

## Mechanisms of Exercise-Induced Glucose Uptake: Evidences and Hypotheses

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**Abstract:** Exercise can increase glucose uptake into skeletal muscle through an insulin-like effect. Mechanisms that control the homeostasis of glucose during muscle contracting, have not been known completely, but in the past several years significant progress about understanding of molecular basis of exercise effects has been provided. A central issue in exercise biology is to elucidate the underlying molecular signaling mechanisms that regulate important metabolic and transcriptional events in skeletal muscle that lead to reduction of blood glucose and improvement of impaired insulin action in diabetic people.

**Key words:** Exercise, diabetes, glucose uptake, glucose transport

### INTRODUCTION

Regular exercise can improve glycemic control. This may be due, in part, to the acute effects of exercise on glucose metabolism as well as training-induced adaptations. Significant number of people in the world suffers from diabetes. This disorder can result from insulin resistance or insulin deficiency that both factors result in impaired transport of glucose into the cells. In people with type II diabetes exercise can reduce blood glucose concentrations mainly because the exercise-induced increase in skeletal muscle glucose uptake is intact even when insulin action is impaired. During dynamic exercise the turnover of ATP in skeletal muscle increases greatly and is fuelled by the catabolism of carbohydrates (intramuscular glycogen, blood glucose) and fatty acid. During exercise in the postabsorptive state, the contribution of blood glucose to ATP resynthesis is initially relatively minor, but as exercise continues and muscle glycogen stores are depleted, the contribution of blood glucose becomes more substantial.

However physical activity mediates glucose transport via an insulin-like effect.

Improvements in glucose tolerance in response to exercise were observed over 20 years ago. Despite positive findings in later years, investigations of the effects of exercise on reduction of blood glucose in humans and experimental animals have yielded inconsistent results. However, it is increasingly clear that lifestyle which includes physical activity will very likely play a major role in the prevention and treatment of type II diabetes and other diseases associated with insulin resistance. In addition, it is equally clear that an understanding at a molecular level of how exercise diminishes insulin resistance and prevents cellular

damage and/or dysfunction will be critical to the success of related efforts (Castaneda *et al.*, 2006; Rose and Richter, 2005; Sakamoto and Goodyear, 2002).

In this review we summarize results of articles that have been chosen among collected researches and reviews via an internet researching on electronic journals of Tabriz university of Medical Sciences and Google sites in the past twenty years. We discuss about signaling and molecular mechanisms that regulate cascade events that lead to exercise-induced reduction of glucose and is initiated with insulin signal transduction and is followed by activation of various enzymes and factors that are caused by alterations in the expression of critical proteins, due to changes in gene transcriptions and alterations in protein synthesis that finally results in increased translocation of glucose transporters and glucose uptake. These effectors include kinases such as PI3-K, MAPK, AMPK and factors such as  $Ca^{2+}$  that have been studied widely in past 2 decade and novel factors that we found a few information about them and more studies is necessary to elucidate their actual role. Also, we explore either insulin-independent mechanisms or additive effects with insulin.

### GLUCOSE UPTAKE

First time Levine and Glostein made efforts to identify molecules and signaling pathways responsible for increased glucose transport during exercise and postexercise, then Bjorntorp and co-workers in the early 1970s reported benefit effects of exercise on stimulation of glucose uptake and following studies confirmed same results in rats (Balon, 2006; Tomas *et al.*, 2002). Identification of glucose transporters directed studies towards recognition of intracellular events and signaling cascade.

Cellular glucose uptake requires transporter proteins because it does not freely permeate the plasma membrane. These transporters are divided in two groups:

- Na<sup>+</sup> dependent D-Glucose Co-Transporters (SGLT) that transport glucose against its concentration gradient. When loss of glucose from isolated intestinal epithelial cells is prevented by blocking GLUTs in the basolateral membrane, the cell can concentrate glucose, taken up via its SGLT, by 30 fold. This transporter has been named hSGLT3 after finding in human DNA sequence of chromosome 22. Findings of Castaneda *et al.* (2000) show that hSGLT3 expression (but not GLUT4) was enhanced in skeletal muscle after 16 weeks of resistance training and its activation with exercise may play a significant role in glycemic control in skeletal muscle (Castaneda *et al.*, 2006; Zierler, 1999).
- Glucose Facilitated Transporter (GLUT) that allows transport of glucose down its concentration gradient (Castaneda *et al.*, 2006).

Isoforms of GLUT have been characterized in mammals that differ by their specificity and affinity for different hexoses. GLUT-1, GLUT-3, GLUT-4 and GLUT-7 are described as high-affinity transporters and GLUT-2 as low-affinity transporter and GLUT-5 is primarily a fructose carrier. High affinity transporters are found in almost every tissue but their expression is higher in cells with high glycolytic activity (Ericsson *et al.*, 2005; Guillet-Deniau *et al.*, 1994; Thorens, 1996). GLUT 12 a novel isoform has similarity to GLUT4 and has been suggested to be insulin sensitive (Ericsson *et al.*, 2005). Results of Douen *et al.* (1990) research in rat suggest that neither insulin nor exercise significantly increased GLUT-1 transporters in the plasma membrane. GLUT-2 is found in tissues carrying large glucose fluxes such as intestine, kidney and liver (Guillet-Deniau *et al.*, 1994; Steenberg *et al.*, 2003). In the human GLUT-3 is expressed predominantly in organs with high glucose requirements such as brain, testis and placenta (Ericsson *et al.*, 2005; Guillet-Deniau *et al.*, 1994). GLUT-7 is localized probably in liver microsomes (Guillet-Deniau *et al.*, 1994).

#### GLUT-4 GLUCOSE TRANSPORTERS

GLUT-4 is the major insulin-responsive glucose transporter isoform and is expressed primarily in striated muscle and adipose tissue (Ericsson *et al.*, 2005; Guillet-Deniau *et al.*, 1994). Many of studies on GLUT-4 have been made in mammals skeletal muscle (specially in rat) but a research by Diaz and Co-workers show that fish GLUT-4 differs from rat GLUT-4 in protein motifs but can translocate to the cell surface in response to insulin and

this difference may explain the relative glucose intolerance observed in fish (Diaz *et al.*, 2007).

The mechanisms of insulin-mediated glucose uptake are complex, however involve with translocation of glucose transporters from intracellular pools to the plasma membrane (Musi *et al.*, 2001; Tomas *et al.*, 2002; Zierath, 2002; Zierler, 1999). Persistent deficiency of insulin leads to reduction in number of GLUT-4 transporters and impaired responsiveness to insulin stimulus. Numerous studies have established that contractions stimulate glucose transport in the complete absence of insulin, therefore maximal effects of exercise and insulin are additive (Fushiki *et al.*, 1989; Henriksen, 2002; Holloszy, 2005; King *et al.*, 1993).

Exercise and insulin stimulate skeletal muscle glucose transport by increasing both the number and activity of glucose transporters (King *et al.*, 1993). They utilize different pathways which lead to the translocation of GLUT-4 to the plasma membrane and transverse tubules (Goodyear *et al.*, 1998; Hirshman *et al.*, 1989; King *et al.*, 1993). Findings of Fushiki *et al.* (1989) and Hirshman *et al.* (1989) suggest that exercise trained rats had significantly more membrane transporters than sedentary rats.

Obese Zucker rat is rodent model that widely has been used to research about obesity-associated insulin resistance (Henriksen, 2002; King *et al.*, 1993). Review of Henriksen regarding studies in Zucker rat suggests that exercise can significantly lower plasma glucose levels and improve the insulin resistance and enhancement of insulin sensitivity of glucose (Henriksen, 2002; King *et al.*, 1993).

First time in 1982 Ruderman's group reported postexercise increase in insulin-sensitivity. They reported that this increase in sensitivity is not just to insulin but also to stimulation of glucose transport by the contraction/hypoxia pathway, because the presence of defect in insulin-induced glucose uptake and GLUT-4 translocation has been proved in both the people with type II diabetes and in obese Zucker rats, despite their total GLUT-4 content has been normal, that suggest a bypass pathway in insulin signaling (Holloszy, 2005). Other conclusion was a hypothesis that different pools of glucose transporters were recruited. This puzzle is yet unresolved, although Coderre in 1995 showed that unique pool is recruited by exercise and insulin (Balon, 2006; Holloszy, 2006; Li *et al.*, 2004; Rose and Richter, 2005).

Even so, both stimuli probably recruit GLUT-4 vesicles that contain vesicle-associated membrane proteins (VAMP) (Rose and Richter, 2005; Torok *et al.*, 2004). These vesicles must dock and fuse the plasma membrane and recent findings suggest that VAMP2 (not VAMP3) is a resident protein of the insulin-sensitive GLUT-4 compartment (Kristiansen *et al.*, 1996; Randhawa *et al.*, 2000; Torok *et al.*, 2004; Li *et al.*, 2001).

Numerous studies have been carried out to distinguish the effects of duration and intensity of exercise on GLUT-4 activity. King *et al.* (1993) reported unlike a maximal insulin stimulus, acute exercise of Zucker rat promotes both transporter translocation and its activation.

Terada reported similar increased expression of GLUT-4 with both "high-intensity and short-time" exercise or "prolonged with low-intensity" exercise (Terada *et al.*, 2001), while Fujimoto has found this enhancement only by high-intensity exercise (Fujimoto *et al.*, 2003).

Since, GLUT-4 plays a critical role in glucose homeostasis, numerous researches have been made about factors that control its expression. Of the effectors, i.e., cAMP, insulin and arachidonic acid-known to down-regulate expression of GLUT-4 *in vitro* and decline in circulating arachidonate level *in vivo* correlated with upregulation of GLUT4 (Ikemoto *et al.*, 1995). A report shows that hyperosmolarity increases its exocytosis (Khayat *et al.*, 1998; Li *et al.*, 2001). Findings of Ryder *et al.* (1999) in GLUT4-deficient mice indicates major role of GLUT4 in exercise-induced glucose uptake, however it shows GLUT4 is not essential for glycogen repletion (Ryder *et al.*, 1999). Previous works suggested that normal GLUT4 content is sufficient for increases in muscle glucose uptake during exercise, while recent studies show that control of exercise-stimulated glucose uptake by GLUT4 is dependent on glucose phosphorylation capacity because when this capacity increased by hexokinase II (HKII) overexpression, GLUT4 availability becomes a marked limitation to exercise-induced glucose uptake (Fueger *et al.*, 2004).

#### EFFECT OF EXERCISE ON GLUCOSE TRANSPORT SIGNALING

The initial trigger for insulin stimulation of glucose transport is the activation and autophosphorylation of insulin receptor (IR) tyrosine protein kinase and subsequent phosphorylation of its downstream effectors, insulin receptor substrate (IRS)1 and IRS2 (Konstantopoulos *et al.*, 2006). Insulin-stimulated p13-K activity is impaired in skeletal muscle from type II diabetic and obese insulin-resistant subjects (Zierath, 2002).

Insulin receptor substrate isoforms (IRS-1 to 4), Gab-1 and Cbl link the initial event of insulin receptor signaling cascade to downstream events. IRS molecules contain multiple tyrosine phosphorylation sites that become phosphorylated after insulin stimulation. IRS-3 and IRS-4 are not expressed in skeletal muscle, but IRS-1 and IRS-2 have selective roles in the regulation of metabolic responses. IRS-1 appears to be the predominant isoform

mediating signal transduction in skeletal muscle and exercise increases its activity (Chibalin *et al.*, 2000; Zierath, 2002).

#### PI3-KINASE AND DOWNSTREAM EFFECTORS

Phosphatidylinositol 3-kinase (PI3-K) is one of the intermediary effectors that plays central role in cellular effect of insulin (Li *et al.*, 2004). It has a catalytic subunit and a regulating subunit. PI3-K associates with tyrosine phosphorylated IRSs after insulin stimulation and catalyzes the formation of phosphatidylinositol-3, 4, 5-triphosphate, [PI-(3,4,5)-P<sub>3</sub>] which finally leads to fusion of GLUT4 vesicles. Subsequent event is activation of protein kinase B (PKB that also known as Akt) and Protein kinase C (PKC) isoforms (Chibalin *et al.*, 2000; Christ-Roberts *et al.*, 2003; Li *et al.*, 2004; Sakamoto and Goodyear, 2000; Torok *et al.*, 2004; Zierath, 2002).

Wortmannin is an agent that inhibits PI3-K activity and subsequently inhibits contraction-induced muscle glucose uptake. A number of studies have demonstrated that PI3-K function is necessary, but not sufficient for insulin-stimulated GLUT4 translocation (Christ-Roberts *et al.*, 2003; Konstantopoulos *et al.*, 2006; Nolte *et al.*, 2003; Somwar *et al.*, 2001).

In contrast to the nondiabetic subjects, it is unlikely that increased IRS-1-associated PI3-K activity is involved in the synergistic effect of exercise on insulin-induced glucose uptake in patients with type II diabetes (Christ-Roberts *et al.*, 2003; Konstantopoulos *et al.*, 2006; Wright *et al.*, 2006).

Several studies have established the involvement of Akt as a downstream target of PI3-K-mediated insulin stimulation of glucose transport (Christ-Roberts *et al.*, 2003; Konstantopoulos *et al.*, 2006; Somwar *et al.*, 2001; Wang *et al.*, 1999; Zierath, 2002). Recent studies suggest that decreased stimulation of Akt isoforms links with insulin resistance (Christ-Roberts *et al.*, 2003; Oku *et al.*, 2001; Li *et al.*, 2001). Results of studies indicate that similar to the PI3-K, exercise synergistically enhances insulin-stimulated Akt serine phosphorylation in the nondiabetic subjects (Christ-Roberts *et al.*, 2003; Konstantopoulos *et al.*, 2006; Rose and Richter, 2005; Wang *et al.*, 1999; Zierath, 2002).

AS160 (a Rab GTP-ase-activating protein) has been reported as a potential but as yet uncharacterized target for Akt or AMPK (Konstantopoulos *et al.*, 2006; Rose and Richter, 2005; Treebak *et al.*, 2006).

Recent studies have identified three kinases rapidly activated by insulin in muscle cells: PI3-K, PKB and p70<sup>S6K</sup>. Blockade of p70<sup>S6K</sup> activity causes the inhibition of protein synthesis in response to insulin (Sakamoto and Goodyear, 2002).

## CA<sup>2+</sup>- PKC HYPOTHESIS

It has been considered that the rise in intracellular Ca<sup>2+</sup> is a critical mediator of increased glucose transport during skeletal muscle contraction and hypoxia (Khayat *et al.*, 1998; Sandstrom *et al.*, 2007). This has been proposed on the basis of inhibition of the stimulation of glucose transport during hypoxia and contraction by Ca<sup>2+</sup> channels blockers (e.g. verapamil) or lower Ca<sup>2+</sup> efflux from the sarcoplasmic reticulum (e.g. dantrolene). Other studies have shown that glucose transport can be increased when cytoplasmic Ca<sup>2+</sup> concentrations are raised using agents such as Caffein, W-7 and Ca<sup>2+</sup> ionophores (Jensen *et al.*, 2007; Khayat *et al.*, 1998). This rise may facilitate the activation of key intracellular signaling molecules. Since Ca<sup>2+</sup> can activate cPKCs and PMA (an activator of cPKC) can increase glucose disposal by distinct mechanism from insulin (Khayat *et al.*, 1998).

Voltage-gated potassium (Kv) channels regulate cell membrane potential (Vm) by controlling the rate of K<sup>+</sup> exit from the cell. Kv1.3 is one of channels that is expressed in insulin-sensitive tissues and its activation can increase peripheral insulin sensitivity independently of body weight. Li *et al.* (2007) showed that inhibition of this channel increases GLUT4 protein at the plasma membrane through a Ca<sup>2+</sup>-dependent process (Wijesekara *et al.*, 2006). At least in adipocytes, intracellular Ca<sup>2+</sup> may play an important role in insulin-stimulated glucose transport (Li *et al.*, 2004). Cell Vm depolarization which initiates muscle contraction, causes a rise in intracellular Ca<sup>2+</sup> concentration and leads to the activation of PKC, of which PKC- $\beta$  isoform likely plays a significant role (Khayat *et al.*, 1998; Li *et al.*, 2004). Ca<sup>2+</sup> also is involved in the regulation of other intracellular proteins such as Calmodulin Kinase (CaMK) and calcineurin (Sakamoto and Goodyear, 2002).

Calmodulin (CaM) is a Ca<sup>2+</sup>-sensing protein, which its inhibition leads to reduced GLUT4 translocation by inhibiting the production of PI- (Barnes *et al.*, 2004; Castaneda *et al.*, 2006; Chibalin *et al.*, 2000) -P<sub>3</sub> without directly affecting IRS-1 or phosphorylation-associated P13-K activity (Yang *et al.*, 2000).

CaMKII is a serine/threonine kinase, which is activated by an increase in cytoplasmic calcium via its association with the ubiquitous calcium receptor, calmodulin. Inhibition of CaMKII by KN62 reduces contraction-stimulated glucose transport (Khayat *et al.*, 1998; Konstantopoulos *et al.*, 2006).

It seems that nitric oxide synthase (NOS) is also activated by Ca<sup>2+</sup>-bound CaM. Production of NOS is increased during exercise (Rose and Richter, 2005).

## AMPK

5'-AMP-activated protein kinase (AMPK) has recently emerged as a potentially key signaling intermediary in the regulation of exercise-induced changes in glucose and lipid metabolism in skeletal muscle.

First time in 1995 Hardie and Winder demonstrated the activation of AMPK by exercise, then in 1997, Merrill and co-workers showed that AICAR (5-Amino-Imidazole Carboxamide Riboside) as an activator of AMPK increases glucose uptake in rat skeletal muscle (Nielsen *et al.*, 2003; Putman *et al.*, 2007; Tomas *et al.*, 2002; Wojtaszewski *et al.*, 2005).

AMPK is a multifunctional serine/threonine protein kinase that acts as important sensor of cellular energy charge and protects cell by acting as a "low fuel warning" system (Nielsen *et al.*, 2003; Rose and Richter, 2005).

AMPK has been identified as a regulator of gene transcription, increasing mitochondrial proteins of oxidative metabolism as well as hexokinase expression in skeletal muscles (Thomson *et al.*, 2008). AMPK consists of a catalytic ( $\alpha$ ) and two regulatory ( $\beta$ ,  $\gamma$ ) subunits, that  $\alpha$ 1 isoform is distributed in different tissues and the  $\alpha$ 2 isoform is primarily expressed in skeletal muscle, heart and liver (Nielsen *et al.*, 2003; Tomas *et al.*, 2002; Whitehead *et al.*, 2001). Allosteric activation of AMPK is mediated by a mechanism, which is sensitive to an increase in cellular AMP/ATP and creatine/phosphocreatine ratio (Koistinen *et al.*, 2003; Nielsen *et al.*, 2003; Rose and Richter, 2005; Sakamoto and Goodyear, 2002). AMPK is also covalently activated by AMPK kinases (AMPKKs) via phosphorylation on Thr<sup>172</sup> of the catalytic  $\alpha$  subunit. Numerous studies have reported an increase in AMPK activation during exercise-contraction, while some studies that have carried out in human or transgenic animals have shown increase in glucose uptake without significant activation of AMPK that suggesting of one or more additional pathways in exercise-induced glucose transport (Nielsen *et al.*, 2003; Rose and Richter, 2005).

Toyoda *et al.* (2006) found that low-intensity exercise activates the  $\alpha$ 1-isoform of AMPK (Toyoda *et al.*, 2006). In contrast, several observations indicate that  $\alpha$ 2-isoform rather than the  $\alpha$ 1-isoform activate in moderate-training (Zierath, 2002), where as some reports have shown the similar activity of both isoforms by high-intensity exercise. Isoform-specific and intensity-dependent effects of exercise and different capacity of muscle fiber types for AMPK signaling must be established in future investigations (Musi *et al.*, 2001; Putman *et al.*, 2007; Rockl *et al.*, 2007; Ryder *et al.*, 2005; Wojtaszewski *et al.*,

2005; Zierath, 2002). Recently, the key role of  $\gamma$ 3-isoform of AMPK in regulating of glycogen accumulation and its probably benefit for prevention or treatment of insulin-resistance has been reported (Barnes *et al.*, 2004; Yu *et al.*, 2006). Clear mechanism of AMPK in exercise-induced glucose uptake is unknown. Collectively, AMPK, like exercise appears to work downstream or independently of PI3-K to improve insulin sensitivity, because using AICAR for activation of AMPK is associated with increase in GLUT4 translocation, without no effect of wortmannin on this translocation (Koistinen *et al.*, 2003; Musi *et al.*, 2001; Rose and Richter, 2005; Sakamoto and Goodyear, 2005). AICAR, also causes the increased phosphorylation of AS160 and ACC (Acetyl-CoA-Carboxylase) as well as AMPK (Thomson *et al.*, 2008; Treebak *et al.*, 2006).

### MAPK

The MAPK (Mitogen-Activated Protein Kinase) family of intracellular signaling cascades are expressed in all eukaryotic cells and include the Extracellular Signal-Regulated Kinase 1 and 2 (ERK 1/2) or P42, ERK5 (or big MAPK), P44 MAPK, the cJun NH<sub>2</sub>-terminal kinase (JNK) and p38MAPK. In 1996 it was first reported that exercise like insulin can activate isoforms of MAPK (Rose and Richter, 2005; Sakamoto and Goodyear, 2002; Zierath, 2002).

The ERK 1/2 cascade is activated primarily by growth factors. They can also translocate to the nucleus for phosphorylation of variety of transcription factors. Activation of ERK1/2 signaling has been reported in animals and human in response to exercise. This signaling is not necessary for the regulation of either contraction-or insulin-stimulated glucose uptake (Sakamoto and Goodyear, 2002). There are few reports that JNK stimulates glycogen synthase activity and its signaling is distinct from that of ERK1/2 (Sakamoto and Goodyear, 2002). Activation of p38 MAPK has been reported in numerous articles (Holloszy, 2005; Sakamoto and Goodyear, 2002). There is isoform-specific regulation of p38 with exercise-contraction such as activation of p38 $\gamma$  after marathon running (Sakamoto and Goodyear, 2002). Also it has been reported that insulin may increase GLUT4 activity via p38 $\alpha$  and/or p38 $\beta$  (Antonescu *et al.*, 2005). This finding is confirmed by using SB203580 (inhibitor of p38MAPK), however this effect on glucose transport may not entirely attributable to inhibition of p38 (Antonescu *et al.*, 2005; Rose and Richter, 2005; Somwar *et al.*, 2002; Sweeney *et al.*, 1999).

### OTHER PUTATIVE MECHANISMS

Exercise provokes the release of regulatory hormones such as catecholamines, which in turn could result in the activation of specific cell surface receptors. A good example is the epinephrine-mediated stimulation of  $\beta$ -adrenergic receptors that leads to the activation of cAMP and protein kinase A (Han and Bonen, 1998; Sakamoto and Goodyear, 2002). Catecholamines have effects on glucose transport in adipocytes, heart and skeletal muscle.

It has been shown that epinephrine translocates GLUT4, while at the same time increasing glucose transport when insulin is absent, or can inhibit glucose transport when insulin is present (Han and Bonen, 1998).

Findings of several studies suggest that norepinephrine-stimulated glucose uptake is mediated by  $\alpha_1$ -adrenergic receptor. Norepinephrine through coupling to Gq (a subunit of the GTP-binding protein) and activation of bradykinin receptors and finally GLUT4 translocation may help to take up glucose to generation ATP for repeated muscle contraction (Kishi *et al.*, 1996). The neurotransmitters calcitonin gene-related peptide, ciliary neurotrophic factor and neuregulin are examples of extracellular messengers that are released by the motor nerve and may act to stimulate intracellular signaling pathways (Sakamoto and Goodyear, 2002; Suarez *et al.*, 2001).

Insulin-like growth factor1 (IGF-1) and fibroblast growth factor may provide an autocrine mechanism for the activation of signaling pathways by exercise (Sakamoto and Goodyear, 2002). IGF-1 is a 70-amino acid peptide that shares a high degree of homology with insulin and both probably mediate their effects through some common intracellular mechanisms, because the maximal stimulation of glucose transport activity by these peptides is not additive (Hokama *et al.*, 1997).

In past years, the relationship between PDGF (Platelet-Derived Growth Factor) and GLUT4 translocation has been investigated. PDGF activates PI3-K and induces actin remodeling, because its major production PI-(Barnes *et al.*, 2004; Castaneda *et al.*, 2006; Chibalin *et al.*, 2000) -P<sub>3</sub> is required in muscle cell for cortical actin remodeling which is in turn necessary for fusion of GLUT4 vesicles (Torok *et al.*, 2004).

Other signals proposed to lead to glucose transport stimulation during muscle contraction are Nitric Oxide (NO) and bradykinin acting through the G protein Gq (Khayat *et al.*, 1998). Insulin stimulates NO synthesis in endothelial cells and this NO may vasodilate and increase

blood flow (Zierler, 1999). NO is released in contracting muscle. A NO synthase (NOS) inhibitor decreased basal and exercise-stimulated glucose uptake but had no effect on insulin-stimulated glucose uptake. This effect may be regulated by a cGMP-mediated mechanism (Kingwell *et al.*, 2002; Sakamoto and Goodyear, 2002).

The role of inflammatory cytokines and oxidative stress on development of insulin resistance has been known (Pederson, 2007; Wei *et al.*, 2008). Interleukin-6 (IL-6) has been proposed as a novel mediator or "exercise factor" of glucose homeostasis during skeletal muscle contraction. IL-6 stimulates the production of anti-inflammatory cytokines and suppresses TNF- $\alpha$  production in humans, since TNF has a role in insulin resistance, it is likely that muscle-derived IL-6 offers protection against this effect. Activation of AMPK by IL-6 appears to play an important role in modulating some of various metabolic effects of IL-6 (Febbraio *et al.*, 2004; Pederson, 2007; Pederson *et al.*, 2007).

Despite data suggesting that administration of IL-6 increases insulin-stimulated glucose disposal, Steenberg and co-workers reported that acute IL-6 administration does not impair whole-body glucose disposal (Steenberg *et al.*, 2003). Pederson *et al.* have proposed that cytokines and other peptides that are produced, expressed and released by muscle fibers and exert either paracrine or endocrine effects should be classified as "myokines". This class includes various factors such as IL-6, IL-8 and IL-15 (Pederson *et al.*, 2007).

NF- $\kappa$ B is a major regulator of transcription and metabolism that as well as MAPK is activated during exercise, however identification of its pathways require future investigations (Kramer and Goodyear, 2007).

Since, adenosine receptor antagonists can improve glucose tolerance, it seems that signaling from adenosine receptors may be a factor contributing to tissue-specific insulin resistance (Crist *et al.*, 1998).

GSK3 (Glycogen Synthase Kinase 3) is a serine/threonine kinase that its activation is altered by exercise and has regulatory role in metabolic and transcriptional processes (Sakamoto and Goodyear, 2002).

PP1G (Glycogen/sarcoplasmic reticulum-associated type 1 Protein Phosphatase) is thought to be essential for regulation of glycogen metabolism under basal conditions and in response to contractile activity but not by insulin (Sakamoto and Goodyear, 2002). Previous reports have contributed a role for mechanical force in activating cellular responses, whereas recently Sandstrom and co-workers reported that mechanical load plays little role in contraction-mediated glucose transport in mouse. Instead, they proposed other factors such the Ca<sup>2+</sup>-dependent alterations in energy turnover and production of ROS (Reactive Oxygen Species) (Sakamoto and Goodyear,

2002; Sandstrom *et al.*, 2007). Their first report was published in 2006 regarding an effective role of ROS in exercise-induced glucose uptake. They have discussed also about possible, an AMPK-independent-antioxidant-mediated and an AMPK-dependent-antioxidant pathways, which may be interesting issue for future investigations (Balon, 2006).

Exercise training is an important stimulator for the increase in skeletal muscle glucose uptake, that probably results from a coordinated increase in rates of glucose delivery (higher capillary perfusion), surface membrane transport and intracellular substrate flux through glycolysis. Despite considerable research, relatively little is known about how exercise/contraction regulates skeletal muscle glucose transport. However, there is evidence that insulin signal transduction through the insulin receptor, insulin receptor substrate (IRS) and phosphorylation of PI3K is enhanced in skeletal muscle. These changes may enhance insulin sensitivity as well as regulate gene expression after exercise (Fig. 1).

Also, it has been demonstrated that the increased glucose transport mainly occurs due to higher amounts of glucose transport protein GLUT4 in surface of membranes. The mechanisms behind this have not been fully elucidated, but may involve several factors that transduce signals relating to changes in the intracellular environment such as higher Ca<sup>2+</sup> concentration that activates CaM and kinases such as CaMK and PK isoforms and NOS. In addition, MAPK signaling to downstream substrate is enhanced, providing a possible molecular mechanism for exercise-induced transcriptional regulation in skeletal muscle.

On the other hand, AMPK is activated when the energy status of the muscle is decreased and its activation can be regarded as a feedback level of regulation (Fig. 2).

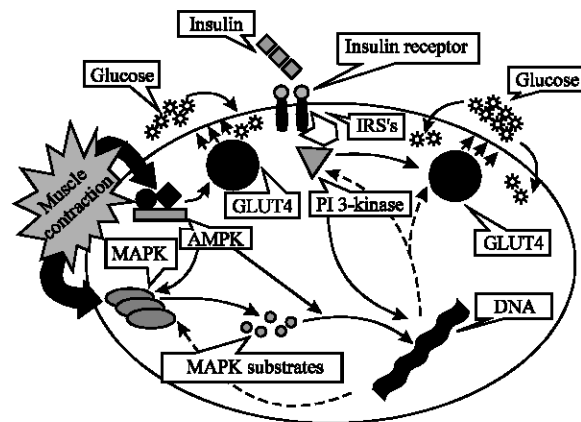


Fig. 1: Exercise training-induced changes in insulin signaling in skeletal muscles

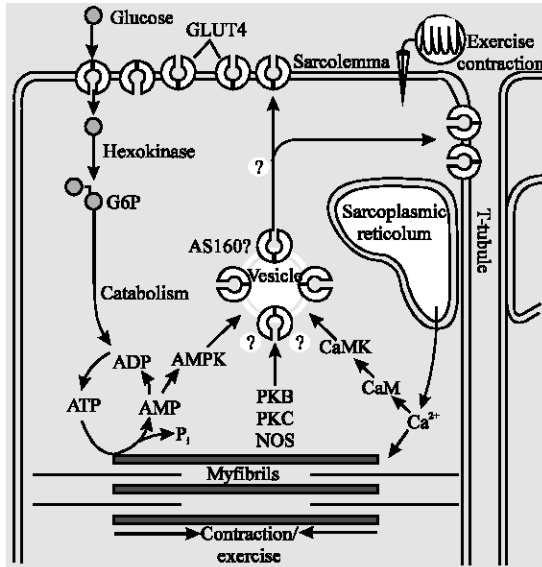


Fig. 2: Putative regulation of glucose uptake and GLUT4 “translocation” in skeletal muscle during exercise/contraction

Although, different systems have been identified regarding insulin-dependent or insulin-independent mechanisms, it has been hypothesized that because insulin and contraction signaling both ultimately result in GLUT4 translocation, there may be convergent signaling intermediators. Degree of activation of each factor depends on exercise duration-intensity and the fiber-type composition of the working muscle.

Future studies must be focused on the identification of pathways or factors that may be achieved through modern biology, comparative genomics, using genetically modified model organisms, combined with bioinformatics approaches, knowledge of human genome sequence and gene-protein array technology to find more information about pathways analysis and relationship between various factors such as MAPK and AMPK or CaM with other components and/or determine the role of novel "exercise factors" such as ROS and molecular targets in this network (Rose and Richter, 2005; Ryder *et al.*, 1999; Ryder *et al.*, 2005; Sakamoto and Goodyear, 2002).

## REFERENCES

Antonescu, C.N., C. Hung, W. Niu, Z. Liu, P.A. Eyers, K.A. Heidenreich, P.J. Bilan and A. Klip, 2005. Reduction of insulin-stimulated glucose uptake in L6 myotubes by the protein kinase inhibitor SB203580 is independent of p38MAPK activity. *Endocrinology*, 146: 3773-3781.

Balon, T.W., 2006. Many pathways are called! Many may be chosen!. *J. Physiol.*, 575: 3.

Barnes, B.R., S. Marklund, T.L. Steiler, M. Walter, G. Hjalml, V. Amarger, M. Mahlapun, Y. Leng, C. Johansson, D. Galuska, K. Lindgren, M. Abrink, D. Stapleton, J.R. Zierath and L. Andersson, 2004. The 5' AMP-activated protein kinase  $\gamma$ 3 isoform has a key role in carbohydrate and lipid metabolism in glycolytic skeletal muscle. *J. Biol. Chem.*, 279: 38441-38447.

Castaneda, F., J.E. Lyane and C. Castaneda, 2006. Skeletal muscle sodium glucose co-transporters in older adults with type 2 diabetes. *Int. J. Med. Sci.*, 3: 84-91.

Chibalin A.V., M. Yu, J.W. Ryder, X.M. Song, D. Galuska, A. Krook, H. Wallberg-Henrikson and J.R. Zierath, 2000. Exercise-induced changes in expression and activity of proteins involved in insulin signal transduction in skeletal muscle: Differential effects of insulin-receptor substrates 1 and 2. *PNAS* 97: 38-43.

Christ-Roberts, C.Y., T. Pratipanawatr, W. Pratipanawatr, R. Berria, R. Belfort and L. Mandarino, 2003. Increased insulin receptor signaling and glycogen synthase activity contribute to the synergistic effect of exercise on insulin action. *J. Applied Physiol.*, 95: 2519-2529.

Crist, G.H., B. Xu, K.F. Lanoue and C.H. Lang, 1998. Tissue-specific effects of *in vivo* adenosine receptor blockade on glucose uptake in Zucker rats. *The FASEB J.* 12: 1301-1308.

Diaz, M., C.N. Antonescu, E. Capilla, A. Klip and J.V. Planas, 2007. Fish glucose transporter (GLUT)-4 differs from rat GLUT4 in its traffic characteristics but can translocate to the cell surface in response to insulin in skeletal muscle cells. *Endocrinology*, 148: 5248-5257.

Douen, A.G., T. Ramlal, S. Rastogi, P.J. Bilan, G.D. Cartee, M. Vranic, J.O. Holloszy and A. Klip, 1990. Exercise induces recruitment of the insulin-responsive glucose transporter. Evidence for distinct intracellular insulin and exercise-recruitable transporter pools in skeletal muscle. *J. Biol. Chem.*, 265: 13427-13430.

Ericsson, A., B. Hamark, T.L. Powell and T. Jansson, 2005. Glucose transporter isoform 4 is expressed in the syncytiotrophoblast of first trimester human placenta. *Human Reprod.*, 20: 521-530.

Febbraio, M.A., N. Hiscock, M. Sacchetti, C.P. Fischer and B.K. Pedersen, 2004. Interleukin-6 is a novel factor mediating glucose homeostasis during skeletal muscle contraction. *Diabetes*, 53: 1643-1648.

- Fueger, P.T., H.S. Hess, K.A. Posey, D.P. Bracy, R.R. Pencek, M.J. Charron and D.H. Wasserman, 2004. Control of exercise-stimulated muscle glucose uptake by GLUT4 is dependent on glucose phosphorylation capacity in the conscious mouse. *J. Biol. Chem.*, 279: 50956-50961.
- Fujimoto, T., J. Kemppainen, K. Kalliokoski, P. Nuutila, M. Ito and J. Knuuti, 2003. Skeletal muscle glucose uptake response to exercise in trained and untrained men. *Med. Sci. Sports Exerc.*, 35: 777-783.
- Fushiki, T., J.A. Wells, E.B. Tapscott and G.L. Dohm, 1989. Changes in glucose transporters in muscle in response to exercise. *Am. J. Physiol. Endocrinol. Metab.*, 256: E580-E587.
- Goodyear, L.J. and B.B. Kahn, 1998. Exercise, glucose transport and insulin sensitivity. *Ann. Rev. Med.*, 49: 235-261.
- Guillet-Deniau, I., A. Leturque and J. Girad, 1994. Expression and cellular localization of glucose transporters (GLUT1, GLUT3, GLUT4) during differentiation of myogenic cells isolated from rat fetuses. *J. Cell. Sci.*, 107: 487-496.
- Han, X.X. and A. Bonen, 1998. Epinephrine translocates GLUT-4 but inhibits insulin-stimulated glucose transport in rat muscle. *Am. J. Physiol.* 274: E700-E707.
- Henriksen, E.J., 2002. Exercise effects of muscle insulin signaling and action. Invited review: Effects of acute exercise and exercise training on insulin resistance. *J. Applied Physiol.*, 93: 1-2.
- Hirshman, M.F., L.J. Wardzala, L.J. Goodyear, S.P. Fuller, E.D. Horton and E.S. Horton, 1989. Exercise training increases the number of glucose transporters in rat adipose cells. *Am. J. Physiol. Endocrinol. Metab.*, 257: E520-E530.
- Hokama J.Y., R.S. Streeper and E.J. Henriksen, 1997. Voluntary exercise training enhances glucose transport in muscle stimulated by insulin-like growth factor I. *J. Applied. Physiol.*, 82: 508-512.
- Holloszy, J.O., 2005. Exercise-induced increase in muscle insulin sensitivity. *J. Applied Physiol.*, 99: 338-343.
- Ikemoto, S., K.S. Thompson, H. Itakura, M.D. Lane and O. Ezaki, 1995. Expression of an insulin-responsive glucose transporter (GLUT4) minigene in transgenic mice: Effect of exercise and role in glucose homeostasis. *PNAS*, 92: 865-869.
- Jensen, T.E., A.J. Rose, Y. Hellsten, J.F.P. Wojtaszewski and E.A. Richter, 2007. Caffeine-induced  $Ca^{2+}$  release increases AMPK-dependent glucose uptake in rodent soleus muscle. *Am. J. Physiol. Endocrinol. Metab.*, 293: E286-E292.
- Khayat, Z.A., T. Tsakiridis, A. Ueyama, R. Somwar, Y. Ebina and A. Klip, 1998. Rapid stimulation of glucose transport by mitochondrial uncoupling depends in part on cytosolic  $Ca^{2+}$  and cPKC. *Am. J. Physiol Cell. Physiol.*, 275: C1487-C1497.
- King, P.A., J.J. Betts, E.D. Horton and E.S. Horton, 1993. Exercise, unlike insulin promotes glucose transporter translocation in obese Zucker rat muscle. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 265: R447-R452.
- Kingwell, B.A., M. Formosa, M. Muhlmann, S.J. Bradley and G. Mc Conell, 2002. Nitric oxide synthase inhibition reduces glucose uptake during exercise in individuals with type 2 diabetes more than in control subjects. *Diabetes*, 51: 2572-2580.
- Kishi, K., H. Hayashi, L. Wang, S. Kamohara, K. Tamaoka, T. Shimizu, F. Ushikubi, S. Narumiya and Y. Ebina, 1996. Gq-coupled receptors transmit the signal for GLUT4 translocation via an insulin-independent pathway. *J. Biol. Chem.*, 271: 26561-26568.
- Koistinen, H.A., D. Galuska, A.V. Chibalin, J. Yang, J.R. Zierath, G.D. Holman and H. Wellberg-Henriksson, 2003. 5-Amino-imidazole carboxamide riboside increases glucose transport and cell-surface GLUT4 content in skeletal muscle from subjects with type 2 diabetes. *Diabetes*, 52: 1066-1072.
- Konstantopoulos, N., S. Marcuccio, S. Kyi, V. Stoichevska, L.A. Castelli, C.W. Ward and S.L. Macaulay, 2006. A purine analog kinase inhibitor, calcium/calmodulin dependent protein kinase II inhibitor 59, reveals a role for calcium/calmodulin dependent protein kinase II in insulin-stimulated glucose transport. *Endocrinology*, 148: 374-385.
- Kramer, H. and L.J. Goodyear, 2007. Exercise, MAPK and  $NF-\kappa B$  signaling in skeletal muscle. *J. Applied Physiol.*, 103: 388-395.
- Kristiansen, S., M. Hargreaves and E.A. Richter, 1996. Exercise-induced increase in glucose transport, GLUT-4 and VAMP-2 in plasma membrane from human muscle. *A. J. Physiol. Endocrinol. Metab.*, 270: E197-E201.
- Li, D., V.K. Randhawa, N. Patel, M. Hayashi and A. Klip, 2001. Hyperosmolarity reduces GLUT4 endocytosis and increases its exocytosis from a VAMP2-independent pool in L6 muscle cells. *J. Biol. Chem.*, 276: 22883-22891.
- Li, Y., P. Wang, J. Xu and G.V. Desir, 2004. Voltage-gated potassium channel Kv 1.3 regulates GLUT4 trafficking to the plasma membrane via a  $Ca^{2+}$ -dependent mechanism. *PNAS*, 10: 13112-3117.



- Musi, N., N. Fujii, M.F. Hirshman, I. Ekberg, S. Froberg, O. Ljungqvist, A. Thorell and L.J. Goodyear, 2001. AMP-activated protein kinase (AMPK) is activated in muscle of subjects with type 2 diabetes during exercise. *Diabetes*, 50: 921-927.
- Nielsen, J.N., S.B. Jorgensen, C. Frosig, B. Viollet, F. Andreelli, S. Vaulont, B. Kiens, E.A. Richter and J.F.P. Wojtaszewski, 2003. A possible role for AMP-activated protein kinase in exercise-induced glucose utilization: insights from humans and transgenic animals. *Biochem. Soc. Trans.*, 31: 186-190.
- Nolte, L.A., D.H. Han, P.A. Hansen, K.A. Hucker and J.O. Holloszy, 2003. A Peroxovanadium compound stimulates muscle glucose transport as powerfully as insulin and contractions combined. *Diabetes*, 52: 1918-1925.
- Oku, A., M. Nawano, K. Ueta, T. Fujita, I. Umebayashi, K. Arakawa, T. Kano-Ishihara, A. Saito, M. Anai, M. Fanaki, M. Kikuchi, Y. Oka and T. Asano, 2001. Inhibitory effect of hyperglycemia on insulin-induced Akt/protein kinase B activation in skeletal muscle. *A. J. Physiol. Endocrinol. Metab.*, 280: E816-E824.
- Pederson, B.K., 2007. Interleukin-6 does/does not have a beneficial role in insulin sensitivity and glucose homeostasis. *J. Applied Physiol.*, 102: 814-816.
- Pederson, B.K., T.C.A. Akerstrom, A.R. Nielsen and C.P. Fisher, 2007. Role of myokines in exercise and metabolism. *J. Applied Physiol.*, 103: 1093-1098.
- Putman C.T., K.J.B. Martins, M.E. Gallo, G.D. Lapaschuk, J.A. Pearcey, I.M. Maclean, R.J. Saranchuk and D. Pette, 2007.  $\alpha$ -Catalytic subunits of 5'AMP-activated protein kinase display fiber-specific expression and are upregulated by chronic low-frequency stimulation in rat muscle. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 293: R1325-R1334.
- Randhawa, V.K., P.J. Bilan, Z.A. Khayat, N. Daneman, Z. Liu, T. Ramlal, A. Volchuk, X.R. Peng, T. Coppola, R. Regazzi, W.S. Trimble and A. Klip, 2000. VAMP2 but not VAMP3/Cellubrevin, mediates insulin-dependent incorporation of GLUT4 into the plasma membrane of L6 Myoblasts. *Molecul. Biol. Cell.*, 11: 2403-2417.
- Rockl, K.S.C., M.F. Hirshman, J. Brandauer, N. Fujii, L.A. Witters and L.J. Goodyear, 2007. Skeletal muscle adaptation to exercise training. *Diabetes*, 56: 2062-2069.
- Rose, A.J. and E.A. Richter, 2005. Skeletal muscle glucose uptake during exercise: How is it regulated? *Physiology*, 20: 260-270.
- Ryder, J.W., Y. Kawano, D. Galaska, R. Fahilman, H. Wallberg, M.J. Charron and J.R. Zierath, 1999. Postexercise glucose uptake and glycogen synthesis in skeletal muscle from GLUT-4-deficient mice. *The FASEB J.*, 13: 2246-2256.
- Ryder, J.W., Y.C. Long, E. Nilsson, E. Mahlapuu and J.R. Zierath, 2005. Effects of calcineurin activation on insulin-AICAR and contraction-induced glucose transport in skeletal muscle. *J. Physiol.*, 567: 379-386.
- Sakamoto, K. and L.J. Goodyear, 2002. Exercise effects on muscle insulin signaling and action. Invited Review: Intracellular signaling in contracting skeletal muscle. *J. Applied Physiol.*, 93: 369-383.
- Sandstrom, M.E., S.J. Zhang, H. Westerblad and A. Katz, 2007. Mechanical load plays little role in contraction-mediated glucose transport in mouse skeletal muscle. *J. Physiol.*, 579: 527-534.
- Somwar, R., W. Niu, D.Y. Kim, G. Sweeney, V.K. Randhawa, C. Huang, T. Ramal and A. Klip, 2001. Defferential effects of phosphatidylinositol 3-Kinase inhibition on intracellular signals regulating GLUT4 translocation and glucose transport. *J. Biol. Chem.*, 276: 46079-46087.
- Somwar, R., S. Koterski, G. Sweeney, R. Sciott, S. Djuric, C. Berg, J. Trevillyan, P.E. Scherer, C.M. Rondinon and A. Klip, 2002. A dominant-negative p38 MAPK mutant and novel selective inhibitors of p38 MAPK reduce insulin-stimulated glucose uptake in 3T3-L1 adipocytes without affecting GLUT4 translocation. *J. Biol. Chem.*, 277: 50386-50395.
- Steenberg, A., C.P. Fischer, M. Sacchetti, C. Keller, T. Osada, P. Schjerling, G. Hall, M.A. Febbraio and B.K. Pedersen, 2003. Acute interleukin-6 administration dose not impair muscle glucose uptake or whole-body glucose disposal in healthy humans. *J. Physiol.*, 548: 631-638.
- Suarez, E., D. Bach, J. Cadefau, M. Palacin, A. Zarzano and A. Guma, 2001. A novel role of neuregulin in skeletal muscle. *J. Biol. Chem.*, 276: 18257-18264.
- Sweeney, G., R. Somwar, T. Ramlal, A. Volchuk, A. Ueyama and A. Klip, 1999. An inhibitor of p38 Mitogen-activated protein kinase prevents insulin-stimulated glucose transport but not glucose transporter translocation in 3T3-L1 adipocytes and L6 myotubes. *J. Biol. Chem.*, 274: 10071-10078.
- Terada, S., T. Yokozeki, K. Kawanaka, K. Ogawa, M. Higuchi, O. Ezaki and I. Tabata, 2001. Effects of high-intensity swimming training on GLUT4 and glucose transport activity in rat skeletal muscle. *J. Applied Physiol.*, 90: 2019-2024.

- Thomson, D.M., S.T. Herway, N. Fillmore, H. Kim, J.D. Brown, J.R. Barrow and W.W. Winder, 2008. AMP-activated protein Kinase phosphorylates transcription factors of the CREB family. *J. Applied Physiol.*, 104: 429-438.
- Thorens, B., 1996. Glucose transporters in the regulation of intestinal, renal and liver glucose fluxes. *Am J Physiol Gastrointest Liver Physiol.*, 270: G541-G553.
- Tomas, E., A. Zorzano and N.B. Ruderman, 2002. Exercise effects on muscle insulin signaling and action. Exercise and insulin signaling: A historical perspective. *J. Applied Physiol.*, 93: 765-772.
- Torok, D., N. Patel, L. JeBailey, S.L. Thong, V.K. Randhawa, A. Klip and A. Rudich, 2006. Insulin but not PDGF relies on actin remodeling and on VAMP2 for GLUT translocation in myoblasts. *J. Cell. Sci.*, 117: 5447-5455.
- Toyoda, T., S. Tanaka, K. Ebihara, H. Masuzaki, K. Hosoda, K. Sato, T. Fushiki, K. Nakao and T. Hayashi, 2006. Low-intensity contraction activates the  $\alpha 1$ -isoform of 5'-AMP-activated protein kinase in rat skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.*, 290: E583-E590.
- Treebak, J.T., S. Glund, A. Deshmukh, D.K. Klein, Y.C. Long, T.E. Jensen, S.B. Jorgensen, B. Viollet, L. Andersson, D. Neumann, T. Wallimann, E.A. Richter, A.V. Chibalin, J.R. Zierath and F.P. Wojtaszewski, 2006. AMPK-Mediated AS 160 phosphorylation in skeletal muscle is dependent on AMPK catalytic and regulatory subunits. *Diabetes*, 55: 2051-2058.
- Wang, Q., R. Somwar, P.J. Bilan, Z. Liu, J. Jin, J.R. Woodgett and A. Klip, 1999. Protein kinase B/Akt participates in GLUT4 translocation by insulin in L6 myoblasts. *Molecul. Cell. Biol.*, 19: 4008-4018.
- Wei, Y., K. Chen, A.T. Whaley-Connell, C.S. Stump, J.A. Ibdah and J.R. Sowers, 2008. Skeletal muscle insulin resistance: Role of inflammatory cytokines and reactive oxygen species. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 294: R673-R680.
- Whitehead, J.P., J.C. Molero, S. Clark, S. Martin, G. Meneilly and D.E. James, 2001. The role of  $Ca^{2+}$  in insulin-stimulated glucose transport in 3T3-L1 cells. *J. Biol. Chem.*, 276: 27816-27824.
- Wijsekara, N., A. Tung, F. Thong and A. Klip, 2006. Muscle cell depolarization induces a gain in surface GLUT4 via reduced endocytosis independently of AMPK. *Am. J. Physiol. Endocrinol. Metab.*, 290: E1276-E1286, 2006
- Wojtaszewski, J.F.P., J.B. Brik, C. Frosig, M. Holten, H. Pilegaard and F. Dela, 2005. 5'AMP activated protein kinase expression in human skeletal muscle: effects of strength training and type 2 diabetes. *J. Physiol.*, 594: 563-573.
- Wright, D.C., P.C. Geiger, D. Han and J.O. Holloszy, 2006. Are tyrosine kinases involved in mediating contraction-stimulated muscle glucose transport? *Am. J. Physiol. Endocrinol. Metab.*, 290: E123-E128.
- Yang, C., R.T. Watson, J.S. Elmendorf, D.B. Sacks and J.E. Pessin, 2000. Calmodulin antagonists inhibit insulin-stimulated GLUT4 (glucose transporter4) translocation by preventing the formation of phosphatidylinositol 3,4,5-Triphosphate in 3T3L1 adipocytes. *Molecul. Endocrinol.*, 14: 317-326.
- Zierath, J.R., 2002. Exercise effects of muscle insulin signaling and action. Invited review: Exercise training-induced change in insulin signaling in skeletal muscle. *J. Applied Physiol.*, 93: 773-781.
- Zierler, K., 1999. Whole body glucose metabolism. *Am. J. Physiol. Endocrinol. Metab.*, 276: E409-E426.