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QUANTITATIVE PROTEOMIC STUDY OF TYPE 2 DIABETIC AND HEALTHY MYOTUBES

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One of the hallmarks of Type 2 Diabetes (T2D) is insulin resistance (IR) in peripheral target tissues. Skeletal muscle is a key tissue site of IR, with evidence of decreased insulin-stimulated glucose uptake and storage as glycogen. Although a number of abnormalities have been identified in skeletal muscle from T2D subjects, the exact molecular mechanism for IR has not been established. Using mass spectrometry we have characterized the basal proteome of myoubes established from T2D subjects and compared them to myoubes from healthy, lean or obese subjects. Myotubes were labeled using iTRAQ as this strategy can be directly implemented on subsequent in vivo studies. Combining Strong Cation Exchange and sensitive mass spectrometry using a combination of different fragmentation strategies on a LC-ESI-LTQ-orbitrap gave a thorough and informative characterization of the myotube proteome. Human myotubes are a valuable model system as the diabetic phenotype is conserved in myotubes established from diabetic subjects. However, the basal proteome of human myotubes had still not been investigated. Therefore, we ran four comprehensive experiments in order to compare the basal proteome of myotubes from obese, lean and obese T2D subjects. We have identified 3593 unique proteins. A total of 32 proteins were found to be upregulated in T2D myotubes, whereas 25 proteins were found to be downregulated. Bioinformatics analysis showed that many enzymes playing key roles in energy-related pathways were upregulated in T2D myotubes whereas structural proteins were downregulated when compared to the two healthy controls. We are now in the process of evaluating these comprehensive datasets.

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PTP1B PLAYS AN ESSENTIAL ROLE IN THE DEVELOPMENT OF AGE-RELATED INSULIN RESISTANCE

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Protein tyrosine phosphatase 1B (PTP1B) is a negative regulator of insulin signaling and a therapeutic target for type 2 diabetes mellitus (T2DM). It is well known that aging is one of the risk factors to increase the susceptibility to metabolic diseases. The purpose of this study was to evaluate the differences in insulin sensitivity between wild-type and PTP1B-/- mice during aging. Wild-type mice showed augmented fat depots, increased adipocyte size and body weight at 15 months of age as compared to young mice at 12 weeks. However, adiposity did not increase in PTP1B-/- mice with aging. Levels of pro-inflammatory cytokines together with the appearance of crown-like structures positive for F4/80 staining were elevated in white adipose tissue (WAT) from wild-type mice with aging. By contrast, levels of pro-inflammatory cytokines and WAT morphology of aged PTP1B-/- mice did not differ from the young controls. ITT tests revealed insulin resistance in 15 month-old wild-type mice compared to mice lacking PTP1B at the same age. Moreover, PTP1B deficiency resulted in a more rapid clearance of glucose as compare to wild-type controls. At the molecular level, both PTP1B mRNA and protein content were up-regulated in liver and muscle from aged wild-type mice compared to the young stage. On the other hand, the expression of p85α was down-regulated in liver and muscle from PTP1B-/- with aging. In both tissues, an age-dependent activation of stress kinases and, conversely, attenuation of insulin receptor tyrosine phosphorylation and its downstream signaling was observed in wild-type mice. However, PTP1B deficiency protected against these effects. Overall, our data provide the possibility to explore the inhibition of PTP1B as a therapeutically approach against age-dependent T2DM.

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PROTEIN-TYROSINE PHOSPHATASE 1 B CONTRIBUTION TO GLYCOGEN METABOLISM

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Protein-tyrosine phosphatase 1 B (PTP1B) is known to be involved in many intracellular signalling processes, mainly acting as a negative regulator on the insulin signalling pathway. As many studies have described, the expression levels of this protein are higher in some insulin-resistant conditions, as obesity and type 2 diabetes. This study focuses on the role of PTP1B on glycogen metabolism in a line of immortalized myocytes deficient in this protein, in basal and under a TNF-α-induced insulin resistant condition. Glycogen content and glycogen synthesis, as well as glycogen synthase (GS) and glycogen phosphorylase (GP) enzymatic activities, were measured. The possible signalling pathways involved were assayed by western blot procedures. Our in vitro studies revealed that insulin positively stimulates GS activity and glycogen content, and this action is enhanced in PTP1B deficient myocytes. We verified the potent glycogenolytic effect of TNF-α, and the inhibitory effect of the cytokine on glycogen synthesis, content, and activation of GS. Moreover, we described a novel role for insulin on GP regulation, so that GP degradation is achieved for low insulin doses. In our model, absence of PTP1B restores insulin action in myocytes pretreated to TNF-α, thus confirming its protective role against an insulin resistant situation, probably owed to an enhanced insulin action on glucose and glycogen metabolism. This could be of special interest in the development of new treatments against type 2 diabetes. We thank the support of CIBERDEM CB07-08-0007 from ISCIII, INSINET S-SAL-159-2006 (Madrid Community) networks, and the project BFU-2008-04043 (Ministry Science and Innovation).