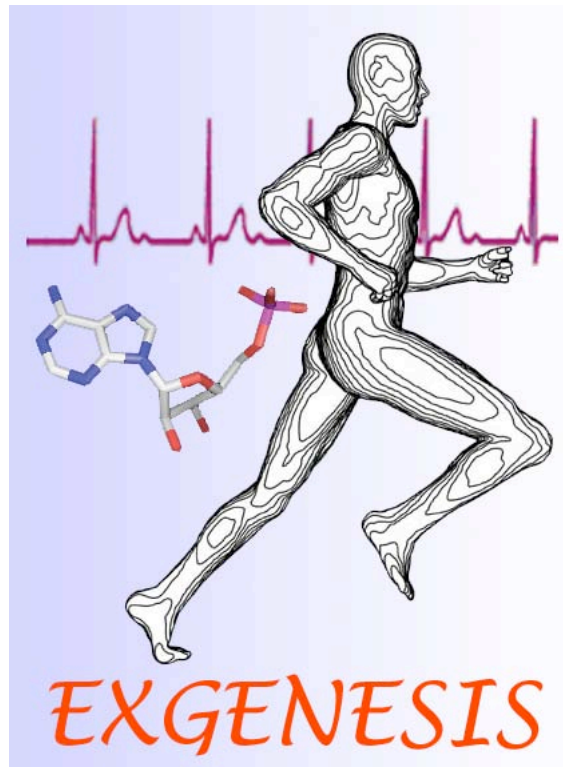


EXGENESIS – health benefits of exercise: identification of genes and signalling pathways involved in effects of exercise on insulin resistance, obesity and the metabolic syndrome

# EXGENESIS



Health benefits of exercise: identification of genes and signalling pathways involved in effects of exercise on insulin resistance, obesity and the metabolic syndrome

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## Introduction

EXGENESIS is a European Framework Programme VI Integrated Project involving a consortium of 26 partners.

The aim of EXGENESIS was to identify the genes and signalling pathways involved in the beneficial effects of exercise on insulin resistance, obesity and the metabolic syndrome. It was already established that regular physical exercise protects the human population against the development of these related metabolic disorders. Our aim was to identify the genes and signalling pathways induced by exercise that accounted for these beneficial effects. Greater insight into these processes and pathways at the molecular level should allow:

- better identification of individuals at risk of developing these conditions, to allow targeting of interventions;
- better design of exercise interventions designed to protect those individuals who are at risk;
- more rational design of new drugs that may mimic the effects of exercise;
- more rational design of policies by decision makers to increase the level of physical activity in the whole population.

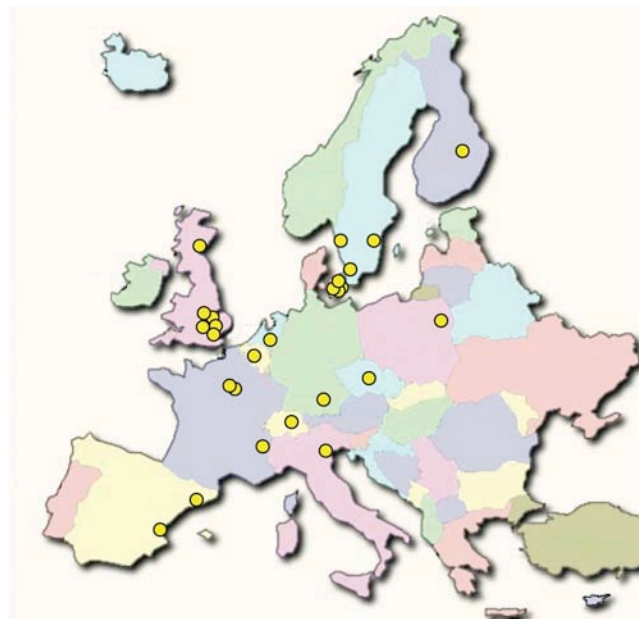
## NATURE OF THE PROBLEM ADDRESSED

Obese humans (BMI >30 kg.m<sup>-2</sup>) have a 20-fold increased risk of developing type 2 diabetes compared with lean individuals. The prevalence of obesity is increasing rapidly, with over 20% of the population in most EU states now having a BMI>30. Not surprisingly, type 2 diabetes is also increasing: its prevalence in the EU was estimated at 3% of the population in 1995, doubling to 6% by 2010. Diabetes causes debilitating complications, and the total costs to the healthcare systems of 8 major EU member states were estimated in 2002 to be €29 billion per annum, or €2800 per patient. While genetic factors increase the risk of developing type 2 diabetes, these cannot explain the rapid increases in its prevalence, which is most likely due to a combination of an ageing population, together with increasing levels of obesity caused by adoption of a sedentary lifestyle with low levels of physical activity, and constant availability of high-calorie, refined foods. The EXGENESIS project is unraveling the genes and signalling pathways underlying the increased prevalence of obesity, type 2 diabetes and the metabolic syndrome in individuals who are physically inactive. It is providing insights into how regular exercise protects against these conditions. These insights should lead to new treatments, as well as encouraging new policies to promote healthier lifestyles.

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## PARTNERS

EXGENESIS is a multidisciplinary consortium with 26 partners (22 academic institutions, 2 pharmaceuticals/food companies, and (in years 1-3) 2 small-to-medium enterprises in biotechnology and scientific instruments) in the 13 member states shown below.



Members of the consortium at one of their annual meetings:



## RESEARCH PROGRAMME

The studies of the consortium are divided between 5 “work-packages”:

- 1) Molecular and Cell Biology (leader: Mark Rider)**
- 2) Animal Models (leader: Pascal Ferré)**
- 3) Integrative Physiology (leader: Erik Richter)**
- 4) Human Pathophysiology (leader: Rainer Rauramaa)**
- 5) Genetics and Epidemiology (leader: Allan Vaag)**

## OBJECTIVES

The specific objectives of the original proposal were to:

- identify and delineate the intracellular signalling pathways that mediate the different effects of exercise in skeletal muscle
- identify the mechanisms leading to release by muscle during exercise of extracellular factors, such as cytokines, that affect insulin resistance and transmit effects of exercise to other sites such as adipose tissue, the liver and endothelial cells, and study their action at those sites
- identify the signalling mechanisms stimulating release of fatty acids and cytokines (adipokines) from adipose tissue during exercise and fasting
- establish whether mutations affecting any of the signalling pathways involved in effects of exercise could predispose to Type 2 diabetes, obesity or the metabolic syndrome in humans
- delineate patterns of expression of genes and proteins in humans at risk of developing Type 2 diabetes and in cohorts of monozygotic and dizygotic twins of known health status
- validate potential new drug targets identified during the research, using small molecule inhibitors or activators, expression of constitutively active or dominant negative mutants, siRNA technology, or gene targeting in mice

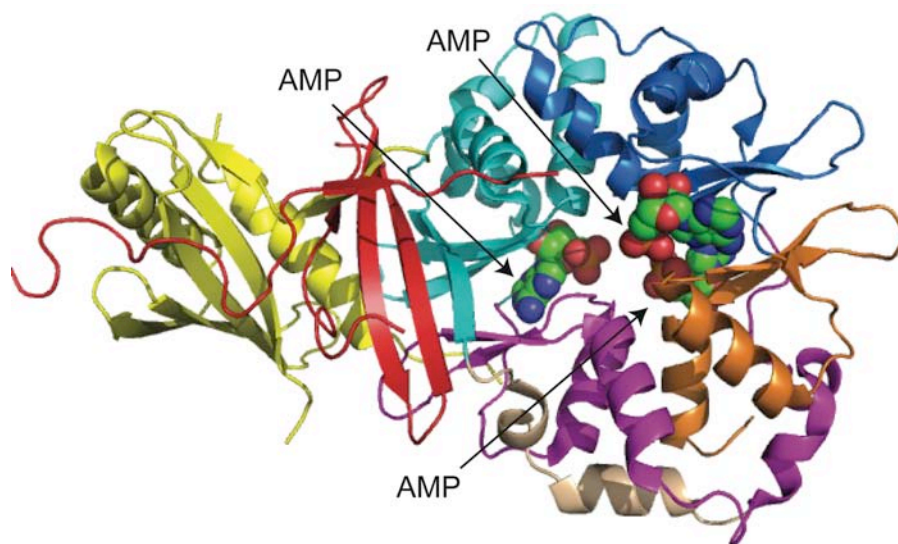


## RESULTS: MOLECULAR AND CELL BIOLOGY

Work-package 1 focuses on the structure, function and downstream targets of AMP-activated protein kinase (AMPK). AMPK acts as a sensor of cellular energy, is switched on by energy depletion in muscle during exercise, and is responsible for many of the metabolic changes that occur in muscle in response to exercise. It has recently been found to be the primary target for the drug metformin, the current front-line drug for treatment of type 2 diabetes (prescribed to 120 million people worldwide), whose molecular target was previously unknown. Metformin activates AMPK indirectly, and more direct activators may be more effective in treatment of diabetes than metformin, while avoiding some of its side effects.

We defined two signalling pathways upstream of AMPK, one involving  $\text{Ca}^{2+}$ -dependent kinases (CaMKKs), the other involving LKB1, a tumour suppressor. This latter discovery introduced the first link between AMPK and cancer, and may help to explain why obese people are more susceptible to the disease. We also showed that AMPK can sense cellular glycogen content, ensuring that muscle glycogen is rapidly replenished after exercise. This is important because glycogen synthesis is an important route by which blood glucose levels, which are elevated in diabetes, can be rapidly reduced. Among novel targets identified for AMPK were proteins involved in glycogen metabolism and smooth muscle contraction.

A particularly important event was the solution of the crystal structure of the core of the mammalian AMPK complex. This structure, which was published in the prestigious journal *Nature*, revealed how the three subunits fit together, and how the natural activator, AMP, binds to the regulatory  $\gamma$  subunit (see below). This provides crucial information that should assist in the development of novel drugs that are direct activators of AMPK.



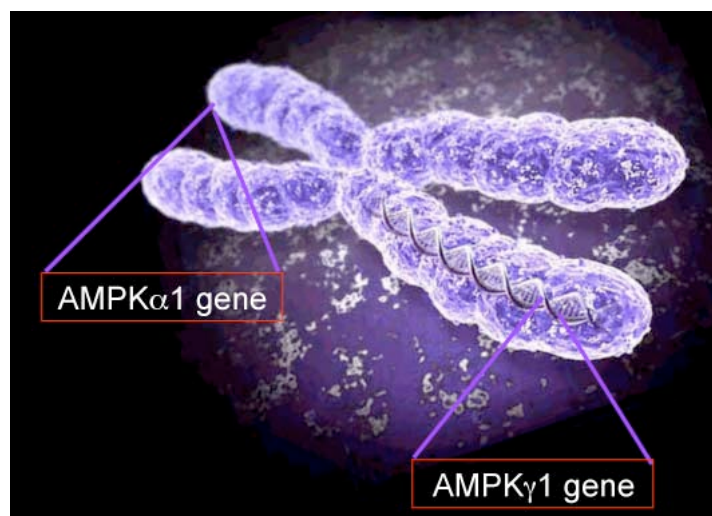
Crystal structure of core of AMPK complex showing location of three AMP molecules

## RESULTS: ANIMAL MODELS

Work-package 2 has focused on the use of animal models, particularly genetically engineered mice, to study two key signalling pathways involved in responses to exercise, i.e. those involving AMPK (see previous page) and interleukin-6 (IL6). A particular focus has been the role of the AMPK system in tissues other than skeletal muscle. Knockout of the  $\alpha 2$  isoform of AMPK in liver caused high blood glucose whereas, conversely, over-expression in mouse liver of a form of  $\alpha 2$  that was activated due to a truncation mutation caused low blood glucose due to reduced liver glucose production (gluconeogenesis). These results, obtained in living mice, are consistent with previous findings in cultured cells suggesting that AMPK activation inhibits gluconeogenesis, a major contributor to the high blood glucose in type 2 diabetes. Interestingly, acute inhibition of AMPK in mouse liver due to expression of a dominant negative mutant led to liver failure caused by endoplasmic reticulum stress. It appears that the acute down-regulation of AMPK may allow an unrestrained synthesis of proteins, cholesterol and fatty acids, causing an overload of the biosynthetic capability of the endoplasmic reticulum.

Using transgenic mice, it was confirmed that activation of AMPK in fat cells had an anti-lipolytic effect, thus reducing the release of fatty acids into the bloodstream. Using adipose cells from human biopsies, evidence was obtained that the same process also operates in humans.

Studies of IL6 deficient mice, which are obese and insulin-resistant, suggest that this may in part be because they have reduced activation of AMPK in skeletal muscle during exercise, and therefore do not “burn off” fatty acids so effectively.



Location of AMPK genes on mouse chromosome 15

## RESULTS: INTEGRATIVE PHYSIOLOGY

Work-package 3 has focused on physiological studies in living animals and in humans. A major finding was the discovery of a previously unrecognized role for the enzyme diacylglycerol kinase- $\delta$  (DGK $\delta$ ) in insulin resistance. Humans with type 2 diabetes exhibited reduced DGK $\delta$  expression and activity in skeletal muscle; similar results were found in diabetic rats. Genetically modified mice in which DGK $\delta$  activity was reduced by 50% displayed increased diacylglycerol content and increased insulin resistance, as well as reduced insulin signaling and glucose transport in muscle. Interestingly, these mice also became obese as they aged.

Women are known to oxidize or “burn” fats at a higher rate during exercise than men, even when matched for the same fitness level. AMPK (see WP1) activates fat oxidation, and is believed to be responsible for increased use of fat during exercise. We therefore tested whether the higher fat oxidation in women was due to increased AMPK activation. Surprisingly, the opposite was the case, with women exhibiting lower degrees of AMPK activation during exercise. Muscles of females contain a higher proportion of oxidative fibres, and are better supplied by blood capillaries than those of men, so their muscles may experience less metabolic stress during exercise, accounting for the reduced activation of AMPK. The higher fat oxidation in females was attributable not to increased AMPK activation but to increased expression of the enzyme hormone sensitive lipase, which is involved in degrading triglycerides, the major storage form of fat.

Very recently, adipose triglyceride lipase (ATGL) was identified as a key enzyme involved in degrading triglycerides in rodent tissues. We were able to detect for the first time the expression and activity of this enzyme in human skeletal muscle, and showed that endurance training increases its level of expression.

Activation of AMPK has been shown to increase production of mitochondria, the “power stations” of the cell. This also occurs during endurance exercise training, and is thought to be important in the protective effects of exercise. The role of AMPK in production of mitochondria was confirmed using two lines of genetically modified mice. Mice in which the major form of AMPK in muscle was knocked out had reduced levels of mitochondria, whereas mice with elevated AMPK activity had increased levels. This may be an important part of the mechanism by which AMPK activation, and physical activity, protect against these “life-style” diseases.

Exercise was known to increase insulin sensitivity of muscle glucose uptake, but the underlying mechanisms were not known. We have provided evidence that the signalling protein TBC1D4 (involved in regulating glucose uptake, also known as AS160) is involved in this process. Four hours after exercise in humans, insulin sensitivity was improved and modification of TBC1D4 by phosphorylation at defined sites was altered. Our results support the idea that phosphorylation of TBC1D4 may be a key event that mediates increased insulin sensitivity following exercise.

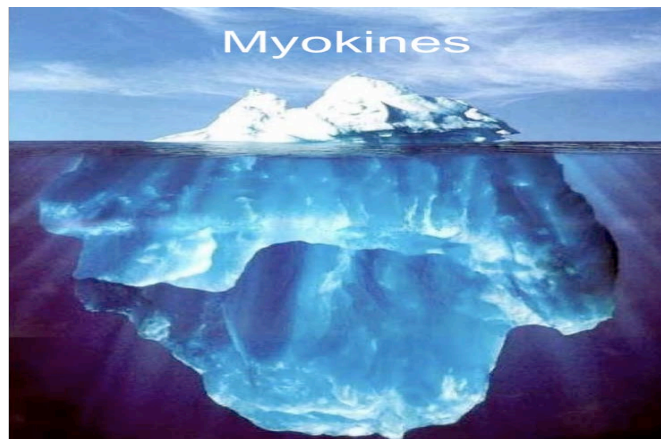
## RESULTS: HUMAN PATHOPHYSIOLOGY

Work-package 4 focuses on the mechanisms whereby regular exercise improves health outcomes, and by which inactivity causes increased risk of chronic diseases.

In one study, a group of young healthy male volunteers decreased their daily activity from the recommended range (10,000 steps) to around 1,500 steps – simply by using motorized transport rather than walking or cycling to work, by avoiding leisure activities involving physical activity, and by using elevators rather than the stairs. In only 2 weeks, they experienced an average 7% increase in intra-abdominal fat mass, with a concomitant decrease in fat-free mass. Moreover, they developed metabolic changes including increased insulin resistance, and a decreased ability to oxidize fats after meals (both associated with type 2 diabetes). This study emphasizes that even short periods of physical inactivity, at a level typical of a major proportion of the population, can have quite deleterious effects.

The Dose-Response to Exercise Training (DR's EXTRA) trial involves a random sample of around 700 men and 700 women in Eastern Finland aged 57-79 years, who are being followed for four years. Within the time frame of EXGENESIS, we were able to demonstrate that BDNF, a growth factor for nerve cells, is a biomarker of impaired memory and general cognitive function in women within this population. Moreover, we found that low levels of BDNF are associated with impaired glucose metabolism. This suggests that reduced BDNF may be a factor involved not only in dementia and depression, but also in type 2 diabetes. This potentially explains why these conditions tend to cluster together. In other studies, we demonstrated: (i) that BDNF is produced by exercising muscle; (ii) that it activates the AMPK system; and (iii) that it stimulates fat oxidation.

It is now clear that skeletal muscle is an endocrine organ producing signaling molecules called myokines. During exercise, these are released into the blood, where they exert effects on muscle and other organs. We have identified novel myokines that may mediate metabolic effects in muscle, on abdominal fat, and on growth of blood vessels. However, these may just be the “tip of the iceberg”:





## RESULTS: GENETICS AND EPIDEMIOLOGY

Work package 5 has studied various cohorts of humans to tease out the role of genetic, epigenetic and environmental factors in determining susceptibility to type 2 diabetes. No less than 16 new gene variants that cause an increased risk of type 2 diabetes were identified. Interestingly, almost all of these genes appear to be involved in control of insulin secretion by the pancreas, with the gene variant having the largest effect on diabetes risk lying within the gene encoding the transcription factor TCF7L2, which is involved in the development of the pancreas. Few of the variants causing increased susceptibility have any potential impact on insulin action in target tissues, so they are unlikely to give rise to insulin resistance. This suggests that insulin resistance, a well-known precursor to type 2 diabetes, is due instead mainly to environmental factors, such as physical inactivity.

The effects of physical inactivity were investigated by analyzing the effects of bed rest in healthy young men. Some of the volunteers, who were retrained after the study, were from groups who are known to be at increased risk of type 2 diabetes due to genetic factors (first-degree relatives of patients with type 2 diabetes) or to environmental factors (subjects born with low birth weight, who may have experienced an adverse intrauterine environment). The bed rest studies showed that all groups and individuals developed severe muscle and liver insulin resistance in response to bed rest, illustrating the significant impact of even a short period of reduced physical activity. Most individuals were able to compensate for this insulin resistance by increasing insulin secretion. Significantly, however, those who were carriers of the TCF7L2 gene variant (which predisposes to type 2 diabetes) were unable to compensate by increasing insulin secretion, rendering these young, non-obese individuals close to a state of overt diabetes. Analyses conducted before and after bed rest identified changes in gene expression that may be responsible for the development of insulin resistance (especially genes involved in mitochondrial function), as well as a number of other genes not previously known to be linked to insulin resistance. Interestingly, many of the gene expression changes were different between the first-degree relatives and low-birth weight individuals compared with the control group, giving clues as to how these groups may respond differently to a lack of physical activity.

Exciting on-going studies within the EXGENESIS consortium also suggest that epigenetic changes, i.e. changes in DNA structure that are acquired after conception due to environmental factors, may also influence susceptibility to type 2 diabetes.

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## CONTACT INFORMATION

Further information about EXGENESIS is available on the EXGENESIS web-site:

[www.dundee.ac.uk/lifesciences/exgenesis/](http://www.dundee.ac.uk/lifesciences/exgenesis/)

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