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G. Florholmen · G. H. Thoresen · A. C. Rustan ·
J. Jensen · G. Christensen · V. Aas

Leukaemia inhibitory factor stimulates glucose transport in isolated cardiomyocytes and induces insulin resistance after chronic exposure

Abstract *Aims/hypothesis:* Hypertrophic and failing hearts have increased utilisation of glucose, but also develop insulin resistance and reduced ability to produce ATP. Increased levels of the IL-6-related cytokine leukaemia inhibitory factor (LIF) are found in failing hearts, and we have recently shown that LIF reduces ATP production in isolated cardiomyocytes. In the present study we investigated effects of LIF on glucose metabolism, and how LIF-treated cells respond to insulin stimulation. *Methods:* Cardiomyocytes were isolated from adult Wistar rats by collagen digestion, maintained in culture for 48 h, and then treated with 1 nmol/l LIF. *Results:* Acute LIF treatment increased deoxyglucose uptake compared with controls, but no additive effect was observed in cardiomyocytes treated with LIF and insulin. The phosphatidylinositol 3-kinase inhibitor wort- mannin did not affect LIF-induced glucose uptake. LIF had no effect on AMP-activated protein kinase phosphorylation. Cardiomyocytes treated with LIF for 48 h did not respond to insulin by increasing deoxyglucose uptake and showed a reduced insulin-mediated uptake of oleic acid and formation of complex lipids compared with control cells. Chronic LIF treatment increased gene expression of the suppressor of cytokine signalling (*Socs*) 3 and reduced expression of solute carrier family 2, member 4 (*Slc2a4*, previously known as glucose transporter 4 [*Glut4*]). In line with these observations, chronic LIF treatment reduced insulin-mediated phosphorylation of both Akt/protein kinase B (PKB) and glycogen synthase kinase (GSK)-3. *Conclusions/interpretation:* Acute LIF treatment increased glucose uptake in isolated cardiomyocytes by a pathway different from that of insulin. Chronic LIF treatment induced insulin resistance, possibly mediated by altered expression of *Socs3* and *Slc2a4*, and impaired insulin-mediated phosphorylation of GSK-3 and Akt/PKB.

Keywords Glucose uptake · Insulin resistance · Isolated cardiomyocytes · Leukaemia inhibitory factor

Abbreviations AMPK: AMP-activated protein kinase · ARC: American Radiolabeled Chemicals · ASM: acid soluble metabolite · CD36: CD36 antigen · DAG: diacylglycerol · ERK: extracellular signal regulated kinase · GSK: glycogen synthase kinase · LIF: leukaemia inhibitory factor · MEK: mitogen-activated ERK-activating kinase · PI: phosphatidylinositol · PKB: protein kinase B · SLC2A1: solute carrier family 2 (facilitated glucose transporter), member 1. Previously known as glucose transporter 1 (GLUT1) · SLC2A4: solute carrier family 2 (facilitated glucose transporter), member 4. Previously known as glucose transporter 4 (GLUT4) · SOCS: suppressor of cytokine signalling · TAG: triacylglycerol

G. Florholmen (✉) · G. Christensen
Institute for Experimental Medical Research,
Surgical building 4th floor,
Ullevaal University Hospital,
0407 Oslo, Norway
e-mail: geir.florholmen@medisin.uio.no
Tel.: +47-23-016800
Fax: +47-23-016799

G. Florholmen · G. Christensen
Center for Heart Failure Research,
University of Oslo,
Oslo, Norway

G. H. Thoresen · A. C. Rustan · V. Aas
Department of Pharmaceutical Biosciences,
School of Pharmacy,
University of Oslo,
Oslo, Norway

J. Jensen
National Institute of Occupational Health,
Oslo, Norway

V. Aas
Faculty of Health Sciences,
Oslo University College,
Oslo, Norway

Introduction

Reduced ability to produce ATP is a hallmark change during development of heart failure [1]. One important change in

energy metabolism is the increased reliance on glucose at the expense of fatty acids. As a result of reduced utilisation of fatty acids, the hypertrophic myocardium has increased consumption of glucose [2].

Increased levels of the IL-6-related cytokine leukaemia inhibitory factor (LIF) is observed in failing hearts, and it is believed that LIF participates in the progression to heart failure [3, 4]. We recently showed that chronic LIF treatment reduced the ability to produce ATP and reduced cellular content of ATP in isolated cardiomyocytes [5]. We [5] and others [6] have found increased glucose uptake following LIF treatment for 48 h. It is not known whether acute LIF treatment has similar effects on the uptake of glucose. Insulin increases glucose uptake in cardiomyocytes by translocating the solute carrier family 2, member 4 (SLC2A4, previously known as glucose transporter 4 [GLUT4]) to the plasma membrane by activation of the phosphatidylinositol (PI) 3-kinase and Akt/protein kinase B (PKB) signalling pathway [7]. It has recently also been demonstrated that the CAP/Cbl pathway contributes to insulin signalling in cardiomyocytes [8]. Insulin resistance is regarded as the inability to increase glucose uptake following insulin stimulation and is associated with changes in intracellular signalling and SLC2A4 translocation [9]. Glucose uptake is also increased by insulin-independent mechanisms such as activation of AMP-activated protein kinase (AMPK) [10].

Hypertrophic hearts develop insulin resistance and reduced SLC2A4 expression [11], and it has been suggested that impaired Akt/PKB signalling contributes to this development [12]. LIF has been shown to induce hypertrophy of cardiomyocytes in vitro [13, 14]. The aim of this study was therefore to investigate effects of acute LIF treatment on glucose uptake, and to detect potential signalling pathways involved. In addition, we examined changes in glucose and fatty acid metabolism with a view to investigating whether chronic LIF treatment influenced the responsiveness of the cardiomyocytes to acute insulin stimulation.

Materials and methods

Cardiomyocyte cultures

Cardiomyocytes from male Wistar rats (250–300 g) (Møllegaard Breeding and Research Center and Bomholgård Breeding and Research Center, Ry, Denmark) were isolated by collagen digestion as described elsewhere [15]. The experiments were conducted in accordance with Regulations on Animal Experimentation under the Norwegian Animal Welfare Act and approved by the Norwegian Animal Research Authority. Isolated cells were maintained in Medium 199 (Sigma, St. Louis, MO, USA) with 2 mg/ml BSA, 2 mmol/l DL-carnitine, 5 mmol/l creatine, 5 mmol/l taurine, 0.1 μmol/l insulin, 0.1 nmol/l triiodothyronine (all from Sigma), 100 U/ml penicillin (Invitrogen, Paisley, UK) and 100 μg/ml streptomycin (Invitrogen) with or without 1 nmol/l LIF (Chemicon, Temecula, CA, USA). This pro-

cedure yields approximately 75% rod-shaped cardiomyocytes after 48 h in culture as previously reported [5]. It has previously been shown that adult cardiomyocytes maintained in culture in a serum-free medium remain insulin responsive [16].

Glucose metabolism

Deoxyglucose transport

Isolated cardiomyocytes were plated on six-well culture dishes (Corning Int., Corning, NY, USA), and measurements of [^{3}H]deoxyglucose uptake were performed as described elsewhere [5]. In brief, the cells were maintained in culture for 48 h with or without 1 nmol/l LIF, washed twice with uptake buffer (in mmol/l) (140 NaCl, 20 HEPES, 5 KCl, 2.5 MgSO$_4$, and 1 CaCl$_2$) and then incubated with the same buffer supplemented with 2-[^{3}H(G)]deoxy-D-glucose (American Radiolabeled Chemicals (ARC), Inc., MO, USA) (37 kBq/ml) for 15 min. The acute effect of LIF (1 nmol/l) on glucose uptake was assessed in cells exposed to LIF only during the 15 min glucose uptake period. Glucose uptake was performed with or without the presence of the mitogen-activated ERK-activating kinase (MEK) 1 inhibitor PD98059 (75 μmol/l; Calbiochem, La Jolla, CA, USA) or the PI3-kinase inhibitor wortmannin (0.5 μmol/l, Sigma). These inhibitors were added to the cells 60 min before measurement of deoxyglucose transport and were also present during the uptake period. In each experiment, a set of control cells was stimulated with 1 μmol/l insulin (Actrapid; Novo-Nordisk, Bagsvaerd, Denmark). Transport of labelled deoxyglucose was measured by liquid scintillation. Non-carrier-mediated transport was determined in the presence of 10 μmol/l cytochalasin B (Sigma) and subtracted from all presented values.

Glucose oxidation

Isolated cardiomyocytes were plated on 12.5 cm^2 flasks (Falcon; Becton Dickinson, Franklin Lakes, NJ, USA) coated with laminin and maintained in culture for 48 h. The control and LIF (1 nmol/l)-treated cells were then incubated with D-[1-^{14}C]glucose (ARC) (7.4 kBq/ml) and 20 mmol/l HEPES. Measurements of glucose oxidation followed procedures described elsewhere [5]. Flasks containing no cells were used to correct for non-specific CO$_2$ trapping.

Lipid metabolism

Oleic acid uptake, distribution and oxidation

Measurement of lipid metabolism was performed in cells maintained in culture for 48 h followed by 4 h incubation with [1-^{14}C]oleic acid (ARC) (18.5 kBq/ml) as previously described [5]. The distribution of oleic acid was determined

by liquid scintillation counting after separation of labelled-cell-associated lipids by thin layer chromatography. β-Oxidation of ^{14}C-oleic acid was measured as acid soluble metabolites (ASMs). Complete oxidation of oleic acid to CO_2 was measured in cardiomyocytes plated on 12.5 cm^2 culture flasks (Falcon). No-cell controls were included. Total oleic acid uptake was calculated as the sum of ASM, the sum of lipids and oxidation to CO_2.

Immunoblotting

Aliquots with 15 μg cell protein (total cell lysate prepared in Laemmli buffer) were separated by SDS-PAGE (10% resolving gel) and transferred to nitrocellulose membranes. The membranes were immunoblotted with antibodies against phosphorylated Akt/PKB (Ser473), phosphorylated extra-cellular signal regulated kinase (ERK)1, ERK2 (p40^{mapk}/p42^{mapk}) (Thr202 plus Tyr204), phosphorylated AMP-kinase α 1/2 (Thr172) or phosphorylated glycogen synthase kinase (GSK)-3α/β (Ser21/9) (all from Cell Signaling, Beverly, MA, USA). Immunoreactive bands were visualised with enhanced chemiluminescence (Amersham Biosciences, Bucks, UK). Quantification of western blots was performed with Gel-Pro Analyzer (ver. 2.0) software.

Real-time quantitative RT-PCR

Total RNA was isolated from cardiomyocytes using an Agilent Total RNA Isolation Kit (Matrix). Glyceraldehyde 3-phosphate dehydrogenase primer sequences were obtained from the RT primer database, entry 192 [17]. Other primers were designed using Primer Express TM Version 2.0 (Applied Biosystems, Foster City, CA, USA) (Table 1). RNA (0.5 μg) was used to prepare cDNA using TaqMan Reverse Transcription Reagents (Applied Biosystems).

Quantification of mRNA was performed using the ABI PRISM 7000 (Applied Biosystems). Assays were performed in triplicates using Sybr Green Master Mix (Applied Biosystems) 30 μmol/l sense and anti-sense primers. Specificity of the assays was confirmed by gel electrophoresis. Gene expression was normalised to glyceraldehyde 3-phosphate dehydrogenase.

Statistics

Groups were compared by the Mann–Whitney rank sum test and Bonferroni corrected where appropriate. A p value<0.05 was considered statistically significant. Data are presented as means±SEM.

Results

Effect of acute LIF treatment on deoxyglucose transport

In the present study we investigated whether acute LIF treatment increased deoxyglucose transport in cardiomyocytes kept in culture for 48 h. Our results showed that in cardiomyocytes treated with LIF (1 nmol/l) for 15 min deoxyglucose transport increased by 35±8% (p<0.01) compared with control cells (Fig. 1). Acute stimulation with insulin (1 μmol/l) increased deoxyglucose transport by 46±12% (p<0.05). No significant difference was found when comparing deoxyglucose transport mediated by acute LIF or insulin treatment. Nor were we able to detect any additive effect when cells were treated with both LIF and insulin compared with control cells (47±15%, p<0.01) (Fig. 1). The measurements of basal glucose uptake were higher while the insulin-mediated uptake was lower than previously reported [18], and may be explained by different experimental conditions.

Table 1 Sequence of primers used in real-time RT-PCR assays

Target	Direction	Sequence	Acc No.
Slc2a1	+	TTGCTGTGGCTGGCTTCTC	NM_138827
	−	CACACAGTTGCTCCACATATTGG	
Slc2a4	+	TGTGGCCTTCTTTGAGATTGG	BC085757
	−	CTGAAGAGCTCGGCCACAA	
Socs3	+	CTGCGCCTCAAGACCTTC	NM_053565
	−	ATCCGCTCTCCTGCAGCTT	
Cd36	+	GACCATCGGCGATGAGAAA	NM_031561
	−	ACCAGGCCCAGGAGCTTTA	
Gapdh	+	ATGATTCTACCCACGGCAAG	−
	−	CTGGAAGATGGTGATGGGTT	

+ forward primers; − reverse primers; *Acc No.* GenBank accession number; *Slc2a1*, solute carrier family 2 (facilitated glucose transporter), member 1 (previously known as glucose transporter 1 [GLUT1]); *Slc2a4*, solute carrier family 2 (facilitated glucose transporter), member 4 (previously known as glucose transporter 4 [GLUT4]); *Socs*, suppressor of cytokine signalling; *Cd36*, fatty acid transporter Cd36 antigen; *Gapdh*, glyceraldehyde 3-phosphate dehydrogenase
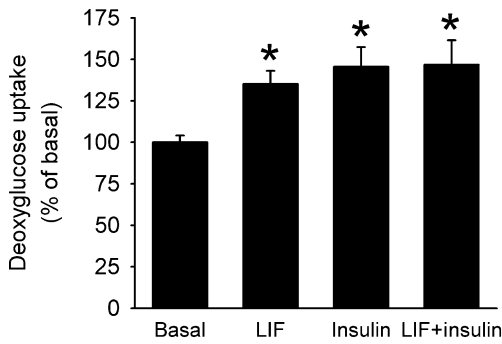
Fig. 1 Glucose transport in cardiomyocytes following acute exposure to LIF or insulin. Cardiomyocytes were kept in culture for 48 h before glucose transport was measured at the basal state and after stimulation with LIF (1 nmol/l) (n=5), insulin (1 μmol/l) (n=5) or the combination of these (n=4) for 15 min. Basal glucose uptake was 5.1±0.4 pmol·mg^{-1}·min^{-1}. Glucose transport is given as percentage of average basal uptake±SEM. *p<0.05 vs basal

Mechanism of the acute effect of LIF on deoxyglucose transport

To the best of our knowledge, it has not previously been reported that LIF has an acute effect on glucose uptake in cardiomyocytes. The mechanism by which LIF increases glucose uptake is therefore not known. We found that LIF-stimulated glucose transport was not inhibited by wortmannin (0.5 μmol/l) (Fig. 2). However, wortmannin did inhibit insulin-induced glucose uptake. We also pre-incubated cells with the MEK1 inhibitor PD98059 (75 μmol/l) [19], which did not inhibit LIF-mediated deoxyglucose uptake (data not shown).

By immunoblotting experiments, we showed that the inhibitors wortmannin and PD98059 prevented LIF-induced phosphorylation of Akt/PKB and ERK1/2, respectively (Fig. 3a). In accord with the measurements of deoxyglucose transport, we observed no additive effect on phosphorylation of Akt/PKB or ERK1/2 in cells treated
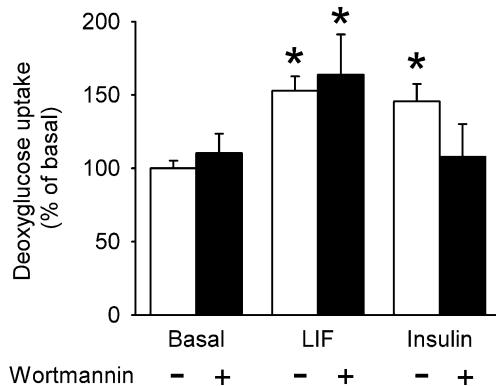

Fig. 2 Effect of wortmannin on acute LIF-stimulated glucose transport. Cardiomyocytes were kept in culture for 48 h before glucose uptake was measured for 15 min in the basal state and after stimulation with LIF (1 nmol/l) or insulin (1 μmol/l). Wortmannin (0.5 μmol/l) was added to the cells 60 min prior to measurements of glucose transport. Basal glucose transport was 5.6±0.5 pmol·mg^{-1}·min^{-1}. Glucose transport is given as percentage of basal uptake without wortmannin, means±SEM, n=5. *p<0.05 vs basal

with both LIF and insulin (Fig. 3b,c). LIF did not alter the phosphorylation levels of AMPK (data not shown).

Effect of chronic LIF treatment on insulin-stimulated deoxyglucose transport

In accord with previous observations [5, 6], basal glucose uptake in the present study was increased by 20±4% after exposure to LIF (1 nmol/l) for 48 h (Fig. 4). Interestingly, chronic LIF treatment induced a state of insulin resistance in the cardiomyocytes. Acute insulin stimulation increased deoxyglucose transport by 30±4% in control cells maintained in culture for 48 h, whereas the insulin response was totally absent following chronic LIF treatment. We then examined whether treatment with insulin induced changes in glucose oxidation to CO_2 in cells exposed to LIF for 48 h. Insulin had
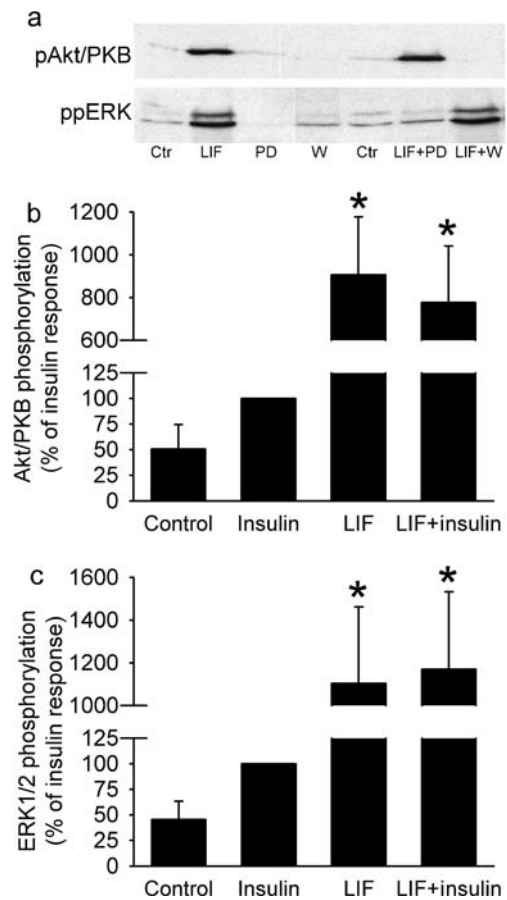

Fig. 3 Effect of acute LIF and insulin on phosphorylation of Akt/PKB and ERK1/2. Cardiomyocytes were kept in culture for 48 h before stimulation with LIF (1 nmol/l), insulin (1 μmol/l) or both LIF and insulin for 15 min. One representative experiment is shown (**a**). Ctr, control; p and pp, phosphorylated. Cell cultures were preincubated with wortmannin (W) (0.5 μmol/l) or PD98059 (PD) (75 μmol/l) for 60 min before LIF or insulin was added. Immunoblot analyses were performed using antibodies against phosphorylated Akt/PKB and phosphorylated ERK1/2. Densitometric analysis of results for Akt/PKB (**b**) and ERK 1/2 (**c**) are from five and six immunoblots, respectively, and presented as percentage of LIF response, means±SEM. *p<0.05 vs control
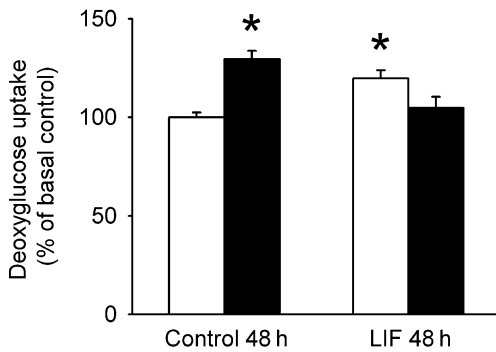
Fig. 4 Glucose transport after chronic exposure to LIF. Cardiomyocytes were incubated with LIF (1 nmol/l) for 48 h before basal (*open bars*) and insulin-stimulated (1 μmol/l) (*closed bars*) glucose transport was measured. Basal glucose transport in control cells was 5.4 ± 0.4 pmol·mg^{-1}·min^{-1}. Results are presented as percentage of basal control, means±SEM from 6–9 experiments. *$p<0.05$ vs basal uptake in control cells

no effect on control cells, nor did we detect changes between control and LIF-treated cells (data not shown).

We next examined whether chronic LIF treatment influenced the ability of insulin to phosphorylate intracellular signalling molecules. Insulin phosphorylated Akt/PKB in control cells maintained in culture for 48 h as expected. However, insulin did not significantly increase Akt/PKB phosphorylation in LIF-treated cardiomyocytes compared with control cells (Fig. 5a). We also observed that chronic LIF treatment prevented insulin-induced GSK-3 phosphorylation (Fig. 5b). Chronic LIF treatment did not alter the insulin-mediated phosphorylation of ERK1/2 (data not shown).

Effect of chronic LIF treatment on insulin-stimulated fatty acid metabolism

Insulin has previously been shown to increase fatty acid uptake in cardiomyocytes [20]. Since we observed reduced glucose uptake by insulin in cardiomyocytes subjected to chronic LIF treatment, we examined the insulin-mediated uptake of fatty acids in these cells. Total oleic acid uptake in control cells was 148.4 ± 14.3 nmol/mg protein (Fig. 6). Acute insulin stimulation increased oleic acid uptake by $29\pm1\%$ ($p<0.05$), which is in agreement with a recent report [20]. Total uptake of oleic acid in cells exposed to LIF was 161.6 ± 19.4 nmol/mg protein, and a $13\pm3\%$ ($p<0.01$) increase was found following stimulation with insulin. The insulin-mediated increase in oleic acid uptake was lower in LIF-treated cells compared with controls ($59\pm9\%$, $p<0.05$). We also found that the intracellular distribution of oleic acid to diacylglycerol (DAG) and triacylglycerol (TAG) was lower in LIF-treated cells following insulin stimulation (59 ± 5 and $46\pm4\%$, respectively, of control values, $p<0.05$). Furthermore, insulin did not alter the intracellular level of free oleic acid, distribution of oleic acid to phospholipids or oleic acid oxidation (assessed as ASM and CO_2).

LIF alters expression of genes involved in glucose metabolism

In order to understand the effects of chronic LIF treatment, we analysed gene expression of proteins involved in glucose and lipid metabolism. Our results showed that gene expression of suppressor of cytokine signalling (*Socs*) 3 was increased by $41\pm12\%$ ($p<0.05$) in cells treated with LIF for 48 h (Fig. 7). Solute carrier family 2, member 1 (*Slc2a1*, previously known as glucose transporter 1 [GLUT1]) gene expression was increased by $39\pm10\%$ ($p<0.05$), while expression of *Slc2a4* was reduced to $53\pm7\%$ ($p<0.05$) of control. LIF did not change the expression of the fatty acid transporter *Cd36*.
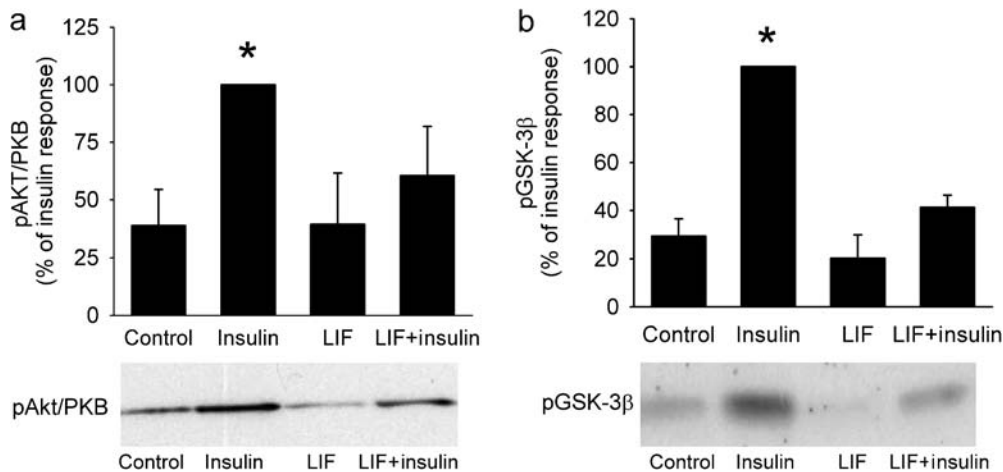

Fig. 5 Effect of chronic LIF treatment on insulin-stimulated phosphorylation of Akt/PKB (**a**) and GSK-3 (**b**). Cardiomyocytes were kept in culture for 48 h with or without the presence of LIF (1 nmol/l). Insulin (1 μmol/l) was added for the last 15 min. One representative blot for each experiment is shown. Densitometric analysis of five (**a**) and three (**b**) immunoblots with antibody against phosphorylated Akt/PKB (pAkt/PKB) (**a**) and GSK-3 (pGSK-3β) (**b**). Data presented as percentage of insulin response, means±SEM. *$p<0.05$ vs control cells
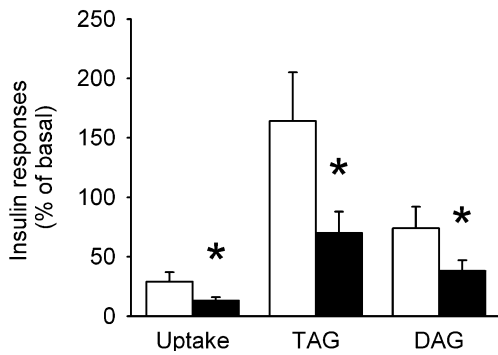
Fig. 6 Effects of insulin on [^{14}C]oleic acid metabolism in cells exposed to LIF for 48 h. Cells were treated with LIF (1 nmol/l) (*filled bars*) for 48 h (untreated cells used as control, open bars) and then incubated with [^{14}C]oleic acid with or without insulin (1 μmol/l) for 4 h. Insulin-mediated changes are calculated as the difference between values obtained when using insulin (1 μmol/l) and no insulin. Values are presented as means±SEM, *n*=4–5, **p*<0.05

Discussion

The aim of this study was to investigate effects of acute LIF treatment on glucose uptake in cardiomyocytes, and to detect potential signalling pathways involved. In addition, we investigated whether chronic LIF treatment influenced the cellular responsiveness to insulin. We show that acute LIF treatment increases glucose uptake in cardiomyocytes through mechanisms that differ from those of insulin-stimulated glucose uptake. Our results also show that chronic exposure to LIF induces insulin resistance. This chronic effect of LIF may be mediated by reduced insulin signalling and transcriptional changes in genes encoding proteins involved in the regulation of glucose uptake.

We found that acute LIF treatment increased glucose uptake in cardiomyocytes. A similar effect was observed with insulin, but there was no additive effect on glucose uptake of LIF and insulin in combination. Interestingly, the uptake mediated by LIF was not significantly different from the uptake induced by insulin. To the best of our knowledge, this is the first time that effects of acute LIF treatment on glucose uptake in cardiomyocytes have been shown. Insulin increases glucose uptake through the PI3-kinase pathway by phosphorylating Akt/PKB [7] or through the CAP/Cbl pathway [21]. Our results showed that the PI3-kinase inhibitor wortmannin did not abolish the LIF-induced glucose uptake, but prevented LIF-stimulated phosphorylation of Akt/PKB. These results suggest that, in contrast to insulin, LIF increases glucose uptake in cardiomyocytes by a PI3-kinase-independent pathway. However, it remains to be investigated whether LIF and insulin share other signalling mechanisms such as the CAP/Cbl pathway [22] to increase glucose uptake.

Insulin-independent pathways also contribute to increase glucose uptake in cardiomyocytes. Activation of AMPK has been reported to increase glucose uptake following ischaemia in cardiomyocytes [23]. However, we found no effect on

AMPK phosphorylation, suggesting that the effects of LIF are independent of this pathway. Activation of mitogen-activated protein kinase pathways also contributes to increased glucose uptake. It was recently shown that sodium nitroprusside increased glucose uptake through activation of ERK1/2 [24]. Our results showed that the MEK 1 inhibitor PD98059 did not prevent the LIF-induced acute uptake of glucose, despite inhibition of ERK1/2 phosphorylation. Since inhibiting the PI3-kinase or ERK1/2 pathways did not abolish the LIF-induced glucose uptake, it remains to be detected which pathways are responsible for these acute effects of LIF.

A novel finding in this study is that chronic LIF treatment induces insulin resistance. This was demonstrated as reduced insulin-mediated glucose uptake in cardiomyocytes exposed to chronic LIF treatment. In addition, chronic LIF treatment reduced the effects of insulin on the uptake of oleic acid. This lower insulin-mediated uptake may contribute to the reduced distribution of oleic acid to DAG and TAG compared with control cells. A potential consequence of the insulin-mediated increase in fatty acid uptake is a build-up of intracellular lipid stores in the cardiomyocytes [20]. Our result suggests, however, that chronic LIF treatment could prevent an accumulation of lipid stores following exposure to insulin by reducing the uptake of fatty acids. Thus, we believe that the reduced responses to insulin are important effects of chronic LIF treatment that may have functional consequences in the cardiomyocytes.

We show two possible mechanisms for the reduced insulin-mediated signalling following chronic exposure to LIF. Firstly, cardiomyocytes treated with LIF increased gene expression of *Socs3*. SOCS proteins modulate cytokine signalling in a negative feedback loop [25]. In addition, SOCS3 directly interacts with residues on the insulin receptor, inhibits tyrosine phosphorylation of insulin recep-
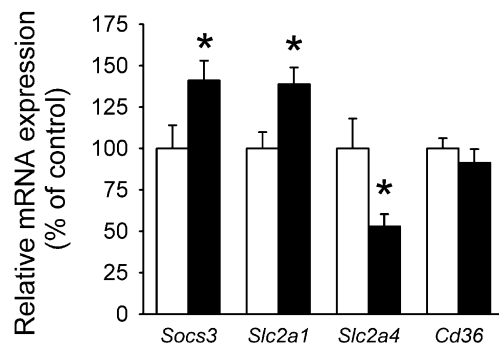

Fig. 7 Effects of LIF on gene expression. Gene expression was analysed in cells treated with LIF (1 nmol/l) (*filled bars*) for 48 h and control cells (untreated) (*open bars*). The levels of gene expression are normalised to the gene encoding glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*). Values are normalised to control±SEM, *n*=4, **p*<0.05 vs control. *Socs3* suppressor of cytokine signalling 3; *Slc2a1*, solute carrier family 2 (facilitated glucose transporter), member 1 (previously known as glucose transporter 1 [GLUT1]); *Slc2a1*, solute carrier family 2 (facilitated glucose transporter), member 4 (previously known as glucose transporter 4 [GLUT4]); *Cd36*, fatty acid transporter *CD36* antigen

tor substrate (IRS)-1 and impairs insulin signalling [26, 27]. Increased expression of the gene encoding SOCS3 following chronic LIF treatment may therefore contribute to the reduced insulin-mediated phosphorylation of Akt/PKB. The second negative effect of LIF on insulin signalling is potentially caused by regulating IRS-1 serine phosphorylation. Our results show that chronic LIF treatment impaired the ability of insulin to phosphorylate and thereby inactivate GSK-3. Active GSK-3 has been shown to serine phosphorylate IRS-1 and impair insulin-mediated signalling [28, 29]. Preventing insulin from phosphorylating GSK-3 may also explain why cardiomyocytes exposed to LIF for 48 h have reduced insulin-mediated signalling.

Reduced expression of the gene encoding the insulin-sensitive glucose transporter SLC2A4 following chronic LIF treatment could also contribute to the induction of insulin resistance in these cardiomyocytes. A recent study found that reduced SLC2A4 translocation to the plasma membrane was partially responsible for induction of insulin resistance in isolated cardiomyocytes [8]. Thus, fewer available SLC2A4 proteins may therefore participate in the development of reduced responsiveness to insulin in the LIF-treated cardiomyocytes. Whether LIF prevents translocation of SLC2A4 to the plasma membrane remains to be investigated. Insulin translocates the fatty acid transporter CD36 to the plasma membrane and thereby participates in increasing fatty acid uptake in cardiomyocytes [20]. Our results showed that LIF did not alter expression of the gene encoding CD36. Further studies are needed to investigate whether LIF alters translocation of CD36 to the plasma membrane or changes expression of other fatty acid transporters.

This study demonstrates that acute and chronic LIF treatment of cardiomyocytes increase glucose uptake. LIF induces cardiomyocyte hypertrophy [13, 14], a process often associated with increased expression of foetal genes [30]. The present study reveals that LIF is also able to induce changes that resemble a foetal cardiomyocyte energy pattern and insulin resistance as found in hypertrophic hearts [11, 31]. Thus, LIF may contribute to increase the basal levels of glucose uptake and reduce the responsiveness of hypertrophic hearts to insulin. A possible limitation in this study is the use of supraphysiological insulin concentrations.

We conclude that acute LIF treatment increases glucose uptake in cardiomyocytes through mechanisms different from those of insulin. Furthermore, chronic LIF treatment alters the acute effects of insulin on uptake of both glucose and fatty acids. LIF may in part induce insulin resistance by altering expression of the genes encoding SOCS3 and SLC2A4, and reduce insulin-mediated phosphorylation of GSK-3 and Akt/PKB.

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