DRUG-INDUCED ADVERSE EVENTS

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Hyperleptinemia contributes to antipsychotic drug–associated obesity and metabolic disorders

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Despite their high degree of effectiveness in the management of psychiatric conditions, exposure to antipsychotic drugs, including olanzapine and risperidone, is frequently associated with substantial weight gain and the development of diabetes. Even before weight gain, a rapid rise in circulating leptin concentrations can be observed in most patients taking antipsychotic drugs. To date, the contribution of this hyperleptinemia to weight gain and metabolic deterioration has not been defined. Here, with an established mouse model that recapitulates antipsychotic drug–induced obesity and insulin resistance, we not only confirm that hyperleptinemia occurs before weight gain but also demonstrate that hyperleptinemia contributes directly to the development of obesity and associated metabolic disorders. By suppressing the rise in leptin through the use of a monoclonal leptin-neutralizing antibody, we effectively prevented weight gain, restored glucose tolerance, and preserved adipose tissue and liver function in antipsychotic drug–treated mice. Mechanistically, suppressing excess leptin resolved local tissue and systemic inflammation typically associated with antipsychotic drug treatment. We conclude that hyperleptinemia is a key contributor to antipsychotic drug–associated weight gain and metabolic deterioration. Leptin suppression may be an effective approach to reducing the undesirable side effects of antipsychotic drugs.

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INTRODUCTION

Psychotic disorders, including schizophrenia, schizoaffective disorders, brief psychotic disorders, and delusional disorders, are major mental health issues that severely burden affected patients (*1*). Therapeutically, several second-generation atypical antipsychotic drugs, including clozapine, ziprasidone, paliperidone, olanzapine, and risperidone, are being used clinically. Although these drugs are highly effective in treating mental health disorders, they are also associated with common side effects, including notable weight gain and hyperglycemia (*2*).

Several different mechanisms, including multiple neuron engagement and inflammatory processes, have been proposed to contribute to the detrimental effects of antipsychotic drugs. Previously, treatment with a serotonin receptor 2C–specific agonist has been shown to reverse olanzapine-induced weight gain and hyperglycemia (*3*). We recently demonstrated that hypothalamic melanocortin 4 receptor (MC4R) participates in risperidone-induced weight gain because acute risperidone treatment inhibits MC4R activity by increasing postsynaptic potassium conductance (*4*). In addition, the involvement of critical hypothalamic appetite regulatory neurons in antipsychotic-induced hyperphagia has been well documented (*5*). Olanzapine treatment decreases proopiomelanocortin (*Pomc*) mRNA expression, POMC neuron numbers, and POMC projections before the onset of obesity (*6*). Moreover, an elevation in the expression of orexygenic neuropeptide Y (Npy) and agouti-related protein (Agrp) has been described (*5*, *7*, *8*). Aside from various neuronal circuits being affected, inflammation has been reported to participate in antipsychotic drug–induced metabolic disorders. Chronic treatment with olanzapine induces inflammatory responses in peripheral tissues and the central nervous system, primarily by promoting macrophage infiltration into adipose tissue. It also increases circulating proinflammatory cytokines, including interleukin-1β (IL-1β), IL-6, IL-8, and tumor necrosis factor–α (TNFα) (*9*, *10*). Furthermore, there is considerable variation in response, with some individuals being highly prone to develop obesity, whereas others gain substantially less weight. More specifically, gene expression profiles in circulating blood cells in both humans and mice with differential weight gain reveal that transcripts elevated in obese-prone individuals relative to obese-resistant participants are enriched for numerous inflammatory and immunomodulatory signaling nodes at baseline before drug exposure (*11*). Moreover, the second-generation tetracycline antibiotic minocycline, which displays robust anti-inflammatory effects,

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mitigates weight gain and hyperglycemia when added to olanzapine treatment (*12*, *13*).

Beyond its role in the regulation of body fat mass, leptin has received much attention over recent years for its proinflammatory actions (*14*). In several clinical studies, circulating leptin has been shown to rapidly increase before the onset of massive weight gain in patients undergoing antipsychotic therapy (*15*, *16*). This raises the question as to whether leptin may be causally involved in weight gain and metabolic deterioration (*17*). Using in vitro–differentiated human adipocytes, the antipsychotic drug clozapine was shown to directly regulate leptin gene expression (*18*). We recently demonstrated that hyperleptinemia is not only a phenomenon associated with weight gain but rather a vastly underestimated driving force for diet-induced obesity. Increasing leptin concentrations, either in a murine transgenic setting or with recombinant leptin administration, accelerates diet-induced obesity and deteriorates glucose intolerance and insulin resistance (*18*–*20*). On the basis of these observations, we hypothesized that antipsychotic drug–induced hyperleptinemia may be a key contributor to therapy-associated weight gain and metabolic dysfunction. By using a well-established mouse model that recapitulates the spectrum of detrimental effects of antipsychotic drug treatment, we show that hyperleptinemia occurs before any increases in body weight. Furthermore, preventing the leptin surge via a monoclonal leptin-neutralizing antibody (LepAb) at least partially mitigates risperidone-induced obesity and prevents metabolic dysfunction.

RESULTS

Olanzapine-induced weight gain is indicated by an increase in leptin expression

Olanzapine can induce substantial weight gain in female mice. However, in response to olanzapine treatment, some female mice are more prone to develop obesity, whereas others are more resistant to weight gain (fig. S1A). It is not clear what differentiates individual mice to the various degrees of weight gain. To answer this question, we analyzed mice that ended up gaining weight ("prone") and mice resistant to weight gain ("resistant") upon exposure to olanzapine. We performed gene analysis in gonadal fat isolated from prone and resistant mice. Compared with resistant mice, the mice prone to weight gain displayed a substantial increase in leptin and a reduction in adiponectin (fig. S1, B and C). Other genes, including *Acc1*, *Fasn,* and *Cpt1a*, were comparable between the prone and resistant mice (fig. S1, D to F). This observation highlights the importance of leptin expression as an indicator of olanzapine-induced weight gain.

Hyperleptinemia occurs before body weight gain in risperidone- and olanzapine-treated mice

To delineate the underlying mechanisms that mediate antipsychotic drug–induced weight gain, we used an established mouse model in which young female mice are fed a high-fat diet (HFD) (45% energy from fat) supplemented with either olanzapine or risperidone (*4*). This mouse model replicates the phenotype of antipsychotic drug–induced weight gain and hyperglycemia. In this model, we initially examined the circulating leptin concentrations in response to acute olanzapine and risperidone treatments. Clinical observations in patients taking antipsychotic drugs indicate a rapid rise in leptin (*21*). Mice administered either olanzapine or risperidone exhibited no acute changes in body weight or food intake (Fig. 1, A and B) but a rapid increase in circulating leptin (Fig. 1C). This increase occurred within 3 days of drug exposure (Fig. 1C). Concomitantly, leptin (*Lep*) gene expression was increased both in subcutaneous adipose tissue (SAT) and gonadal adipose tissue (GAT) depots (Fig. 1, D and E). We further observed a reduction in adiponectin gene expression, albeit only in SAT, indicating fat depot–specific regulation (Fig. 1, F and G). In addition, a marked increase in total adipose tissue macrophages, as assessed by gene expression of the macrophage marker F4/80 (*Adgre1*), was evident in both fat depots (Fig. 1, H and I), with risperidone exerting a more pronounced proinflammatory effect than olanzapine. On the basis of these data, we selected risperidone to further explore the contributions of leptin to the undesired side effects of antipsychotic drug treatment on body weight and systemic metabolism.

Leptin neutralization reduces risperidone-induced weight gain and improves glucose tolerance

If hyperleptinemia contributes to antipsychotic drug–induced weight gain and glucose intolerance, then we would expect that neutralizing excess leptin can prevent these undesired side effects. We examined the efficacy of our homemade LepAb toward reducing the activity of leptin signaling. With human embryonic kidney (HEK) 293 cells stably transfected with the long form of the leptin receptor, we stimulated the cells in the absence and presence of leptin and different doses of LepAb and measured the abundance of phospho–signal transducers and activators of transcription 3 (p-STAT3). The results indicate that our customized LepAb effectively blocked leptin signaling in a dose-dependent manner (fig. S2A). On the basis of these in vitro studies, we selected an optimal concentration for in vivo studies. We also tested whether the acute treatment with LepAb affected insulin sensitivity in obese mice. We performed hyperinsulinemic euglycemic clamps (HIECs) before and after acute LepAb treatment in HFD-fed mice. Before treatment, all mice were clamped at similar glycemia and displayed a similar glucose infusion rate (fig. S2B). However, after acute antibody treatment (16 hours), the mice receiving LepAb displayed a marked increase in the glucose infusion rate to maintain similar glycemia (fig. S2B), indicating an increase in insulin sensitivity. These results indicate that our LepAb can effectively bind circulating leptin and modulate leptin signaling in a highly acute setting.

We applied this approach to female mice that were fed an HFD supplemented with risperidone. As expected, female mice on risperidone treatment gained far more weight than mice fed an HFD alone (Fig. 2A). The rapid weight gain induced by risperidone was likely caused by a substantial increase in daily and cumulative food consumption (Fig. 2, B and C). In this setting, LepAb administration to mice on a risperidone-supplemented diet reduced body weight gain, in part by reducing food intake (Fig. 2, A to C). Risperidone treatment has previously been shown to lead to an impairment in glucose tolerance in mice (*22*, *23*). In line with these previous observations, mice on a risperidone-supplemented diet exhibited glucose intolerance, a phenomenon that was fully reversed by LepAb treatment (Fig. 2D). Collectively, these observations indicate that risperidone-induced hyperleptinemia directly promotes weight gain and glucose intolerance.

Because treatment with the LepAb led to a partial prevention of risperidone-induced weight gain, we wondered whether leptin neutralization would produce similar effects in mice with preexisting

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obesity due to risperidone treatment. We exposed female mice to an HFD and risperidone for 4 weeks to allow the mice to develop obesity. Only at that point, we initiated exposure to the LepAb [or control immunoglobulin G (IgG)] in these obese mice. Our results indicate that leptin neutralization showed similar effects in slowing down further weight gain (Fig. 2E), reducing daily food intake and food accumulation over the remaining risperidone treatment period (Fig. 2, F and G). Moreover, compared with IgG-treated mice, the obese mice exposed to LepAb had greatly increased glucose tolerance (Fig. 2H). These observations support that reducing leptin can counteract the side effects of induced antipsychotic drugs at various stages of risperidone treatment.

Because acute exposure to risperidone elicited a pronounced increase in systemic leptin concentrations, we also measured leptin, adiponectin, and insulin dynamics after chronic drug treatment. Our analyses revealed that leptin (*Lep*) gene expression was increased in both SAT and gonadal fat (GWAT) (Fig. 3, A and B) and that circulating leptin was also substantially elevated after chronic risperidone exposure (Fig. 3C). After LepAb treatment, this surge in leptin gene expression and circulating concentrations was largely blunted (Fig. 3, A to C). Risperidone treatment also caused a marked reduction of adiponectin (*Adipoq*) gene expression in SAT, which was successfully restored by LepAb treatment (Fig. 3D). In contrast, adiponectin gene expression and circulating concentrations in GAT were not markedly altered (Fig. 3, E and F).

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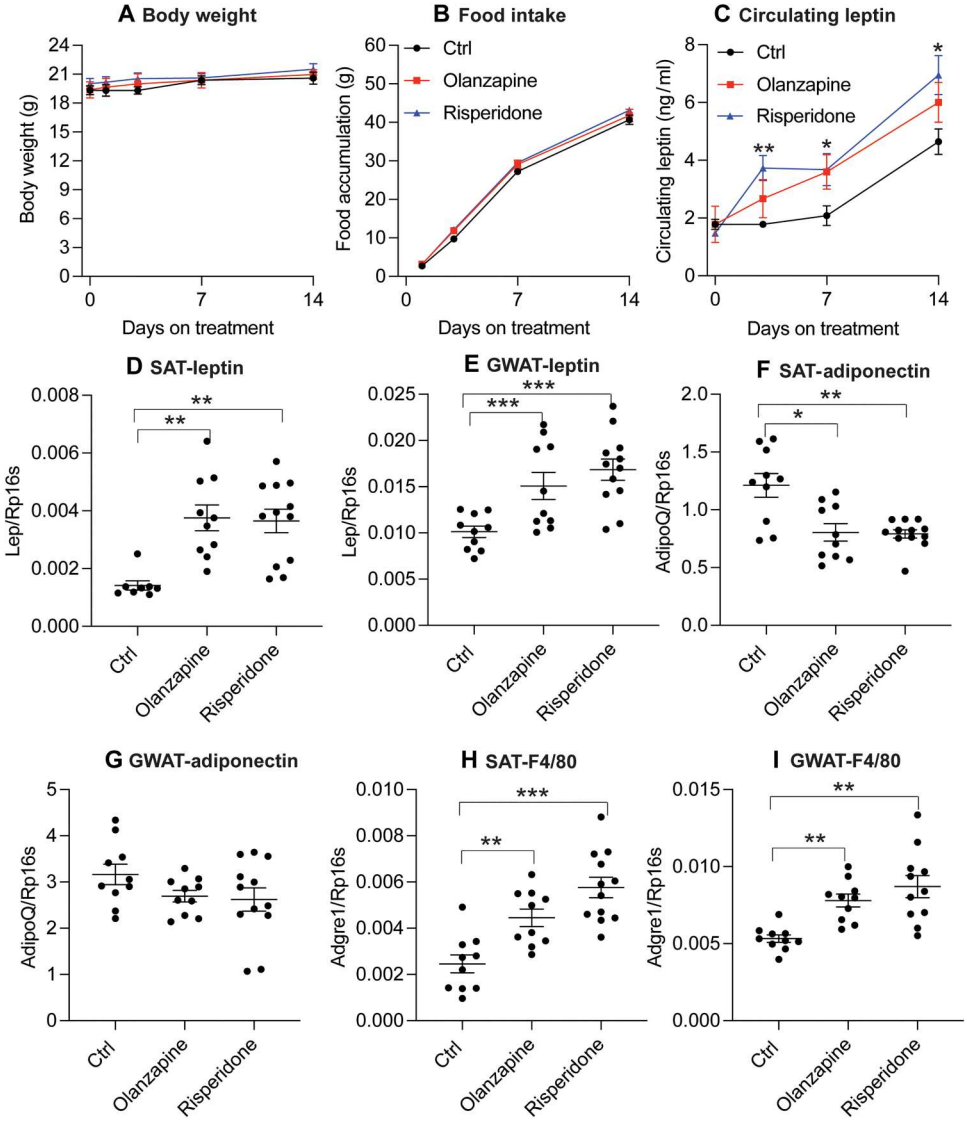

Fig. 1. Effect of acute olanzapine and risperidone treatment on body weight, food intake, and circulating leptin. Female mice ($n = 5$ per group) were placed on an HFD alone (Ctrl) or an HFD supplemented with olanzapine or risperidone for 2 weeks. Body weight, food intake, and circulating leptin concentrations were measured at the indicated time points. At the end of the experiment, SAT and GAT were collected for gene expression analysis. (**A**) Body weight; (**B**) food intake; (**C**) circulating leptin; (**D**) leptin gene expression in SAT; (**E**) adiponectin gene expression in SAT; (**F**) F4/80 expression in SAT; (**G**) leptin expression in GAT; (**H**) adiponectin gene expression in GAT; (**I**) F4/80 expression in GAT. Data are means ± SEM. Student's *t* test or one-way ANOVA: $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ for olanzapine versus Ctrl or risperidone versus Ctrl.

With regard to insulin, risperidone induced a notable increase in the circulating concentration of insulin, reflecting prevailing systemic insulin resistance (Fig. 3G). LepAb treatment also potently reversed this hyperinsulinemia, although it only partially alleviated weight gain. These results suggest the existence of a weight-independent effect of leptin neutralization on glucose tolerance and insulin sensitivity.

Risperidone-induced hyperleptinemia promotes liver fibrosis and whitening of brown fat

Several studies have shown that leptin plays a crucial role in the development of liver fibrosis in response to chronic liver injury (*24*, *25*). We thus investigated whether the hyperleptinemia caused by risperidone treatment also promoted liver fibrosis and furthermore assessed its involvement in other common forms of obesity-associated tissue dysfunction such as brown fat whitening. We observed severe liver fibrosis in response to chronic risperidone treatment as indicated by a substantial increase in the expression of fibrogenic genes such as collagen type 1 alpha 1 chain (*Col1a1*), *Col4a4*, and smooth muscle actin (SMA; *Acta2*) (Fig. 4, A to C). This was substantiated by trichrome staining, with a clear-cut darker blue stain in risperidone-treated mice, reflecting exacerbated tissue fibrosis (Fig. 4D). LepAb treatment completely prevented risperidone-driven liver fibrosis (Fig. 4, A to D). Risperidone also caused a considerable increase in hepatic steatosis as judged by lipid droplet accumulation, which was partially reversed by LepAb treatment.

Obesity coincides with a whitening of brown fat in mice (*26*). The role of obesity-associated hyperleptinemia in this process has, however, remained controversial (*27*). Mice that were fed a risperidone-supplemented diet exhibited a more pronounced whitening of brown fat as indicated by a decrease in the expression of specific brown fat markers, such as *Prdm16*, peroxisome proliferator-activated receptor-gamma coactivator-1 alpha (Pgc1α) (*Ppargc1a*), and *Ucp1* (Fig. 4, E to G), concomitant with increased accumulation of larger lipid droplets (Fig. 4H). Similar to what we observed in the liver, these effects of risperidone treatment on brown fat were mostly prevented by application of the LepAb.

Risperidone-induced hyperleptinemia increases local tissue and systemic inflammation

Inflammation is considered a driving force in the development of antipsychotic drug–induced metabolic disorders (*28*). Because leptin constitutes a key regulator of immune function (*29*), the hyperleptinemia triggered by antipsychotic drug treatment may be an underappreciated factor in establishing systemic inflammation and metabolic deregulation. In the context of obesity, GWAT undergoes massive expansion and severe inflammation, which is often considered to contribute immediately to systemic inflammation.

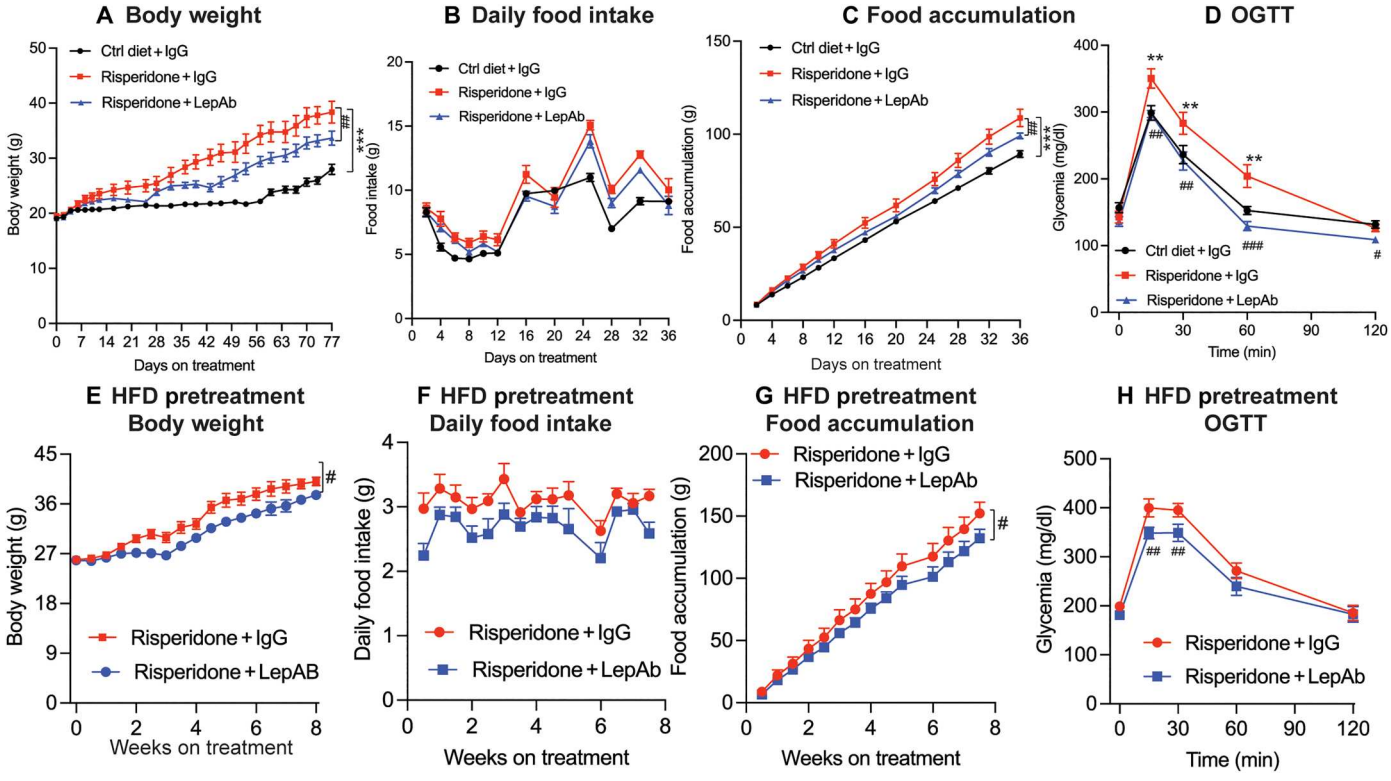

Fig. 2. Effect of risperidone and risperidone + LepAb on body weight, food intake, and glucose tolerance. Female mice ($n = 9$) were placed on an HFD alone (Ctrl) or an HFD supplemented with risperidone. Mice were treated with either control IgG or LepAb twice weekly. Body weight and food intake were measured. Glucose tolerance was measured by a GTT at the end of the experiment. (**A**) Body weight; (**B**) daily food intake; (**C**) cumulative food intake; (**D**) glucose tolerance. A second cohort of female mice ($n = 8$ per group) was placed on an HFD plus risperidone diet for 4 weeks to achieve substantial obesity. The mice were then injected with IgG or LepAb for another 8 weeks. (**E**) Body weight; (**F**) daily food intake; (**G**) food accumulation; (**H**) oral glucose tolerance test (OGTT). Data are means ± SEM. Student's *t* test or one-way ANOVA: $^{**}P < 0.01$ and $^{***}P < 0.001$ for risperidone + IgG versus Ctrl diet + IgG; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ for risperidone + LepAb versus risperidone + IgG.

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Accompanying weight gain, chronic risperidone treatment increased the expression of inflammation markers such as F4/80 (*Adgre1*), monocyte chemoattractant protein-1 (MCP1) (*Ccl2*), and *Ccl4* in GAT (Fig. 5, A to C). GWAT from risperidone-treated mice furthermore displayed increased macrophage infiltration, as evidenced by enhanced galectin-3 (Mac2) staining (Fig. 5D). LepAb treatment effectively reduced the expression of these inflammation markers and macrophage infiltration in GWAT, highlighting the profound role that hyperleptinemia plays in the development and maintenance of GWAT inflammation.

Beyond GWAT, the liver is similarly affected by obesity-associated inflammatory processes. In line with this, mice fed a risperidone-supplemented diet also displayed increased expression of inflammation markers in the liver, which was successfully alleviated

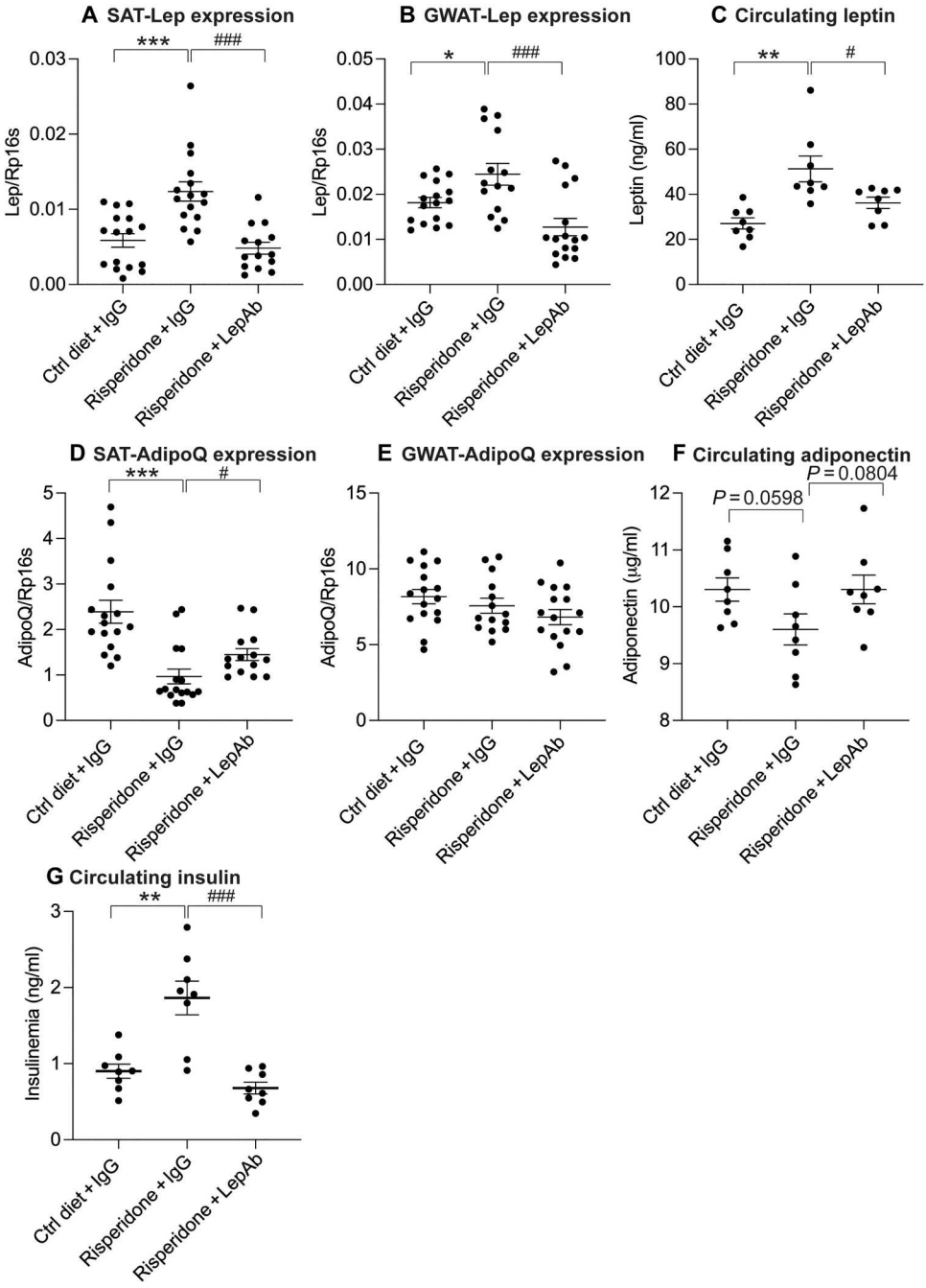

Fig. 3. Circulating leptin, adiponectin, and insulin concentrations in response to risperidone and risperidone + LepAb treatment. (**A**) Leptin expression in mouse SAT; (**B**) leptin expression in mouse GAT; (**C**) circulating leptin; (**D**) adiponectin expression in SAT; (**E**) adiponectin expression in GAT; (**F**) circulating adiponectin; and (**G**) circulating insulin in the fed state. Data are means ± SEM. Student's *t* test: **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 for risperidone + IgG versus Ctrl diet + IgG; #*P* < 0.05 and ###*P* < 0.001 for risperidone + LepAb versus risperidone + IgG.

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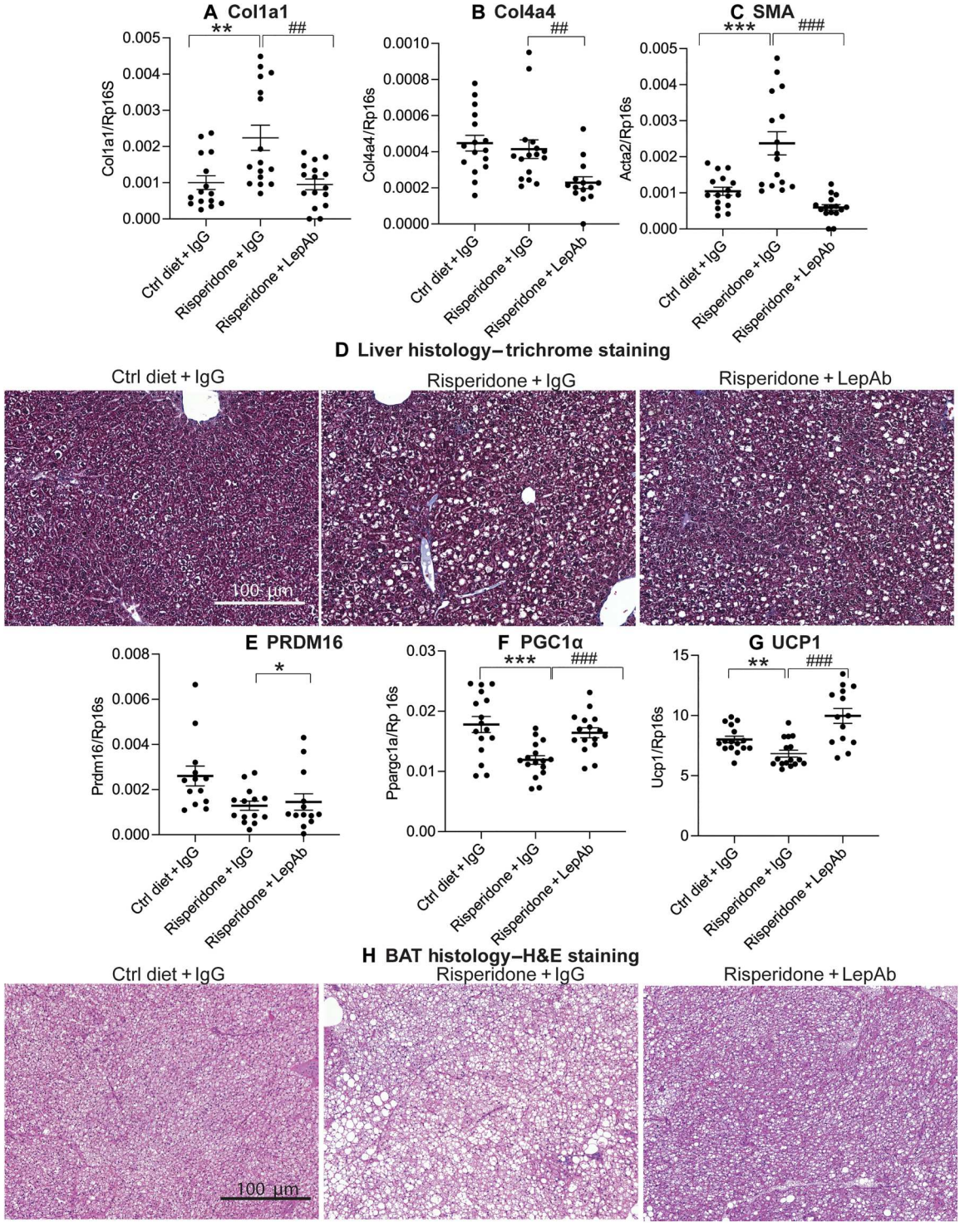

Fig. 4. Effects of risperidone and risperidone + LepAb on liver and brown adipose tissue function. (**A**) *Col1a1*, (**B**) *Col4a4*, and (**C**) *Acta2* expression in mouse liver. (**D**) Trichrome staining of liver. (**E**) *Prdm16*, (**F**) *Pgc1a*, and (**G**) *Ucp1* expression in brown fat (BAT). (**H**) Hematoxylin and eosin (H&E) staining of brown fat. Data are means ± SEM. Student's *t* test: **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 for risperidone + IgG versus Ctrl diet + IgG; ##*P* < 0.01, and ###*P* < 0.001 for risperidone + LepAb versus risperidone + IgG.

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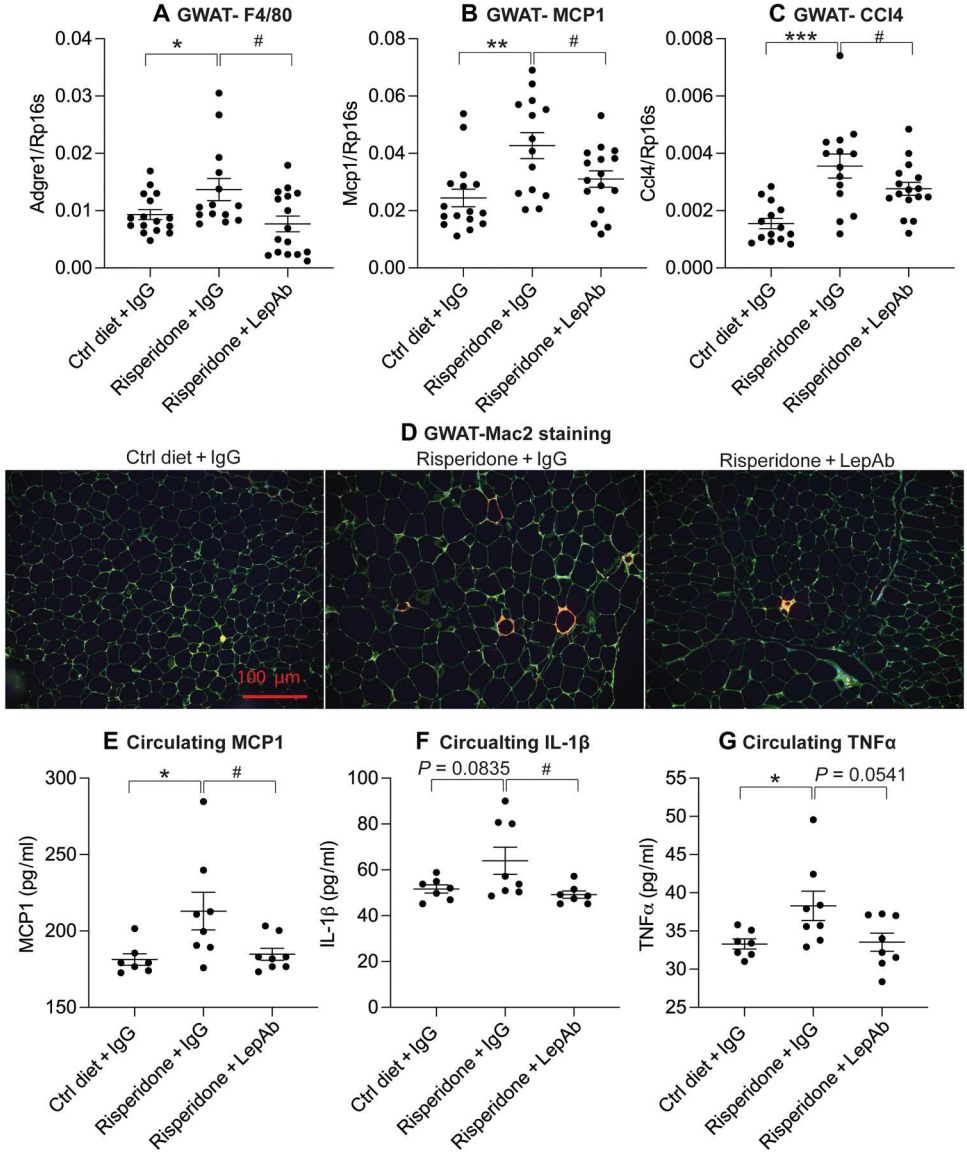

Fig. 5. Adipose tissue and systemic inflammation in response to risperidone and risperidone + LepAb. (**A**) *Adgre1* expression in mouse GAT; (**B**) *Mcp1* expression in GAT; (**C**) *Ccl4* expression in GAT; (**D**) Mac2 staining in GAT; (**E**) circulating MCP1; (**F**) circulating IL-1β; (**G**) circulating TNFα. Data are means ± SEM. Student's *t* test: **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 for risperidone + IgG versus Ctrl diet + IgG; #*P* < 0.05 for risperidone + LepAb versus risperidone + IgG.

by LepAb treatment (fig. S3, A to D). Risperidone-treated mice also displayed enhanced hepatic staining of F4/80, which was reduced by leptin neutralization as well (fig. S3, A to D). Last, reduced hepatic SMA staining in LepAb-treated mice further confirmed its protective effects against liver fibrosis.

Accompanying increased tissue inflammation, the circulating concentrations of inflammatory cytokines such as MCP1, IL-1β, and TNFα were elevated in risperidone-treated mice (Fig. 5, E to G). This treatment-induced elevation of inflammation markers was equally prevented by LepAb treatment (Fig. 5, E to G). Collectively, these observations robustly support a model in which risperidone-induced hyperleptinemia directly contributes to local tissue and systemic inflammation, ultimately resulting in diverse metabolic manifestations.

Leptin neutralization dampens hypothalamic inflammation

It has previously been shown that risperidone treatment is associated with a reduction of POMC expression in neuron populations critically involved in the regulation of energy balance (*30*). Related to this, we have shown that leptin reduction restores leptin sensitivity in the hypothalamus (*20*, *31*). With these previous findings in mind, we investigated whether LepAb treatment could restore the reduced hypothalamic Pomc expression in risperidone-treated mice. Whereas risperidone treatment was associated with a robust reduction of hypothalamic *Agrp* and *Pomc* gene expression, leptin neutralization by LepAb application normalized the expression of these factors (Fig. 6, A to C). We also observed a marked increase in hypothalamic inflammation gene expression in risperidone-treated mice, against which LepAb treatment exerted similarly potent protective effects (Fig. 6, D to F). Together, these

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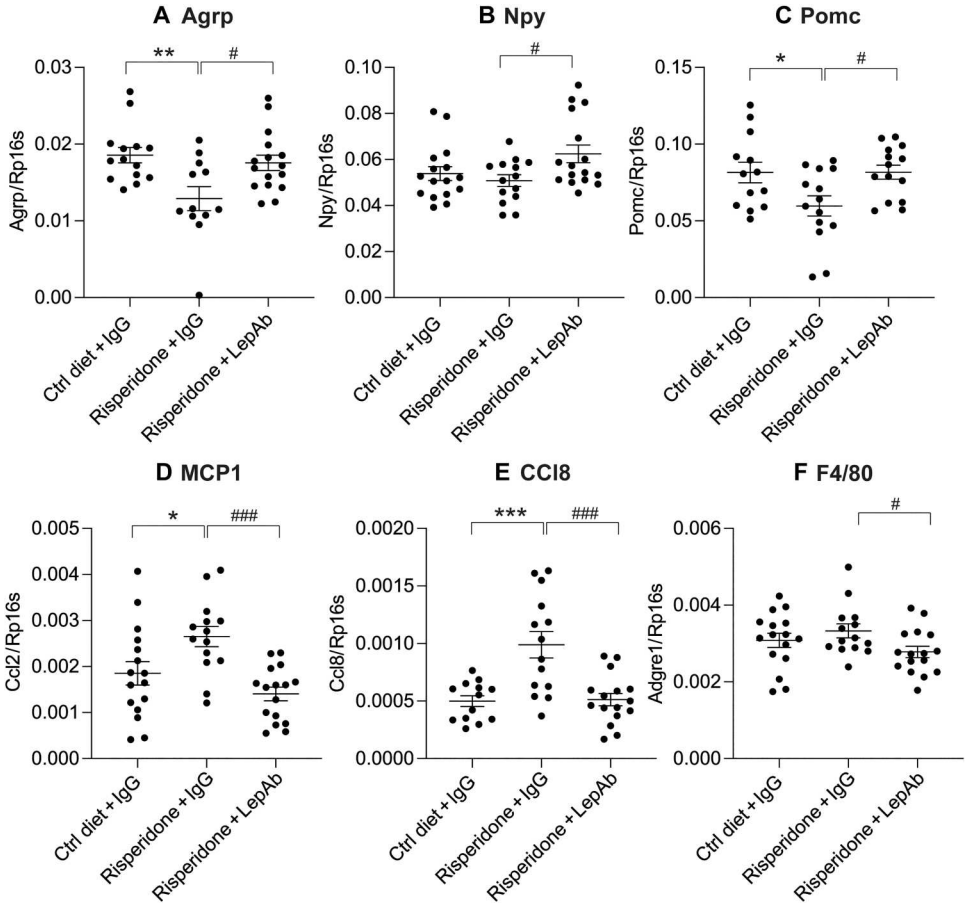

Fig. 6. Effects of risperidone and risperidone + LepAb on hypothalamic gene expression. (**A**) Mouse *Agrp* expression; (**B**) *Npy* expression; (**C**) *Pomc* expression; (**D**) *Mcp1* expression; (**E**) *Ccl8* expression; (**F**) *Adgre1* expression. Data are means ± SEM. Student's *t* test: **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 for risperidone + IgG versus Ctrl diet + IgG; #*P* < 0.05 and ###*P* < 0.001 for risperidone + LepAb versus risperidone + IgG.

observations further support the notion that risperidone-induced hyperleptinemia is a driving force for central and peripheral systemic inflammation as well as central dysregulation of energy balance.

Risperidone treatment is associated with widespread mammary duct development

A salient side effect of risperidone is that it markedly increases the plasma concentrations of prolactin in patients (*32*). Prolactin, however, is also a key factor in the formation of the mammary ductal network during pregnancy (*33*, *34*). After chronic risperidone treatment, we observed the formation of structures, similar to those observed during pregnancy-induced mammary gland development, in the subcutaneous fat (SWAT) of nonpregnant mice (Fig. 7A). These structures were not present in control mice that were fed HFD alone. Histological staining of cluster of differentiation 31 (CD31) confirmed that these structures also exhibited cellular resemblances to mammary ducts, consistent with widespread features of mammary gland development induced by antipsychotic drug exposure (Fig. 7B). LepAb treatment partially suppressed the development of these mammary gland–like structures in SWAT (Fig. 7, A and B). Previous reports implicated prolactin in this process of inducing mammary gland expansion during pregnancy (*33*). We therefore wondered whether our observed expansion of these mammary gland structures is associated with an increase in circulating prolactin. To this end, we measured circulating prolactin concentrations in mice with and without risperidone treatment. As expected, we found that risperidone significantly increased circulating prolactin ($P < 0.001$), and this increase was partially blocked by leptin neutralization (Fig. 7C). These observations suggest that leptin neutralization may be beneficial in reducing hyperprolactinemia-induced mammary gland development in response to risperidone treatment.

Reducing circulating leptin acts similarly in olanzapine-treated female mice

Because both risperidone and olanzapine show similar effects in inducing circulating leptin, we wondered whether the increased circulating leptin would be a universal mechanism associated with antipsychotic drug–associated weight gain and glucose intolerance. We therefore administered olanzapine to female mice instead of risperidone. We initiated the IgG and LepAb injections at the time of olanzapine administration. Similar to our observations made with risperidone, LepAb effectively prevented olanzapine-induced weight gain (fig. S4A) by reducing daily food intake and food accumulation over the course of the olanzapine treatment period (fig. S4, B and C). In addition, LepAb reversed olanzapine-induced glucose

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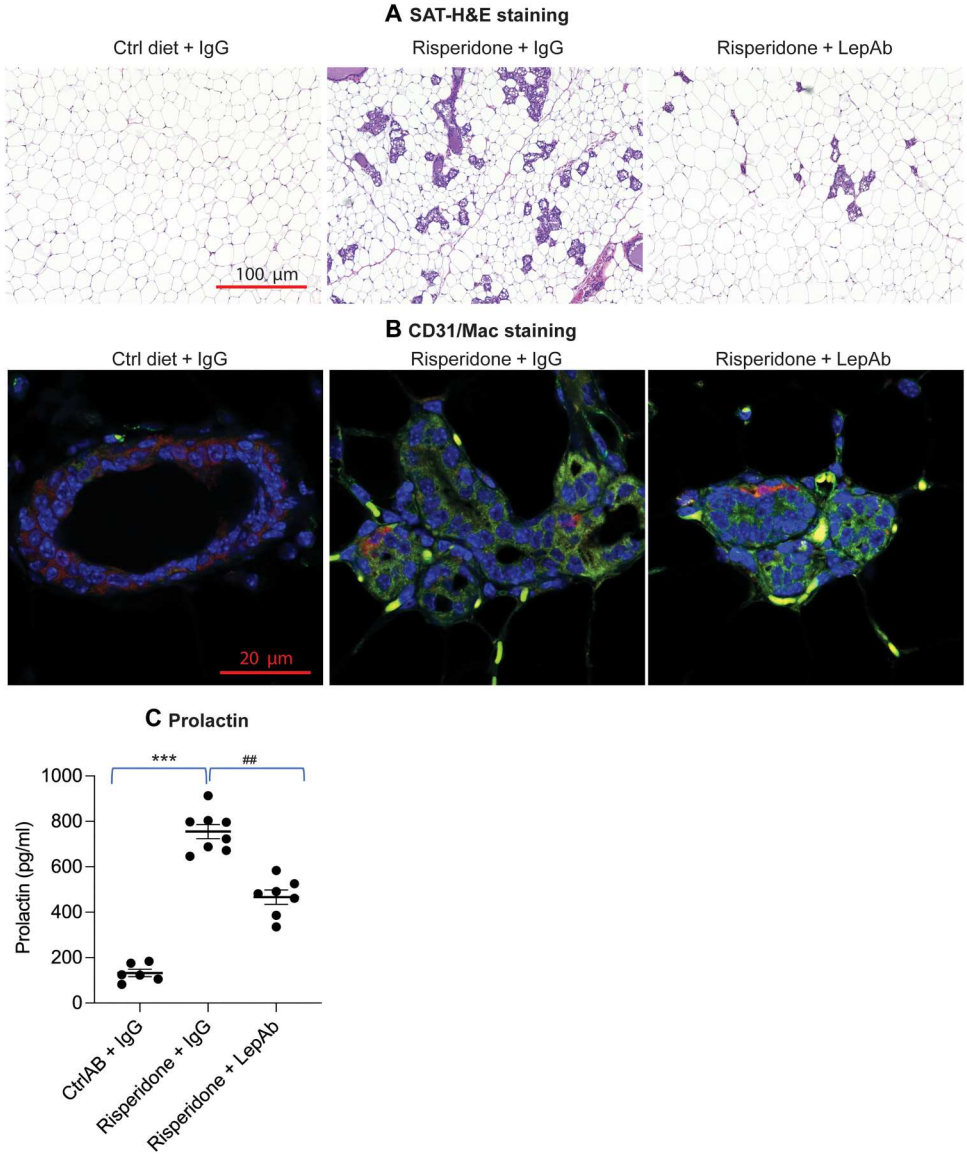

Fig. 7. Risperidone induces mammary gland development. (**A**) H&E staining of mouse SAT; (**B**) CD31/Mac2 staining of mammary duct–like structures in mouse SAT. CD31 stained as green and Mac2 stained as red; (**C**) prolactin concentrations. Data are means ± SEM. Student's *t* test: ***$P < 0.001$ for risperidone + IgG versus Ctrl diet + IgG; ###$P < 0.001$ for risperidone + LepAb versus risperidone + IgG.

intolerance (fig. S4D). Furthermore, we found that leptin neutralization significantly alleviated liver fibrosis–associated gene expression (fig. S4E) and inflammation-associated gene expression in adipose tissue (fig. S4F) in olanzapine-treated mice, similar to the effects observed in risperidone-treated mice. These observations further support the notion that antipsychotic drug–induced hyperleptinemia contributes to weight gain and glucose intolerance. Thus, a leptin reduction strategy, induced by a LepAb, offers a potential means to treat antipsychotic drug–associated side effects.

DISCUSSION

Antipsychotic drugs are highly effective in alleviating symptoms of psychosis. However, they are also generally associated with a series of common side effects, including massive weight gain, development and of progression of diabetes, and liver disease (*35*). The mechanisms underlying these unwanted side effects are largely unknown. As we show here, antipsychotic drug exposure is associated with increased circulating leptin concentrations even before weight gain occurs, proposing an involvement of leptin in mediating the drugs' side effects. However, to date, this proposition has not been examined experimentally. The system we used here reproduces central side effects of antipsychotic drug exposure. Taking advantage of this preclinical model, we provide evidence that the antipsychotic drug–induced hyperleptinemia contributes to weight gain, glucose intolerance, and tissue dysfunction. More specifically, we show that suppressing leptin signaling through application of a monoclonal LepAb constitutes an effective approach to alleviating

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antipsychotic drug–induced weight gain and reverse several other detrimental aspects of these drugs, overall improving tissue and systemic functions.

The overall impact of hyperleptinemia on body weight regulation remains somewhat controversial. Leptin transgenic mice display high circulating leptin as early as the embryonic stage, which results in an extremely lean phenotype with improved glucose tolerance, improved insulin sensitivity, and reduced systemic triglyceride-rich lipoprotein concentrations (*27*, *36*). These studies, however, are confounded by considerable developmental impact of hyperleptinemia. Recently, we and others demonstrated that hyperleptinemia can promote diet-induced obesity and glucose intolerance (*19*, *20*), which aligns well with a model that implicates hyperleptinemia directly in the development of leptin resistance (*37*). On the basis of our observations and those of others in the field, we were encouraged to examine the contribution of antipsychotic drug–induced hyperleptinemia into weight gain and metabolic deterioration. Our results strongly support the concept that antipsychotic drug–induced hyperleptinemia is a driving force behind the observed resulting body weight gain. We demonstrate that suppressing leptin signaling systemically is an effective intervention that reverses several side effects of preclinical antipsychotic drug treatment. Our study further supports the importance of leptin neutralization in the context of common obesity, further substantiating our previous studies.

One of the mechanisms by which hyperleptinemia and antipsychotic drugs promote body weight gain may lie in the induction of systemic inflammation. Given that leptin is a potent regulator of immune function, hyperleptinemia promotes a high degree of monocyte proliferation and subsequent maturation of these cells to macrophages, which can effectively infiltrate adipose tissue and the liver, culminating in local tissue and systemic inflammation. Here, we observed enhanced inflammation in several tissues in treated mice, specifically in adipose tissue, the liver, and the hypothalamus. Neutralizing circulating leptin effectively prevents these inflammatory processes, leading to restored tissue homeostasis and improved glucose tolerance.

It is well established that hyperleptinemia can induce robust suppressor of cytokine signaling 3 (SOCS3) expression and phosphorylation in the hypothalamus to impair specific aspects of insulin and leptin signaling (*38*). However, although risperidone too has previously been shown to up-regulate SOCS3 expression in cells (*39*), it has remained unknown whether hypothalamic SOCS3 up-regulation in response to risperidone treatment critically depends on the hyperleptinemia this drug induces. If so, then neutralizing circulating leptin could be a viable approach to restoring normal SOCS3 expression. Beyond this, we show here that mice consuming a risperidone-supplemented diet exhibited a substantial reduction in hypothalamic *Pomc* and *Agrp* expression, which was normalized by leptin neutralization. What constitutes possible mechanism(s) associated with the change of *Pomc* and *Agrp*? Reduced inflammation in the hypothalamic region may be the major driver. As previously reported, leptin is a master of regulator of innate and adaptive immune responses. Antipsychotic drug–induced hyperleptinemia overactivates the immune system, leading to peripheral and central inflammation, which, in turn, affects *Pomc* and *Agrp* gene expression. As a result, normalization of leptin concentrations by a LepAb effectively reduces inflammatory states in adipose tissues and the central nervous system and normalizes the expression of *Pomc* and *Agrp*. In addition, another notable observation is that the changes of *Pomc* and *Agrp* are in the same direction. Generally speaking, POMC and AGRP act in an opposite fashion: Fasting conditions stimulate *Agrp* expression, whereas fed conditions promote *Pomc* expression. In general, one would expect to see changes of *Pomc* and *Agrp* expression in opposite directions, which is seen in some, but not all, publications. Celastrol induces leptin sensitivity promoting weight loss but regulates the expression of *Pomc* and *Agrp* in the same direction, similar to our current observations (*40*). On the basis of these observations, we conclude that antipsychotic drug–induced obesity and associated metabolic disorders have their roots in hyperleptinemia. Hence, our proposed leptin neutralization strategy may be a possible addition to current antipsychotic therapy in the clinic.

The observation that the mouse model we use here also exhibits enhanced mammary gland development furthermore suggests that it can serve as a useful preclinical system to study this side effect of antipsychotic drug treatment. Furthermore, the widespread mammary gland development observed in nonpregnant young female mice on antipsychotics is a possible drawback of using this drug in treating younger patients, especially children. Currently, we do not have any data on the use of risperidone and the incidence of breast cancer. Because of the increased mammary gland development upon drug treatment, this issue deserves further evaluation.

There are limitations of our study. Here, we established a mouse model recapturing many of the side effects of antipsychotic drugs in the clinic. As with all preclinical models, there are limitations to each model. Although we believe that the model presented here reflects many responses to the drugs seen clinically as far as metabolic changes are concerned, we will have to await validation of the key premise (leptin as a driver of weight gain and insulin resistance) in the clinic. To determine the appropriate dose of antipsychotic compounds, we performed studies with various amounts of risperidone and olanzapine and settled on a minimal dosage that gives us the metabolic changes seen in our study here. However, compared with the doses conventionally used in the clinic, the doses used here in mice was considerably higher. To establish a preclinical model reflecting the clinical readouts, there are no other alternatives at present. Another limitation of our study is that only female, but not male, mice respond to antipsychotic drug exposure. Male mice on the same drug regimen fail to display a substantial increase in body weight. Future work is warranted to address these limitations.

In conclusion, antipsychotic drug therapy induces high concentrations of circulating leptin before massive weight gain. This leptin surge is a driving force for the development of obesity, tissue dysfunction, and impaired glucose tolerance, which likely occurs primarily through increased systemic inflammation. Leptin neutralization in the context of antipsychotic drug treatment is greatly beneficial to the management of the weight gain and metabolic dysfunction. In the future, the addition of LepAbs to antipsychotic drug regimens may serve to prevent metabolic side effects in patients treated for psychotic conditions.

MATERIALS AND METHODS

Study design

The aim of this study is to investigate the contribution of antipsychotic drug–induced hyperleptinemia in weight gain and its associated metabolic disorders. To explore our hypothesis, we selected a

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mouse model that mimics the side effects of antipsychotic drug in humans. In line with our observations and previous publications, only female mice were used in this study. We administrated two common antipsychotic drugs (risperidone and olanzapine) to the mice, in the absence or presence of our LepAb. In each experiment, age-matched female mice were randomly assigned to experimental groups. All the female mice were fed a diet supplemented either with risperidone or olanzapine, followed by IgG- or LepAb-neutralizing antibody injection. Food intake and body weight were monitored. Investigators were not blinded to genotyping or treatment group at Touchstone Diabetes Center, Dallas, Texas, USA. Mice were maintained, and studies were performed according to protocols approved by the Institutional Animal Care and Use Committee of University of Texas Southwestern Medical Center. Standard food or special diet was provided ad libitum throughout the experiments. We replicated experiments at least once to ensure biological reproducibility and adequate statistical analysis for comparisons.

Animals

All animal experimental protocols were approved by the Institutional Animal Care and Use Committee of University of Texas Southwestern Medical Center at Dallas. Mice were housed under standard laboratory conditions (12-hour on/off; lights on at 6:00 a.m.) in a temperature-controlled environment with food and water available ad libitum. Female mice (catalog no. 000664) were obtained from the Jackson Laboratory laboratory at about 8 weeks of age.

Antibody preparation

The parental LepAb was isolated from a phage displayed human single-chain variable fragments (ScFv) antibody library. To avoid immunogenicity against the human antibody during long-term and multiple dosing treatment in mice, we mouserized the parental LepAb. Mouserization was accomplished using a combined Kabat/IMGT complementarity-determining regions (CDR)–grafting method. The variable fragment heavy chain (VH) and variable fragment light chain (VL) DNA sequences of the parental antibody were blasted against the mouse germline gene sequence database with IgBLAST or IMGT/V-QUEST. The most similar mouse germline VH and VL sequences were selected as templates. The CDRs defined by Kabat/IMGT were grafted onto the framework regions of corresponding templates. The CDR-grafted VH and VL were cloned into mouse IgG1 and light-chain backbone to express the full-length antibody. Antibody expression and purification were based on protocols described previously. Monoclonal anti-LepAb and isotype control (IgG) were transiently expressed in ExpiHEK293 cells in shake flask cultures according to the manufacturer′s protocol (Thermo Fisher Scientific, Invitrogen). Briefly, variable heavy and light sequences (patent no. 17/124,481; US2021-0188970-A1) were constructed into two separate expression vectors for cotransfection and expression in HEK293 cells using polyethylenimine (Sigma-Aldrich) to mediate cell transfection. Cell culture supernatants were harvested by centrifugation at 4000*g* for 10 min after 7 days of culturing in a shaker incubator with 8% CO_2 and 80% humidity. Monoclonal antibodies secreted in cultures were purified using protein A affinity resin (Repligen) as described previously.

Mouse treatment

After 1 week of acclimation in the mouse room, female mice were placed either on control HFD diet (D09092903, Research Diet), olanzapine diet (D12040807, Research Diet), or risperidone diet (D09092903, Research Diet) for various periods, as indicated in figures. For experiment related to LepAb injection, there were three groups of mice: control diet with control IgG (I-536, Leinco Technologies) injection, risperidone diet with control IgG injection, and risperidone diet with monoclonal LepAb injection (lot #20200821 with an endotoxin concentration of 4.66 EU/mg of Ig). The control IgG and LepAb were given at a dose of 1 mg/kg of body weight at a frequency of twice a week (Monday and Thursday).

Food intake and body weight

To measure food intake and body weight gain, all female mice were singly housed. Before each experiment, mice were acclimated in the single cage for at least 1 week to reduce stress. Food intake and body weight were measured before each injection.

Glucose tolerance and insulin tolerance tests

Glucose tolerance tests (GTTs) were performed as previously described (*41*). For the GTTs, mice were fasted for 4 to 6 hours in the morning and then orally gavaged 2 g of glucose/kg of body weight [dissolved in phosphate-buffered saline (PBS)] (catalog no. 806552, Sigma-Aldrich). Blood glucose was measured using a contour glucometer.

HIEC studies

Clamp studies were performed as previously described (*42*).

Blood parameters

Blood was taken from fed animals in the morning, allowed to clot, and centrifuged for 5 min at 8000*g* to isolate serum for multiple analyses. Leptin and adiponectin were measured using appropriate enzyme-linked immunosorbent assay kits (#90080, Crystal Chem, and #EZMADP-60K, Thermo Fisher Scientific). For the leptin measurement, we first removed IgG-bounded leptin by precipitating the plasma with anti-mouse IgG beads. After centrifugation, the supernatant was used for leptin measurement. We referred to this leptin as "free" leptin.

Reverse transcription quantitative polymerase chain reaction

RNA was extracted from fresh or frozen tissues by homogenization in TRIzol reagent (Thermo Fisher Scientific) as previously described (*43*). cDNA was prepared using the iScript Reverse Transcription kit (Bio-Rad), and analyses were performed using PowerUp SYBR Green Master Mix (Thermo Fisher Scientific) on a QuantStudio 6 system (Thermo Fisher Scientific). Most reverse transcription quantitative polymerase chain reaction primers were from Harvard PrimerBank (https://pga.mgh.harvard.edu/primerbank/). Relative expression was calculated using the comparative threshold cycle method and normalized to the housekeeping gene *Rps16*.

Histology

Histology was performed as previously described (*44*). Briefly, adipose tissue and liver tissues were collected and fixed overnight

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in 10% PBS-buffered formalin and thereafter stored in 50% ethanol. Tissues were further processed by the University of Texas Southwestern Medical Center (UTSW) Molecular Pathology Core.

Cell culture and Western blots

HEK293 cells (catalog no. CRL-1573, American Type Culture Collection) were transfected with lentivirus to allow stable expression of long-form leptin receptor (LepRb). This cell line was seeded into a six-well plate and reached 80% confluence. The cells were then treated with Dulbecco's modified Eagle's medium without serum for 6 hours and incubated in the absence and presence of 10 ng of leptin and different doses of LepAb. Before adding to the wells, leptin and LepAbs were mixed together in a 1.5-ml Eppendorf tubes with gentle shaking for 1 hour. Then, the mixture was added into the well for 10 min. After that, the wells were washed with cold PBS twice and then stored for further analysis. Western blot was performed as previously done. The antibody p-Stat3 was obtained from Cell Signaling Technology (catalog no. 9145, RRID: AB_2491009) with a dilution of 1:1000.

Immunofluorescence

Immunofluorescence was performed as previously described (*45*). Briefly, formalin-fixed, paraffin-embedded sections from adipose tissues or liver were blocked in 1× phosphate-buffered saline with 0.1% Tween 20 (PBST) containing 5% bovine serum albumin. Primary antibodies used were perilipin (1:500 dilution; #NB100-60554, Novus; RRID: AB_922242), CD31 (1:250 dilution; #ab124432, Abcam; RRID: N/A), and Mac2 (1:500 dilution; #125401, BioLegend; RRID: AB_1134237). Secondary antibodies (1:250 dilution) used were Alexa Fluor 488 or Alexa Fluor 594 donkey anti-rabbit IgG both heavy and light (H+L) chains or Alexa Fluor 488 or Alexa Fluor 594 donkey anti-goat IgG (H+L) (Thermo Fisher Scientific). Slides were counterstained with 4′,6-diamidino-2-phenylindole. Fluorescent images were acquired using an AxioObserver Epifluorescence Microscope (Zeiss) or FSX100 microscope (Olympus).

Statistical analysis

For all animal studies, we designed experiments to address the parameter(s) of interest with utmost efforts to minimize and control for confounding variables such as mouse strain, gender, and age, tissue sampling time of day, fed/fasted state, diet composition, and light cycle. On the basis of our previous experience, we can use five animals per group to achieve sufficient statistical power to detect significant differences for measures of RNA, protein, and metabolites. For studies that require mice to undergo surgery (as for clamp studies), eight animals were used to account for the variability that occurs because of differences in recovery time and experienced stress. All values were expressed as the means ± SEM. Pairwise comparison of means was accomplished under the assumptions of normality using Student's *t* test (two-sided) for comparison of two groups. One-way or two-way analysis of variance (ANOVA) was used for comparisons of more than two groups. $P \leq 0.05$ was regarded as statistically significant.

Supplementary Materials

This PDF file includes:
Figs. S1 to S4

Other Supplementary Material for this manuscript includes the following:
Data file S1
MDAR Reproducibility Checklist

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Acknowledgments

Funding: This work was supported by U.S. National Institutes of Health grants R01-DK55758, R01-DK099110, RC2-DK118620, R01-DK127274, R01-DK131537, and P01-AG051459 (to P.E.S.), U.S. National Institutes of Health grants R01-DK118725 and R01-DK088423 (to J.K.E.), U.S. National Institutes of Health grants R01 DK114036 and DK130892 (to C.L.), and U.S. National Institutes of Health grants R01 DK117872 (to O.O.). In addition, this study was also supported by the Cancer Prevention and Research Institute of Texas (CPRIT) Grants RP190561 and Welch Foundation grant no. AU-0042-20030616 (to Z.A.). S.Z. was supported by the U.S. National Institutes of Health grant R00-AG068239, and S.Z. holds a Voelcker Fund Young Investigator Pilot Award from the Max and Minnie Tomerlin Voelcker Fund. Y.Z. was supported by the U.S. National Institutes of Health grant R01-DK136619 and R01-DK136532; L.L. was supported by AHA Postdoc Fellowship 23POST1019715; Q.Z. was supported by AHA Career Development Award 855170. Research reported in this publication was supported by the UTSWNORC grant under NIDDK/NIH award number P30-DK127984. **Author contributions:** Conceptualization: P. E.S. and S.Z. designed and conceptualized the entire study. S.Z., Q.L., and L.L. performed experiments using risperidone and olanzapine to treat the female mice. W.X., N.Z., and Z.A. generated LepAb used in this study. L.S., X.S., J.-B.F., C. Li, N.J., Z.X., M.-Y.W., and J.B. performed gene expression analysis in different tissues and immunostaining. S.C. performed HIEC studies. D.Z., R.Z., and O.O. performed experiments to characterize olanzapine-prone and -resistant mice. Q.Z. performed the staining of CD31/Mac 2 staining in mouse SAT. S.C.W. performed the brain dissection. G.L. measured prolactin in mice. S.Z. wrote the first draft of the manuscript. Z. Z., Y.Z., X.H, L.S., J.-B.F., C.M.K., J.K.E, O.O., C.L., and P.E.S participated in discussion and revised the manuscript; P.E.S. supervised the entire study. **Competing interests:** The authors declare that they have no competing interests. **Data and materials availability:** All data associated with this study can be found in the paper or the Supplementary Materials. All materials and mouse models are available through request to P.E.S. (philipp.scherer@utsouthwestern.edu). Raw data are provided in data file S1.

Submitted 12 September 2022
Resubmitted 29 August 2023
Accepted 17 October 2023
Published 22 November 2023
10.1126/scitranslmed.ade8460

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Science Translational Medicine

Hyperleptinemia contributes to antipsychotic drug–associated obesity and metabolic disorders

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Sci. Transl. Med. **15** (723), eade8460. DOI: 10.1126/scitranslmed.ade8460

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