Neural control of hepatic lipid metabolism: A (patho)physiological perspective
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THE EFFECT OF PHARMACOLOGICAL BLOCKADE OF THE SYMPATHETIC NERVOUS SYSTEM ON SERUM TRIGLYCERIDES IN HUMAN SUBJECTS


* These authors contributed equally.
ABSTRACT

Animal experimental studies indicate that blocking sympathetic activity towards the liver could be beneficial in lowering circulating triglyceride concentrations. In this study, we investigated if the β-adrenergic receptor blocker, propranolol, widely used for the treatment of cardiovascular disease, decreases serum triglyceride concentration in humans. To this end, we performed a randomized clinical study in healthy post-menopausal women receiving the non-selective β-blocker propranolol or no medication for 12 weeks. Our results show that propranolol, already at a low dose, increases triglyceride concentrations without significant effects on plasma cholesterol. Review of the literature shows that, although many studies have methodological limitations, propranolol increases triglycerides and decreases HDL-cholesterol concentrations. The mechanism of this effect is not yet fully understood, although it has been hypothesized that decreased adipose tissue lipoprotein lipase activity is involved.
INTRODUCTION

Increasing evidence shows that several measures of high sympathetic activity correlate with components of the metabolic syndrome, including hypertriglyceridemia (1,2). Animal studies using surgical sympathetic denervation have shown promising effects in lowering secretion by the liver of triglycerides packed in very low density lipoproteins (VLDL-TG) (3,4). Therefore, it can be hypothesized that blocking sympathetic activity is beneficial in the treatment of dyslipidemia. Compared with surgery, pharmacological sympathetic blockade represents a less invasive method which could possibly have the same effects in humans.

β-adrenergic receptor blockers, widely used for the treatment of cardiovascular disease, block the action of adrenaline and noradrenaline on the β-adrenergic receptors, thus making the post-synaptic neurons or parenchymal cells less sensitive to the input of the sympathetic nervous system. Three types of β-adrenergic receptors are known: β1, β2 and β3 receptors. β1-adrenergic receptors are located mainly in the heart and kidneys, β2-adrenergic receptors are located mainly in the lungs, gastrointestinal tract, liver, uterus, vascular smooth muscle, and skeletal muscle, and β3-adrenergic receptors are located mainly in adipocytes. β-blockers can be divided into several categories depending on their specificity for the β-adrenergic receptor. All β-blockers approved for human use antagonize the β1 receptor, either selectively or in combination with the β2 receptor and most β-blockers are prescribed to treat cardiovascular disease.

The liver is the central organ in processing free fatty acids and in controlling the secretion of triglycerides in VLDL-TG particles. In the human liver, mainly β2-adrenergic receptors are present (5). To investigate if pharmacological sympathetic blockade has a beneficial effect on plasma triglyceride levels we performed a randomized clinical study in healthy post-menopausal women receiving the non-selective β-blocker propranolol or no medication for 12 weeks. Subsequently, we performed a literature search on the relationship between plasma lipids and propranolol.

MATERIALS AND METHODS

Analysis of lipid metabolism in healthy post-menopausal women receiving propranolol

Subjects

Sixteen healthy post-menopausal female subjects were recruited from the Amsterdam region, the Netherlands, through advertisement. The protocol was approved by the Medical Ethical Committee of the Academic Medical Center in Amsterdam. All participants provided written informed consent before participation.

Study design

This randomized controlled trial was carried out at the Academic Medical Center (AMC), University of Amsterdam. At baseline, participants were interviewed concerning their medical history of cardiovascular disease (CVD), dyslipidemia, smoking and use of lipid lowering drugs. Body weight, height and blood pressure were measured, electrocardiography was
performed and an overnight fasted venous blood sample was taken. Participants were randomly assigned by block randomization to receive propranolol 80 mg retard once daily or no medication for the duration of the study (12 weeks). Subsequent visits were planned at 4 weeks, 8 weeks and 12 weeks and at every visit an overnight fasted venous blood sample was taken.

**Analytical procedures**

All serum samples were collected in the morning between 7:00 and 9:00 h after an overnight fast. Serum concentrations of total cholesterol, HDL-cholesterol and triglycerides were measured on a Roche Modular autoanalyzer using standard colorimetric techniques at the central laboratory of the AMC, University of Amsterdam. LDL-cholesterol was calculated with the Friedewald formula (

\[ \text{LDL (mmol/L)} = \text{[Cholesterol total]} - \text{[HDL]} - (0.45 \times \text{[Triglycerides]}). \]

**Statistical analysis**

We used SPSS version 20 (IBM) for the statistical analysis. All measures are reported using the mean and standard error of mean. To test for differences between the two groups at baseline, an unpaired t-test was performed. Differences between the groups were investigated in a linear mixed model including the factors Time, Treatment and the interaction between Time*Treatment without correction for baseline measurements. A natural log transformation was performed for triglycerides and total cholesterol. For triglycerides, a random intercept was included to take into account the differences in starting levels between the patients. The significance level was set at p = 0.05. Assumptions of the model were met.

**Literature search**

We performed a search in Medline (December 2012) with synonyms for triglycerides and propranolol. Additionally, we scanned the reference lists of all relevant studies. The papers that prospectively investigated the effects of propranolol on triglycerides, separate from other medication, were selected if reporting on the dose of propranolol and the absolute concentrations of triglycerides either before and after treatment, or between a propranolol and placebo/no treatment group. Furthermore, the effects of propranolol on total cholesterol, HDL-cholesterol and LDL-cholesterol were recorded. In papers reporting on the concentrations of triglycerides or cholesterol in mg/dl, the concentrations were converted to mmol/l (Triglycerides mmol/l = mg/dL x 0.0113; Cholesterol mmol/L = mg/dL x 0.0259). When standard deviation (SD) was used to report on the error, standard error of the mean (SEM) was calculated (SEM = SD/√n).

**RESULTS**

**Analysis of lipid metabolism in healthy post-menopausal women receiving propranolol**

The baseline characteristics of the subjects are presented in Table 1. None of the patients had a history of cardiovascular disease or dyslipidemia. No patients used statins. One patient in the propranolol group smoked. There were no significant differences between the groups.
Participants receiving propranolol for 12 weeks showed an increase in serum triglyceride concentrations compared to control subjects as apparent from a significant interaction effect ($\text{Time} p = 0.075$; $\text{Time}\times\text{Treatment} p = 0.001$; $\text{Treatment} p = 0.956$) (Figure 1A). The mean baseline corrected difference between the control and propranolol groups at the end of the experiment was 0.82 mmol/l. Changes in total cholesterol concentrations were not different between the groups ($\text{Time} p = 0.973$; $\text{Time}\times\text{Treatment} p = 0.547$; $\text{Treatment} p = 0.650$) (Figure 1B). Likewise, HDL-cholesterol ($\text{Time} p = 0.388$; $\text{Time}\times\text{Treatment} p = 0.165$; Table 1).

<table>
<thead>
<tr>
<th>Table 1. Baseline characteristics</th>
<th>Values are shown in mean ± SEM.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Control (n = 8)</td>
</tr>
<tr>
<td></td>
<td>53 ± 1</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>Blood pressure systolic (mmHg)</td>
<td>124 ± 4</td>
</tr>
<tr>
<td>Blood pressure diastolic (mmHg)</td>
<td>73 ± 4</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.47 ± 0.29</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.96 ± 0.28</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.68 ± 0.31</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.62 ± 0.14</td>
</tr>
</tbody>
</table>

Figure 1 A-D. Baseline corrected serum lipid profile of control participants (open circles) and participants receiving propranolol (closed squares) measured every 4 weeks for the total duration of 12 weeks. Values are shown in mean ± SEM.
Treatment $p = 0.783$) and LDL-cholesterol ($\text{Time} p = 0.709; \text{Time} \times \text{Treatment} p = 0.425; \text{Treatment} p = 0.742$) did not reveal significant differences between the groups (Figure 1 C and D).

**Literature search**

A summary of the 16 selected papers is shown in Table 2 and Figure 2 (6-21). All studies reported triglycerides and some reported total cholesterol, HDL-cholesterol and LDL-cholesterol concentrations. Studies were performed in a before-after design ($n = 7$), cross-over design with placebo ($n = 7$), observational design comparing propranolol with no treatment ($n = 1$) or randomized controlled trial ($n = 1$). The study populations of these studies, except for the randomized controlled trial by Shulman et al. (19) ($n = 1432$), were relatively small with a median of 17 subjects (range 7-53). The large study by Shulman et al. (19) used non-fasted samples. Seven studies included male patients exclusively, six studies included both sexes, and in three studies the ratio of males to females is unknown. Patients with hypertension ($n = 8$), cardiovascular disease ($n = 7$) or combined hypertension and dyslipidemia ($n = 1$) were included in the studies. Only one study investigated healthy males, aged 24 to 37 years (11). The doses of propranolol ranged from 40-640 daily, given from 1 week up to 24 months.

Nine out of sixteen studies showed a significant increase of triglycerides after treatment with propranolol. Four out of sixteen studies reported a decrease in total cholesterol levels, eight out of twelve studies reported a decrease in HDL-cholesterol levels and two out of ten studies reported a decrease in LDL-cholesterol.

**DISCUSSION**

In this randomized controlled study we found that propranolol, already at a low dose, increases triglyceride concentrations in normolipidemic, normotensive women within 12 weeks without significant effects on plasma cholesterol. This shows that non-selective pharmacological blockade of sympathetic activity in healthy women has unfavourable effects in terms of the lipid spectrum.

Review of the literature on propranolol and triglycerides shows that most studies reported an increase of plasma triglycerides, including a large cohort of patients in the β-blocker heart attack trial (6,9,10,12,13,14,15,18,19). It should however be noted that many studies have methodological limitations. For example, studies were performed in a ‘before and after treatment’ design, neglecting effects of inclusion in a study. Furthermore, mainly patients with hypertension, coronary artery disease or dyslipidemia were studied. In the β-blocker heart attack trial (19) the average non-fasted level of triglycerides in the placebo group was $= 2.5$ mmol/l. The average level of triglycerides in our study is in the low risk category (< 1.70 mmol/l; American Heart Association). Five out of the nine studies from our literature search with average baseline values below 1.70 mmol/l showed a significant increase of triglycerides between 0.19 and 0.91 mmol/l and are therefore comparable to our study (6,9,10,12,18). However, only one previous study measured the effect of propranolol in healthy subjects and they did not find an effect of propranolol given 1-2 weeks in healthy
Table 2. Overview of studies on the effects of propranolol on triglycerides (TG), total cholesterol (Total-C), HDL-cholesterol (HDL-C) and LDL-cholesterol (LDL-C).

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>P/C</th>
<th>M/F</th>
<th>Patient group</th>
<th>Mg daily (doses)</th>
<th>Duration</th>
<th>TG</th>
<th>Total-C</th>
<th>HDL-C</th>
<th>LDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andreasen et al. 1984</td>
<td>BA</td>
<td>25/NA</td>
<td>13/12</td>
<td>Hypertension</td>
<td>160-640 (4)</td>
<td>4 weeks</td>
<td>↑</td>
<td>↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bernik et al. 2005</td>
<td>CO</td>
<td>11/NA</td>
<td>14/10 PM</td>
<td>Hypertension</td>
<td>160 (2)</td>
<td>8 weeks</td>
<td>NS</td>
<td>↓</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Birnbaum et al. 1983</td>
<td>CO</td>
<td>20/NA</td>
<td>U</td>
<td>Chronic angina pectoris</td>
<td>90-240 (3)</td>
<td>6 weeks</td>
<td>NS</td>
<td>↓</td>
<td>NS</td>
<td>↓</td>
</tr>
<tr>
<td>Day et al. 1979</td>
<td>BA</td>
<td>16/NA</td>
<td>16/0</td>
<td>Hypertension</td>
<td>120-480 (3)</td>
<td>6 months</td>
<td>↑</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day et al. 1982</td>
<td>BA</td>
<td>53/NA</td>
<td>U</td>
<td>Hypertension</td>
<td>160 (2)</td>
<td>3 months</td>
<td>↑</td>
<td>NS</td>
<td>↓</td>
<td>NS</td>
</tr>
<tr>
<td>Durrington et al. 1985</td>
<td>CO</td>
<td>11/NA</td>
<td>11/0</td>
<td>Healthy (age 24-37)</td>
<td>160 (2)</td>
<td>1-2 weeks</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Fogari et al. 1989</td>
<td>CO</td>
<td>17/NA</td>
<td>17/0</td>
<td>Hypertension</td>
<td>160 (1)</td>
<td>24 months</td>
<td>↑</td>
<td>NS</td>
<td>↓</td>
<td>NS</td>
</tr>
<tr>
<td>Fogari et al. 1999</td>
<td>CO</td>
<td>37/NA</td>
<td>37/0</td>
<td>Hypertension and dyslipidemia</td>
<td>160 (1)</td>
<td>18 months</td>
<td>↑</td>
<td>NS</td>
<td>↓</td>
<td>NS</td>
</tr>
<tr>
<td>Leren et al. 1980</td>
<td>BA</td>
<td>23/NA</td>
<td>23/0</td>
<td>Hypertension</td>
<td>80-160 (2)</td>
<td>8 weeks</td>
<td>↑</td>
<td>NS</td>
<td>↓</td>
<td>NS</td>
</tr>
<tr>
<td>Miller et al. 1987</td>
<td>BA</td>
<td>7/NA</td>
<td>4/3</td>
<td>Angina pectoris</td>
<td>80-160 (2)</td>
<td>1 year</td>
<td>↑</td>
<td>NS</td>
<td>↓</td>
<td>NS</td>
</tr>
<tr>
<td>Murphy et al. 1984</td>
<td>CO</td>
<td>9/NA</td>
<td>6/3</td>
<td>Hypertension or angina pectoris</td>
<td>320 (2)</td>
<td>12 weeks</td>
<td>NS</td>
<td>↓</td>
<td>NS</td>
<td>↓</td>
</tr>
<tr>
<td>Northcote et al. 1987</td>
<td>BA</td>
<td>21/NA</td>
<td>21/0</td>
<td>Ischaemic heart disease</td>
<td>80 (U)</td>
<td>1 year</td>
<td>NS</td>
<td>NS</td>
<td>↓</td>
<td>NS</td>
</tr>
<tr>
<td>Shaw et al. 1978</td>
<td>CO</td>
<td>17/NA</td>
<td>U</td>
<td>Hypertension</td>
<td>160-240 (1)</td>
<td>4 weeks</td>
<td>↑</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shulman et al. 1983</td>
<td>RCT</td>
<td>1432/1442</td>
<td>1198/234</td>
<td>Myocardial infarction</td>
<td>180-240 (U)</td>
<td>12 months</td>
<td>↑</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streja et al. 1978</td>
<td>OS</td>
<td>16/21</td>
<td>16/0</td>
<td>Coronary artery disease</td>
<td>40-240 (U)</td>
<td>U</td>
<td>NS</td>
<td>NS</td>
<td>↓</td>
<td>NS</td>
</tr>
<tr>
<td>Tanaka et al. 1976</td>
<td>BA</td>
<td>10/NA</td>
<td>5/5 PM</td>
<td>Stroke</td>
<td>60-120 (3)</td>
<td>7 weeks</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P/C: number of patients in propranol (P) group or control group (C); NA: control group not applicable; M/F: male/female ratio in propranolol group; BA: before and after design; CO: cross-over design starting with placebo; RCT: randomized controlled trial with placebo group; OS: Observational study comparing propranolol and no treatment; PM: post-menopausal women; U: unknown parameter; NS: not significantly changed; ↑ = significant increase after treatment compared to before treatment or placebo/no treatment group; ↓ = significant decrease after treatment compared to before treatment or placebo/no treatment group.
Figure 2. Triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol before treatment with propranol or in the placebo/no treatment group (open bars) compared to the concentrations after treatment with propranolol (closed bars). All data are shown in mmol/l as mean±SEM except for the studies by Bernik et al. and Shulman et al. where no SD or SEM is reported. * Significant difference reported in paper.
Figure 2. (Continued).
men (11). Our study in post-menopausal women who received propranolol for 12 weeks, did find an increase in triglycerides. The difference between our study and the study performed in healthy males could be due to a gender difference or the duration of treatment. However, the effect in our study was already apparent after 4 weeks. Two studies investigated the effect of gender on lipid concentrations. Andreasen et al. (6) found that the increase of plasma triglycerides with propranolol was only significant in women, whereas the large study by Shulman et al. (19) found no statistically significant gender differences.

In our study we find no changes in total cholesterol, HDL-cholesterol and LDL-cholesterol concentrations. However, previous studies in patients with hypertension or coronary artery disease did observe a decrease of HDL-cholesterol concentrations (10,12,13,14,15,17,19,20). After 12 months of follow up in the β-blocker heart attack trial (19) the treatment group, as compared to the placebo group, had 6% to 8% lower HDL-cholesterol concentrations. Possibly, our study is underpowered to detect a difference in HDL-cholesterol concentrations. Only few studies report a decrease of total cholesterol (6,7,8,16) or LDL-cholesterol concentrations (8,16).

In summary, there is mounting evidence that treatment with propranolol increases plasma triglycerides and decreases HDL-cholesterol concentrations. Although propranolol is the non-selective β-blocker studied most frequently, the non-selective β-blocker sotalol has been shown to have highly similar effects (22).

A few studies focused on the mechanism of β-blockers’ effects on lipid metabolism by comparing selective and non-selective β-blockers to elucidate which receptor is involved. Day et al. (10) compared the β1-blockers atenolol and metoprolol with the non-selective β-blocker propranolol. All β-blockers caused increased triglyceride levels, although propranolol caused a higher increase in triglyceride levels compared to the β1 selective drugs. HDL-cholesterol significantly decreased in all groups, with no changes in total cholesterol and LDL-cholesterol concentrations. Furthermore, when studying two β-blockers possessing intrinsic sympathicomimetic properties, acebutolol and pindolol, it was found that they do not increase triglyceride levels (23-26). Especially pindolol is known for its high sympathicomimetic properties. In summary, most β1-blockers and non-selective β-blockers are known to increase triglycerides and decrease HDL-cholesterol concentrations. The non-selective β-blocker with strong sympathicomimetic properties, pindolol, was found to be neutral.

The question arises which organ(s) is involved in causing increased plasma triglyceride concentrations. This could theoretically be increased synthesis of triglycerides by the liver in VLDL-TG particles, decreased clearance of triglycerides by the tissues, or a combination of both. Human studies have found decreased clearance of triglycerides after β-blockade with propranolol (10,11). Furthermore, most studies reported an increase in VLDL-TG combined with a decrease in HDL-cholesterol. These results suggest that all the changes might be mediated through inhibition of lipoprotein lipase (LPL) activity at the level of the adipocyte, as inhibition of LPL results in a decreased uptake of triglycerides. During β-blockade with propranolol, plasma catecholamine levels are increased and in vitro catecholamines appear to inactivate LPL (27,28). Therefore, α-adrenergic stimulation, rather then β-blockade might
suppress LPL during propranolol treatment, with secondary reduction of HDL-cholesterol and rising levels of triglyceride concentrations. Based on this hypothesis, α-blockade could have an opposite effect compared to β-blockade. Studies comparing the α-blocker prazosin and the β-blocker propranol indeed found opposite effects (14,29). Prazosin caused decreased triglyceride levels and increased HDL-cholesterol levels and it was proposed this was caused by activation of lipoprotein lipase activity (30).

Few studies have directly measured LPL activity after propranolol. LPL is located at the capillary endothelium and can be measured in plasma after intravenous injection of heparin, which removes it from its binding site on the capillary wall. Tanaka et al. (21) indeed found a significant reduction of post-heparin lipolytic activity after 8 weeks of treatment with propranolol. However, a study in rats measuring LPL activity and LPL mRNA either after a single dose or after four weeks of treatment with propranolol found no effect on these parameters, nor on plasma triglyceride concentrations (31). Therefore, the proposed mechanism for the increased triglyceride concentrations after β-blockade, i.e., a decreased clearance of triglycerides by α-adrenergic inhibition of LPL, is still unresolved.

In contrast to some animal studies showing a beneficiary effect on the secretion of VLDL-triglycerides from the liver after a surgical selective hepatic sympathetic denervation (3,4), our randomized trial in normotensive, normolipidemic subjects shows an opposite effect, confirming previous observations on propranolol and triglycerides. In conclusion, there is mounting evidence that treatment with propranolol increases plasma triglycerides and decreases HDL-cholesterol concentrations.

REFERENCES


