrial transcription factor A, are under the control of nuclear respiratory factors 1 and 2. In addition, the transcription factor PPARα controls the expression of many of the fatty acids and β-oxidation genes. For activation these transcription factors depend on co-activators such as PPARγ co-activator α (PGC1α) to become active. The expression of all mitochondrial genes is coordinated by PGC1α. In a very neat model of tissue-engineered muscle, transfected with PGC1α, the role of mechanical, electrical and hormonal stimuli can now be examined independently. In these engineered muscles it has been shown that overexpression of PGC1α increases GLUT4 and cytochrome C expression and increases fatigue resistance.

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The signals from exercise that trigger events causing mitochondrial biogenesis and GLUT4 induction were discussed by Dr Ojuka (Ojuka, 2004). Clearly, during high-intensity exercise AMPK is involved; however, during exercise at moderate intensity AMPK is unchanged. Intermittent increases in sarcoplasmic Ca stimulate mitochondrial biogenesis. A number of signalling molecules are affected by changes in Ca, including CaMK, myocyte-enhancing factor 2, PGC1 and nuclear respiratory factors 1 and 2, but mitochondrial transcription factor A is also increased. One of the candidate genes regulating mitochondrial biogenesis that is under control of Ca is CaMK IV. Indeed, it has been shown that overexpression of CaMK IV results in increases in GLUT4 content as well as in increased mitochondrial density. These observations have led to the hypothesis that following steady-state exercise at moderate intensity, where activation of AMPK is minimal, cyclic increases in sarcoplasmic Ca levels primarily trigger the adaptation that increases mitochondrial biogenesis and GLUT4 content. It has been hypothesized that during strenuous exercise the adaptive stimulus includes both activation of AMPK and cyclic increases in sarcoplasmic Ca levels.

In-depth analyses of how Ca and calmodulin kinases regulate mitochondrial biogenesis were made in the lecture by Dr Chin (Chin, 2004). In her lecture Dr Chin stressed the notion that CaMK have multiple downstream targets and thus serve multifunctional processes. Determinants of CaMK specificity include the localization and isoform of the CaMK and the duration, amplitude and frequency of elevation of sarcoplasmic Ca. In line with this dependence on cyclic changes in Ca, repetitive contractile activity activates CaMKII, induces hypertrophy and increases oxidative capacity. Thus, Ca-dependent transcriptional regulation plays a pivotal role in modulating muscle fibre typology and metabolic capacity. Interestingly, the γ isoform of CaMKII appears to be involved in slow to fast transitions in muscle fibre during ageing and denervation. CaMKIIγ is up regulated in aged and denervated soleus muscle but not in fast-twitch anterior tibialis muscle.

Mitochondrial biogenesis largely depends on the ability to import nuclear-encoded proteins into the mitochondria, a topic covered by Dr Hood (Hood & Joseph, 2004). The import rate of proteins into the mitochondria, which is a vital step in mitochondrial assembly, depends on the level of precursor protein present, but can be modulated by, for example, chronic contractile activity resulting in a doubling of the import rate with the same level of precursor proteins present. While protein import rates are of importance they probably do not limit biogenesis, as indicated by normal to higher protein import rates in patients with mitochondrial myopathies. The exercise-inducible increase in mitochondrial protein import rates indicates the feasibility of using exercise as a model for improving potential import defects that could contribute to mitochondrial myopathies. Components of the mitochondrial import machinery, such as translocases of the outer membrane (outer membrane import receptor protein Tom20), are probably regulated by PGC1α.

Diet- and exercise-induced changes in the nuclear-encoded mitochondrial uncoupling protein 3 (UCP3) were reported by Dr Schrauwen (Schrauwen & Hesselink, 2004). Recent data indicate that despite frequently-reported associations between UCP3 and energy efficiency, the primary role of UCP3 is not uncoupling *per se* but rather the outward transport of fatty acid anions or lipid peroxides from the mitochondrial matrix. Thus, the matrix can be protected against the deleterious effects of reactive oxygen species and mitochondrial DNA damage can be prevented. As a side effect of the export of fatty acid anions or peroxides in exchange for a proton mild uncoupling occurs, explaining the observed relationship between UCP3 and energy efficiency. Lack of UCP3 together with reduced fat oxidative capacity and high circulatory fatty acid levels (as in type 2 diabetes) increases the mitochondrial fat load and may result in oxidative damage of the mitochondria (lipotoxicity). Thus, the primary physiological role of UCP3 may be to prevent mitochondrial dysfunction as a result of lipotoxicity.

New methodologies and insights into the regulation of fat metabolism during exercise

In this symposium the focus was on the use of intramyocellular lipids (IMCL) as a substrate during exercise in health and disease and the regulation of the lipases involved. One of the speakers in this symposium was Morten Donsmark from Copenhagen, who was the winner of the Young Investigator Award on Biochemistry of Exercise 2003 given by the IRGBE. The award was made in recognition of Dr Donsmark's work on the regulation of hormone-sensitive lipase (HSL) in muscle by contractions.

In his lecture on the use of IMCL during exercise Dr van Loon first outlined the existing controversy about whether or not IMCL are indeed used during exercise. It appears that when biochemical extraction of lipids is used to quantify IMCL levels the data reported are inconsistent, while the use of tracers, histochemistry and proton magnetic resonance spectroscopy clearly indicate that IMCL can be a substrate during exercise. During prolonged exercise there is a marked drop in IMCL in all muscle fibres, with the most prominent decline being found in type I fibres. Repletion of IMCL is affected by post-exercise diet composition; a low-fat high-carbohydrate diet results in incomplete restoration of IMCL levels for at least 48 h while a 'normal' diet induces a marked repletion of IMCL within 48 h post exercise. As the relative contribution of IMCL progressively declines during prolonged exercise whereas plasma fatty acid levels increase, the effect of plasma fatty acid levels on IMCL oxidation has been investigated. Pharmacological blocking of lipolysis with acipimox has shown that blunted plasma fatty acid levels are associated with increased use of IMCL, suggesting that elevated plasma fatty acid levels may impair IMCL oxidation. Thus, the impaired utilization of IMCL in obese subjects and subjects with type 2 diabetes could be a result of elevated plasma fatty acid levels. Thus, reducing plasma fatty acid levels in obese subjects and subjects with type 2 diabetes, combined with increased energy expenditure, could be a tool for increasing IMCL oxidation and reducing insulin resistance.

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In his award-winning contribution Dr Donsmark (Donsmark *et al*. 2004) discussed data obtained in rats on the regulation and role of HSL, the enzyme that hydrolyses IMCL. HSL activity can be increased by adrenaline via stimulation of β-adrenergic receptors and protein kinase A. Contractile activity stimulates HSL, mediated by PKC, partly via the extracellular-regulated protein kinase pathway. Adrenaline and contractions have a partly additive effect on the hydrolysis of triacylglycerols, but not diacylglycerol, by HSL. Interestingly, endurance training decreases adrenaline-mediated HSL activity but increases contraction-mediated HSL activation in muscle.

The majority of these findings were confirmed in the presentation by Dr Spriet (Watt & Spriet, 2004), who discussed the role of HSL in man. Despite activation of HSL in human subjects by infusion of adrenaline, these changes are often opposite to those occurring during exercise. This finding indicates that during exercise other (intramuscular) regulatory processes may be of more importance in the regulation of HSL activity than adrenaline. In relation to substrate availability and HSL activation, it has been shown that during exercise for 2 h at 60 % $V_{O_2\,max}$ HSL activation and fat oxidation are lower with glucose ingestion than without glucose, possibly as a result of lower adrenaline and higher insulin levels in the glucose trial. Activation of HSL during exercise decreases over time both with high-intensity exercise (90 % $V_{O_2\,max}$ for 10 min) and with moderate-intensity exercise (60 % $V_{O_2\,max}$ for 2 h), while in the same time period adrenaline levels increase. It appears, therefore, that HSL activity is not predictive of the rate of fat oxidation (and IMTG lipolysis). Putative downstream points of regulation of HSL include: allosteric regulation by, for example, long-chain fatty acyl-CoA esters; movement of the HSL complex to the lipid droplet; the presence of perilipin, which prevents direct access of the lipase to the lipid droplet.

The last speaker in this session was Dr Blaak (Blaak, 2004), who discussed the disturbances in fat metabolism in diabetes and obesity. In her lecture she related the accumulation of IMCL to the putative role of muscular fatty acid uptake, fatty acid oxidation and the imbalance between esterification, lipolysis and oxidation. Data showing decreased levels of the cytosolic fatty acid-binding protein in subjects with type 2 diabetes seem to indicate a decrease in the capacity to take up fatty acids. Together with data on arterio–venous differences across the human forearm that show a decrease in fatty acid uptake under conditions of β-adrenergic stimulation, these findings suggest decreased rather than increased fatty acid uptake in subjects with type 2 diabetes. Also, the rate of plasma-derived fat oxidation is decreased in the muscle of subjects with diabetes. A lifestyle intervention programme, comprising regular physical activity and the consumption of a more-healthy diet and weight loss, reverses reduced rates of fat oxidation. The restored rates of fat oxidation are paralleled by decreased acetyl-CoA carboxylase 2 mRNA levels. These effects are only observed if diet and exercise are combined, but are not observed after weight loss alone.

Muscle hypertrophy: the signals of insulin, amino acids and exercise

This symposium focused on muscle plasticity and how muscle mass can be modulated by exercise and exercise-related signals. The first lecture by Dr Esser (Hornberger & Esser, 2004) provided a nice overview of the effect of mechanical loading on muscle-cell gene expression, starting from the concept of mechano-transduction. She briefly outlined the different effects of uniaxial *v*. multiaxial strain on gene expression and protein synthesis. When C2C12 cells are subjected to different strain regimens, using a novel device, both uniaxial and multiaxial strain induce activation of the Akt/protein kinase B (PKB) and extracellular-regulated protein kinase signalling pathways, whereas multiaxial strain also affects p70 and GS kinase 3. The use of cytochalasin D to disrupt the actin cytoskeleton results in inhibition of ribosomal S6 kinase phosphorylation without affecting Akt/PKB signalling. This finding indicates that mechanical strain can, partly via the actin cytoskeleton, activate distinct mechano-sensory–mechano-transduction pathways, and activation to these pathways is specific to the types of mechanical forces applied. Extending the model to an *in vitro* strain system to study protein synthesis reveals that increased protein synthesis as a result of strain is linked to the phosphatidylinositol 3-kinase (PI3K) pathway, but the signalling is different from that described for insulin. These experimental models will be valuable tools in future studies of mechano-transduction and gene expression. This field is important, as mechanical (un)loading is inevitably linked to daily life (in)activity and little is known about the importance of the direction and level of an applied mechanical load in relation to muscle growth.

The importance of insulin-like growth factor (IGF-1) in muscle growth was outlined by Dr Machida (Machida & Booth, 2004), in terms of the mechanism, within the framework of ageing-induced sarcopenia. The working hypothesis was that aged skeletal muscles show defective regrowth because of the absence of an endogenous growth factor, putatively IGF-1. Indeed, IGF-1 activates the PI3K/Akt pathway, down regulates cell-cycle inhibitor $p27^{Kip1}$ and increases satellite-cell proliferative potential. Examination of the role of forkhead transcription factor 1 has been used to identify the mechanisms by which IGF-1 down regulates $p27^{Kip1}$. Data from the luciferase reporter gene assay show that forced expression of forkhead transcription factor 1 increases $p27^{Kip1}$ promotor activity and that IGF-1 inhibits the induction via phosphorylation of forkhead transcription factor 1.

Next to regrowth of aged muscle by activating satellite cells, hypertrophy of muscle may also occur via Ca-dependent signalling molecules. This topic was covered by Dr Michel (Michel *et al.* 2004), who unfortunately was not able to present the lecture but found an outstanding stand-in in Dr Chin. Intact calcineurin–nuclear factor of activated T cells signalling suffices to induce slow-muscle gene expression and is sufficient to induce muscle hypertrophy during functional overload but not under normal weight-bearing conditions, raising the question of what additional regulatory protein is needed for *in vivo* muscle growth.

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Putatively, calcineurin may exert its effect via myostatin, a negative regulator of muscle mass. Myostatin antibody treatment of Bl6 mice results in increased muscle mass. If myostatin antibody treatment is used in KK-A^y/a mice, increased muscle mass is paralleled by reduced levels of serum triacylglycerols, LDL, fatty acids and HbA_1C, showing the potential benefits of increased muscle mass in insulin-resistant prone phenotypes. In isolated myocytes IGF-1-induced hypertrophy is partially suppressed after treatment with cyclosporin A, a calcineurin inhibitor, suggesting that IGF-1-induced hypertrophy is also affected by calcineurin. In the search for a hypothetical link between IGF-1, calcineurin and mechanical overload-induced hypertrophy, dystrophin and utrophin, two proteins located in the trans-sarcolemmal focal adhesions and therefore involved in mechano-transduction, have been investigated. Utrophin is hierarchically expressed in the different muscle fibre types (most abundant in type I fibres, least abundant in type IIb). Fascinatingly, inhibition of calcineurin by cyclosporin A reduces utrophin mRNA levels markedly, while in the calcineurin transgenic mice utrophin gene expression is markedly increased. This finding may indicate that increased mechanical loading is sensed in the focal adhesions by, for example, utrophin, with calcineurin as a downstream target for overload-induced hypertrophy. This work again highlights the relevance of mechano-transduction and the need to explore this field in future studies of load-induced muscle hypertrophy.

In the lecture by Dr Kimball (Bolster *et al*. 2004) the integration of signalling by insulin, amino acids and exercise was discussed. Increased activation of the PI3K–mammalian target of rapamycin signalling pathway is involved in the signalling of insulin, amino acids and exercise, but the precise site(s) in the PI3K–mammalian target of rapamycin pathway at which these triggers exert their effect are still the subject of extensive studies. The positive effect of resistance exercise on protein synthesis may occur directly via PI3K, phosphatidylinositol-dependent protein kinase or Akt/PKB. If Akt/PKB is the main target, increased protein synthesis may occur via activation of GS kinase 3 and the translation initiation factor eukaryotic initiation factor 2B. Alternatively, Akt/PKB may exert its effect directly via mammalian target of rapamycin. Resistance exercise in rats does not affect phosphorylation, and hence activation of GS kinase 3 and eukaryotic initiation factor 2B, in the first 60 min post exercise, while activation of downstream targets of the Akt/PKB–mammalian target of rapamycin pathway are markedly increased within 10 min post exercise. This finding indicates that increased translation of mRNA encoding ribosomal proteins and translation elongation factors is implicated in the increased protein synthesis after resistance exercise.

Since muscle mass is the net result of protein synthesis and protein degradation, it is also important to have detailed information on how protein degradation is involved in muscle plasticity. This topic was covered by Dr Attaix (Taillandier *et al*. 2004). The ubiquitin–proteasome-dependent proteolytic machinery is one of the major proteolytic systems in the skeletal muscle. In rat soleus muscle increased ubiquitin proteasome-dependent proteolysis is involved in muscle wasting during unloading and also during muscle recovery after reloading the muscle. This finding clearly points to a role for proteolysis in muscle plasticity in cycles of loading and unloading. In addition, in old rats in the postprandial state proteasome-dependent proteolysis is not inhibited, in contrast with observations in adult rats. A leucine-supplemented diet restores the defective postprandial inhibition of the proteasome-dependent proteolysis in aged rats but has no effect in adult rats. This work again emphasizes the importance of the ubiquitin-dependent proteasome system in the remodelling of muscle during disease, disuse, contractile activity and training, and hence during muscle recovery from physical inactivity.

Adipose tissue–liver–muscle interactions leading to insulin resistance

In the last session of the meeting some of the inter-organ communications relating to insulin resistance were discussed. In the first lecture the concept of metabolic (in)flexibility was discussed by Dr Storlien (Storlien *et al*. 2004). Metabolic flexibility is defined as the capacity for use of, and the ability to rapidly and appropriately transit between, fat and carbohydrate fuels. Disturbances in metabolic flexibility (metabolic inflexibility) may contribute to the metabolic syndrome, with insulin resistance as a major outcome. The five critical tissues involved are the brain, pancreas, liver, skeletal muscle and adipose tissue, with adipose tissue and the mitochondrial oxidative capacity playing a pivotal role. Lipotoxicity is a likely contributor to β-cell dysfunction and deterioration of mitochondrial oxidative capacity. Hence, restoration of metabolic flexibility should include the reduction of fatty acid availability via loss of excess body weight, pharmaceutical intervention with, for example, PPARα or PPARγ agonists, or activation of the main cofactor for mitochondrial biogenesis, PGC1α or PGC1β.

In the next lecture the physiological regulation of fatty acid availability was described by Dr Stich (Stich & Berlan, 2004). The most important physiological conditions affecting fatty acid availability include: the resting post-absorptive state, the resting postprandial state and physical exercise (either in the post-absorptive state or combined with food intake). The anti-lipolytic activity of adrenaline, mediated via α2 adrenoreceptors, is involved in the regulation of lipolysis during exercise. Adipocyte hypertrophy (as in the obese state) positively correlates with the number of α2 adrenoreceptors, facilitating the higher anti-lipolytic effect of adrenaline observed in obese subjects. In obese women 12 weeks of aerobic training does not affect the responsiveness of adipose tissue to the β2 and α2 component of adrenaline action. Training also fails to affect the levels of β2 and α2 adrenergic receptor and HSL expression in the adipose tissue of these obese women. Thus, in obesity the responsiveness of lipolysis is impaired (lower adipose tissue-derived fatty acid mobilization). The impaired responsiveness of lipolysis under basal conditions could be a protective mechanism to prevent excess levels of circulatory fatty acids.

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In the lecture by Dr Dohm (Hulver & Dohm, 2004) the intriguing molecular link between muscle fat accumulation and insulin resistance was highlighted. In the insulin-resistant state both IMCL and fatty acyl-CoA levels are increased and high levels of fatty acyl-CoA esters impede insulin signalling via diminished activity of insulin receptor tyrosine kinase. Insulin receptor substrate 1 activity is markedly increased if it is phosphorylated at the tyrosine residues, whereas phosphorylation of serine and threonine residues has been associated with insulin resistance. Phosphorylation of insulin receptor substrate 1 at serine and threonine residues is affected by the activity of different protein kinase C isoforms; the β, δ and θ isoforms of PKC are increased when muscle strips from obese patients are stimulated with insulin. In addition, activation of PKC causes insulin resistance, possibly via phosphorylation of insulin receptor substrate 1 at the serine and threonine residues, whereas the opposite effect is achieved after inhibition of PKC. Similarly, overexpressing PKCβ causes insulin resistance, and the knocking-out of PKCβ results in improved insulin signalling. These findings highlight the important role of PKC isoforms in impeding insulin signalling, possibly by activation of the PKC isoforms either by long-chain fatty acyl-CoA esters or by diacylglycerol. A putative cause of the increased levels of long-chain fatty acyl-CoA esters or diacylglycerol could be a reduced fat oxidative capacity. However, increased levels of fatty acyl-CoA have also been reported in muscles with a normal fat oxidative capacity. Hence, although the involvement of PKC isoforms in the development of insulin resistance has been established, the conditions in which increased fatty acyl-CoA levels activate PKC and the origin of the increased levels of diacylglycerol and fatty acyl-CoA remains to be determined.

In the last lecture of the meeting Dr Ruderman (Tomas *et al*. 2004) discussed the metabolic and hormonal links between adipose tissue and skeletal muscle. It has recently become evident that adipose tissue should be viewed as an endocrine organ, given the number of hormone-like substances it releases. The roles of two of these substances, leptin and adiponectin, and the way in which they act on muscle were discussed. The effect of leptin on muscle is at least partially accounted for indirectly via the central nervous system, but direct effects have also been reported. In rat soleus muscle AMPKα2 is activated after an injection of leptin; however, after denervation the activation of AMPK is absent. In extensor digitorum longus muscle incubation with the globular head of adiponectin increases AMPK activity, decreases malonyl-CoA and increases glucose uptake. The lack of effect when the full-length adiponectin is used may be because skeletal muscle expresses the receptor for the globular head of adiponectin but not for the full-length adiponectin. IL6, which is released from the muscle during and after exercise, also activates AMPK in rat extensor digitorum longus, an effect also reported in F422A adipocytes. Thus, the overall picture of interaction of adipocyte-derived adipokines and cytokines and muscle-derived cytokines has become increasingly complex, but at present it is clear that: leptin exerts its effect on skeletal muscle both directly and via the central nervous system; adiponectin has a direct effect on muscle and muscle metabolism; IL6, either derived from the adipocytes or from the contracting muscle, can also affect substrate metabolism, both in the muscle and in the adipose tissue.

Closing comments

Thanks to a series of outstanding lectures and fruitful discussions the 12th conference of the IRGBE on 'The biochemical basis for the health effects of exercise' was an excellent meeting. In the meeting state-of-the-art knowledge on how physical exercise exerts its effects on health was presented. The fast-growing number of identified pathways and processes involved in the health effects of physical exercise that were discussed during the meeting will help to develop tailored physical activity regimens for use in the prevention of inactivity-induced deterioration of health.

In his closing words the chair of the IRGBE, Dr Poortmans, expressed his gratitude to all speakers and contributors to the conference and to the organizing committee. Importantly, he also kindly invited everyone to the next conference of the IRGBE, which will be in October 2006 in Seoul, South Korea.

Suggestions for topics to be discussed can be sent to: Marc.francaux@edph.ucl.ac.be.

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