Opening Lecture

The biochemical basis of the health effects of exercise: an integrative view

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Physical inactivity–gene interactions result in changes in gene expression, leading to phenotypic changes in the skeletal muscle cell. A subpopulation of those genes that show changes in expression during physical inactivity are candidates for the environment–gene interactions that cross a threshold of biological significance such that overt clinical disease occurs. AMP kinase, GLUT4 and myosin heavy chain IIx are proposed as candidates for physical inactivity-modulated genes that have an altered function that may trigger a crossing of a threshold to disease. Future experiments will be needed to test the validity of the ideas presented.

Gene–environment interaction: Physical activity levels: Sedentary lifestyle

Åstrand & Rodahl (1986) wrote that approximately 100 % of human biological existence was dominated by outdoor physical activity, consisting of hunting, foraging for food and other life necessities. They indicated that while man adapted to that style of life, the human population has developed into an urbanized highly-technological society. If human genes evolved to support the hunting and foraging lifestyle, then it is only logical to assume that physically-active individuals would have the 'normal' gene expression profile. Based on this evolutionary definition of 'normal', physically-active individuals should be considered as the control group for research purposes. Further, sedentary individuals should then be considered the treatment group because physical inactivity is not the normal condition for the human genome. Physical inactivity superimposed on genes selected for a lifestyle of hunting and foraging for food disrupts the evolved environment–gene interaction, defined as changes in the expression of a gene induced by an external factor. Genes predisposing to chronic health conditions pass the threshold of overt clinical disease more frequently in physically-inactive individuals (Chakravarthy & Booth, 2003), but the molecular

mechanisms are poorly defined. Conversely, physically-active populations retain the normally evolved environment–gene interaction.

Environment–gene interaction

It has been known for millennia that an environmental factor, such as physical inactivity, can alter gene expression. The early Greeks observed that those individuals who did not lift weights had smaller muscle masses than those who did (Atha, 1981). More recently, it has been observed that cage-housed animals have muscles that appear to be whiter than those of their physically-active counterparts in the wild, which have skeletal muscles with a redder colour (see Holloszy, 1967). Holloszy (1967) has shown that rats that have been housed in cages without access to a motor-driven treadmill have whiter muscles than their cage mates who have run on motor-driven treadmills for 2 h daily. These examples of the appearance of phenotypic changes with physical inactivity in man and animals indicate that changes in the expression of genes must have occurred, supporting the contention that the environmental factor of

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physical inactivity does interact with an unidentified pool of genes to alter gene expression. Indeed, recent reports have shown that the transcription of specific genes increases during and immediately following an exercise bout in human skeletal muscle (Pilegaard *et al.* 2000). Further, the cellular levels of 121 mRNA (identified genes and expressed sequence tags) are perturbed by only 12 h of decreased activity (Bey *et al.* 2003), narrowing the exercise–physical inactivity interaction to a time frame that begins within hours.

The application of the concept of environment–gene interaction has been enunciated by Beaudet *et al.* (1995), who wrote that any given individual will inherit a particular combination of disease-susceptibility genes. Disease-susceptibility genes produce some relative risk that may combine with an environmental component to cross a 'threshold' of biological significance, such that the individual is affected with overt clinical disease. Tiret (2002) stated that there is accumulating evidence that most of the susceptibility genes to common diseases do not have a primary causal role in the predisposition to disease. Rather, he contended that susceptibility genes act as responders, or modifiers, to exogenous factors such as stress, environment, disease and drug intake. Physical inactivity is an environmental factor that must be interacting with disease-susceptibility genes (Booth *et al.* 2002; Chakravarthy & Booth, 2003). Based on an overwhelming number of publications reporting that physical exercise lowers the risk of chronic disease, it has been proposed that those genes that predispose individuals to increased chronic health problems and that have an altered detrimental expression as a result of physical inactivity be termed 'genes producing physical-inactivity diseases' (Chakravarthy & Booth, 2003).

Candidates for genes producing inactivity-related diseases

For the purposes of the present review, the *Webster's Dictionary* (Random House, 1999) definition of disease has been adopted, which reads: 'a disordered or incorrectly functioning organ, part, structure, or system of the body resulting from the effect of genetic or developmental errors, infection, poisons, nutritional deficiency or imbalance, toxicity, or unfavourable environmental factors; illness; sickness; ailment.' As physical inactivity is an environmental factor and is associated with an increased risk of many chronic diseases, any change in inactivity-associated gene expression is thus associated with the increased prevalence of chronic diseases. However, it remains to be determined whether changes in the expression of specific genes by physical inactivity are causal, secondary or coincidental associations. The possibility of a few potential candidate genes being the causal links from physical inactivity to disease will be reviewed.

Activity dictates mitochondrial gene expression

Morgan *et al.* (1971) have demonstrated that O_2 consumption and the activities of succinate dehydrogenase, NADH dehydrogenase and cytochrome oxidase increase per g mitochondrial protein from the quadriceps muscle of men who have undergone 1 month of training (2 h of daily pedalling on a cycle ergometer). In addition, the volume density of mitochondria and the concentration of mitochondrial protein per g muscle are increased in the trained men. Conversely, trained subjects who have detrained exhibit 18 and 39 % decreases in mitochondrial density (using citrate synthase and succinate dehydrogenase activities as markers of mitochondrial concentration) after 12 and 56 d of detraining respectively (Coyle *et al.* 1984). Thus, in skeletal muscle the expression of genes producing mitochondrial proteins is markedly affected by the habitual levels of physical activity, with diminished activity lowering gene expression for mitochondrial proteins within days.

Low mitochondrial gene expression increases the risk of many chronic diseases

Petersen *et al.* (2003) have proposed the hypothesis that an age-associated decline in mitochondrial oxidative and phosphorylation activity contributes to insulin resistance in the elderly. This postulate is based on their data showing an inverse correlation between *in vivo* rates of mitochondrial oxidative activity (via ^{13}C NMR and ^{31}P NMR) and insulin resistance in the elderly. It is presumed that the elderly were more physically inactive than the young subjects in the Petersen *et al.* (2003) study because physical inactivity is the major determinant of insulin resistance in the elderly (see p. 201). Kirwan *et al.* (1993) have shown that regular exercise effectively reduces hyperinsulinaemia and improves insulin action in 65-year-old subjects to levels typical of young individuals; the 65-year-old subjects had trained at 80 % of their maximal heart rate (45 min/d for 4 d/week) during the 9-month programme. Following the training programme the subjects underwent a hyperglycaemic clamp procedure, during which the rise in mean plasma insulin was attenuated by 23 % (Kirwan *et al.* 1993), implying enhanced insulin sensitivity. Unfortunately, the improvement in insulin sensitivity gained with physical activity can be reversed during a short period of physical inactivity. Master athletes (defined as individuals in their 7th decade of life who either run 88 km/week or cycle 209 km/week), 61 years old and running an average of 12·9 km/d, appear to be protected against the aging-related changes in glucose tolerance, as during an oral glucose tolerance test their plasma glucose and insulin levels have been shown to be not significantly different from those of young lean sedentary men (Rogers *et al.* 1990). After 10 d of inactivity, however, glucose tolerance was found to be impaired in four of the fourteen master athletes, the impairment being sufficiently marked in two of the subjects to be classified as impaired glucose tolerance. Additional human studies have reported that decreases in oral glucose tolerance and increases in fasting plasma glucose and insulin concentrations occur within 3 d of commencing continuous bed rest (Lipman *et al.* 1972; Smorawinski *et al.* 2000). It can be concluded from these reports that a large portion of increased insulin resistance with aging is a result of physical inactivity, not an aging-programmed change in insulin signalling. Thus, the following questions can be posed: how does the myocyte

sense and transduce a signal, such as reduced mitochondrial ATP production during 3–10 d of physical inactivity, to the human genome; how does this signal then alter gene expression in such a manner that it produces a sufficient change in environment–gene interaction to initiate a crossing of a 'threshold' of biological significance that would lead to overt clinical disease if continued for an extended time period.

AMP-activated protein kinase as a candidate gene for inactivity-related diseases

Increases in contractile activity transiently activate AMP-activated protein kinase (AMPK) in skeletal muscle (Hutber *et al*. 1997). Conversely, the exercise-induced oscillations in AMPK activity are absent during physical inactivity. One suggested function of AMPK in skeletal muscle is that it is a 'fuel' sensor (Ruderman *et al*. 1999) that transduces signals that alter the activity of multiple transcription factors (Leff, 2003). Increases in AMPK activity inhibit lipid, carbohydrate and protein synthesis while generating ATP through the stimulation of fatty acid oxidation and glucose uptake via the activation of GLUT4; AMPK also up regulates the expression of GLUT4, hexokinase and mitochondrial enzymes (Hardie *et al*. 2003). Pharmacological activation of AMPK by 5-amino-imidazole-4-carboxamide-1-β-D-riboside increases GLUT4 transcription by a mechanism that requires response elements within 895 bp of the human GLUT4 proximal promoter, and this process may be cooperatively mediated by myocyte-enhancing factor 2 (Zheng *et al*. 2001). Thus, AMPK is a candidate for a gene that undergoes environmentally-induced down regulation as a result of physical inactivity, thus altering signals that interact with disease-susceptibility genes and leading to an increased risk of metabolic chronic diseases.

GLUT4 gene expression is decreased by physical inactivity, increasing the risk of disease

Cell-surface GLUT4 levels and glucose transport in skeletal muscle are proportional regardless of whether transport is stimulated with insulin or muscular contractions, or both (Holloszy, 2003). NMR spectroscopy techniques have revealed that defects in insulin-stimulated muscle glycogen synthesis are responsible for most of the insulin resistance observed in skeletal muscle of patients with type 2 diabetes, and this abnormality can be attributed to defects in insulin-stimulated muscle GLUT4–glucose transport activity (Petersen & Shulman, 2002). Thus, low expression of GLUT4 protein in skeletal muscle can be considered to contribute to skeletal muscle insulin resistance. Physical inactivity decreases the biological activity of GLUT4 in skeletal muscle, both by preventing pulses of GLUT4 being translocated to the sarcolemma (see Holloszy, 2003) and by decreasing total GLUT4 protein quantity. For example, the soleus and red vastus lateralis muscles of sedentary rats have 29 and 59 % less GLUT4 protein content respectively than those of rats with 6 weeks of treadmill training (Neufer *et al*. 1992). Additionally, the GLUT4 values return to approximately control values after 7 d of detraining (Neufer *et al*. 1992). A second report has indicated that increases in GLUT4 protein and in insulin-stimulated glucose transport are completely reversed to sedentary levels within 40 h of the last exercise bout, after both 5 d and 5 weeks of training (Host *et al*. 1998). A recent study (MacLean *et al*. 2002) has shown that regulation of GLUT4 expression is associated with an environmental factor, i.e. exercise. The exercise-induced increase in the transcription of the human *GLUT4* gene appears to be mediated, at least in part, by one or more response elements within −895 bp of the promoter, with at least one essential element lying between −895 and −730 bp (MacLean *et al*. 2002). Thus, the environmental factor, physical inactivity, interacts with the *GLUT4* gene to reduce its transcription. Subsequently, decreased insulin sensitivity for glucose uptake into skeletal muscle occurs with the longer-term consequence, in some cases, that a 'threshold' of biological significance will be crossed, such that the individual is affected with the diseases elicited by insulin resistance.

Physical inactivity increases myosin heavy chain IIx expression

Myosin heavy chain (MHC) is a key regulatory protein in the contractile apparatus within skeletal muscle. Adult human skeletal muscle primarily expresses three MHC proteins and each of these proteins can be described by its contractile speed and metabolic properties: type I (slow oxidative); type IIa (fast oxidative); type IIx (fast glycolytic). Fast-contracting fibres have both high and low numbers of mitochondria (i.e. high and low oxidative respectively) and each of the MHC is expressed in varying proportions in muscle. For example, the human soleus muscle, a postural muscle that is highly recruited during locomotion and while standing, is composed of approximately 69 % type I MHC, 18 % type IIa and 13 % type IIx (Gregory *et al*. 2001). On the other hand, the vastus lateralis muscle, which is not recruited to the same extent as the soleus muscle during locomotion, comprises approximately 47 % type I MHC, 21 % type IIa and 32 % type IIx (Gregory *et al*. 2001). A review by Mujika & Padilla (2001) has contributed to the understanding of the differences in MHC among muscles. Their conclusion was that the effects of training cessation on muscle fibre distribution appear to be dependent on the duration of the inactivity period, i.e. in most studies fibre type distribution does not change with ≤32 weeks of sedentary levels of ambulatory activity. For example, Coyle *et al*. (1984) observed no change in the percentage of type I fibres after 84 d of detraining in subjects who had previously averaged 10 years of endurance training. However, the percentage of type IIa fibres was found to decline from 43 to 26 while that of type IIx fibres increased from 3 to 19 in these subjects. A longer detraining period may be needed to decrease the percentage of type I fibres in previously-trained individuals. It has been shown that after 42 months of detraining the percentages of type I fibres in the proximal arm and leg muscles of athletes decrease by 16 and 14 % respectively (Larsson & Ansved, 1985). However, subjects who increase their activity level through

systematic daily physical training over an approximately 4-year period show no significant changes in the percentages of fibre types in either arm or leg muscles. It was suggested by Larsson & Ansved (1985) that the smaller net change in physical activity level caused by training as compared with detraining accounts for the difference in response. The conclusion was that the results showed that the percentages of fibre types in intact human skeletal muscle are not exclusively determined by heredity, but may also be influenced by environmental factors, such as physical activity level.

A reduction in skeletal muscle loading below normal sedentary levels of ambulatory activity has been reported to produce changes in the percentage of fibre types in some studies. For example, the most severe circumstance of skeletal muscle disuse occurs as the result of spinal cord injury. Biopsies taken from patients 6–24 weeks after spinal cord injury have revealed a decrease in type IIa MHC from 41 to 30%, an increase in type IIx MHC from 28 to 36% and no change in type I MHC in the vastus lateralis muscle (Castro et al. 1999). In contrast, while 37 d of bed rest does lead to atrophy it does not significantly alter MHC protein expression (Andersen et al. 1999). However, decreases occur in the mRNA for type I (from 53 to 40%) and IIa (from approximately 38 to 19%) MHC while there is an increase (from 4·5 to 19%) in IIx MHC mRNA in 28-year-old subjects (Andersen et al. 1999). Limb immobilization also induces a state of muscle disuse that leads to shifts in fibre type. Eight athletes who had undergone surgery for knee injuries showed a drop in the percentage of type I fibres from 54 to 43 after surgery and immobilization (Haggmark et al. 1986). In another study (Hortobagyi et al. 2000) it was found that 3 weeks of leg immobilization and subsequent exercise training affects the three MHC fibre types as follows: type I fibres are reduced (by 9%) and increased (by 13%) respectively; the percentage of type IIa fibres does not change and is increased by 7% respectively; type IIx fibres are increased (by 7%) and then decreased (by 11%) respectively. Furthermore, immobilization induces a decrease (28%) in type I MHC mRNA and an increase (200%) in type IIx mRNA (Hortobagyi et al. 2000). As there have been numerous reports of a direct association between the percentage of type IIx fibres and obesity or type 2 diabetes (see Tanner et al. 2002), consideration will be given to the possibility that this association is not causal for metabolic disorders, but rather is a consequence of physical inactivity lowering the levels of mitochondria and the oxidative protein concentrations in skeletal muscle.

Myosin heavy chain IIx as a possible gene marker of physical frailty induced by aging

While there is sufficient data to show a switch from type I to IIx mRNA in the soleus muscle of young rats when the environmental factor, load-bearing activity by the muscle, is removed (Jänkälä et al. 1997; Pattison et al. 2003), in old rats this environment–gene interaction is attenuated (Pattison et al. 2003). A relevant observation allows the speculation that defective expression of MHC IIx might contribute to the previously documented preferential atrophy of type 2 muscle fibres in the elderly (Lexell et al. 1988). Muscle atrophy in the aged, if severe enough, leads to reduced strength and physical frailty. Thus, aged skeletal muscle shows the loss of a normal environment–gene interaction present in young animals by revealing an aging-associated defect in MHC IIx gene expression. Overall, the expression of MHC IIx is paradoxical; an increased expression is predictive of metabolic dysfunction in young animals and a decreased expression is predictive of physical frailty in old animals.

Conclusion

The biological existence of humans has been dominated by physical activity, which is reflected in the human genome. Physical activity is the normal physiological condition and it provides the proper environment–gene interaction. Conversely, physical inactivity is an improper environment–gene interaction that leads to negative phenotypic changes within the muscle cell. The genes that show changed expression during periods of physical inactivity are candidates for the environment–gene interactions that cross a threshold of biological significance such that overt clinical disease occurs. AMPK, GLUT4 and MHC IIx are candidates for genes producing inactivity-related diseases.

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