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Indispensable role of lipoprotein bound-ApoE in adipogenesis and endocytosis induced by postprandial TRL

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ABSTRACT

Diet-associated obesity is coexisted with postprandial hypertriglyceridemia that indicates increased number of triglyceride-rich lipoproteins (TRL). This study aimed to investigate the effect of postprandial TRL-bound apolipoprotein E (ApoE) on adipogenesis and potential mechanisms. 3T3-L1 cells were cultured with (i) human TRL (h-TRL) with or without insulin, or (ii) TRL from wild type mice (WT-TRL) or ApoE knock-out mice (EKO-TRL) and insulin. The differentiating adipocytes were incubated with different kinds of TRL labeled by red fluorescence and confocal microscopy was performed. Receptor associated protein (RAP), heparin or both were added to inhibit low density lipoprotein receptor family receptors, heparan sulfate proteoglycan or both, respectively. With the aid of insulin, postprandial h-TRL or WT-TRL, instead of EKO-TRL, successfully induced adipogenesis. Confocal microscopy revealed red fluorescence in the differentiating adipocytes treated with h-TRL or WT-TRL, but not with EKO-TRL. RAP markedly reduced red fluorescence within the differentiating adipocytes, while heparin had little impact. The low density lipoprotein receptor related protein 1 protein showed upward trend with the increase of TRL concentrations. Taken together, lipoprotein-bound ApoE was required in both postprandial TRL-induced adipogenesis and TRL endocytosis by the differentiating adipocytes, the latter could be partially through low density lipoprotein receptor family dependent-pathway.

1. Introduction

Obesity has turned into a worldwide health problem, particularly in developing countries like China [1]. It's a major risk factor for chronic diseases ranging from cardiovascular disease to cancers [2–6]. Obesity is a condition of expanded mass of white adipose tissue (WAT), which is caused by adipocyte hypertrophy (cell size increase) and hyperplasia (cell number increase). The latter is adipogenesis, which involves preadipocytes proliferation and their differentiation into mature adipocytes. Although adipocyte number stays constant in adults, approximately 10% of fat cells are annually renewed, indicating that the adipocyte number is tightly controlled in a dynamic balance [7]. Adipogenesis can be induced by some chemicals *in vitro*. However, there is little information about other naturally *in vivo* adipogenic factors.

New evidence showed that high fat diet-induced obesity involves adipogenesis [8]. High fat diet is followed by postprandial hypertriglyceridemia which is mild and transient in healthy subjects while prominent and prolonged in obese subjects [9]. Postprandial hypertriglyceridemia represents increased number of triglyceride-rich lipoproteins (TRL), a group of heterogeneous lipoproteins of very low density lipoproteins (VLDL), chylomicrons and their remnants. VLDL isolated from fasting plasma enabled preadipocytes to differentiate into adipocytes [10]. A high-fat meal containing 800 calories is demonstrated to produce prominent postprandial hypertriglyceridemia in Chinese subjects [9]. The potential adipogenetic effect of postprandial TRL is unknown.

Cell surface lipoprotein receptors play an important role in diet-

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induced obesity in animal studies. Inactivation of low density lipoprotein (LDL) receptor family members resulted in resistance to dietary fat-induced obesity [11,12]. Cell surface heparan sulfate proteoglycans (HSPG) also contribute to intracellular lipid accumulation in adipocytes during adipogenesis [13]. These receptors can bind and internalize TRL particles via their ligand – apolipoprotein E (ApoE) carried on lipoproteins. Receptor-mediated uptake of the whole VLDL particles played an important role in adipogenesis [10]. However, the potential role of ApoE in postprandial TRL-induced adipogenesis and the responsible receptor for the internalization of postprandial TRL are not clear.

In the present study, we investigated (i) the adipogenic effect of postprandial TRL from hypertriglyceridemic subjects or mice, (ii) if adipogenesis and the uptake of postprandial TRL were dependent on lipoprotein-bound ApoE, (iii) the possible receptor for postprandial TRL endocytosis by the differentiating adipocytes, (iv) the expression of lipogenesis and lipolysis genes in postprandial TRL-induced adipogenesis.

2. Material and methods

2.1. Animals

Breeding pairs of wild type (WT) C57BL/6 and ApoE-knock out (EKO) mice (on a C57BL/6 genetic background) were obtained from the Jackson Laboratory (Bar Harbor, Me), housed under specific pathogen-free conditions, and maintained in static microisolator cages. WT or EKO mice (8–10 weeks old) were fasted for six hours prior to receiving an intragastric load of olive oil (10 mL/kg body weight). Four hours later, venous blood samples were collected in sterile tubes containing Na_2EDTA for wild type TRL (WT-TRL) or ApoE-deficient TRL (EKO-TRL) isolation. The housing and care of all animals and all procedures used in these studies were performed in accordance with the regulations of the Animal Ethics Committee of Central South University.

2.2. Human subjects and study meal

Eighteen hypertriglyceridemic individuals (fasting serum total triglycerides > 1.7 mmol/L, 38–48 years of age, 13 male/5 female, body mass index 23.1 ± 2.4 kg/m^2) were recruited under informed written consent according to a protocol approved by the ethics committee of the Second Xiangya Hospital. Patients on lipid-lowering or anti-inflammatory medications or with a fasting blood glucose >6.1 mmol/L were excluded. After a 12-h fast, these volunteers underwent a standardized oral fat tolerance test [14]. Four hours later, venous blood samples were collected for human TRL (h-TRL) isolation.

2.3. ApoE genotype determination

Genomic DNA was amplified using Taq DNA Polymerase kit, the polymerase chain reaction (PCR) thermalcycler along with primers for ApoE. Reaction condition: 95 °C for 5 min followed by 32 cycles of 95 °C for 70 s, 58 °C for 70 s and 72 °C for 70 s, finished with 72 °C for 10 min. The PCR products were digested at 37 °C for more than 3 h with *Hha*I restriction enzyme. DNA fragments were separated by 8% polyacrylamide gel. ApoE genotypes were determined by scoring for unique combination of fragment sizes as described by Hixson and Venier [15].

2.4. TRL isolation and characterization

The plasma samples were ultracentrifuged (d < 1.006 g/mL) to isolate TRL as previously described [14]. The prepared lipoproteins were dialyzed, concentrated and sterilized. Protein concentration was measured by BCA method. Final concentrations of the prepared lipoproteins were 1.5–2.9 mg protein/mL.

2.5. Cell culture and adipogenic differentiation

3T3-L1 preadipocytes were cultured in DMEM with 10% FBS and 100 units/mL of penicillin-streptomycin. Two-day post-confluent 3T3-L1 preadipocytes (designated day 0) were used for adipogenic inductions.

2.6. Oil red O staining

Cells were stained with Oil Red O at room temperature. After microscopic examination, the Oil Red O in cells were extracted by 100% isopropyl alcohol and measured for absorbance at 520 nm.

2.7. Isolation of total RNA and quantitative real-time PCR

Total RNA from cells was prepared using RNeasy Mini Kit. First-strand cDNA was synthesized from equal amounts of total RNA using SuperScript III First-Strand Synthesis Kit. The real-time PCR was performed using iQ SYBR Green Supermix on an ABI PRISM 7300 System. All reactions were done in triplicates. The relative amount of mRNA was calculated using the comparative threshold cycle ($2^{-\hat{A}\hat{A}CT}$) method. Mouse GAPDH mRNA was used as the invariant control.

2.8. Western blot analysis

Cell lysate extractions were prepared with Mammalian Protein Extraction Reagent according to the manufacturers' protocols. Lysates were quantitated using a BCA kit. Equal amounts of protein were subjected to SDS-PAGE and transferred to nitrocellulose membrane. Membranes were incubated with primary antibodies. Membranes were washed with phosphate-buffered saline (0.1% Tween-20) and incubated with secondary antibodies. The Odyssey Infrared Imaging System was used for image detection. The normalization control was β-actin.

2.9. Confocal microscopy analysis

TRL were conjugated to atto 565 NHS ester (red). EKO-TRL were incubated with recombinant human ApoE protein (10:3 in protein weight) in reaction buffer (0.9% NaCl, 20 mmol/L Tris and 0.001% EDTA, pH 7.4) for 1 h at 37 °C. The mixture was ultracentrifuged to remove unbound recombinant ApoE and immediately added into cultured cells.

For the uptake assay, day 0 preadipocytes are co-cultured with 0.5 mM of 3-isobutyl-1-methylxanthine (M), 1 μM of dexamethasone (D) and 10 μg/mL of insulin (I) until day 3, and maintained in DMEM (10% FBS) plus 10 μg/mL of insulin until day 5. Cells were incubated with DMEM (0.1% BSA) containing 50 mg/L Atto-565 NHS eater-labeled TRL and 1000 mg/L unlabeled TRL, fixed and used for immunofluorescence assay.

2.10. Statistical analysis

Data were represented as the mean ± standard deviation. Statistical analysis was done by one-way analysis of variance (ANOVA). When the ANOVA was significant, post hoc least significant difference and Duncan's t-tests were performed. GraphPad Prism v5.0 software was used for statistical analysis. $P < 0.05$ (two-sided) was considered statistically significant.

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