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14. ABSTRACT Atypical antipsychotics (AAP) are prescribed to patients with autism spectrum disorders with symptoms of aggression or agitation, stereotypic behavior, temper tantrums and self-injury. Although AAP can ameliorate some mental or behavioral dysfunctions, they have serious metabolic side-effects that include weight gain, fat accretion, the metabolic syndrome, and increased risk of diabetes and cardiovascular disease. The current dogma is that the metabolic side effects of AAP are attributed to their action on neuronal circuits the brain, primarily via dopaminergic and serotonergic receptors. However, we discovered expression of such receptors in human and rodent adipocytes and demonstrated that administration of AAP to animal models caused significant weight gain and increased adiposity. This led us to propose that these receptors are directly targeted by AAP. Recent <i>In vitro</i> studies using human adipocytes and rat adipose tissue explants demonstrated multiple direct effects of AAP on adipose tissue. These include increase preadipocyte proliferation, augmentation of adipocyte size, suppression of basal lipolysis, and alterations in key lipogenic and lipolytic enzyme gene expression. We conclude that AAP-induced metabolic dysregulation is caused, in part, by their direct action on adipose tissue, most likely via local dopamine and serotonin receptor subtypes.					
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Introduction

Atypical antipsychotics (AAP) are used chronically to treat millions of pediatric, adult, and geriatric patients with schizophrenia, bipolar disorder, autism, major depression, and post-traumatic stress disorder (1,2). While most drugs alleviate neurobehavioral symptoms, many of the AAPs cause serious metabolic side-effects such as weight gain, metabolic syndrome and increased mortality due to cardiovascular disease (3-5). The precise targets of AAPs as metabolic disruptors are unclear, but they are known to bind primarily to dopamine and serotonin receptors (1, 6). The current dogma is that AAPs bind to these receptors within the brain. However, the discovery of dopamine (7) and serotonin (8) receptors expression in adipose tissue, lead us to propose that these drugs can activate those receptors as well.

Among the most widely prescribed AAP, olanzapine (Zyprexa) and clozapine (Clozaril) carry the greatest risk of metabolic disturbances, quetiapine

(Seroquel) and risperidone (Risperdal) have an intermediate risk, while ziprasidone (Geodon) and aripiprazole (Abilify) confer lower risks (9). **Table 1** shows the relative effects of three AAP on weight gain, glucose homeostasis and dyslipidemia. For our studies, we selected Olanzapine and Ziprasidone which represent high and low risk of the metabolic syndrome.

Table 1: Metabolic disturbances associated with selected antipsychotics

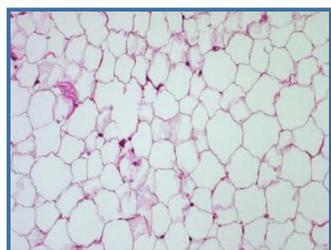
	Weight Gain	Glucose Abnormalities	Dyslipidemia	Metabolic Syndrome
Olanzapine	High	High	High	High
Risperidone	Medium	Medium-Low	Low	Medium
Ziprasidone	Low	Low	Low	Low

Hypothesis: Direct effects of AAP on selected functions of the adipocytes contribute to weight gain, fat accretion and metabolic dysregulation.

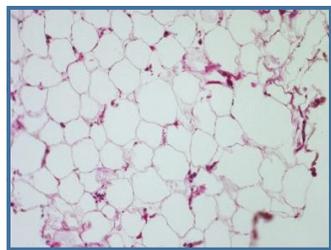
Data

AAP directly Increase the size of rat adipocytes

Subcutaneous adipose explants from Sprague Dawley male rats (N=8) were incubated in DMEM/F12



Control



Olanzapine (100 nM)

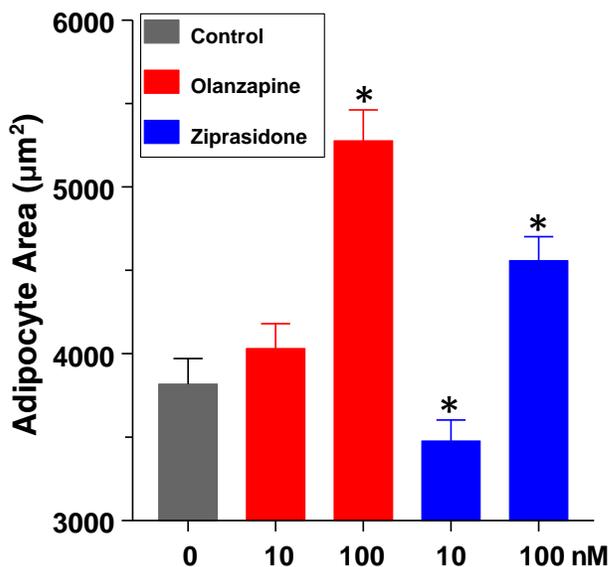


Fig 1: Direct effects of Olanzapine and Ziprasidone on rat sc adipocyte size. Explants were incubated with vehicle (control), Olanzapine or Ziprasidone for 7 days. Adipocyte size analysis was done on H&E-stained sections (N=8).

Ziprasidone caused a small, but significant, reduction in adipocyte size. These data demonstrate that the observed *in vivo* effect of these antipsychotics on body weight and body fat in rats, reported last year, were due, at least in part, to their direct action on the adipocytes, causing their enlargement. We are currently planning to conduct the same experiments using human adipose explants.

AAP directly Increased the proliferation of human preadipocytes

We previously cloned a unique human adipocyte cell line, named LS14, from a patient with

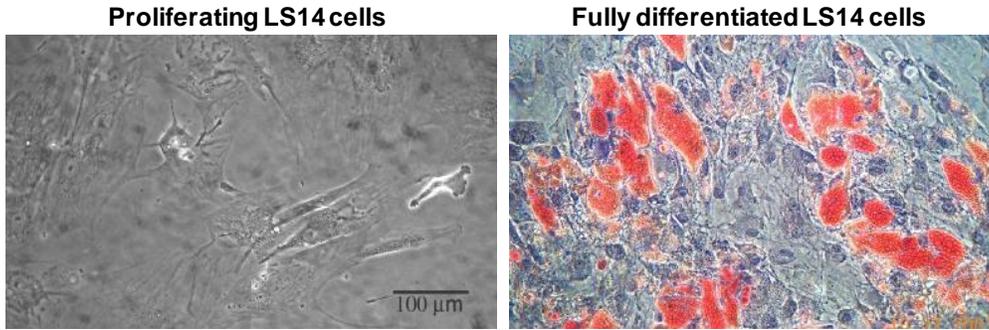


Fig 2: *Left panel:* photographs of proliferating LS14 preadipocytes. *Right panel:* 10 days after induction of differentiation, showing marked changes in cell shape and lipid accumulation, as determined by staining with Oil-Red-O.

liposarcoma (10). These spontaneously immortalized cells have been maintained in culture for many generations. Extensive characterization revealed that they resemble visceral adipocytes, and can be used as a cellular model for studying both: proliferating preadipocytes and fully differentiated mature adipocytes. **Fig 2** shows photographs of these cells before and after adipogenesis.

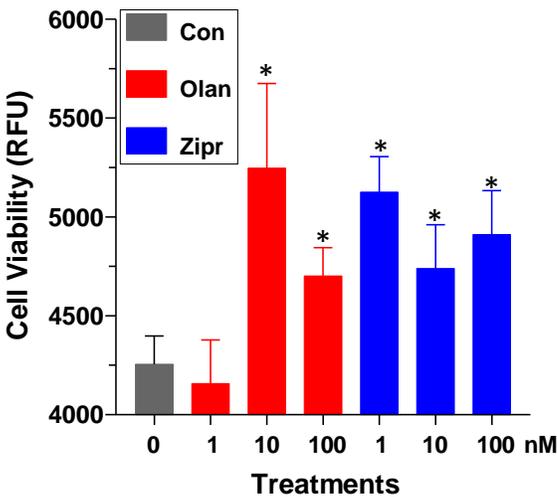


Fig 3: AAP increase the proliferation of human preadipocytes. LS14 cells were incubated with Olanzapine or Ziprasidone for 72 hrs. Cell viability was determined by the Resazurin assay. RFU: relative fluorescence units.

Using LS14 cells as preadipocytes, we examined the effects of AAP on cell proliferation. Cells were incubated with the drugs for 72 hrs, and cell proliferation was then determined by the Resazurin assay. **Fig 3** shows that Ziprasidone increased cell proliferation at all doses tested while Olanzapine was effective as 10 and 100 nM but not at 1 nM. These data indicate that in addition to expanding fat mass by adipocyte enlargement, AAP are also capable of increasing the pool of preadipocytes that eventually differentiate into lipid filled mature adipocytes.

Suppression of basal lipolysis by AAP

We next examined the direct effects of AAP on lipolysis, using either isolated primary human mature sc adipocytes, or primary human preadipocytes which were induced to differentiate in culture. Cells were incubated with the drugs for 72 hrs, and after media replacement, conditioned media were collected for 4 hrs and analyzed for glycerol by a colorimetric assay. As evident in **Fig 4**, Olanzapine caused dose-dependent inhibition of lipolysis in differentiated preadipocytes, having a similar effect in mature adipocytes except at the higher dose. Ziprasidone was less effective than Olanzapine. These data indicate that the suppression of basal lipolysis by

differentiate in culture. Cells were incubated with the drugs for 72 hrs, and after media replacement, conditioned media were collected for 4 hrs and analyzed for glycerol by a colorimetric assay. As evident in **Fig 4**, Olanzapine caused dose-dependent inhibition of lipolysis in differentiated preadipocytes, having a similar effect in mature adipocytes except at the higher dose. Ziprasidone was less effective than Olanzapine. These data indicate that the suppression of basal lipolysis by

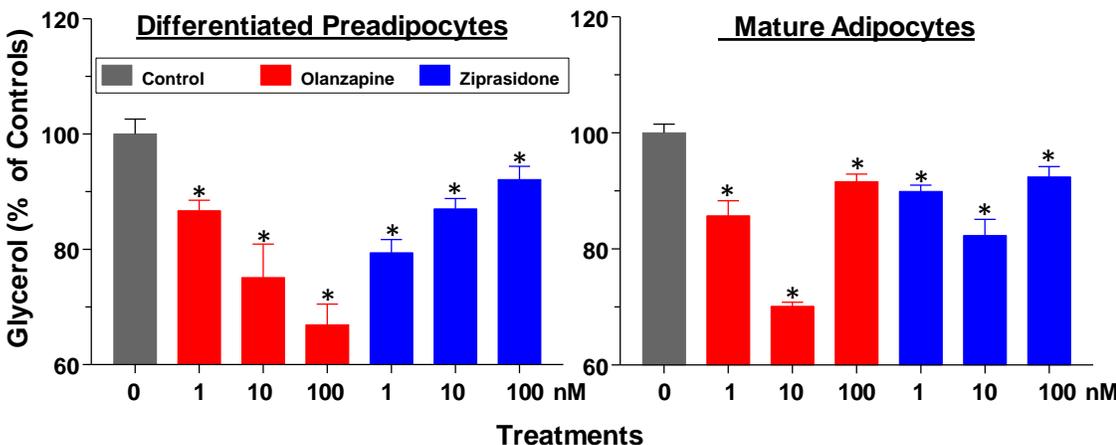


Fig 4: Suppression of basal lipolysis in differentiated human preadipocytes (*left panel*) or isolated mature adipocytes (*right panel*). Cells were incubated with Olanzapine or Ziprasidone for 72 hrs. Conditioned media collected after 4 hr were analyzed for glycerol release by a colorimetric assay.

AAP likely contribute to fat accumulation and enlargement of the adipocytes. The non-linear dose-dependence effects suggest activation of various receptors at different doses, an issue that should be examined in future studies by conducting knockout studies of selected dopaminergic and serotonergic receptors.

Regulation of gene expression by AAP

Mature sc primary human adipocytes, harvested from 3 patients undergoing abdominoplasty, were incubated with 100 nM Olanzapine or Ziprasidone for 72 hrs. Total RNA was isolated, reversed transcribed and the cDNA was analyzed by custom-designed RNA arrays with 21 metabolic-related

Table 1: Regulation of gene expression in mature human adipocytes (3 patients)

Gene	<u>Olanzapine</u>		<u>Ziprasidone</u>	
	Regulation	Range (fold change)	Regulation	Range (fold change)
PPARG	↑	1 - 1.7	nc	--
SREBF1	↑	1.2 - 3.7	nc	--
ATGL	↑	1.2 - 6.4	↑	1.3 - 1.5
HSL	↓	1.9 - 8.5	↓	4.8 - 9.7
LPL	↑	4.5 - 26.8	↑	4.0 - 11.8
FASN	↑	1.1 - 5.2	↑	1.3 - 1.5

genes; β -2 microglobulin (B2M) and hypoxanthine phosphoribosyl transferase (HPRT) were used as reference genes. Because of the very large variations among patients, data are presented in **Table 1** as range of fold changes, with nc designating no change. Notably, either drug caused little, if any, changes in the expression of two transcription factors which regulate adipogenesis: PPAR γ and SREBF1. On the other hand, the lipogenic enzyme fatty acid synthase (FAS) increased up to 5-fold by Olanzapine, while hormone sensitive lipase (HSL) was markedly decreased by both drugs. A major increase, up to 25-fold, was also evident in lipoprotein lipase (LPL), which provides free fatty acids to the adipocytes, while adipose triglyceride lipase (ATGL) was moderately increased by Olanzapine, but not by Ziprasidone. Additional studies, using more samples, different doses of AAP, and different times of treatment are needed before a conclusive picture emerges.

Key Research Accomplishments

- ❖ AAP exert direct actions on fat accretion by increasing preadipocyte proliferation and augmenting the size of mature adipocyte.
- ❖ Enhanced fat accumulation caused by AAP is also due to the suppression of basal lipolysis.
- ❖ AAP alter the expression of enzymes associated with lipogenesis and lipolysis.
- ❖ As predicted, Olanzapine is more potent than Ziprasidone in affecting metabolic functions of the adipocytes.
- ❖ Collectively, these studies support our major hypothesis that AAP-induced metabolic alterations in patients are due, in part, to their direct action on the adipocytes.

Reportable Outcome

Presentations in Scientific Meetings:

- ❖ Ben-Jonathan and Hugo: **Direct actions of antipsychotics: A cause for metabolic dysregulation.** The 7th International Congress of psychopharmacology, Antalya, Turkey, April 2014 (Appendix 1)
- ❖ Hugo, Sakai, Phillips, Fox, Premkumar, and Ben-Jonathan: **Direct effects of weight-inducing antipsychotics on adipose tissue from humans and rats.** The annual meeting of the Endocrine Society, Chicago, IL, June 2014. (Appendix 2).

Conclusion

In the last three years, we have accomplished a significant amount of our proposed research although several critical experiments still await to be performed and/or be analyzed. So far, all our data strongly support our hypothesis on the direct action of atypical antipsychotic on adipose tissue, which lead to weight gain, increased adiposity and the metabolic syndrome.

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Direct actions of antipsychotics on adipocytes: A cause for metabolic dysregulation

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Second generation antipsychotics (SGA) are prescribed to millions of patients with neurobehavioral disorders such as schizophrenia, bipolar disorder, [major depression](#), posttraumatic stress disorder, and autism. SGAs have been generally preferred over first generation antipsychotics because they cause fewer extrapyramidal side effects. Although SGAs can alleviate psychiatric symptoms, many of these drugs have serious metabolic side-effects such as weight gain, development of the metabolic syndrome, [diabetes](#), and cardiovascular disease. The primary therapeutic targets of SGAs within the brain are dopamine (DAR) and [serotonin](#) (5-HTR) receptor subtypes. The mechanisms underlying the metabolic [side effects](#) of SGAs are not completely understood, but are generally attributed to their central action. While analyzing the regulation of prolactin, produced in human adipose tissue, we unexpectedly discovered expression of functional DAR and 5-HTR subtypes in adipocytes. This observation led us to question whether undesirable metabolic side effects effects of certain SGAs are due, at least in part, to their direct action on adipocytes. For *in vitro* studies, we used primary human adipocytes and a human adipocyte cell line, LS14, developed in our laboratory. For *in vivo* studies, we used Sprague Dawley female rats treated orally with olanzapine or ziprasidone. Adipose tissue explants and adipocytes were incubated with olanzapine, risperidone and ziprasidone at pharmacologically-relevant concentrations for various times. These treatments differentially suppressed the release of leptin, a satiety hormone, and adiponectin, an insulin sensitizing hormone, inhibited lipolysis, and stimulated preadipocyte proliferation. Using a PCR array, we found that the above SGAs increased the expression of lipoprotein lipase and fatty acid synthase, key enzymes in the process of lipogenesis. Collectively, the SGA-induced changes result in increased adiposity. Treatment of rats with the SGAs resulted in a marked and rapid suppression of leptin and adiponectin expression in subcutaneous fat tissue, concomitant with increased food intake, weight gain, and adipocyte enlargement. In conclusion, direct actions of SGAs on DAR and possibly 5-HTR subtypes in adipocytes contribute to the ensuing metabolic disturbances. We propose that human adipocytes could be integrated into the screening paradigm of candidate new drugs for the identification of undesirable metabolic alterations prior to costly animal studies and clinical trials. The long term goal is to provide safer, yet effective, antipsychotic drugs to patients requiring treatment with such medications.

Keywords: metabolic dysregulation, antipsychotics, adipocytes

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Presenter : Eric R Hugo (Eric.Hugo@uc.edu)

Direct Effects of Weight-Inducing Antipsychotics on Adipose Tissue from Humans and Rats

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Abstract Text:

Background: Second generation antipsychotics (SGA) are prescribed to millions of patients with neuropsychiatric disorders. Although SGAs can ameliorate mental dysfunctions, they have serious metabolic side-effects such as weight gain, the metabolic syndrome, and increased risk of diabetes and cardiovascular disease. The primary therapeutic targets of SGAs are dopamine (DAR) and serotonin (5-HTR) receptors. The current dogma is that metabolic side effects of SGAs are attributed to their action on the brain. We recently discovered expression of functional DAR and 5-HTR subtypes in human and rodent adipocytes and speculated that these receptors are targeted by SGA.

Hypothesis: Direct effects of SGA on selected adipocyte functions contribute to weight gain and metabolic dysregulation.

Methods: *in vitro* model: Subcutaneous (sc) adipose explants and mature adipocytes were harvested from patients undergoing abdominoplasty. Both sc and periovarian (visceral) explants were obtained from female Sprague-Dawley rats. Samples were incubated with 1-100 nM olanzapine or ziprasidone for 72 h and analyzed for lipolysis by glycerol release and for a panel of metabolic-related genes by qRT-PCR. *In vivo* model: Rats were treated with olanzapine or ziprasidone in cookie dough for 3 or 7 days. Food intake, body weight, and fat accumulation (by NMR) were determined. Periovarian and sc adipose explants were analyzed by qRT-PCR for the gene panel as above. Serum leptin and adiponectin were determined by ELISA.

Results: Olanzapine, and to a lesser extent ziprasidone, caused marked suppression of leptin and adiponectin, and modest suppression of basal and isoproterenol-stimulated lipolysis from human sc adipose explants and mature adipocytes, respectively. Treatment of rats with SGA rapidly increased food intake and body weight and a delayed increase in fat accumulation. In sc fat, SGA caused over 5-fold suppression of key lipases, leptin, adiponectin, and PPAR γ , but a significant stimulation of SREBP.

Conclusion: SGA-induced metabolic dysregulation is caused, in part, by their direct action on adipose tissue, presumably via the local DAR and/or 5-HTR subtypes. We suggest that adipocytes should be integrated into the screening paradigm of candidate new antipsychotics to identify undesirable metabolic characteristics prior to costly animal studies and clinical trials. The long term goal is to provide safer drugs to patients requiring treatment with these medications.