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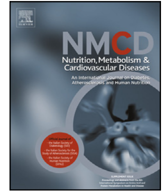

Impaired stimulation of glucose transport in cardiac myocytes exposed to very low-density lipoproteins

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Abstract We recently observed that free fatty acids impair the stimulation of glucose transport into cardiomyocytes in response to either insulin or metabolic stress. *In vivo*, fatty acids for the myocardium are mostly obtained from triglyceride-rich lipoproteins (chylomicrons and Very Low-Density Lipoproteins). We therefore determined whether exposure of cardiac myocytes to VLDL resulted in impaired basal and stimulated glucose transport. Primary adult rat cardiac myocytes were chronically exposed to VLDL before glucose uptake was measured in response to insulin or metabolic stress, provoked by the mitochondrial ATP synthase inhibitor oligomycin. Exposure of cardiac myocytes to VLDL reduced both insulin-and oligomycin-stimulated glucose uptake. The reduction of glucose uptake was associated with a moderately reduced tyrosine phosphorylation of the insulin receptor. No reduction of the phosphorylation of the downstream effectors of insulin signaling Akt and AS160 was however observed. Similarly only a modest reduction of the activating phosphorylation of the AMP-activated kinase (AMPK) was observed in response to oligomycin. Similar to our previous observations with free fatty acids, inhibition of fatty acid oxidation restored oligomycin-stimulated glucose uptake. In conclusions, VLDL-derived fatty acids impair stimulated glucose transport in cardiac myocytes by a mechanism that seems to be mediated by a fatty acid oxidation intermediate. Thus, in the clinical context of the metabolic syndrome high VLDL may contribute to enhancement of ischemic injury by reduction of metabolic stress-stimulated glucose uptake.
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Introduction

The metabolic syndrome is a leading cause of mortality and morbidity in the Western hemisphere. The metabolic syndrome increases not only the prevalence but also the severity of myocardial infarction [8]. Stimulation of myocardial glucose transport in response to metabolic stress has been shown to be important for post-ischemic recuperation of function and survival of the myocardium [26,28]. Thus impairment of the glucose transport response to metabolic stress could underlie the increased susceptibility of the myocardium to ischemia and reperfusion injury. This response shares several mechanisms with the stimulation of glucose transport in response to insulin [21], which is impaired in the metabolic syndrome.

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Dyslipidemia is a hallmark of the metabolic syndrome, and includes elevated circulating free fatty acids and very low-density lipoproteins (VLDL) [15]. We have recently shown that chronic exposure of cardiac myocytes to free fatty acids (FFA) resulted in a marked impairment of the stimulation of glucose transport, in response to either insulin or metabolic stress [3]. Notably, the mechanisms appear to be distinct from those by which free fatty acids induce insulin resistance in skeletal muscle myocytes.

VLDL have also been shown to induce insulin-resistance in terms of glucose transport in skeletal muscle cells [24]. Lipoproteins deliver fatty acids to muscle cells, a mechanism that is primordial for energy production by cardiac myocytes [5]; however it could be shown that the lipoprotein receptor interaction, not the provision of fatty acids, mediates insulin resistance in skeletal muscle cells [24]. Therefore, in the present study, we determined whether exposure of cardiac myocytes to VLDL had an impact on basal and stimulated glucose transport.

Materials and methods

Animals

We obtained male Sprague Dawley rats (100–110 g) from Charles Rivers France (L'Arbresle, France). The ethical committee of the Geneva University School of Medicine and the Geneva State Veterinary Office approved the study protocol, which conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Very low-density lipoproteins

Human very low-density lipoproteins (VLDL) were obtained from the sera of healthy volunteers, by differential centrifugation [14] and suspended in sterile phosphate buffer saline (PBS) containing 10^{-5} M EDTA. VLDL from several donors were pooled, stored at 4 °C and used within one week. All donors gave informed consent regarding the utilization of sera, and the Ethical Committee for Human Research of the Geneva University Hospitals approved the protocol.

Adult rat cardiomyocytes culture

Adult rat cardiomyocytes were isolated as previously described [2] by retrograde perfusion of the hearts with collagenase (type II; Worthington) [9,10]. Cardiomyocytes were separated from non-myocyte cardiac cells by preplating the whole cell suspension for 90 min on untreated plastic, to which non-myocyte cells, but not cardiomyocytes, readily adhere. Cardiomyocytes were plated in M199 medium containing 5.5 mM glucose supplemented with 20 mM creatine, 100 μM cytosine-β-D-arabinofuranoside, 100 nM 9-cis retinoic acid and 20% fetal calf serum (FCS) in dishes previously coated with 0.1% gelatin for 4 h and incubated overnight with complete culture medium. VLDL, PBS and pharmacological agents or their solvents were added to the medium at the time of plating (day 0). The culture medium was renewed every 2–3 days, and subsequent analyses were performed on day 7. At this time point control cardiomyocytes display a well differentiated phenotype with stable insulin responsiveness [25].

Determination of 2-deoxy-D-glucose uptake

Glucose transport was estimated by measuring 2-deoxyglucose (2-DG) uptake, as previously described [2,20]. Briefly, cardiomyocytes were incubated in M199 containing 10 nM [2,6-^{3}H]-2-DG (ANAWA) (1–2 μCi/ml) and 5.5 mM cold glucose at 37 °C for 1 h, in the presence or absence of glucose transport agonists. Glucose transport agonists used were insulin (10^{-10} to 10^{-6} M), or oligomycin (10^{-6} M) to induce metabolic stress. Glucose transport in response to hypoxia was also measured in M199, with the 2-DG concentration reduced to 1 nM, to avoid toxic effects of 2-DG over the long incubation periods in hypoxia experiments. Hypoxia was maintained for 6 h in an airtight Billups-Rothenberg chamber flushed with 10% CO_2, 90% N_2; control incubations were conducted for the same time in 5% CO_2 in air at 37 °C.

2-DG uptake was stopped by three washes with ice-cold PBS before lysis in 1 ml 0.1 M NaOH. Twenty-microliter aliquots were taken for protein content determination and the remaining NaOH lysate assayed for radioactivity in a TriCARB 1900TR liquid scintillation analyzer (Packard). Results are expressed as fold stimulation of 2-DG uptake with respect to control unstimulated cardiomyocytes.

Intracellular signaling analysis

After preincubation in serum-free M199 for 30 min, cardiomyocytes were stimulated with glucose transport agonists for 10 min (insulin; 10^{-10} to 10^{-6} M) or 20 min (oligomycin; 10^{-6} M). Stimulations were terminated by three washes in ice-cold PBS before solubilizing cells in 200 μl lysis buffer containing 150 mM NaCl, 50 mM Tris–HCl (pH7.5), 1 mM EDTA, 0.5% sodium deoxycholate, 1% Igepal CA 630, Halt protease and phosphatase inhibitor Cocktail (Pierce, Thermo Scientific). Proteins (50 μg) from each sample were separated on SDS-PAGE gels and transferred onto polyvinylidene difluoride membranes. Western blots were immunoprobed with antibodies for phosphorylated and total signaling intermediates. The following primary antibodies were used: Akt (#9272; 1:1000), phosphoSer^{473}-Akt (#9271; 1:1000), phosphoThr^{308}-Akt (#9275; 1:1000), AMPKα (#2532; 1:1000), phospho Thr^{172}-AMPKα (#2531; 1:1000), phospho-(Ser/Thr)-Akt substrate (#9611; 1:1000), phosphoSer^{235}/Ser^{236} S6 ribosomal protein (#4858; 1:1000), S6 ribosomal protein (#2217; 1:1000) and Insulin Receptor β (#3025; 1:1000) from Cell Signaling Technologies, AS160 (#07-741; 1:1000) and phosphotyrosine (#05-777; 1:1000) from Millipore, GLUT1 (#ab652; 1:1000), GLUT4 (#ab654; 1:5000) and GLUT8 (#ab169779; 1:1000) from Abcam, α-

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