Divergent Effects of Oxytocin Treatment of Obese Diabetic Mice on Adiposity and Diabetes

Jordi Altirriba, Anne-Laure Poher, Aurélie Caillon, Denis Arsenijevic, Christelle Veyrat-Durebex, Jacqueline Lyautey, Abdul Dulloo, and Françoise Rohner-Jeanrenaud

Laboratory of Metabolism (J.A., A.-L.P., A.C., C.V.-D., J.L., F.R.-J.), Department of Internal Medicine Specialties, Faculty of Medicine, University of Geneva, Geneva 1211, Switzerland; and Department of Medicine/Physiology (D.A., A.D.), University of Fribourg, Fribourg 1700, Switzerland

Oxytocin has been suggested as a novel therapeutic against obesity, because it induces weight loss and improves glucose tolerance in diet-induced obese rodents. A recent clinical pilot study confirmed the oxytocin-induced weight-reducing effect in obese nondiabetic subjects. Nevertheless, the mechanisms involved and the impact on the main comorbidity associated with obesity, type 2 diabetes, are unknown. Lean and *ob*/*ob* mice (model of obesity, hyperinsulinemia, and diabetes) were treated for 2 weeks with different doses of oxytocin, analogues with longer half-life (carbetocin) or higher oxytocin receptor specificity ([Thr4,Gly7]-oxytocin). Food and water intake, body weight, and glycemia were measured daily. Glucose, insulin, and pyruvate tolerance, body composition, several hormones, metabolites, gene expression, as well as enzyme activities were determined. Although no effect of oxytocin on the main parameters was observed in lean mice, the treatment dose-dependently reduced food intake and body weight gain in *ob*/*ob* animals. Carbetocin behaved similarly to oxytocin, whereas [Thr4,Gly7]-oxytocin (TGOT) and a low oxytocin dose decreased body weight gain without affecting food intake. The body weight gain-reducing effect was limited to the fat mass only, with decreased lipid uptake, lipogenesis, and inflammation, combined with increased futile cycling in abdominal adipose tissue. Surprisingly, oxytocin treatment of *ob*/*ob* mice was accompanied by a worsening of basal glycemia and glucose tolerance, likely due to increased corticosterone levels and stimulation of hepatic gluconeogenesis. These results impose careful selection of the conditions in which oxytocin treatment should be beneficial for obesity and its comorbidities, and their relevance for human pathology needs to be determined.

According to the World Health Organization, obesity is one of the 5 leading causes of mortality due to its well-known comorbidities, affecting subjects across all income groups. Thus, around 2.8 million people die worldwide due to overweight or obesity (1). One of these comorbidities is type 2 diabetes, the most predominant form of diabetes (ie, 90%–95% of patients with diabetes) (2).

Oxytocin is a hypothalamic nonapeptide synthesized in the magnocellular neurons of the paraventricular and supraoptic nuclei projecting to the pituitary, where it reaches the peripheral circulation, as well as in the parvocellular neurons of the paraventricular nucleus projecting to other brain regions (3). Peripheral oxytocin synthesis has also been described (4). Classically, oxytocin has been involved in uterine contractions during labor and milk ejection during lactation, and its major applications in clinics is for labor induction (5) and postpartum hemorrhage (6). Further studies show that oxytocin is involved in several other functions, such as natriuresis, insulin and glucagon secretion, thermoregulation, social and sexual behavior, food intake, body weight, etc. (for an exhaustive review, see Ref. 4). Collectively, these data indicate growing interest for this neuropeptide and its potential therapeutic applications. In particular, several clinical studies are currently

Abbreviations: DIO, diet-induced obese; eWAT, epididymal white adipose tissue; FASN, fatty acid synthase; MRI, magnetic resonance imaging; NEFA, nonesterified fatty acid; OXTR, oxytocin receptor; PF, pair-fed; TG, triglyceride; TGOT, [Thr4,Gly7]-oxytocin.

performed to test the efficiency of oxytocin treatment in the fields of autism (7) and obesity (8). The reasons why oxytocin has been suggested as a novel therapeutic agent against obesity are the following: 1) it induces weight loss and improves glucose tolerance in chronically treated diet-induced obese (DIO) rodents (8–12); 2) it acutely decreases food intake and body weight in leptin-deficient Zucker and Koletsky rats, upon administration of a single dose (11, 13); and 3) it decreases body weight in lean and obese nondiabetic patients (8, 14). However, despite these promising observations, the mechanisms involved in the beneficial oxytocin effects on body weight homeostasis, as well as the impact of the treatment on the development of type 2 diabetes, are unclear.

The aim of the present study was to determine whether a chronic oxytocin treatment has beneficial effects in *ob/ob* mice. This model was selected, because it presents a more severe phenotype than DIO mice and rats, with more extreme obesity, as well as higher basal glycemia and insulinemia, altogether representing a model of a more advanced stage in the development of type 2 diabetes. Although the treatment was mostly ineffective in lean mice, it dose-dependently reduced food intake and body weight gain in *ob/ob* mice. The latter effect was limited to the fat mass only, with decreased lipid uptake, lipogenesis, and inflammation, combined with increased futile cycling. Surprisingly, oxytocin treatment or the use of oxytocin analogues worsened basal glycemia and glucose tolerance, likely due to increased corticosterone levels and stimulation of hepatic gluconeogenesis.

Materials and Methods

Mice

The principles of laboratory animal care were followed (European and local government guidelines) and protocols were approved by the Geneva Cantonal Veterinary Office. Eight-week-old C57BL/6JRj (referred to as C57BL6/J) and B6.V-Lepob/JRj (referred to as *ob/ob*) male mice (Janvier) were treated for 14 days with oxytocin (PolyPeptide) at 5, 50, or 150 nmol/d, carbetocin (Bachem) at 50 nmol/d, [Thr4,Gly7]-oxytocin (TGOT) (Abgent) at 50 nmol/d, or the solvent (saline, NaCl 0.9%) given sc via osmotic pumps (Alzet). Mice were distributed in the different groups by stratified random allocation, in order to obtain no statistical difference of body weight and glycemia among the groups, at the beginning of the treatment. Body weight, food, and water intake were measured daily, and glycemia (Accu-Check; Roche) was determined every second day at 10 AM. A saline-treated pair-fed (PF) group was included with a series of saline- and oxytocin-treated animals. The pair-feeding protocol consisted in providing the PF mice with the mean quantity of food consumed the day before by the oxytocin-treated group. Glucose (1 g/kg; Bichsel), pyruvate (2 g/kg; Sigma), or insulin (0.75 UI/kg; Actrapid HM, Novo Nordisk) ip tests were performed at day 10 of treatments, after 5 hours of food deprivation (10 AM to 3 PM). At the beginning and the end of the treatment, body composition was analyzed by magnetic resonance imaging (MRI) (EchoMRI), and data are represented as the difference between the 2 sets of values. Epididymal white adipose tissue (eWAT) and liver were fixed in 10% (vol/vol) formalin solution (Sigma) overnight at 4°C, dehydrated, and embedded in paraffin before sectioning, or embedded in OCT (optimum cutting temperature) (Thermo Fisher Scientific) and frozen for subsequent analyses.

Plasma and hepatic measurements

At the end of the treatment, trunk blood was collected in EDTA tubes (Becton Dickinson), and aprotinin (Axon Lab AG) was added at 400 kIU/mL. Plasma insulin (Mercodia), glucagon (Merck), corticosterone (Immunodiagnostic Systems), nonesterified fatty acids (NEFA) (Wako Diagnostics), glycerol (Sigma), triglyceride (TG) (Biomérieux), and leptin (Bertin Pharma) levels were measured. Plasma oxytocin concentrations were also determined (Enzo Life Sciences) with a previous extraction step (Strata C18-E; Phenomenex), as recommended by the manufacturer. Hepatic TG content (Biomérieux) was measured after chloroform:methanol extraction, and hepatic glycogen (Sigma) was determined according to the manufacturer protocol.

RNA isolation and real-time PCR

Total RNA was isolated using the RNeasy Lipid Tissue Mini kit (QIAGEN), with DNAse (deoxyribonuclease) treatment in column. RNA integrity was analyzed using a lab-on-a-chip (Agilent Technologies). RNA was reverse transcribed using M-MLV (Moloney murine leukemia virus reverse transcriptase) reverse transcriptase (Thermo Fisher Scientific). Real-time PCR was carried out in a StepOne Real Time System (Thermo Fisher Scientific), using the SYBR Green fluorophore (Roche). A standard curve for each primer set was generated from serial dilutions of cDNA. PCR products were verified by dissociation curve analysis, using the StepOne software (Thermo Fisher Scientific). Expression levels were normalized to ribosomal protein S29 (*Rps29*) and represented as percent of the saline-treated group (set at 100%). Primer sequences were designed by the Primer Express software (Thermo Fisher Scientific) and are provided in Supplemental Table 1.

Fatty acid synthase (FASN) activity

FASN activity was measured according to the protocol described previously (15).

Histological studies

eWAT sections were deparaffinated and rehydrated, applying primary antibody against MAC-2 (CL8942AP) and secondary antirat-Cy3 (Jackson ImmunoResearch). Hoechst 33258 was used as nuclear marker (Sigma). Liver sections were stained with hematoxylin and eosin or Oil-Red-O (Sigma). Images were taken using a Mirax Desk and an Axioskop 2 (Carl Zeiss). Total cell counting was performed using the ImageJ plugin cell counter (16) on the nuclear Hoechst stained blue channel images. MAC-2-positive cells were counted manually on the merged images stained with the MAC-2 antibody and the Hoechst nuclear marker. The number of cells counted was 7409 ± 825 for C57BL6/J and 12 866 ± 2109 for *ob/ob* mice.

Statistical analysis

Quantitative data are expressed as mean ± SEM. The statistical significance was determined by the Student's *t* test (2 groups) or by ANOVA (more than 2 groups), with a Tukey's post hoc test. Lon-

gitudinal data with several groups, such as Δ-glycemia, were analyzed by generalized linear models and Tukey's post hoc test. Analyses were performed with SPSS (IBM) and R (17).

Results

Phenotype of obese/diabetic and lean mice treated with oxytocin

In obese diabetic *ob/ob* mice, the oxytocin treatment had a clear effect in preventing body weight gain (Figure 1A). Both food and water intake were reduced, the effects being more marked during the first than the second week of the study (Figure 1, C and G). Food efficiency, the ratio of body weight gain per unit food intake over the whole treatment expressed as percent was decreased in oxytocin-treated obese mice (Figure 1E). Surprisingly, basal glycemia measured in the fed state was increased in the oxytocin-treated group (Figure 1I). Among the various circulating parameters measured at the end of the treat-

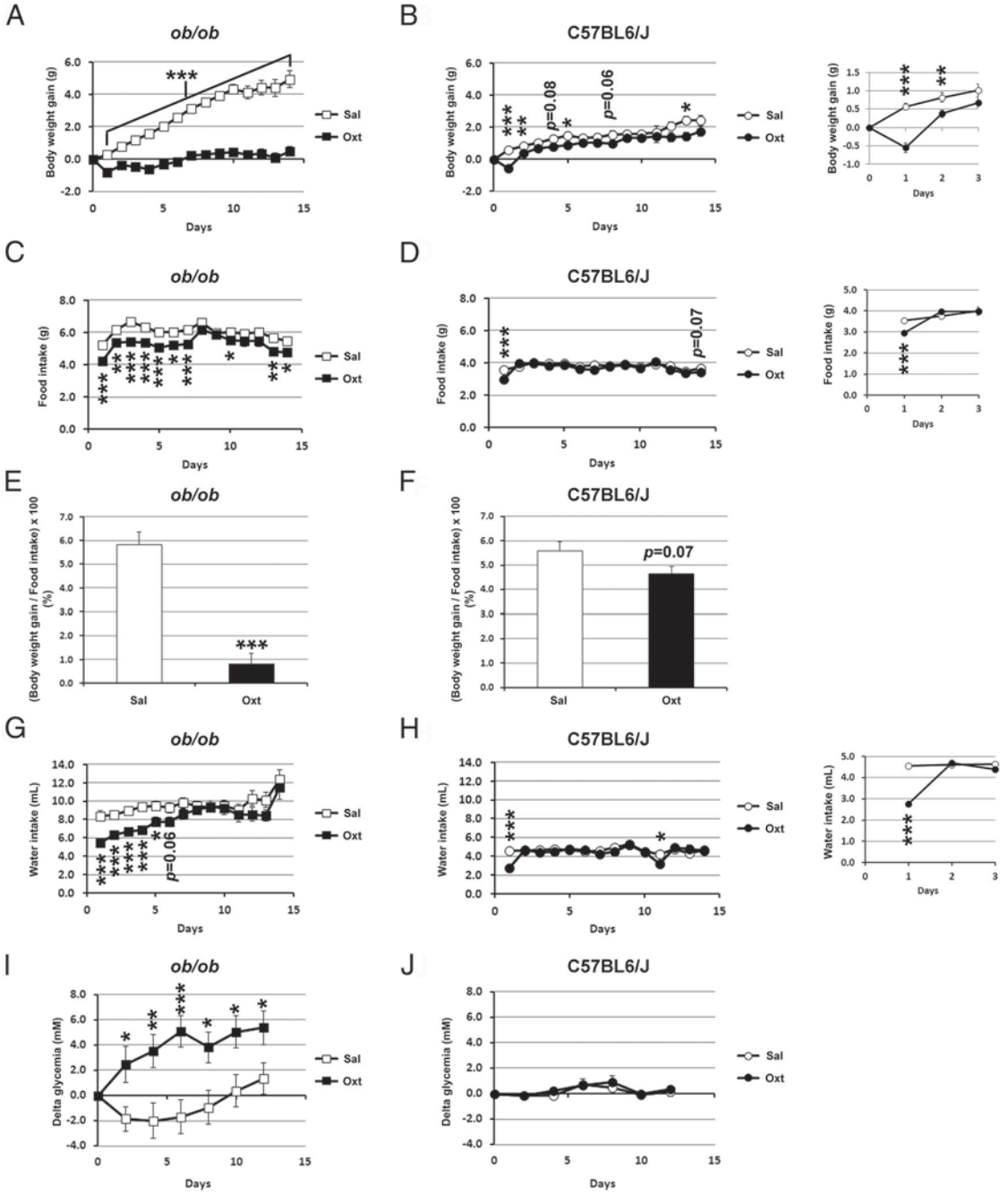

Figure 1. Oxytocin effects on body weight, food and water intake, as well as glycemia in lean and *ob/ob* mice. Δ-Body weight (A and B), food intake (C and D), food efficiency (E and F), water intake (G and H), and Δ-glycemia (I and J) of *ob/ob* (A, C, E, G, and I) or C57BL6/J (B, D, F, H, and J) mice sc treated with oxytocin (50 nmol/d) or vehicle (saline) during 14 days. Next to B, D, and H, magnification of the effects observed during the first 3 days of treatments in C57BL6/J mice. *, $P < .05$; **, $P < .01$; ***, $P < .001$. *ob/ob*, n = 22–38 and C57BL6/J, n = 13–19.

ment, none was altered, except for blood glucose, which exhibited a tendency to be higher ($P = .07$), and for corticosterone levels, measured after 5 hours of food deprivation (referred to as "fast corticosterone"), which were significantly higher in the oxytocin- than in the saline-treated group (Table 1). Even though oxytocin is envisaged as a treatment against obesity, its effects were also determined in C57BL6/J mice, the lean nondiabetic controls of *ob/ob* mice with similar genetic background. In these mice, the oxytocin treatment induced a transient decrease in food and water intake during the first day, which led to a decrease in body weight (Figure 1, B, D, and H). Subsequently, body weight gain was similar in saline- and oxytocin-treated mice, with a trend to decreased food efficiency (Figure 1F). When measured daily in the fed state, basal glycemia of lean mice was unaltered by the oxytocin treatment (Figure 1J). In contrast, after a 5-hour food deprivation ("fast glucose"), glycemia was increased in the oxytocin-treated group, with a concomitant elevation in insulinemia. Circulating corticosterone, glycerol, NEFA, and TG levels were similar in both groups, whereas leptinemia and glucagon levels were higher in the oxytocin-treated animals (Table 2).

Given that the oxytocin treatment decreased food intake, either transiently in C57BL6/J, or in a more sustained way in *ob/ob* mice, the expression of the hypothalamic neuropeptides from the first-order leptin-sensitive neurons was measured. As expected, the expression of the orexigenic neuropeptides *Npy* and *Agrp* was higher, whereas that of the anorexigenic neuropeptide *Pomc* was lower in *ob/ob* than in lean C57BL6/J mice. The oxytocin treatment was without any effect on these parameters in both groups (Supplemental Figure 1).

Table 1. Biochemical Parameters Measured at the End of the Treatment in *ob/ob* Mice Subcutaneously Treated with Oxytocin (50 nmol/d) or Vehicle (Saline) During 14 Days

	ob/ob	
	Saline	**Oxytocin**
Fed glucose (mM)	17.4 ± 1.1	19.6 ± 0.9
Fast glucose (mM)	10.9 ± 1.3	14.6 ± 1.4 ($P = .07$)
Fed insulin (pmol/L)	2679.8 ± 842.6	2330.1 ± 479.3
Fast insulin (pmol/L)	2439.6 ± 402.8	2967.1 ± 336.6
Glucagon (pg/mL)	132.6 ± 8.4	117.7 ± 11.2
Fed corticosterone (ng/mL)	175.6 ± 41.6	263.2 ± 46.5
Fast corticosterone (ng/mL)	247.0 ± 32.7	448.4 ± 52.6[a]
Glycerol (μg/mL)	49.5 ± 13.0	50.9 ± 12.1
NEFA (mM)	0.33 ± 0.02	0.30 ± 0.03
TGs (g/L)	0.63 ± 0.04	0.65 ± 0.05

Abbreviations: Oxt-HD, oxytocin-"high dose"; Oxt-LD, oxytocin-"low dose". n = 6–17.
[a], $P < .05$; [b], $P < .01$

Table 2. Biochemical Parameters Measured at the End of the Treatment in C57BL6/J Mice Subcutaneously Treated with Oxytocin (50 nmol/d) or Vehicle (Saline) During 14 Days

	C57BL6	
	Saline	**Oxytocin**
Fed glucose (mM)	8.5 ± 0.3	9.3 ± 0.3 ($P = .09$)
Fast glucose (mM)	8.5 ± 0.2	9.5 ± 0.3[a]
Fed Insulin (pmol/L)	54.9 ± 5.1	66.8 ± 4.8
Fast Insulin (pmol/L)	68.5 ± 10.1	121.1 ± 16.6[a]
Glucagon (pg/mL)	58.3 ± 3.7	72.3 ± 3.7[b]
Leptin (ng/mL)	0.7 ± 0.1	1.2 ± 0.1[b]
Fed corticosterone (ng/mL)	30.4 ± 5.7	25.0 ± 13.6
Fast corticosterone (ng/mL)	92.2 ± 21.4	54.0 ± 4.8
Glycerol (μg/mL)	28.8 ± 1.5	37.2 ± 3.9
NEFA (mM)	0.23 ± 0.02	0.32 ± 0.04
TGs (g/L)	0.84 ± 0.08	0.90 ± 0.10

n = 4–13.
[a], $P < .05$; [b], $P < .01$

Specificity and dose dependency of oxytocin effects in obese/diabetic mice

A saline-infused control group of *ob/ob* mice receiving the same amount of food as the oxytocin-treated animals was included in the study (PF group), demonstrating that the oxytocin-induced prevention of body weight gain was only partly, but not exclusively, due to a decrease in food intake. Indeed, the final body weight gain of PF mice was higher than that of oxytocin-treated animals (Figure 2A), and food efficiency of oxytocin-treated mice was lower than that of both the saline and the PF control groups (Figure 2B).

The dose of oxytocin administered to the mice (50 nmol/d) was the same as that used in previous studies (9, 12). This dose produced a maximal effect, because increasing it by 3-fold (Oxt-HD, 150 nmol/d) did not further decrease body weight gain (Figure 2C). When the 50 nmol/d oxytocin dose was reduced by 10-fold (Oxt-LD, 5 nmol/d), the body weight gain was lower than that of saline-infused controls (Figure 2C), without affecting food intake (Figure 2E).

The next step consisted in treating obese/diabetic mice with the longer half-life oxytocin analog, frequently used in clinics, carbetocin at a dose of 50 nmol/d (18). As can be seen on Figure 2D, carbetocin treatment produced a similar decrease in body weight gain as oxytocin itself. Furthermore, despite clear differences in affinity of oxytocin for its own receptor and for one of the vasopressin receptors (oxytocin affinity for oxytocin receptor [OXTR], 0.83nM; V1a, 20.38nM; V1b, 36.32nM) (19),

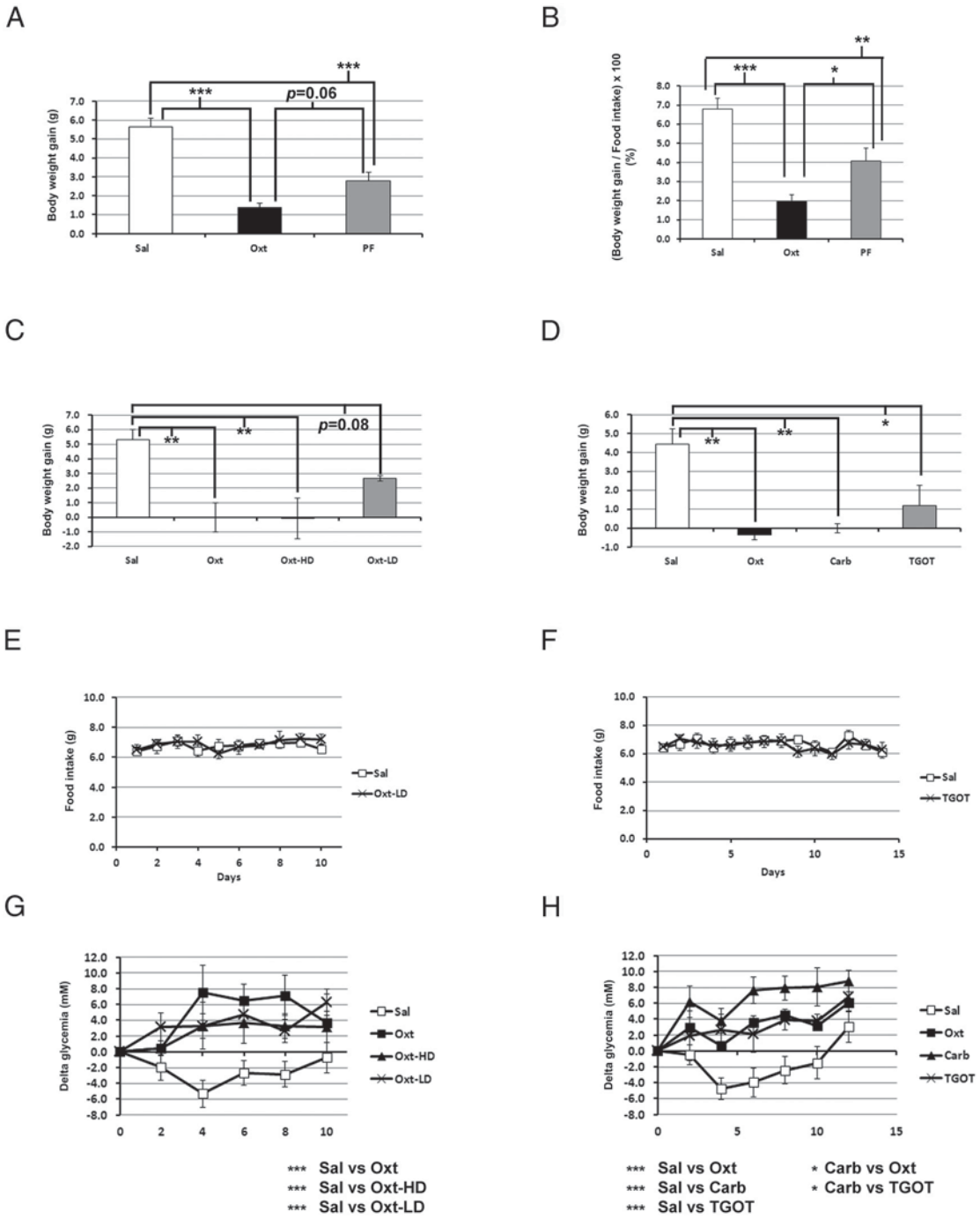

Figure 2. Effects of pair-feeding, different doses of oxytocin, and oxytocin analogues on body weight, food intake, and glycemia in *ob/ob* mice. Δ-Body weight (A) and food efficiency (B) of *ob/ob* mice treated with vehicle (saline), oxytocin (50 nmol/d), or vehicle (saline) but receiving the same amount of food as oxytocin-treated mice (PF group) after 14 days of treatment; n = 6–7. Δ-Body weight (C), food intake (E), and Δ glycemia (G) of *ob/ob* mice treated with vehicle (saline), oxytocin (Oxt) (50 nmol/d), oxytocin low dose (Oxt-LD) (5 nmol/d), or oxytocin high dose (Oxt-HD) (150 nmol/d) during 10 days; n = 3–8. Δ-Body weight (D), food intake (F), and Δ glycemia (H) of *ob/ob* mice treated with vehicle (saline), oxytocin (Oxt) (50 nmol/d), carbetocin (Carb) (50 nmol/d), or TGOT (50 nmol/d) during 14 days; n = 5–11. *, $P < .05$; **, $P < .01$; ***, $P < .001$.

it was possible that the oxytocin effect was, at least partly, mediated by unspecific activation of the vasopressin signaling pathway. To address this issue, we treated another series of mice with an analog of oxytocin characterized by a much lower affinity for the vasopressin than for the OXTR (TGOT, affinity for OXTR, 0.04nM; V1a, >1000nM; V1b, >10 000nM) (19). TGOT produced a similar effect as the low oxytocin dose on body weight gain, as well as on food intake (ie, no change in this parameter) (Figure 2, D and F). This effect was, however, not different from that observed in response to the high oxytocin dose or to carbetocin.

Regarding the profile of basal glycemia, it was similarly increased in all the groups receiving oxytocin (5, 50, and 150 nmol/d) or analogues (TGOT and carbetocin) compared with controls (Figure 2, G and H).

Oxytocin effects on adipose tissue

Using MRI, we observed that the effects of oxytocin on body weight in *ob/ob* mice were exclusively related to decreased fat mass gain (Figure 3A). This was confirmed by weighing the epididymal fat pad, which was smaller in the

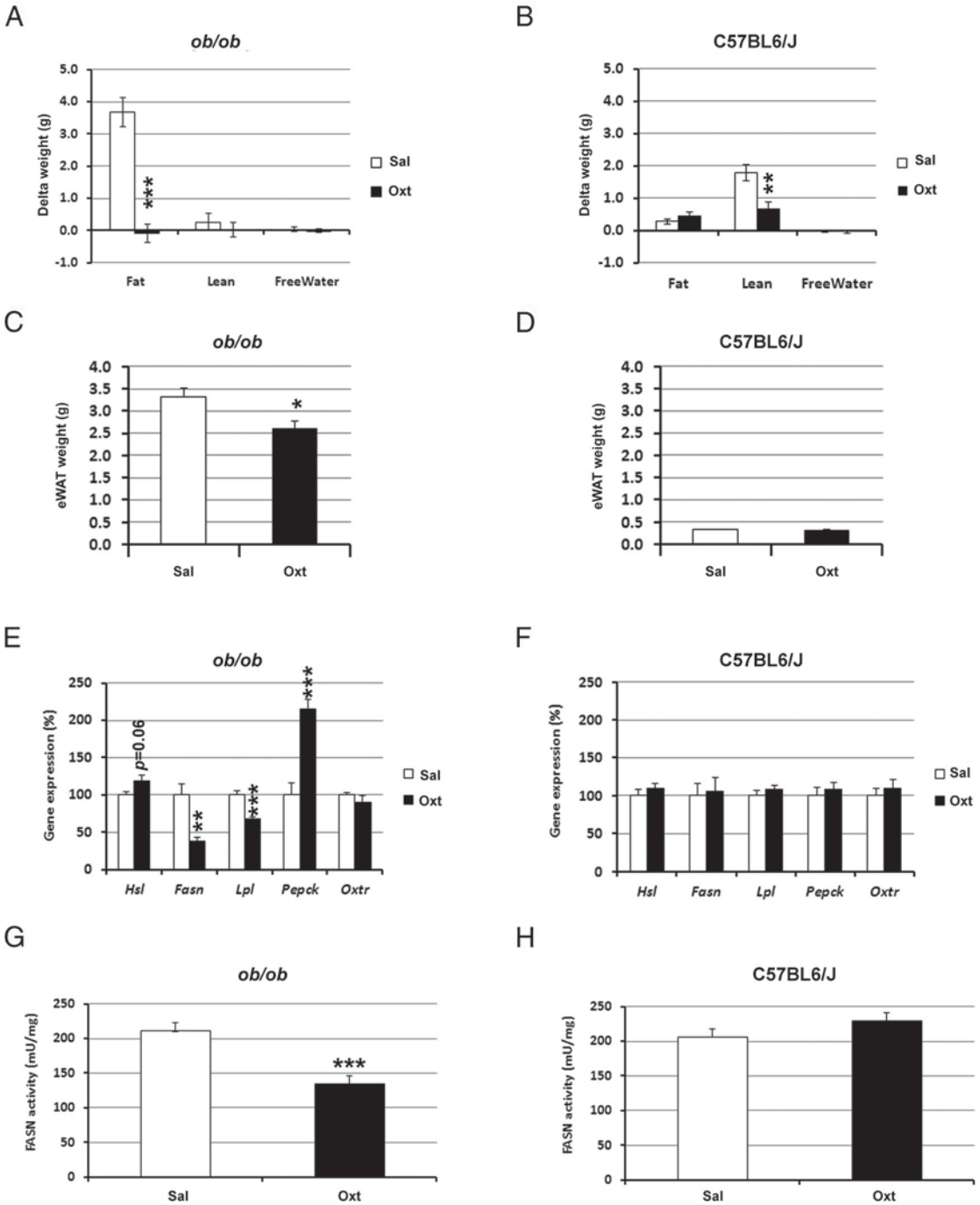

Figure 3. Oxytocin effects on body composition and eWAT. A and B, Δ-Body composition between the beginning and the end of the treatment measured by MRI. C and D, eWAT weight at the end of the treatment. E and F, eWAT gene expression of lipolytic (*Hsl*), lipogenic (*Fasn*), lipid uptake (*Lpl*), and glyceroneogenic (*Pepck*) enzymes, as well as of the OXTR (*Oxtr*), considering the levels in the saline-treated group as 100%. G and H, FASN activity in the eWAT. Mice were *ob/ob* (A, C, E, and G) or C57BL6/J (B, D, F, and H) sc treated with oxytocin (50 nmol/d) or vehicle (saline) during 14 days. *, $P < .05$; **, $P < .01$; ***, $P < .001$. *ob/ob*, n = 17–20 (A) and n = 6–7 (C, E, and G); and C57BL6/J, n = 12–13 (B) and n = 4–8 (D, F, and H).

oxytocin- than in the saline-infused group (Figure 3C). This effect was independent of food intake (Supplemental Figure 2, A and B). Surprisingly, in C57BL6/J mice, oxytocin treatment significantly reduced the lean, without affecting the fat mass (Figure 3, B and D).

The mechanisms responsible for the decrease in fat mass gain in *ob/ob* mice were investigated by measuring the gene expression of various enzymes involved in lipid metabolism in adipose tissue, as well as the activity of the most relevant one. The results suggested that oxytocin treatment decreased lipogenesis (decreased *Fasn* expression and FASN activity), lipid uptake (decreased *Lpl* expression), increased lipolysis (higher *Hsl* expression), and glyceroneogenesis (higher *Pepck-C* expression, which increases glycerol-3-phosphate required for NEFA reesterification into TG), indicating increased futile cycling, without modifying the OXTR levels (Figure 3, E and G). These effects on lipid metabolism were food intake independent (Supplemental Figure 2, C and D) and were not observed in the lean C57BL6/J animals (Figure 3, F and H).

Finally, adipose tissue inflammation was measured by quantifying the amount of macrophages in the epididymal fat depot (eWAT), using immunohistochemistry or gene expression levels. This showed that oxytocin treatment of *ob/ob* mice reduced the infiltration of adipose tissue by macrophages (Figure 4, A and B), independently from changes in food intake (Supplemental Figure 2, E and F), whereas it had no effect in lean C57BL6/J animals (Figure 4, C and D).

Oxytocin effects on the liver

Oxytocin was not able to improve the steatosis present in the liver of *ob/ob* mice, as demonstrated by similar histology (hematoxylin and eosin and Oil-Red-O staining) and TG content in oxytocin- and saline-treated mice (Figure 5, A–C). As expected, these parameters were also unchanged by food restriction (ie, pair-feeding) of *ob/ob* mice (Supplemental Figure 2, G–I). Interestingly, the gene expression levels of various enzymes related to glucose metabolism revealed that *G6pc* expression was increased by the oxytocin treatment, suggesting enhanced gluconeogenesis (Figure 5G). This effect was food intake independent (Supplemental Figure 2J). It was further substantiated by the results of a pyruvate tolerance test, indicating higher glycemia values in the oxytocin- than in the saline-treated group, in response to pyruvate administration (Figure 5I).

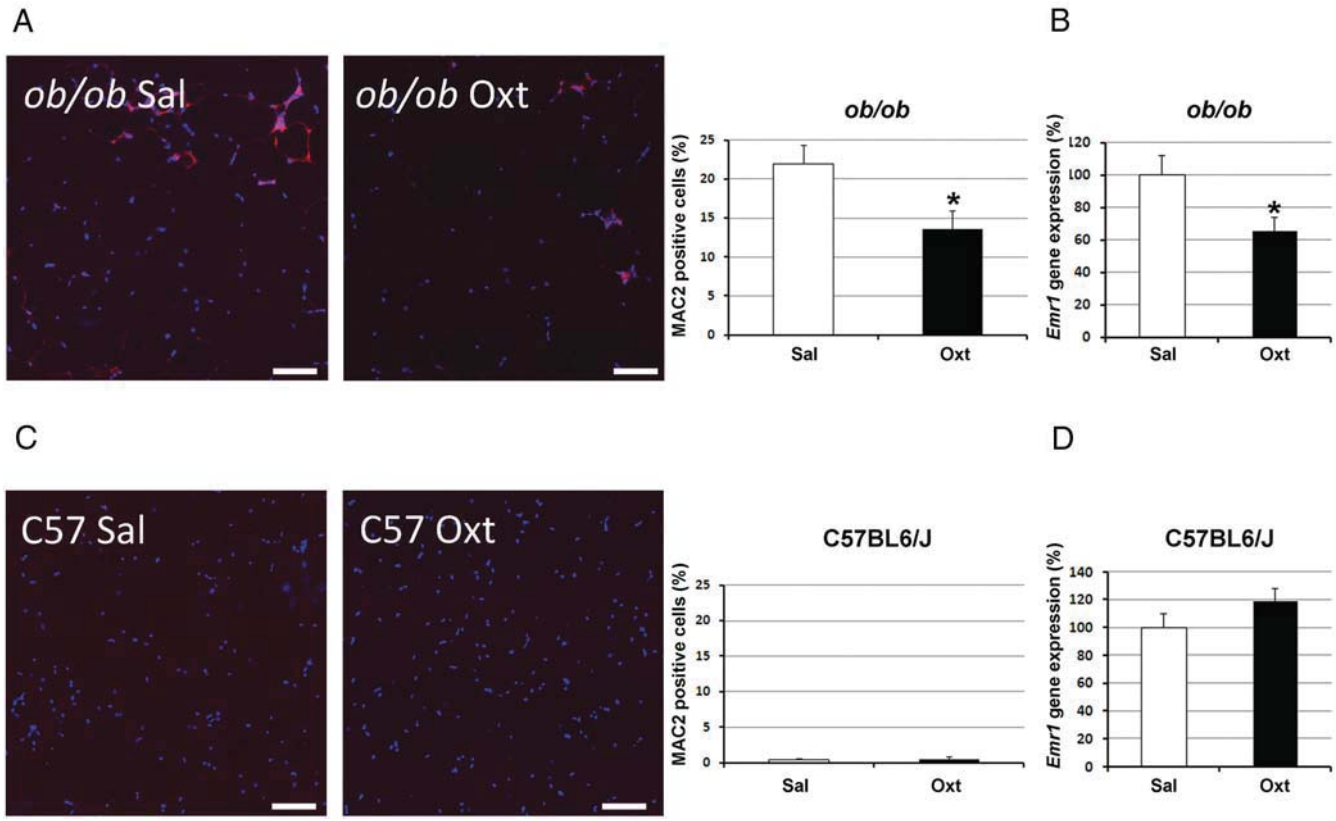

Figure 4. Oxytocin and inflammation in eWAT. A and C, Representative merged immunofluorescence images of the macrophage marker MAC-2 in the red channel and the nuclear marker Hoechst 33258 in the blue channel and quantification of the immunofluorescence in percent of MAC-2-positive cells over all cells present on the slice. Scale white bar, 100 um. B and D, eWAT gene expression of the macrophage marker *Emr1a* (*F4/80*), considering the levels in the saline-treated group as 100%. Mice were *ob/ob* (A and B) or C57BL6/J (C and D) sc treated with oxytocin (50 nmol/d) or vehicle (saline) during 14 days. *, $P < .05$; n = 6–8.

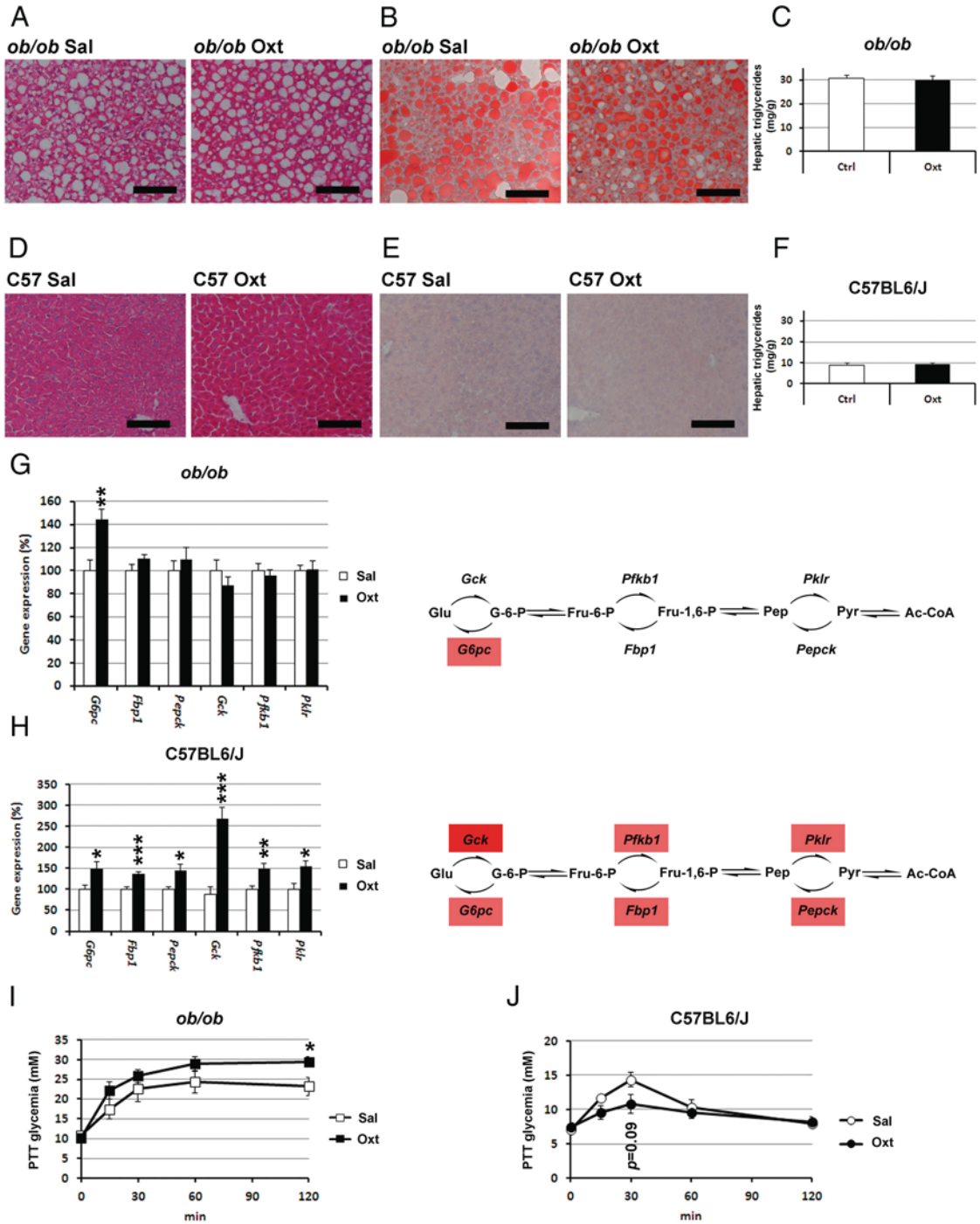

Figure 5. Oxytocin effects on the liver. A, B, D, and E, Representative images of hematoxylin and eosin (A and D) and oil red O (B and E) staining in liver sections of *ob/ob* (A and B) or C57BL6/J (D and E) mice sc treated with oxytocin (50 nmol/d) or vehicle (saline) during 14 days. Scale black bar, 100 um. C and F, Hepatic TG content of *ob/ob* (C) and C57BL6/J (F) mice; n = 7–8. G and H, Hepatic gene expression of glycolytic (*Gck*, *Pfkb1*, and *Pklr*) and gluconeogenic (*G6pc*, *Fbp1*, and *Pepck*) enzymes of *ob/ob* (G) and C57BL6/J (H) mice, considering the levels in the saline-treated group as 100%. Representation of glycolysis and gluconeogenesis, highlighting the up-regulated enzymes on a red background; n = 7–8. I and J, Glycemia during a pyruvate tolerance test in *ob/ob* (I) and C57BL6/J (J) mice; n = 4–6. *, $P < .05$; **, $P < .01$; ***, $P < .001$.

On the basis of the reported observation that oxytocin inactivates glycogen synthase in isolated hepatocytes (20), we measured the gene expression levels of glycogen synthase (*Gys2*) and phosphorylase (*Pygl*), as well as the total liver glycogen content. None of these parameters was affected by the oxytocin treatment of *ob/ob* mice (Supplemental Figure 3).

In lean nondiabetic C57BL6/J mice, neither the histology nor the TG content was affected by the oxytocin treatment (Figure 5, D–F). The expression of genes involved in

glucose metabolism suggested the occurrence of a concomitant increase in both gluconeogenesis (*G6pc*, *Fbp1*, and *Pepck*) and glycolysis (*Gck*, *Pfkb1*, and *Pklr*) (Figure 5H). In these mice, the pyruvate tolerance test was similar in both the treated and the untreated group, with a trend toward lower glycemia under oxytocin treatment (Figure 5J). Regarding the liver glycogen content, it was decreased by the treatment with an increase in the gene expression of glycogen phosphorylase (*Pygl*) (Supplemental Figure 3).

Oxytocin effects on glucose metabolism

Oxytocin treatment of *ob/ob* mice worsened glucose tolerance, without affecting insulin levels (which were unaltered by the glucose load) (Figure 6, A and C). These effects were independent of food intake (Supplemental Figure 2, K and L). In C57BL6/J mice, glucose tolerance was unaffected (Figure 6B), but oxytocin treatment increased basal insulinemia (Figure 6D). Finally, oxytocin treatment of *ob/ob* mice slightly worsened insulin tolerance (Figure 6E), without any effect on this parameter in the C57BL6/J group (Figure 6F).

Discussion

The present work addressed the effects of chronic oxytocin treatment on both body weight and glucose metabolism as well as their underlying mechanisms, in a mice model of marked obesity and diabetes.

Regarding body weight, previous work showed that acute oxytocin administration in obese Zucker or Koletsky rats lacking the leptin receptor (11, 13), or chronic

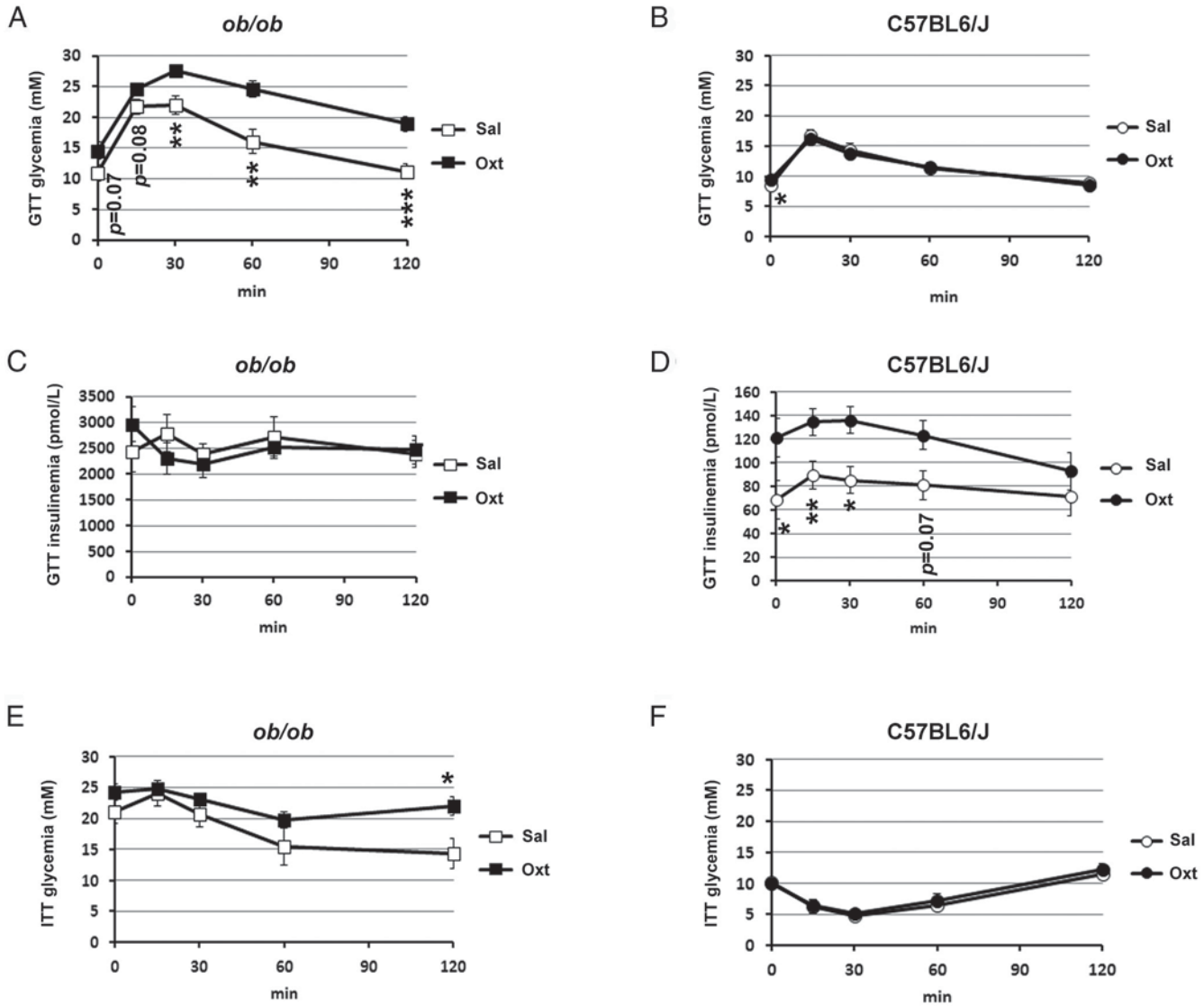

Figure 6. Oxytocin effects on glucose metabolism. Glycemia (A and B) and insulinemia (C and D) during a glucose tolerance test in *ob/ob* (A and C) or C57BL6/J (B and D) mice sc treated with oxytocin (50 nmol/d) or vehicle (saline) during 14 days. E and F, Glycemia during an insulin tolerance test in *ob/ob* (E) and C57BL6/J (F) mice; n = 15–17 (A), n = 6–8 (B–E), and n = 4 (F). *, $P < .05$; **, $P < .01$; ***, $P < .001$.

oxytocin treatment of DIO rodents, decreased food intake and body weight (9, 10, 12). Our work extended these results by demonstrating that chronic oxytocin treatment decreased body weight gain in leptin-deficient mice, a mouse model of more severe obesity than that of high-fat-fed rodents. Our results further showed that the oxytocin effects on body weight were dose-dependent, reaching a plateau at 50 nmol/d, as well as partly independent from changes in food intake. Also, a higher dose of oxytocin or an analog with a longer half-life (carbetocin) (21) did not increase the effects observed, whereas a low dose decreased body weight gain, without affecting food intake.

Oxytocin and vasopressin receptors display high homology, because oxytocin and vasopressin are 2 nonapeptides differing only in 2 amino acids (19). According to in vitro affinity and activation studies, oxytocin could potentially activate the vasopressin receptors (19), suggesting that part of the effects observed could be mediated not only by the OXTR activation but also by a cross-activation of the vasopressin receptor. For this reason, we treated another group of *ob/ob* mice with an oxytocin analog, TGOT, presenting an affinity for the OXTR, which was 5 orders of magnitude higher than for the V1a and V1b receptors (19). The results showed that, similar to the effects of the low oxytocin dose, TGOT reduced body weight without affecting food intake. It could therefore be suggested that activation of the vasopressin receptor is partly mediating the oxytocin-induced effects on food intake, in keeping with a previously reported study (22). However, these data should be taken with caution, because the TGOT metabolism and agonist activity in mouse tissues are not well characterized and because the dynamics of its transport through the blood-brain barrier is unknown.

When considering lean mice and rats, oxytocin administration was shown to decrease food intake and body weight in acute experiments (12, 23, 24). We observed that a 2-week oxytocin treatment of lean mice decreased food intake and body weight during the first 24 hours of the treatment only, returning to normal values immediately thereafter. Overall, the body weight gain of oxytocin- and saline-treated mice was essentially similar over 2 weeks. The potential reasons underlying the different responses of lean and obese mice to the same treatment will be discussed below.

One of the most interesting findings of the present study is that adipose tissue appears to be the main target of the oxytocin effects in obese mice. Indeed, the decreased body weight gain of *ob/ob* animals was due to a specific decrease in the fat mass gain, without any change in lean mass. That adipose tissue is a target of oxytocin action was not totally unexpected, given that the OXTR is expressed in this tissue at a similar level as in most of the classical oxytocin target tissues (Supplemental Figure 4) (25, 26). However, regarding oxytocin effects on this tissue, conflicting data were previously reported, because both increased lipogenesis (27–29) and lipolysis (9, 29) were observed, depending on the dose tested (29). When investigating the possible molecular mechanisms underlying the oxytocin effects in adipose tissue in our model, we found that it involved decreased lipid uptake, lipogenesis, and increased futile cycling (increase in both lipolysis and glyceroneogenesis). Reduced lipogenesis is likely to represent the main mechanism, as suggested by the amplitude of the decreased mRNA and protein activity of FASN.

It is noteworthy that oxytocin is located downstream of leptin action, possibly mediating some of the leptin effects (30, 31). Leptin is known to deplete the TG content of adipose tissue without causing a concomitant rise in circulating free fatty acids by suppressing de novo lipogenesis and activating both futile cycling (lipolysis and glyceroneogenesis) (32). Most of these effects are observed in oxytocin-treated *ob/ob* mice, in keeping with the concept that oxytocin may mediate leptin effects on lipid metabolism in adipose tissue.

Another interesting observation was that oxytocin treatment of obese mice reduced the macrophage infiltration of adipose tissue. This will be studied in more details in further experiments. Given the first conclusion of our study that chronic oxytocin treatment reduces body weight gain, mainly by acting on adipose tissue, the question of the different responsiveness of lean and obese mice can be raised. Several reasons could be envisaged, including the next ones: 1) OXTR signaling; *ob/ob* mice exhibited markedly higher *Oxtr* expression than C57BL6/J mice at the level of adipose tissue (Supplemental Figure 4B); and 2) adipose tissue mass; adipose tissue of obese mice not only overexpressed the *Oxtr*, but its overall mass was much higher than in lean mice (fat mass, 1.91 $\pm$ 0.06 g in lean and 21.85 $\pm$ 0.50 g in obese mice; $P < .001$, n = 25–63).

It should be added that plasma oxytocin levels were similar in lean and obese mice (Supplemental Figure 4C). This is in contrast to what was reported in Zucker rats, in which circulating oxytocin levels of obese were lower than those of lean animals (26). In such a situation, oxytocin administration was expected to be more efficient in obese than in lean rats, because it partly reestablished normal oxytocin signaling. This was not the case in *ob/ob* mice.

Unlike its effects in adipose tissue of *ob/ob* mice described in the present study, as well as in contrast with previously reported results in DIO mice (12), oxytocin treatment had no effect on hepatic steatosis. One of the reasons for the discrepancy between the 2 models is the

magnitude of the defect. Indeed, hepatic steatosis is much more severe in *ob/ob* than in DIO mice (liver weight of *ob/ob* around 3 times higher than liver weight of DIO mice). Another potential explanation, which needs to be addressed in future studies, is that oxytocin-induced improvement of hepatic steatosis is a leptin-dependent process.

Regarding the impact of the oxytocin treatment on glucose metabolism, previous studies performed in DIO rodents treated with oxytocin showed improvements in insulin sensitivity and glucose tolerance (9, 12, 33). Furthermore, improved glucose tolerance was also observed in response to oxytocin in mice made diabetic for 4 days by streptozotocin administration (8). However, in that particular model, it appeared that oxytocin was preventing β-cell death from streptozotocin toxicity, thereby improving glucose tolerance. These results are in keeping with the antioxidant properties of oxytocin reported previously (34, 35), given that streptozotocin mediates β-cell death by the production of reactive oxygen species. In a clinical trial with obese nondiabetic subjects, no effect of chronic oxytocin treatment on glucose and insulin levels was reported (8). Results pertaining to these measurements in diabetic obese or nonobese patients are expected and will be of utmost importance. However, they have not been published yet. Therefore, the available literature on the effects of oxytocin on glucose metabolism in rodents was mainly gathered in the prediabetic state, with a moderate hyperinsulinemia and a mild hyperglycemia, and there are no data in diabetic patients as yet. The present study evaluated the oxytocin effects on glucose metabolism one step further, using diabetic mice, which are markedly hyperinsulinemic, glucose intolerant and insulin resistant. Given the available above-mentioned literature, our own data on the oxytocin-mediated improvement of glucose intolerance and insulin sensitivity in DIO rats (9), in particular, and our present observation of decreased oxytocin-induced adiposity in *ob/ob* mice, we expected the treatment to bring about an improvement of glucose metabolism. Surprisingly, the results showed that oxytocin administration worsened the basal glycemia, glucose tolerance and insulin sensitivity. The reasons underlying the discrepancy between these results and those we published in DIO rats (9) could be related to a species difference. However, this seems unlikely due to other data reported in the literature, showing oxytocin-mediated improvement of glucose intolerance in DIO mice (12, 33).

Some of the hypotheses possibly explaining the deleterious effects of oxytocin treatment in *ob/ob* mice can be summarized as follows:

1) Incapacity of β-cells to increase insulin secretion. In lean mice, we observed that oxytocin treatment resulted in increased circulating insulin and glucagon levels, in keeping with previous data obtained in vivo (36). However, *ob/ob* mice are highly hyperinsulinemic and hyperglucagonemic and the treatment did not alter these levels, possibly due to the fact that pancreatic α- and β-cells have reached their maximal secretory capacity.

2) Decreased lipogenesis. Oxytocin treatment of *ob/ob* mice decreased lipogenesis, because *Fasn* expression and FASN activity were decreased. In these animals, de novo lipogenesis from glucose is likely to represent an important pathway, using a substantial amount of substrate. A decrease in the activity of this pathway could therefore be responsible for increased basal plasma glucose levels.

3) Hypothalamic–pituitary–adrenal (HPA) axis and hepatic glucose production. *ob/ob* mice exhibited increased basal corticosterone levels (around 6 times higher than levels of C57BL6/J control mice). These levels were further increased by the oxytocin treatment, especially after a mild stress, such as 5 hours of diurnal food deprivation, time at which the glucose tolerance test was performed. The oxytocin treatment had no effect on plasma corticosterone levels in lean mice (with a trend toward a decrease). In *ob/ob* mice, the oxytocin-induced increase in corticosterone levels is likely responsible for the occurrence of enhanced gluconeogenesis, in keeping with our observation of an increased hepatic *G6pc* expression in these animals. Similarly, in humans, excess cortisol in Cushing syndrome is well known to increase gluconeogenesis, consequently leading to diabetes (37).

Although the liver is not supposed to express the OXTR (38), it cannot be excluded that oxytocin could have direct effects on hepatocytes, increasing gluconeogenesis, as was once reported ex vivo, although more than 25 years ago (39). It is also worth mentioning that corticosterone-induced stimulation of gluconeogenesis does not take place exclusively in the liver but also in the kidney and intestine (40, 41). These parameters were not determined in the present study. Finally, glycogen metabolism was unaffected in *ob/ob* mice, whereas oxytocin treatment brought about an increase in glycogenolysis and consequent decrease in liver glycogen content in lean mice that may be linked to their increased plasma glucagon levels (42).

Considering the present data, as well as the available literature, it appears that the oxytocin effects on glucose metabolism may depend on the status of the leptin signaling process. Thus, in the absence of leptin (*ob/ob* mice, present study), or of leptin signaling (ZDF *fa/fa* rats, our unpublished data), oxytocin impairs glucose metabolism, whereas it improves it in the presence of leptin, even in the face of leptin resistance, as occurs in DIO rats and mice (9, 12, 33). Also, a single administration of oxytocin was reported to decrease postprandial glycemia, as well as cor-

tisol levels in lean human subjects with normal leptin signaling (14). It can therefore be proposed that defective glucose metabolism after oxytocin treatment of *ob/ob* mice could, at least partly, be due to leptin deficiency. This may be linked to the recent demonstration that leptin is able to restore hyperglycemia in models of type 1 diabetes by decreasing the activity of the hypothalamic–pituitary–adrenal (HPA) axis, leading to decreased gluconeogenesis (43). Clearly, much additional work is needed to clarify this complex issue, which involves many different players, including hypothalamic neuropeptides.

4) Involvement of the autonomic nervous system. Although not evaluated in the present study, a role of the autonomic nervous system in the oxytocin-mediated deleterious effects on glucose metabolism cannot be excluded. Actually, it could be linked to the modulation of the different players just mentioned.

In conclusion, oxytocin treatment decreases food intake and adiposity in severely obese mice lacking leptin, although it worsens glucose metabolism, probably due to an increase in corticosterone levels and resulting enhanced hepatic glucose production. Together with previously reported data, these results suggest that the oxytocin-mediated effects in decreasing fat mass are independent of leptin, whereas the beneficial impact on glucose metabolism requires the presence of leptin. Considering the regulation of lipid metabolism in adipose tissue, our data are compatible with a role of oxytocin as a mediator of leptin action in this tissue. In addition to bringing new knowledge about the neuroendocrine regulation of body weight homeostasis, these data prompt the careful selection of the conditions in which oxytocin treatment should be beneficial for human obesity and its comorbidities.

Acknowledgments

We thank Professor C. Wollheim for helpful discussion and M.O. Boldi for help with the statistics.

Address all correspondence and requests for reprints to: Jordi Altirriba, PhD, Laboratory of Metabolism, Department of Internal Medicine Specialties, Faculty of Medicine, University of Geneva, 1 Rue Michel-Servet, CH-1211 Geneva, Switzerland. E-mail: jorge.altirriba@unige.ch.

This work was supported by the Swiss National Science Foundation Grant 31003A_134919/1 as well as the European Foundation for the Study of Diabetes and the Fondation Romande pour la Recherche sur le Diabète.

Disclosure Summary: F.R.-J. has a patent application (PCT/IB2011/052156) covering novel therapeutic uses of oxytocin. J.A., A.-L.P., A.C., D.A., C.V.-D., J.L., and A.D. have nothing to disclose.

References

1. **World Health Organization.** *Global Health Risks: Mortality and Burden of Disease Attributable to Selected Major Risks*. Geneva, Switzerland; 2009. http://www.who.int/healthinfo/global_burden_disease/GlobalHealthRisks_report_full.pdf.
2. **American Diabetes Association.** Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2014;37(suppl 1):S81–S90.
3. **Meyer-Lindenberg A, Domes G, Kirsch P, Heinrichs M.** Oxytocin and vasopressin in the human brain: social neuropeptides for translational medicine. *Nat Rev Neurosci*. 2011;12(9):524–538.
4. **Gimpl G, Fahrenholz F.** The oxytocin receptor system: structure, function, and regulation. *Physiol Rev*. 2001;81(2):629–683.
5. **Kenyon S, Tokumasu H, Dowswell T, Pledge D, Mori R.** High-dose versus low-dose oxytocin for augmentation of delayed labour. *Cochrane Database Syst Rev*. 2013;7:CD007201.
6. **Gizzo S, Patrelli TS, Gangi SD, et al.** Which uterotonic is better to prevent the postpartum hemorrhage? Latest news in terms of clinical efficacy, side effects, and contraindications: a systematic review. *Reprod Sci*. 2013;20(9):1011–1019.
7. **Bakermans-Kranenburg MJ.** van IJMH. Sniffing around oxytocin: review and meta-analyses of trials in healthy and clinical groups with implications for pharmacotherapy. *Transl Psychiatry*. 2013;3:e258.
8. **Zhang H, Wu C, Chen Q, et al.** Treatment of obesity and diabetes using oxytocin or analogs in patients and mouse models. *PLoS One*. 2013;8(5):e61477.
9. **Deblon N, Veyrat-Durebex C, Bourgoin L, et al.** Mechanisms of the anti-obesity effects of oxytocin in diet-induced obese rats. *PLoS One*. 2011;6(9):e25565.
10. **Zhang G, Bai H, Zhang H, et al.** Neuropeptide exocytosis involving synaptotagmin-4 and oxytocin in hypothalamic programming of body weight and energy balance. *Neuron*. 2011;69(3):523–535.
11. **Morton GJ, Thatcher BS, Reidelberger RD, et al.** Peripheral oxytocin suppresses food intake and causes weight loss in diet-induced obese rats. *Am J Physiol Endocrinol Metab*. 2012;302(1):E134–E144.
12. **Maejima Y, Iwasaki Y, Yamahara Y, Kodaira M, Sedbazar U, Yada T.** Peripheral oxytocin treatment ameliorates obesity by reducing food intake and visceral fat mass. *Aging (Albany NY)*. 2011;3(12):1169–1177.
13. **Maejima Y, Sedbazar U, Suyama S, et al.** Nesfatin-1-regulated oxytocinergic signaling in the paraventricular nucleus causes anorexia through a leptin-independent melanocortin pathway. *Cell Metab*. 2009;10(5):355–365.
14. **Ott V, Finlayson G, Lehnert H, et al.** Oxytocin reduces reward-driven food intake in humans. *Diabetes*. 2013;62(10):3418–3425.
15. **Penicaud L, Ferre P, Assimacopoulos-Jeannet F, et al.** Increased gene expression of lipogenic enzymes and glucose transporter in white adipose tissue of suckling and weaned obese Zucker rats. *Biochem J*. 1991;279(pt 1):303–308.
16. **Schneider CA, Rasband WS, Eliceiri KW.** NIH Image to ImageJ: 25 years of image analysis. *Nat Methods*. 2012;9(7):671–675.
17. **R Core Team** R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2013. http://www.R-project.org.
18. **Cort N, Einarsson S, Schams D, Vilhardt H.** Blood concentrations of oxytocin equivalents after single injections of deamino-1-monocarba-[2-O-methyltyrosine]-oxytocin in lactating sows. *Am J Vet Res*. 1981;42(10):1804–1806.
19. **Busnelli M, Bulgheroni E, Manning M, Kleinau G, Chini B.** Selective and potent agonists and antagonists for investigating the role of mouse oxytocin receptors. *J Pharmacol Exp Ther*. 2013;346(2):318–327.
20. **Ariño J, Bosch F, Gómez-Foix AM, Guinovart JJ.** Oxytocin inactivates and phosphorylates rat hepatocyte glycogen synthase. *Biochem J*. 1989;261(3):827–830.
21. **Hunter DJ, Schulz P, Wassenaar W.** Effect of carbetocin, a long-

acting oxytocin analog on the postpartum uterus. *Clin Pharmacol Ther*. 1992;52(1):60–67.
22. **Langhans W, Delprete E, Scharrer E.** Mechanisms of vasopressin's anorectic effect. *Physiol Behav*. 1991;49(1):169–176.
23. **Arletti R, Benelli A, Bertolini A.** Influence of oxytocin on feeding behavior in the rat. *Peptides*. 1989;10(1):89–93.
24. **Arletti R, Benelli A, Bertolini A.** Oxytocin inhibits food and fluid intake in rats. *Physiol Behav*. 1990;48(6):825–830.
25. **Boland D, Goren HJ.** Binding and structural properties of oxytocin receptors in isolated rat epididymal adipocytes. *Regul Pept*. 1987; 18(1):7–18.
26. **Wu C, Orozco C, Boyer J, et al.** BioGPS: an extensible and customizable portal for querying and organizing gene annotation resources. *Genome Biol*. 2009;10(11):R130.
27. **Goren HJ, Hanif K, Dudley R, Hollenberg MD, Lederis K.** Adenosine modulation of fat cell responsiveness to insulin and oxytocin. *Regul Pept*. 1986;16(2):125–134.
28. **Eriksson H, Björgell P, Akerlund M, Hauksson A, Melin P.** Effects of a tocolytic oxytocin analogue on lipid and carbohydrate metabolism. *Gynecol Obstet Invest*. 1990;29(2):97–100.
29. **Muchmore DB, Little SA, de Haën C.** A dual mechanism of action of ocytocin in rat epididymal fat cells. *J Biol Chem*. 1981;256(1): 365–372.
30. **Perello M, Raingo J.** Leptin activates oxytocin neurons of the hypothalamic paraventricular nucleus in both control and diet-induced obese rodents. *PLoS One*. 2013;8(3):e59625.
31. **Blevins JE, Schwartz MW, Baskin DG.** Evidence that paraventricular nucleus oxytocin neurons link hypothalamic leptin action to caudal brain stem nuclei controlling meal size. *Am J Physiol Regul Integr Comp Physiol*. 2004;287(1):R87–R96.
32. **Buettner C, Muse ED, Cheng A, et al.** Leptin controls adipose tissue lipogenesis via central, STAT3-independent mechanisms. *Nat Med*. 2008;14(6):667–675.
33. **Zhang G, Cai D.** Circadian intervention of obesity development via resting-stage feeding manipulation or oxytocin treatment. *Am J Physiol Endocrinol Metab*. 2011;301(5):E1004–E1012.
34. **Rashed LA, Hashem RM, Soliman HM.** Oxytocin inhibits NADPH oxidase and P38 MAPK in cisplatin-induced nephrotoxicity. *Biomed Pharmacother*. 2011;65(7):474–480.
35. **Szkudelski T.** The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiol Res*. 2001;50(6):537–546.
36. **Gao ZY, Drews G, Henquin JC.** Mechanisms of the stimulation of insulin release by oxytocin in normal mouse islets. *Biochem J*. 1991; 276(pt 1):169–174.
37. **Pivonello R, De Leo M, Vitale P, et al.** Pathophysiology of diabetes mellitus in Cushing's syndrome. *Neuroendocrinology*. 2010; 92(suppl 1):77–81.
38. **Kusui C, Kimura T, Ogita K, et al.** DNA methylation of the human oxytocin receptor gene promoter regulates tissue-specific gene suppression. *Biochem Biophys Res Commun*. 2001;289(3):681–686.
39. **Whitton PD, Rodrigues LM, Hems DA.** Stimulation by vasopressin, angiotensin and oxytocin of gluconeogenesis in hepatocyte suspensions. *Biochem J*. 1978;176(3):893–898.
40. **Katz N, Brinkmann A, Jungermann K.** Compensatory increase of the gluconeogenic capacity of rat kidney after partial hepatectomy. *Hoppe Seylers Z Physiol Chem*. 1979;360(1):51–57.
41. **Habold C, Foltzer-Jourdainne C, Le Maho Y, Lignot JH, Oudart H.** Intestinal gluconeogenesis and glucose transport according to body fuel availability in rats. *J Physiol*. 2005;566(pt 2):575–586.
42. **Cherrington AD, Chiasson JL, Liljenquist JE, Lacy WW, Park CR.** Control of hepatic glucose output by glucagon and insulin in the intact dog. *Biochem Soc Symp*. 1978(43):31–45.
43. **Perry RJ, Zhang XM, Zhang D, et al.** Leptin reverses diabetes by suppression of the hypothalamic-pituitary-adrenal axis. *Nat Med*. 2014;20(7):759–763.