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Temperature induced modulation of lipid oxidation and lipid accumulation in palmitate-mediated 3T3-L1 adipocytes and 3T3-L1 adipocytes

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ABSTRACT

Human skin temperature can vary widely depending on anatomical location and ambient temperature. It is also known that local changes in skin and subcutaneous temperature can affect fat metabolism. This study aimed to explore the potential effects of surrounding thermal environment on fat by investigating cell viability, lipid oxidation, and lipid accumulation in 3T3-L1 adipocytes and palmitate-treated adipocytes after 4 h incubation. No significant differences of viability in 3T3-L1 adipocytes were detected under different temperature conditions. Despite no significant increase being observed under warm temperature (39 °C) conditions, a similarly significant suppression of intracellular reactive oxygen species (ROS) and lipid peroxidation were found in 3T3-L1 adipocytes and palmitate-treated adipocytes under 4 h exposure to cooler temperatures of 31–33 °C ($P < 0.01$). ROS, chemically reactive molecules containing oxygen, are currently understood to be a major contributor to oxidantive stress in obesity. Additionally, cooler temperatures (31–33 °C) could improve the size of lipid droplets in 3T3-L1 adipocytes ($P < 0.01$), but no significant effect was generated by temperature change on lipid droplets in palmitate-treated adipocytes. In the palmitate-induced adiposity model, although excessive ROS and lipid peroxidation has been attenuated by temperature decrease ($P < 0.01$), it still does not positively modulate lipid droplet size ($P > 0.05$) and remedy the palmitate damage induced cell death ($P < 0.01$). These findings provide preliminary support for potential interventions based on temperature manipulation for cell metabolism of adipocytes.

1. Introduction

Although humans are regarded as homeotherms (able to maintain a constant temperature), in fact, different parts of the body have different body temperatures, with a wide range of variations from below 27 °C to over 39 °C (Luchakov and Nozdrachev, 2009). The temperature differences depend on whether heat is taken up through oral means, inside a body cavity, on the surface of the skin in terms of different body parts (head vs. toe) or influenced by the microclimate of the skin and thermal environment near the skin (Luchakov and Nozdrachev, 2009; Pal and Pal, 1990; Saxena, 1983). It is well-known that regional changes in skin and subcutaneous temperature can affect fat metabolism. Earlier publication from Phinney et al. has been reported that the proportions of saturated and unsaturated fatty acids are significant different from abdomen, inner thigh and outer thigh, due to the temperature may have an effect on the selection process determining the variation in adipose fatty acid composition with anatomical location (Phinney et al., 1994). In addition, recent studies have indicated that regional body temperatures of humans are different for those who are obese and have a normal weight mainly because their body composition is different (Chudecka et al., 2014). Evidences also have shown that obese individuals have a lower abdominal temperature than those of normal weight (Savastano et al., 2009).

The main physiological function of adipose tissue is the regulation of blood glucose and lipid metabolism, insulin action, energy balance, and inflammation (Guilherme et al., 2008; Rajala and Scherer, 2003; Rosen and Spiegelman, 2006). Human body fat includes essential body fat and nonessential body fat. Essential fat contributes to maintaining the normal metabolism for regulating temperature, absorbing shock, and regulating essential body nutrients. Nonessential fat is the outcome of intake of more calories

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than you need. Too much nonessential fat accumulated always causes excess fat and triggers obesity and related metabolic syndromes (Koster et al., 2010; Sierra-Johnson et al., 2008). Elevation of excess saturated free fatty acids (SFFAs), such as palmitate, has been demonstrated to increase oxidative stress and cause insulin resistance (Epps-Fung et al., 1997; Hardy et al., 1991; Kennedy et al., 2009). Over the last decade, oxidative stress in excessive adipose tissue has been hypothesized to be a primary mechanism conducive to the pathogenesis and progression of obesity and obesity-related metabolism, such as diabetes and cardiovascular disease (Boden, 1997; Furukawa et al., 2004; Houstis et al., 2006; Van Gaal et al., 2006).

Data from Slikker et al. have suggested that glutathione peroxidase (GSH-Px) significantly increases at lower temperature (32–35 °C) in Chinese hamster ovary (CHO) cells, which indicated that the cooler temperature reduced the risk of oxidative stress-induced cellular damage. It has also been found that cooler temperatures (32–35 °C) reduced oxidative DNA damage and DNA damage-triggered pro-death signaling events against ischaemic brain injuries (Ji et al., 2007; Slikker et al., 2001). Slightly higher temperatures for the body temperature to reach a level between 39 °C and 42 °C has been supported as a novel treatment to control oxidative stress in obesity-related metabolic syndromes (Okada et al., 2004). Whole-body hyperthermia may be expected as a new potential therapy for diabetes (Medina-Navarro and Guerrero-Linares, 2009; Okada et al., 2004). Recent study has demonstrated that fat cells could directly sense temperature in the range from 27 °C to 39 °C to activate thermogenesis in a cell-autonomous manner (Ye et al., 2013).

Acting as an insulating layer beneath the skin, it is more likely that the subcutaneous adipose tissue experiences changes directly in the surrounding thermal environment; thus a change of surrounding thermal living conditions is becoming a main concern for influencing oxidative stress in adipose tissue, and is potentially a novel strategy to modulate adipose tissue-related health. Palmitate has been used as an incentive to provoke differentiated 3T3-L1 adipocytes in terms of mimicking an oxidative stress-related obese adiposity model (Hunnicutt et al., 1994; McCall et al., 2010; Wen et al., 2011). The aim of this study was to investigate how the variation of surrounding thermal living conditions affects lipid oxidation, lipid accumulation and cell viability in differentiated 3T3-L1 adipose and palmitate-treated 3T3-L1 adipocytes, and to explore the potential mechanism involved.

2. Materials and methods

2.1. Materials

3T3-L1 cell lines were purchased from American Type Culture Collection (ATCC). The 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)−2 h-tetrazolium-5-carboxanilide (XTT), phenazine methosulfate (PMS) and Oil red O were purchased from Sigma (USA). Reactive oxygen species (ROS) and the lipid peroxidation malondialdehyde (MAD) assay kits were purchased from the Beyotime Institute of Biotechnology (Jiangsu, China).

2.2. 3T3-L1 Preadipocytes culture and differentiation

The 3T3-L1 preadipocytes were cultured in 10% newborn calf serum with 100 U/ml penicillin and 100 μg/ml streptomycin (1 × P/S) (Gibco, USA) and incubated at 37 °C in 5% CO_2. To induce differentiation, 3T3-L1 preadipocytes were allowed to grow to confluence and treated with differentiation cocktail methylxanthine, dexamethasone and insulin (MDI) treatment (Gregoire et al., 1998). 3T3-L1 preadipocytes were cultured in differentiation medium containing 0.5 mM 3-Isobutyl-1-methylxanthine (IBMX, Sigma), 1 μM Dexamethasone (Dex, Sigma) and 10 μg/ml insulin (Sigma). After 48 h of exposure to differentiation medium, cells were changed to exposure to the medium with 10 μg/ml insulin for another 48 h. Then, cells were maintained for an additional 4 days in DMEM with 10% fetal calf serum to achieve full differentiation for further experiments.

2.3. Palmitate treatment

Palmitate (Sigma) was used as an incentive to provoke differentiated 3T3-L1 adipocytes in terms of establishing an adipose tissue model with obesity and insulin resistance (Epps-Fung et al., 1997; Shankar et al., 2008; Yeop Han et al., 2010). Briefly, 3T3-L1 adipocytes were incubated with the palmitate solutions (0.3 mM final concentration) containing 2% (w/v) free bovine serum albumin (BSA, Sigma) in serum-free DMEM for 24 h before treated with different temperature.

2.4. Cell viability

3T3-L1 adipocytes with or without exposure to palmitate were seeded at a density of 1×10^5 cells/well in 96-wells plate and kept in the humidified incubator with 37 °C, 5% CO_2 for 24 h. After incubation, cells were changed to be incubated to a range of temperature (31–39 °C) in the incubator of SANYO MCO175 with 5% CO_2 for 4 h (Ye et al., 2013). XTT was performed after different temperature treatment. In brief, the cells were washed with PBS and incubated for 4 h with 50 μL XTT solution. The absorbance was measured using a micro-plate reader (Bio Rad, USA) at a wavelength of 475 nm (reference wavelength 660 nm).

2.5. Intracellular reactive oxygen species

Intracellular ROS productions were investigated by means of a fluorescent oxidant sensing probe, DCFH-DA. Fully differentiated adipocytes were seeded into 96-wells and 6-wells plate with a density of 1×10^5 cells/well and 1×10^6 cells/well, respectively; 24 h later, cells were exposed to a range of temperature (31–39 °C) in culture for 4 h. Thereafter, the medium was removed and replaced with serum-free medium, and 100 μL of 10 μM DCFH-DA was added to each well. Cells were incubated for 30 min and then the data of intracellular oxidation with ROS were obtained quantitatively by using fluorescence and absorbance reader (Spectrafluor plus, Tecan, USA) and qualitatively by using fluorescent microscopy (Leica, DM4000B, Germany).

2.6. Lipid peroxidation

Fully differentiated adipocytes were seeded into 6-wells with a density of 1×10^6 cells/well, respectively. 24 h later, cells were exposed to a range of temperature (31–39 °C) in culture. Cells then were homogenized on ice in 300 ml of the MDA Lysis Buffer and the homogenates were centrifuged at 1600*g* at 4 °C for 10 min. The supernatants were collected and determined with lipid peroxidation MDA assay kit. A 200 μl of thiobarbituric acid (TBA) reagent was added to 100 μl of the sperm suspension. The mixture was treated at 100 °C for 15 min. After cooling, the suspension was centrifuged (1000*g*; 10 min) and the supernatant was separated. Pipette 200 ml from each reaction mixture into a 96-wells plate for analysis. For colorimetric assays, the absorbances were measured by micro-plate reader (Bio Rad, USA) at 532 nm for MDA.

2.7. Lipid droplet size

Fully differentiated adipocytes were washed with PBS and then