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HDL-Cholesterol Increase Associated to Triglycerides Degradation *in vitro*

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The effect of muscle tissue from rats trained by swimming on the extracellular degradation of triglyceride (TG) rich particles has been studied *in vitro*. During incubation, there is a progressive decline of the TG concentration in the incubation medium. At the end of the incubation period (90 min), a significantly reduction in the TG levels ($p < 0.05$) is associated with a significant increase in the high-density lipoprotein (HDL) cholesterol level ($p < 0.005$). There are no significant changes in total cholesterol levels. The correlation of the TG decline with the HDL-cholesterol increase is significant ($r = 0.695$, $p < 0.05$, $n = 25$). The experimental model used here could be of great interest for the *in vitro* study of factors affecting lipid levels in plasma.

Key words: HDL-cholesterol, Triglycerides, Lipoprotein lipase.

There exists evidence that physical exercise reduces the risk of coronary heart disease (1). Major studies have reached the conclusion that physical exercise raises the high-density-lipoprotein (HDL) cholesterol levels in plasma and reduces triglyceride (TG) levels (5).

The activity of certain enzymes, related to lipid metabolism, directly affects plasma lipid concentrations. Lipoprotein lipase (LPL) is responsible for the plasma TG catabolism, and catalyzes the hydrolysis of these lipids to release fatty acids that cells use as a source of energy. This enzyme is present in muscle, adipose tissue and plasma (luminal side of blood vessels). It can act enzymatically on the very-low-density-lipoprotein (VLDL) via formation of first intermediate density and then low-density-lipoproteins (9), and can also promote lipid transfer, from chilomicrons and VLDL to HDL. Reduced LPL activity leads to insufficient TG degradation in type-1 hypertriglyceridaemia.

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The present work studies the influence of exercise on LPL activity and its possible role in the extracellular degradation of TG rich particles. The model muscle tissue used was obtained by biopsy from the hind quarters of trained rats incubated with human serum.

Materials and Methods

Female Wistar rats were used, having an approximate weight of 150 g at the start of training.

The exercise consisted of swimming in a tank of 1.5 x 1 x 1 m dimensions, at 35 °C, with a maximum of 10 rats per tank. They were submitted to three 30 min sessions daily with 5 min rest periods, 5 days a week, for 6 weeks (7). The training was progressive with an increase of 1 min per session per day. On the last day of the experiment, the rats are sacrificed by cervical dislocation, followed by the inmediate resection of gastronemic muscle from the hind quarters. Ten sedentary rats were used as controls.

Human serum was obtained from healthy 22 ± 2 year-old volunteers by extraction from the cubital vein at the level of the elbow flexion. As no coagulant was used, the samples were centrifuged immediately at 2500 rpm for 10 min to separate the serum. Serum from various subjects was pooled to form a homogeneous sample, which was assayed for TG, total cholesterol and HDL-cholesterol.

The incubation of 200 mg of finely chopped muscle in 1 ml of serum was performed in a thermostatic shaking bath at 37 °C, oxygenated every 5 minutes during 2 min, with carbogen 5 % CO_2 : 95 % O_2). The supernatant was collected after 0, 30 and 90 min of incubation for plasma lipid assays.

The TG, cholesterol and HDL-cholesterol concentrations were determined by

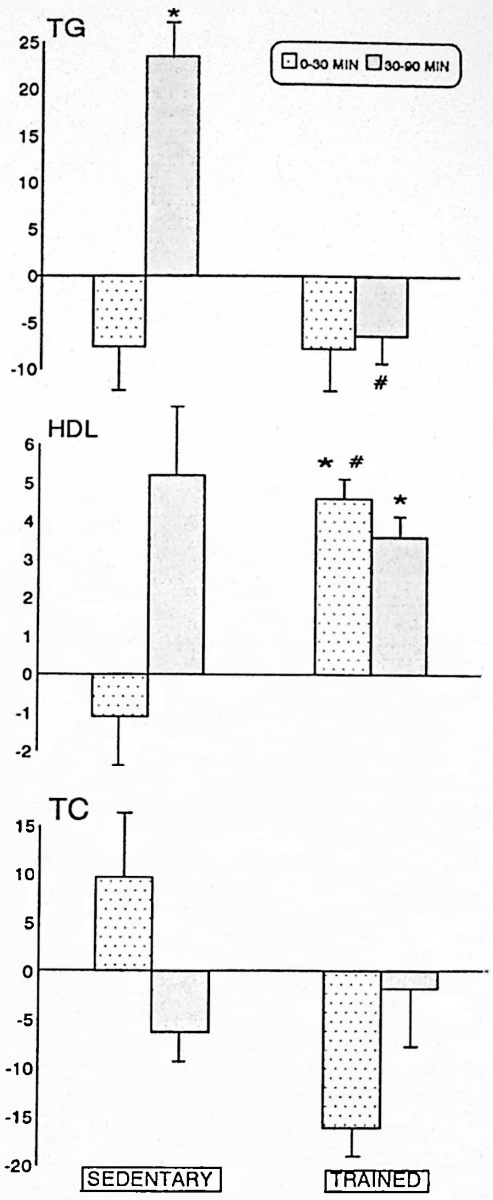

Fig. 1. *Triglyceride (TG), HDL-cholesterol (HDL) and total cholesterol (TC) over basal (time 0) increments measured after 30 and 90 min of incubation of sedentary and trained rat muscle tissue with human plasma.*
* $p < 0.05$ relative to time 0.
# $p < 0.05$ trained vs sedentary.

spectrophotometric based methods using Böehringer Mannheim commercial kits.

The results are expressed as mean ± SEM. Statistical evaluation of the results was by mean of the ANOVA test.

Results

Figure 1 shows the over-basal (time 0) increments in lipid parameters measured at minutes 30 and 90 of *in vitro* incubation of muscle tissue from 10 sedentary and 25 trained rats with human serum. When the muscle used was from trained rats, increment TG levels underwent a statistical significant reduction at the end of incubation. There were no statistically significant changes in total cholesterol increments. The HDL-cholesterol increments with muscle of trained rats rose significantly risen at the end of incubation (90 min). When the muscle incubated was from sedentary rats, a significant increment of TG levels at 90 min was observed, probably due to the release of muscle TG to the incubation medium. The increment of HDL-C at the end of incubation period was no significant.

The analysis of the correlation between the fall in the TG levels and the rise in the HDL-cholesterol levels in trained rats, showed a statistically significant negative correlation ($r = -0.695$, $p < 0.05$, $n = 25$).

Discussion

The mechanism giving rise to the changes observed in plasma lipid levels as a consequence of exercise is still not entirely clear, but it is generally admitted that the enzyme LPL plays a major role in the process (4, 5). In several papers, a correlation between LPL activity and HDL-cholesterol levels has been demonstrated (3, 9). The inverse correlation between the TG and HDL-cholesterol levels found by other authors (2) in clinical studies and in professional cyclists (8) suggests that the generation of HDL particles associated with the intravascular degradation of TG-rich lipoproteins could explain the rise in HDL levels in cyclists during a prolong cycling race by stages (6). As the cyclists take in food in the course of a stage, many chilomicrons and VLDL's might well be metabolized directly by the muscle during the exercise itself in the presence of high LPL levels. This process would be associated with the generation of HDL particles (5). Various studies have shown a correlation between LPL activity and HDL-cholesterol levels. Our *in vitro* results demonstrate that the change in the lipoprotein profile of plasma (the rise in HDL-cholesterol and fall in TG levels) found in trained individuals could partially be explained by such intravascular degradation, since the incubation of muscle from our trained rats with human serum produced alterations that were similar to those obtained in aerobic sports people. There also exists a significant correlation between the TG decline and the HDL-cholesterol rise.

The *in vitro* system presented here could provide a relatively simple model to study the factors affecting plasma lipid levels.

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B. JIMENO, M. A. G. ZUBELDIA, M. G. GOMEZ, J. E. CAMPILLO y P. MENA. *Incremento de colesterol en HDL asociado al consumo de triglicéridos* in vitro. Rev. esp. Fisiol. (J. Physiol. Biochem.), 51 (2), 101-104, 1995.

Se estudian *in vitro* los efectos del tejido muscular de ratas entrenadas, sobre la degradación extracelular de partículas ricas en triglicéridos. Durante la incubación se produce un progresivo descenso de la concentración de triglicéridos en el medio de incubación y al final (90 minutos) se observa un descenso significativo ($p < 0,05$), asociado a un incremento significativo de la concentración de colesterol en HDL ($p < 0,005$). No hay modificaciones significativas en las cifras de colesterol total. El descenso de triglicéridos se correlaciona significativamente con el aumento de colesterol en HDL ($r = 0,695$, $p < 0,05$, $n = 25$). El sistema experimental propuesto puede ser de gran interés para el estudio *in vitro* de los factores que influyen sobre los lípidos plasmáticos.

Palabras clave: Colesterol en HDL, Triglicéridos, Lipoproteína lipasa.

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