AuPS/ASB Meeting - Newcastle 2007

Symposium: Insulin signaling in health and disease

Monday 3 December 2007 – Hunter Room

Chair: Mark Febbraio

Molecular mechanisms underpinning enhanced insulin action by prior exercise

J.F.P. Wojtaszewski, Copenhagen Muscle Research Centre, Institute of Exercise and Sport Sciences, University of Copenhagen, 13 Universitetsparken, DK 2100, Copenhagen, Denmark. (Introduced by M. Febbraio)

Type 2 diabetes mellitus (T2DM) has become one of the most prevailing diseases world-wide. A hall mark of T2DM is skeletal muscle insulin resistance to increase glucose uptake. Skeletal muscle is responsible for clearing most of the available glucose and increasing insulin sensitivity within this tissue is thought to be a beneficial treatment/prevention strategy. Interestingly, like physical activity, some of the anti-diabetic drugs used today are now known to activate 5′AMP activated protein kinase (AMPK). It is the working hypothesis that AMPK may promote insulin sensitivity not only in response to exercise but also to anti-diabetic drugs targeting skeletal muscle.

Following an acute bout of exercise, glucose transport decreases gradually whereas the sensitivity of muscle to insulin increases. This effect of exercise can persist for several days, even after full glycogen repletion. The mechanism(s) for this increased insulin sensitivity is at present not known, but apparently it is not linked to changes in activity of the proteins involved in the proximal part of the classical phosphatidylinositol 3 kinase insulin signalling cascade.

It is possible that the increase in post-exercise induced insulin sensitivity in skeletal muscle may come about through modulation of further down-stream elements in the insulin signalling pathway. Interestingly, one such element may be the recently discovered Akt substrate of 160 kDa (AS160). AS160 is involved in the insulin regulated glucose transport in skeletal muscle, and AS160 was recently shown to be regulated by various other kinases, including AMPK. The interplay between stimuli such as exercise (AMPK) and insulin (Akt) on the regulation of AS160 suggests that AS160 may be a point of signal convergence leading to enhanced insulin sensitivity post exercise.

Planet Akt. Are there alternate life forms in the insulin action galaxy?

D.E. James, Diabetes and Obesity Research Program 384 Victoria Street, Darlinghurst, NSW 2010, Australia.
(Introduced by Mark Febbraio)

The Ser/Thr kinase Akt has been shown to play an important role in the insulin dependent trafficking of GLUT4 in both muscle and fat cells. This is based upon the use of inhibitors of components in the pathway that are upstream of Akt, the expression of either dominant inhibitory or constitutively active versions of Akt in cells or suppression of Akt in physiologically relevant tissues using either siRNA or knock out mice. Despite considerable evidence for the role of Akt in GLUT4 trafficking it remains unclear if activation of Akt alone is sufficient to active glucose transport for a number of reasons. First, suppression of Akt expression is not accompanied by complete suppression of insulin action. Second, a role for other insulin responsive pathways such as Rac, aPKC and c-Cbl/TC10 have been proposed. Third, the steps required to elicit increased glucose transport in response to insulin are complex and numerous and it has been suggested that insulin may both increase GLUT4 exocytosis, reduce GLUT4 endocytosis as well as activate the intrinsic activity of the transporter itself and so one can envisage a role for multiple signalling pathways in this complex process. One of the most important observations to suggest that Akt acts alone in the insulin action pathway is the observation that overexpression of a constitutively active version of Akt in adipocytes results in increased GLUT4 at the plasma membrane. However, this experiment is confounded by both the amplitude and duration of Akt signalling that was evoked prior to assessment of GLUT4 trafficking. In the present study we have used a dimerisation strategy to transiently activate Akt in adipocytes. We have found that Akt becomes active within 5 min after addition of the dimerisation agent to the cells as indicated by measurement of phosphorylation of downstream targets such as AS160. Moreover, cell surface levels of GLUT4 and cellular glucose transport were significantly increased following addition of the dimeriser following similar kinetics to that observed with insulin addition alone. We have been unable to find any evidence for alternate pathways in response to the dimeriser in these cells. These data suggest that Akt is the major signalling intermediate activated by insulin in order to stimulate glucose transport in adipocytes. These data do not support a major role for other signalling pathways in insulin mediated glucose transport in the adipocyte.

The retinoic acid receptor-related orphan nuclear receptor α regulates adiposity and lipid homeostasis

P. Lau, R. Fitzsimmons, S. Suryaprakash and G.E.O. Muscat , Institute for Molecular Bioscience, University of Queensland, Qld 4072, Australia. (Introduced by M. Febbraio)

The nuclear hormone receptor (NR) superfamily encodes hormone-dependent DNA binding proteins that convert metabolic, nutritional and pathophysiological signals into gene regulation. The retinoic acid receptor-related orphan nuclear receptor α (ROR alpha) is a member of the NR superfamily, however, the endogenous native molecule(s) and/or synthetic compounds that regulate the activity/function of this orphan NR are unknown. *In vitro* and *in vivo* studies have implicated RORα in the regulation of lipid homeostasis. Insights into the function of this nuclear receptor have been gained from the analysis of staggerer mice (sg/sg) that have impaired RORα function and dyslipidemia. We utilized this mouse model to investigate the role of RORα in the control of adiposity, and the adaptation to changes in dietary status in the liver, adipose and skeletal muscle. Our initial studies indicate that RORα4 is the predominant isoform in wild-type mice, and that expression of RORα (1 and 4) transcripts are significantly attenuated in the liver, white adipose and skeletal muscle of staggerer mice. Moreover, we identify changes in gene expression that regulate several critical aspects of lipid metabolism in the major mass metabolic tissues. The dysfunctional RORα expression (and function) coupled to these changes in gene expression leads to changes in the physiological response to energy dense/high fat diets.

Skeletal muscle fat metabolism and insulin resistance: old targets, new players

M.J. Watt, St Vincents Institute, 9 Princes St, Fitzroy, VIC 3065, Australia.

Insulin resistance and type 2 diabetes are frequently accompanied by triglyceride (TG) accumulation in skeletal muscle. However, it is not known whether TG deposition in skeletal muscle causes insulin resistance or whether reducing TG improves insulin sensitivity. We addressed these questions using loss and gain-of-function approaches. Acyl CoA:diacylglycerol acyltransferase 2 (DGAT2), an enzyme that catalyses the final step in TG biosynthesis, was genetically overexpressed in the skeletal muscle of mice by using the muscle creatine kinase promoter. This resulted in enhanced TG deposition in skeletal muscle and was associated with insulin resistance as assessed by whole body insulin and glucose tolerance tests and reduced 2-deoxyglucose uptake in isolated muscle. This occurred independent of changes in other lipid metabolites and serine / threonine kinases known to interfere with insulin signal transduction. We next tested whether increasing TG turnover would prevent fat-induced insulin resistance. To do this we overexpressed the novel triglyceride lipase, adipose triglyceride lipase (ATGL) in skeletal muscle myotubes by retroviral transfection and in rat tibialis anterior by electroporation of an ATGL vector. Anaesthesia was induced with 5% and maintained with 1–2% halothane in oxygen, the surgical site was irrigated with bupivicaine (0.5 mg/100 g) before closure, and 5 mg/kg ketoprofen was administered to provide postoperative analgesia. ATGL overexpression in myotubes increased the oxidation of fatty acids liberated from TG, but resulted in diglyceride and ceramide accumulation. These responses in cells were largely recapitulated in rats overexpressing ATGL. When ATGL protein expression and TG hydrolase activity in obese, insulin resistant rats were restored to levels observed in lean rats, TG content was reduced; however, the insulin resistance induced by the high fat diet persisted as assessed by hyperinsulinemic-euglycemic clamp. Collectively, these studies indicate that TG accumulation can cause insulin resistance and that short-term turnover of TG by ATGL overexpression does not protect against fat-induced insulin resistance.