



Professional article

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## REGULATION OF GLUCOSE METABOLISM BY INSULIN IN CARDIOMYOCYTES

### SUMMARY

Cardiac function is improved during ischemia by stimulating glucose metabolism and subsequent decreasing of fatty acid (FA) oxidation. The impairment of heart glucose metabolism may contribute to the heart dysfunction and cardiomyopathy.

Glucose transport is one of the first steps in insulin-stimulated glucose uptake. Glucose entry into cells is a process that requires the involvement of a carrier protein in order to facilitate the movement of glucose across the plasma membrane. In cardiomyocytes (CMY), insulin-stimulated glucose disposal is mediated *via* translocation of glucose transporters (GLUTs): GLUT4 and GLUT1.

The major mechanism by which insulin regulates GLUT4 translocation and stimulation of glycogen synthesis in CMY is through activation of the protein kinase B (PKB) *via* phosphoinositol 3 kinase (PI3-K). In addition, insulin stimulates GLUT4 translocation and increases glucose uptake in CMY *via* PI3-K independent pathway by Cbl proto-oncoprotein phosphorylation. Combined activity of both pathways is required for GLUT4 translocation in CMY.

Insulin independent pathways, like AMP-activated protein kinase (AMPK) pathway, also contributes to increased glucose uptake in CMY and PKB, and AMPK activity are inversely correlated during myocardial ischemia, although the influence of insulin on AMPK cardiac signaling would contradict previous observations. It has been reported that *via* PKB, insulin has an ability to inactivate AMPK. Inhibition of AMPK by insulin may be a contributory mechanism to the observation that cardiac FA oxidation is inhibited by insulin. Understanding how these two kinases interact at the molecular level in response to insulin may provide insights into how insulin is cardioprotective against ischemia.

**Key words:** cardiomyocytes, glucose metabolism, insulin, signaling pathways, myocardial ischemia

### MOLECULAR MECHANISMS OF INSULIN SIGNALING IN CARDIOMYOCYTES

Insulin is a hormone of endocrine pancreas whose action is initiated through the binding to and

activation of its cell surface receptor – insulin receptor (IR) (1). Insulin increases cardiac contractility and may have an antiapoptotic effect on cardiomyocytes (CMY). Insulin has a number of acute metabolic actions on the heart: promotes glycogen synthesis, promotes glycolysis and glucose

oxidation, inhibits fatty acid (FA) oxidation and it is one of the factors responsible for glucose uptake stimulation in CMY (2, 3). Hypoxia and contraction are also involved in glucose uptake stimulation in CMY (4).

Numerous studies have documented the sequence of events in insulin signal transduction cascades emanating from cell surface receptors and a great progress in understanding these cascades in CMY has been accomplished in recent years.

Activation of the IR includes autophosphorylation on numerous tyrosine (Tyr) residues and these phosphorylated Tyr provide docking sites for intracellular proteins like IR substrate 1 (IRS-1), or proto-oncoprotein Cbl which also becomes phosphorylated on Tyr residues (1).

Phosphorylated IRS-1 binds to intracellular signaling proteins containing src-homology 2 (SH2) domains. One of the proteins with SH2 domain is the lipid kinase- phosphoinositol 3 kinase (PI3-K) (1). Several distinct classes of PI3-K exist and based on their functional characteristics they have been classified into three classes (5). In CMY, the fundamental importance of IA PI3-K, most extensively characterized class of PI3-K, has been documented. IA PI3-K exists as a heterodimer consisting of regulatory subunit p85 and catalytic subunit p110. Association of the p85 regulatory subunit of PI3-K, which contains two SH2 domains, with Tyr phosphorylated IRS-1, activates the p110 catalytic subunit of the PI3-K and it is currently taught that this is the major mechanism by which insulin regulates downstream signaling via PI3-K (6).

Activated PI3-K promotes the phosphorylation of phosphatidylinositol-3,4-bisphosphate (PI(3,4)P<sub>2</sub>) and increases the cellular level of phosphatidylinositol-3,4,5-triphosphate (PI(3,4,5)P<sub>3</sub>) which recruits and facilitates the activation of downstream target Serin (Ser)/Threonin (Thr) kinase – protein kinase B (PKB) and additionally the phosphoinositol dependent kinase (PDK) (6).

PKB is subsequently phosphorylated at both Thr<sup>308</sup> and Ser<sup>473</sup> and becomes fully activated by PDK. After activation, temporal changes in PKB localization occur. From the cytoplasm PKB moves to a membrane proximal position and ultimately accumulating in the nucleus (7). Multiple binding proteins and intracellular substrates for PKB have been identified (7).

In addition, insulin stimulates GLUT4 translocation and increases glucose uptake in CMY via PI3-K independent pathway which involves Thr phosphorylation of Cbl proto-oncoprotein and its recruitment to a lipid raft-located complex containing flotillin and adapter protein CAP.

Combined activity of both pathways is required for GLUT4 translocation in CMY (1, 8, 9).

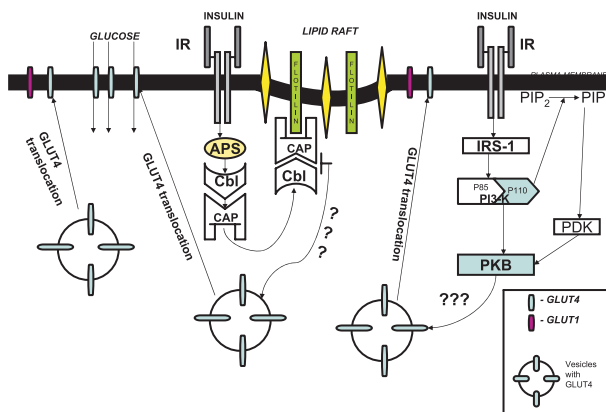


Figure 1.

*Insulin Signaling In Adult Cardiomyocytes- IR-insulin receptor; APS-adaptor protein; Cbl-proto-oncoprotein; CAP-Cbl associated protein; IRS-1-insulin receptor substrate; PI3K-phosphatidylinositol 3 kinase; PDK-phosphoinositol dependent kinase; PKB-protein kinase B; PIP2-phosphatidylinositol-3,4-bisphosphate; PIP3- phosphatidylinositol-3,4,5-triphosphate; GLUT4, GLUT1-glucose transporters 1 and 2.*

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## ROLE OF INSULIN IN GLUCOSE UPTAKE IN CMY

Glucose metabolism is the main energy source for the heart and it is very important in pathophysiological conditions such as ischemia (10). Significant amount of glucose derived ATP is used for maintaining proper cardiac functions (11). Reduced ability to produce ATP appears during development of heart failure. Change in energy metabolism occurs: reliance on glucose is increased at the expense of FA. As a result of reduced utilization of FA, the hypertrophic myocardium has increased consumption of glucose (12). Cardiac function is improved during ischemia by stimulating glucose metabolism and subsequent decrease in FA oxidation, and the impairment of heart glucose metabolism in diabetes mellitus may contribute to the mechanical dysfunction and cardiomyopathy observed in this disease (10).

Glucose transport is one of the first steps in insulin stimulated glucose uptake and it is a rate-limiting step in whole glucose metabolism. Glucose entry into cells is a process that requires the involvement of a carrier protein in order to facilitate the movement of glucose across the plasma membrane of a cell and they are identified as glucose transporters (GLUTs) (1). In CMY, insulin stimulated glucose disposal is mediated via translocation of GLUT4 from intracellular storage

site to the plasma membrane and GLUT1 firstly mediates basal, rather than insulin mediated glucose uptake (8). Impairment of the mechanisms responsible for this translocation leads to insulin resistance (8) and it is well-known that basal as well as insulin stimulated glucose transport in the diabetic heart is reduced due to either insulin resistance or insulin deficiency (10).

Insulin independent pathways also contribute to increased glucose uptake in CMY (12). AMP-activated protein kinase (AMPK) as a signaling intermediate, is a major regulator of cardiac energy substrate use, stimulating both glucose uptake and utilization of the FA oxidation (2). The activity of AMPK is actually regulated by changes in the intracellular ratios of ATP and AMP; CP and creatine, as well as changes in intracellular pH (13). Pharmacological activation of AMPK can be achieved and increased glucose transport independent from insulin signaling (13).

Since inhibition of PI3-K, using chemical inhibitors of PI3-K, blocks insulin-stimulated GLUT4 translocation and glucose uptake, it has been suggested that activation of PI3-K is necessary for insulin-stimulated glucose uptake (1).

PKB activated via PI3-K seems to be critical for insulin effects on a wide range of processes such as GLUT4 translocation, stimulation of glycogen

synthesis and protein synthesis, and gene expression (14).

It has been shown that PKB and AMPK activities are inversely correlated during myocardial ischemia. AMPK has been reported to increase glucose uptake following ischemia in CMY and PKB has been inactivated. Although the influence of insulin on AMPK cardiac signaling would contradict previous observations, it has been reported that via PKB, insulin has an ability to inactivate AMPK, although the mechanism is currently unknown (11). It is possible that decreased phosphorylation of AMPK by PKB occurs either by inactivation of the upstream AMPK kinase or by stimulation of the AMPK phosphatase. Alternatively, activated PKB may directly phosphorylate AMPK on the separate site, which may prevent subsequent phosphorylation by AMPK kinase. Inhibition of AMPK phosphorylation, by insulin may be a contributory mechanism to the observation that cardiac FA oxidation is inhibited by insulin (2, 11).

There is a cross talk between the AMPK and PKB pathways, understanding how these two kinases interact at a molecular level in response to insulin, which may provide insights into why and how glucose and insulin are cardioprotective against ischemia (11).

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## REGULACIJA METABOLIZMA GLUKOZE INSULINOM U KARDIOMIOCITIMA

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### SAŽETAK

Funkciju srca, tokom stanja ishemije, moguće je poboljšati stimulacijom metabolizma glukoze i usled toga nastalim smanjenjem oksidacije masnih kiselina (MK). Poremećaji u metabolizmu glukoze uzrokuju poremećaje u radu srca i dovode do kardiomiopatije.

Transport glukoze u ćelije jedan je od prvih koraka u insulinom stimulisano preuzimanju glukoze u ćelije srca-kardiomiocite (CMY). Insulinom stimulisano ulazak glukoze u ćelije odvija se uz pomoć proteina nosača koji olakšavaju transport glukoze kroz ćelijsku membranu i oni su identifikovani kao glukozni transporteri (GLUT). U CMY lokalizovani su GLUT1 i GLUT4. Insulinom stimulisano preuzimanje glukoze i sinteza glikogena u CMY, uglavnom se odvijaju signalnim putem, koji uključuje aktivaciju protein kinaze B (PKB) preko fosfoinozitol 3 kinaze (PI3K). Takođe, insulin stimuliše translokaciju GLUT4 u CMY signalnim putem pomoću fosforilacije Cbl proto-onkoproteina. Aktivnost oba signalna puta neophodna je za translokaciju GLUT4 u CMY.

Insulin nezavisni put, takodje je uključen u transport glukoze u CMY. On se ostvaruje preko aktivacije AMP-om aktivirane protein kinaze (AMPK), koju inaktiviraju insulinom aktivirana PKB, što dovodi do inhibicije oksidacije MK u CMY. Aktivnosti enzima PKB i AMPK su u inverznoj relaciji tokom ishemije srca. Rasvetljavanje molekularnog mehanizma interakcije AMPK i PKB pod delovanjem insulina u CMY doprineće objašnjenju protektivne uloge insulina u procesima ishemije srca.

**Ključne reči:** kardiomiociti, metabolizam glukoze, insulin, signalni putevi, ishemija srca