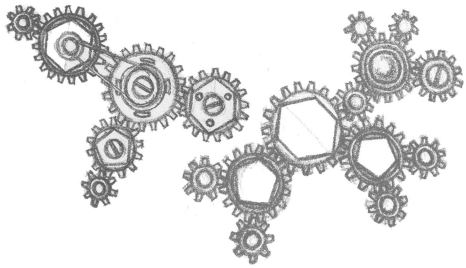

Chapter 8:

Humans

A low TSH profile predicts Olanzapine-induced weight gain and relief by Topiramate in healthy male volunteers.

S.S. Evers[2], A.A. van Vliet[1], B. van Vugt[1], A.J.W. Scheurink[2], G. van Dijk[2,3]

[1.]PRA Health Sciences, Zuidlaren, The Netherlands.
[2]University of Groningen, Dept. of Neuroendocrinology, Groningen, The Netherlands
[3]University of Groningen, ESRIG Center for Isotope Analysis, Groningen, The Netherlands

Abstract

Second generation antipsychotics, like olanzapine (OLZ), have become the first line drug treatment for patients with psychotic disorders, specifically schizophrenia. However, OLZ treatment is often associated with body weight gain, hyperglycemia, insulin resistance, elevated appetite and food intake, elevated cholesterol levels, and acute hypothermia. Therefore, the search for an adjunctive treatment to inhibit OLZ's negative metabolic effects has been of major interest. One potential candidate is the anticonvulsant Topiramate (TPM) used for the treatment of epilepsy, but also known for its weight reducing effects. Several studies have indeed reported the efficacy of adjunctive TPM administration to OLZ treatment, but whether comorbidities associated with OLZ treatment are affected by TPM is less clear.

The aim of this study was to investigate in healthy male volunteers whether adjunctive TPM administration opposes OLZ-induced weight gain over the course of 14 days also counters the metabolic co-morbidities associated with OLZ treatment. In addition, we investigated behavioral, endocrine and metabolic characteristics as potential predictive factors for weight regulation and/or metabolic derangements associated with OLZ and TPM treatment.

While adjunctive TPM indeed reduced OLZ-induced weight gain (with carbohydrate intake at breakfast as a major contributor), metabolic derangements associated with OLZ treatment were not affected by TPM. Using multiple regression analysis, cumulative food intake interacting with morning OLZ levels most profoundly explained variation in BW gain over the course of drug treatment, without a discrimination between body fat or body fat free mass as contributing factors. Neither TPM treatment, or its circulating levels, contributed to variation in BW gain. Besides food intake, also daily plasma TSH levels explained variation in OLZ-induced BW gain. In fact, individuals with a low daily plasma TSH profile already before the start of drug treatment were most sensitive to OLZ-induced weight gain, whereas individuals with a high daily plasma TSH profile did not gain weight over 14 days of OLZ treatment. Furthermore, individuals with a low plasma TSH profile were also those that were sensitive to adjunctive TPM treatment blocking BW gain and reducing OLZ's negative effects on HOMA-IR and meal-induced insulin responses. Because improvement of brief psychiatric rating scores (BPRS) are associated with OLZ-induced body weight gain, but body weight gain is a threat to compliance, adjunctive TPM treatment might be a solution especially for individuals most responsive to OLZ's beneficial effects on treating schizophrenia.

Key words: Olanzapine, Topiramate, body weight, thyrotropin, TSH, T3, T4, HOMA-IR, insulin, glucose, body temperature.

Introduction

Second generation ('atypical') antipsychotics have become the first line drug treatment for patients with psychotic disorders, specifically schizophrenia [1]. Among these, Olanzapine (OLZ) is commonly used and known for its clinical efficacy, but it is unfortunately associated with a number of metabolic side effects threatening treatment compliance [2]. Specifically, OLZ causes body weight (BW) weight gain and a number of metabolic changes collectively called the metabolic syndrome [3,4]. These changes include hyperglycemia, insulin resistance, elevated appetite and food intake, elevated cholesterol levels, and acute hypothermia [5-10].

Body weight gain as a result of OLZ treatment can be remarkably large and, together with the metabolic effects, increases the risk for Diabetes Mellitus (DM). Individuals suffering from schizophrenia already have a predisposition for developing DM without treatment with antipsychotic agents [11] and metabolic syndrome and cardiovascular diseases are important causes of morbidity and mortality among patients with severe mental illnesses [12]. Therefore the search for an adjunctive treatment to reduce OLZ-induced body weight gain, and concomitant reduction of metabolic side effects, without affecting clinical efficacy, is of major interest.

It was recently discovered that topiramate (TPM), an anticonvulsant prescribed mainly for the treatment of epilepsy, but also used as a drug for weight loss, is capable of controlling olanzapine-induced weight gain in schizophrenic patients without aggravation of psychotic symptoms [13,14]. TPM is thought to have its anticonvulsant effects through a blockage of voltage-dependent sodium channels, antagonism of AMPA/kainate subtype of the glutamate receptor, an augmentation of gamma-aminobutyrate acid (GABA) activity for some subtypes of the GABA-A receptors and inhibition of the carbonic anhydrase enzyme, mainly isozymes II and IV [15]. Which of the mentioned mechanisms causes weight loss is still unknown. Nonetheless, co-administration of TPM with OLZ blunted weight gain, caused a reduction of appetite, improved insulin sensitivity and a caused decreases in fasting blood glucose, total cholesterol, triglycerides, high-density lipoprotein (HDL)-cholesterol and leptin levels in schizophrenic patients [14].

Although the phenomenon of weight gain by OLZ is well recognized, not all individuals are equally prone to these adverse side effects [16], and a precise understanding of the mechanisms fails. OLZ is a thienobenzodiazepin derivate and it is thought to exert its effects through antagonism of the serotonergic 5HT2,3,6 and dopaminergic D1,2 receptors [17,18]. OLZ is also known to antagonize histamine, muscarine and α-adrenergic receptors [19]. A high affinity for the 5-HT2C and the

histamine H1 receptors, while antagonizing the peripheral M3 muscarinic receptor and effects on central 5-HT2C receptors, may potentially be related to treatment of emergent diabetes observed independent of obesity [20]. Furthermore, OLZ affects a number of neuroendocrine factors released from the pineal, pituitary, gonadal, adrenal, or thyroid glands [21-23]. Despite this knowledge regarding potential mechanisms responsible for the metabolic side effects, there is little known about the within-population variation of responsiveness of such endocrine pathways to OLZ treatment. When a specific pathway can be identified as a predictor for drug-induced side effects, it should be possible to decrease the occurrence of unwanted side-effects, either by excluding patients that are at a high-risk for developing negative side-effects or potentially by treating these patients with a specific adjuvant countering these side-effects.

The first aim of this study was to investigate the potency and mechanisms by which adjunctive TPM administration reduces OLZ-induced body weight gain and related metabolic side effects in healthy male volunteers. Secondly, to investigate whether baseline parameters can be identified as predictive factors explaining OLZ-induced BW gain and co-morbidities. The data reported here confirm that TPM attenuates OLZ-induced body weight gain. Subsequently, increased body weight gain was found to be the main denominator of metabolic derangements, like increased HOMA-IR levels. Finally, a low daily plasma TSH profile before starting OLZ treatment highly predicted OLZ-induced weight gain. In addition, adjunctive TPM treatment was particularly effective in inhibiting OLZ-induced BW gain, and attenuating OLZ-induced metabolic side effects, also in subjects with low daily plasma TSH profile prior to treatment. Altogether, the data suggest that low plasma TSH levels can serve as a predictor for the responsiveness to OLZ treatment in individuals, who then also benefit mostly from adjunctive TPM treatment in order to attenuate the metabolic side effects associated with OLZ treatment.

Methods

Study design: This was a randomized, double-blind, placebo-controlled clinical study conducted at PRA Clinics in Zuidlaren in The Netherlands. The independent ethics committee Foundation BEBO in Assen, the Netherlands, approved the clinical study protocol. All subjects provided written informed consent before participation and the study was conducted in accordance with the principles of the Declaration of Helsinki, and with the laws and regulations of the Netherlands. This work was performed within the framework of the Dutch Top Institute Pharma project: T2-105. EudraCT number: 2010-019664-37.

Study procedures: Study medication was administered double blind by personnel not involved in the preparation of the medication.

Treatments: Each subject participated in a 14-day treatment period in house and was dosed 10 mg OLZ o.d. with TPM (or placebo) at 25 mg b.i.d. during Days 1-6 and 50 mg b.i.d. during Days 7-14. Recovery from effects was assessed in a follow-up visit on Day 28 after washout of all medication had occurred.

This multiple dose evaluation was conducted in two cohorts of 15 healthy male volunteers each.

It was assumed that the trial medication OLZ plus TPM could have strong CNS effects such as extreme sedation and sleepiness, which might cause subjects to drop-out and make the results impossible to evaluate. For that reason a gradual increment in dosing of TPM was chosen in this study. Drug doses were selected on the basis of prior dose-response studies consistent with this study [24], in order to minimize side effects and yet still reach clinically relevant therapeutic levels.

Subject demographics and disposition: Thirty healthy male subjects were randomized to receive OLZ plus placebo (n=15) or OLZ plus TPM (n=15) treatment. Baseline demographics and characteristics (Table 1) were comparable across treatment groups. Key exclusion criteria were evidence of clinically relevant pathology, history of psychiatric diseases, significant psychopathology in first grade family members, and positive screen on drugs of abuse or alcohol intake of more than 24 units of alcohol per week.

Body weight was measured at Day -2 (baseline), 13 and 28 at 9:00 am just before drug dosing. Caloric intake was recorded continuously on a daily basis. Besides the standardized meals subjects were allowed to eat ad libitum.

Table 1: Descriptive statistics of demographic characteristics

Variable	Classes	n (%)	mean	stdev	CV	median	min	max
Age		30	36	11	30%	37	20	54
Weight (kg), at screening		30	84,1	11,8	14%	82,5	66,3	112,2
Height (cm)		30	181	8	4%	180	170	199
Body Mass Index		30	25,5	2,5	10%	25,5	21,7	29,6
Gender	Male	30 (100%)						
Ethnicity	White	24 (80%)						
	Asian	2 (7%)						
	Black	2 (7%)						
	White/black	2 (7%)						

Laboratory investigations: Blood samples for assessment of OLZ levels were taken on day 1 of treatment, four hours after dosing at 9:00 am, at day 6 (at 9:00 am at dosing and four hours later) and at day 13 (at 9:00 am at dosing and four hours later). Levels of TPM were assessed only at days 6 and 13 at 9:00am (for assessment of baseline level). Blood samples for glucose, insulin and C-peptide were taken just prior to dosing at 9:00 am and at 0.5, 1, 1.5, 2, 2.5 and 3 h after a meal tolerance test 1hr post dosage (MTT; Ensure Plus® challenge) on Days -1, 6, 14 and 28 (the prior to dosing time point on Days -1 and 28 was chosen at a time similar to the time points on dosing days). For determination of glucose, insulin and C-peptide, blood samples of 4 mL per time point were taken into sodium-heparin tubes. Analysis of serum glucose, insulin, C-peptide, total cholesterol, high-density lipoprotein-bound cholesterol [HDL-cholesterol], low-density lipoprotein-bound cholesterol (LDL-cholesterol), serum triglycerides were analyzed in blood samples on days -1, 6, 14, 28 and performed according to the following methods:

OLZ and TPM levels were measured by HPLC-MS from whole blood (0.5ml) at the University Medical Center Groningen, Dept. of Clinical Pharmacy and Pharmacology.

Serum glucose: quantitative determination of glucose in serum was done on the ADVIA 1800 Chemistry systems via the enzymatic method by Slein1,2 using hexokinase and glucose-6-phosphate dehydrogenase enzymes. Normal range: 4.1-5.9 mmol/L, critical value (CV) 1.1-2.3 %.

Insulin: for quantitative determination of Insulin in serum the ADVIA Centaur XP Insulin assay was used, a two-site sandwich immunoassay using direct chemiluminescent technology which uses constant amounts of two antibodies. Sensitivity and Assay Range was 0.5–300 mU/L. Normal range: 3.0-25.0 mU/L l, CV 6.3 -7.5 %

C-peptide: for quantitative determination of C-peptide in serum the IMMULITE 2000 Systems on heparinized plasma was used. Normal range 0.9-7.1 ng/ml (0.3 -2.4 nmol/ L; 298-2,350 pmol/L), CV within-run 1.7%–2.3%.

Total cholesterol: for quantitative determination of total cholesterol in serum on the ADVIA 1800 Chemistry systems was used according to the CHOL_2 method which is based on an enzymatic method using cholesterol esterase and cholesterol oxidase conversion followed by a Trinder endpoint. Normal values < 5.18 mmol/l, CV 0.5-1.1%

HDL-cholesterol: for quantitative determination of HDL cholesterol in serum and plasma the on the ADVIA 1800 Chemistry systems was used according to the Direct-

HDL Cholesterol method that measures HDL cholesterol without prior separation, based on an enzymatic method developed by Izawa, Okada and Matsui. Normal values >1.6 mmol/l, CV 2.1-2.5 %

LDL-cholesterol: for quantitative determination of LDL cholesterol in serum the ADVIA 1800 Chemistry systems was used. Normal values < 2.6 mmol/l, CV 0.5-1.1%

Triglycerides were measured on the ADVIA 1800 Chemistry systems according to the method based on the Fossati three-step enzymatic reaction with a Trinder endpoint. The single-reagent procedure quantitates the total triglycerides including the mono and diglycerides and the free glycerol. Normal value: < 2.83 mmol/l .

Daily energy expenditure was calculated using the doubly-labeled water method as previously described by Speakman [25]. Determination of $^{2}H/^{1}H$ and $^{18}O/^{16}O$ ratios in saliva and blood samples were performed at the Center for Isotope Research, University of Groningen. Samples were prepared by micro-distillation in a vacuum line trapping the emerging water vapor in a cooled (with liquid nitrogen) glass vial. Water samples from these glass vials were then automatically injected into a Hekatech High Temperature Pyrolysis unit [26], in which the injected water reacted with glassy carbon at a temperature of 1420 °C. The resultant H_2 and CO gasses, emerging into a continuous helium flow through the system, were then led through a gas chromatography column to separate the two gases in time and finally fed into a GVI Isoprime Isotope Ratio Mass Spectrometer for the analysis of $\delta^{18}O$ and $\delta^{2}H$. Typical relative duplicate differences were below 2.5% for $\delta^{2}H$, and 1% for $\delta^{18}O$.

Homeostasis model assessment of insulin resistance (HOMA-IR) was estimated as described by Matthews et al [27]. (HOMA-IR = fasting insulin (mU/l)*fasting plasma glucose (mmol/l)/22.5). These were calculated either as absolute levels or as percent change %HOMA-IR from baseline (at day -1).

Thyroid hormone activity. Because particular emphasis is put in this study on the role of thyroid hormones in treatment efficacy of OLZ and TPM, plasma levels of TSH, T3 and T4 were determined at several days (-1, 1, 6, 13, and 14) during the study at time points 0, 3, 5, and 7 hours after drug administration at 9:00 AM. Because circadian patterns show a nocturnal rise in these hormones [28], and decline in the morning , an area under the curve (AUC) for each individual on the days of sampling was calculated (from 0-7 hrs after drug dosing) and used as the parameter for plasma thyroid hormone profile (=AUC of daily thyroid hormone levels). Besides the absolute AUCs also the percent change (%AUC) from baseline (at day -1) was assessed.

Thyrotropin (TSH): for quantitative determination of thyroid-stimulating hormone (TSH, thyrotropin) in serum, heparinized plasma, and EDTA plasma the ADVIA Centaur

XP TSH3-Ultra assay was used, a third-generation assay that employs anti-FITC monoclonal antibody covalently bound to paramagnetic particles, an FITC-labeled anti-TSH capture monoclonal antibody, and a tracer consisting of a proprietary acridinium ester and an anti-TSH mAb antibody conjugated to bovine serum albumin (BSA) for chemiluminescent detection. Normal for adults: 0.55–4.78 µIU/mL (mIU/L); CV 5.13-6.64%.

Triiodothyronine (T3): for quantitative determination of T3 in serum the ADVIA Centaur XP systems was used. Sensitivity and Assay Range was 0.15–12.3 nmol/L nmol/L. Normal values 0.92–2.79 nMol/l; CV 1.84-3.44 %. Thyroxine (T4): for quantitative determination of T4 in serum the ADVIA Centaur XP systems was used. Sensitivity and Assay Range was 3.9 – 387 nmol/L; normal range: 58.1 to 140.6 nmol/L).

Statistical analysis was performed using SPSS20.0. Within group testing was done with paired t-test (2-tailed), between groups analyses were performed using rm- or oneway-ANOVA post hoc LSD. Relations between parameters were performed either using Pearson 2-tailed correlation (R^2) or by using linear regression analyses (ANOVA). Stepwise multiple regression analysis was used to define which parameters best explained the variation of the dependent variable of interest. To test for normal distribution the Shapiro-Wilk test was performed. Differences were considered significant at $P<0.05$. Graphs were illustrated using Graphpad Prism 5.0. Results are expressed as average ± sem, unless stated otherwise.

Results

1. Circulating OLZ and TPM levels.

Circulating OLZ levels were increased on day 1 at 4 hrs after dosing at 9:00AM, and steadily increased on day 6 (both at baseline right before dosing at 9:00AM and 4hrs later) and day 13, the latter reflecting the fluctuation at day 6 at slightly higher levels (see fig. 1A). Levels of TPM at dosing at 9:00AM were increased on day 6 and further increased on day 13 reflecting an increased dosing from 25 mg to 50 mg b.i.d at day 7 till the end of treatment (see fig. 1B). Adjunctive TPM administration did not have an effect on circulating OLZ levels as is illustrated in fig. 1A.

2. General effects of OLZ and adjunctive TPM treatment

Pre-treatment body weight (at day -2) was 85.1±12.7 kg for the OLZ group and 84.3±12.3 kg for the OLZ+TPM group (table 2). Figure 2a shows the changes in body weight (ΔBW) over the course of treatment. Within group analyses revealed that the

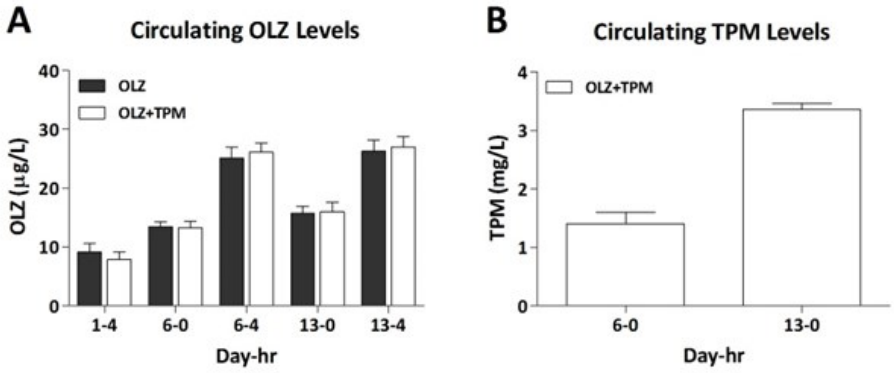

Fig. 1: Circulating Olanzapine and Topiramate levels. A) OLZ levels assessed at day 1 (4hrs after administration at 9:00AM), day 6 and day 13 (both 0/4hrs after dosing at 9:00AM). B) TPM levels were measured at day 6 and 13 at 9:00AM. A) Adjunctive TPM treatment did not affect circulating OLZ levels. B) Circulating TPM levels (n=15) rose from day 6 towards day 13.

Table 2: OLZ and OLZ+TPM, group averages±sem

	Day	OLZ	OLZ+TPM
BW (kg)	-2	85.1±12.7	84.3±12.3
	13	86.6±11.9#	84.6±13.3
	28	87.5±12.4##	85.3±12.9##
Δ BW (kg)	13	2.40±0.53#	1.08±0.37
Adiposity (%)	13	19.65±1.50	19.14±1.37
Daily Caloric Intake (kcal)	Avg	2652±105	2487±114
Carbohydrate Breakfast (kcal)	Avg	93.7±4.9	78.8±4.6*
Energy Expenditure (kcal/day)	6-13	2324±90	2309±125
HOMA-IR	-1	1.96±0.23	2.12±0.31
	14	3.16±0.31	2.84±0.38
Insulin (AUC)	-1	127±22	114±17
	14	154±28	171±32
Glucose (AUC)	-1	37.0±1.2	37,6±1.1
	14	39.0±1.1	38,9±0.7
Δ Temperature (°C)	1	-0.48±0.09	-0.29±0.08

Avg: Daily average; AUC: Area under the curve.
#P<0.05, paired t-test compared to day -2
##P<0.05, paired t-test compared to day -2 and day 13
*P<0.05, oneway-ANOVA, OLZ > OLZ+TPM

OLZ-treated subjects gained weight over 13 days of treatment (P=0.023; t_{14}=-2.562, paired t-test), whereas OLZ+TPM treated subjects did not significantly increase weight over 13 days of treatment compared to their body weight at arrival (P=0.538; t_{14}=-0.631, paired t-test). Both groups had, compared to their initial body weight, an increased body weight at day 28 (OLZ: P<0.01, t_{14}=-4.522; OLZ+TPM: P<0.05, t_{14}=-2.895), which was fourteen days after treatment termination. Both groups also had an increase in body weight at day 28 compared to day 13 of treatment (OLZ: P<0.01, t_{14}=-3.514; OLZ+TPM: P<0.05, t_{14}=-2.369). Between groups, no difference was observed over the duration of treatment (rm-ANOVA) or between single time points.

The magnitudes of ΔBW (at day 13 vs day -1) segregated according to tertiles (n=10 each) of "gain", "stable" and "loose" (according to Ascher-Svanum *et al* [30]) irrespective of treatment are shown in Figure 2B. We observed that subjects treated with OLZ vs those treated with OLZ+TPM were differently distributed over the three ΔBW groups (mean rank: 18.83/12.17; P<0.05, Mann-Whitney U). Specifically, the Gain group consisted of more OLZ-treated than OLZ+TPM treated subjects, whereas the Loose group consisted predominantly of OLZ+TPM treated subjects (see panel 2C). While the segregation was done according to ΔBW on day 13, repeated measures ANOVA revealed that ΔBW differed significantly between all three groups over the entire period ($F_{4,54}$=23.289, P<0.001; rm-ANOVA post hoc LSD), as is illustrated in fig. 2B.

Besides the effect of adjunctive TPM treatment to attenuate OLZ-induced BW gain, a treatment effect was found on the amount of carbohydrate intake during breakfast over the course of drug treatment (P<0.05, $F_{1,29}$=4.855, oneway-ANOVA), which was significantly elevated in the OLZ-treated subjects compared to the OLZ+TPM treated subjects. As displayed in table 2, no main effects were found of adjunctive TPM treatment on adiposity, daily caloric intake, daily energy expenditure (assessed by DLW analysis), HOMA-IR, AUC-glucose, AUC-insulin, and temperature response, or any other assessed parameter not listed in table 2.

3. Thyroid profiles.

Over the course of OLZ treatment, AUC-TSH remained rather stable, and adjunctive TPM treatment did not significantly alter these responses. Closer inspection revealed that the total data set at baseline was bimodally distributed (P<0.01, df_{28}=.879, Shapiro-Wilk), with subjects having either high or low AUC-TSH values at day -1 almost equally distributed over the OLZ and OLZ+TPM groups. Because of this finding we decided to group them accordingly in the OLZ and OLZ+TPM groups into high and low AUC-TSH subgroups. On day -1, the magnitude of the AUC-TSH value was highly

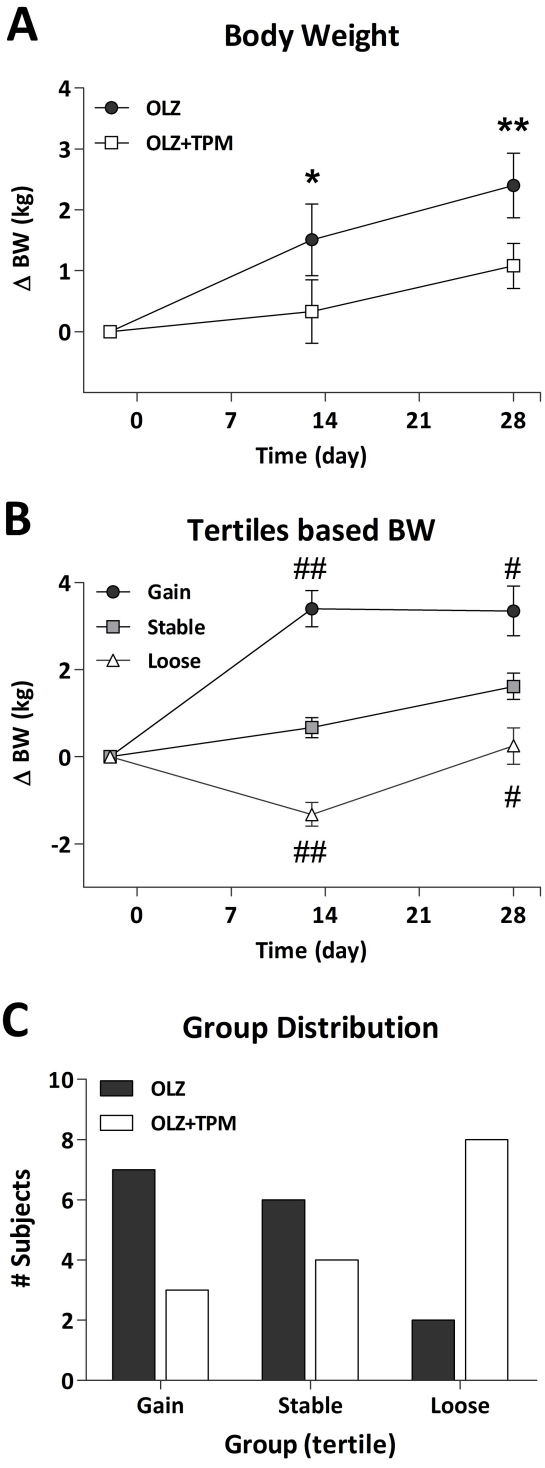

Fig. 2: Delta body weight. A). Changes in body weight (ΔBW) represented in the OLZ and OLZ+TPM over the course of 14 day treatment, and over the course of the wash-out on day 28. OLZ treated subjects showed an increase in BW after 13 days of treatment compared to initial BW (*P<0.05; paired t-test). Compared to day -2 both groups showed increased BW at day 28 (**P<0.01, *P<0.05; paired t-test), fourteen days after treatment termination. B) The thirty subjects segregated into groups of n=10 each that either gained body weight (Gain), remained a stable body weight (Stable), and those that lost body weight (Loose) irrespective of treatment. This segregation illustrating the variation in ΔBW responses between these groups (P<0.001, rm-ANOVA post hoc LSD) irrespective of treatment, and is among others relevant with respect to the number of OLZ and OLZ+TPM subjects in each group as shown in panel C. Compared to the Stable group the Gain and Loose group showed respectively increased or decreased body weights at day 13 and 28 ($^{\#}$P<0.05, $^{\#\#}$P<0.01, oneway-ANOVA post hoc LSD). C) Subjects treated with OLZ vs those treated with OLZ+TPM were differently distributed over the three ΔBW groups (mean rank: 18.83/12.17; P<0.05, Mann-Whitney U). Specifically, the Gain group consisted predominantly of OLZ-treated subjects (OLZ/TPM: 7/3), whereas the Loose group was largely constituted by OLZ+TPM treated subjects (2/8). The Stable group showed an equal distribution of subjects from both treatment groups (6/4).

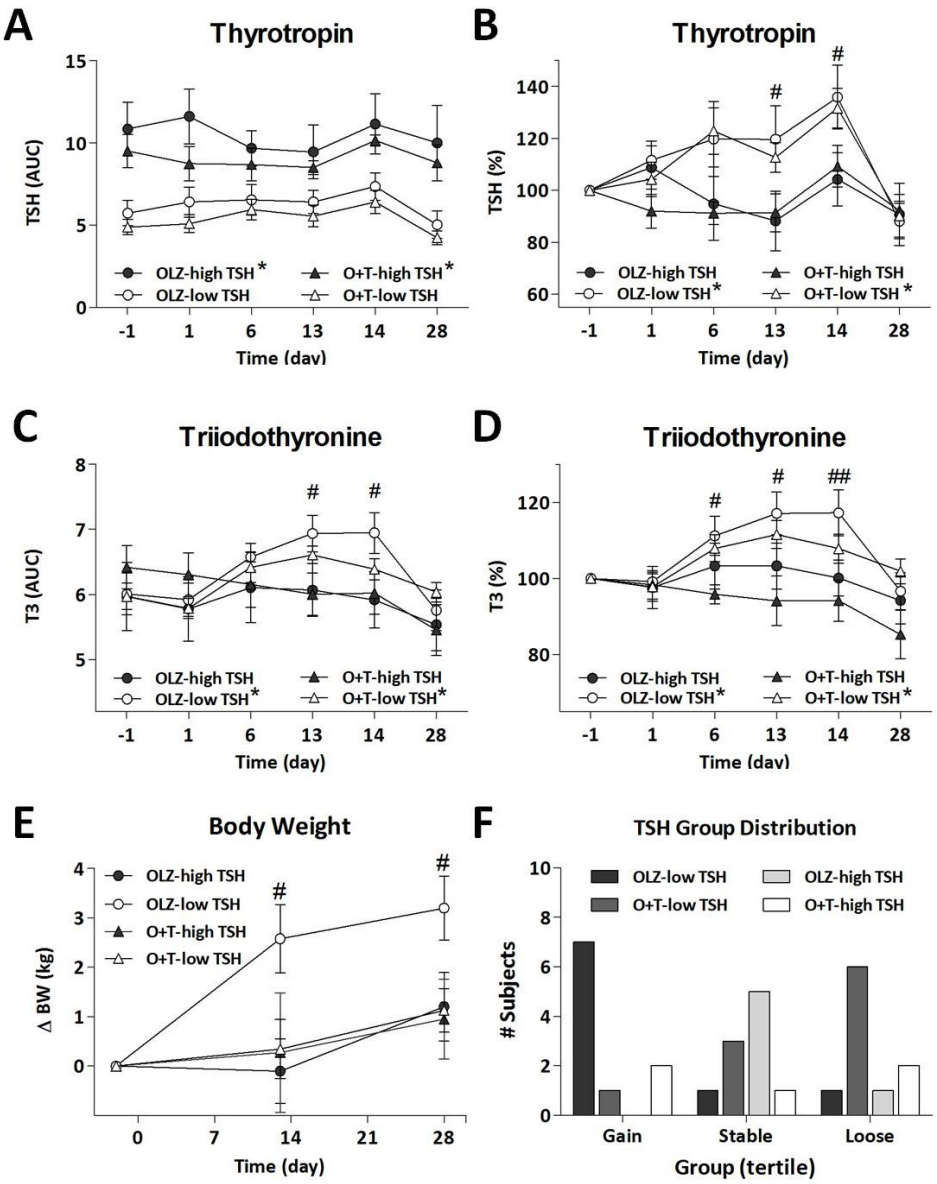

Fig. 3: Thyroid AUC values during treatment. A) Thyrotropin (TSH) AUC values divided in high or low AUC-TSH subgroups at day -1 were different over time (*P<0.01, rm-ANOVA). TPM did not affect AUC-TSH values during treatment. B) Percentual change of AUC-TSH levels relative to day -1 (%AUC-TSH) values were increased in the AUC-TSH low subgroups during treatment compared to the AUC-TSH high subgroups (*P<0.05, rm-ANOVA); between groups an increase was observed at day 13 and 14 in AUC-TSH low subgroups compared to the AUC-TSH high subgroups ($^{\#}$P<0.05, oneway-ANOVA). C) Triiodothyronine (T3) AUCs divided on the basis of high/low AUC-TSH subgroups assessed at day -1 revealed an increase of AUC-T3 values during

correlated to the height of the plasma TSH concentration at t=0 (9:00 am day -1: P<0.001, r=.783, Pearson 2-tailed). Meaning that basal circulating TSH levels were predictive of the total AUC-TSH measured of 7 hours (see methods). Statistical analyses revealed that the OLZ and OLZ+TPM groups both with high AUC-TSH values at day -1 had continuously high AUC-TSH values during the study period compared to both low AUC-TSH groups (P<0.01, $F_{5,130}$=3.350; rm-ANOVA, figure 3A). Viewing the percentage change of the AUC-TSH value (%AUC-TSH) during OLZ/TPM treatment relative to the baseline AUC-TSH at day -1, this revealed that in both low AUC-TSH groups the %AUC-TSH value increased during the study (P<0.01, $F_{5,130}$=3.374; rm-ANOVA, figure 3B), irrespective of TPM treatment.

AUC-T3 values were not affected by TPM treatment, and baseline levels were not bimodally distributed as in the case of AUC-TSH profiles (fig 3C). Nevertheless, when segregated according to "high" and "low" AUC-TSH values as mentioned above, profound elevations in AUC-T3 profiles during treatment were found in the low AUC-TSH subgroups compared to both high AUC-TSH subgroups (P<0.01, $F_{5,130}$=3.917, rmANOVA). In particular the low AUC-TSH group treated with OLZ showed a higher AUC-T3 value compared to the high AUC-TSH group treated with OLZ at day 13 (P<0.05, $F_{3,29}$=2.428, post hoc LSD) and at day 14 (P<0.05, $F_{3,29}$=2.375, post hoc LSD, see figure 3C). During the study the %AUC-T3 value during treatment (figure 3D) was only increased in the low AUC-TSH subgroups (P<0.01, $F_{5,130}$=3.574, rmANOVA). Within-group analyses did not reveal a change in %AUC-T3 values in the high AUC-TSH groups, whereas in the low AUC-TSH groups (and particular when treated with OLZ alone) within-group effects of increased %AUC-T3 values were observed. AUC-T4 segregated according to the low or high AUC-TSH groups at day -1 did not reveal differences between these groups (see appendix figure 1).

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treatment in the low AUC-TSH groups (*P<0.01, rm-ANOVA). D) %AUC-T3 values were increased during treatment in the AUC-TSH low subgroups (*P<0.01, rm-ANOVA). Within groups increases compared to day -1 of AUC-T3 values were found at day 6, 13, and 14 ($^{\#}$P<0.05, paired t-test). E) Segregation of subjects into OLZ or OLZ+TPM groups with high or low baseline daily AUC-TSH profiles. The high AUC-TSH profiles in both treatment groups were not associated with increased ΔBW. In contrast, subjects with a low baseline AUC-TSH profile increased ΔBW when treated with OLZ, but not when treated with OLZ+TPM ($^{\#}$P<0.05, post hoc LSD). At day 28, the OLZ-high group increased ΔBW compared to all other groups ($^{\#}$P<0.05, post hoc LSD). F) Segregation of individuals with either high or low AUC-TSH levels over the gain, stable and loose BW groups revealed that subjects in the gain group consisted of significantly more AUC-TSH low subjects treated with OLZ relative to other subgroups. In contrast, the loose group consisted predominantly of subjects from the AUC-TSH group adjunctively treated with TPM (P<0.01, Mann Whitney U). No difference in rank distribution was observed for the AUC-TSH high groups.

When adding the AUC-TSH profile at day -1 as a covariate to the ANOVA analyses, this resulted in a difference in ΔBW at day 13 between OLZ and OLZ+TPM ($P<0.05$, $F_{1,27}=4.484$, ANCOVA). This effect was found even though AUC-TSH profiles at day -1 were not significantly different between groups (OLZ=6.40±0.92 AUC; OLZ+TPM=5.27±0.76 AUC). Inspection of ΔBW in the AUC-TSH high and low groups revealed that only subjects with a low baseline AUC-TSH profile gained weight on OLZ (OLZ-low), whereas subjects with a high baseline AUC-TSH profile did not gain weight (OLZ-high) ($P<0.001$, $F_{1,29}=28.455$, oneway-ANOVA). Adjunctive TPM treatment reduced ΔBW in the subjects based on a low TSH profile (TPM-low) compared to the OLZ-treated subjects with a low baseline AUC-TSH profile. Repeated measures analyses revealed that ΔBW in the OLZ-low group was higher compared to all other groups ($P<0.05$, $F_{6,52}=2.710$, rm-ANOVA post hoc LSD). Specifically, at day 13 ΔBW was increased in OLZ-low TSH compared to OLZ-high TSH ($P<0.05$) and TPM-low TSH groups ($P<0.05$, $F_{3,29}=3.052$, ANOVA post hoc LSD); at day 28 of the study OLZ-low TSH subjects showed increased ΔBW compared to all other groups ($P<0.05$, $F_{3,29}=3.232$, ANOVA post hoc LSD).

Ranking the AUC-TSH profiles over the BW based tertiles (fig 3F; Gain, Stable, Loose) revealed that the "Gain" group consisted predominantly of AUC-TSH low OLZ treated subjects, whereas the "Loose" group consisted largely of AUC-TSH low TPM subjects (mean rank: 11.50/5.67; $P<0.01$, Mann Whitney U). Subjects belonging to the AUC-TSH high groups did not differ in group distribution (mean rank: 7.83/8.25; $P=0.838$).

Finally, there was a positive correlation in the OLZ treated group between ΔBW (kg) at day 13 and the increase of %AUC-TSH value at day 13 ($R^2=0.542$; $P<0.01$, $F_{1,13}=15.379$, ANOVA), which was absent in the TPM treated group. A similar, but weaker, correlation between ΔBW (kg) and the %AUC-T3 value at day 13 was observed also exclusively in the OLZ treated group ($R^2=0.289$; $P<0.05$, $F_{1,13}=5.289$, ANOVA).

4. Temperature regulation.

Between OLZ and OLZ+TPM treatment groups, rm-ANOVA analyses did not reveal a difference in the hypothermic response (ΔT) observed at day 1 of treatment. The overall pattern of body temperature responses revealed that the first day of treatment caused suppression in core temperature, which was lost on the last day of treatment. The OLZ treated group showed a within-group decrease in ΔT at day 1 compared to day -1 ($P<0.01$, $t_{14}=4.927$) as well as the OLZ+TPM treated group ($P<0.05$, $t_{14}=2.851$, paired t-test), but no between-group difference was observed ($P=0.117$, $F_{1,28}=2.622$, oneway-ANOVA). For all subjects it applied that no

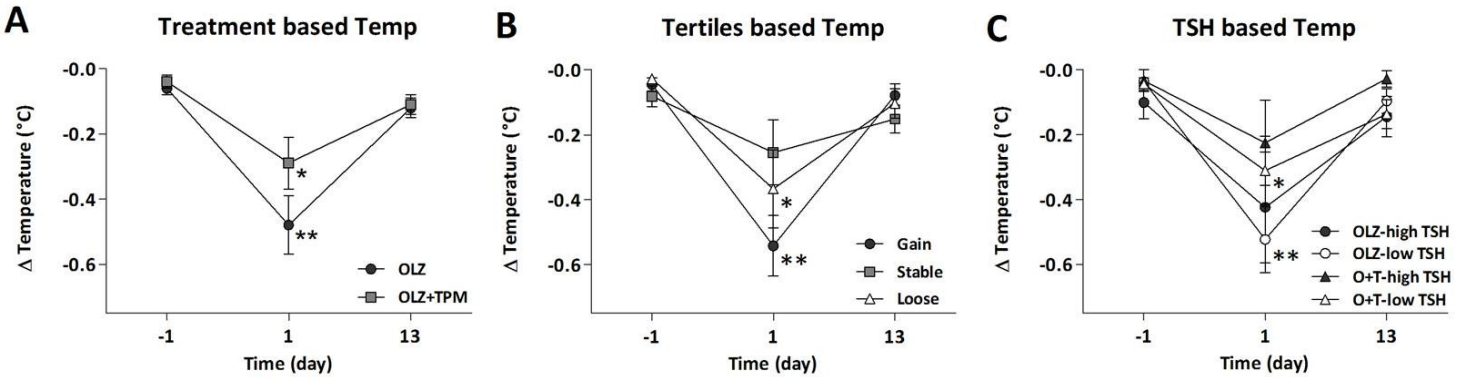

Fig. 4: Temperature responses. A) ΔTemperature of OLZ and OLZ+TPM treated groups; both groups showed a temperature decrease at day 1 of treatment compared to day -1. The hypothermic response was absent after 13 days of treatment. C) ΔTemperature response based on body weight gain tertiles revealed that both the Gain and the Low group decreased Δtemperature at day 1 compared to day -1, whereas temperature was not affected in the Stable group. B) ΔTemperature responses of subgroups based on baseline AUC-TSH values at day -1. Only the low AUC-TSH groups showed a within-group reduction of Δtemperature at day 1 compared to day -1 of treatment (*P<0.05, **P<0.01, paired t-test).

Table 4: Multiple regression analyses showing stepwise parameters explaining the observed variation of the dependent variable.

Dependent:	ΔBW (kg)			Δ HOMA			Δ Insulin (AUC)			ΔGlucose (AUC)			Food Intake (kcal)		
	Ind.	F	P	Ind.	F	P	Ind.	F	P	Ind.	F	P	Ind.	F	P
1 ($F_{1,27}$)	TSH	6.551	.016	ΔBW	10.599	.003	ΔBW	7.110	.013	Gluc	22.857	.000	OLZ	9.515	.005
2 ($F_{2,26}$)	FFA	8.726	.001				LDL	6.114	.007	Cpep	14.925	.000	Temp	8.215	.002

List of independent variables put into the analyses to explain the variation observed in the dependent variables:
Treatment, circulating OLZ levels, circulating TPM levels, BW at arrival, Δ BW (kg) at day 13, temperature response at day 1 (AUC), baseline HOMA-IR, baseline insulin/glucose/C-peptide response (AUC), baseline TSH/T3/T4 response (AUC), baseline FFA/TGC/LDL/HDL/Chol levels, baseline melatonin/oxyntomodulin/glucagon levels.
Ind.: independent variable; AUC: area under the curve.

hypothermic response was observed at day 13 of treatment (see fig. 4A).

Within groups analyses of the body weight gain based groups showed a decrease in ΔT at day 1 compared to day -1 in the Gain ($P<0.01$, $t_9=5.195$, paired t-test) and Loose ($P<0.05$, $t_9=3.052$, paired t-test) group, but not in the Stable group. In addition, a quadratic (U-shaped) correlation was observed between ΔT and body weight gain ($P<0.05$, $F_{2,28}=4.129$, ANOVA), showing both individuals loosing and gaining weight had the greater temperature reductions, whereas subjects with a relatively stable body weight during treatment did not show a hypothermic response at day 1 of treatment (see fig. 4B).

Based on the AUC-TSH values at day -1, within group analyses revealed that ΔT (fig. 4C) was decreased at day 1 compared to day -1 in both OLZ-low AUC-TSH ($P<0.01$, $t_8=5.143$, paired t-test) and TPM-low AUC-TSH individuals ($P<0.05$, $t_9=2.466$, paired t-test). A trend was observed for a lower ΔT in the OLZ-high AUC-TSH group ($P=0.071$, $t_5=2.281$, paired t-test) surprisingly, both TSH-high groups did not show a significant change in temperature response between day -1 and day 1, due to high within group variation. In addition, a negative correlation was observed between ΔT (day 1) and the percentage increase of AUC-T3 at day 14 ($P<0.05$, $r=-0.375$; Pearson 2-tailed).

5. Multiple regression analyses

To further explore factors during treatment potentially contributing to the observed variation in ΔBW we performed a stepwise multiple regression analysis using parameters assessed during drug treatment, which we considered to be related to body weight fluctuations as independent variables (i.e., plasma OLZ/TPM levels, OLZ-induced hypothermia, energy intake/expenditure/excess, age, AUC-TSH/AUC-T3/AUC -T4 profiles as well as their percentage change compared to day -1), as well as treatment. From this analyses we found that total cumulative food intake was the main factor explaining the variation of ΔBW on day 13 of treatment ($P<0.001$, $F_{1,27}=30.945$, ANOVA, $R^2=.534$), and the model also revealed that it could be improved by the addition of morning plasma OLZ levels taken at day 13 of treatment ($P<0.001$, $F_{2,26}=22.622$, ANOVA, $R^2=.635$). Beside circulating OLZ levels, the strongest factor improving the regression model in explaining the variation in body weight gain, in addition to total food intake, was the percentage increase of the AUC-TSH value at day 13 ($P<0.001$, $F_{2,26}=24.684$, ANOVA; $R^2=.655$). As mentioned above, the percentage increase of AUC-TSH levels at day 13 were positively correlated with ΔBW on day 13 in the OLZ group, but also overall ($P<0.05$, $F_{1,29}=7.326$, ANOVA; $R^2=.207$). Finally, the variation in ΔBW could not be further explained by adding TPM treatment or plasma levels of TPM into the regression model. Besides ΔBW, variation in total food intake

was best explained by the circulating OLZ levels at day 13 at 4hrs after drug administration ($P<0.01$, $F_{1,27}=9.515$, ANOVA; $R^2=.261$) and the model was improved by the temperature response after drug administration at the first day of treatment ($P<0.01$, $F_{2,26}=8.215$, ANOVA; $R^2=.387$). Remarkably, both circulating OLZ levels on day 13 at 4hrs ($F_{1,29}=10.169$, $P<0.01$, ANOVA; $R^2=.266$) and ΔT(AUC) at day 1 ($F_{1,29}=8.060$, $P<0.01$, ANOVA; $R^2=.230$) were negatively correlated to total FI. This implies that both higher circulating OLZ levels and a greater hypothermic response are associated with lower food intake. In contrast, only in the TPM treated group a negative correlation was observed between circulating OLZ levels and ΔT at day 1 ($F_{1,13}=5.174$, $P<0.05$, ANOVA; $R^2=.301$). The latter is of interest because we consider the hypothermic reaction to acute OLZ treatment as a pharmacological readout to the subject's responsiveness (Evers *et al*, submitted).

In a second analysis, we explored whether any of these factors before the start of drug treatment (day -1) could contribute to explain the variation in ΔBW. We only added baseline parameters in the stepwise multiple regression analyses (see table 3). The model revealed that the AUC-TSH value at day -1 best explained the variation in ΔBW ($P<0.05$, $F_{1,27}=6.551$, ANOVA; $R^2=.195$), but the model improved considerably in combination with baseline levels of free fatty acids ($P<0.01$, $F_{2,26}=8.726$, ANOVA; $R^2=.313$). In addition, stepwise multiple regression also showed that a combination of the AUC-TSH value at day -1 and treatment improved the model of explaining observed ΔBW ($P<0.05$, $F_{2,27}=5.029$, ANOVA; $R^2=.271$). Comparable effects were not observed with respect to T3 or T4 profiles.

In a third analysis, we explored potential factors contributing to the variation in the change (i.e., compared to day -1) of meal-induced excursions of plasma insulin (ΔInsulin) and glucose (Δglucose) levels as well as its HOMA-index (ΔHOMA), which we considered the dependent variables mostly reflecting OLZ's adverse metabolic side effects. Whereas variation in ΔHOMA at day 14 could not be explained by any of the parameters included in the model, variation in ΔHOMA at day 6 was to some extent explained by ΔBW ($P<0.01$, $F_{1,27}=10.599$, ANOVA; $R^2=.282$). ΔInsulin at day 14 was best explained by ΔBW too ($P<0.05$, $F_{1,27}=7.110$, ANOVA; $R^2=.208$), but was improved considerably by addition of morning LDL levels ($P<0.01$, $F_{2,26}=6.114$, ANOVA; $R^2=.320$). Unlike the variation in ΔHOMA and ΔInsulin, the variation in ΔGlucose was not explained by any parameter during treatment, except for its own baseline response at day-1 (i.e., which is indicative of the stability of glucose regulation through insulin secretion). The figures of the meal-induced excursions (HOMA, glucose, insulin, and C-peptide) can be found in Appendix 2.

In summary, the stepwise multiple regression analyses revealed four parameters

(TSH, FI, circulating OLZ levels, and temperature response) to be most relevant in explaining the variation in ΔBW in response to OLZ and adjunctive TPM treatment.

Conclusions

Treatment.

The data described here show that BW gain found in OLZ treated subjects is attenuated by adjunctive TPM treatment. Beside this TPM effect the only effect observed between treatment groups was a decrease of carbohydrate intake during breakfast, however, because subjects did not have a breakfast during baseline conditions (due to the meal tolerance test on day -1), it is impossible to say if this difference did not already exist between groups at baseline.

Circulating OLZ levels.

To rule out, or acknowledge, that pharmacodynamical interactions between OLZ and TPM formed the basis of the observed results, we measured circulating OLZ levels at three stages of the study, reflecting C max and steady state levels of OLZ. This showed that adjunctive TPM treatment in humans at these concentrations did not have an effect on circulating OLZ levels or vice versa.

Nonetheless a distinct correlation with circulating OLZ levels was observed. Namely, circulating OLZ levels were correlated with a decrease of total food intake, whereas food intake was also negatively correlated with a greater hypothermic response at day 1. However, higher OLZ levels were only correlated with greater hypothermic response in the adjunctive TPM group. A possible mechanism could be that TPM via stimulating GABA neurons at the level of the raphe pallidus blocks sympathetic outflow to the brown adipose tissue and suppresses counter regulatory mechanisms to increase body temperature [29]. This may result in a direct relation between OLZ levels and temperature loss in the adjunctive TPM treated group, whereas in the OLZ (only) treated group counter-regulatory mechanisms may be up-regulated and mask the correlation. Although not measured in this study, higher circulating OLZ levels could also be related to reduced food intake through increased sedation or nausea, and consequently decrease the motivation to eat. Furthermore, adjunctive TPM treatment might have had an additive effect to the (in)tolerability of OLZ treatment and therefore reduced food intake.

Body weight gain as a factor.

Because treatment did not cause major differences in other metabolic parameters except for within-group body weight gain, we redistributed the subjects into three equal sized groups (independent of treatment): Gain, Stable, and Loose (as Ascher-

Svanum *et al* [30]). The group distribution, as presented in table 2, revealed that the Gain group consisted more of OLZ treated than OLZ+TPM treated subjects (7/10), whereas the Loose group predominantly consisted of OLZ+TPM treated subjects (8/10), adding to the hypothesis that TPM attenuates OLZ-induced weight gain.

Based on weight gain, the data showed that an increased weight gain is correlated with an increase of HOMA-IR and Δinsulin response. Surprisingly, the Stable group also displayed an increase in HOMA-IR during treatment and already had increased insulin and C-peptide responses prior to treatment, which persisted throughout the duration of the study. Showing that OLZ-related increases of HOMA-IR and insulin responses are not *per se* body weight gain related. The Loose group, however, showed no increase of HOMA-IR or insulin responses, revealing beneficial effects of adjunctive TPM treatment in protecting against OLZ-induced insulin resistance.

Temperature responses.

Within the different groups it became clear that TPM did not have an effect on OLZ-induced temperature loss, because a hypothermic response at day 1 was observed in both treatment groups. Remarkably, on day 13 of treatment a hypothermic response after drug administration was absent in all treated subjects. The latter indicates an adaptive response to treatment, possibly related to increases of TSH and T3 levels during treatment.

Additionally, a more pronounced effect on temperature was observed in the weight gain based groups, showing a hypothermic effect in the Gain and Loose group, whereas the Stable group was not susceptible to OLZ-induced hypothermia. Interestingly, the absence of a hypothermic response, in combination with a high baseline insulin or C-peptide response, might be a marker for individuals that are susceptible to OLZ-induced insulin resistance independent of weight gain.

TSH profile as a predictive factor

The multiple regression analyses revealed that the variation in BW gain during treatment was best explained by food intake during treatment and that plasma OLZ levels contributed further to this model, but the AUC-TSH value at day -1 was the best predictor of OLZ-induced body weight gain. In contrast to food intake showing a positive correlation with body weight gain, a negative correlation between body weight gain and the baseline AUC-TSH value was observed. By adding the baseline AUC-TSH value as a covariate to treatment, a difference between OLZ and adjunctive TPM treatment in body weight gain was observed. By dividing the treatment groups into low and high AUC-TSH subgroups it became clear that those individuals with a

low AUC-TSH gained weight on OLZ, while subjects with a high AUC-TSH did not (see fig. 3E). In addition, both the low and high AUC-TSH groups on adjunctive TPM treatment did not gain weight.

Discussion

This study was performed in a relatively small group of healthy male volunteers, therefore, -unlike animal studies- a high within-group variation between subjects could be expected related to drug induced weight alterations. Our study revealed that this variation in responsiveness to OLZ-induced weight gain was predicted by the magnitude of the baseline daily decline in plasma TSH values (calculated as area under the curve, AUC-TSH), even in a relatively small group size. Specifically, by segregating individuals into groups with relatively low and high AUC-TSH profiles before the start of drug treatment we observed that subjects with low AUC-TSH values (63% of subjects) were not only susceptible to OLZ-induced weight gain, but were also responsive to adjunctive TPM treatment blocking weight gain (see fig. 3E/F). Together this might potentially offer the use of basal TSH levels (in drug naïve/free patients) as a predictor for treatment outcome, as well as a predictor for successful adjunctive TPM treatment. It remains to be evaluated whether the AUC-TSH profiles also have predictive value for the affective domains on which OLZ and TPM have effects. However, such an effect might be anticipated based on the finding in the early 1980's by Langer et al (1982) that patients with a 'blunted' TSH response to intravenous TRH administration had a better treatment outcome, based on an improvement of the brief psychiatric rating scale (BPRS), compared to subjects with a relatively high TSH response. From this, Langer et al (1982) concluded that the TSH response to TRH challenges could predict treatment outcome in the use of antidepressants and neuroleptic agents [31,32]. Furthermore, they reported that during neuroleptic treatment the TSH response was 'deblunted' and this increased TSH response also correlated to an improved treatment outcome [32]. Whereas in our study we did not perform a TRH challenge, these outcomes do relate to the lower baseline TSH profile and increased TSH and T3 profiles during treatment observed in the low-TSH groups of our study.

Others [16,33] have reported that only a subpopulation of schizophrenic patients is susceptible to OLZ-induced rapid weight gain, and additionally found that OLZ-induced weight gain is also associated with an improvement of the BPRS scale. Altogether it suggests that weight gain is a predictor for treatment responsiveness/efficacy. Unfortunately, body weight gain is also a major threat for treatment compliance. Therefore our observation that specifically subjects susceptible for weight gain are also responsive to adjunctive TPM treatment is of key importance:

i.e., blocking weight gain and protecting HOMA-IR and insulin levels, and possibly improving treatment compliance within a group responsive to the psychiatric benefits of OLZ treatment.

Mechanisms

The basal TSH levels prior to treatment within our subjects were within the normal range for adults: 0.55–4.78 µIU/mL (mIU/L), but as mentioned afore were not normally distributed. Because only the low AUC-TSH subjects increased circulating TSH levels during treatment the overall group range of basal TSH levels stayed within the normal range and therefore a group effect could not be observed.

TSH release from the adeno-pituitary is stimulated by TRH, produced from neurons of the paraventricular hypothalamus, and TSH synthesis and release is inhibited by dopamine via D2 receptors at the level of the pituitary [34]. We propose that low AUC-TSH subjects have an increased dopaminergic sensitivity/activity, compared to high AUC-TSH subjects, and possibly have increased D2-receptor expression at the level of the pituitary. As a consequence TSH release is predominantly regulated via the dopaminergic pathway inhibiting TSH release. High AUC-TSH subjects have lower D2-receptor expression; therefore TRH is the dominant pathway stimulating TSH secretion. The effect of OLZ would therefore be stronger in the low AUC-

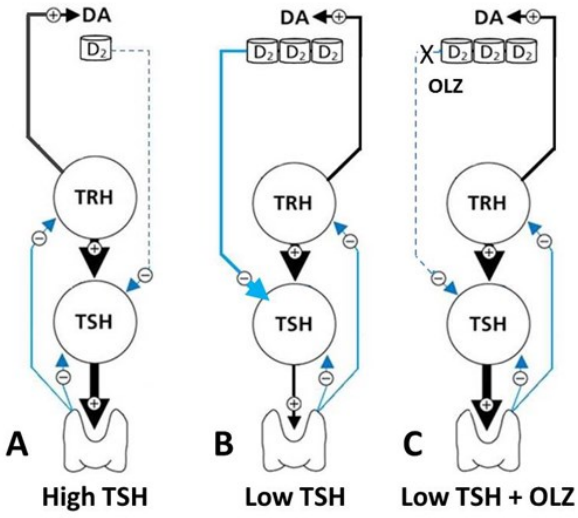

Fig. 5: TSH regulation. A) Individuals with lower D2 receptor density, at the level of the anterior pituitary, have a lower negative input from dopamine on TSH secretion, resulting in higher circulating TSH levels. B) Individuals with low circulating levels of TSH have a stronger negative dopaminergic input, due to high D2 receptor density, attenuating TSH secretion. C) OLZ blocks the D2 negative signal on TSH production, therefore circulating TSH levels increase during OLZ treatment. Due to endogenously high D2 receptor expression, the low TSH individuals are more sensitive to OLZ's effect on removing the dopaminergic brake on TSH secretion.

TSH subjects, because it would remove the brake on TSH production and consequently increases circulating TSH (see fig. 5).

From another perspective, Raskind et al [21] found in rats that OLZ reduced nighttime melatonin levels, and that melatonin replacement therapy blocked OLZ-induced weight gain. Similar results of melatonin's effectiveness in attenuating OLZ-induced weight gain have since been reported by Modabbernia et al [35] in first-episode schizophrenic patients. Like melatonin, circadian TSH levels peak during the night and decrease during day time [36] and it has been reported to be higher in the obese compared to the lean subjects [37]. Vriend et al [38] found that melatonin suppresses thiourea-induced TSH release and suggested that melatonin stimulates the sensitivity of the pituitary to T3/T4 feedback inhibition of TSH. Furthermore, melatonin induced a reduction of prolactin levels, whereas OLZ is associated with increased prolactin levels also via inhibition of the D2-receptor [39]. In our study, we did not find any specific differences between circulating melatonin levels at bed or wake-up time between any of the groups (data not shown). However, melatonin has also been shown to exhibit anticonvulsant properties [40,41], and both TPM and melatonin provide neuroprotection against hypoxia [42]. In analogy with Vriend et al [38], suggesting a role for melatonin on the pituitary, it is possible that TPM also increased the sensitivity of the pituitary to TSH and T3/T4. Following that hypothesis, both subgroups of low AUC-TSH groups showed increased TSH and T3 profiles during treatment, but only in the TPM treated subjects this increase might have been effective as a counter-regulatory response attenuating OLZ-induced weight gain. In addition, McCann et al (1986) reported the inhibition of TSH secretion by intravenously administrated bicucculine, a $GABA_A$ antagonist, which suggests a possible stimulatory effect on TSH secretion by orally administered TPM. In contrast, intracerebroventricular (icv) administration of GABA lowered serum TSH and this effect could be blocked by pimozide, a D2 receptor antagonist. This indicates that the role of GABA, and the possible effects of TPM, on the thyroid axis are variable and depends on the site of action [43].

Finally, the increase of TSH and T3 could be an adaptive response to the hypothermic effect of OLZ, because cold exposure is a stimulator of TSH secretion [44]. This explains that no significant decrease of body temperature was observed after 13 days of treatment. Segregating all subjects into 3 groups of equal size consisting of those who gained (Gain) body weight during treatment, lost (Loose) body weight during treatment, and those who remained stable (Stable) showed that especially in the Gain and Loose group a hypothermic response at day 1 of treatment was observed, both groups mainly consisting of low-TSH subjects in which an increase of TSH and T3 profiles were observed during treatment. By other measures, the segregation into

body weight “Gain”, “Loose” and “Stable” ones was remarkably insightful since the Gainers were significantly more often those that were not treated with TPM, whereas the body weight Losers were significantly more often those that were treated with TPM. The regression analysis revealed that changes in body weight during treatment most strongly explained the variation in changes in HOMA, and meal-induced insulin excursion during treatment, while plasma OLZ levels and energy intake mostly explained the variation in body weight gain during treatment. Finally, it is important to note that a subset of subjects did not gain weight on OLZ treatment and did not benefit from TPM treatment, namely the Stable group. However, this subgroup, mainly constituted of subjects with a high TSH profile, did display increased HOMA and insulin levels during treatment. As mentioned, other studies have shown that gaining weight and TSH responsiveness during drug treatment are related to improvement of the BPRS scale [16,33]. Therefore, OLZ, and presumably other SGAs, should arguably not be the first line of treatment in this group we defined as “Stable”, which can be recognized by high TSH baseline levels and lacking a hypothermic response at the first day of OLZ treatment.

Summary and Limitations

In this study we observed that the baseline daily AUC-TSH profile could potentially be used as a marker to identify individuals susceptible to OLZ-induced weight gain, and that this parameters could also be used as a predictor for the responsiveness to adjunctive TPM treatment blocking weight gain.

Other studies already have reported on the beneficial effects of TPM against OLZ-induced weight gain. One of the more extensive studies, performed by Vieta et al (2004), reported that beneficial effects of adjunctive TPM treatment was optimal when titrated to app. 300mg/day, and concomitantly reducing OLZ dosing below 10mg/day [45]. The idea of adjunctive TPM treatment to reduce OLZ dosing without losing treatment efficacy is important, given the negative side effects OLZ, e.g. hypothermia and sedation. We did not find an interaction between pharmacodynamics of OLZ and TPM at the concentration used in this study, but interactions at higher dosages should not be ruled out. In fact, high dosing of TPM in rats increased circulating OLZ levels significantly, this profoundly augmented OLZ-induced metabolic side effects (Evers et al, in prep). Precaution and well-adjusted titration of drug dosages should be advised in the use of adjunctive TPM treatment, as was shown in a case study where OLZ and adjunctive TPM treatment could lead to acute hyperthermia in high ambient temperatures [46]. Finally, Vieta et al (2004) reported an improvement of the BPRS score in subjects co-treated with TPM [45]. This finally results in two questions that have to be addressed in future studies: 1)

does adjunctive TPM treatment have additive clinical efficacy to OLZ treatment, or – derived from our study and the report of Kinon et al [16] – 2) is TPM predominantly effective in inhibiting OLZ-induced weight gain in individuals most responsive to OLZ's clinical efficacy?

Acknowledgments

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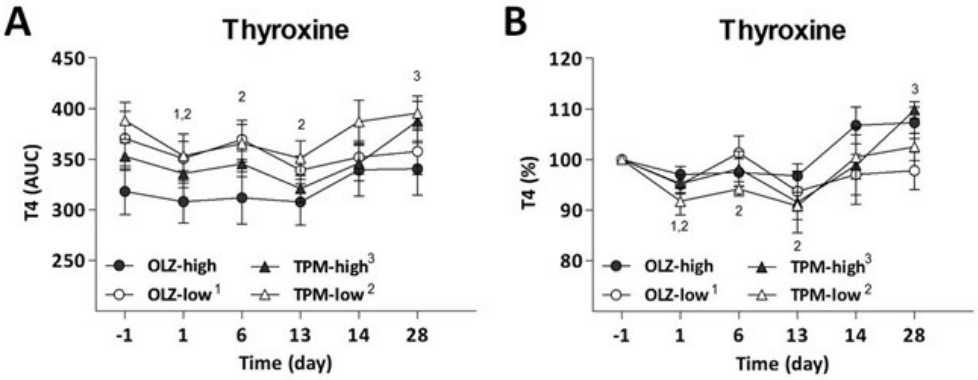
Appendix

1. Thyroxine (T4) responses during treatment

In contrast to TSH and T3 profiles (fig. 3), T4 profiles did not increase during treatment compared to baseline in the subjects belonging to the low AUC-TSH subgroups (see App. Fig. 1). Unlike baseline TSH profiles, T4 profiles at day -1 were normally distributed, however, a trend of lower T4 was observed in the low AUC-TSH subgroups (P=0.083, $F_{1,29}$=3.241, oneway-ANOVA) .



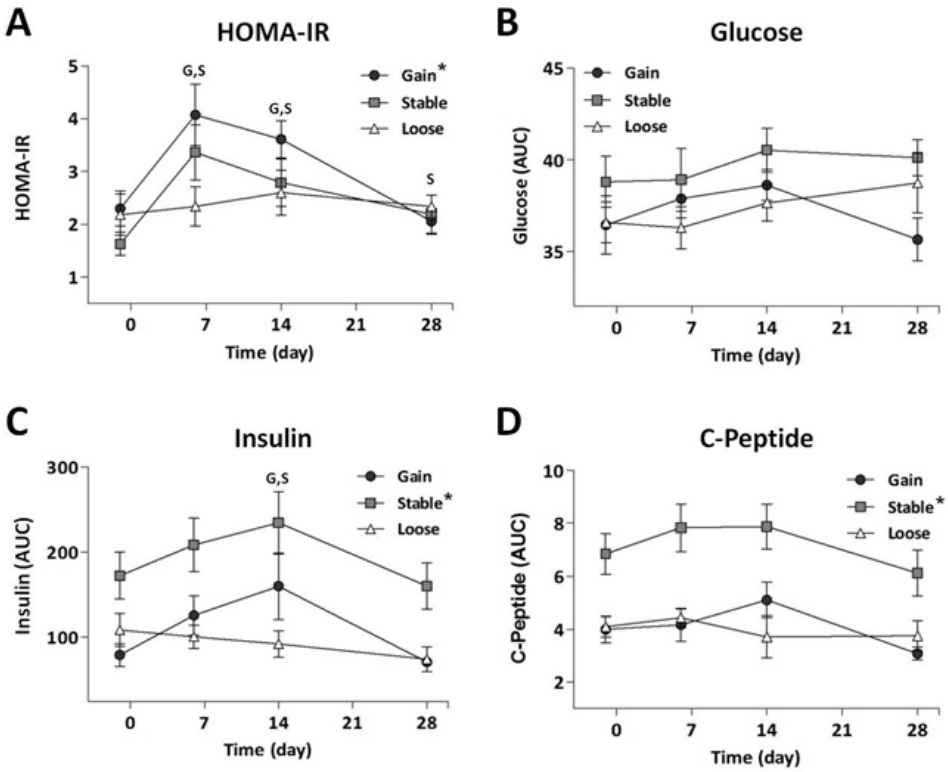
App. Fig 1. Thyroxine (T4) profiles during treatment. A) Thyroxine (T4) AUCs during treatment were not different between high/low AUC-TSH subgroups. Compared to day -1, within-group analyses observed a decrease in the OLZ-low[1] and TPM-low[2] groups at day 1, 6, and 13; and an increase at day 28 in the TPM-high[3] group ([1,2,3]P<0.05, paired t-test). B) No differences between TSH-low/high groups were found in %AUC-T4 values during treatment; only with-in group differences compared to baseline were observed ([1,2,3]P<0.05, paired t-test).

2. Glucose and Insulin regulation based on body weight gain

As already mentioned above, no main effects were found of treatment on HOMA-IR, nor glucose or insulin responses during the MTT. Since baseline HOMA-IR levels (see table 2) were not normally distributed ($P<0.01$, $df_{28}=0.866$, Shapiro-Wilk), we decided to segregate them according to the ΔBW tertiles as mentioned above, based on the fact that glucose homeostasis is linked to alterations in BW. Over time, HOMA-IR levels remained unaltered in the Loose group, whereas compared to day -1 an increase of HOMA-IR scores was observed in the Gain (day 6: $P<0.01$; day 14: $P<0.05$, paired t-test) and in the Stable group (day 6: $P<0.01$; day 14: $P<0.01$; day 28: $P<0.05$, paired t-test; see App. fig 2A). No differences between Gain-Stable and Loose groups were observed in the AUC-glucose profiles during the MTT (meal tolerance test) over the duration of the study (App. fig 2B).

Like baseline HOMA-IR, also the AUC-insulin value at baseline (day-1) was not normally distributed ($P<0.01$, $df_{28}=.913$, Shapiro-Wilk). Within group analyses showed no change in AUC-insulin values over the duration of the study in the Loose group, whereas compared to day -1 both the Gain and the Stable group had increased AUC-insulin values at day 14 ($P<0.05$. paired t-test). AUC-insulin values over the duration of the study (analyzed by between-subjects rm-ANOVA) were higher in the Stable group compared to both the Gain and Loose groups ($P<0.01$, $F_{2,27}=8.383$; post hoc LSD; App. fig 2C).

The plasma C-peptide response during the MTT was higher in the Stable group over the duration of the study compared to both the Gain ($P<0.01$) and Loose ($P<0.01$; $F_{2,27}=11.183$, rm-ANOVA post hoc LSD), and showed already at baseline to be not normally distributed ($P<0.05$, $df_{28}=.913$, Shapiro-Wilk). However, no differences of within group changes were observed in C-peptide responses over the duration of the study (App. fig 2D).



App Fig. 2: Insulin and glucose regulation. A) HOMA-IR was increased over the duration of the study in the Gain group compared to the Loose group (*P<0.05, rm-ANOVA), both the Gain and Stable group showed a within group increase of HOMA-IR at day 6 of treatment compared to day -1 (^{G,S}P<0.05, paired t-test). B) Glucose responses were not different between or within group over the duration of the study. C) Insulin responses were higher in the Stable group (*P<0.01, rm-ANOVA) over the duration of the study compared to the Gain and Loos group. Both the Gain and Stable group showed a within-group increase of insulin response at day 14 of treatment (^{G,S}P<0.05, paired t-test). D) C-Peptide responses were higher over the duration of the study in the Stable group compared to both the Gain and Loose group (*P<0.01, rm-ANOVA).

3. Correlations

Appendix Table 1 shows the correlations between measured parameters during treatment and Group (treatment), circulating OLZ levels on day 13 at 4hrs post drug administration, ΔBW at day 13 of treatment, and average daily food intake (kcal). Appendix Table 2 shows the correlations between thyroids responses and ΔBW at day 13 and average daily food intake (kcal) during treatment per treatment group (OLZ or OLZ+TPM). Appendix Table 3 shows the correlations of measured parameters between groups based on BW tertiles.

Table 1: Correlations between group, OLZ levels, body weight gain, and food intake.

		Group	OLZ levels Day 13_4hrs	Body Weight Δ Day13	Food Intake Average
Group: OLZ vs OLZ+TPM	Pearson Correlation	1	.052	-.274	-.197
	Sig. (2-tailed)		.784	.143	.296
OLZ levels Day 13_4hrs	Pearson Correlation	.052	1	-.170	-.514**
	Sig. (2-tailed)	.784		.370	.004
Body weight Δ Day 13	Pearson Correlation	-.274	-.170	1	.720**
	Sig. (2-tailed)	.143	.370		.000
Food Intake Average	Pearson Correlation	-.197	-.514**	.720**	1
	Sig. (2-tailed)	.296	.004	.000	
Carbohydrate Breakfast	Pearson Correlation	-.384*	-.131	.469**	.686**
	Sig. (2-tailed)	.036	.489	.009	.000
Carbohydrate Diet	Pearson Correlation	-.144	-.402*	.568**	.888**
	Sig. (2-tailed)	.449	.028	.001	.000
Fat Diet	Pearson Correlation	-.166	-.514**	.718**	.895**
	Sig. (2-tailed)	.382	.004	.000	.000
Protein Diet	Pearson Correlation	-.172	-.556**	.598**	.886**
	Sig. (2-tailed)	.365	.001	.000	.000
Energy Excess Week 1	Pearson Correlation	-.244	-.152	.641**	.551**
	Sig. (2-tailed)	.202	.430	.000	.002
Energy Excess Week 2	Pearson Correlation	-.261	-.300	.377*	.389*
	Sig. (2-tailed)	.163	.108	.040	.034
Cholesterol Day 14	Pearson Correlation	-.201	-.008	.406*	.143
	Sig. (2-tailed)	.286	.968	.026	.452
LDL Day 14	Pearson Correlation	-.150	-.099	.418*	.187
	Sig. (2-tailed)	.429	.603	.021	.323
HDL Day 14	Pearson Correlation	.000	-.239	.433*	.373*
	Sig. (2-tailed)	1.000	.204	.017	.043
HOMA-IR Day 6	Pearson Correlation	.020	-.283	.555**	.369*
	Sig. (2-tailed)	.916	.129	.001	.045
Insulin Δ Day 14	Pearson Correlation	.078	-.011	.447*	.484**
	Sig. (2-tailed)	.683	.952	.013	.007
T3 % Day 13	Pearson Correlation	-.126	-.048	.411*	.340
	Sig. (2-tailed)	.509	.801	.024	.066
T4 % Day 13	Pearson Correlation	-.226	.138	-.424*	-.364*
	Sig. (2-tailed)	.229	.467	.020	.048
TSH% Day 14	Pearson Correlation	.042	.058	.511**	.211
	Sig. (2-tailed)	.827	.760	.004	.264
Temperature AUC Day 1	Pearson Correlation	.213	.268	-.156	-.481**
	Sig. (2-tailed)	.268	.161	.420	.008

*Correlation is significant at the level P<0.05 (2-tailed)
**Correlation is significant at the level P<0.05 (2-tailed)

App. Table 2: Correlations with thyroid responses

		OLZ ΔBW Day13	OLZ+TPM ΔBW Day13	OLZ Food Intake	OLZ+TPM Food Intake
TSH (AUC)	Pearson Correlation	-.654**	-.197	-.174	-.011
Day -1	Sig. (2-tailed)	.008	.482	.535	.968
TSH (AUC)	Pearson Correlation	-.562*	-.043	-.197	.195
Day 1	Sig. (2-tailed)	.026	.878	.482	.486
TSH (AUC)	Pearson Correlation	-.595*	-.177	-.152	.121
Day 6	Sig. (2-tailed)	.019	.528	.588	.667
TSH (%)	Pearson Correlation	.538*	.405	.294	.203
Day 6	Sig. (2-tailed)	.039	.135	.287	.469
TSH (%)	Pearson Correlation	.611*	.221	.231	.203
Day 13	Sig. (2-tailed)	.016	.429	.407	.469
TSH (%)	Pearson Correlation	.736**	.239	.339	.153
Day14	Sig. (2-tailed)	.002	.391	.217	.586
T3 (AUC)	Pearson Correlation	.561*	.511	.043	.384
Day 13	Sig. (2-tailed)	.030	.052	.879	.157
T3 (AUC)	Pearson Correlation	.593*	.344	.106	.287
Day 14	Sig. (2-tailed)	.020	.209	.708	.299
T3 (%)	Pearson Correlation	.538*	.224	.537*	.088
Day 14	Sig. (2-tailed)	.039	.422	.039	.755
T4 (AUC)	Pearson Correlation	.041	-.547*	-.187	-.627*
Day 13	Sig. (2-tailed)	.885	.035	.504	.012
T4 (AUC)	Pearson Correlation	-.141	-.524*	-.277	-.536*
Day 14	Sig. (2-tailed)	.616	.037	.318	.039
T4 (%)	Pearson Correlation	-.570*	-.401	-.403	-.084
Day 1	Sig. (2-tailed)	.027	.138	.136	.766
T4 (%)	Pearson Correlation	-.311	-.751**	-.229	-.601*
Day 13	Sig. (2-tailed)	.259	.001	.412	.018
T4 (%)	Pearson Correlation	-.525*	-.598*	-.337	-.388
Day 14	Sig. (2-tailed)	.044	.019	.219	.153

*Correlation is significant at the level P<0.05 (2-tailed)
**Correlation is significant at the level P<0.05 (2-tailed)

App. Table 3: Correlations with body weight gain based on tertiles

		Gain-Stable-Loose (n=30)	Gain vs Loose (n=20)	Gain vs Stable (n=20)	Stable vs Loose (n=20)
Group	Pearson Correlation	-,408*	-,503*	-,105	-,408
OLZ=0, OLZ+TPM=1	Sig. (2-tailed)	,025	,024	,660	,074
Food intake	Pearson Correlation	,717**	,792**	,661**	,364
Average	Sig. (2-tailed)	,000	,000	,002	,115
Energy	Pearson Correlation	,556**	,600**	,282	,556*
Excess Wk1	Sig. (2-tailed)	,002	,005	,241	,014
Energy	Pearson Correlation	,377*	,577**	,316	,099
Excess Wk2	Sig. (2-tailed)	,040	,008	,175	,677
Carbohydrate	Pearson Correlation	,555**	,640**	,196	,568**
Breakfast	Sig. (2-tailed)	,001	,002	,407	,009
Diet	Pearson Correlation	,559**	,599**	,388	,444*
Carbohydrate	Sig. (2-tailed)	,001	,005	,091	,050
Diet	Pearson Correlation	,716**	,825**	,743**	,160
Fat	Sig. (2-tailed)	,000	,000	,000	,499
Diet	Pearson Correlation	,604**	,665**	,436	,428
Protein	Sig. (2-tailed)	,000	,001	,055	,060
TGC	Pearson Correlation	,353	,452*	,045	,424
% Day 6	Sig. (2-tailed)	,056	,045	,850	,063
FFA	Pearson Correlation	-,377*	-,468*	-,148	-,335
Baseline	Sig. (2-tailed)	,040	,037	,534	,149
FFA	Pearson Correlation	-,364*	-,454*	-,444*	-,036
Day 14	Sig. (2-tailed)	,048	,044	,050	,881
Cholesterol	Pearson Correlation	,415*	,499*	,022	,485*
% Day 14	Sig. (2-tailed)	,023	,025	,926	,030
HOMA-IR	Pearson Correlation	,486**	,570**	,015	,608**
Δ Day 6	Sig. (2-tailed)	,006	,009	,949	,004
Insulin	Pearson Correlation	-,161	-,282	-,579**	,406
AUC baseline	Sig. (2-tailed)	,394	,228	,007	,076
Insulin	Pearson Correlation	,245	,355	-,312	,645**
AUC Day 14	Sig. (2-tailed)	,191	,125	,181	,002
C-peptide	Pearson Correlation	-,018	-,035	-,590**	,602**
AUC baseline	Sig. (2-tailed)	,925	,884	,006	,005
C-peptide	Pearson Correlation	,197	,301	-,516*	,644**
AUC Day 14	Sig. (2-tailed)	,297	,197	,020	,002
Temperature	Pearson Correlation	-,222	-,269	-,444*	,176
Δ Day 1	Sig. (2-tailed)	,247	,265	,050	,472

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

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