Original article

MOLECULAR METABOLISM

Adipose tissue glycogen accumulation is associated with obesity-linked inflammation in humans

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ABSTRACT

Objective: Glycogen metabolism has emerged as a mediator in the control of energy homeostasis and studies in murine models reveal that adipose tissue might contain glycogen stores. Here we investigated the physio(patho)logical role of glycogen in human adipose tissue in the context of obesity and insulin resistance.

Methods: We studied glucose metabolic flux of hypoxic human adipoctyes by nuclear magnetic resonance and mass spectrometry-based metabolic approaches. Glycogen synthesis and glycogen content in response to hypoxia was analyzed in human adipocytes and macrophages. To explore the metabolic effects of enforced glycogen deposition in adipocytes and macrophages, we overexpressed PTG, the only glycogen-associated regulatory subunit (PP1-GTS) reported in murine adipocytes. Adipose tissue gene expression analysis was performed on wild type and homozygous PTG KO male mice. Finally, glycogen metabolism gene expression and glycogen accumulation was analyzed in adipose tissue, mature adipocytes and resident macrophages from lean and obese subjects with different degrees of insulin resistance in 2 independent cohorts.

Results: We show that hypoxia modulates glucose metabolic flux in human adipocytes and macrophages and promotes glycogenesis. Enforced glycogen deposition by overexpression of PTG re-orients adipocyte secretion to a pro-inflammatory response linked to insulin resistance and monocyte/lymphocyte migration. Furthermore, glycogen accumulation is associated with inhibition of mTORC1 signaling and increased basal autophagy flux, correlating with greater leptin release in glycogen-loaded adipocytes. PTG-KO mice have reduced expression of key inflammatory genes in adipose tissue and PTG overexpression in M0 macrophages induces a pro-inflammatory and glycolytic M1 phenotype. Increased glycogen synthase expression correlates with glycogen deposition in subcutaneous adipose tissue of obese patients. Glycogen content in subcutaneous mature adipocytes is associated with BMI and leptin expression.

Conclusion: Our data establish glycogen mishandling in adipose tissue as a potential key feature of inflammatory-related metabolic stress in human obesity.

Keywords Glycogen; Adipocyte; Macrophage; Autophagy; Obesity; Insulin resistance

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Received September 17, 2015 • Revision received October 2, 2015 • Accepted October 9, 2015 • Available online 16 October 2015

http://dx.doi.org/10.1016/j.molmet.2015.10.001

1. INTRODUCTION

Adipose tissue is a highly complex metabolic organ with essential roles in handling and storing excess nutrients and preventing ectopic lipid accumulation in other organs. Excess lipid storage in adipose tissue leads to changes in its metabolic and endocrine functions, resulting in the generation of stress signals and the derangement of carbohydrate metabolism, among others [1]. In this setting, not only adipocytes but also infiltrating adipose tissue macrophages (ATMs), among other immune cells, participate in the chronic low-grade inflammation that occurs in the adipose tissue of obese individuals [2].

While triglyceride stored in adipose tissue is considered the principal energy reserve in mammals, glucose can also be stored as glycogen, primarily in liver and skeletal muscle, for mobilization during times of energy deficit. In humans, hepatic and skeletal muscle glycogen deposition is decreased under pathologic conditions associated with obesity, such as insulin resistance and type 2 diabetes [3–7], and is a hallmark of metabolic stress.

Glycogen synthesis is dynamically regulated by insulin through coordinated dephosphorylation of the key glycogenic enzyme glycogen synthase (GS) by the serine/threonine phosphatase PP1, which results in its activation, and the glycogenolytic enzyme glycogen phosphorylase (GP), which is inactivated [8]. The PP1 catalytic subunit (PP1c) is targeted to glycogen particles by glycogen-associated targeting subunits (PP1-GTS), including PPP1R3A (G_M, R_{GL}), PPP1R3B (G_L), PPP1R3C (PTG, or protein targeting to glycogen), PPP1R3D (PPP1R6) and PPP1R3E. PP1-GTS exhibit tissue and species-specific expression and play critical roles in the hormonal regulation of cellular glycogen metabolism [9–11].

Although present in low quantities, studies in murine models have shown that adipose tissue can store glycogen [12,13]. *Pp1r3c* was identified in a two-hybrid screen of a 3T3–L1 adipocyte library [14] and, to date, remains the only reported PP1-GTS expressed in murine adipocytes. Transgenic overexpression of PTG in adipose tissue increases glucose flux into the glycogen synthesis pathway, indicating that adipocytes are capable of storing high levels of glycogen. Interestingly, although adipocyte function appeared to be maintained in these animals, leptin, but not adiponectin, protein content in adipose tissue was increased, and the associated hyperleptinemia was independent of fat mass [15]. Further studies showed that upon caloric excess-induced expansion of adipose tissue mass, the elevated levels of glycogen in this model inhibited the mobilization of triglyceride and impeded weight loss following the return to chow feeding [16].

There is a paucity of research on the potential physio(patho)logical role of glycogen metabolism in adipose tissue. We hypothesized that obesity redirects glucose metabolic flux into glycogen synthesis in human adipocytes. Here we show that hypoxia, which has been linked to obesity-related adipose tissue dysfunction, increases glucose uptake and stimulates glycogen synthesis in adipocytes. Glycogen-loaded adipocytes exhibit increased autophagic flux, which directly impacts their endocrine secretory function. Furthermore, enforced glycogen deposition by overexpression of PTG in macrophages promotes polarization towards the M1 pro-inflammatory phenotype. Studies with human clinical samples confirm the interplay between autophagy and glycogen storage and show that human obesity is associated with glycogen deposition in adipocytes. Overall, our data demonstrate that glycogen accumulation in adipocytes and macrophages contributes to adipose tissue inflammation, and might underlie the metabolic alterations in obesity.

2. METHODS

2.1. In vitro cell cultures

The SGBS cell line, provided by Dr. Wabitsch (University of Ulm, Germany) and Lisa-2 cells, provided by Dr. Möller (University of Ulm, Germany), were used as cellular models of human subcutaneous and visceral pre-adipocytes, respectively, and were induced to differentiate as described [17]. THP-1 cells (a human monocytic cell line; ATCC, Rockville, MD) were induced to differentiate to macrophages with PMA as previously described [18]. The human myogenic cell line LHCN-M2 was used as a cellular model of human myoblasts. For migration experiments, monocytic THP-1 cells and Jurkat cells (human *T cell* lymphoblast-like *cell* line; ATCC, Rockville, MD) were grown in suspension. hASCs were isolated from the adipose tissue of lean patients (BMI 22.5 $\pm$ 0.3) following published protocols [19]. For hypoxia experiments, fully differentiated cells were cultured in a modular incubator flushed with 2% O_2, 93% N_2, and 5% CO_2. As controls, cells were cultured in a standard incubator (21% O_2 and 5% CO_2). Human adipose tissue-derived macrophages were isolated from the stromal-vascular fraction as previously described [20].

2.2. Adenoviral transduction

Cells were infected 7 days after induction of differentiation with an adenovirus expressing murine PTG (Ad-PTG) [21] or GFP (Ad-GFP) under the control of the CMV promoter [10]. Adenoviral infection was carried out for 2 h at a multiplicity of infection (moi) of 50. One day after infection, culture medium was depleted of insulin and FBS and metabolic experiments were performed 24 h later.

2.3. Gene expression analysis

Total RNA was extracted from adipose tissue/cells using the RNeasy Lipid Tissue Midi Kit (Qiagen Science, Hilden, Germany). Total RNA quantity was measured at 260 nm and purity was assessed by the OD260/OD280 ratio. One microgram of RNA was reverse transcribed with random primers using the Reverse Transcription System (Applied Byosistems, Foster City, CA). Quantitative gene expression was evaluated by Real-time PCR (qPCR) on a 7900HT Fast Real-Time PCR System using TaqMan® Gene Expression Assays (Applied Biosystems) for *in vitro* experiments and TaqMan® Low Density Arrays (Applied Biosystems, microfluidic cards) for studies with human samples (See Supplementary Experimental Procedures for all evaluated genes). Results were calculated using the comparative Ct method (2-$\Delta\Delta$Ct), and expressed relative to the expression of the housekeeping genes cyclophilin 1A (*PPIA*) (Hs 04194521_s1) and 18S (Hs 03928985_g1).

2.4. Glycogen synthesis rate

Cells were incubated with 20 mM [U-14C]glucose (0.05 μCi/μmol). To extract glycogen, cell monolayers were scraped into 100 μl of 30% (w/v) KOH and homogenates were boiled for 15 min. Homogenates were spotted onto Whatman 3 MM paper, and glycogen was precipitated by immersing the paper in ice-cold 66% (v/v) ethanol. Radioactivity in dried papers was counted in a beta-radiation counter. Glycogen synthesis rate was calculated as nanomoles of glucose incorporated per milligram of protein, and results were expressed as the percentage of stimulation over basal (control = 100) [10].

2.5. Glycogen content determination

Cell monolayers were scraped in 30% (wt/vol.) KOH. Homogenates were spotted onto Whatman 3 MM paper, and glycogen was precipitated in ice-cold 66% (vol./vol.) ethanol. The papers were incubated in

