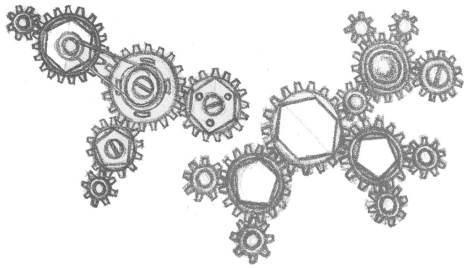

Chapter 2:

Pharmacokinetics

Topiramate affects pharmacokinetics of Olanzapine in rats, with consequences for glucose and body temperature homeostasis.

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Abstract

Human studies revealed that the antipsychotic Olanzapine (OLZ) stimulates weight gain and decreases insulin sensitivity. In contrast, the anticonvulsant Topiramate (TPM) reduces body weight and improves insulin sensitivity. Several case- and controlled-studies in humans have reported the beneficial effect of adjunctive TPM treatment in reducing OLZ-induced weight gain and restoring insulin sensitivity, but may cause derangements in body temperature regulation. Because it was reported that OLZ dosing decreased when co-administered with TPM, we hypothesized that TPM affects OLZ pharmacokinetics or pharmacodynamics. Therefore, several combinations of different OLZ and TPM doses were given intragastrically to male Wistar rats, and we assessed the circulating drug levels for 24 hours after administration. Concomitantly with drug administration animals received an intragastric glucose load to measure the acute effects of both drugs on glucose and insulin regulation. In addition, we measured core body temperature continuously and assessed circulating glucocorticoid levels. We observed that TPM increased peak OLZ levels and increased the half-life time of circulating OLZ. Circulating OLZ levels, independent of adjunctive TPM administration, were directly correlated to hypothermia (and the latter to corticosterone levels), and increased glucose levels 60 minutes post administration. In contrast, circulating insulin levels were negatively correlated with increased OLZ levels 15 minutes post administration. Although not assessed here, we attribute these acute effects of OLZ on glucose homeostasis to delayed gastric emptying, rather than changes in insulin signaling. Our study may have pharmacokinetic implications for adjunctive TPM treatment during OLZ therapy in humans, especially in relation to TPM's effectiveness in reducing OLZ-induced weight gain.

Keywords:

Olanzapine, Topiramate, pharmacokinetic, hypothermia, corticosterone, glucose-insulin regulation, rat

Introduction

Second generation antipsychotics (SGAs), like Olanzapine (OLZ), have become the first line of treatment in schizophrenia over the past decades. However, OLZ is infamous for its weight increasing and diabetogenic side effects [1-4]. Therefore, other agents have been studied in the attempt to specifically reduce the metabolic side effects of OLZ, without interfering with OLZ's antipsychotic abilities. One of these agents is Topiramate (TPM). TPM is developed as an anticonvulsant known to block AMPA/kainate-gated ion and sodium channels and stimulates GABA receptors [5], but is also an antagonist of carbonic anhydrase [6]. TPM reduces body weight during long-term treatment and has accordingly been shown to improve glucose and insulin regulation [7,8]. A second possible benefit of TPM, as an adjunctive to OLZ treatment, is its mood stabilizing property and its effectiveness in treating bipolar disorder [9,10]. Several case and controlled human studies reported about the effectiveness of TPM to manage OLZ-induced weight gain [11-16]. The most recent study performed by Narula et al [17] demonstrated not only that TPM, as an adjunctive, attenuated OLZ-induced weight gain, it also improved Positive and Negative Syndrome (PANS) score. These results relate to the work of Vieta et al [15], who already showed that the combined treatment of OLZ and TPM improved Young Mania Rate Scale (YMRS), the 17-item Hamilton Depression Scale (HDS), and the Modified Clinical Global Impressions for Bipolar Disorder (CGI-BP-M). Because Vieta et al's study was set-up such that optimal OLZ and TPM drug dosing was titrated according to their optimal efficacies, this led to a finally lower dose of OLZ than normally provided with increasing TPM doses [15]. This gradual reduction in OLZ dosing possibly added to the tolerability and reduced unwanted side effects (e.g. somnolence, nausea, or fatigue), and might be related to TPM's mood stabilizing properties. One possible mechanism underlying the increased efficacy of OLZ by TPM is that TPM affects OLZ pharmacokinetics and therefore OLZ dosing needed to be decreased in the study of Vieta [15].

The pharmacokinetics of the first generation antipsychotic haloperidol (HAL), of which the oxidation is known to be mediated by CYP3A4, has been reported to be affected by TPM (which is an inhibitor of CYP3A4) [18], with a significant increase of 15% of area under the curve (AUC) of circulating HAL levels when co-administered with TPM [19]. OLZ is not oxidized by CYP3A4, but by cytochrome P450 (CYP) isoenzymes 1A2 and 2D6, and TPM is said not to inhibit these proteins, which would argument against possible effects of TPM on OLZ pharmacokinetics [16]. However, OLZ is also cleared by other processes (including direct conjugation and excretion via the UDP-glucuronosyltransferase (UGT) system [20]), which might hypothetically be

affected by TPM [21].

Because Migliardi et al [22] found that baseline circulating levels of OLZ assessed 10-12 hrs after drug dosing did not increase when administered in combination with TPM, we hypothesized that peak circulatory drug levels and drug half-life time of OLZ and TPM would influence one another. Specifically, we investigated acute doses of OLZ (10 and 20mg/kg) and the adjunctive administration of TPM (50 or 100mg/kg) on circulating OLZ and TPM levels in rats. If pharmacokinetics of OLZ is affected by TPM, one would expect that OLZ's actions on glucose and insulin kinetics [23], hypothermia [24], and circulating corticosterone levels [25] would be affected by TPM too. The concentrations of OLZ and TPM used in our study were based on a publication of Albaugh et al [23], where TPM treatment in rats was able to reduce OLZ-induced adiposity and BW gain after 5 days of treatment. In their study they showed that circulating glucose and insulin levels were reduced after acute OLZ treatment during an oral glucose tolerance test (OGTT), whereas an increased insulin response was observed during OGTT after chronic OLZ treatment. This latter is consistent with OLZs diabetogenic liability seen throughout literature [26,27]. Unfortunately, Albaugh et al [23] did not investigate the effects of adjunctive TPM treatment on OLZ's efficacy to potentially lower insulin responses during the OGTT.

In our study we could confirm that OLZ's pharmacokinetics changed considerably in rats with adjunctive administration of TPM. Enhanced circulating levels of OLZ by TPM probably contributed to the enhanced hypothermic reaction and increased corticosterone levels compared to when OLZ was given without TPM. Similar to Albaugh et al [23], we also found decreased glucose and insulin levels during an IGGTT after OLZ administration (potentially due to reduced gastrointestinal glucose absorption), and these effects were also enhanced by TPM. TPM administration alone did not have any effects on aforementioned parameters, demonstrating that TPM's effect is mediated by primarily altering OLZ's pharmacokinetics and potentially by altering its efficacy.

Methods

Animals and surgery

Eighteen male Wistar rats (Harlan, Horst, NL), weighing 459±6g, were used in this experiment. Upon arrival, they were housed individually in a 24x24x36cm Plexiglas cage with wood chip bedding, a wooden gnawing stick, paper nesting material, under a 12-12hr light-dark cycle (lights off at 11:00hr), and with *ad libitum* access to

standard chow (AB diets, Woerden, NL) and water. One week after acclimatization, they underwent surgery (under high O2-low CO2 isoflurane inhalation anesthesia) during which a gastric cannula (1.40mm OD, 0.80mm ID) was inserted in the corpus of the stomach [[28]] and a second cannula (1.40mm OD, 0.80mm ID) was inserted in the right jugular vein [[29]]. The intragastric cannula was used for drug administration, whereas the jugular vein cannula allowed stress free blood sampling, according to methodologies described elsewhere [[30]]. Both cannulas were subcutaneously guided to the head, where they were exteriorized via a bent 19G stainless steel metal sleeve, which was fixed to the skull by surgical stainless steel screws and dental cement. An analgesic (0.1 mg/kg Finadyne diluted in 0.1 ml/kg saline) was administered s.c. 15 minutes before animals were taken off anesthesia. Both exteriorized jugular vein and gastric cannulas were closed by plastic caps made of a piece of flame-sealed PE100 tubing, and were rinsed twice a week starting 2 days after surgery to prevent blockage. The gastric cannula was rinsed with 0.5 ml saline; obstruction of the jugular vein cannula was prevented by using a 55% PVP solution. Animals were also equipped with an abdominal telemetric transponder (model TA10TA-F40, Data Sciences, St. Paul, MN) to measure core body temperature continuously. These and all other procedures are approved by the animal ethical committee of the University of Groningen.

Drug administration

Pure powdered Olanzapine (Fournier Laboratory, France) and Topiramate (Hannover, Germany) were kindly provided by Abbott laboratories. Olanzapine was dissolved, after acidification using 1 M HCl, in 0.9% NaCl saline at a final concentration of 0.51mg/ml (OLZ 10 mg/kg) and 1.02mg/ml (OLZ 20mg/kg) and adjusted to pH 6.5-7 using 1 M NaOH. Topiramate was dissolved, after alkalization using 1M NaOH, 0.9% NaCl saline at a final concentration of 2.55mg/ml (TPM 50 mg/kg) and 5.1mg/ml (TPM 100 mg/kg) and readjusted to ph 7.4 using 1M HCl. Final solutions consisted of saline or any of the possible drug combinations, resulting in 8 different treatment groups: Control, OLZ10, OLZ20, TPM50, TPM100, OLZ10/TPM50, OLZ10/TPM100, OLZ20/TPM50, and OLZ20/TPM100. Finally, 150 mg/ml glucose was added to all drug solutions at the start of each study and was administered during a 9 min constant infusion (1ml/min) via the gastric cannula, while animals could freely move in their home cage.

Intragastric Glucose Tolerance Test

IG-GTT was performed at the start of the dark phase (11AM). Prior to the start of IG-GTT, animals (n=6) were fasted for 4 hours. A baseline blood sample was drawn 10

minutes (t=-15) before the start (t=0) of glucose and drug infusion. Blood samples (0.2 ml) were taken at time points -10, 0, 5, 10, 15, 20, 25, 30, 40, and 60 minutes. Blood samples were immediately put on ice during IG-GTT in vials containing 10µl EDTA (0.09 g/ml). Whole blood samples of 50µl diluted in 450µl 2% heparin solution were stored at -20°C until analysis of glucose concentrations by the ferry-cyanide method [31] in a *Technicon* auto analyzer. The remaining blood samples were centrifuged (15min, 2500 rpm, 4°C) and plasma was collected and stored at -20°C until insulin determination. Plasma insulin levels were measured in duplicates using a commercial radioimmunoassay kit (Rat Insulin, ^{125}I-Insulin Cat# RI-13K, Linco Reasearch, Nucli Lab, NL).

Experimental set up

All animals participated in three different challenges and were divided randomly over each group, the only restriction we added was that the first challenge always consisted of the lowest dosing and increased with every other challenge. Between two challenges animals were allowed one week of recovery. Every treatment resulted in a group size of n=6 per drug dosing. A challenge consisted of an IG-GTT and extra blood samples (0.2ml) for drug analyses were drawn at 2, 4, 6, and 24 hours after the start of drug administration. Circulating OLZ and TPM levels were determined from plasma samples at t=5, 10, 15, 30min, 1, 2, 4, 6, and 24hr after drug administration using HPLC (Fournier Laboratories S.A., Dijon, France). During each challenge core body temperature was measured every 10 min. Circulating corticosterone levels were determined using plasma samples collected at -10, 0, 15, 30, and 60 minutes during the IGGTT) with the commercial available ImmuChem Cort ^{125}I-RIA KIT (MP Biomedicals, Germany GmbH, Eschwegge, D).

Data analyses

All data are expressed as average±sem. OLZ levels are expressed as ng/ml; TPM levels are expressed as µg/ml.

The elimination half-life time ($t_{1/2}$) is calculated using the following formula calculating the elimination rate constant (k_{elim}):

$$k_{elim} = \frac{\ln(C_{peak}) - \ln(C_{trough})}{t_{interval}}$$,and $$t_{1/2} = \frac{\ln(2)}{k_{elim}}$$; or: $$t_{1/2} = \frac{t_{interval} * \ln(2)}{\ln(C_{peak}/C_{trough})}$$

where C_{peak} is the peak concentration of the decaying substance , C_{trough} is the concentration not yet decayed after time *t* (time interval). Because concentration at 24hr after administration was close to zero in all instances, we used the drug concentration measured at t=6hr as C_{trough}.

Core body temperature changes are expressed as Δ body temperature (°C) relative to the start of drug infusion (T=0hrs; Δ B.Temp=0°C). Core body temperature is assessed every 5 minutes, for illustrative purposes we used the core body temperature averaged over 30 minute periods. Glucose and insulin responses are expressed as ΔmM and Δng/ml resp., relative to levels assessed at baseline (t=0 minutes). To show the observed difference in shape of the glucose and insulin responses, the total $AUC_{0\text{-}60min}$ is divided into the percentage of $AUC_{0\text{-}20min}$, $AUC_{20\text{-}40min}$, and $AUC_{40\text{-}60min}$. With this we illustrate the distribution of contribution to the total AUC. Corticosterone levels are expressed as ng/ml. Statistics was performed in SPSS20 using oneway-ANOVA (post hoc LSD) to show differences between groups; repeated measures (rm)ANOVA (post hoc LSD) was used to show differences between groups over time dependent measurements. General Linear Model (GLM) rmANOVA was used to calculate overall drug effects irrespective of dosing. Pearson 2-tailed correlations were performed to show correlations between variables. Data is assumed to be significant at $P<0.05$. Graphs were prepared using Graphpad Prism 5.0.

Results

After 9 min of intragastric infusion, overall circulating OLZ levels (fig 1A) were increased in OLZ20 compared to OLZ10 ($F_{9,90}=8.131$, $P<0.001$, rmANOVA). Also peak levels ($OLZ10_{max}=319\pm35$ ng/ml, $OLZ20_{max}=916\pm151$ ng/ml; $F_{1,11}=14.750$, $P<0.01$, oneway-ANOVA) were higher in OLZ20 than in OLZ10. Overall circulating TPM levels (fig 1B) were increased in TPM100 compared to TPM50 ($F_{9,90}=25.359$, $P<0.001$, rmANOVA) as well as peak levels ($TPM50_{max}=29.9\pm2.4$ µg/ml, $TPM100_{max}=67.5\pm4.9$ µg/ml; $F_{1,11}=47.698$, $P<0.0001$, oneway-ANOVA). Overall circulating TPM (50 or 100) levels did not change when co-administered with OLZ (10 or 20; data not shown).

When OLZ 10 was co-administered with TPM50 overall circulating OLZ levels increased (fig 1C) compared to singly administered OLZ10 ($P<0.05$) and increased even more when co-administered with TPM100 ($P<0.01$, $F_{18,126}=3.256$, rmANOVA post hoc LSD). GLM rmANOVA revealed a TPM effect, with increased overall OLZ20 levels compared to OLZ20 without TPM ($P<0.01$, $F_{9,135}=3.163$, GLM rmANOVA). Post-hoc analyses did not reveal significant effects of TPM treatment on OLZ20 levels on individual time points.

As shown in table 1 peak OLZ levels in OLZ10 were increased when co-administered with TPM50 ($P<0.05$) and TPM100 ($P<0.01$, $F_{2,16}=5.501$, oneway-ANOVA post hoc LSD). Half-life time ($T_{1/2}$) of circulating OLZ10 levels were increased by TPM50 ($P<0.05$) and TPM100 ($P<0.01$, $F_{2,16}=7.022$,oneway-ANOVA post hoc LSD). Whereas circulating OLZ peak levels in OLZ20 were not significantly increased by TPM50 or TPM100, $T_{1/2}$ of circulating OLZ20 did not increase by the co-administration of TPM50, but did increase by the co-administration of TPM100 ($P<0.01$, $F_{2,17}=7.901$, oneway-ANOVA post hoc LSD). $T_{1/2}$ of OLZ20 was also increased compared to OLZ10 ($P<0.05$, oneway-ANOVA post hoc LSD). As mentioned, peak TPM levels were not affected by OLZ co-administration, but $T_{1/2}$ of circulating TPM50 was increased by the co-administration of OLZ10 ($P<0.05$) and OLZ20 ($P<0.05$, $F_{2,15}=5.586$. oneway-ANOVA

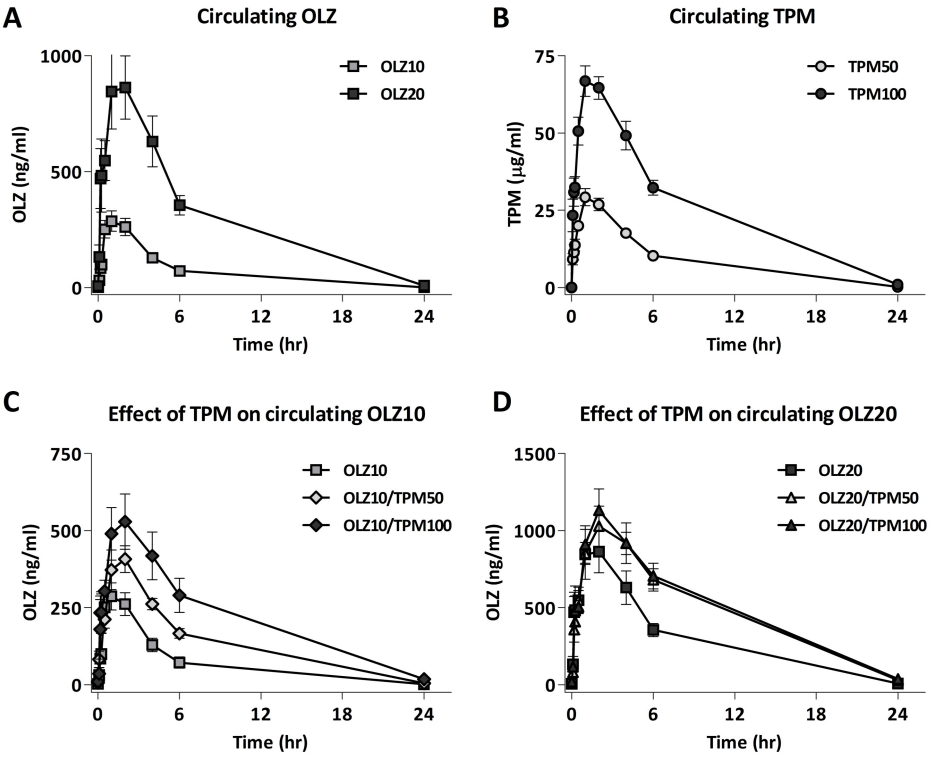

Fig 1: Circulating OLZ and/or TPM levels. A) Dose response curves of circulating OLZ levels; OLZ10 caused lower overall circulating OLZ levels compared to OLZ 20 ($P<0.01$, rmANOVA). B) Dose response curves of circulating TPM levels; TPM50 caused lower circulating TPM levels compared to TPM100 ($P<0.01$, rmANOVA). C) Circulating OLZ (10 mg/kg) levels increased when co-administered with TPM50 ($P<0.05$, rmANOVA post hoc LSD) and TPM 100 ($P<0.05$, rmANOVA post hoc LSD). D) Circulating OLZ (20 mg/kg) levels were increased after co-administration of TPM ($P<0.05$, GLM rmANOVA).

post hoc LSD), whereas $T_{1/2}$ of circulating TPM100 was only increased by co-administration of OLZ10 ($P<0.05$, $F_{2,16}=3.023$, oneway-ANOVA post hoc LSD).

Table 1: Circulating OLZ and TPM peak levels and half-life time.

OLZ levels (ng/ml)	TPM			$T_{1/2}$ (hr)	TPM	
	0	50	100	0	50	100
OLZ10	319 ± 35	447 ± 42[a]	566 ± 87[a]	2.2 ±0.3	3.4 ± 0.4[a]	4.1 ± 0.5[A]
OLZ20	916 ± 151	1024 ± 96	1183 ± 122	3.4 ± 0.3[a]	4.5 ± 0.2	5.8 ± 0.6[B]
TPM levels (µg/ml)	**OLZ**			**$T_{1/2}$ (hr)**	**OLZ**	
	0	**10**	**20**	**0**	**10**	**20**
TPM50	29.9 ± 2.4	30.3 ± 1,4	24.5 ± 1.6	3.2 ± 0.3	4.3 ± 0.4[c]	4.6 ± 0.4[c]
TPM100	67.5 ± 4.9	68.1 ± 4.4	59.2 ±4.3	3.9 ± 0.2	4.9 ± 0.4[d]	4.2 ± 0.3

[a]P<0.05, compared to OLZ10; [A]P<0.01, compared to OLZ10; [B]P<0.01, compared to OLZ20; [C]P<0.001, compared to TPM50; [d]P<0.05, compared to TPM100.

OLZ-induced hypothermia

Temperature responses to drug administration are shown in figure 2. Olanzapine dose dependently decreased body temperature after administration ($F_{48,360}=12.226$, rmANOVA; OLZ 10: P<0.01, OLZ20: P<0.001). As shown in table 2 maximal temperature drop (ΔT_{Max}) was dose dependently enhanced ($F_{2,17}=10.092$, oneway-ANOVA) by OLZ treatment; OLZ10 (P<0.05) and OLZ20 (P<0.001) compared to Control treatment, and between OLZ-treated groups (P<0.05). Core body temperature or ΔT_{Max} was not different in the TPM-treated groups compared to Controls. The combination OLZ10/TPM100 decreased body temperature ($F_{48,360}=12.962$, rmANOVA) stronger than OLZ10 alone (P<0.001) or the OLZ10/TPM50 combination (P<0.001). ΔT_{Max} following OLZ10 (P<0.05), OLZ10/TPM50 (P<0.01), and OLZ10/TPM100 (P<0.001) treatments was increased compared to Control ($F_{3,23}=23.883$, oneway-ANOVA post hoc LSD); ΔT_{Max} following OLZ10/TPM100 administration was increased compared to both OLZ10 (P<0.001) and OLZ10/TPM50 (P<0.001). Also in the OLZ20 treated groups only OLZ20/TPM100 increased the hypothermic response compared to OLZ20 ($P<0.05$, $F_{48,336}=2.806$, rmANOVA post hoc LSD). All OLZ20 treated groups showed an increased ΔT_{Max} compared to Control (OLZ20, OLZ20/TPM50: P<0.01; OLZ20/TPM100: P<0.001, $F_{3,22}=15.607$, oneway-ANOVA post hoc LSD). OLZ20 (P<0.01) and OLZ20/TPM50 (P<0.05) caused a lower ΔT_{Max} compared to OLZ20/TPM100. Pearson's correlation revealed that ΔT_{Max} was negatively correlated with circulating OLZ_{peak} levels ($r=-0.736$, $R^2=0.542$, P<0.001, Pearson 2-tailed), whereas circulating TPM_{peak} levels were not correlated to ΔT_{Max} ($r=-0.290$, $R^2=0.084$, P=0.102, Pearson 2-tailed).

Table 2: Maximal temperature drop after drug administration.

Δ $Temp_{Max}$ (°C)	TPM		
	0	50	100
Control	-1.3 ± 0.2	-1.2 ± 0.12	-1.1 ± 0.2
OLZ10	-2.4 ± 0.2^{aC}	-2.8 ± 0.44^{AC}	-5.1 ± 0.4^{AA}
OLZ20	-3.7 ± 0.6^{AbD}	-4.2 ± 0.60^{Ad}	-6.0 ± 0.5^{AA}

^{a}P<0.05, compared to Control; ^{A}P<0.01, compared to Control; ^{AA}P<0.001, compared to Control; ^{b}P<0.05, compared to OLZ10; ^{C}P<0.001, compared to OLZ10/TPM100; ^{d}P<0.05, compared to OLZ20/TPM100 ; ^{D}P<0.01, compared to OLZ20/TPM100, oneway-ANOVA post hoc LSD.

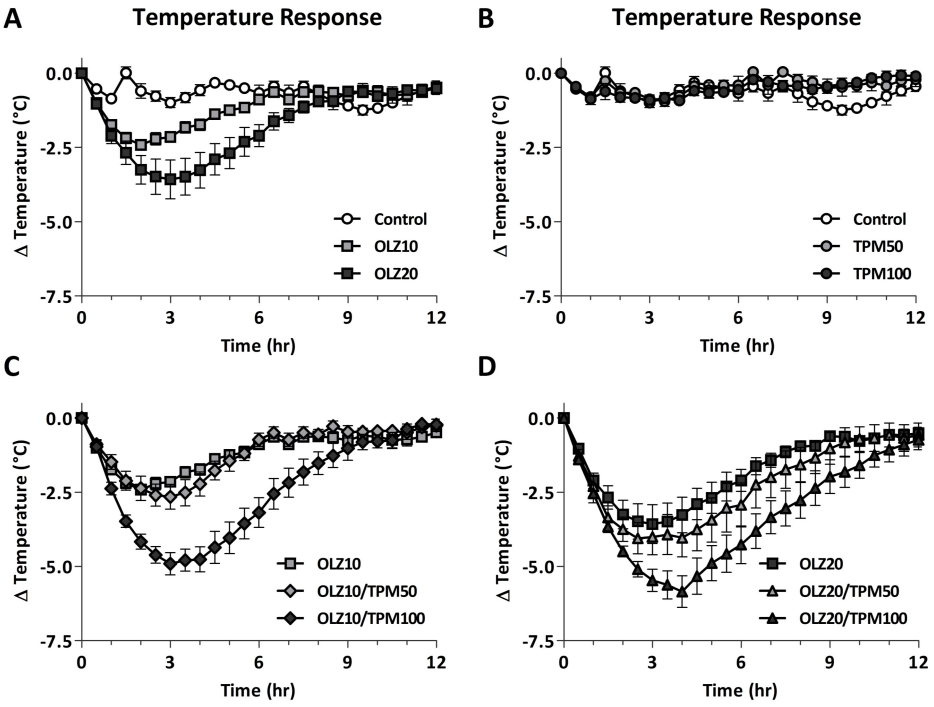

Fig 2: Acute temperature response after OLZ and/or TPM administration. a) OLZ induced a dose-related hypothermia (P<0.01, rmANOVA). b) TPM administration did not affect temperature regulation. c) TPM100 amplified OLZ10 induced hypothermia (P<0.01, rmANOVA). d) TPM100 amplified OLZ20 induced hypothermia (P<0.05, rmANOVA).

Glucose Responses

Circulating glucose levels during IG-GTT (fig. 3) were altered by OLZ treatment ($P<0.001$, $F_{8,128}=3.956$, GLM rmANOVA), but post hoc analyses did not identify difference in overall glucose responses between groups. During the first 20 minutes of the IVGTT, OLZ decreased glucose levels ($F_{2,17}=6.586$, oneway-ANOVA) resulting in a reduced percentage of $AUC_{0\text{-}20min}$ in both the OLZ10 ($P<0.05$) and the OLZ20 ($P<0.01$) treated groups, as shown in fig. 5A. During the next 20 minute interval (from 20-40min) no differences were observed by OLZ treatment on glucose levels, nor between any of the other groups (data not shown). From 40 till 60 minutes after the start of glucose infusion the percentage of $AUC_{40\text{-}60min}$ was increased ($F_{2,17}=4.469$, oneway-ANOVA) in both OLZ10 ($P<0.05$) and OLZ20 ($P<0.05$,) compared to Controls. TPM (50 and 100) did not affect the glucose response compared to Controls. The percentage of AUC during the first and last 20 minutes of the glucose response was not different between Controls and TPM50; whereas compared to TPM50 a decrease in the percentage of $AUC_{0\text{-}20min}$ ($P<0.01$, $F_{2,17}=4.698$, oneway-ANOVA post hoc LSD) and an increase in the percentage of $AUC_{40\text{-}60min}$ was observed in the TPM100 ($P<0.05$, $F_{2,17}=3.679$, oneway-ANOVA post hoc LSD). Compared to OLZ10 alone, adding TPM50 or TPM100 to OLZ10 did not change the overall glucose response from 0-60min. Compared to Control, OLZ10/TPM50 ($P<0.05$) and OLZ10/TPM100 ($P<0.001$, $F_{3,22}=6.142$, oneway-ANOVA) treatment caused a reduction of the percentage of $AUC_{0\text{-}20min}$ of the glucose response, and like OLZ10 the bulk of the AUC was delayed towards the last 20 minutes (i.e., $AUC_{40\text{-}60min}$) of the assessed response compared to Controls (OLZ10/TPM50: $P<0.01$; OLZ10/TPM100: $P<0.001$, $F_{3,22}=6.264$, oneway-ANOVA post hoc LSD). TPM did not have an additional effect to OLZ20 treatment on overall glucose responses, but both OZL20/TPM50 and OLZ20/TPM100 had altered glucose responses compared to Controls ($P<0.001$, $F_{8,168}=4.208$, GLM rmANOVA). Like OLZ20, both OLZ20/TPM50 ($P<0.01$) and OLZ20/TPM100 ($P<0.01$, $F_{3,23}=5.123$, oneway-ANOVA post hoc LSD) decreased the percentage of $AUC_{0\text{-}20min}$ and increased the percentage of $AUC_{40\text{-}60min}$ compared to Controls (OLZ20/TPM50, OLZ20/TPM100: $P<0.001$, $F_{3,23}=6.985$, oneway-ANOVA post hoc LSD).

Insulin Response

Overall circulating insulin levels during the IGGTT were decreased in the OLZ10 treated group compared to Controls ($P<0.05$, $F_{16,120}=1.752$, rmANOVA post hoc LSD; table 3). However, the insulin response of the OLZ20 group was not affected when compared to Controls (fig. 4A). Total $AUC_{0\text{-}60min}$ was decreased in OLZ10 compared to both Controls ($P<0.05$) and OLZ20 ($P<0.05$, $F_{2,17}=3.302$, oneway-ANOVA post hoc

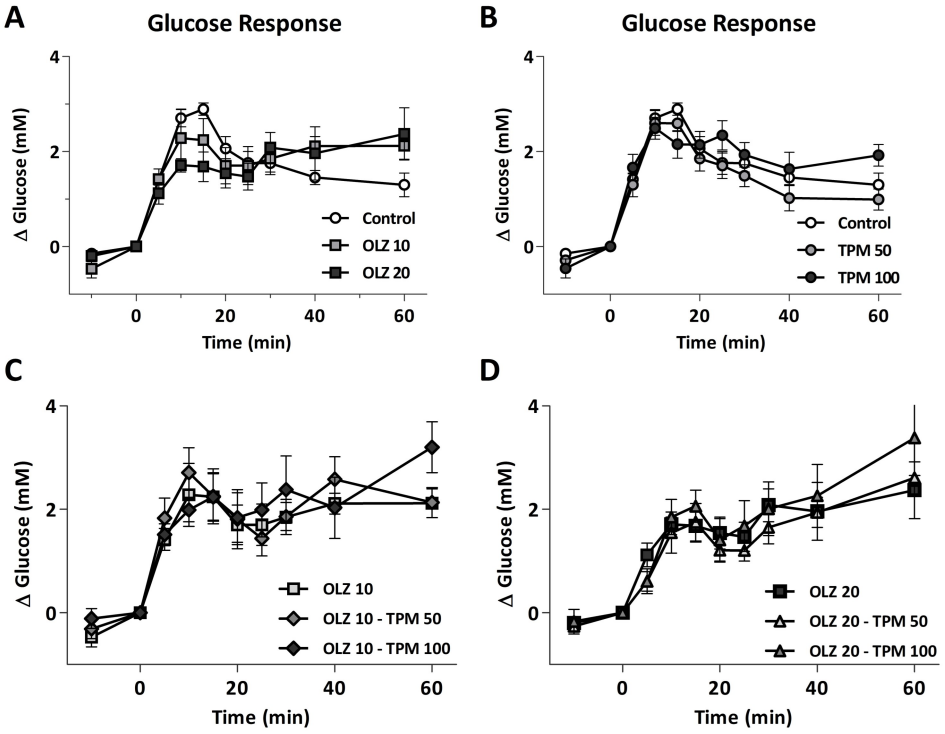

Fig. 3: Acute effect of Olanzapine and Topiramate on circulating glucose levels during an intragastric glucose tolerance test. a) Glucose levels after OLZ 10 and OLZ 20 administration. b) Glucose levels after TPM 50 and TPM 100 administration. c) Glucose levels after co-administration of TPM50/100 with OLZ 10. d) Glucose levels after co-administration of TPM50/100 with OLZ 20. RM-ANOVA post-hoc test did not reveal any significant differences between groups during the period between 0 to 60 min after glucose administration.

LSD), as is shown in table 3. The weight of the percentage of AUC (fig.5B), however, was not changed between OLZ-treated groups and Controls. TPM50 and TPM100 did not significantly affect the insulin response compared to Controls (fig. 4B). In addition, total $AUC_{0\text{-}60min}$ was not different between TPM-treated groups and Controls. However, the percentage of $AUC_{0\text{-}20min}$ revealed an increase following TPM50 treatment compared to both Controls ($P<0.05$) and TPM100 treatment ($P<0.05$, $F_{2,17}=4.285$, oneway-ANOVA). No effects were observed on the percentage of $AUC_{20\text{-}40min}$, whereas the percentage of $AUC_{40\text{-}60min}$ following TPM50 treatment was decreased only compared to TPM100 ($P<0.01$, $F_{2,17}=4.918$, oneway-ANOVA post hoc LSD). Compared to Controls, all groups treated with OLZ10 irrespective of TPM treatment (fig. 4C) showed a change in insulin response ($P<0.05$, $F_{24,152}=1.699$, rmANOVA post hoc LSD). Like OLZ10, both OLZ10/TPM50 and OLZ10/TPM100 decreased total $AUC_{0\text{-}60min}$ compared to Controls ($P<0.05$, $F_{3,22}=2.452$, oneway-ANOVA

Table 3: Area under the Curve: Insulin response.

		TPM		
AUC_{Ins}		0	50	100
OLZ	0	494 ± 58	502 ± 42	445 ± 47
	10	340 ± 30*	339 ± 39*	336 ± 73*
	20	497 ± 56	406 ± 43	451 ± 77

*P<0.05, compared to Control, TPM50, and OLZ20 (oneway-ANOVA post hoc LSD.

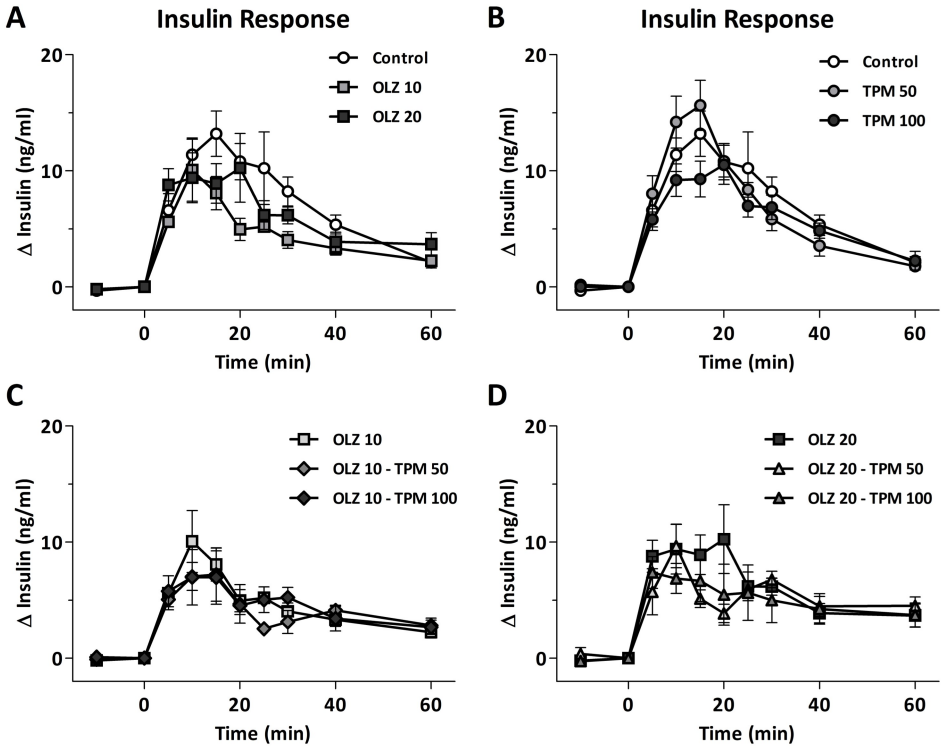

Fig 4: Acute effect of OLZ and TPM on circulating insulin levels during an intragastric glucose tolerance test. A) Circulating insulin levels after OLZ 10 and OLZ 20 administration. B) Circulating insulin levels after TPM 50 and TPM 100 administration. C) Circulating insulin levels after co-administration of OLZ10 with TPM50/100. D) Circulating insulin levels after co -administration of OLZ20 with TPM50/100.

post hoc LSD). The percentage of $AUC_{0\text{-}20min}$ of OLZ10/TPM50 and TPM100 were not different compared to control or OLZ10, but the $AUC_{40\text{-}60min}$ of OLZ10/TPM50 ($P<0.01$) and OLZ/TPM100 ($P<0.05$, $F_{3,22}=3.540$, oneway-ANOVA post hoc LSD) were increased compared to Control. Again no effects were found on $AUC_{20\text{-}40min}$. The insulin responses of the OLZ20 treated groups (fig. 4D) were not significantly different compared to Control treatment. Like OLZ20, total $AUC_{0\text{-}60min}$ following OLZ20/TPM50 and OLZ20/TPM100 treatments were not different from Controls. In addition, the percentage of $AUC_{0\text{-}20min}$ in the OLZ20 treated groups did not show a difference compared to Controls. In contrast to OLZ20, the percentage of $AUC_{40\text{-}60min}$ were increased in OLZ20/TPM50 ($P<0.01$) and OLZ20/TPM100 ($P<0.01$, $F_{3,23}=5.627$, oneway -ANOVA post hoc LSD) compared to Controls.

To identify potential relations between glucose and insulin responses, all glucose and insulin levels within each treatment group were correlated. Pearson's correlations revealed that circulating glucose and insulin levels correlated in Control ($P<0.001$), TPM50 ($P<0.001$), TPM100 ($P<0.01$), OLZ10 ($P<0.01$), and OLZ10/TPM50 ($P<0.05$), whereas in the remaining treatment groups circulating glucose and insulin levels were not correlated (table 4).

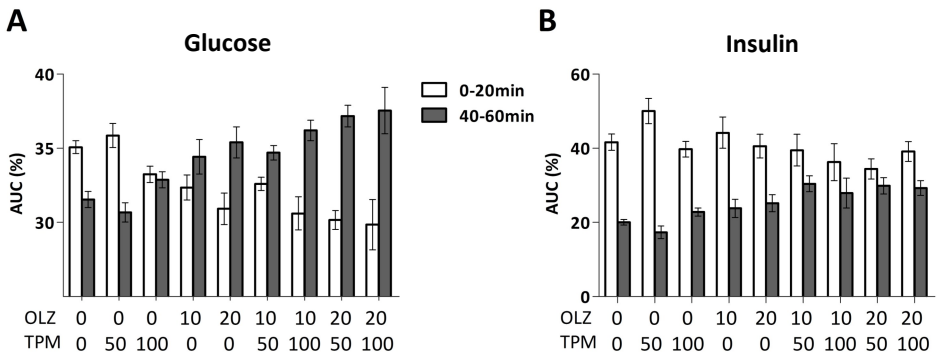

Fig. 5: Glucose and insulin area under the curve expressed as a percentage of the total AUC of the first (white bars) and last (grey bars) 20 minutes of the response by (combinations of) OLZ and TPM. A) Area under the curve glucose response: $\%AUC_{0\text{-}20min}$ of Control > OLZ10[a], OLZ20[b], OLZ10/TPM50[a], OLZ10/TPM100[c], OLZ20/TPM50[b], and OLZ20/TPM100[b]. $\%AUC_{40\text{-}60min}$ of Control < TPM100[a], OLZ10[a], OLZ20[a], OLZ10/TPM50[b], OLZ10/TPM100[b], OLZ20/TPM50[c], and OLZ20/TPM100[c]. B) Area under the curve insulin response: $\%AUC_{0\text{-}20min}$ of TPM50 > Control[a], TPM100[a]; $\%AUC_{40\text{-}60min}$ of TPM50 < TPM100[b]; $\%AUC_{40\text{-}60min}$ of Control < OLZ10/TPM50[b], OLZ10/TPM100[a], OLZ20/TPM50[a], and OLZ20/TPM100[b]. ([a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$; oneway-ANOVA post hoc LSD).

Table 4: Correlation between glucose and insulin levels

		TPM		
r-values		**0**	**50**	**100**
OLZ	0	0,687***	0,793***	0,535**
	10	0,514**	0,467*	-0,311
	20	0,164	0,005	0,342

***P<0.001, **P<0.01, *P<0.05: Pearson Correlation (2-tailed)

Corticosterone response

Circulating corticosterone levels during IGGTT, as presented in fig.6, were stable over the 60 minutes period in the Control group, whereas corticosterone levels were increased in the OLZ10 and OLZ20 ($P<0.01$, $F_{6,45}=3.157$, rmANOVA post hoc LSD) compared to Controls (fig. 6A). Corticosterone levels were increased at 30 minutes in OLZ20 ($P<0.05$, $F_{2,17}=3.696$, oneway-ANOVA post hoc LSD), and at 60 minutes after start of infusion in both OLZ10 and OLZ20 ($P<0.01$, $F_{2,17}=8.359$, oneway-ANOVA post hoc LSD) compared to Control. No dose response relations of OLZ with circulating corticosterone levels were observed. Both TPM50 and TPM100 did not have a significant effect on circulating corticosterone levels during IGGTT (fig. 6B). Nonetheless, at 15 minutes post infusion TPM100 increased corticosterone levels compared to Controls ($P<0.05$, $F_{2,17}=2.608$, oneway-ANOVA post hoc LSD). Both OLZ10/TPM50 and OLZ10/TPM100 increased circulating corticosterone levels (fig. 6C) compared to Control ($P<0.001$) and singly administered OLZ10 ($P<0.05$, $F_{9,57}=2.715$, rmANOVA post hoc LSD). Circulating corticosterone levels were increased in both OLZ10/TPM50 and OLZ10/TPM100 compared to Controls ($P<0.01$) and OLZ10 ($P<0.05$, $F_{3,22}=6.619$, oneway-ANOVA post hoc LSD) at 15min post infusion. Corticosterone levels were increased compared to Controls in all three OLZ10 treated groups at 30 minutes ($P<0.01$, $F_{3,22}=8.996$, oneway-ANOVA post hoc LSD) and 60 minutes ($P<0.001$, $F_{3,22}=20.048$, oneway-ANOVA post hoc LSD). In addition, circulating corticosterone levels in OLZ10 were lower at 60 minutes compared to OLZ10/TPM50 ($P<0.05$). All OLZ20 treated groups (fig.6D) increased circulating corticosterone levels compared to Control ($P<0.01$, $F_{9,60}=4.780$, rmANOVA post hoc LSD), the co-administration of TPM to OLZ20 did not have an additive effect on corticosterone levels. Control circulating corticosterone levels were lower compared to OLZ20/ TPM100 ($P<0.01$, $F_{3,23}=3.064$, oneway-ANOVA post hoc LSD) at 15 minutes. All three

OLZ20 groups had increased circulating corticosterone levels compared to control at 30 minutes ($P<0.01$, $F_{3,23}=3.733$, oneway-ANOVA post hoc LSD) and at 60 minutes ($P<0.001$, $F_{3,23}=14.370$, oneway-ANOVA post hoc LSD) post drug administration. Circulating corticosterone levels 60 minutes after drug administration were negatively correlated with ΔT_{60min} ($r=-0.544$, $R^2=0.296$, $P<0.001$, Pearson 2-tailed), and positively correlated with circulating OLZ_{60min} levels ($r=0.622$, $R^2=0.387$, $P<0.001$, Pearson 2-tailed), $Gluc_{60min}$ levels ($r=0.474$, $R^2=0.225$, $P<0.001$, Pearson 2-tailed), Ins_{60min} levels ($r=0.451$, $R^2=0.203$, $P<0.01$, Pearson 2-tailed), but no correlation was found with circulating TPM_{60min} levels (table 5).

Table 5 shows the correlation between assessed parameters at 15 and 60 minutes after administration. Overall glucose and insulin levels were correlated at both 15

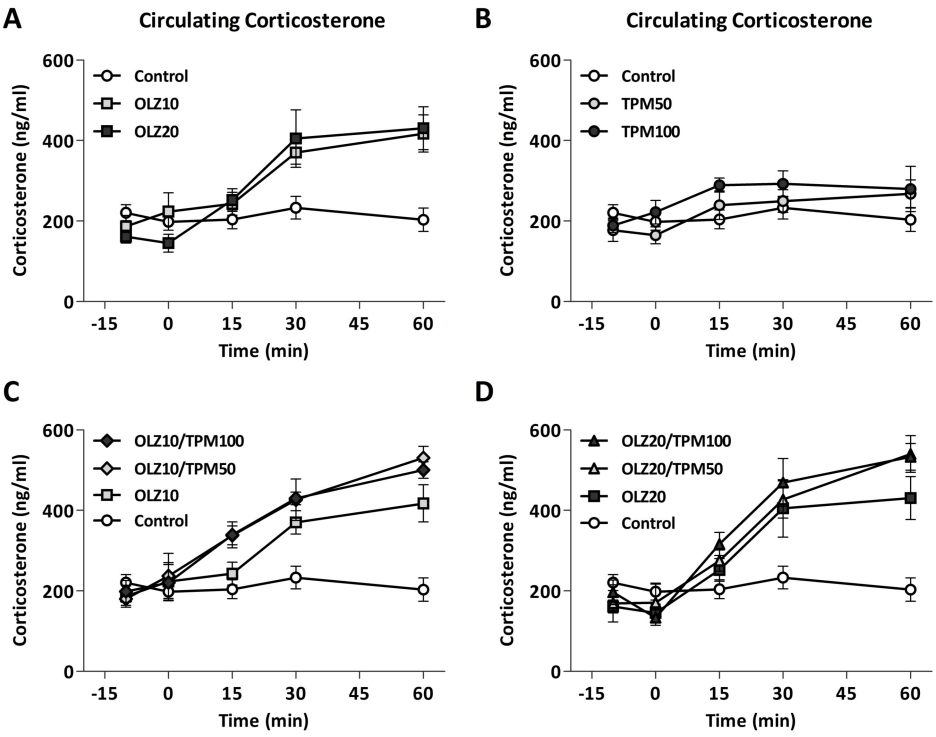

Fig 6: Circulating corticosterone levels during IGGTT after OLZ and TPM co-administration. A) OLZ (10 and 20) significantly increased circulating corticosterone levels compared to Controls ($P<0.01$, rmANOVA post hoc). B) Only TPM 100 increased corticosterone levels compared to Controls at 15min ($P<0.05$; oneway-ANOVA post hoc). C) Corticosterone levels after co-administration of TPM (50-100mg/kg) with OLZ10 were increased compared to Controls ($P<0.01$) and OLZ10 ($P<0.05$, rmANOVA post hoc). D) Corticosterone levels after co-administration of TPM (50-100mg/kg) with OLZ20 were increased compared to Controls ($P<0.01$; rm-ANOVA post hoc).

minutes (P<0.001) and 60 minutes (P<0.001). Unlike Gluc15min, Gluc60min was correlated with circulating OLZ60min levels (P<0.001), Δ Temp.60min (P<0.001), and Cort60min (P<0.001). Ins15min was negatively correlated with OLZ15min (P<0.05) and was positively correlated with Δ Temp.15min (P<0.01), but conversely Ins60min had a positive correlation with OLZ60min (P0<0.01) and a negative correlation with Δ Temp.60min (P<0.01), and was also positively correlated with Cort60min (P<0.01). TPM15min was only correlated with Cort15min, whereas TPM60min showed no correlation with any of the measured parameters. OLZ15min was, additionally to Ins15min, negatively correlated with Δ Temp.15min (P<0.01) and was positively correlated with Cort15min. OLZ60min, Δ Temp.60min, and Cort60min were correlated with all other parameters except to TPM60min.

Conclusions and discussion

In this study we observed that TPM increased circulating OLZ levels. Especially in the OLZ10 group both doses of TPM (50 and 100 mg/kg) increased circulating OLZ levels. In the OLZ20 group a TPM effect was observed, but no differences were found between TPM doses in increasing circulating OLZ. In contrast, OLZ did not affect circulating TPM levels (table 1). Not only did adjunctive TPM increase OLZ peak levels, it also caused a longer half-life time of OLZ in circulation. These data demonstrate that TPM has a specific effect, at least under these doses and circumstances, to increase circulating OLZ levels. The underlying mechanisms are yet to be discovered, but may be related to decreased excretion (e.g. via UDP-glucuronosyltransferase

Table 5: Correlations between parameters measured at 15 and 60 minutes post administration.

60min \ 15min		Glucose	Insulin	Olanzapine	Topiramate	Δ Temperature	Corticosterone
Glucose	Corr.		.469**	-.124	-.027	.258	.027
	Sig.		0.000	0.377	0.849	0.062	0.847
Insulin	Corr.	.582**		-.289*	-.082	.454**	-.054
	Sig.	0.000		0.036	0.565	0.001	0.699
Olanzapine	Corr.	.477**	.388**		.003	-.581**	.347*
	Sig.	0.000	0.004		0.986	0.000	0.011
Topiramate	Corr.	.239	.001	-.036		.004	.389**
	Sig.	0.092	0.995	0.803		0.981	0.005
Δ Temperature	Corr.	-.550**	-.357**	-.763**	-.057		-.280*
	Sig.	0.000	0.009	0.000	0.689		0.043
Corticosterone	Corr.	.474**	.451**	.622**	.040	-.544**	
	Sig.	0.000	0.001	0.000	0.783	0.000	

Above the diagonal line: correlations at 15 minutes post administration; under the diagonal line: correlations at 60 minutes post administration
Correlation is significant at *P<0.05 or **P<0.01 (Pearson Correlation 2-tailed).

pathway) rather than reductions in oxidative capacity of OLZ by TPM.

The increase of OLZ in the circulation by adjunctive TPM administration was reflected in the hypothermic response, which was in fact correlated to the circulating OLZ levels, whether or not boosted by adjunctive TPM administration. The highest dose of TPM (100mg/kg) was particularly potent to amplify the hypothermic response, which indicates non-linear effects of combined OLZ and TPM on body temperature regulation.

Several case reports have mentioned hypothermia in human subjects treated with OLZ, especially during the first days of treatment or when dosage needed to be increased because of return of psychosis scores [32-35]. Cases of hypothermia have been reported even after long-term (seemingly well-tolerated) OLZ treatment [36]. Our animal data suggest that especially at the start of adjunctive TPM treatment caution is advised related to OLZ-induced hypothermia. One case study reports about the adjunctive effect of TPM on OLZ regarding thermoregulation, and observed – in contrast to our observations- a hyperthermic effect [37]. However, in this case report, described by Strawn et al [37], the almost fatal hyperthermia (42.2°C) was observed in an adolescent girl treated with 15mg/day OLZ and 200mg TPM twice a day, after she had been riding her bike during a high ambient temperature (32°C/90°F). Apparently, combined OLZ and TPM treatment compromises thermoregulatory heat loss (by sweating), which may be related to TPM's antagonistic effect on carbonic anhydrase and OLZ's antagonistic properties for the muscarin 3 receptor [38]. In a meta-analysis of Knudsen et al [39] on the adjunctive TPM effects to enhance the risk of hypothermia most commonly associated with valproic acid therapy, it was suggested that carbonic anhydrase antagonism by TPM could lead to metabolic acidosis via a decrease of HCO_3^-. In turn this would affect $GABA_A$ conductance sensitive to pH and mediate hypothermia [40]. Another possible mechanism via which TPM enhances OLZ-induced hypothermia is that brown adipose tissue (BAT) thermogenesis, which is sympathetically regulated from the raphe pallidus (RPa), is increased by microinjection of the $GABA_A$ antagonist bicuculline in the RPa [41]. Stefanidis et al [42] demonstrated that OLZ blocks BAT thermogenesis. At the level of the RPa, TPM might enhance the induced hypothermia by blocking counter-regulatory mechanisms, including attenuation of sympathetic outflow via $GABA_A$ neurons. Thus, besides the increased circulating levels of OLZ induced by TPM, which causes profound hypothermia, TPM at the dose of 100mg/kg could also disable counter-regulatory responses to compensate OLZ-induced hypothermia resulting in an enhanced hypothermic response.

In this study, we observed that the hypothermic response induced by OLZ and

adjunctive TPM administration was correlated to increased levels of circulating corticosterone. The absence of a correlation between circulating corticosterone levels and circulating OLZ levels may be explained by a ceiling effect in which the corticosterone response was already maximally stimulated at the lower dose of OLZ with adjunctive TPM50. It seems plausible to assume that the corticosterone response was aimed at compensating OLZ-induced hypothermia, analogous to the corticosterone release after administration of the hypothermic 5-$HT1_A$ agonist 8-OH-DPAT [43]. In the latter case, raising corticosterone levels by exogenous administration was indeed capable of attenuating 8-OH-DPAT induced hypothermia [44]. Whether the observed ceiling effect to raise endogenous corticosterone release is the result of TPM inhibiting the HPA axis remains to be investigated (although some evidence exists that this is the case, see Yehuda et al, 2004 [45]). Albaugh et al previously observed that acute OLZ and adjunctive TPM treatment blunts glucose and insulin responses [23], and our data is largely consistent with these findings. While these results may be explained to indicate that OLZ and/or TPM increase glucose utilization and/or insulin sensitivity, this idea would conflict with other studies showing that OLZ attenuates insulin signaling [25,46]. Closer inspection of our data revealed that the bulk of especially the glucose AUC of the OLZ and adjunctive TPM treated groups shifted from the first 20 minutes ($AUC_{0\text{-}20min}$) to the last 20 minutes ($AUC_{40\text{-}60min}$) of the glucose response. Correlations also revealed that in the control and TPM groups increased glucose levels were highly correlated with increased insulin levels, however, this correlation was lost when OLZ was added in combination with TPM. However, increased glucose levels at 60 minutes after administration were correlated with increased OLZ levels. Surprisingly, increased OLZ levels were negatively correlated with insulin levels at 15 minutes after administration. Therefore, we hypothesized that reduced glucose uptake from the gastrointestinal (GI) tract by OLZ was the key mechanism by which OLZ affected glucose and insulin responses. In a follow-up study (not presented here) we indeed found that OLZ dose dependently disrupts glucose uptake from the gut, primarily via the attenuation of GI peristalsis as a result of OLZs antagonistic properties at the 5-$HT2_{A/C}$ and M3 receptor [47]. While other studies need to address OLZ's effect on gastrointestinal functioning, it is worthy to mention here that SGAs with relatively high anticholinergic affinity, like OLZ, are related to ileal obstruction [48] induced by a failure of peristalsis. Whereas the blunted glucose peak in the OLZ and adjunctive TPM treated groups could be the result of attenuated glucose uptake from the GI-tract, the hyperglycemia observed between 40 and 60 minutes could be a consequence of corticosterone-induced gluconeogenesis and peripheral insulin resistance. Girault et al [25] demonstrated that during intragastric OLZ infusion (3mg/hr) both circulating

corticosterone levels and endogenous glucose production increased; while simultaneously a decrease of hepatic insulin sensitivity was observed. In addition, Johnson et al [49] showed that OLZ in vitro is capable of blocking carbachol-induced insulin secretion via M3 receptor antagonism. It is possible in our study that between 40 and 60 minutes during the glucose response OLZ blocked insulin secretion, resulting in the comparatively low insulin levels, and reduced hepatic glucose uptake while increasing endogenous glucose production resulting in the observed hyperglycemia.

One of the differences in the study of Albaugh et al [23] and ours is that in their study OLZ was already administered one day before the start of the glucose tolerance test and consequently baseline glucose levels were already decreased after 5hrs fasting in their OLZ treated group compared to controls, which might have affected the glucose response during the oral glucose tolerance test. The disadvantage of administering glucose via the GI tract is that one does not control for glucose uptake into the circulation, especially when discrimination between endogenous glucose production and administered glucose is not taken into account. As a result one cannot be certain if the amount of insulin secretion is based on an equal amount of glucose in the circulation and therefore conclusions about insulin sensitivity cannot be drawn.

In this study, we observed that that TPM alone did not have an acute effect on circulating glucose and insulin levels compared to Controls. Although TPM has been shown to enhance insulin sensitivity by interfering directly with the insulin signaling cascade, it may be possible that that beneficial effect on glucose regulation and insulin sensitivity after chronic treatment is also mediated by TPM-induced weight loss [7,8].

In conclusion, we found that adjunctive TPM treatment in rats caused marked increases in circulating OLZ levels. The mechanism via which this occurs still needs to be elucidated, but it is unlikely that hepatic drug metabolization by cytochrome P540 isozymes are involved. It is out of the scope of this article to conclude which mechanisms may play a role, and if any, they could also be different in rats from humans. Furthermore, the impact of OLZ in combination with TPM on thermoregulation could possibly put patients at risk under thermal stress conditions, like extreme heat or cold. Our study also shows that the half-life time of OLZ is increased by adjunctive TPM administration, which grants the possibility to reduce OLZ dosing when co-administered with TPM, like Vieta et al's study already showed [15]. TPM's mood stabilizing properties and beneficial weight reducing effect, in combination with the positive reporting in the existing literature, still makes it a suitable candidate as adjunctive to OLZ treatment.

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