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# Alpha-syntrophin deficient mice are protected from adipocyte hypertrophy and ectopic triglyceride deposition in obesity

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ABSTRACT

Alpha-syntrophin (SNTA) is a molecular adapter protein which is expressed in adipocytes. Knock-down of SNTA in 3T3-L1 preadipocytes increases cell proliferation, and differentiated adipocytes display small lipid droplets. These effects are both characteristics of healthy adipose tissue growth which is associated with metabolic improvements in obesity. To evaluate a role of SNTA in adipose tissue morphology and obesity associated metabolic dysfunction, SNTA deficient mice were fed a standard chow or a high fat diet. Mice deficient of SNTA had less fat mass and smaller adipocytes in obesity when compared to control animals. Accordingly, these animals did not develop liver steatosis and did not store excess triglycerides in skeletal muscle upon high fat diet feeding. SNTA −/− animals were protected from hyperinsulinemia and hepatic insulin resistance. Of note, body-weight, food uptake, and serum lipids were normal in the SNTA null mice. SNTA was induced in adipose tissues but not in the liver of diet induced obese and ob/ob mice. In human subcutaneous and visceral fat of seven patients SNTA was similarly expressed and was not associated with body mass index. Current data demonstrate beneficial effects of SNTA deficiency in obesity which is partly attributed to smaller adipocytes and reduced white adipose tissue mass. Higher SNTA protein in fat depots of obese mice may contribute to adipose tissue hypertrophy and ectopic lipid deposition which has to be confirmed in humans.

## 1. Introduction

Adipose tissue has a central role in the physiology of the organism which becomes apparent in diseases associated with too much and too little fat mass (Buechler et al., 2015; Freitas and Carvalho, 2013; Sun et al., 2011). Adipocytes are a major storage site for triglycerides and cholesterol. In obesity adipocyte number and size are increased to satisfy the needs of elevated fat deposition. Healthy adipose tissue expansion is characterized by the recruitment of adipocyte precursors that differentiate into small, insulin sensitive adipocytes. Pathological fat mass growth is associated with inappropriate recruitment and proliferation of fat cell progenitor cells. Fat pad expansion occurs through extensive enlargement of resident adipocytes and is associated with adipocyte death, hypoxia, fibrosis and inflammation. Fatty acid surplus is stored in muscle and liver and contributes to insulin resistance which is a major cause for metabolic diseases associated with obesity (Buechler et al., 2015, 2011; Sun et al., 2011, 2013; Wang et al., 2013).

The adapter protein alpha-syntrophin (SNTA) is a component of the dystrophin associated glycoprotein complex (DAPC) in skeletal muscle (Bhat et al., 2013). SNTA deficient animals display skeletal muscle hypertrophy and aberrant neuromuscular junctions (Adams et al., 2004; Hosaka et al., 2002). Skeletal muscle SNTA is reduced in diabetic rats and patients with gestational diabetes (Boyle et al., 2014; Mulvey et al., 2005). Neuronal nitric oxide synthase (nNOS) binds to SNTA and is low in muscle of diabetic rats (Mulvey et al., 2005) and type 2 diabetic patients (Krause et al., 2014). This is not associated with signs of muscle pathology but may contribute to inappropriate insulin responsiveness (Mulvey et al., 2005). SNTA deficiency in mice alters cellular distribution of nNOS but does not affect the contractile properties of muscles (Kameya et al., 1999).

Recently, our group has shown that SNTA is expressed in adipocytes and its knock-down enhances 3 T3-L1 preadipocyte proliferation. Low SNTA in 3T3-L1 preadipocytes does, however, not grossly affect differentiation to mature cells. Proteins like adiponectin and caveolin-1, which are induced during adipogenesis (Scherer et al., 1994; Weigert et al., 2008), are at normal levels. Interestingly, adipocytes with low

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SNTA form smaller lipid droplets. In 3T3-L1 adipocytes SNTA knock-down does neither change insulin signaling nor basal or beta-adrenergic induced lipolysis (Eisinger et al., 2016). Thus enhanced lipolysis does most likely not contribute to the formation of small lipid droplets in 3T3-L1 adipocytes with low SNTA. SNTA binds to guanine nucleotide-binding protein alpha-subunits which are downstream of β-adrenergic receptors (Okumura et al., 2008). Knock-down of SNTA in COS-7 cells enhances isoproterenol induced cAMP production and lipolysis (Okumura et al., 2008). Different effect of SNTA deficiency on lipolysis in COS-7 cells and adipocytes suggests that the biologic function of this adapter protein is cell type specific.

Here we intend to unravel the function of SNTA in the development of metabolic pathologies using SNTA deficient mice fed a high fat diet. Indeed, SNTA −/− animals display reduced fat pad weights, adipocyte hyperplasia and are protected from ectopic lipid deposition. Adipocytes in white fat are nearly entirely filled by a single lipid droplet which thereby determines cell volume (Sun et al., 2011) suggesting that smaller adipocytes in fat pads of SNTA −/− mice are related to the reduced size of the lipid droplets.

Cellular pathways of adipose tissue growth affect the pathogenesis of metabolic disturbances in obesity (Guilherme et al., 2008; Sun et al., 2011, 2013). SNTA deficiency favors healthy adipose tissue enlargement and this may at least in part protect these animals from metabolic dysfunction in obesity.

## 2. Material and methods

### 2.1. Animal handling

The male SNTA −/− mice and the corresponding wild type mice were from The Jackson Laboratory (Bar Harbor, USA). Fourteen weeks old animals were fed ad libitum a control diet (ssniff® EF acc. D12450B (I) mod., SD) or a high fat diet (ssniff® EF R/M, D12451, 42% of energy from fat, HFD) for 14 weeks (n = 6 per group). Musculus quadriceps femoris, liver, white adipose tissues and serum were collected from mice fasted overnight. Tissues were also obtained from six mice (fasted for 12 h) 10 min after intraperitoneal injection of insulin (1 mU/g body weight) or water as control. The 13 week old ob/ob mice (n = 5) and the five control animals were killed after an overnight fast. Procedures were approved by the University of Regensburg Laboratory Animal Committee and complied with the German Law on Animal Protection and the Institute for Laboratory Animal Research Guide for the Care and Use of Laboratory Animals, 1999. Experiments were conducted according to institutional and governmental regulations for animal use (Government of the Oberpfalz and Unterfranken).

### 2.2. Human adipose tissues

Paired samples of visceral and subcutaneous adipose tissues of 7 patients (4 males) were obtained from patients undergoing surgery. The median age was 62 (22–76) years, the median body mass index was 25.6 (24.0–29.7) kg/m$^2$. The study protocol was approved by the local ethics committee and was carried out in accordance with the Helsinki guidelines. All patients gave written informed consent.

### 2.3. GeneChip analysis

*The Mouse Gene 2.1.* ST Array (Affymetrix) was hybridized with RNA from subcutaneous fat of 5 wild type (WT) and 5 SNTA −/− mice kept on SD and 5 WT and 4 SNTA −/− mice fed a HFD. The Ambion WT Expression Kit and Affymetrix WT Terminal Labeling and Hybridization procedure were used according to the suppliers´ suggestions. Data were analyzed using the Affymetrix Command Console and Expression Console. Differences were calculated by unpaired Student's *t*-test (Kompetenzzentrum für Fluoreszente Bioanalytik, Regensburg, Germany). Leptin is well described to be induced in fat tissues in obesity (Rasouli and Kern, 2008) and the respective *p*-value for this difference ($p = 0.00019$) was chosen as cut off value.

### 2.4. Immunoblot, ELISA and PCR

Immunoblot and real-time RT-PCR analysis were performed as described (Krautbauer et al., 2014). Primers used are listed in Table S1. SNTA antibody has been described (Peters et al., 1997). Antibodies for APPL1, ATGL, AMPK, Akt, pAkt, caveolin-1, cyclophilin A, p44/42, FABP4, FAS, GAPDH, HSL, LC3B, PARP-1, perilipin, PPARγ, Rab5 and SCD1 were from New England Biolabs GmbH (Frankfurt am Main, Germany). ABCA1 and adipophilin antibodies were from Abcam (Cambridge, UK). SR-BI antibody was from Biomol (Hamburg, Germany). SREBP1c antibody was from Thermo Fisher Scientific (Schwerte, Germany). Antibody to eNOS was from Merck Millipore (Schwalbach, Germany). CD163 antibody was from Bio-Rad (Munich, Germany) and catalase antibody from Santa Cruz Biotechnology (Dallas, Texas, USA). MnSOD antibody was from Thermo Fisher Scientific (Schwerte, Germany). Quantification was done using Image J software (Schneider et al., 2012). Chemerin ELISA was from R&D Systems.

### 2.5. Assays

Glucose, proinsulin and insulin were measured as described (Eisinger et al., 2014a,b). Data of the WT mice have partly been published (Eisinger et al., 2014a,b). The Homeostasis model assessment **(**HOMA**)** index was calculated using the formula: [fasting glucose (mmol/L) x fasting insulin (mU/L)]/22.5. Malondialdehyde was measured by a colorimetric assay from Abcam (Lipid Peroxidation (MDA) Assay Kit).

### 2.6. Quantification of lipids

Triglycerides were measured using GPO-PAP micro-test (Roche, Mannheim, Germany) and cholesterol by an assay from Diaglobal (Berlin, Germany). Fatty acids were analyzed by gas chromatography coupled to mass spectrometry (GC–MS) (Ecker et al., 2012). Sphingosine based ceramides (Cer d18:1) and hexosylceramides (HexCer d18:1) were analyzed by using a fragment ion of *m/z* 264 (Liebisch et al., 1999). Hepatic lipids are given as nmol/mg wet weight.

### 2.7. Hematoxylin & eosin staining

Tissues were fixed in 4% formalin solution, embedded in paraffin and sectioned at a thickness of 5 µm. After deparaffinization with Histol (Carl Roth, Karlsruhe, Germany) (two washes 10 min each) and rehydration in ethanol (100%, 96% and 70% ethanol: two washes for 5 min each) and one wash for 5 min in distilled water, the slides were incubated in hematoxylin solution (Carl Roth, Karlsruhe, Germany) for 2 min. After washing with water, the slides were stained with Eosin G solution (Carl Roth, Karlsruhe, Germany) for 5 min. Slides were dehydrated by sequential transfer through an increasing alcohol gradient (70%, 96% and 100%, two washes for 2 min each) and subsequently incubated with Histol (two times for 5 min each). Entellan (Merck Chemicals GmbH, Darmstadt, Germany) was used as mounting medium.

### 2.8. Statistical analysis

Data are shown as box plots. Statistical differences were analyzed by Mann-Whitney *U* Test (SPSS Statistics 21.0 program), one-way ANOVA with posthoc Bonferroni or Student's *t*-test (Ms Excel). Except for GeneChip experiments a value of $p < 0.05$ was regarded as significant.