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Glucose deprivation accelerates VLDL receptor-mediated TG-rich lipoprotein uptake by AMPK activation in skeletal muscle cells

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Abstract

Glucose and fatty acids are major energy sources in skeletal muscle. Very low-density lipoprotein receptor (VLDL-R), which is highly expressed in heart, skeletal muscle and adipose tissue, plays a crucial role in metabolism of triglyceride (TG)-rich lipoproteins. To explore energy switching between glucose and fatty acids, we studied expression of VLDL-R and lipoprotein uptake in rat L6 myoblasts. L-Glucose or D-glucose deprivation in the medium noticeably induced the AMPK (AMP-activated protein kinase) activation and VLDL-R expression. Dose-dependent induction of VLDL-R expression was observed when D-glucose was less than 4.2 mM. The same phenomenon was also observed in rat primary skeletal myoblasts and cultured vascular smooth muscle cells. The uptake of β-VLDL but not LDL was accompanied by induction of VLDL-R expression. Our study suggests that the VLDL-R-mediated uptake of TG-rich lipoproteins might compensate for glucose shortfall through AMPK activation in skeletal muscle.
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Nutritional state and chronic metabolic disease such as diabetes mellitus affect serum glucose and lipid levels. In skeletal muscle, glucose and long chain fatty acids (LCFA) are utilized as energy substrates. Insulin-stimulated glucose transport into skeletal muscle is mediated by the glucose transporter GLUT4 [1]. However, LCFA uptake likely involves both passive diffusion and active protein-mediated transporters. Fatty acid translocase (FAT)/CD36, plasma membrane-bound fatty acid binding protein (FABPpm) and fatty acid transport protein (FATP) are well described as plasma membrane-associated proteins for LCFA transport into cells [2]. In the uptake of fatty acids (FAs) from triglyceride (TG)-rich lipoproteins (chylomicron [CM] and very low-density lipoprotein [VLDL]), hydrolysis of the TG from CM and VLDL by lipoprotein lipase (LPL) is a key process. However, the precise mechanism of the uptake of fatty acids from TG-rich lipoproteins is not fully understood.

The VLDL receptor is a member of the low-density lipoprotein (LDL) receptor family. It is expressed abundantly in heart, skeletal muscle, and adipose tissue, which are all active in fatty acid metabolism [3]. *In vitro* studies have shown that VLDL receptor binds and internalizes TG-rich lipoproteins such as VLDL and β-migrating VLDL (β-VLDL) but does not bind LDL. These findings suggest that TG-rich lipoproteins are taken up in peripheral tissues through the VLDL receptor pathway. Subsequently, several lines of evidence indicating the existence of this

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VLDL receptor pathway for fatty acid transport into heart, skeletal muscle and adipose tissue have been reported. In an *in vivo* study, VLDL receptor knockout mice were protected from obesity due to high-fat diet or the *ob*/*ob* (obese) genotype in mice [4]. Furthermore, uptake of [^{3}H]TG-derived free FA was decreased in adipose tissue and skeletal muscle in VLDL receptor knockout mice compared with wild type mice [5]. We have also reported that β-VLDL was taken up through the VLDL receptor pathway in cultured rat cardiomyocytes and vascular smooth muscle cells (SMCs) [6–8].

In skeletal muscle, glucose deprivation induces a change in the main energy substrate from glucose to FAs. Although the adaptation to glucose deprivation is indispensable for muscle cells to survive, this mechanism is not fully understood. The present study examined *in vitro* the association of VLDL receptor expression with this adaptation. During glucose deprivation VLDL receptor expression was up-regulated, and the uptake of β-VLDL increased simultaneously through AMPK (AMP-activated protein kinase) cascade. Those findings indicate that the VLDL receptor is necessary for this adaptation during glucose deprivation, and that the VLDL receptor pathway for delivery of FAs from TG-rich lipoprotein might be one of the energy sources in skeletal muscle.

Materials and methods

Cell culture. Rat L6 myoblasts were purchased from Health Science Research Resources Bank (Osaka, Japan). Human THP-1 monocytic leukemia cells were obtained from American Type Culture Collection (Manassas, VA, USA). Primary cultures of vascular SMCs were obtained from the media of cultured thoracic aortas of male Sprague–Dawley (SD) rats as described previously [7]. Cells were grown in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA, USA) for L6 myoblasts and SMCs or RPMI-1640 (Invitrogen) for THP-1 cells, supplemented with 10% heat-inactivated fetal calf serum (FCS; MP Biomedicals, Irvine, CA, USA) including antibiotics (100 U/mL penicillin, 100 mg/mL streptomycin sulfate) in a humidified atmosphere of 5% CO_2 at 37 °C. To obtain THP-1 macrophages, THP-1 cells were incubated at a concentration of 200 nM phorbol-12-myristate-13-acetate (PMA; Wako Pure Chemicals, Osaka, Japan) for 3 days. To vary the glucose concentration in the medium, L-glucose or D-glucose (Sigma–Aldrich, St. Louis, MO, USA) with 2 mM sodium pyruvate (Sigma–Aldrich) was supplemented in DMEM without glucose (Invitrogen).

Isolation of rat primary skeletal myoblasts. Skeletal muscles were harvested from the lower limbs of 1- or 2-day-old SD rats, and minced into small pieces with scissors. After dissociation with 0.35% trypsin (Invitrogen) and 0.07% collagenase (Wako, Osaka, Japan) at 37 °C for 5 min, the cells were washed and resuspended in DMEM supplemented with 10% FCS. The cell suspension was filtered to remove undigested tissue and plated onto culture dishes for 45 min. The non-attached cells were removed and plated onto other culture dishes. The isolated skeletal myoblasts were grown in 10% FCS-DMEM. Cell passages were performed at less than 70% density to avoid myogenic differentiation at higher densities. The skeletal myoblasts that underwent 1–2 passages were used in the experiments.

Antibodies and chemicals. Polyclonal antibody (VR2) against the VLDL receptor that reacts with human, rabbit, rat, and mouse VLDL receptor protein was generated as described previously [9,10]. Phospho-AMPK-α Thr 172 and AMPK-α antibody were purchased from Cell Signaling Technology (Denver, CO, USA). Anti-β-actin polyclonal antibody was purchased from BioLegend (San Diego, CA, USA). Hybridoma cells producing monoclonal anti-human LRP-1 were purchased from the American Type Culture Collection. Metformin was provided from Dainippon Sumitomo Pharma Co. (Osaka Japan).

Protein isolation and Western blot analysis. Cellular proteins were prepared according to a standard method and measured according to the method of Bradford et al. [11]. SDS–PAGE was performed on the fractions with 7.0% slab gels that contained 0.1% SDS. Total cell protein (30 μg/lane) was applied. Detection of antibodies was performed using a second antibody and was visualized by enhanced chemiluminescence (ECL; GE Healthcare, Buckinghamshire, England).

RNA isolation and Northern blot analysis. Total cellular RNA was isolated according to the guanidinium thiocyanate–phenol–chloroform extraction method. Total RNA (20 μg) was electrophoresed in a denaturing formaldehyde–agarose gel and transferred to a Zeta-probe (Bio-Rad Laboratories, Hercules, CA, USA) filter via capillary transfer. After UV cross-linking, the filter was pre-hybridized and then hybridized with rat VLDL receptor cDNA fragments that were labeled with [α-^{32}P]dCTP (GE) according to the random primer method using the Random Primer DNA Labeling Kit Ver. 2 (Takara Bio, Osaka, Japan) as previously described [9,10].

Quantitative real time PCR. cDNA was produced from total RNA (5 μg) via reverse transcription using SuperScript III reverse transcriptase (Invitrogen). The target genes were amplified and analyzed using Taqman® probes and ABI Prism7000 SDS Software (Applied Biosystems, Foster City, CA, USA). The expression values of target genes were normalized with that of 18 S rRNA.

Lipoprotein preparation. Rabbit β-VLDL ($d < 1.006$ g/mL) was isolated from the plasma of Japanese white rabbits that were fed a diet containing 0.5% cholesterol for 4 weeks. Human VLDL ($d < 1.006$ g/mL) and LDL ($1.019 < d < 1.063$ g/mL) were isolated from the plasma of fasting healthy individuals. Lipoproteins were subjected to second ultracentrifugation, isolated at the same density, and exhaustively dialyzed against 150 mM NaCl and 0.24 mM EDTA (pH 7.4). Protein concentration was measured according to the method of Lowry [12].

Oil red-O staining, DiI and ^{125}I-labeled lipoprotein. For oil red-O staining, L6 myoblasts were seeded onto multi-well slides and incubated with VLDL, β-VLDL or LDL for 24 h. The cells were washed with phosphate-buffered saline (PBS), fixed with formaldehyde, and stained with oil red-O and hematoxylin [10]. VLDL, β-VLDL, and LDL were labeled with 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indocarbocyanine perchlorate (DiI; Molecular Probes, Carlsbad, CA, USA) as described previously [3]. To evaluate the uptake of DiI-labeled lipoproteins, cells were incubated in the medium containing a specified glucose concentration for 24 h, washed with PBS, and then incubated with 5% lipoprotein-depleted serum ([LPDS]-DMEM) containing 2.5 μg/mL of DiI-labeled lipoproteins for 3 h at 37 °C. The uptake of DiI-lipoprotein was detected by fluorescence microscopy. β-VLDL and LDL were also labeled with ^{125}I using Bolton and Hunter reagents (GE), and cell association was measured as described previously [3,13].

Statistical analysis. Data are expressed as means ± SEM. For comparisons between multiple groups, we determined the significance of the difference between group means by ANOVA using the least significant difference for multiple comparisons. Differences with p values < 0.05 were considered to be statistically significant.

Results

Glucose deprivation increased expression of the VLDL receptor

To explore VLDL receptor expression in skeletal muscle during glucose deprivation, rat L6 myoblasts were incubated for 24 h in a medium whose glucose concentration was adjusted to 30 mM L-glucose (the optical isomer of

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