Gut hormones - Novel tools in the treatment of insulin resistance

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Chapter 6

Obinepitide, a novel Y2/Y4 agonist, and TM30339, a novel Y4 agonist, reduce insulin resistance in high-fat-fed C57Bl/6J mice

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In preparation

Abstract

Obinepitide, a dual-analogue of peptide YY_{3-36} and pancreatic polypeptide (PP), and TM30339, a PP analogue, were developed as anti-obesity drugs. Pre-clinical studies have shown long-term effects on decreased body weight in human and animals. Here, we further evaluate the potential of these drugs in terms of insulin sensitivity of glucose and lipid metabolism. Diet-induced insulin resistant C57Bl/6J mice were daily subcutaneously injected with Obinepitide (0.3 and 3.0 mg/kg), TM30339 (0.3 and 3.0 mg/kg), or vehicle for 4 weeks. Glucose and glycerol turnover were determined during a hyperinsulinemic euglycemic clamp. Very low density lipoprotein-triglyceride (VLDL-TG) production was determined in basal and hyperinsulinemic conditions after intravenous injection of tyloxapol. 3.0 mg/kg Obinepitide and both doses of TM30339 significantly enhanced insulin mediated glucose disposal (Obinepitide: 100 ± 26, $p<0.01$; 0.3 mg/kg TM30339: 90 ± 25, $p<0.05$; 3.0 mg/kg TM30339: 116 ± 19, $p<0.01$; control: 62 ± 25 % stimulation). In addition, both drugs affected insulin's capacity to inhibit endogenous glucose production (Obinepitide: 51 ± 24, $p<0.05$; 0.3 mg/kg TM30339: 60 ± 23, $p<0.01$; 3.0 mg/kg TM30339: 68 ± 24, $P<0.01$; control: 30 ± 26 % inhibition). Insulin's capacity to inhibit glycerol turnover was not affected by any of the treatments. In basal conditions, none of the drugs affected VLDL-TG production. However, during insulin infusion VLDL-TG production was decreased in 3.0 mg/kg treated mice only (3.0 mg/kg TM30339: 134 ± 17; control: 167 ± 29 µmol/h/kg; $p<0.01$). The data suggest that Obinepitide and TM30339 appear to be useful tools to improve glucose metabolism in diet-induced insulin resistant C57Bl/6J mice.

Introduction

Peptide YY (PYY) and pancreatic polypeptide (PP) are closely related 36-amino acid peptides. PYY is synthesized in the intestinal L-cells, which are predominantly located in the distal part of the gastrointestinal tract. PP is synthesized in the so-called F-cells, located in the pancreatic islet of Langerhans. Both PYY and PP are released into the circulation in response to nutrient intake in proportion to caloric intake[1, 2]. PYY circulates primarily as the N-terminally truncated form of the full length peptide, PYY_{3-36}[3]. After being released PYY_{3-36} and PP bind and activate a class of G-protein coupled receptors belonging to the neuropeptide Y receptor family. To date 5 subtypes have been identified: Y1R, Y2R, Y4R, Y5R, and Y6R[4]. PYY_{3-36} is known to exert its effects predominantly via the Y2R, which are highly expressed in the arcuate nucleus[5], whereas PP effects are thought to be mediated via the Y4R subtype, expressed at many sides in the brain[6].

The central Y receptors are involved in the control of a diverse set of regulatory processes, including appetite and body weight. Food intake is significantly reduced after peripheral administration of PYY_{3-36} and PP in both rodents and humans (7-10). Chronic PYY_{3-36} administration reduces body weight[11, 12]. Moreover, PYY overexpression protects against diet-induced obesity in mice, whereas ablation of PYY leads to the development of obesity[13, 14]. In addition to their roles in feeding and body weight regulation, PYY_{3-36} and PP affect insulin sensitivity in animal models of obesity and type 2 diabetes[12, 15-17]. Specifically, for PYY_{3-36} this is the result of an enhanced insulin mediated glucose disposal [16, 17].

The aforementioned studies suggest that the Y receptors are interesting targets for the treatment of obesity and type 2 diabetes mellitus (T2DM). Selective Y2/Y4 agonists may have metabolic effects similar to those of PYY_{3-36} and PP. In this respect, Obinepitide (dual Y2/Y4 receptor agonist) and TM30339 (Y4 receptor agonist), were developed by 7TM Pharma (Copenhagen, Denmark) as anti-obesity drugs[18, 19]. Pre-clinical studies have shown that Obinepitide reduces the body weight of DIO mice more than native PYY_{3-36}. Furthermore, daily sc injections of Obinepitide in obese humans inhibited food intake at a statistically significant level up to 9 h after dosing[20]. TM30339 also reduces the body weight of DIO mice[21].

Here, we further explore the pharmacological characteristics of Obinepitide and TM30339 in terms of their metabolic effects. In particular, we evaluate the chronic impact of these compounds on insulin sensitivity of glucose and very low density lipoprotein-triglyceride (VLDL-TG) metabolism in diet-induced insulin resistant C57Bl/6 mice. Overproduction of VLDL-TG, which is a prominent feature of diabetic dyslipidemia (as the proximate cause of hypertriglyceridemia), contributes significantly to cardiovascular risk in T2DM patients[22]. Neuropeptide Y (NPY), also acting via the Y receptors, has been shown to stimulate VLDL production[23, 24]. Since PYY_{3-36} inhibits NPY expression via presynaptic Y2

receptors[7, 25] (and possibly PYY exerts the same effects on NPY via the Y4R), we therefore hypothesized that Obinepitide and TM30339 would inhibit VLDL-TG production.

Methods

Animals. 12 week old male C57BL/6J mice (Charles River, Maastricht, The Netherlands) were housed in a temperature-controlled room on a 12-hour light-dark cycle (lights on from 7:00 – 19:00) and fed a high fat diet (44 energy% fat derived from bovine fat, Hope Farms, Woerden, The Netherlands) for 16 weeks with free access to water to induce insulin resistance[26]. All animal experiments were approved by the Animal Ethics Committee from the Leiden University Medical Center, Leiden, The Netherlands.

Drugs. Obinepitide and TM30339 were supplied by 7TM Pharma. These peptide analogues of pancreatic polypeptide were synthesized by solid phase peptide synthesis (SPPS) and purified by reversed phase high performance liquid chromatography. The peptides were dissolved in sterile 0.9% saline for injection and the solution pH adjusted to approximately pH 7.4 before being filtered through a 0.22 µm filter.

Treatment. Mice were injected subcutaneously (sc) with phosphate buffered saline (PBS) twice daily (BID) for 11 days for habituation to daily injections. Thereafter, at 12 weeks of high-fat-feeding, the mice were divided into 2 x 5 groups. The groups were matched in fasting condition for body weight and plasma glucose levels for clamp experiments and body weight and plasma TG levels for VLDL production experiments (period of fasting was 16 hours for clamp study and 4 hours for VLDL study). Each group received sc injections of Obinepitide BID (0.3 or 3.0 mg/kg), TM30339 once daily (OD) (0.3 mg/kg or 3.0 mg/kg), or saline BID for 4 weeks. The first dose was given at the beginning of the light period and the second dose directly before the lights went out. TM30339 was administered at the end of the light period. Mice receiving TM30339 also received vehicle on the first dosing occasion to mirror vehicle control. Before, after 2 weeks, and by the end of treatment, body weight, plasma glucose, insulin, free fatty acids (FFA), and TG concentrations were determined in fasting condition. On the day of study, mice received a last sc dosing of Obinepitide (0.3 or 3.0 mg/kg), TM30339 (0.3 or 3.0 mg/kg), or saline 20 min prior to the experiments.

Hyperinsulinemic euglycemic clamp. Mice had free access to high fat food and water until 16 hours prior to the clamp (i.e. 17.00h the day before the clamp). Hyperinsulinemic euglycemic clamps were performed under 6.25 mg/kg acepromazine (Alfasan, Woerden, The Netherlands), 6.25 mg/kg midazolam (Roche, Mijdrecht, The Netherlands), and 0.3125 mg/kg fentanyl (Janssen-Cilag, Tilburg, The Netherlands) anesthesia to quantify glucose

and glycerol turnover. First, basal rates of glucose and glycerol turnover were determined by giving a primed (p) continuous (c) intravenous (iv) infusion of [1-^{14}C]-glucose (p: 0.2 µCi; c: 0.3 µCi/h, Amersham, Little Chalfont, U.K.) and [1-(3)-^{3}H]-glycerol (p: 0.6 µCi; c: 0.9 µCi/h, Amersham) for 60 minutes. Subsequently, insulin (Actrapid, Novo Nordisk, Denmark) was administered in a primed (4.5 mU) continuous (6.8 mU/h) iv infusion for 90 minutes to attain steady state circulating insulin levels of ~4 ng/ml. A variable iv infusion of a 12.5% D-glucose solution was used to maintain euglycemia, as determined at 10 min intervals via tail bleeding (< 3 µl, Accu-chek, Sensor Comfort, Roche Diagnostics GmbH, Mannheim, Germany). Blood samples (60 µl) were taken via tail bleeding during the basal period (after 50 and 60 min) and during the clamp period (20 and 10 minutes prior to- and at the end of the clamp) to determine the plasma concentration of glucose, glycerol, FFA, and insulin and plasma [1-^{14}C]-glucose and [1-(3)-^{3}H]-glycerol specific activities. At the end of the clamp, VLDL-TG production was quantified.

VLDL-production. Basal VLDL production experiments were performed after a 4 hour fast (09.00-13.00h) and under 6.25 mg/kg acepromazine (Alfasan), 6.25 mg/kg midazolam (Roche), and 0.3125 mg/kg fentanyl (Janssen-Cilag) anesthesia. Mice received an iv injection of 100 µl PBS containing 150 µCi Tran^{35}S label (GE Healthcare, Little Chalfont, U.K.) to label newly produced apoB. After 10 minutes, the animals received a sc dosing of Obinepitide (0.3 or 3.0 mg/kg), TM30339 (0.3 or 3.0 mg/kg), or saline. Twenty minutes thereafter, mice received a 15% (by volume) iv injection of tyloxapol (500 mg/kg body weight; Triton WR-1339, Sigma-Aldrich Corp., St. Louis, MO, US) to prevent systemic lipolysis of newly secreted hepatic VLDL-TG. Blood samples were taken before (time point 0) and 15, 30, 60, and 90 min after tyloxapol injection. Thirty minutes after the last sampling, mice were sacrificed by cervical dislocation and exsanguinated via the retro-orbital plexus.

Hyperinsulinemic VLDL-TG production was determined during continuous insulin infusion (6.8 mU/h) directly after the clamp study. Mice received a 15% (by volume) iv injection of tyloxapol (500 mg/kg body weight; Triton WR-1339, Sigma). Blood samples were taken before (time point 0) and 10, 20, 40, and 60 min after tyloxapol injection. After the last sampling, mice were sacrificed

DEXA scan. Body composition was measured by dual-energy X-ray absorptiometry (DEXA) using the Norland pDEXA Sabre X-Ray Bone Densitometer (Norland, Hampshire, UK). During measuring, mice were anesthetized with a mixture of 6.25 mg/kg acepromazine (Alfasan), 6.25 mg/kg midazolam (Roche), and 0.3125 mg/kg fentanyl (Janssen-Cilag).

Analytical procedures. Commercially available kits were used to determine plasma levels of glucose, FFA, TG (Instruchemie, Delfzijl, The Netherlands), and free glycerol (Sigma). Plasma insulin concentration was measured by ELISA (Crystal Chem. Inc., Downers Grove, IL, USA). Total plasma [1-^{14}C]-glucose and [1-(3)-^{3}H]-glycerol were determined in 8 µl plasma and in supernatants after trichloroacetic acid (20%) precipitation and water evaporation to eliminate tritiated water. VLDL was quantitatively isolated from plasma after density gradient ultracentrifugation at d<1.006 g/ml by aspiration. VLDL-apoB was selectively precipitated with 2-propanol and counted for incorporated ^{35}S.

Calculations. Turnover rates of glucose (µmol/min/kg) were calculated in basal and hyperinsulinemic conditions as the rate of tracer infusion (dpm/min) divided by the plasma specific activity of [1-^{14}C]-glucose and [1-(3)-^{3}H]-glycerol (dpm/µmol). The ratio was corrected for body weight. Endogenous glucose production (EGP) was calculated as the difference between glucose disappearance and the infusion rate of glucose. Hepatic VLDL-TG production rates (µmol/h/kg) were calculated from the linear increase in plasma TG concentrations in time.

Statistical analysis. Statistical analysis was performed using SPSS (version 16.0; SPSS Inc., Chicago, IL, USA). Differences between groups were determined with the Kruskal–Wallis non-parametric test for *k* independent samples. When significant differences were found, the Mann–Whitney non-parametric test was used as a follow-up test to determine differences between two independent groups. A p-value of less than 0.05 was considered statistically significant. Data are presented as mean ± SD.

Results

Body weight and DEXA scan. 3.0 mg/kg Obinepitide and both doses of TM30339 significantly reduced body weight (figure 1). The decrease was most prominent during the first week of treatment. However, the difference with control animals remained till the end of the treatment period. Body weight reduction was fully accounted for by a reduction in fat mass, as shown by DEXA scan analysis (figure 2).

Glucose metabolism

Body weight and plasma parameters. Fasting body weights, plasma glucose, and insulin concentrations before and during treatment and in hyperinsulinemic conditions are shown in table 1. Chronic administration of 3.0 mg/kg Obinepitide and both doses of TM30339

reduced fasting plasma glucose levels. Basal plasma insulin levels were not affected by any of the treatments.

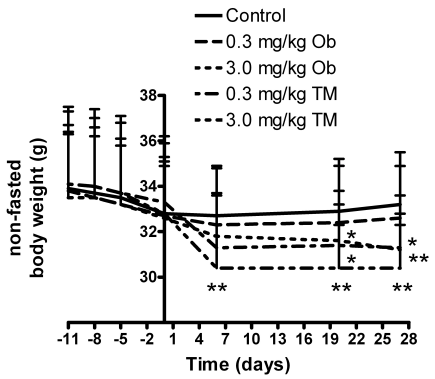

Figure 1. Non-fasted bodyweight of mice after treatment with Obinepitide (Ob) (0.3 or 3.0 mg/kg), TM30339 (TM) (0.3 or 3.0 mg/kg), or vehicle for 4 weeks. Values represent mean ± SD for at least 21 mice per group. *p <0.05 vs. control. **p <0.01 vs. control.

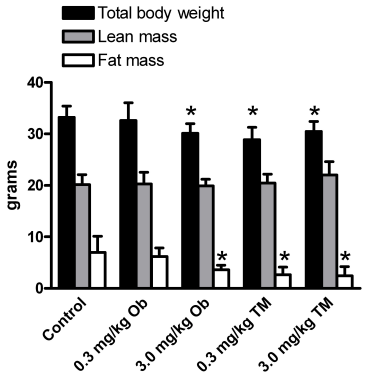

Figure 2. Body composition of mice after treatment with Obinepitide (Ob) (0.3 or 3.0 mg/kg), TM30339 (TM) (0.3 or 3.0 mg/kg), or vehicle for 4 weeks. Values represent mean ± SD for at least 6 mice per group. *p <0.05 vs. control.

Hyperinsulinemic euglycemic clamp. Basal glucose turnover, glucose disposal and EGP in hyperinsulinemic conditions after 4 weeks of treatment are shown in table 2. In basal conditions, there was no difference in glucose turnover between groups. Chronic administration of 3.0 mg/kg Obinepitide and both doses of TM30339 increased the rate of glucose infusion necessary to maintain euglycemia during insulin administration, indicating that both drugs enhanced whole body insulin sensitivity (figure 3).

Glucose disposal during insulin infusion was significantly increased in 3.0 mg/kg Obinepitide and 3.0 mg/kg TM30339 treated mice (table 2). If expressed as a percentage from baseline values, also 0.3 mg/kg TM30339 treatment increased insulin's action to stimulate glucose disposal (figure 4). Glucose production during insulin infusion was significantly decreased in TM30339 treated animals (table 2). If expressed as a percentage from baseline values, the highest dose of Obinepitide also reinforced the capacity of insulin to suppress EGP (figure 5). The lowest dose of Obinepitide did not significantly affect glucose turnover.

Table 1. Plasma parameters in basal (fasting) condition and during hyperinsulinemia, before and during chronic treatment with Obinepitide (BID) (0.3 or 3.0 mg/kg), TM30339 (OD) (0.3 or 3.0 mg/kg), or control. Values represent mean ± SD for at least 10 mice per group. **p<0.01 vs. control.

		Control (saline BID)	Obinepitide (BID)		TM30339 (OD)	
			0.3 mg/kg	3.0 mg/kg	0.3 mg/kg	3.0 mg/kg
Body weight (g)	Start treatment	31.2 ± 2.5	30.9 ± 2.1	31.3 ± 2.5	31.1 ± 2.9	31.3 ± 3.0
	2 wks treatment	31.3 ± 2.2	30.1 ± 1.9	29.5 ± 1.0	28.8 ± 1.8**	28.2 ± 1.4**
	4 wks treatment	31.5 ± 2.3	30.3 ± 1.5	28.6 ± 0.6**	28.6 ± 1.5**	27.9 ± 1.2**
	Hyperinsulinemic	-	-	-	-	-
Glucose (mmol/l)	Start treatment	6.9 ± 1.2	6.7 ± 0.6	6.7 ± 1.0	7.0 ± 1.0	6.5 ± 0.9
	2 wks treatment	6.8 ± 1.1	6.3 ± 0.8	5.0 ± 1.8**	5.6 ± 0.9**	5.1 ± 1.2**
	4 wks treatment	6.0 ± 1.3	6.0 ± 1.5	5.1 ± 0.7**	5.0 ± 0.6**	4.8 ± 0.6**
	Hyperinsulinemic	5.9 ± 1.3	6.2 ± 1.1	5.4 ± 0.8	5.1 ± 0.7	4.7 ± 0.6
Insulin (ng/ml)	Start treatment	0.5 ± 0.3	0.5 ± 0.2	0.4 ± 0.2	0.5 ± 0.2	0.5 ± 0.3
	2 wks treatment	0.5 ± 0.2	0.5 ± 0.2	0.3 ± 0.2	0.5 ± 0.3	0.3 ± 0.2
	4 wks treatment	0.7 ± 0.3	0.6 ± 0.3	0.4 ± 0.2	0.6 ± 0.4	0.4 ± 0.2
	Hyperinsulinemic	3.9 ± 1.1	4.1 ± 1.1	3.5 ± 0.7	3.6 ± 1.0	3.4 ± 0.9
FFA (mmol/l)	Start treatment	0.9 ± 0.2	0.8 ± 0.2	1.0 ± 0.3	0.9 ± 0.2	0.9 ± 0.2
	2 wks treatment	0.9 ± 0.2	0.9 ± 0.2	1.0 ± 0.2	0.9 ± 0.2	0.8 ± 0.3
	4 wks treatment	0.8 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	0.8 ± 0.3	0.7 ± 0.2
	Hyperinsulinemic	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1

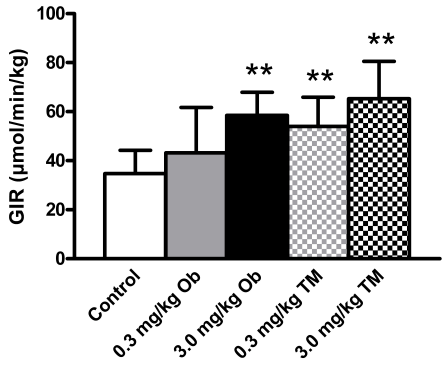

Figure 3. Glucose infusion rate (GIR) during a hyperinsulinemic euglycemic clamp in mice after treatment with Obinepitide (Ob) (0.3 or 3.0 mg/kg), TM30339 (TM) (0.3 or 3.0 mg/kg), or control for 4 weeks. Values represent mean ± SD for at least 10 mice per group. **p <0.01 vs. control.

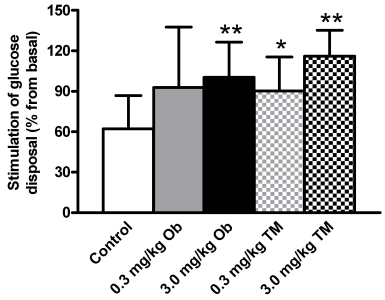

Figure 4. Stimulation of glucose disposal during a hyperinsulinemic euglycemic clamp in mice after treatment with Obinepitide (Ob) (0.3 or 3.0 mg/kg), TM30339 (TM) (0.3 or 3.0 mg/kg), or control for 4 weeks. Values represent mean ± SD for at least 10 mice per group. *p <0.05 vs. control. **p <0.01 vs. control.

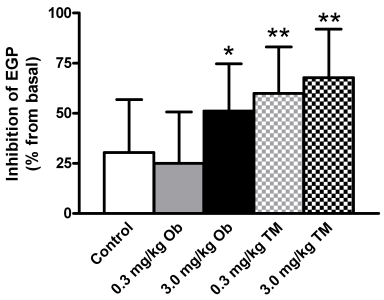

Figure 5. Inhibition of endogenous glucose production (EGP) during a hyperinsulinemic euglycemic clamp in mice after treatment with Obinepitide (Ob) (0.3 or 3.0 mg/kg), TM30339 (TM) (0.3 or 3.0 mg/kg), or control for 4 weeks. Values represent mean ± SD for at least 10 mice per group. *p <0.05 vs. control. **p <0.01 vs. control.

Table 2. Glucose turnover in basal and hyperinsulinemic conditions in mice that received treatment for 4 weeks with Obinepitide (BID) (0.3 or 3.0 mg/kg), TM30339 (OD) (0.3 or 3.0 mg/kg), or control. Values represent mean ± SD for at least 10 mice per group. *p<0.05 vs. control **p<0.01 vs. control.

	Control (saline BID)	Obinepitide (BID)		TM30339 (OD)	
		0.3 mg/kg	3.0 mg/kg	0.3 mg/kg	3.0 mg/kg
Basal glucose turnover (µmol/min/kg)	38.6 ± 7.8	38.7 ± 8.8	39.2 ± 7.4	36.7 ± 5.7	34.8 ± 5.0
Insulin mediated glucose disposal (µmol/min/kg)	62.4 ± 15.0	74.0 ± 22.9	77.4 ± 11.2*	68.9 ± 7.4	74.9 ± 10.3*
Hyperinsulinemic endogenous glucose production (µmol/min/kg)	27.9 ± 12.5	31.6 ± 15.7	19.8 ± 11.6	14.1 ± 7.6**	10.9 ± 8.3**

VLDL metabolism

Neither drug affected VLDL-apoB or VLDL-TG production rate in basal (fasting) conditions (table 3). Remarkably, both drugs appeared to elevate plasma TG concentrations in basal condition, where the increase was statistically significant (compared to control) for the highest dose of each drug. During insulin infusion, plasma TG concentrations rose more slowly after tyloxapol in mice that received chronic injections of 3.0 mg/kg TM30339 (figure 6), which translates into a decreased VLDL-TG production rate in these animals (table 3).

Table 3. Bodyweight, plasma TG levels and VLDL production rate in basal (fasting) condition and during hyperinsulinemia before and during chronic treatment with Obinepitide (BID) (0.3 or 3.0 mg/kg), TM30339 (OD) (0.3 or 3.0 mg/kg), or control. Values represent mean ± SD for at least 7 mice per group. *p <0.05 vs. control. **p<0.01 vs. control. #p <0.01 vs. start treatment.

		Control (saline BID)	Obinepitide (BID)		TM30339 (OD)	
			0.3 mg/kg	3.0 mg/kg	0.3 mg/kg	3.0 mg/kg
Body weight (g)	Start treatment	31.0 ± 2.2	31.7 ± 3.9	31.0 ± 2.4	31.4 ± 3.8	31.4 ± 4.4
	2 wks treatment	31.4 ± 2.2	31.5 ± 3.2	30.1 ± 1.8	29.8 ± 2.9	28.8 ± 2.9
	4 wks treatment	32.4 ± 2.1	32.2 ± 3.5	29.8 ± 1.6	30.1 ± 3.1	29.7 ± 3.0
TG (mmol/l)	Start treatment	0.54 ± 0.11	0.53 ± 0.10	0.49 ± 0.12	0.50 ± 0.13	0.46 ± 0.11
	2 wks treatment	0.62 ± 0.07	0.61 ± 0.07	0.58 ± 0.08	0.55 ± 0.16	0.40 ± 0.08
	4 wks treatment	0.66 ± 0.17	0.79 ± 0.20#	0.99 ± 0.18**#	0.85 ± 0.18#	0.88 ± 0.17*#
VLDL-apoB production ($x10^3$ dpm/ml/h)	Basal	56 ± 14	59 ± 21	58 ± 13	66 ± 14	65 ± 17
VLDL-TG production (µmol/h/kg)	Basal	282 ± 49	282 ± 69	263 ± 37	291 ± 47	273 ± 48
	Hyperinsulinemic	167 ± 29	169 ± 48	159 ± 10	172 ± 27	134 ± 17**

Lipolysis

Obinepitide or TM30339 did not affect lipolysis (glycerol R_a) (figure 7) or circulating FFA concentrations in basal (fasting) conditions (table 1). Although insulin infusion reduced glycerol turnover significantly in the 3.0 mg/kg Obinepitide and TM30339 groups, insulin's capacity to suppress glycerol turnover (expressed as percentage of baseline values) was not affected by any of the interventions (data not shown). Also, the decline of plasma FFA concentrations induced by insulin was not affected by either drug (table 1).

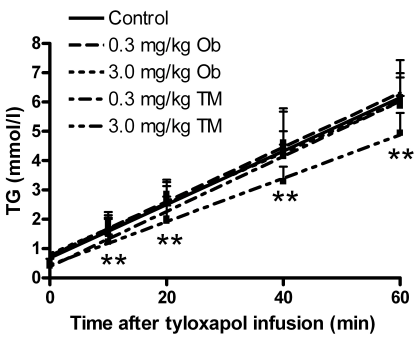

Figure 6. Triglyceride (TG) concentration after tyloxapol injection in hyperinsulinemic conditions in mice after treatment with Obinepitide (0.3 or 3.0 mg/kg), TM30339 (0.3 or 3.0 mg/kg), or vehicle for 4 weeks. Values represent mean ± SD for at least 10 mice per group. **p <0.01 vs. control.

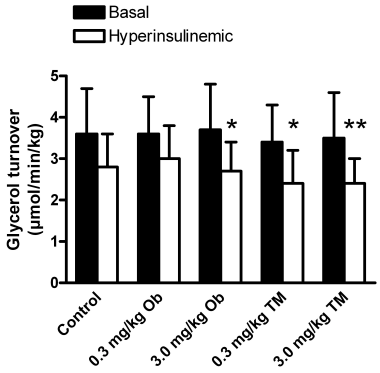

Figure 7. Glycerol turnover during a hyperinsulinemic euglycemic clamp in mice after treatment with Obinepitide (0.3 or 3.0 mg/kg), TM30339 (0.3 or 3.0 mg/kg), or vehicle for 4 weeks. Values represent mean ± SD for at least 10 mice per group. *p <0.05 vs. basal. **p <0.01 vs. basal.

Discussion

This study shows that Obinepitide and TM30339 may be useful tools to improve glucose metabolism in diet-induced insulin resistant C57Bl/6J mice. In particular, chronic administration of Obinepitide (3.0 mg/kg) and TM30339 (0.3 and 3.0 mg/kg) reduced fasting plasma glucose in these animals. Moreover, chronic treatment with these drugs clearly enhanced whole body insulin sensitivity. Both compounds can reinforce insulin's capacity to stimulate glucose disposal and inhibit glucose production. In addition, insulin's capacity to inhibit VLDL-TG production was reinforced by chronic treatment with 3.0 mg/kg TM30339.

The beneficial effects on body weight corroborate the results of previous pre-clinical studies investigating the anti-obesity potential of Obinepitide and TM30339[20, 21]. All weight was lost in the first week of treatment, which is consistent with earlier findings documenting a transient effect on food intake and body weight of chronic PYY_{3-36} treatment[11, 27]. Clearly, in time, the body compensates via yet unknown mechanisms to restore energy homeostasis. However, despite the loss of this acute effect, body weight remained lower during treatment with either drug. The decline of body weight was fully accounted for by a reduction of fat mass. Neither Obinepitide nor TM30339 affected lipolysis or insulin's capacity to suppress this process and, accordingly, neither drug changed plasma FFA concentrations. Therefore, the reduction of fat mass can not be

attributed to a lipolytic effect of the drugs. Alternatively, the decline of fat mass may have been the result of a shift in the balance of fuel use in favor of fat oxidation[11, 17].

Besides affecting body weight, both drugs clearly ameliorated insulin resistance. In particular, Obinepitide enhanced insulin mediated glucose disposal, an observation that is in line with previous findings of chronic PYY_{3-36} treatment[16, 17]. Interestingly, our findings also show that agonism of Y4R reinforces insulin's ability to promote glucose disposal, indicating a novel role for this receptor. Moreover, both drugs reinforced insulin action to inhibit EGP. Previous studies showed that activation of Y2 receptors by PYY_{3-36} reinforces insulin mediated glucose disposal, but not its capacity to suppress EGP. Therefore, the impact of Obinepitide on EPG might be mediated by the Y4R. In this respect, it is interesting to note that PP infusion in patients with chronic pancreatitis, a condition accompanied by decreased PP levels, ameliorates insulin resistance of EGP[28].

The mechanism underlying the effects of both compounds on insulin sensitivity of glucose metabolism remains to be established. A single injection of both drugs did not affect glucose metabolism in the same experimental context (data not shown). It is conceivable that the drugs work via their impact on food intake and/or body weight[29]. However, chronic PYY_{3-36} administration enhances insulin-mediated glucose disposal via a mechanism that is independent of food intake and body weight[17]. Whatever the mechanism, and highly significant, the present data suggest that chronic treatment with Obinepitide or TM30339 has beneficial metabolic effects in insulin resistant DIO mice, which holds promise for their potential as tools to treat obesity and type 2 diabetes in humans.

These drugs do not appear to modulate VLDL metabolism in basal conditions. However, insulin's capacity to inhibit VLDL-TG production was reinforced by chronic treatment with 3.0 mg/kg TM30339, suggesting that the drug may be able to improve postprandial control of TG production. It is rather surprising that only the highest dose of TM30339 was effective, since the lower dose of TM30339 and 3.0 mg/kg Obinepitide have a similar impact on body weight and glucose metabolism. The mechanism underlying the capacity of TM30339 to reinforce insulin action to reduce VLDL-TG production needs to be clarified in more detail.

Unexpectedly, both Obinepitide and TM30339 increased fasting plasma TG levels after 4 weeks of treatment. In view of the fact that VLDL-TG production in basal condition was not affected by either one of the drugs, this observation suggests that both compounds hamper clearance of VLDL particles from the circulation. Since hypertriglyceridemia is a cardiovascular risk factor, this feature of the drugs is worrisome if confirmed in future (clinical) experiments. It is important to note that comparison between plasma TG levels at 2 vs. 4 weeks of intervention is difficult, since the timing of

drug administration was somewhat different. At 2 weeks, the last injection was given 4 hours before blood sampling, compared to 20 minutes before blood sampling during the VLDL production experiment. The latter may indicate that Obinepitide and TM30339 acutely increased plasma TG levels. Increased TG levels have been observed previously in chow-fed transgenic mice over-expressing PYY[14]. When these mice were put on a high-fat diet, TG levels decreased to the level observed in control animals. PYY over-expressing mice on an ob/ob background have decreased plasma TG levels compared to controls. These observations suggest that the metabolic background modulates the impact of Y2R activation (Y2R is the primary receptor of PYY_{3-36}) on TG levels. Therefore, it is conceivable that Obinepitide and/or TM30339 impact on plasma TG concentrations in obese animal models differently than in the animal model used here (and perhaps obese humans as well).

In conclusion, the current study indicates that Obinepitide and TM30339 reduce body weight and ameliorate insulin resistance of glucose metabolism in C57Bl/6J mice maintained on a high fat diet. The drugs did not impact on lipolysis, and only the highest dose of TM30339 reinforced the capacity of insulin to inhibit VLDL-TG production. These data hold promise for the potential of these drugs as tools to treat obesity and type 2 diabetes mellitus in humans.

This research was supported by a grant from 7TM Pharma.

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