Glycobiology in cardiometabolic homeostasis
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THE EFFECT OF A DIODOXYRONINE MIMETIC ON INSULIN SENSITIVITY IN MALE CARDIOMETABOLIC PATIENTS: A DOUBLE-BLIND RANDOMIZED CONTROLLED TRIAL


Submitted
CHAPTER 5

ABSTRACT

Context Our world is under the spell of obesity and their associated cardiometabolic co-morbidities. Since thyroid hormone mimetics are capable of uncoupling the beneficial metabolic effects of thyroid hormones from their deleterious effects on heart, bone and muscle, this class of drug is considered as adjacent therapeutics to weight-lowering strategies.

Objective - This study investigated the safety and efficacy of TRC150094, a thyroid hormone mimetic.

Design - This 4-week, randomized, placebo-controlled, double-blind trial was conducted in India and The Netherlands. Hyperinsulinemic euglycemic clamp and 1H-Magnetic Resonance Spectroscopy (MRS) were performed before and after treatment.

Subjects - In total 40 male subjects aged 30 to 65 years and characterized by a metabolic syndrome were included of whom none withdrew from the study after randomization.

Intervention - Subjects were randomized at a 1:1 ratio to receive either TRC150094 dosed at 50 mg or placebo once daily for 4 weeks.

Main Outcome Measure - Primary efficacy was assessed via the change in hepatic or peripheral insulin sensitivity from baseline to week 4.

Results - TRC150094 dosed 50mg once daily was safe and well tolerated, however, insulin sensitivity, hepatic fat content and lipid profiles did not improve following 4 weeks of TRC150094 administration.

Conclusions - Collectively, these data show that, in contrast to the potent metabolic effects in experimental models, TRC150094 at a dose of 50mg daily does not improve the metabolic homeostasis in subjects at an increased cardiometabolic risk. Further studies are needed to evaluate whether TRC150094 has beneficial effects in patients with more severe metabolic derangement, such as overt diabetes mellitus and hypertriglyceridemia.
INTRODUCTION
Despite the growing awareness of the detrimental impact of obesity on global health, the pandemic still shows no signs of abating. Currently, two thirds of the world’s population lives in countries where obesity-associated co-morbidity is the leading cause of premature death. Hence, there is an immense, unmet medical need for safe and effective therapies aimed at preventing the cardiometabolic sequelae associated with central adiposity, which can be implemented on top of weight-lowering strategies. Among the potential candidates, thyroid hormones (TH) have been shown to increase basal energy expenditure and oxygen consumption leading to a reduction in body weight with concomitant favorable improvements in lipid and carbohydrate metabolism. In a clinical setting, however, TH have failed predominantly due to cardiotoxicity, as well as bone and muscle toxicity. Subsequently, selective TH analogs were designed in an effort to retain the beneficial effects whilst avoiding the toxic side effects. Analogs of TH with a 22-fold higher affinity for the hepatic thyroid hormone receptor beta (TRβ) than the ‘ubiquitous’ TRα isoform were reported to lower low density lipoprotein cholesterol (LDLc) by approximately 30% without significant heart, muscle or bone toxicity. Though, the first data with this compound were promising, the Eprotirome program had to be discontinued due to the observation of increased cartilage damage following prolonged exposure to Eprotirome in dogs.

More recently, a mimetic of diiodothyronine (T2) - TRC150094 – was studied in a phase I study (data not published). TRC150094 has a very low potency for both TR isoforms when compared to T3, the active form of TH. The mechanism of action of T2 has been attributed to a direct, receptor-independent interaction of T2 with mitochondria. In preclinical studies, TRC150094 was shown to stimulate mitochondrial fatty acid oxidation (FAO) which led to a reduction of visceral adiposity in Wistar rats. In line, TRC150094 improved glucose tolerance and hepatic steatosis in obese Zucker spontaneously hypertensive fatty (ZSF1) rats with a concomitant reduction in plasma cholesterol and triglycerides in ZSF1 rats. Most importantly, TRC150094 was not associated with any adverse safety signal in experimental models up to 24 weeks. In phase I clinical studies, once daily oral administration of TRC150094 at doses of 50mg and 150mg for 28 days were well tolerated without any adverse safety signals in the obese subjects.

In the present study, we set out to evaluate the effect of TRC150094 on insulin sensitivity, liver fat content and lipid profile, as well as on safety markers in obese male subjects with an increased cardiometabolic risk.
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METHODS

Study design
This randomized, placebo-controlled, double-blind trial was conducted at 2 sites and was approved by the local Institutional Review board at Veeda Clinical Research, India and at the Academic Medical Centre (AMC), The Netherlands. The trial was conducted according to the principles of the International Conference on Harmonisation–Good Clinical Practice guidelines, and externally monitored by an independent contract research organization and registered on clinicaltrials.gov (NCT01408667). All participants provided written informed consent. In total, 40 subjects were enrolled; 20 subjects at Veeda Clinical Research, Ahmedabad, India, and 20 subjects at AMC, Amsterdam, The Netherlands. Each subject attended the study center for 5 visits; 1 screening visit, 2 study visits (1 baseline and 1 end of treatment), 1 intermediate safety visit and 1 post-study follow-up visit. During all visits physical examination, vital signs, safety biochemistry and laboratory investigations were performed and evaluated by a physician blinded for treatment allocation. Before and after treatment a hyperinsulinemic euglycemic clamp and $^1$H-Magnetic Resonance Spectroscopy (MRS) were performed (details are provided in the Supplementary Information 1). The primary efficacy variable was the change in insulin sensitivity from baseline to week 4. Secondary efficacy variables were changes in hepatic fat content (IHTG) and lipid profile. Safety assessments included documentation of adverse events, blood pressure, heart rate, body temperature, weight and laboratory tests, including thyroid and liver-function tests.

Patient selection
Eligible subjects were male, aged 30 to 65 years, and characterized by a metabolic syndrome based on the following criteria: increased waist circumference (Indian ≥90cm, Caucasian ≥102 cm), blood pressure ≥130/85 mmHg or use of antihypertensive drugs, fasting glucose >5.5 mmol/l - 11.0 mmol/l and fasting insulin level ≥10 mU/mL. Subjects were considered not eligible in case of history of somatic illness, including neoplasm, endocrine or neurologic disorders, active infection, unstable weight 3 months prior to inclusion or recent surgical procedure within 3 months of the study initiation; respectively systolic and diastolic blood pressure of ≥160mmhg or ≥100mmHg, impaired kidney function (eGFR <60 mL/min/1.73m$^2$ as evaluated by MDRD method) or impaired liver function (ALT or AST >3 x ULN) at screening. After screening, subjects were randomized at a 1:1 ratio to receive either TRC150094 dosed at 50 mg or placebo once daily for 28 days.

Statistical analysis
Continuous data were analysed with parametric or non-parametric tests depending on the data distribution verified by the Shapiro-Wilk test. Within-group comparisons of pre- and post-treatment values were performed using the paired samples Student t-test or Wilcoxon signed ranks test.
Between-group comparisons of the relative changes were performed using the unpaired samples t-test or Mann–Whitney U test. Data for qualitative variables are presented as incidence rates (N, number and percent). The data of continuous variables were summarized using measures of central tendency (i.e. mean, median) and dispersion (i.e. standard deviation, range). Statistical analysis was performed using SPSS 19.0 (SPSS, Chicago, IL, USA).

RESULTS

Baseline characteristics

From November 2011 through May 2012 we randomly assigned 40 men to TRC150094 (n = 20) or placebo (n = 20), all of whom completed the study protocol (Figure S 1). At baseline, clinical characteristics were comparable between TRC150094 and placebo group (Table 1). Baseline characteristics were also comparable between Indian and Caucasian subjects except for BMI, fasting insulin and FFA (Table S 1).

Table 1 – Characteristics of Study Subjects at Baseline

<table>
<thead>
<tr>
<th></th>
<th>TRC150094 (N = 20)</th>
<th>Placebo (N = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>49 ± 11</td>
<td>50 ± 10</td>
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<tr>
<td>Weight, kg</td>
<td>102 ± 18</td>
<td>103 ± 19</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>33.3 ± 4.5</td>
<td>33.6 ± 4.9</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>112.5 ± 12</td>
<td>114.7 ± 11.8</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>5.6 ± 1</td>
<td>5.3 ± 0.6</td>
</tr>
<tr>
<td>Fasting plasma insulin, mU/L</td>
<td>12 ± 7</td>
<td>14 ± 8</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.9 ± 1.8</td>
<td>3.5 ± 2.4</td>
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<tr>
<td>Cholesterol, mmol/L</td>
<td>4.63 ± 1.03</td>
<td>4.90 ± 0.74</td>
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<tr>
<td>HDLc</td>
<td>0.94 ± 0.24</td>
<td>1.01 ± 0.32</td>
</tr>
<tr>
<td>LDLc</td>
<td>2.91 ± 0.82</td>
<td>3.13 ± 0.71</td>
</tr>
<tr>
<td>TG</td>
<td>1.60 ± 1.06</td>
<td>1.67 ± 0.72</td>
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<tr>
<td>Fasting free fatty acids, mmol/L</td>
<td>0.51 ± 0.10</td>
<td>0.52 ± 0.19</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>140 ± 8</td>
<td>137 ± 10</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>88 ± 4</td>
<td>87 ± 7</td>
</tr>
</tbody>
</table>

NOTE. Values are expressed as mean ± standard deviation. No significant differences in clinical variables were found between TRC and Placebo group at baseline, p < 0.05. The body mass index is the weight in kilograms divided by the square of the height in meters. HDLc, high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol; TG, triglycerides.
Safety analyses

No serious adverse events were reported and no subjects withdrew from the study after enrolment. The total number of adverse events during the study was similar among the study groups (8 adverse events in both groups). The majority of these events were mild (81%) or moderate (19%). Supplementary Table 2 lists the number, intensity, relationship to treatment and type of adverse events that occurred during the study. No changes in vital signs were observed; blood pressure, heart rate, body temperature and weight remained stable throughout the study (Table 2). Liver function tests including ALT, AST and GGT did not change after TRC150094 treatment (Table 2). A marginal increase in FT4 in the treatment arm was observed, however, there was no concomitant reduction in TSH (Table 2).

Table 2 – Safety Analyses of Study Subjects at Baseline and After 4 Weeks

<table>
<thead>
<tr>
<th></th>
<th>TRC150094 (N = 20)</th>
<th>Placebo (N = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Week 4</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>140 ± 8</td>
<td>140 ± 9</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>88 ± 4</td>
<td>88 ± 5</td>
</tr>
<tr>
<td>Pulse, beats/min</td>
<td>76 ± 10</td>
<td>74 ± 9</td>
</tr>
<tr>
<td>Body temperature, °C</td>
<td>36.6 ± 0.5</td>
<td>36.4 ± 0.5</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>101 ± 18</td>
<td>103 ± 21</td>
</tr>
<tr>
<td>Liver function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>40 ± 22</td>
<td>39 ± 20</td>
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<tr>
<td>AST, U/L</td>
<td>29 ± 12</td>
<td>27 ± 11</td>
</tr>
<tr>
<td>GGT, IU/L</td>
<td>44 ± 31</td>
<td>43 ± 28</td>
</tr>
<tr>
<td>Thyroid function</td>
<td></td>
<td></td>
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<tr>
<td>FT3, pmol/L</td>
<td>4.27 ± 1.02</td>
<td>4.88 ± 1.29</td>
</tr>
<tr>
<td>FT4, pmol/L</td>
<td>11.70 ± 2.12</td>
<td>12.57 ± 1.96</td>
</tr>
<tr>
<td>TSH, mIU/L</td>
<td>2.10 ± 0.00</td>
<td>2.23 ± 1.14</td>
</tr>
</tbody>
</table>

NOTE. Values are expressed as mean ± standard deviation.

* Nonparametric test show a significant increased T3 in TRC150094 group (p= 0.005) and significant decrease in placebo group (p =0.049). Also, a significant increase in FT4 after TRC150094 treatment (p=0.025).

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase; FT3, free trio-iodothyronine; FT4, free thyroxine; TSH, thyroid stimulating hormone (thyrotropin).
Efficacy analyses in subjects at increased cardiometabolic risk

Effect of TRC150094 on insulin sensitivity

At baseline, male subjects were characterized by markedly impaired hepatic and peripheral insulin sensitivity, compared to reference values observed in healthy, non-obese control subjects (Figure 1 A-B). Hepatic insulin sensitivity was expressed as the suppression of Endogenous Glucose Production (EGP). After TRC150094 administration there was no improvement in suppression of endogenous glucose production (mean EGP suppression from 59.5% to 62.1%; p = 0.477) (Figure 1 A), whereas peripheral insulin sensitivity (expressed as the rate of glucose disappearance (Rd)) was not altered upon TRC150094 administration (mean Rd from 28.8 to 26.4 μmo/kg·min⁻¹; p = 0.185) (Figure 1 B). Although T2’s mechanism of action is expected to stimulate lipolysis and FAO, TRC150094 administration did not result in differences in fasting plasma FFA (mean FFA from 0.51 to 0.51 mmol/L, p= 0.887) or in insulin-mediated suppression of lipolysis (lipolysis suppression from 57% to 54%, p = 0.102) (Figure 1 C). Overview of efficacy results in glucose kinetics, lipolysis and glucoregulatory hormones at baseline and after TRC administration are provided in Supplementary Table 3.

Effect of TRC150094 on IHTG content and lipid profile

Intrahepatic triglyceride (IHTG) content was measured with ¹H MRS. At baseline, mean IHTG content was 10.6% (± 6.4%) in the whole group. After 4-weeks of treatment, IHTG was unaltered in both the TRC150094 and the placebo group (Figure 1). Similarly, no change in lipid profile, i.e. total cholesterol, LDLc, HDLc or TG, was detected in the patients on either TRC150094 or placebo (Figure 1). Responses divided per treatment arm are provided in Supplementary Table 4.

Subgroup analysis in subjects with severe metabolic derangement

To evaluate whether the response differed between subjects with mild and severe metabolic derangement, we analyzed the subjects with a mean TG above 1.64 mmol/L compared to those below the mean plasma TG. Subgroup analysis showed a numerical reduction of IHTG content in the highest TG group (absolute IHTG from 12.7% (± 3.9) to 11.8% (± 4.3) (p=0.378) with a relative IHTG change of −6.3% (p=0.682), which did not reach statistical significance. In the subjects below mean TG, IHTG levels remained stable (absolute IHTG from 9.9% (± 6.9) to 10.4% (± 8.1), as reflected by a relative IHTG change of +3.6% (p=0.759). Changes in hepatic and peripheral insulin sensitivity were not significantly different between upper versus lower TG groups. Finally, TG levels decreased in the upper TG group following TRC150094 (from 2.66 ± 1.17 to 2.26 ± 1.31 mmol/L, p =0.012) whereas no change was observed in the lower TG group. See supplementary Table 5 for an overview of the subgroup analyses.
Figure 1 – Efficacy data TRC150094 in males with increased cardiometabolic risk. A-D: Box plots of hepatic insulin sensitivity (suppression of EGP %), peripheral insulin sensitivity (Rd umol·kg$^{-1}$·min$^{-1}$), hepatic fat content (IHTG %) and insulin mediated suppression of lipolysis (suppression of lipolysis %) before after TRC administration. Blue background depicts reference values in healthy population, based on historical data$^{12-15}$. E: Bar graph of lipid profile showing no improvement after TRC150094 administration.
DISCUSSION

In the present study we show that short-term administration of TRC150094 dosed 50mg once daily is safe and well tolerated. Neither hepatic, nor peripheral insulin sensitivity improved in subjects at an increased cardiometabolic risk. In line, IHTG content and plasma lipid profiles were not altered following 4 weeks of TRC150094 administration. A subgroup analysis in subjects with TG levels above the mean did reveal a significant reduction in TG levels but no changes in insulin sensitivity nor IHTG. Collectively, these data show that, in contrast to the potent metabolic effects in experimental models, TRC150094 at a dose of 50mg daily does not improve the metabolic homeostasis in subjects at an increased cardiometabolic risk. Further studies are needed to evaluate whether TRC150094 may have an effect in subjects with more severe metabolic derangement, such as overt diabetes mellitus and hypertriglyceridemia.

Insulin sensitivity

In the present study all enrolled subjects were characterized by decreased EGP suppression as well as decreased peripheral glucose disposal rate, indicative of the presence of both hepatic and peripheral insulin resistance. Also, intrahepatic fat accumulation as assessed via IHTG content showed hepatic steatosis in all subjects. Following 4 weeks of TRC150094 administration at a dose of 50mg once daily, neither hepatic nor peripheral insulin sensitivity changed. In line, hepatic fat content and lipid profile were unaltered. This apparent discrepancy between the marked impact of TRC150094 on glycemic profile, hepatic fat accumulation and serum lipids in experimental protocols and the absence of any change in the present clinical study may have several explanations, consisting of the mechanism of action, the dose and concentration of TRC150094.

First, the mechanism of action of di-iodothyronine (T2). The biologically active thyroid hormone tri-iodothyronine (T3) exerts its effects via specific nuclear receptors; namely TR α and β. T2 has a 50-400 times lower affinity for TR than T3, making it unlikely that TR activation contributes to the effects of T2. Extensive preclinical work, however, did substantiate a rapid effect of T2 on energy expenditure in rats, which were shown to be mediated by direct effects on mitochondria independent of classical nuclear thyroid receptors. The T2 mimetic TRC150094, which is also associated with minimal TR transcriptional activation, was observed to increase whole body mitochondrial fatty acid oxidation (FAO) and resting metabolic rate (RMR) in rats, leading to marked improvements of glucose and lipid homeostasis. In contrast, data on the (patho)physiological relevance of T2 in humans are absent. In fact, proof for a receptor-independent effect of T2 in humans is lacking altogether. In the present study, TRC150094 also failed to increase FAO in terms of decreased plasma FFA and less insulin mediated suppression of lipolysis. As this study was of shorter duration i.e. 28 days, we can not exclude the possibility that the receptor-independent activities of T2 may become apparent in humans after a longer duration.
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Secondly, in the current study 50 mg once daily dosed was selected based on drug exposure (AUC) as well as safety and tolerability data obtained from earlier animal models and a phase I Multiple Ascending Dose study in humans (data not published). In previous animal studies, the plasma exposure in which significant effects on insulin sensitivity and hepatic lipid content were observed ranged between 3.8 to 11.8 µg*h/ml. Steady state exposure of TRC150094 in humans was observed between 2.7 to 8.0 µg*h/ml after administration of 50mg once a day for 28 days. Nevertheless, the selected dose of TRC150094 50 mg once daily may have been insufficient. This could be explained by human equivalent dose (HED) calculation from animal efficacy studies. The current dose of 0.5 mg/kg (mean weight approx 100kg) in humans may be at the lower end, since conversion for drug dosage between rats and humans indicates a HED of approximately 4 mg/kg. Thus efficacy of TRC150094 at a higher dose of 75 to 100 mg once daily needs further exploration in future studies.

IHTG and plasma TG changes

Following 4 weeks of TRC150094 administration we did not observe changes in hepatic and serum lipids. The absence of a reduction in hepatic fat can in part explain the lack of an effect on insulin sensitivity, although the association between hepatic fat content and insulin sensitivity is ambiguous. Subgroup analyses included subjects with severe metabolic derangement identified via plasma TG >1.64 mmol/l at baseline. Whereas the ‘high-TG’ subjects showed comparable hepatic fat content levels at baseline compared to the low TG subjects, following TRC150094 administration hepatic fat content following TRC150094 administration was reduced numerically by 6% in the high TG subjects versus no change in the subjects with lower TG levels. In line, hepatic and peripheral insulin sensitivity did show a trend towards improvement (respectively +2.69% and +3.61%). Thus, it cannot be ruled out that an effect may have been observed in case of selection of metabolic syndrome patients with markedly elevated TG levels.

Safety and tolerability

Overall, TRC150094 administration was well tolerated and showed no safety concerns. The modest changes in FT4 were unexpected, since the affinity of TRC150094 for TR α and β is extremely low. Most importantly, we can exclude any biological relevance since no decrease in TSH was observed. Besides, other clinical manifestations of TR activation were absent, such as changes in blood pressure, heart rate, body temperature and body weight following TRC150094 administration.

In conclusion, in the present phase 2, randomized double-blind controlled trial we show that TRC150094 did not improve insulin sensitivity and lipid metabolism or decrease hepatic steatosis in obese insulin resistant subjects with an increased cardiometabolic risk. Since subgroup analysis in subjects with high triglyceride levels provided a trend towards improvement, future studies should address the potential impact of TRC150094 administration at higher dose, particularly in patients at a high cardiometabolic risk with elevated TG levels.
REFERENCE LIST

7. Per Bengtsson. KARO BIO Terminates the Eprotirome Program. 2012
SUPPLEMENTAL MATERIAL

Supplementary Information 1 – Methods

A. Hyperinsulinemic euglycemic clamp

Prior to the study day, all subjects refrained from vigorous exercise for 48 hours. After an overnight fast, subjects were admitted to the metabolic ward of the study centre at 07:15 hours. A catheter was inserted in an antecubital vein for infusion of stable isotope tracers, insulin and glucose. Another catheter was inserted into a contralateral hand vein and kept in a thermoregulated (60°C) Plexiglas box for sampling of arterialised venous blood. Saline was infused as NaCl 0.9% at a rate of 50 ml/h to sustain catheter patency. [6,6-²H₂]glucose and [1,1,2,3,3-²H₅]glycerol were infused as tracers (>99% enriched; Cambridge Isotopes, Andover, MA, USA) to study glucose kinetics and lipolysis (total triacylglycerol hydrolysis), respectively. At time 0 (08:30 hours) blood samples were drawn for determination of background enrichments, where after a continuous infusion of isotopes was started ([6,6-²H₂]glucose and [1,1,2,3,3-²H₅]glycerol, both at a rate of 0.11 μmol*kg⁻¹*min⁻¹, with a priming dose equivalent to 80 min of infusion) and continued until the end of study. After an equilibration time of 150 min, three blood samples were taken for the measurement of isotope enrichments and one for the measurement of glucoregulatory hormones and NEFA. Thereafter, a two-step hyperinsulinaemic–euglycaemic clamp was started. A continuous infusion of insulin (Actrapid 100 U/ml; Novo Nordisk Farma, Alphen aan de Rijn, the Netherlands) was started for 130 min at the rate of 20 mU [m² body surface area]⁻¹*min⁻¹, followed by an infusion of insulin at a rate of 60 mU [m² body surface area]⁻¹*min⁻¹ for another 130 min. Plasma glucose levels were measured every 10 min at the bedside. Glucose was infused as 20% glucose at a variable rate, to maintain a plasma glucose concentration of 5.0 mmol/l. [6,6-²H₂]glucose was added to the 20% glucose solution to achieve glucose enrichments of 1% to approximate the values for enrichment reached in plasma and thereby minimise changes in isotopic enrichment due to changes in the infusion rate of exogenous glucose. During the last 40 min of both hyperinsulinaemic periods, blood samples were drawn at 5 min intervals for determination of isotope enrichments and glucoregulatory hormones.

During the study day, all subjects remained fasted but were allowed to drink water.

B. Glucose and glucoregulatory hormones measurements

Plasma glucose concentrations were measured with the glucose oxidase method using a YSI analyzer. [6,6-²H₂]glucose enrichment (tracer-to-tracee ratio) was measured as reported earlier with an intra-assay variation of 0.5–1% and an inter-assay variation of 1% and a detection limit of 0.04%. [1,1,2,3,3-²H₅]glycerol enrichment was determined with an intra-assay variation of 1–3% for glycerol and 4% for [1,1,2,3,3-²H₅]glycerol, and inter-assay variation of 2–3% for glycerol and 7% for [1,1,2,3,3-²H₅]glycerol, as reported earlier. Insulin was determined on an Immulite 2000 system (Diagnostic Products, Los Angeles, CA, USA). Insulin was measured with a chemiluminescent immunometric assay with intra-assay variation of 3–6%, inter-assay variation of 4–6% and detection limit of 15 pmol/l. Calculations and statistics HOMA of insulin resistance (HOMA-IR) was calculated using the formula described previously by Matthews et al. Endogenous glucose production (EGP)
and peripheral glucose uptake (rate of disappearance [Rd]) were calculated using the modified forms of the Steele equations. EGP and Rd were expressed as μmol kg⁻¹min⁻¹. Insulin clearance was calculated as the rate of insulin infusion (mU [m² body surface area]⁻¹ min⁻¹) divided by the mean plasma insulin concentration during the clamp. Lipolysis (glycerol turnover) was calculated using formulas for steady-state kinetics adapted for stable isotopes and was expressed as μmol kg⁻¹min⁻¹. Lipolysis was assessed as percentage change in rate of appearance of glycerol from the basal to low dose insulin-stimulated state. Plasma FFA concentrations were measured with an enzymatic colorimetric method (NEFA-C test kit; Wako Chemicals GmbH, Neuss, Germany) (intra-assay variation 1%, total-assay variation 4-15%; detection limit 0.02 mmol/L).

C. ¹H MRS

¹H-MRS spectra were acquired using a 3.0 T Intera (Philips, Best, the Netherlands). During the measurements, subjects remained in the supine position within the MRI scanner. IHTG content was obtained using single-voxel ¹H-MRS, using a body array coil as the transmitter and phased surface coils as receivers. MRS measurements were acquired during breathhold, using single-voxel stimulated acquisition mode (TE/TR 20/3.000 ms, six acquisitions). Volumes of interest in the liver were located away from major vascular structures and bile ducts. Voxel size was 27 mm³. The water and fat resonance peaks, located at 4.65 and 1.3 ppm, were integrated using jMRUI software, and relative fat content was expressed as the ratio of the fat peak area over the cumulative water and fat peak areas. Calculated peak areas of water and fat were corrected for T2 relaxation (T2water, 34 ms; T2fat, 68 ms32) and the percentage hepatic fat content was calculated.33
T2 ANALOGUE IN METABOLIC MEN

Supplementary Figure 1 - Overview of study scheme. * Screenfailures in specific; due to malignancy in history, ECG abnormalities at screening, too low fasting insulin or glucose, haemoglobin, age and/or withdrawing of consent after screening.
### Supplementary Table 1 – Characteristics of Study Subjects at Baseline Between Ethnicities

<table>
<thead>
<tr>
<th></th>
<th>Indian (N = 20)</th>
<th>Caucasian (N = 20)</th>
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<tbody>
<tr>
<td></td>
<td>TRC150094 (N = 10)</td>
<td>Placebo (N = 10)</td>
</tr>
<tr>
<td>Age, y</td>
<td>41 ± 7</td>
<td>43 ± 6</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>87 ± 7</td>
<td>90 ± 11</td>
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<td>Body mass index, kg/m²</td>
<td>30.2 ± 2.7</td>
<td>31.1 ± 3.3</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>105 ± 6</td>
<td>107 ± 7</td>
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<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>5.7 ± 1.3</td>
<td>5.3 ± 0.7</td>
</tr>
<tr>
<td>Fasting plasma insulin, mU/L *</td>
<td>8 ± 3</td>
<td>13 ± 9</td>
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<tr>
<td>HOMA-IR</td>
<td>2.0 ± 1.0</td>
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<td>Cholesterol, mmol/L</td>
<td>4.68 ± 1.09</td>
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<tr>
<td>HDLc</td>
<td>1.02 ± 0.30</td>
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<td>LDLc</td>
<td>2.89 ± 0.81</td>
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<td>TG</td>
<td>1.50 ± 1.16</td>
<td>1.91 ± 0.91</td>
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<tr>
<td>Plasma free fatty acids, mmol/L *</td>
<td>0.52 ± 0.12</td>
<td>0.40 ± 0.09</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>139 ± 2</td>
<td>139 ± 2</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>89 ± 1</td>
<td>89 ± 1</td>
</tr>
</tbody>
</table>

**NOTE.** Values are expressed as mean ± standard deviation.

*Baseline characteristics were comparable between Indian and Caucasian subjects except for BMI, fasting insulin and FFA. The body mass index is the weight in kilograms divided by the square of the height in meters. HDLc, high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol; TG, triglycerides.
Supplementary Table 2 – Number of patients with adverse events

<table>
<thead>
<tr>
<th></th>
<th>TRC150094 (N = 20)</th>
<th>Placebo (N = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serious adverse event</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Adverse event</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Intensity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Moderate</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Relationship to TRC150094/Placebo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not likely</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Possible</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Probable</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Event</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Back pain</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Blurred vision</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Dry mouth</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Flu like symptoms</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Headache</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Heartburn</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Hip pain</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Increased appetite</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Insomnia</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Polyuria</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Rash</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
### Chapter 5

 Supplementary Table 3 – Glucose Kinetics, Glucoregulatory hormones in TRC and Placebo Group at Baseline and After 4 Weeks Treatment

<table>
<thead>
<tr>
<th></th>
<th>TRC (N = 20)</th>
<th>Placebo (N = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>5.2 (4.1-8.4)</td>
<td>5.5 (4.5-8.9)</td>
</tr>
<tr>
<td>Step 1</td>
<td>5.1 (4.7-6.4)</td>
<td>5.1 (4.8-5.8)</td>
</tr>
<tr>
<td>Step 2</td>
<td>5.0 (4.8-5.5)</td>
<td>5.1 (4.6-5.4)</td>
</tr>
<tr>
<td>Insulin, μU/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>11 (2-28)</td>
<td>11 (4-29)</td>
</tr>
<tr>
<td>Step 1</td>
<td>32 (19-68)</td>
<td>30 (17-81)</td>
</tr>
<tr>
<td>Step 2</td>
<td>96 (62-225)</td>
<td>97 (54-197)</td>
</tr>
<tr>
<td>EGP, μmol/kg/min⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>9.1 (7.5-13.4)</td>
<td>9.0 (7.4-13.4)</td>
</tr>
<tr>
<td>Step 1</td>
<td>3.9 (1.2-3.8)</td>
<td>2.7 (0.6-7.7)</td>
</tr>
<tr>
<td>Step 2</td>
<td>60 (52-67)</td>
<td>62 (55-70)</td>
</tr>
<tr>
<td>Egpr suppr, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>27.5 (11.7-48.0)</td>
<td>24.2 (11.1-46.9)</td>
</tr>
<tr>
<td>Plasma FFA, mmol/L</td>
<td>0.51 (0.36-0.77)</td>
<td>0.51 (0.26-0.67)</td>
</tr>
<tr>
<td>Lipolysis, μmol/kg/min⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>2.1 (1.2-3.2)</td>
<td>2.2 (1.3-4.6)</td>
</tr>
<tr>
<td>Step 1</td>
<td>0.9 (0.6-1.7)</td>
<td>1.0 (0.6-3.2)</td>
</tr>
<tr>
<td>Lipolysis suppr, %</td>
<td>57 (32-77)</td>
<td>54 (20-77)</td>
</tr>
</tbody>
</table>

NOTE. Values are expressed medians (minimum – maximum).
Step 1 is measurements during low dose insulin infusion and step 2 is measurements during high dose insulin infusion during hyperinsulinemic euglycemic clamp.

 Supplementary Table 4 – IHTG content and lipid profiles in TRC and Placebo Group at Baseline and Week 4

<table>
<thead>
<tr>
<th></th>
<th>TRC (N = 20)</th>
<th>Placebo (N = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IHTG content, %</td>
<td>10.8 ± 6.1</td>
<td>10.9 ± 6.9</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tot al cholesterol, mmol/L</td>
<td>4.63 ± 1.03</td>
<td>4.58 ± 1.02</td>
</tr>
<tr>
<td>LDLc</td>
<td>2.91 ± 0.82</td>
<td>2.81 ± 0.79</td>
</tr>
<tr>
<td>HDLc</td>
<td>0.94 ± 0.24</td>
<td>0.97 ± 0.21</td>
</tr>
<tr>
<td>TG</td>
<td>1.61 ± 1.06</td>
<td>1.75 ± 1.00</td>
</tr>
</tbody>
</table>

NOTE. Values are expressed mean ± standard deviation.
Supplementary Table 5 – Subgroup analyses in Subjects with High TG

<table>
<thead>
<tr>
<th></th>
<th>TRC (N = 7)</th>
<th>Placebo (N = 8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>4 weeks</td>
<td></td>
</tr>
<tr>
<td>EGP suppr, %</td>
<td>56.2 ± 10.5</td>
<td>58.3 ± 15.4</td>
<td>+ 2.69</td>
</tr>
<tr>
<td>Rd, µmol/kg/min</td>
<td>25.9 ± 8.1</td>
<td>27.0 ± 11.4</td>
<td>+ 3.61</td>
</tr>
<tr>
<td>IHTG, %</td>
<td>12.7 ± 3.9</td>
<td>11.8 ± 4.3</td>
<td>- 6.31</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>4.87 ± 1.33</td>
<td>4.60 ± 1.52</td>
<td>- 6.46</td>
</tr>
<tr>
<td>LDLc</td>
<td>2.79 ± 1.08</td>
<td>2.67 ± 1.14</td>
<td>- 4.60</td>
</tr>
<tr>
<td>HDLc</td>
<td>0.86 ± 0.12</td>
<td>0.90 ± 0.11</td>
<td>+ 5.89</td>
</tr>
<tr>
<td>TG</td>
<td>2.66 ± 1.17</td>
<td>2.26 ± 1.31</td>
<td>- 18.6</td>
</tr>
<tr>
<td></td>
<td>70.0 ± 17.2</td>
<td>64.4 ± 17.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>26.5 ± 5.9</td>
<td>25.5 ± 5.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.3 ± 4.0</td>
<td>9.5 ± 5.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.13 ± 0.98</td>
<td>5.23 ± 0.76</td>
<td>+ 2.93</td>
</tr>
<tr>
<td></td>
<td>3.25 ± 0.81</td>
<td>3.29 ± 0.69</td>
<td>- 0.22</td>
</tr>
<tr>
<td></td>
<td>0.82 ± 0.13</td>
<td>0.83 ± 0.19</td>
<td>+ 2.44</td>
</tr>
<tr>
<td></td>
<td>2.33 ± 0.66</td>
<td>2.47 ± 0.61</td>
<td>+ 7.23</td>
</tr>
</tbody>
</table>

Subgroup analyses of subjects with serum TG >1.64 mmol/l at baseline. NOTE. Values are expressed mean ± standard deviation. Relative change is the mean relative change in percentage.