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Changes in the GLUT4 Expression by Acute Exercise, Exercise Training and Detraining in Experimental Models

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Abstract

There is a direct correlation between an increase in insulin sensitivity and increased cell surface GLUT4 content. Acute exercise promotes glucose-transport stimulation that is independent of AMPK and CaMKII insulin-signaling. In turn, post-exercise glucose uptake occurs through changes in components of the insulin signaling cascade involving alterations in downstream mediators such as TBC1D1, TBC1D4/AS160 and p38 MAPK. However, the effects of acute exercise can be reversed within 18–24 hours and appear to be dependent on muscle glycogen levels. Exercise training results in adaptations that facilitate insulin-mediated glucose uptake and are regulated by different mechanisms. It leads to changes in gene expression and greater blood flow and signaling and changes in GLUT4 protein exocytosis and endocytosis. But when exercise training is discontinued GLUT4 tend to return to baseline levels. We have demonstrated in our laboratory that one-week detraining is sufficient to reduce GLUT4 in the heart and adipocytes while this same effect was seen in the gastrocnemius muscle within 2 weeks of training. The present study aimed to review how acute exercise, exercise training, and detraining affect mainly GLUT4 translocation to the insulin-sensitive cell surface.

Keywords: Glucose transporter type 4, Acute exercise, Exercise training, Insulin resistance

Introduction

Glucose transporter 4 (GLUT4) is mainly expressed in insulin-sensitive cells such as adipose tissue and skeletal muscle cells and cardiomyocytes [1]. The main function of this protein is to facilitate glucose uptake into these cells and maintain control of blood glucose levels. GLUT4 protein in the basal state is stored in intracellular vesicles and their translocation to the plasma membrane occurs mainly by insulin action [2] or through insulin-independent pathway during muscle contraction. Insulin resistance may result from impaired insulin signal transduction leading to decreased GLUT4 translocation [3,4] and/or diminished capacity for GLUT4 synthesis [5]. In addition, other factors may also contribute to insulin resistance including reduced blood flow and muscle mass, as well as changes in the proportion of muscle fiber types and in intramuscular oxidative pathways [6].

Acute exercise leads to increased glucose transport even in the presence of very low levels of circulating insulin [7]. During muscle contraction two mechanisms contribute to increasing GLUT4 translocation to the cell surface: activation of the 5'-adenosine monophosphate-activated protein kinase (AMPK) [8] and calcium/calmodulin-dependent protein kinase (CaMK) II [9]. When the acute effects of exercise on glucose transport have disappeared, there are changes in insulin sensitivity [10] that appear to be dependent on muscle glycogen levels [11]. In particular, TBC1D1, TBC1D4/AS160 and p38 MAPK that remain phosphorylated for hours after exercise [12,13], probably activated by residual AMPK of muscle contraction, may contribute to increased insulin sensitivity after exercise. However, these effects may be reversed within 18–24 hours [14,15].

Aerobic [16] and/or resistance exercise training [17] are known to improve insulin sensitivity and have other beneficial effects on blood pressure, heart rate, heart rate variability, and chemoreceptor and arterial baroreceptor reflex sensitivity [18,19]. Given these benefits exercise training has been successfully used in the treatment of diabetic patients [20]. This adaptation is a result of marked increase in glucose transport, which is mostly attributed to greater mobilization of GLUT4-containing vesicles to the cell surface through insulin-dependent [21] and insulin-independent pathways [11]. Besides this effect on GLUT4 trafficking, exercise training also increases transcription factors involved in GLUT4 gene expression and, consequently, increased intracellular GLUT4 stores [22]. Exercise training has also anti-inflammatory effects, which can modify insulin-mediated glucose uptake [23]. However, these benefits are gradually lost after cessation of exercise training. The time course of this phenomenon is still controversial and these changes are affected by several factors. The purpose of this study was to review aspects related to GLUT4 modulation and translocation to the cell surface in response to acute exercise, exercise training, and detraining focusing on studies using experimental models.

GLUT4 and Insulin Resistance

Glucose transport in mammalian tissues occurs primarily by facilitated diffusion, a process that uses a carrier protein for transport of a substrate across a membrane into cells. These facilitative glucose transporters (GLUTs) are a family of proteins that were denominated in chronological order of characterization [24] and are expressed in tissue and cells with different regulatory and kinetic properties that reflect their roles in cellular metabolism.

GLUT4 is the most abundant glucose transporter in the body [1]. In baseline status, i.e. unstimulated cells, GLUT4 is stored intracellularly

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in specialized compartments called GLUT4 storage vesicles (GSV), which participate in the GLUT4 cycles to and from the plasma membrane, through slow exocytosis from GSV, fast endocytosis from the cell surface [25-28]. The synthesis of GLUT4 occurs through the expression of the gene SLC2A4 (Solute carrier 2a4 gene that codifies GLUT4 protein) [29] and can determine the amount of protein stored in the GSV.

The main action of insulin is to increase glucose uptake in insulin-sensitive tissues, balancing blood and intra-cellular glucose levels, through increased GLUT4 content at the cell surface. The insulin-mediated glucose uptake is carried out by binding this hormone with its membrane receptors and transmitting its signal to the interior of cells. The insulin-mediated GLUT4 translocation to plasma membrane includes the phosphatidylinositol-3-kinase (PI3K) complex. The c-Cbl-associating protein (CAP/c-Cbl) pathway and its regulation of TC10 downstream also seems involved in GLUT4 translocation to plasma membrane [30]. Decreased insulin action on insulin-sensitive tissues may occur by several mechanisms: low concentration and/or kinase activity of proteins related to insulin signaling, leading to insufficient recruitment of GLUT4 to the plasma membrane, despite normal GLUT4 expression [4]; low capacity of GLUT4 synthesis, even if the rate of translocation of vesicles containing this protein is preserved [5]; and, changes in the rate of GLUT4 exocytosis and endocytosis [31], determined by failure of insulin-derived signals. Thus, insulin resistance is characterized by a reduction of the biological effect of this hormone [32]. Studies have shown a marked relation between insulin resistance and cardiovascular risk factors, among them obesity and sedentary lifestyle [33,34].

The consumption of a high fat diet, even for a short period of time, leads to insulin resistance by reducing the insulin signaling pathway [35]. Thus, experimental models of obesity have been used to elucidate the pathophysiological mechanisms that contribute to the genesis of insulin resistance. High fat diet induced-obesity model has shown that rats subjected to a high fat diet for 3 months developed weight gain, especially epididymal fat, hyperglycemia, hyperinsulinemia and insulin resistance [36]. The decrease of insulin action was related to protein tyrosine phosphatase 1B (PTP1B). Its increased expression and activation inhibits tyrosine phosphorylation of insulin receptors and their substrates. This is reinforced by experiments with PTP1B knockout mice, which showed high tyrosine phosphorylation of insulin receptors and their substrates, and improved insulin sensitivity as compared to wild mice [37].

Decreased insulin-mediated glucose uptake related to obesity is frequently accompanied by an inflammatory state, characteristic of this condition [38]. The increase in adipocyte size is associated with the release of free fatty acids from the adipose tissue and increased production of reactive oxygen species [39], which may promote the production and release of proinflammatory cytokines such as interleukin-6 (IL-6), serum amyloid A protein (SAA) and monocyte chemoattractant protein-1 (MCP-1) [40]. Cytokines released into the bloodstream act as mediators in the migration of monocytes into the adipose tissue [41]. After migration, these cells differentiate into macrophages, which release cytokines, especially tumor necrosis factor-alpha (TNF-α) [42]. When the signaling pathway of TNF-α is activated, intermediate substrates, like serine kinase c-Jun NH2-terminal (JNK) influence the phosphorylation of IRS1, decreasing the insulin signal transduction [43]. Increased TNF-α expression leads to decreased phosphorylation of insulin receptors and their substrates, contributing to the genesis of insulin resistance related to obesity [44]. In addition to impaired insulin signal transduction, this cytokine also inhibits the transcription factors of the SLC2A4 gene [42], reducing the expression and, consequently, the intracellular protein stores.

Moreover, there is data suggesting that autonomic changes modulate hormonal and immune function, by inducing release of bioactive molecules which are probably involved in the development of cardio metabolic profile changes [45,46]. In fact, Hellstrom examined evidence that development of a diverse group of diseases, such as diabetes, hypertension, and heart disease, is favored by increased sympathetic neural outflow resulting in endothelial dysfunction, dyslipidemia,

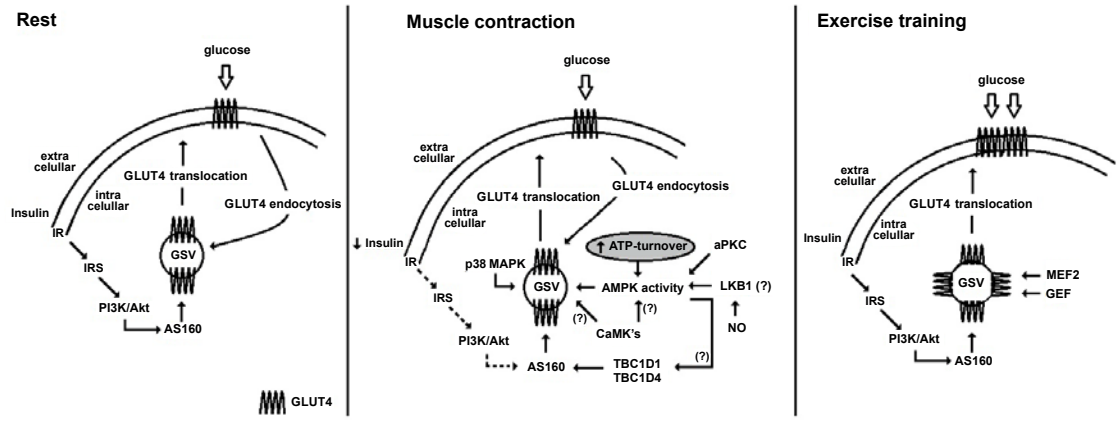

Figure 1: Putative metabolic pathways leading to changes in GLUT4 expression in the skeletal muscle following acute exercise and exercise training. Most GLUT4 is stored in intracellular vesicles (GSV = GLUT4 storage vesicles) in the muscle at rest and their translocation to the plasma membrane occurs mainly by insulin action; after glucose transport, GLUT4 is endocytosed from the cell surface. Muscle contraction leads to an increase in glucose uptake by the working muscle evidenced by gain of GLUT4 at cell surface, which does not involve any signals proximal to the insulin receptor substrate (IRS), phosphatidyl inositol 3-kinase (PI3K) or Akt. Instead, this gain occurs by increased AMPK activity that stems from increased ATP-turnover. CaMK protein is also involved in GLUT4 trafficking, although it is unclear whether CaMK is AMPK-dependent or not. Together, these mechanisms increase cellular glucose uptake even in the presence of very low circulating levels of insulin. When the acute effects of exercise on glucose transport have disappeared there are changes in insulin sensitivity that appear to be dependent on TBC1D1, TBC1D4/AS160 and p38 MAPK, which remain phosphorylated for hours after exercise, probably activated by residual AMPK of muscle contraction. As for chronic effects of exercise, evidence suggests there are adaptations in the pre-translational and post-translational levels, in particular MEF2 and GEF, which was shown to increase intracellular GLUT4 and improve insulin sensitivity.

inflammation and insulin resistance [47]. In female fructose fed rats we observed increased arterial pressure and a positive correlation between insulin resistance and cardiac vagal effect attenuation [48]. Recently, we tested the time course of the effect of fructose given in the drinking water in mice, showing: 1) insulin resistance, increased plasma levels of cholesterol, triglycerides and leptin after 60 days of fructose; 2) increase in systolic and mean arterial pressure associated with cardiac and vascular sympathetic increased modulation and spontaneous baroreflex attenuation from day 15 of fructose. The key finding was that dysfunction of cardiovascular autonomic control occurred prior to any metabolic changes [49].

In fact, besides the impairment of extra and intracellular glucose homeostasis, insulin resistance is commonly associated with arterial hypertension and is *per se* a predictor of cardiovascular events [50]. Katayama et al. (1997) showed impaired glucose tolerance in spontaneously hypertensive rats (SHR). Interestingly, GLUT4 in the plasma membrane of the gastrocnemius was elevated in SHR, as compared to control rats (Wistar). However, with 20 weeks of age, SHR normalized glucose tolerance and GLUT4 expression, as compared with Wistar rats. It is possible that the impaired glucose tolerance of SHR have other causes not related to GLUT4, or at least, that GLUT4 is not the only factor contributing to metabolic abnormalities seen in this animal model [51]. This may involve the transport of glucose in the plasma membrane and not the increased levels of GLUT4 at the cell surface. This phenomenon may be explained in part by glucose not binding to GLUT4 on the cell surface, slowing its entry into these cells [52].

Effects of Acute Exercise and Training on GLUT4

Exercise can induce acute metabolic benefits that are different from those induced by training (Figure 1). An acute bout of exercise, through muscle contraction, requires several metabolic changes to supply adenosine triphosphate (ATP), including the use of muscle glycogen. The energy demands of an acute exercise require increased glucose uptake in muscle cells, mainly to muscular glycogen resynthesizing. Thus, acute exercise leads to changes in glucose uptake that occur through insulin-independent pathways [53,54] to ensure an adequate glucose supply to muscle cells. Moreover, acute exercise induces adjustments in the GLUT4 cycling as increased GLUT4 exocytosis and/or lower GLUT4 endocytosis [55,56].

It was observed following the acute effects of exercise a persistent increase in glucose transport for hours that appears to be dependent on the working muscle, [57]. This effect is associated to increased GLUT4 translocation, probably stimulated by downstream TBC1D1 and TBC1D4/AS160 phosphorylation, which is activated by the remaining AMPK activity of muscle contraction [12,13]. Increased glucose uptake after muscle contraction can also be explained by an acute increase in p38 MAPK activation, which remained increased even 3 h after muscle contraction in the soleus and epitrochlearis muscles of rats [58]. Together, TBC1D1, TBC1D4/AS160 and p38 MAPK remain phosphorylated for hours after exercise and may contribute to increased insulin sensitivity after exercise.

In contrast, adaptations in the pre-translational and post-translational levels are observed as chronic effects of exercise. Hence, over time, it leads to enhanced insulin action, either increased expression and activity of insulin-signaling protein kinases and other insulin-independent pathways such as increased GLUT4 transcription factors, resulting in an increase in intracellular GLUT4 stores.

Acute Effects of Exercise

Some studies suggest there are different intracellular pools of GLUT4, one stimulated by insulin and one stimulated by exercise [59,60]. Furthermore, studies in insulin receptor knockout mice showed exercise-mediated increase in glucose uptake and glycogen synthase activity in vivo as compared to animals in the control group [61]. These facts supports the idea that the acute exercise can activate molecular pathways of GSV mobilization that are not, at least in part, insulin-dependent. In insulin-independent pathways, two proteins have a major role in the mobilization of GLUT4: AMPK and CaMKII.

AMPK is activated by AMP-binding resulting from an increase in ATP-turnover and evidence from experiments using an AMP-mimetic compound TBC1D1, 5-aminoidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR), shows that AMPK may be sufficient to increase glucose transport [62,63], and this effect is related to muscle fiber type [64,65]. Kurth et al. (1999) have showed increased glucose uptake observed with AMPK activation by AICAR in perfused rat hindlimb muscles is due to an increase in the translocation of GLUT4 to surface membranes [8].

One of the mechanisms for GLUT4 trafficking may be the interaction of AMPK with AS160, a protein of the PI3K pathway [66]. The administration of AICAR was found to be associated with increased AS160 phosphorylation together with increased AMPK activity. In contrast, after the administration of Wortmannin, a PI3K inhibitor, AS160 activity was completely inhibited [67]. The same was evidenced in other studies [68]. Thus, it is admissible to think that acute exercise-induced increased AMPK activity can be involved with AS160 phosphorylation, and in this case, it is independent of the insulin signaling.

Besides the acute effects of exercise on AMPK-induced GLUT4 trafficking, a positive correlation was observed between acute exercise-induced increase in CaMKII levels and glucose transport in muscle cells [69]. Evidence obtained using subcontraction concentrations of caffeine, which release Ca^{2+} from the sarcoplasmic reticulum and activate CaMKII, has shown that inhibition of this protein prevents an increase in Ca^{2+} -induced glucose transport [70,71]. Although studies [72,73] have shown that caffeine-stimulated glucose transport is highly AMPK-dependent, there is evidence demonstrating that contraction-induced skeletal muscle glucose uptake involving Ca^{2+} /calmodulin-dependent protein kinase is independent of AMPK [74]. Witczak et al. later demonstrated using a CaMKII inhibitory peptide transfected into tibialis anterior muscles by in vivo electroporation that this peptide did not either change GLUT4 expression or impair contraction-induced increases in the phosphorylation of AMPK or TBC1D1 and TBC1D4 on AS160 phosphorylation [75]. This evidence supports that CaMKII plays a critical role in the regulation of contraction-induced glucose uptake.

Other proteins such as aPKC (atypical PKC) have been investigated as modulators of GLUT4 translocation in response to acute exercise [13]. Chen et al. (2002) found that glucose uptake in L6 muscle cells (cultured muscle fibers) resulted from protein kinase activation including the isoforms PKC-ζ and PKC-λ among others. A possible explanation for this association would be an interaction of PKC with the AMPK signaling pathway that in turn stimulates GLUT4 translocation [76].

Nitric oxide (NO) has also been investigated as a potential factor to induce increased glucose uptake into muscle cells as an adjustment to acute exercise. Roberts et al. (1997) used nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO, in the gastrocnemius muscle of

Sprague-Dawley rats undergo acute exercise and acute exercise plus L-NAME. After the one bout of exercise (treadmill exercise for 45 min at high intensity), a group received L-NAME in the drinking water (1g/mL). The group that only exercised without L-NAME showed increased glucose uptake and GLUT4 in the plasma membrane of the gastrocnemius muscle as compared to the control group. The group that exercised plus L-NAME did not show any changes in either glucose uptake or the amount of GLUT4 at the cell surface compared to controls [77]. One possible explanation for the effect of NO on insulin-mediated glucose uptake would be an interaction with the AMPK pathway [78], in particular α2-AMPK site that seems to have a more relevant role in the regulation of glucose transport than α1-AMPK [79,80].

Another aspect may be related to S-nitrosation of the proteins involved in the insulin signaling cascade, which reduces insulin action [81-82]. This hypothesis is supported by experiments in Wistar rats submitted to high-fat diet-induced obesity, showing high levels of iNOS and S-nitrosation of IR β subunit, IRS1 and Akt, changes that were reversed by acute exercise. The improvements were ascribed to increased AMPK activity that negatively modulates iNOS levels and subsequent S-nitrosation of the proteins involved in insulin signaling [83].

Thus, it seems clear that several insulin-independent pathways are activated in response to acute exercise. The major pathways are those mediated by AMPK protein activation and are dependent on the muscle fiber type.

Chronic Effects of Exercise

Exercise-induced chronic effects on GLUT4 occur primarily through pre-translational mechanisms, which favor the increase of intracellular stores of GLUT4 protein. Besides this beneficial adaptation, exercise training also determines changes in molecular pathways that induce GLUT4 translocation. The changes of exercise training can also be observed in the total GLUT4 protein levels, i.e. GLUT4 expression at cell surface enriched of microsomal fraction.

In this context, Neufer et al. (1992) studied Wistar rats that were submitted to exercise training on a treadmill for 1 day, 1 week or 6 weeks (1.9 km/h, 2 hours a day, 6 days a week). Biopsy samples of soleus and vastus lateralis muscles (red and white fibers) were examined and compared with those of animals that did not exercise. There was no difference in the amount of GLUT4 in the muscles after 1 day or 1 week of exercise, suggesting that exercise load was insufficient. Exercise training for 6 weeks resulted in increased levels of GLUT4 in the plasma membrane of soleus (1.4 times) and in the oxidative muscle, but not in the glycolytic fibers of the vastus lateralis muscle (1.7 times). Probably cell requirements related to cellular oxidative capacity modulates GLUT4 translocation, as mentioned above. Another explanation would be that dynamic aerobic training was not enough to exercise or even activate glycolytic fibers of the vastus lateralis muscle [84].

To study different types of exercise training, 6-week-old Sprague-Dawley rats were submitted to resistance training (3 sets of lifting of 75% one-repetition maximum, 10 repetitions, 3 days a week for 12 weeks) or aerobic training (1.9 km/h, 15% incline, 45 min a day, 3 days a week for 12 weeks) and compared with a sedentary group. After training periods, rats received an insulin infusion and samples of the soleus, plantar and oxidative and glycolytic fibers of the gastrocnemius and quadriceps muscles were collected. There was a similar increase in glucose uptake in the groups submitted to resistance and aerobic training and both had higher glucose uptake than the sedentary group. There was a higher rate of glucose transport in rats subjected to the resistance training, probably due to an increase in the amount of GLUT4 at the cell surface [17].

Our group found a similar result in SHR and WKY rats. There was no difference in GLUT4 content in the plasma membrane of the heart, gastrocnemius muscle and epididymal fat of SHR submitted to exercise training (treadmill, 1h/day, 5 days/week for 10 weeks) as compared to WKY rats [85]. Song et al. (1998) reported no difference in GLUT4 expression in samples of gastrocnemius muscle in response to swim training for 4 weeks between stroke-prone spontaneously hypertensive (SHRSP) rats, which are characteristically resistant to insulin, and WKY rats [86].

Luciano et al. (2002) described improved glucose tolerance and increased total GLUT4 expression in the gastrocnemius muscle of Wistar rats submitted to a 6 week regimen of swimming [21]. These changes were attributed to an increase in insulin signaling due to greater phosphorylation of IRS1 and IRS2 substrates. PI3K kinase activity associated with IRS1 and IRS2 as well as serine phosphorylation of Akt protein also increased in trained animals, as compared to sedentary ones. Chibalin et al. (2000) reported that Wistar rats submitted to swim training had increased GLUT4 expression in the epitrochlearis muscles. These adaptations resulted in part from increased tyrosine phosphorylation of insulin receptors and its IRS1 and IRS2 substrates, as well as its association with PI3K protein [87]. Akt activity also increased after exercise training. On the other hand, only the expression levels of insulin receptors were high. Interestingly, the expression of IRS1 substrate after 5 days of swim training was reduced, despite an increased IRS1 phosphorylation; as IRS1 and IRS2 responded differently to exercise training; these two molecules may have different roles and regulations in insulin signaling.

Data from our group showed that chronic treatment with L-NAME induced not only hemodynamic impairment but also insulin resistance, which was not reversed after exercise training at the baseline [88]. These results suggest an important role of NO not only in the development of insulin resistance at baseline, but also in adaptive responses to exercise training.

Another hypothesis for increased GLUT4 at the cell surface in response to exercise training may be effects of galanin. Galanin is a neuroendocrine peptide and an important hormone in insulin sensitivity modulation [89]. He et al. (2011) used an antagonist of galanin (M35) and a regimen of 4-week swim training (60 min per day) to determine whether increased galanin would elevate GLUT4 concentration in the plasma membrane of hind-limb skeletal-muscle of streptozotocin-induced diabetic rats. It was shown that plasma galanin levels after swim training were higher as compared with the sedentary control group. The antagonization of galanin reduced glucose disappearance rate of euglycemic hyperinsulinemic clamp tests when compared with diabetic controls, but swimming enhanced insulin sensitivity in all trained groups. Moreover, M35 treatment reduced GLUT4 concentration and mRNA levels compared with the diabetic control group. In contrast, all trained groups showed an increase of the GLUT4 mRNA expression and GLUT4 protein level of hind-limb skeletal-muscle [90]. Considering these results, endogenous galanin may enhance glucose disappearance rate by increased GLUT4 content in the skeletal muscle's plasma membrane.

Finally, the increase in GLUT4 at the cell surface in response to exercise training may also suggest an effect on GLUT4 mRNA. SLC2A4 gene transcription is activated by two main factors: myocyte enhancer

factor 2 (MEF2) [91] and GLUT4 enhancer factor (GEF) [92]. The rest of MEF2 is related to class II histone deacetylase 5 gene isoform (HDAC5), a molecule that represses SLC2A4 gene transcription. On the other hand, AMPK protein activation through muscle contraction requires HDAC5 phosphorylation resulting in MEF2 release [93]. Hence, another interesting aspect of increased AMPK activity in response to exercise, although not directly related to GLUT4 trafficking to the plasma membrane, is its interaction with transcriptional activation of SLC2A4 gene via MEF2D activity [22], which could increase intracellular GLUT4 stores. However, it is unclear whether a single bout of exercise is strong enough to functionally affect GLUT4 intracellular stores or it is a chronic positive effect of exercise training.

Collectively, data suggest that dynamic aerobic training and/or resistance lead to increased GLUT4 expression levels in the insulin-sensitive cells. This fact increases the uptake of insulin-mediated glucose, providing an appropriate glycemic balance between tissue and plasma in the resting state.

Effects of Exercise Detraining on GLUT4

Although exercise training is beneficial, increasing insulin sensitivity, this adaptation is transient. When this practice is stopped or the stimulus of exercise training is not sufficient to lead to further physiological adaptations, the trend is for the transport of glucose to return back to baseline values [94,95]. It has been demonstrated that the metabolic improvements decline with different degrees during detraining time course [96]. However, most of the studies were made with athletes or healthy subjects and there are only few works focusing on the association of detraining and disease.

Furthermore, in our laboratory, using SHR, we found that after 1 and 2 weeks of exercise detraining, animals remain with the improvement in insulin sensitivity (whole-body insulin sensitivity measured by the insulin tolerance test – ITT) and lower blood pressure levels determined by exercise training on the treadmill for 10 weeks. However, the reversal of the increased expression of GLUT4 occurred after one week of detraining in the heart and adipose tissue, and after two weeks in skeletal muscle (gastrocnemius) [95]. Other authors showed that one week is sufficient to reverse the benefits on GLUT4 in skeletal muscle [84]. However, the difference might be the type of fiber used. This hypothesis suggests that different molecular mechanisms governing the process of exercise detraining is mediated by a tissue-specific modulation of expression of GLUT4. Neufer et al. (1992) analyzed samples of the soleus and vastus lateralis (red fibers) which are essentially oxidative [97], while our research was performed with samples of the gastrocnemius with no separation of white and red fibers. Other explanation would be that the effect of exercise detraining is dependent on the extension of the exercise training. Our training regimen was based on 5 days per week for 10 weeks (50 sessions). Neufer et al. (1992) used 6 days a week for 6 weeks (36 sessions).

To study the effects of exercise detraining on insulin receptors the epitrochlearis muscle of Fischer rats was analyzed 29 and 53 hours after cessation of 3-weeks of voluntary wheel running. GLUT4 protein levels in the plasma membrane of epitrochlearis muscle returned to sedentary levels (reduction of 29%) 53 hours after the cessation of the physical activity. This fact could be partially explained by decrease in tyrosine phosphorylation and protein level of IR (β-subunit) as well as Akt phosphorylation activity. All these variables also returned to sedentary levels after the same period of detraining (53 hours) [98].

Reynolds et al. (2000) observed the effects of swimming (5 days or 5 weeks) or treadmill (5 weeks), followed by 1 or 2 days of detraining period [99]. The amount of GLUT4 in the plasma membrane remained high during 24 hours after training in all groups trained, but returned to baseline levels 48 hours after the last bout of exercise, regardless of the period or exercise regimen. One possible explanation is an adaptation of the GLUT4 half-life, which can be regulated by pre- and post-translational mechanisms. The increased half-life also results from a decrease in the rate of degradation of the protein; in this case, the rate of reversal of the adaptation would occur more rapidly than its development [94].

Mostarda et al. (2009) evaluated the effect of 3-week detraining after 10 weeks of training in streptozotocin-induced diabetic rats. Despite evidence showing that GLUT4 expression returns to pre-training levels within 48 hours to 1 week [84,95,99], they found that, after 3 weeks of detraining, glucose levels remained similar to those in the trained group [100]. These data show that glucose clearance cannot be explained only by GLUT4 expression levels as it was demonstrated.

Although the biological effects of exercise detraining on glucose metabolism are evident, the molecular mechanisms of this down-regulation have not been totally understood, especially on insulin-independent pathways. Studies are needed to determine the involvement and time-course of these mechanisms to lead to effective prevention management and treatment of insulin resistance and its consequences.

Conclusions

Based on the assumptions presented, we conclude that: 1) acute exercise increases GLUT4 expression by parallel insulin signaling pathways; 2) most mechanisms implicated involve activation of AMPK and/or CaMKII; 3) AMPK-mediated glucose uptake is higher in fast-twitch muscle fibers; 4) increased glucose uptake following acute exercise may also involve changes in GLUT4 exocytosis and endocytosis rates; 5) improved insulin sensitivity following acute exercise results from adaptations linked to TBC1D1, TBC1D4/AS160 and p38 MAPK, which remain active for hours after muscle contraction cessation; and finally 6) adaptations in the pre-translational and post-translational levels are regarded as chronic effects of exercise. On the other hand, cessation of exercise training leads to a decrease in the amount of GLUT4 in the plasma membrane in animal models. We conclude that: 1) training-induced beneficial effects on GLUT4 expression can be reversed within 48 h to 1 week following training cessation; 2) the effects of detraining on GLUT4 expression may involve pre- and post-translational mechanisms; and 3) glucose clearance cannot be explained only by GLUT4 levels, at least not in insulin-resistant rats.

This review shows the importance of acute and chronic exercise in changes in GLUT4 expression by insulin-independent pathways, which remain intact even in individuals with insulin resistance.

Competing Interests

The authors declare that they have no competing interests.

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