

γ -synuclein is a novel player in the control of body lipid metabolism

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Abbreviations: SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor; WAT, white adipose tissue; HFD, high fat diet; ATGL, adipose triglyceride lipase; SNAP, synaptosomal-associated protein; CNS, central nervous system; PNS, peripheral nervous system; BAT, brown adipose tissue; TAG, triacylglycerol; HSL, hormone sensitive lipase; VAMP, vesicle-associated membrane protein; PUFA, polyunsaturated fatty acids

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Synucleins are a family of homologous, predominantly neuronal proteins known for their involvement in synaptic transmission and neurodegeneration. γ -synuclein is predominantly localized in axons and presynaptic terminals of selected populations of peripheral and central neurons but is also highly expressed in human white adipose tissue (WAT) and increased in obesity. We have recently shown that γ -synuclein is nutritionally regulated in murine adipocytes while its loss protects mice from high fat diet (HFD)-induced obesity and associated metabolic complications. This protection was coupled with increased adipocyte lipolysis, lipid oxidation, and energy expenditure in HFD-fed γ -synuclein-null mutant compared with wild-type mice. Cellular studies suggest that relocalization of ATGL to the lipid droplet in γ -synuclein-deficient adipocytes may contribute to increased lipolysis in these cells. Loss of γ -synuclein in adipocytes also attenuates the assembly of SNARE complexes, an important component of lipid droplet fusion machinery, possibly due to reduced chaperoning of SNAP-23 to the assembling SNARE complex by γ -synuclein. Together our data suggests that not only is γ -synuclein a novel regulator of lipid handling in adipocytes but also that the deficiency of this protein has a significant effect on whole body energy expenditure.

The synucleins (α -, β -, and γ -) are a family of highly homologous and chordate-specific proteins, with a pattern of overlapping expression throughout the nervous system. Whereas α - and β -synuclein are restricted to presynaptic terminals in the CNS,

γ -synuclein is abundant in the presynaptic terminals and axons of motor neurons and sensory neurons of the PNS where it is expressed from the earliest stages of neural development,¹ although expression can also be found in certain populations of neurons localized in other regions of the CNS.^{2,3} We have shown that in addition to neural tissues, γ -synuclein is highly expressed in murine white adipose tissue (WAT), with other groups reproducing this finding in humans and pigs, and that expression in humans is nutritionally regulated.^{4,5} We did not observe detectable expression of the other two synucleins in any depots of murine WAT suggesting that γ -synuclein is the major, if not the only, synuclein protein expressed in this tissue.

We demonstrated that in murine WAT γ -synuclein is expressed principally by the mature white adipocytes, and like in human WAT, is up and downregulated upon increased fat consumption in the diet and caloric restriction, respectively. These observations suggested a role of γ -synuclein in white adipocyte biology and stimulated us to pursue further in vitro and, particularly in vivo studies. The latter were facilitated by the availability of mice with constituent deletion of the γ -synuclein gene that had been previously produced in our laboratory.⁶ These animals were viable, fertile, and did not display any obvious neurological or metabolic phenotype, although there were subtle alterations in certain neuronal populations,⁶⁻⁹ including changes in lipid profiles and fatty acid patterns.¹⁰ In our recent publication in the *Proceedings of the National Academy of Sciences*¹¹ we reported that knockout of γ -synuclein rescued mice from the increased adiposity and metabolic derangements associated

with high fat diet feeding including hyperinsulinemia and hepatic steatosis, the latter being strongly linked to hepatic insulin resistance and dysregulated glucose homeostasis. This rescue was associated with increased energy expenditure and lipid oxidation, and decreased adipocyte size. We also found that specifically re-expressing γ -synuclein in the fat pads of null mutant mice using lentiviral vectors reverses the observed decrease in adipocyte size. Critically, this reversal was only seen in those adipocytes re-expressing γ -synuclein and not in non-transduced cells in the same depot, indicating that this aspect of the *in vivo* phenotype is cell-autonomous. Further investigations demonstrated that γ -synuclein loss caused increased expression of lipid oxidation markers in brown adipose tissue (BAT) as well as increased lipolysis in white adipocytes that may be explained by an observed increase of the lipase, ATGL. Interestingly, knockdown of γ -synuclein in differentiated 3T3-L1 adipocytes resulted in a dramatic redistribution of predominantly cytosolic ATGL enzyme to the lipid droplet surface, and that γ -synuclein and ATGL are capable of interacting, at least *in vitro*. This suggests that one function of γ -synuclein in mature adipocytes may be to sequester ATGL away from the lipid droplet when these cells are under basal conditions. As such the loss of γ -synuclein, while not actively stimulating lipolysis, facilitates the process by placing ATGL in close proximity to its substrate thereby enhancing lipolysis under any given condition.

We also found that γ -synuclein is expressed in brown adipocytes, although at a considerably lower level compared with that in white adipocytes. It is possible that γ -synuclein deficiency in BAT was at least partially responsible for the alterations seen in BAT lipid oxidation and overall increased energy expenditure in γ -synuclein null mice. Indeed studies of adipose selective overexpression of ATGL suggest that increased lipolysis in WAT and BAT increases lipid oxidation and energy expenditure.¹² It appears likely that this results at least partly from activation of oxidative gene expression in BAT downstream of products of lipolysis in this tissue. Conversely, adipose-specific ablation of ATGL results in a loss of

BAT-like phenotype with this tissue adopting a more WAT like appearance and function.¹³ Indeed similar effects have also been observed in liver and macrophages.^{14,15} The beneficial metabolic effects we observe in γ -synuclein null mice may therefore result at least partly from increased supply of lipid substrates for oxidation from WAT or almost exclusively due to increased activation of oxidative capacity in the BAT itself. The high capacity of BAT for oxidising lipids and its ability to dissipate energy through uncoupled respiration has led to significant interest in increasing the BAT:WAT ratio or inducing more BAT-like characteristics in WAT adipocytes as a potential therapy for metabolic disease.¹⁶ Were this achievable it would evidently offer a route to dispose of excess lipids, reduce adiposity and protect other tissues from harmful ectopic lipid accumulation. We did not find evidence either of increased mass of BAT nor browning of WAT depots, with the appearance of so-called “beige” or “brite” adipocytes, in γ -synuclein-deficient mice. However, we cannot exclude that this could also contribute to the increased energy expenditure and lipid oxidation we reported. Clearly further studies are required to determine the relative contributions of WAT and BAT to the physiological effects we observe in this model.

The studies highlighted above regarding TAG lipolysis imply that increasing this by increasing ATGL activity could be beneficial in metabolic disease. However, it should be noted that an opposite effect on WAT lipolysis, *i.e.*, its downregulation by decreasing the levels or activity of hormone sensitive lipase (HSL), have also been reported to ameliorate obesity associated metabolic parameters, particularly insulin sensitivity.¹⁷ Due to compensatory mechanisms weight gain is also not exacerbated under these conditions. Overall this implies that the effects of altering lipolysis on metabolic health are complex and whether γ -synuclein offers a plausible means to influence this positively will depend on teasing apart the tissue specific effects and molecular details of where it acts in the process.

As γ -synuclein expression is not exclusive to adipose tissue, it is also possible that loss of γ -synuclein expression in other cell

types plays a role. Levels of γ -synuclein in liver and skeletal muscle are undetectable however one cannot entirely exclude the possibility that the protein is only present in certain conditions and that its loss may alter lipid oxidation in these tissues. More likely to be important is the significant neuronal expression of γ -synuclein. Hence its loss in relevant neuronal populations may contribute to some aspects of the phenotype we observed. On this note, two papers have revealed that hypothalamic expression of γ -synuclein is substantially downregulated in mice fasted for 48 h but restored to nearly *ad libitum* fed levels by leptin treatment in both the paraventricular and arcuate nucleus of the hypothalamus, a region well known for its modulation of downstream metabolic effects.^{18,19} Given that adipose tissue lipolysis is subject to significant regulation by the nervous system, neuronal γ -synuclein could plausibly influence lipolytic rates *in vivo*. We therefore cannot at present exclude a role for an effect of γ -synuclein deficiency in the brain, nor any compensatory mechanisms activated in non-adipose tissues in response to global γ -synuclein knockout that could contribute to other aspects of the phenotype such as the increased energy expenditure or decreased hepatic steatosis. However, several of the experiments described in our publication demonstrate that at least part of the observed effect on body lipid metabolism is caused by cell autonomous loss of γ -synuclein in adipose tissue. Clearly, as a future direction, this issue should be addressed and, hopefully resolved in studies of mice with conditional knockout of γ -synuclein specifically in adipocytes.

An intriguing link between the function of α -synuclein in neuronal synapses and γ -synuclein in adipocytes might also explain how γ -synuclein affects lipolysis and the size of mature adipocytes in conditions of nutrient excess. Burre and colleagues have shown that in neuronal synapses, α -synuclein enhances neurotransmitter exocytosis by regulation of synaptic vesicle fusion with the cell membrane via promoting the assembly of SNARE complexes from its subunits, namely vesicular SNARE protein vesicle-associated membrane protein 2 (VAMP-2/synaptobrevin) and two target membrane-associated SNARE (tSNARE) proteins,

syntaxin-1 and synaptosomal-associated protein 25 (SNAP-25).²⁰ These complexes play a pivotal role in synaptic vesicle docking and fusion pore formation at the plasma membrane, during the process of neurotransmitter release (reviewed in refs. 21 and 22). In this paper, the function of α -synuclein as a promoter of SNARE complex assembly was suggested to be particularly important during periods of increased synaptic activity. Functional homologs of synaptic proteins, namely the vSNARE protein VAMP-4 and tSNARE proteins syntaxin-5 and SNAP-23, form SNARE complexes in adipocytes. These complexes are involved in fusion between neutral TAG packaged within amphipathic lipoproteins and the lipid droplet phospholipid monolayer, the process that was suggested to underlie lipid accumulation in the adipocyte and consequent increase in its size.^{23–26} More broadly, recent screens of genes affecting lipid droplet formation, fusion and morphology in insect cells and yeast have revealed roles in these processes for several proteins involved in vesicle trafficking.^{27,28} In our publication we have shown that in conditions of increased lipid supply, i.e., in HFD-fed mice, the lack of γ -synuclein substantially attenuates assembly of SNARE complexes in adipocytes. We also demonstrated that γ -synuclein and the tSNARE SNAP-23 colocalize in the cytosol of cells differentiating into adipocytes in culture, and that this colocalization is partially lost upon oleate treatment. Findings that were not included in this publication also suggested that γ -synuclein and SNAP-23 can interact in vitro, without γ -synuclein being a constituent of assembled SNARE complexes. We hypothesize that γ -synuclein chaperones SNAP-23 to the forming SNARE complex during lipid partitioning in adipocytes. It is therefore possible that the decreased SNARE complex formation seen in γ -synuclein null adipocytes reduces TAG incorporation into the lipid droplet contributing to the decreased adipocyte size, and overall, the decreased adiposity in mice. In itself, a decreased ability to appropriately store lipids would be likely to worsen rather than improve metabolic disease in the face of nutrient excess. This is most aptly illustrated in human syndromes of lipodystrophy.²⁹ However, if

γ -synuclein is acting pleiotropically to simultaneously increase lipid oxidation this may offset these potentially negative consequences of decreased lipid storage in WAT. It is also possible that the increased lipolysis we observe may partly reflect the increased availability of TAG, which has not been appropriately incorporated into the core of the lipid droplet. This TAG would then be subsequently hydrolysed, with this process requiring compensatory increases in ATGL protein levels (summarized in Fig. 1). This inefficiency would be particularly apparent in situations of increased turnover, for example when WAT is exposed to increased dietary lipids. This scenario would be similar to what was observed in α -synuclein-deficient neurons, in the way that the prominent defect was seen during times of increased work load, in this case, upon increased synaptic firing. Overall our data suggest that both α -synuclein and γ -synuclein share the ability to potentiate SNARE-mediated fusion in physiological and/or environmental conditions requiring increased efficiency of cellular mechanisms that depend on this fusion, the former important for synaptic transmission in neuronal synapses and the latter important for lipid droplet maintenance and expansion in adipocytes. At present only a few studies have assessed the importance of SNARE proteins in adipocyte lipid droplet formation and turnover and further investigation both in vitro and in vivo will be required to clarify their importance and roles.

The wider involvement of synuclein family members in the upregulation of membrane vesicle fusion via SNARE complexes is supported by findings over the past decade that describe the nature of the interactions of synucleins with lipid membranes as well as the possible chaperoning of other proteins. The link between synucleins and lipid biochemistry is well documented. α -synuclein has been shown to interact with phospholipid vesicles in vitro, with intracellular lipid droplets formed in cells loaded with lipids, and can accumulate at lipid rafts in cultured cells.^{30–34} Alterations in lipid metabolism have also been demonstrated in the brains of α -synuclein null mutant mice.^{35,36} Links between synucleins and polyunsaturated fatty acids (PUFA) have also been

documented, including direct in vitro interaction of α -synuclein with PUFA.^{37,38} There is also a large body of evidence suggesting chaperone activity for all three synucleins.^{39–44} There is the highest degree of amino acid similarity between synucleins within the first 85 residues, the region known to be responsible for interaction with lipids. However γ -synuclein differs considerably at the C-terminus from the other two family members.^{1,2,45} It appears that this diversion in C-terminal sequence confers some key differences in structure from the other two synucleins, and this is thought to mediate functional variations between the three proteins, with differences in γ -synuclein function expected to result from different protein–protein interactions mediated by its C-terminus.^{46,47} It therefore seems feasible that each synuclein is able to bind certain lipids species not always in the same cell type, with their C-terminus responsible for the ability to bind different protein groups to facilitate various cellular outcomes.

We have shown that loss of γ -synuclein function in white adipocytes causes alterations in lipid handling, ultimately leading to changes in whole-body metabolic outcome, and so it is possible that γ -synuclein plays a role in the development of human obesity and related disorders. Overall our initial study implies that inhibiting γ -synuclein expression or function might reduce adipose mass in obesity and reduce at least some aspects of associated metabolic disease. At present it is not clear whether insulin sensitivity per se is improved in mice lacking γ -synuclein although responses to insulin and glucose challenges suggest that this is plausible and worthy of future investigation. Similarly, future studies will be needed to reveal whether more subtly increasing or decreasing γ -synuclein in physiological or pathophysiological states might affect adiposity, insulin sensitivity, and lipid metabolism in rodents and in humans. This work has also shown that γ -synuclein is an important player in adipocyte biology, and has helped to highlight the potential importance of SNARE complex formation in appropriate adipocyte lipid storage. Although this is still an emerging concept in the field, it will be interesting to see if other modulators of SNARE complexes,

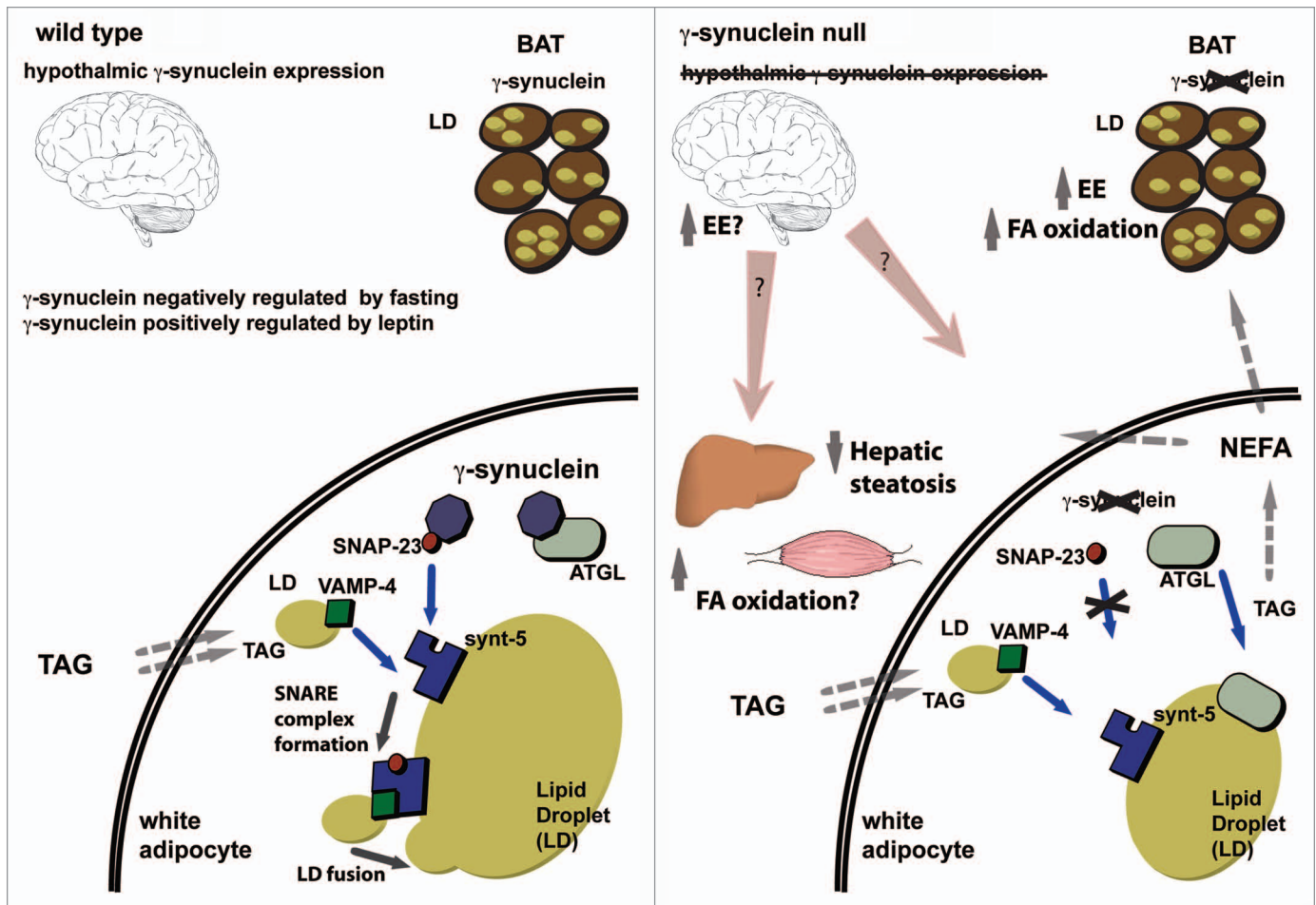


Figure 1. Possible role of γ -synuclein in white adipocytes and consequences of γ -synuclein deficiency. A proposed model for the role of γ -synuclein in metabolic tissues in times of energy surplus. When the lipid supply to WAT is low (e.g., in low fat diet-fed mice) and the amount of newly synthesized TAGs that need to be stored is limited, a basal level of SNARE complex assembly matches the demand for lipid droplet fusion and therefore γ -synuclein levels do not significantly impact upon adipocyte lipid storage. However, when adipocytes attempt to store large quantities of TAG due to the increased supply of lipids in the diet (e.g., in HFD-fed mice) the ability of γ -synuclein to potentiate SNARE-mediated fusion of lipid droplets may become limiting for these fusion events and the accumulation of TAG (left panel). In γ -synuclein-deficient adipocytes (right panel) the inability to potentiate SNARE complex assembly may restrict the rate of lipid droplet fusion. Deficiency of γ -synuclein also increases the accumulation of ATGL on the lipid droplet, possibly due to a loss of sequestration by γ -synuclein in the cytosol. This relocalization of ATGL potentiates the hydrolysis of its substrate TAG. NEFA released from the adipocyte is oxidized in BAT and possibly also liver and skeletal muscle. This increase in fat metabolism could either be due to compensatory responses in these tissues or due to deficiency of γ -synuclein either directly (in the case of BAT, where this protein is expressed although at relatively low level) or indirectly (liver and skeletal muscle, which are γ -synuclein-negative tissues). Loss of γ -synuclein centrally did not increase physical activity in mice but may alter neuronal inputs to peripheral tissues, particularly BAT and WAT, influencing whole body NEFA oxidation and energy expenditure (EE).

as well as alterations to the complexes themselves, are able to strongly influence lipid storage in adipocytes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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