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Lipid synthesis is promoted by hypoxic adipocyte-derived exosomes in 3T3-L1 cells

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ABSTRACT

Hypoxia occurs within adipose tissues as a result of adipocyte hypertrophy and is associated with adipocyte dysfunction in obesity. Here, we examined whether hypoxia affects the characteristics of adipocyte-derived exosomes. Exosomes are nanovesicles secreted from most cell types as an information carrier between donor and recipient cells, containing a variety of proteins as well as genetic materials. Cultured differentiated 3T3-L1 adipocytes were exposed to hypoxic conditions and the protein content of the exosomes produced from these cells was compared by quantitative proteomic analysis. A total of 231 proteins were identified in the adipocyte-derived exosomes. Some of these proteins showed altered expression levels under hypoxic conditions. These results were confirmed by immunoblot analysis. Especially, hypoxic adipocyte-released exosomes were enriched in enzymes related to *de novo* lipogenesis such as acetyl-CoA carboxylase, glucose-6-phosphate dehydrogenase, and fatty acid synthase (FASN). The total amount of proteins secreted from exosomes increased by 3–4-fold under hypoxic conditions. Moreover, hypoxia-derived exosomes promoted lipid accumulation in recipient 3T3-L1 adipocytes, compared with those produced under normoxic conditions. FASN levels were increased in undifferentiated 3T3-L1 cells treated with FASN-containing hypoxic adipocytes-derived exosomes. This is a study to characterize the proteomic profiles of adipocyte-derived exosomes. Exosomal proteins derived from hypoxic adipocytes may affect lipogenic activity in neighboring preadipocytes and adipocytes.

1. Introduction

Adipose tissues store excess energy in the form of lipids [1,2]. The tissues are the largest energy reserve in mammals and are capable of accommodating prolonged nutrient excess by altering their mass. However, abnormal or excess accumulation of lipids in adipose tissues causes obesity, which may impair health [3–5].

Adipose tissue expansion occurs when adipocyte numbers and size increase, which is known as hyperplasia and hypertrophy, respectively [6]. Limiting adipocyte hyperplasia leads to lipid accumulation in existing adipocytes, resulting in hypertrophy. Uptake of exogenous lipids or synthesis of endogenous lipids in the cytosol causes hypertrophy. Smaller adipocytes may be more likely to synthesize fatty acids endogenously (*de novo* lipogenesis) to begin the lipids accumulation process, while uptake of exogenous fatty acids is more predominant in developing cells [7].

De novo lipogenesis [8] is the process in which non-lipid precursors are converted to fatty acids, and requires acetyl-CoA, which is generated during various metabolic processes. Acetyl-CoA provides the carbon atoms necessary for fatty acid synthesis. It is converted to malonyl-CoA, and the rate-limiting steps in *de novo* lipogenesis are catalyzed mainly by acetyl-CoA carboxylase (ACC). Successive

Abbreviations: ACN, acetonitrile; ACC, acetyl-CoA carboxylase; FASN, fatty acid synthase; FBS, fetal bovine serum; G6PD, glucose-6-phosphate dehydrogenase; GAPDH, glyceraldehyde-phosphate dehydrogenase; Hsc70, heat shock cognate 71 kDa protein; Hsp, heat shock protein; Hsp72, heat shock 71 kDa protein 1A; HIF-1α, hypoxia-inducible factor-1α; WT, wild-type.

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malonyl-CoA molecules, which serve as a two-carbon donor, are added to acetyl-CoA by the multi-functional enzyme complex, fatty acid synthase (FASN). Glucose-6-phosphate dehydrogenase (G6PD) is a key enzyme that supplies the cellular NADPH required for lipid biosynthesis.

Adipocytes have a limited capacity to accumulate lipid droplets. When adipocytes suffer from lipid overload, hypoxia develops; the reduction in oxygen tension is directly linked to adipocyte dysfunction. To avoid lipid overload and the associated cellular stress in adipose tissues, expression of enzymes related to *de novo* lipogenesis is reduced [9]. Additionally, adipocytes do not increase in size in a synchronized fashion [10]. Small adipocytes and preadipocytes can act as reservoirs by increasing their storage capacity when larger adipocytes no longer accommodate increased lipid storage. However, how adipocytes without lipid overload are activated to store excess energy remains unknown. Adipocytes communicate with each other and with other tissues [11], but the types of communication between stressed larger adipocytes under hypoxic stress and non-stressed, less hypoxic adipocytes are unknown. Three types of signals are known to control communication between adipocytes [11]: cell-to-cell contact, soluble factors, and exosomes.

Exosomes are small 50–150 μm membrane vesicles secreted from most cell types [12]; they play an important role as information carriers between donor and recipient cells. Exosomes contain a wide variety of cytosolic contents as well as membranous components from donor cells, including genetic materials, lipids, and proteins, which determine the types of information carried [13,14]. Exosome content is thought to reflect the conditions surrounding the donor cells [15]. Exosomes could fuse with and transfer their internal contents into the cytosol of recipient cells [14]. Upon interacting with exosomes and receiving the internal contents, recipient cells undergo morphological and physiological changes, including cancer metastasis, angiogenesis, and cell differentiation [16–19]. Adipocytes also secrete exosomes [20]; however, the characteristics of adipocyte-derived exosomes are poorly understood, particularly under pathological conditions.

In this study, we first conducted quantitative proteomic analysis in 3T3-L1 adipocyte-derived exosomes. We demonstrated that multiple enzymes related to *de novo* lipogenesis were enriched in exosomes secreted under hypoxic conditions. These exosomes may promote lipid accumulation by transferring lipogenic enzymes into recipient cells.

2. Materials and methods

2.1. Reagents, cell lines, and animals

Detailed material information can be found in the data supplement.

2.2. Exosome purification

Donor cells (3T3-L1 cells or HEK 293T cells) were cultured in DMEM (4500 mg/L glucose) supplemented with 10% exosomes-depleted fetal bovine serum (FBS). Exosomes were depleted of FBS by 12 h ultracentrifugation at 100,000*g*, 4 °C. Exosomes were prepared from cell supernatants using sequential centrifugation and filtration steps. Briefly, cell supernatants were diluted in an equal volume of phosphate-buffered saline (PBS) and centrifuged for 30 min at 2000*g*, 4 °C. The supernatants were centrifuged for 60 min at 10,000*g*, 4 °C. Next, the supernatants were ultracentrifuged for 3 h at 100,000*g*, 4 °C. Pellets were resuspended in a large volume of PBS and the suspension was filtered through a 0.22-μm filter. Exosomes were pelleted by 3 h of ultracentrifugation at 100,000*g*, 4 °C. Total amount of exosomes was determined by using the BCA assay (Thermo Scientific, Rockford, IL, USA). Purified exosomes were resuspended in PBS and stored at −80 °C until use.

2.3. Electron microscopy analysis

PBS-resuspended exosomes were deposited onto formvar/carbon-coated EM grids (EMJapan Co., Ltd., Tokyo, Japan). Membranes were allowed to absorb for 10 min in a dry environment; excess liquid was removed gently using absorbent paper. Exosome-coated grids were stained with 1% uranyl acetate and the preparations were examined under a transmission electron microscope (TEM; H-7500, JEOL Ltd., Tokyo, Japan).

2.4. Proteomic analysis of purified exosomes

Exosomes purified from supernatants of 3T3-L1 adipocytes under normoxic (20% O_2) or hypoxic (1% O_2) for 48 h were solubilized in 0.8% RapiGest SF (Waters, Milford, MA, USA) and lysed by 3 freeze–thaw cycles in liquid nitrogen under sonication. Next, 60 μg of exosomal proteins were reduced, alkylated, digested with trypsin, and labeled with 2-plex iTRAQ reagents (AB Sciex, Framingham, MA, USA) according to the manufacturer's instructions with minor modifications. After the labeling reaction (114, Normoxia; 116, hypoxia), the 2 samples were pooled and 10 μL of 20% (v/v) trifluoroacetic acid was added to cleave the RapiGest. Samples were vortexed, incubated at 37 °C for 1 h and centrifuged. Supernatants were purified using a cation exchange column (AB Sciex) using standard procedures, as previously described [21]. Briefly, mobile phase A contained 98% water (2% acetonitrile (ACN), 0.1% formic acid) and mobile phase B contained 70% ACN (0.1% formic acid, 30% water). The column effluent was introduced into a spray chamber through a tapered stainless steel emitter and directly electrosprayed into the QSTAR System ion trap mass spectrometer in positive mode for nanoESI-tandem mass spectrometry (MS/MS) analysis. Each sample was run for 150 min. Protein identification was performed using Analyst QS Software 2.0 (AB Sciex) in positive-ion mode. Both data sets were processed using ProteinPilot Software 2.0.1 with the Paragon™ search algorithm (AB Sciex). MS/MS data were searched against the NCBI database using a *Mus musculus* taxonomy filter. The minimum threshold for protein identification was set at a protein score of 0.47, corresponding to a confidence level >66% and 1% false discovery rate.

2.5. Immunoblot analysis

Detailed material information can be found in the data supplement.

2.6. Exosomes taken up by 3T3-L1 cells

For red fluorescent labeling of cells, we incubated HEK 293T cells with PKH26 (Sigma–Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. 3T3-L1 preadipocytes were cultured in media containing PKH26 exosomes.

2.7. Statistical analysis

All data are presented as means ± SD. Data were analyzed using paired Student's *t*-test. $P < 0.05$ was considered significant.

3. Results

3.1. Serum exosomes are increased in obese animals

To examine whether obesity affects serum exosomes, serum exosomes from leptin-deficient (ob/ob) obesity mice and wild-type

(WT) mice were isolated. Exosomes from ob/ob mouse serum contained more protein amount than WT mouse serum (Fig. 1A). Notably, the amount of exosomal protein in ob/ob mice was similar to that in WT mice after compensating for body weight (Fig. 1B), indicating that the increase of serum exosomes in ob/ob mice was due to increased body weight.

3.2. Hypoxia enhances exosome secretion in 3T3-L1 adipocytes

Next, using 3T3-L1 cells as a model of differentiated-adipocytes, we examined whether adipocytes secrete exsomes. Conditioned medium were collected from pre-differentiated or differentiated 3T3-L1 cells after 24–48 h culture under normoxic conditions. Sequential centrifugation steps with increasing centrifugal forces up to 100,000*g* yielded a greasy pellet, which contained the exosomes (Fig. 1C). TEM analysis revealed that both conditioned medium from pre-differentiated and differentiated 3T3-L1 cells contained 50–150 μm intact membrane vesicles with typical exosome morphology (Fig. 1D).

Substantial evidence indicates that hypoxia develops in adipose tissue as the tissue mass expands, contributing to adipocyte dysfunction in obese animals [22]. Therefore, we examined the effect of hypoxia on exosome secretion of 3T3-L1 adipocytes. Expression of hypoxia-inducible factor-1α (HIF-1α), a regulator of hypoxic response, was markedly up-regulated under hypoxic conditions (Fig. 1E). As in normoxic 3T3-L1 adipocytes, 3T3-L1 adipocytes under hypoxic conditions secreted exosomes (Fig. 1E). Additionaly, 3T3-L1 adipocytes under hypoxic conditions showed a 3–4-fold increase in exosome secretion based on protein levels (Fig. 1F).

3.3. Exosomes from hypoxic 3T3-L1 adipocytes contain more lipogenic enzymes

To investigate whether hypoxic stress is reflected in exosomal protein content, we performed silver staining. Interestingly, a different protein band pattern was observed in 3T3-L1 adipocyte-derived exosomes under normoxic and hypoxic conditions (Supplementary Fig. 1). Additionally, we performed iTRAQ-based quantitative proteomic analysis. 3T3-L1 adipocyte-derived exosomes purified from normoxic or hypoxic culture conditions were analyzed using liquid chromatography–MS/MS. A total of 231 proteins were identified, with measured weighted median protein ratios reported in the Supplementary Tables 1–3 (ratio of hypoxia to normoxia, 116:114). A higher than 1.2-fold increase or less than 0.8-fold decrease was considered biologically significant. Among these, 75 and 67 exosomal proteins were up-regulated and down-regulated under hypoxic conditions, respectively. (Fig. 2A, and Supplementary Table 1A–C).

To confirm the quantitative proteomics data, the differential abundance of a subset of proteins was investigated using

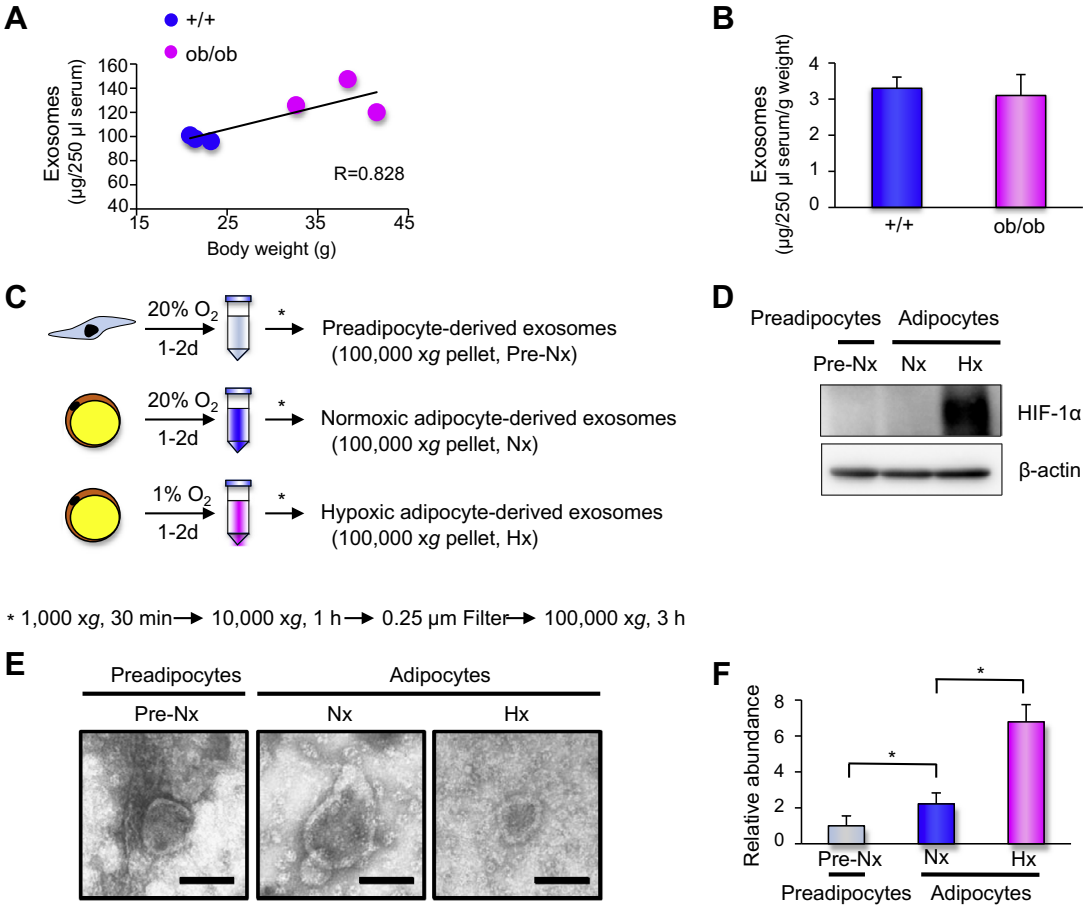

Fig. 1. Characterization of adipocyte-derived exosomes under hypoxic conditions. (A) Serum exosomes were purified from 7-week-old WT (+/+) and ob/ob mice using ExoQuick™ reagent and exosomal proteins per unit serum were determined. The graph represents the ratio of protein in serum exosomes for each group. Values are mean ± SD (each $n = 3$). $^*P < 0.01$. (B) Data in (A) are corrected by body weight. Values are mean ± SD. (C) Flow chart of differential centrifugation-based protocol for exosome purification from culture supernatants. (D) Expression level of HIF-1α protein in 3T3-L1 preadipocytes and adipocytes cultured under normoxic or hypoxic conditions for 24 h. (E) Morphology of 3T3-L1 preadipocyte- and adipocyte-derived exosomes from normoxic or hypoxic conditions visualized by electron microscopy. Scale bar, 100 nm. (F) Protein concentrations in preadipocyte- and adipocyte-derived exosomes generated under normoxic or hypoxic conditions. The graph represents the ratio of normoxic exosomal proteins to hypoxic proteins. Values are mean ± SD ($n = 3$). $^*P < 0.01$. 3T3-L1 adipocytes 10 days post differentiation were used for assays in (C)–(F).

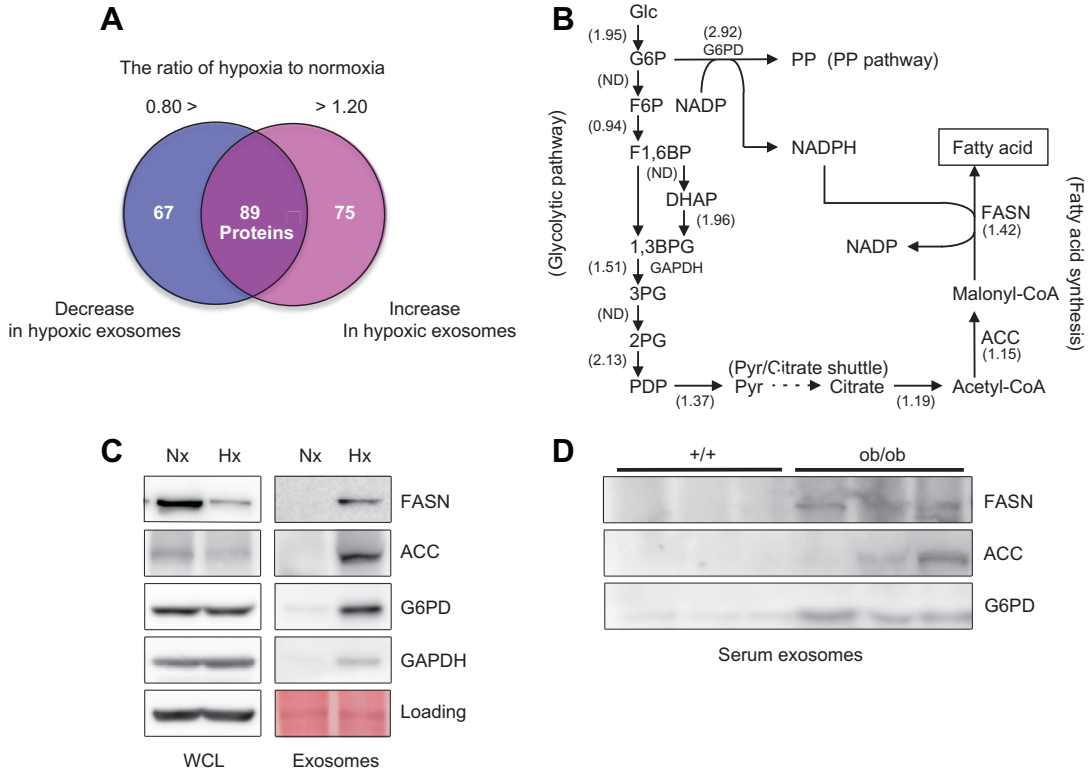

Fig. 2. Proteomic analysis of adipocyte-derived exosomes from normoxic or hypoxic conditions. (A) Comparison of proteins identified in adipocyte-derived exosomes under normoxic or hypoxic conditions. A total of 231 proteins were identified; a ratio under 0.80 was considered a decrease, while that over 1.20 was considered an increase. (B) Metabolic pathways leading to fatty acid production and its related enzymes. (C) FASN, ACC, and G6PD expression levels in whole cell lysates (WCL) and exosomes by Western blot analysis. ImmunoGold is a control blot of exosomal proteins. (D) FASN, G6PD, and GAPDH expression levels in serum exosomes from 7-week-old WT (+/+) and ob/ob mice by Western blot analysis.

Table 1
Up-regulated proteins in hypoxic adipocyte-derived exosomes, compared to control (>1.20-fold).

Accession numbers	Protein names	Unique peptdes detected	Sequencecoverage%	116:114 (Nx:Hx)	Expectation-value
gi|93102409	Fatty acid synthase	18	19	1.42	1.70E-22
gi|31981562	Pyruvate kinase isozymes M1/M2 isoform 1	6	22	1.37	1.32E-05
gi|33859482	Elongation factor 2	5	8	1.53	1.34E-05
gi|70794816	Uncharacterized protein LOC433182	5	21	1.34	3.62E-05
gi|6679937	Glyceraldehyde-3-phosphate dehydrogenase	4	21	1.51	1.91E-05
gi|6678483	Ubiquitin-like modifier-activating enzyme 1 isoform 1	4	10	1.51	2.67E-06
gi|6755901	Tubulin alpha-1A chain	4	18	1.41	5.03E-05
gi|309264022	PREDICTED: 40S ribosomal protein SA-like	3	19	1.88	2.15E-04
gi|31980648	ATP synthase subunit beta, mitochondrial precursor	3	10	1.81	1.04E-03
gi|52353955	D-3-phosphoglycerate dehydrogenase	3	8	1.68	8.92E-03

This table included up-regulated proteins having at least 3 unique peptides with ⩾99% confidence using ProteinPilot 2.0 software. Accession numbers are from the NCBI database. For additional information, see Supplementary Tables 1A and 2).

immunoblot analysis. In agreement with the proteomics data, an increase in heat shock protein (Hsp) 90 kDa –alpha, and –beta (Hsp90α, Hsp90β) and heat shock cognate 71 kDa protein (Hsc70) was found. In exosomes from 3T3-L1 adipocytes cultured under hypoxic conditions, heat shock 71 kDa protein 1A (Hsp72) was slightly decreased under hypoxic conditions, and Hsp60 was not detected, in agreement with our quantitative proteomics analysis.

From the proteomic analysis, we found that FASN was identified with significantly high confidence (Tables 1 and Supplementary Tables 1A and 2), and thereby focused on this enzyme in later analysis. Moreover, some of enzymes that could lead to fatty acids production were included among the proteins increased in hypoxic adipocyte-derived exosomes (Fig. 2B). In agreement with the proteomics data, an increase in FASN and G6PD was confirmed by immunoblot analysis. The ACC level was also increased in hypoxic adipocyte-derived exosomes, although the level was not statistically significant in proteomic analysis (Fig. 2C).

We next investigated whether such lipogenic factors were increased in serum exosomes, although serum contains both adipocyte-derived exosomes and a wide variety of tissue- and cell-derived exosomes. Serum exosomes from ob/ob mice showed increased protein levels of FASN, ACC and G6PD, compared with age- and sex-matched WT mice (Fig. 3D). Adipose tissue from WT and ob/ob mice expressed similar protein levels of FASN, ACC and G6PD (Supplementary Fig. 3).

3.4. Hypoxic adipocyte-derived exosomes promote lipogenesis

We examined whether 3T3-L1 adipocyte-derived exosomes induce changes in recipient cells, particularly regarding FASN. 3T3-L1

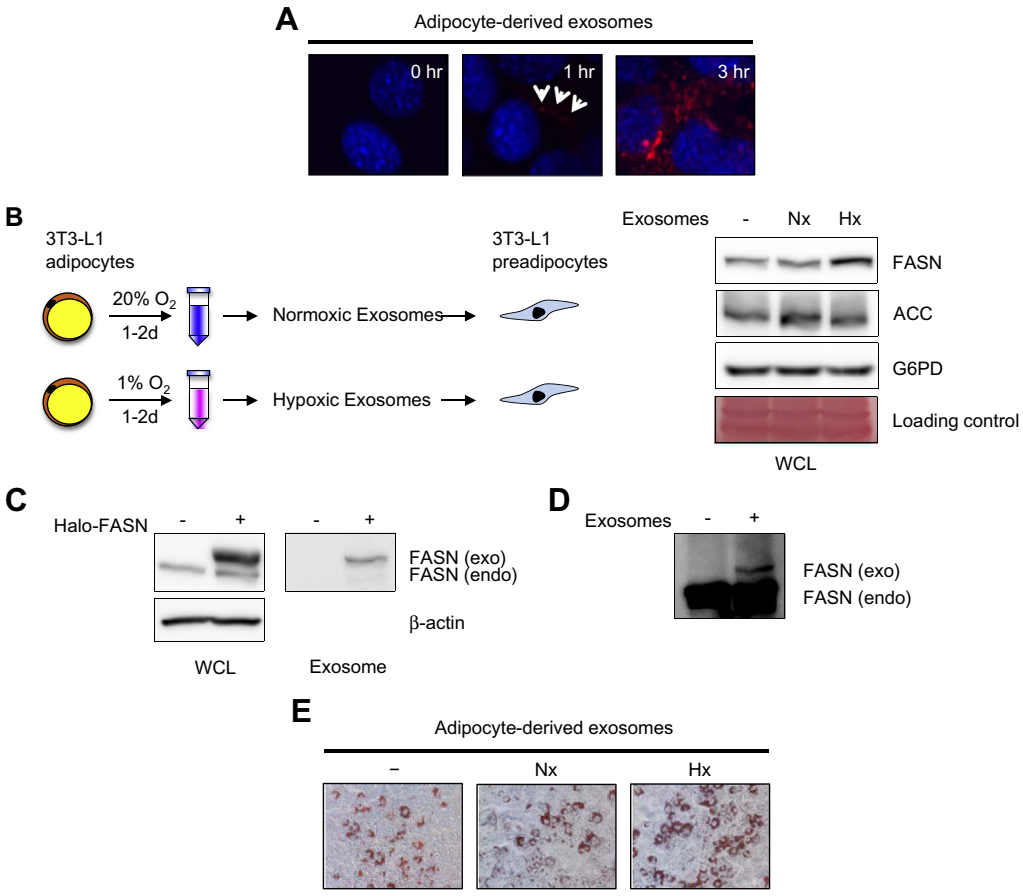

Fig. 3. Increased iipid accumulation in adipocytes treated with hypoxic adipocyte-derived exosomes. (A) Time-dependent uptake of adipocyte-derived exosomes in 3T3-L1 preadipocytes. PKH26-labeled exosomes (*red*) were added to 3T3-L1 cells and incubated as indicated. Cells were fixed and stained for nuclei (DAPI, *blue*). (B) Lysates from 3T3-L1 preadipocytes after incubation with exosomes from differentiated 3T3-L1 adipocytes cultured under normoxic or hypoxic conditions for 48 h were subjected to Western blot analysis using the indicated antibodies (FASN, ACC, and G6PD). (C) HEK 293T cells were transfected with plasmid encoding Halo-FASN. In Western blotting analysis, the same amounts of cell lysates (20 μg) and exosomes (1 μg) were loaded into the indicated lanes. (D) Lysates from 3T3-L1 preadipocytes after incubation with exosomes from HEK 293T cells transfected with plasmid encoding Halo-FASN were subjected to Western blot analysis using the anti-FASN antibody. (E) 3T3-L1 cells were induced to differentiate after confluence with a cocktail of hormones/steroids as described in the Section 2 for 6 days. 100 μg of adipocyte-derived exosomes from normoxic or hypoxic conditions was added to culture medium every 2 days. Original magnificaiton 100×.

preadipocytes were selected as recipient cells because this cell line accumulates cytosolic lipid droplets upon differentiation to adipocytes and its ease of use in assessing promoted lipogenesis.

First, we examined whether 3T3-L1 adipocyte-derived exosomes under normoxic conditions are internalized by 3T3-L1 preadipocytes. Exosomes labeled with PKH26 dye were added to 3T3-L1 preadipocytes. An efficient increase in the PKH26-derived signal from 3T3-L1 preadipocytes occurred in a time-dependent manner (Fig. 3A). Confocal microscopy confirmed that exosomes were inside 3T3-L1 preadipocytes rather than attached to the cell surface, suggesting that 3T3-L1 adipocyte-derived exosomes were taken in by 3T3-L1 preadipocytes.

We next examined whether proteins carried in exosomes can be transferred between donor and recipient cells. Recipient 3T3-L1 preadipocytes with 3T3-L1 adipocyte-derived exosomes were harvested from normoxic or hypoxic conditions. FASN level was elevated in recipient cells cultured with exosomes from hypoxic 3T3-L1 adipocytes (Fig. 3B). However, G6PD and ACC levels in recipient cells were not increased. To demonstrate that the increased FASN level was not a result of increased endogenous FASN expression but due to exosomal transfer, Halo-tagged FASN was expressed in human embryonic kidney (HEK 293T) cells, and exosomes were purified from untransfected or Halo-FASN-transfected cells. Exosomes from Halo-tagged FASN-overexpressed HEK 293T cells contained Halo-tagged FASN protein (Fig. 3c). 3T3-L1 cells incubated with purified exosomes including Halo-tagged FASN confirmed the transfer of Halo-FASN protein in exosomes into 3T3-L1 cells (Fig. 3D). These results suggest that exosomal FASN is transferred into recipient cells.

To determine whether exosomes from hypoxic 3T3-L1 adipocytes modify adipose differentiation in 3T3-L1 cells, we first examined the effect of exosomes maintained in cell culture during adipose differentiation. Normoxic exosomes did not show remarkable changes in lipid accumulation, while hypoxic exosome treatment of 3T3-L1 cells during preadipocyte differentiation enhanced lipogenesis and induced deposits of lipid droplets (Fig. 3D).

4. Discussion

Cellular stress conditions are reflected in the content of cell-derived exosomes [15]. Exosomes contain genetic materials and protein from the cell of origin, and thus depend on the stresses of the donor cells at the time of exosome biogenesis. Exosomes modulate the physiological functions of recipient cells through the transfer of RNA and proteins [14]. Exosomes exposed to some stress have been suggested to generally induce tolerance against

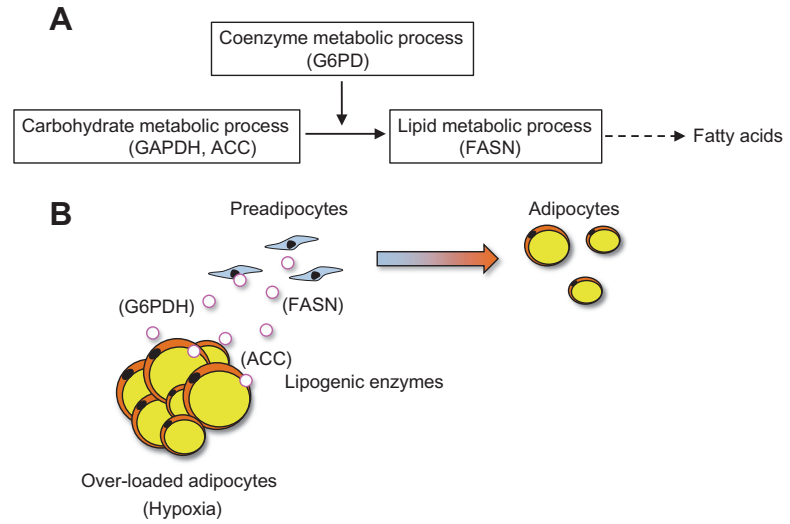

Fig. 4. Schematic representation of this study. (A) Relationship between ACC, G6PD, and FASN and *de novo* lipogenesis. (B) Promotion of lipid accumulation in preadipocytes or small adipocytes due to hypoxic adipocyte-derived exosomes.

further stresses in recipient cells [23]. Although adipocytes secrete exosomes [20], the functions of adipocyte-derived exosomes in pathological situations are poorly understood.

We here characterized the proteome profiles of mouse 3T3-L1 adipocyte-derived exosomes and evaluated the effect of hypoxia on adipocyte-derived exosomes as a stress model mimicking hypertrophied adipocytes. Lipid overload and subsequent hypertrophy of adipocytes triggers hypoxia due to insufficient blood supply [24]. We also demonstrated that both protein amounts and composition in adipocyte-derived exosomes varied significantly depending on the oxygen pressure under which cells were cultured. This suggests that adipocytes employ exosome-mediated cell communication to transfer information under hypoxic conditions. Using electron microscopy, isolated vesicles were found to have characteristisc that werre typical of exosomes [25]. Several exosome marker proteins, such as Hsp70, CD81 [26], and flotillin [27] were also detected in proteomics data. The amount of secreted exosome-associated proteins increased by 3–4-fold from adipocytes under hypoxic conditions. A recent study reported that several cell lines increase the number of exosomes under hypoxia [28], while others do not, even when exposing to hypoxia [18]. Whether increased exosome-associated proteins in hypoxic adipocytes are due to an increased number of exosomes or increased protein content in exosomes remains unknown. Using proteomic methods, we found that hypoxic adipocyte-derived exosomes showed much different protein profiles compared with normoxic exosomes. We subjected exosomal proteins to quantitative proteomics and identified 231 proteins. Seventy-five proteins were increased and 67 proteins were decreased in hypoxic 3T3-L1 adipocyte-derived exosomes. Analysis of enriched GO-terms showed that proteins related to metabolic processes were significantly increased in hypoxic adipocyte-derived exosomes. Interestingly, enzymes related to *de novo* lipogenesis such as G6PD, ACC, and FASN [8] were increased.

We found that adipocyte-derived exosomes from hypoxic conditions, which contain lipogenic enzymes, promote lipid accumulation in non-stressed, normoxic cells. Multi-enzyme complex FASN catalyzes palmitic acid synthesis from acetyl-CoA and ACC-produced malonyl-CoA in the presence of G6PD-produced NADPH (Fig. 4A). Therefore, upon exosomal transfer, donor hypoxic adipocytes may transfer the "lipogenic system", in which fatty acid synthesis is encoded, to recipient cells. As a result, donor cells decrease lipogenic enzymes, while recipient cells may increase these enzymes (Fig. 4B). Because the levels of FASN [29], G6PD [30], and ACC [31] are positively correlated with lipid synthesis in adipocytes, exosomal transfer of these enzymes promotes lipid synthesis in recipient cells. In our study, the FASN level in recipient 3T3-L1 preadipocytes was increased after treatment with hypoxic 3T3-L1 adipocyte-derived exosomes. However, the levels of G6PD and ACC were not increased. The reason may be because the high base-line level of G6PD and ACC interrupted the detection of exosome-derived proteins. Moreover, even a slight increase in G6PD has been reported to promote lipogenesis in 3T3-L1 cells [32].

In addition, we found that exosomes purified from ob/ob mice contain detectable FASN, ACC, and G6PD levels compared with those in WT mice. Thus, these exosomal proteins reflect metabolic stress in mammals and may be novel biomarkers, although the origin of exosomes may be non-adipose tissues. Serum FASN is elevated in patients with insulin resistance, although they exosomes have not been examined [33]. Future studies should be conducted to determine how exosomal proteins are incorporated in exosomes and the mechanisms involved.

In conclusion, adipocyte-derived exosomes are potential information carriers under pathological conditions that transfer internal proteins into neighboring cells. Adipocyte-derived exosomes are potential targets for obesity-associated adipose dysfunction.

Conflict of interest and funding

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.01.183.

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