Primary cultures of Human skeletal muscle cells as a model for system for studying obesity-induced diabetes

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INTRODUCTION

The skeletal muscle is of major site for body glucose disposal, especially under conditions of insulin stimulation (1). Insulin deficiency (i.e. starvation, insulin resistance) decreased glucose utilization, which is compensated by increasing supply and utilization of available lipid-derived substrates non-esterified fatty acids (NEFA), triglyceride (TAG) and ketone bodies] (2). The pyruvate dehydrogenase complex is a major target for substrate competition, particularly in skeletal muscle where PDC has a purely energetic role (3). Increased circulating FFAs have been proposed as the mediator of PDK4 expression and it has been suggested that this might be a PPAR-dependent process (4;5) The PPARs are nuclear hormone receptors which regulate transcription of genes involved in fat oxidation and storage, and certain long-chain fatty acids, in particular polyunsaturated fatty acids, are potent activators for both PPAR and PPAR (6).

This is a very important aspect of human metabolism, since insulin resistance is a serious risk factor for subsequent development of cardiovascular disease and diabetes, and the specific intracellular pathway(s)

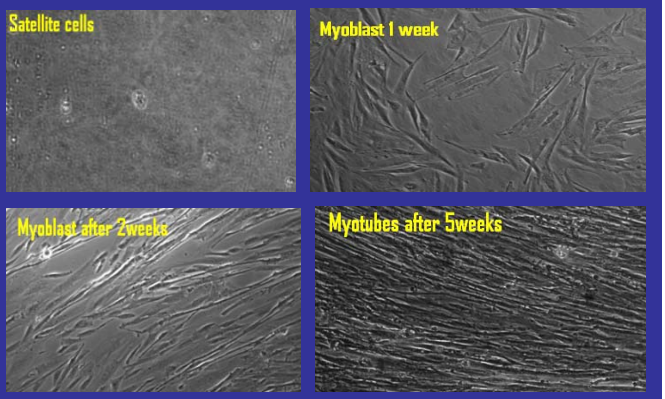
In this project we developed human primary myocyte culture to validate effects of insulin and fatty acids on expression of key metabolic genes and comparing the findings with data obtained from human *in vivo* studies.

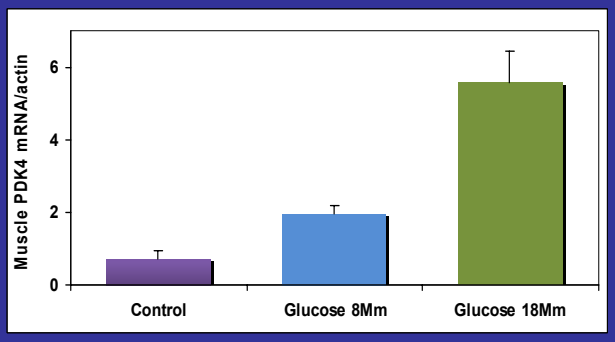
Expression profiling of adipose tissue in high and low fat-oxidising obese human subjects, has been carried out using microarray technology. This powerful technique allows us to visualise how the regulation of metabolic genes influences the observed phenotypes, and provides us with targets for therapeutic intervention. It also gives us the opportunity to use *in silico* modelling of biological systems to test hypotheses.

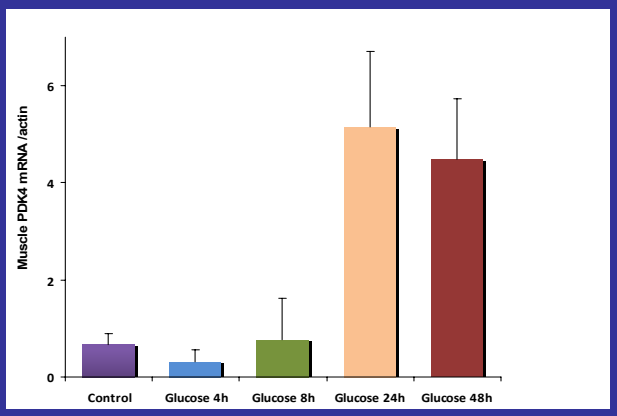
Material and methods:

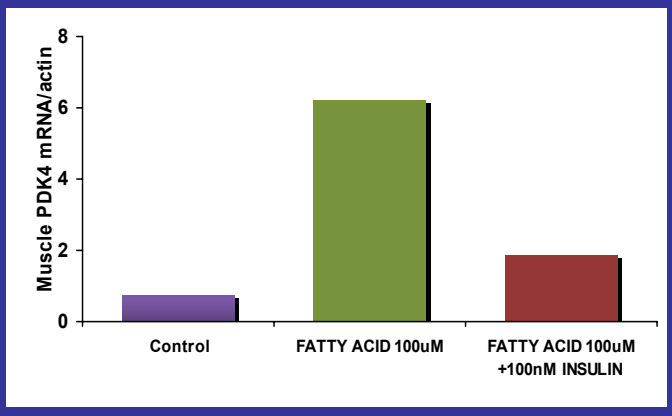
The development of primary cell lines from small skeletal muscle samples obtained from (<40 years old) healthy male volunteers using the biopsy needle technique healthy individuals. Biopsies have been taken in David Greenfield Human Physiology Unit facility. The unit is one of the leading centres of its kind in the country and utilises the muscle biopsy technique, which derives from Professor Eric Hultman's research in the 1960's. Satellite cells were isolated from the muscle tissue obtained from each volunteer by trypsin digestion and were grown to confluent myoblasts and differentiated into myotubes in growth medium.

Differentiated cells (myotubes) were treated with mixure of FFA acid in the presence and absence of insulin and both PPAR and PPAR agonist and antagonist. The effects of FFAs and ligands for nuclear receptors proposed to be involved in the regulation of metabolic genes were assessed by real time PCR and reporter gene analysis.









RESPONSE OF PDK4 GENE EXPRESSION TO GLUCOSE, FATTY ACIDS AND INSULIN IN DIFFERENTIATED PRIMARY HUMAN MYOTUBES

Primary Human myotubes in culture respond to Metabolic and hormonal stimuli in the same way As human skeletal muscle in vivo

Reference

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