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# A Comparison of the Effects of Troglitazone and Vitamin E on the Fatty Acid Composition of Serum Phospholipids in an Experimental Model of Insulin Resistance

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## Summary

The aim of this study was to investigate the effects of troglitazone (TRO) – a new insulin-sensitizing agent – on some metabolic parameters in an experimental model of hypertriglyceridemia and insulin resistance, hereditary hypertriglyceridemic rats, and to compare its effects with those of vitamin E, an antioxidant agent. Three groups of the above rats were fed diets with a high content of sucrose (70 % of energy as sucrose) for four weeks. The first group was supplemented with TRO (120 mg/kg diet), the second one with vitamin E (500 mg/kg diet), and the third group served as the control. Vitamin E supplementation did not lower serum triglycerides (2.42±0.41 *vs.* 3.39±0.37 mmol/l, N.S.) while TRO did (1.87±0.24 *vs.* 3.39±0.37 mmol/l, $p<0.01$). Neither TRO nor vitamin E influenced the serum levels of free fatty acids (FFA). Both drugs influenced the spectrum of fatty acids in serum phospholipids – TRO increased the levels of polyunsaturated fatty acids (PUFA) n-6 (36.04±1.61 *vs.* 19.65±1.56 mol %, $p<0.001$), vitamin E increased the levels of PUFA n-3 (13.30±0.87 *vs.* 6.79±0.87 mol %, $p<0.001$) and decreased the levels of saturated fatty acids (32.97±0.58 *vs.* 51.45±4.01 mol %, $p<0.01$). In conclusion, TRO lowered the level of serum triglycerides but vitamin E did not have this effect in hypertriglyceridemic rats. Compared with TRO, vitamin E had a different effect on the spectrum of fatty acids in serum phospholipids.

**Key words**
Troglitazone • Vitamin E • Fatty acids • Serum phospholipids • Insulin resistance

## Introduction

The syndrome of insulin resistance, in which hypertriglyceridemia is associated with hyperinsulinemia, increased availability of free fatty acids and elevated blood pressure, is considered to be one of the risk factors in the development of cardiovascular diseases and noninsulin-dependent diabetes mellitus (Reaven 1992, 1995). There is growing evidence that changes in fatty acid distribution and the process of lipid peroxidation might be involved in many of the key events in the pathogenesis of cardiovascular diseases (Witztum 1994). Previously, we found increased lipoprotein oxidability and aortic lipid peroxidation in an experimental model of the insulin resistance syndrome (Kazdová *et al.* 1997). Recently, troglitazone, which belongs to the family of insulin-sensitizing agents called thiazolidinediones, was found to improve insulin resistance. It has been



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demonstrated that troglitazone (TRO) and other thiazolidinediones can serve as ligands for PPAR gamma (Lehmann *et al.* 1995) and stimulate its transcriptional activity (Lenhard *et al.* 1997). It has been shown in several reports that TRO can increase the sensitivity of skeletal muscles *via* increased expression of the glucose transporters GLUT1 and GLUT4 and inhibit the activity of lipoprotein lipase in adipocytes and, in this way, decrease the levels of free fatty acids (Ciaraldi and Henry 1997, Horikoshi and Yoshioka 1998). Thiazolidinediones share a thiazolidine-2,4-dione structure which is responsible for the majority of pharmacological actions. TRO was synthesized by alpha-tocopherol (vitamin E) chain substitution in an attempt to produce a drug with an antioxidant effect. TRO and vitamin E have a similar scavenging effect on reactive oxygen species (Inoue *et al.* 1997). In this study, we investigated the effect of TRO on serum triglycerides, free fatty acids, oral glucose tolerance test as a parameter of insulin resistance, and serum phospholipid fatty acid composition in hereditary hypertriglyceridemic rats. This line of rats, selected in our laboratory from rats of the Wistar strain, exhibits insulin resistance, hyperinsulinemia and elevated blood pressure (Vrána and Kazdová 1990). The effect of TRO was compared with that of vitamin E therapy.

## Methods

Three groups of adult (3 months old) female hereditary hypertriglyceridemic rats (HHTg), weighing 240-260 g, were fed diets rich in sucrose (70 % of energy as sucrose) for four weeks. The first group (n=7) was supplemented with TRO (120 mg/kg diet), the second group (n=7) with vitamin E (500 mg/kg diet), the third one (n=7) served as the controls. The rats were killed by decapitation in the postprandial state, mixed blood from the neck vessels was collected, and the serum separated. The levels of TG and FFA in serum were measured by enzymatic kits (Lachema, Czech Republic and Boehringer, Germany, respectively). For the oral glucose tolerance test (OGTT), performed 3 days before decapitation, plasma glucose was determined by a glucose oxidase method (Lachema, Czech Republic). After overnight fasting, the rats received 30 % glucose (1 ml/kg b.w.) by oral gavage. Blood samples were withdrawn from the tail immediately prior to and 30, 60 and 120 min after glucose administration. From these measurements, we calculated the area under the curve (AUC). Serum lipids were extracted according to Folch (Folch *et al.* 1957). Lipid classes were separated by thin layer chromatography using hexane-diethylether-acetic acid (80:20:3, v/v) as a solvent system. Fatty acids in serum phospholipids were converted to methyl esters using 1 % solution of Na in methanol. The fatty acid methyl esters were eluted with hexane. Gas chromatography of the fatty acid methyl esters was performed on a GS 5890A (Hewlett Packard, USA) instrument equipped with a flame-ionization detector. A carbowax fused silica capillary column (25 m x 0.25 mm i.d.) was used. The column temperature was 150–225 °C (2 °C/min), hydrogen was used as the carrier gas. Individual peaks of fatty acid methyl ester were identified by comparing retention times with those of authentic standards (Sigma, Czech Republic). The composition of serum FA (spectrum of 17 main fatty acids) was analyzed. All data are expressed as means ± S.E.M. For expression of influence of troglitazone and vitamin E we used Bonferroni adjustment of significance levels. $P<0.05$ was considered statistically significant. Statistical analysis of differences was carried out by the BMDP program.

## Results

Neither TRO nor vitamin E supplementation influenced the body weight of the animals. The levels of TG and FFA in the serum are shown in Table 1.

**Table 1.** Levels of serum triglycerides (TG) and free fatty acids (FFA)

| Group of rats | Serum TG (mmol/l) | Significance * | Serum FFA (mmol/l) | Significance * |
|---|---|---|---|---|
| *HHTg control* | 3.39±0.37 | - | 0.71±0.07 | - |
| *HHTg + TRO* | 1.87±0.24 | $p<0.05$ | 0.63±0.07 | N.S. |
| *HHTg + vitamin E* | 2.42±0.41 | N.S. | 0.69±0.17 | N.S. |

*Values represent means ± S.E.M. *The groups of HHTg+TRO and HHTg+vitamin E are compared to the HHTg control group.*

Although treatment with TRO decreased the level of TG, vitamin E supplementation did not. The decreases in the serum FFA levels caused by TRO or vitamin E were not significant compared with the control group.

The fatty acid (FA) composition in serum phospholipids is shown in Table 2. Analyses of the 17 main FA were done. FA were divided into four groups, which means the sums of saturated (Σ SFA), monounsaturated (Σ MUFA), polyunsaturated n-6 (Σ n6), and polyunsaturated n-3 (Σ n3) fatty acids. The change caused by TRO is significant only in the percentage of arachidonic acid (20:4n6) which increased (20.88±1.47 *vs.* 14.24±1.67 mol %, $p<0.05$).

**Table 2**: The profile of FA in serum phospholipids.

| FA | HHTg control (mol %) | HHTg + TRO (mol %) | Significance * | HHTG+vit. E (mol %) | Significance * |
|---|---|---|---|---|---|
| *14:0* | 0.29±0.19 | 0.10±0.03 | N.S. | 0.04±0.02 | N.S. |
| *16:0* | 13.19±1.95 | 9.46±2.48 | N.S. | 1.83±0.30 | p<0.01 |
| *16:1n7* | 1.26±.38 | 0.83±2.28 | N.S. | 0.08±0.02 | p<0.01 |
| *18:0* | 37.79±2.85 | 33.55±2.01 | N.S. | 30.86±0.78 | N.S. |
| *18:1n9* | 7.96±1.41 | 5.85±0.30 | N.S. | 6.05±0.28 | N.S. |
| *18:1n7* | 2.43±0.24 | 1.96±0.17 | N.S. | 2.58±0.16 | N.S. |
| *18:2n6* | 10.21±0.71 | 9.46±1.03 | N.S. | 7.53±0.50 | p<0.05 |
| *18:3n6* | 0.16±0.01 | 0.16±0.04 | N.S. | 0.20±0.04 | N.S. |
| *18:3n3* | 0.27±0.14 | 0.50±0.26 | N.S. | 0.27±0.15 | N.S. |
| *20:0* | 0.18±0.03 | 0.33±0.11 | N.S. | 0.25±0.03 | N.S. |
| *20:1n9* | 0.27±0.04 | 1.36±0.51 | N.S. | 0.78±0.07 | p<0.01 |
| *20:2n6* | 0.72±0.15 | 0.88±0.14 | N.S. | 1.28±0.17 | N.S. |
| *20:3n6* | 2.58±0.59 | 2.94±0.39 | N.S. | 3.97±0.29 | N.S. |
| *20:4n6* | 14.24±1.67 | 20.88±1.47 | p<0.05 | 30.06±0.38 | p<0.01 |
| *20:5n3* | 4.07±0.43 | 5.02±0.47 | N.S. | 6.35±0.45 | p<0.01 |
| *22:4n6* | 1.91±0.39 | 1.72±1.03 | N.S. | 1.21±0.47 | N.S. |
| *22:6n3* | 2.45±0.44 | 4.98±1.98 | N.S. | 6.69±0.86 | p<0.01 |
| *Σ SFA* | 51.45±4.01 | 43.44±3.98 | N.S. | 32.97±0.58 | p<0.01 |
| *Σ MUFA* | 11.93±1.85 | 10.01±0.68 | N.S. | 9.48±0.47 | N.S. |
| *Σ PUFA n6* | 29.65±1.56 | 36.04±1.61 | p<0.05 | 44.27±0.64 | p<0.01 |
| *Σ PUFA n3* | 6.79±0.87 | 10.50±2.37 | N.S. | 13.30±0.87 | p<0.01 |
| *ΣSFA/ ΣUFA* | 1.47±0.27 | 0.82±0.12 | N.S. | 0.77±0.04 | p<0.05 |
| *Σ n6/ Σ n3* | 3.02±0.19 | 4.18±0.56 | N.S. | 1.57±0.11 | p<0.05 |

*Values represent means ± S.E.M. *The groups of HHTg+TRO and HHTg+vitamin E are compared to the HHTg control group.*

In spite of small non-significant changes, the whole group of PUFA n-6 increased significantly compared with the control group (36.04±1.61 *vs.* 29.65±1.56 mol %, $p<0.05$). The saturated/unsaturated ratio and the polyunsaturated n-6/polyunsaturated n-3 ratio did not change significantly. Vitamin E supplementation had a different effect. It changed the profile of seven FA. There was a decrease in the level of saturated FA (32.97±0.58 *vs.* 51.45±4.01 mol %, $p<0.01$) and an increase in the polyunsaturated n-3 group (13.30±0.78 *vs.* 6.79±0.87 mol %, $p<0.01$). The saturated/unsaturated ratio decreased (0.77±0.04 *vs.* 1.47±0.27, $p<0.05$) as did the polyunsaturated n-6/polyunsaturated n-3 ratio (1.57±0.11 *vs.* 3.02±0.19, $p<0.05$).

To assess the insulin sensitivity, we performed the oral glucose tolerance test. The values expressed as the area under the curve (AUC) are shown in Table 3. Fasting blood glucose was decreased by vitamin E treatment but not by TRO. Neither TRO nor vitamin E supplementation influenced AUC.

**Table 3.** Fasting blood glucose level (FBG) and oral glucose tolerance test expressed as AUC in HHTg rats.

| Group of rats | FBG (mmol/l) | Significance * | AUC | Significance * |
|---|---|---|---|---|
| *HHTg control* | 4.979±0.265 | - | 706±29.5 | - |
| *HHTg + TRO* | 4.382±0.118 | N.S. | 685±19.2 | N.S. |
| *HHTg + vitamin E* | 4.073±0.119 | $p<0.01$ | 673±26.7 | N.S. |

*Values represent means ± S.E.M. *The groups of HHTg+TRO and HHTg+vitamin E are compared to the HHTg control group.*

## Discussion

TRO was found to decrease plasma triglycerides in various insulin-resistant experimental models (Komers and Vrána 1998). In our study, TRO treatment decreased the level of serum triglycerides, but vitamin E treatment did not. Both drugs shared a part of the alpha-tocopherol molecule, but vitamin E does not have the thiazolidin-2,4-dione structure which seems to be responsible for this pharmacological effect. In some studies, TRO has been shown to decrease the levels of free fatty acids (FFA) in diabetic rats (Sreenan *et al.* 1996, Mokuda and Sakamoto 1998). The decreases in serum FFA in our study were not significant. It might be particularly due to a different experimental model employed or to different doses of TRO administration. The response of hereditary hypertriglyceridemic rats to insulin action is impaired (Vrána *et al.* 1993). It can be particularly explained by the low levels of the glucose transporters GLUT4 in this line (Šeböková *et al.* 1995). TRO has an insulin-sensitizing effect (Kumar *et al.* 1998, Day 1999). This mechanism may include increasing GLUT1 expression in insulin-resistant subjects (Park *et al.* 1998) and raising the number of preadipocytes – both the differentiation rate and the percentage of proliferation – in rats and humans (Adams *et al.* 1997, Okuno *et al.* 1998). In our study with hypertriglyceridemic female rats and a 120 mg of TRO/kg diet, we have not found any improvement in glucose tolerance. Although TRO improved carbohydrate and lipid metabolism in various experimental models with insulin resistance (for review see Komers and Vrána 1998), our results are different. There might be several reasons for it: different dose of the drug, duration of supplementation, gender or age of animals. Vitamin E decreased FBG. Similar results were described by Paolisso *et al.* (1993) who supplemented elderly Type-II diabetic patients with vitamin E. The fatty acid composition in the serum or in any tissues reflects the activity of esterifying or elongating enzymes, the quantities of dietary FA, and protection of FA against damage by free radicals (Fernandes and Venkatraman 1993, Berry 1997). Vitamin E, an antioxidant agent, increased the levels of polyunsaturated n-3 fatty acids and decreased the saturated/unsaturated ratio. This means that the high serum level of vitamin E decreased oxidative stress, which is present in insulin-resistant rats (Kazdová *et al.* 1996), and protects polyunsaturated FA against damage by free radicals. The low saturated/unsaturated ratio may be convenient because n-3 fatty acids are precursors to many metabolites which improve certain metabolic disorders associated with insulin resistance (Ginter 1988). In our study, TRO increased the level of arachidonic acid (20:4 n6), finally resulting in increased levels of polyunsaturated n-6 fatty acids. It has been reported that TRO may inhibit stearoyl-CoA desaturase 1 enzyme activity by repressing its gene expression in adipocytes (Kurebayashi *et al.* 1997). Stearoyl-CoA desaturase catalyses the desaturation of palmitic and stearic acids to palmitoleic and oleic acids, respectively. In our study, we did not find lower levels of palmitoleic (16:1 n7) or oleic (18:1 n9) acid and the product/precursor ratios (16:1 n7/16:0 and 18:1 n9/18:0) were not significantly different in the TRO-treated group. The results indicate that despite the presence of a part of alpha-tocopherol in its molecule, TRO seems to work differently. Its effect on the composition of FA was – in terms of their atherogenicity – less beneficial than that of vitamin E.

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