Original Article

MOLECULAR METABOLISM

Adipocyte lipid synthesis coupled to neuronal control of thermogenic programming

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ABSTRACT

Background: The de novo biosynthesis of fatty acids (DNL) through fatty acid synthase (FASN) in adipocytes is exquisitely regulated by nutrients, hormones, fasting, and obesity in mice and humans. However, the functions of DNL in adipocyte biology and in the regulation of systemic glucose homeostasis are not fully understood.

Methods & results: Here we show adipocyte DNL controls crosstalk to localized sympathetic neurons that mediate expansion of beige/brite adipocytes within inguinal white adipose tissue (iWAT). Induced deletion of FASN in white and brown adipocytes of mature mice (iAdFASNKO mice) enhanced glucose tolerance, UCP1 expression, and cAMP signaling in iWAT. Consistent with induction of adipose sympathetic nerve activity, iAdFASNKO mice displayed markedly increased neuronal tyrosine hydroxylase (TH) and neuropeptide Y (NPY) content in iWAT. In contrast, brown adipose tissue (BAT) of iAdFASNKO mice showed no increase in TH or NPY, nor did FASN deletion selectively in brown adipocytes (UCP1-FASNKO mice) cause these effects in iWAT.

Conclusions: These results demonstrate that downregulation of fatty acid synthesis via FASN depletion in white adipocytes of mature mice can stimulate neuronal signaling to control thermogenic programming in iWAT.

Keywords Adipocytes; de novo lipogenesis; iWAT browning; Glucose homeostasis; Sympathetic nerve activation

1. INTRODUCTION

Adipose tissue metabolism plays a paramount role in regulating energy balance and metabolic homeostasis, and its disruption is thus closely associated with metabolic diseases, such as obesity and diabetes [1–5]. Several studies have demonstrated major beneficial effects of implantation of adipocytes or adipose tissues derived from insulin sensitive mice into obese animals [5–8]. Three predominant pathways have been suggested whereby adipose tissue exerts such control over whole body glucose homeostasis. One hypothesis proposes a key role for sequestering triglyceride (TG) within adipocytes, reducing systemic "lipotoxicity" by keeping lipids away from disrupting sensitive pathways in other tissues such as liver and muscle [3,9]. A second concept focuses on extensive evidence that adipocytes display endocrine functions that play a pivotal role in controlling whole body metabolism [1,2,10,11]. Subsequent to the discoveries of the secreted adipose proteins leptin [12,13] and adiponectin [14], many other adipocyte-derived peptides that may modulate multiple organs (e.g., liver, brain, pancreas, skeletal muscle, and heart) important for the maintenance of energy balance and prevention of metabolic dysfunction have been identified [2,10,15–17]. A third concept of how adipose tissue might regulate systemic metabolic homeostasis invokes relatively new findings that brown adipose tissue (BAT) and "beige/brite" adipocytes within mouse and human white adipose tissues (WAT) might be major sites of fatty acid oxidation and energy expenditure [4,8,18]. These three concepts are not mutually exclusive, and, together, these pathways may operate synergistically to optimize adipose tissue depot influence on overall metabolic flux in mammals.

Initial work on adipocyte metabolism in obesity revealed that the biosynthesis of palmitate from acetyl CoA, denoted de novo lipogenesis (DNL), was markedly reduced in the insulin resistant state [19,20] in spite of near normal glucose uptake [21]. These data suggested a possible role of DNL disruption in insulin resistance of adipocytes that was extended to human adipose tissue [22,23]. These findings were initially puzzling, because DNL contributes only a few percent of fatty acids to the TG that accumulates in the lipid droplets of adipocytes [24–26]. More recently, however, a number of adipose-derived putative bioactive lipids that arise from DNL [27,28] have been identified,

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including palmitoleate [29], the novel fatty acid—hydroxy—fatty acid (FAHFAs) lipids [30], and the recently identified PPARγ ligand alkyl ether-lipid (1-*O*-octadecenyl-2-palmitoyl-3-glycerophosphocholine) [31]. Beside biosynthesis of fatty acids, the DNL pathway is a rich source of metabolites known to regulate cellular processes, ranging from gene transcription to protein post-translational modifications (e.g., protein acetylation and palmitoylation) [32—36]. Nonetheless, experimental genetic manipulation of this pathway in adipocytes has produced conflicting results in terms of systemic effects [29,31,37,38], and the role of adipocyte DNL in both adipose function and systemic metabolic regulation remains unclear.

The aim of the present studies was to clarify the physiological role of adipocyte DNL by generating mouse models in which adipocyte FASN could be depleted after the animals reach maturity, thereby eliminating possible confounding effects of FASN knockout during mouse development. To this end, we generated a tamoxifen (TAM)-inducible adipose-specific FASN knockout mouse line (iAdFASNKO) in which FASN deletion and inhibition of DNL in adipocytes is initiated in adult mice. Analysis of these mice following FASN deletion revealed improved glucose tolerance and showed strong iWAT browning, with enhanced UCP1 and thermogenic gene expression, as previously found in constitutive adipocyte FASN knockout mice [31]. Surprisingly, however, depletion of FASN in primary white adipocytes in vitro failed to modulate UCP1 or other genes that are characteristically expressed at high levels in BAT, indicating that a cell autonomous mechanism is not involved and that an intact animal is required for the browning effect. This interpretation proved correct, as a markedly increased sympathetic innervation of iWAT was observed in response to induced FASN depletion in adipocytes of mature mice.

2. MATERIALS AND METHODS

2.1. Animal studies

Four-week old male C57BL/6J (WT) and B6.V-Lepob/J (ob/ob) mice were obtained from Jackson Laboratory. Mice were housed on a 12 h light/dark schedule and had free access to water and food, except when indicated. WT mice were fed a high fat diet (HFD) that contained 60% calories from lipids (Research Diets, D12492). Mice with conditional $FASN^{flox/flox}$ alleles were generated as described elsewhere [31]. To inactivate FASN in adult mice, homozygous $FASN^{flox/flox}$ animals were crossed to Adiponectin-Cre-ERT2 mice (a generous gift from Dr. Evan Rosen). At eight-weeks of age, both $FASN^{flox/flox}$ and $FASN^{flox/flox}$-Adiponectin-Cre-ERT2 (iAdFASNKO) were then treated once a day via intraperitoneal (i.p.) injection with 1—2 mg TAM dissolved in corn oil for 6 consecutive days. $FASN^{flox/flox}$ animals were also crossed with UCP1-Cre mice (Jackson Laboratory stock number 024670) to generate the UCP1-FASNKO that specifically delete FASN in brown adipocytes. For deletion of FASN in cultured primary adipocytes, $FASN^{flox/flox}$ mice were crossed with the TAM-inducible UBC-Cre-ERT2 mice (B6.Cg-Tg(UBC-cre/ERT2)1Ejb/2J mice, stock number 008085) from Jackson Laboratories.

All of the studies performed were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Massachusetts Medical School.

2.2. Metabolic studies

Glucose and insulin tolerance tests were performed on WT, iAdFASNKO, or UCP1-FASNKO mice as indicated. Glucose (1 g/kg) was administrated by i.p. injection. Blood samples were drawn from the tail vein at the indicated times, and glycemia was determined using a Breeze 2 glucose meter (Bayer and alpha-trak). For the effect of FASN deficiency on glucose tolerance, mice were first fed with chow or HFD for 4 weeks and then injected with vehicle or TAM to induce FASN deletion. Plasma insulin levels were measured with Millipore insulin ELISA.

2.3. Lipogenesis assay

To assess lipogenesis in vivo, hyperinsulinemic-euglycemic clamps were performed following an overnight fast, a 2-h hyperinsulinemic (insulin at 150 mU kg−1 body weight priming followed by 2.5 mU kg−1 min−1)-euglycemic clamp was conducted in awake mice using [^{14}C]-U-glucose to assess glucose metabolism in adipose tissue depots as described previously [39]. Tissues were then harvested, total neutral lipids extracted, and incorporation of glucose into TG or fatty acids determined as previously described [40]. For lipogenesis ex vivo, adipose tissue explants were incubated with labeling media containing 2% FA-free BSA, 1% (v/v) Pen/Strep, 0.5 mM D-Glucose, 0.5 mM Sodium Acetate, 2 mM sodium pyruvate, 2 mM glutamine, 2 μCi/mL ^{14}C-U-glucose. Insulin at 1 μM was added to insulin-stimulated conditions. Adipose tissue explants were incubated at 37 °C in a humidified incubator (5% CO2) for 4.5 h before lipid extraction. Glucose incorporation into TG or into fatty acids was then determined as previously described [40]. To determine lipogenesis in cultured primary adipocytes, cells were incubated with labeling media with or without insulin for 4.5 h and total neutral lipid extracted as described.

2.4. Westerns blots

For in vivo insulin stimulated Akt phosphorylation, mice were fasted for 4 h, injected with phosphate-buffered saline (PBS) or insulin (1 IU/kg), and 15 min after the injection, tissues were harvested. For protein expression analyses, tissues were homogenized in lysis buffer (20 mM HEPES [pH 7.4], 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.1% SDS, 10% glycerol, 0.5% sodium deoxycholate) that had been supplemented with Halt protease and phosphatase inhibitors (Thermo Pierce). Samples from tissue lysates were then resolved by SDS-PAGE, and immunoblots were performed using standard protocols. Membranes were blotted with the following antibodies: AKT (S473 and total), ACLY, ACC, GAPDH, pHSL-S660, HSL, NPY, and perilipin (Cell Signaling Technology); FASN (BD Biosciences); UCP1 (Alpha Diagnostics); phospho-perilipin-S522 (Vala Sciences); TH (Abcam and Millipore), Tubulin (Sigma—Aldrich). Best results for western blotting of TH were obtained with Millipore-MAB318 antibody. For the ex vivo insulin signaling experiments, adipose tissue explants were incubated in Dulbecco's Modified Eagle Medium (DMEM) media supplemented with 0.5 mM glucose, 2 mM sodium pyruvate and 2 mM glutamine, in the absence or presence of 1 μM insulin for 30 min. Adipose tissue explants were then homogenized and total cell lysate immunoblotted with total and phospho-Akt.

2.5. Adipocyte isolation

For detection of FASN protein on isolated adipocytes, adipose tissues from control or iAdFASNKO were minced and digested, and the adipocytes were isolated as described previously [41].

2.6. Histological analysis

For the immunohistochemistry (IHC) and immunofluorescence (IF) of adipose tissue analyses, tissue samples were fixed in 4% paraformaldehyde and embedded in paraffin. Sectioned slides were then stained for H&E, UCP1 (Abcam) or for TH (Abcam) and DAPI, at UMASS Medical School Morphology Core. For immunofluorescence analyzes, photos from the stained adipose tissue sections were taken with an Axiovert 35 Zeiss microscopy (Zeiss, Germany) equipped with an Axiocam CCl camera at indicated magnification.