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Short Communication

# Alterations in triglyceride rich lipoproteins are related to endothelial dysfunction in metabolic syndrome

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ABSTRACT

Our aim was to analyze the effect of circulating triglyceride rich lipoprotein (TRL) on endothelial function in metabolic syndrome (MetS).

**Methods:** We studied 40 patients with MetS (ATPIII), divided into those presenting normal endothelial function ($n = 19$) and those with endothelial dysfunction ($n = 21$) by means of the evaluation of pulse wave velocity, before and after brachial artery ischemia. In fasting serum we measured lipid and lipoprotein profile, insulin and glucose (HOMA-IR). Moreover, isolated TRL ($d < 1006$ g/l) were chemically characterized. In parallel, using randomly selected TRL from MetS patients with endothelial dysfunction ($n = 6$) and MetS patients with normal endothelial function ($n = 6$), the ability of TRL to inhibit ACh-induced vasorelaxation ($10^{-9}$–$10^{-5}$ mM) on aortic rings previously pre-contracted by noradrenaline ($10^{-8}$ mM) was evaluated.

**Results:** Interestingly, TRL isolated from MetS patients presenting endothelial dysfunction showed triglyceride over-enrichment ($59.1 \pm 4.8$ *vs.* $54.1 \pm 4.7\%$; $p = 0.04$), even after adjusting by potential confounders ($p = 0.05$). In addition, while TRL resulting from both MetS groups significantly inhibited endothelium dependent vasorelaxation ($p < 0.001$), TRL from MetS patients with endothelial dysfunction showed a strong tendency to a greater inhibition of vasorelaxation ($p = 0.06$). Moreover, TRL-triglyceride (%) showed a strong tendency to correlate with the grade of vasorelaxation inhibition exerted by TRL ($r = 0.60$; $p = 0.05$).

**Conclusion:** These results, taken together, would allow inferring for the first time that the predominance of triglyceride over-enriched TRL in circulation in MetS would induce endothelial dysfunction, contributing to the inherent cardiovascular risk of MetS.



## 1. Introduction

Metabolic syndrome (MetS) is a nowadays common disorder, leading to an increased risk for cardiovascular disease and type 2 diabetes. MetS is characterized by elevated fasting triglyceride and low high-density lipoprotein cholesterol levels, predominance of small dense LDL sub-fraction with higher atherogenic capacity, and postprandial accumulation of triglyceride rich lipoproteins (TRLs) [1].

Endothelial dysfunction is one of the first steps involved in the pathophysiology of atherosclerosis, leading to cardiovascular disease. Currently, it is possible to evaluate endothelial function by means of non-invasive methods. In a recent publication, the authors suggest that an alternative to evaluate endothelial function is to measure carotid-radial pulse wave velocity (PWV), previous and post-ischemia of brachial artery [2].

In previous reports, we observed that the presence of insulin-resistance was related with the predominance of modified TRL in circulation, which were triglyceride over-enriched and larger in size [3,4]. Nevertheless, to date it is not known whether these TRL exert or not endothelial dysfunction.

Our aim was to study the effect on endothelial function of circulating TRL isolated from MetS patients.

## 2. Materials and methods

Forty untreated patients with diagnosis of MetS (ATPIII criteria) [5] were consecutively selected among those who attended at the Clinical University Hospital, University of Buenos Aires, Argentina.

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Written informed consent was required from all the participants to be included in the study, which was approved by the Ethics Committee of the Faculty of Pharmacy and Biochemistry, University of Buenos Aires.

Patients were derived to the Laboratory of Hypertension, Cardiology Division from the same Hospital, for the evaluation of endothelial function by means of carotid-radial PWV, measured before and after the induction of ischemia on the brachial artery. Carotid-radial post-ischemia changes were considered normal after a PWV reduction ≥5% from baseline and abnormal when the reduction was <5% or with a paradoxical response (increasing PWV) [2]. Thus, MetS patients were divided into two groups: one group with those patients with normal endothelial function ($n = 19$) and other group with those patients who presented altered endothelial function ($n = 21$). No significant differences were observed in gender proportion between groups ($p = 0.7634$).

After 12 h fasting, blood was drawn from all patients. In serum, total cholesterol, triglycerides and glucose were measured using standardized methods (Roche Diagnostics, Mannheim, Germany) in a Cobas 6000 autoanalyser, CV intra-assay < 1.9%, CV inter-assay < 2.4%, averaging CV values of these parameters. HDL and LDL cholesterol were determined by homogeneous assays (Roche Diagnostics, Mannheim, Germany), CV intra-assay < 1.5% and CV inter-assay < 2.0%. Insulin was measured with Immulite/Immulite 1000 Insulin (Siemens, USA). In order to estimate insulin-resistance, the HOMA-IR index was calculated [6].

Moreover, TRL were isolated from serum by ultracentrifugation ($\delta < 1006$ g/l) and characterized in their components: triglycerides and cholesterol were measured by the previously mentioned methods, phospholipids were assessed by measuring the phosphorous in the dry residue after lipid extraction [7] and proteins by Lowry method. The sum of all them in mg/dl was considered as total circulating TRL mass, and percentage composition of each component was calculated.

### 2.1. Effect of TRL on endothelium: an in vitro assay

In order to determine the direct effect of TRL in MetS on the endothelium, we implemented an *in vitro* endothelial function assay as previously described [8]. Randomly selecting six isolated TRL from MetS patients with altered endothelial function and six TRL isolated from MetS patients with normal endothelial function. Briefly, the assay consisted in the incubation of each isolated TRL with aorta rings surgically obtained from Sprague–Dawley rats.

Acetylcholine-mediated relaxation of aorta rings (Acetylcholine concentration; $10^{-9}$–$10^{-5}$ mM), previously contracted with noradrenaline ($10^{-8}$ mM), was determined both in the presence and in the absence of TRL. In each case dose-response curves were obtained, representing the endothelium-mediated relaxation of aorta rings, one in absence of TRL (blank curve) and the other in the presence of each studied TRL.

The maximum relaxation induced by acetylcholine was expressed as the percentage of the obtained relaxation, regarding the maximum contraction induced by noradrenaline (100% of contraction or 0% of relaxation). Each curve obtained in the presence of TRL was compared to each respective blank curve (obtained without TRL), and the difference between the percentage of maximum relaxation of both curves was considered as the percentage of inhibition of endothelial relaxation, exerted by TRL.

All procedures were carried out according to the National Institute of Health Guide for the Care and Use of Laboratory Animals [9] and the protocol was approved by the Institutional Committee for the Care and Use of Laboratory Animals of the Faculty of Pharmacy and Biochemistry, University of Buenos Aires.

### 2.2. Statistical analysis

Results were expressed as mean ± SD or median (range) for normal or skewed data, respectively. Differences between groups were tested using the unpaired Student's *T*-test for normally distributed data and Mann-Whitney U Test for skewed data. Pearson analysis was used to determine correlations between parameters. All analyses were performed using SPSS 17.0 software. *p* values <0.05 were considered significant.

## 3. Results

Table 1 shows clinical and biochemical characteristics of patients with MetS, divided into two groups according they presented normal or altered endothelial function. No differences in age and waist circumference were observed between groups ($p > 0.1409$). However, patients with altered endothelial function presented increased HOMA-IR ($p = 0.002$), without differences in lipid-lipoprotein profile between groups ($p > 0.129$). Moreover, neither systolic blood pressure nor diastolic blood pressure differed between groups ($p > 0.352$).

TRL isolated from patients presenting altered endothelial function showed triglyceride over-enrichment in comparison to TRL from patients with normal endothelial function [altered endothelial function: 59.1 ± 4.8 *vs.* normal function: 54.1 ± 4.7%; $p = 0.04$]. This difference practically maintained significance after adjusting by HOMA-IR, systolic blood pressure, total cholesterol and LDL-cholesterol as co-variables ($F = 7.2$; $p = 0.05$). No differences were observed in the other components. Additionally, patients with altered endothelial function showed higher total TRL mass [altered endothelial function: 125.1 ± 76.8 *vs.* normal function: 112.5 ± 41.8 mg/dl; $p = 0.048$], however this difference was no longer significant after adjusting by HOMA-IR, systolic blood pressure, total cholesterol and LDL-cholesterol as co-variables ($F = 1.7$; $p = 0.25$).

Furthermore, endothelial dysfunction exerted by TRL was evaluated by means of the *in vitro* assay from which dose-response curves were plotted. Fig. 1 shows the dose-response curves in the absence and in the presence of TRL isolated from MetS patients with normal and altered endothelial function. In comparison to the control curve, TRL from both MetS groups significantly inhibited endothelium relaxation ($p < 0.001$), meaning that both groups of TRL induced endothelial dysfunction. However, comparing TRL from both MetS groups, those from patients with altered endothelial function showed a strong tendency to a greater inhibition of endothelium relaxation ($p = 0.06$).

Triglyceride content in TRL presented a strong tendency to directly correlate with the grade of inhibition of relaxation exerted by TRL ($r = 0.60$; $p = 0.05$).

**Table 1**
Clinical and biochemical characteristics of patients with metabolic syndrome, divided whether they presented normal or altered endothelial function determined by carotid-radial pulse wave velocity (PWV) measured before and after the induction of ischemia on the brachial artery.

| | Normal function ($n = 19$) | Altered function ($n = 21$) |
|---|---|---|
| Age (years) | 59 ± 10 | 57 ± 12 |
| Waist (cm) | 102.1 ± 10.1 | 105.0 ± 7.2 |
| BMI (kg/m$^2$) | 31.2 ± 6.1 | 33.9 ± 7.7 |
| Glucose (mg/dl) | 97 ± 14 | 116 ± 23* |
| Insulin (μU/ml) | 6.0 (2.1–12.6) | 19.7 (7.5–29.2)* |
| HOMA-IR | 1.5 (0.5–3.9) | 6.6 (2.9–8.9)* |
| Total cholesterol (mg/dl) | 192 ± 29 | 203 ± 34 |
| Triglycerides (mg/dl) | 128 ± 31 | 141 ± 77 |
| LDL-cholesterol (mg/dl) | 125 ± 27 | 137 ± 31 |
| HDL-cholesterol (mg/dl) | 47 ± 9 | 44 ± 8 |
| Non-HDL-cholesterol (mg/dl) | 138 ± 30 | 156 ± 41 |
| Systolic blood pressure (mm Hg) | 135 ± 25 | 130 ± 13 |
| Diastolic blood pressure (mm Hg) | 80 ± 14 | 77 ± 6 |

Carotid-radial post-ischemia changes were considered normal after a PWV reduction of 5% or more from baseline and abnormal when the reduction was <5% or with a paradoxical response (increasing PWV). Data expressed as mean ± SD for normally distributed variables and median (range) for skewed distributed variables. Student's *T*-test for parametric data and Mann Whitney *U* test for non parametric data. BMI: body mass index. HOMA-IR: homeostasis model assessment for insulin resistance index.

* $p < 0.05$.

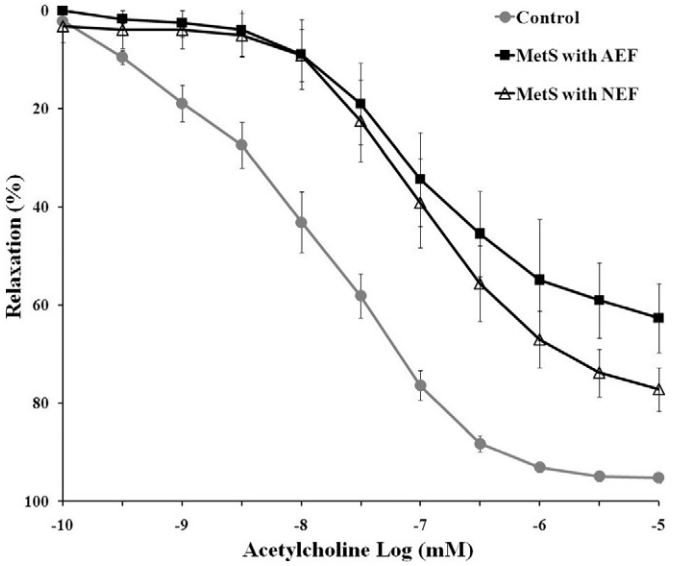


**Fig. 1.** Effect of triglyceride rich lipoproteins (TRLs) on endothelium function: relaxation percentage curves as function of the logarithm of accumulated acetylcholine dose. Mean concentration–response curves to acetylcholine obtained in the absence of lipoproteins (grey curve with circles), and in the presence of TRL isolated from patients with metabolic syndrome (MetS) with altered endothelial function (AEF) (closed squares; 0.15 mg protein/ml), and TRL from MetS patients with normal endothelial function (NEF) (open triangles; 0.15 mg protein/ml).

## 4. Discussion

In the present study we evaluated the action of the circulating TRL from MetS patients on endothelial function. TRL were studied in patients with normal and abnormal endothelial function, and isolated TRL from sub-groups of patients were implemented in an *in vitro* bioassay to assess their effect on endothelial function. It is the first time that altered TRL from MetS patients would demonstrate to impair endothelial function.

Several studies have established the existence of an association between insulin-resistance and alterations in endothelial function [10–12]. In an animal model, Sukumar P et al. show that the increase oxidative stress, produced in insulin-resistant states, affects nitric oxide bioavailability contributing to endothelial dysfunction [11]. In humans, most of the evidence demonstrates endothelial dysfunction in insulin-resistant patients by means of several detection methodologies of variable invasiveness [10,12].

The carotid-radial PWV-measured before and after the induction of ischemia on the brachial artery-allowed the sub-division of patients into those with normal and those with abnormal endothelial function. This method represents an alternative and useful non-invasive tool for endothelial function evaluation [2]. In our studied population, MetS patients with endothelial dysfunction were more insulin-resistant than those with normal endothelial function, evidenced by increased HOMA-IR.

While modified LDL causes endothelial dysfunction through inhibition of endothelium-dependent relaxation, by decreasing nitric oxide bioavailability [13], some contradictions still exist regarding whether TRL exert or not endothelial dysfunction [14,15].

It has been already demonstrated that TRL in MetS present alterations in their size and composition [4,16]. In this study, the TRL particles from MetS patients with post-ischemia PWV reduction below 5% or with a paradoxical response presented higher triglyceride content; even after adjusting by other factors such as insulin-resistance. In a previous study we have demonstrated that qualitative alterations in isolated VLDL from donors were associated with greater endothelial dysfunction measured *in vitro* [8]. Herein, TRL from the two MetS groups were randomly selected for the measurement of endothelial relaxation by adding acetylcholine. The *in vitro* bioassays revealed that TRL from MetS with endothelial dysfunction tended to exert greater inhibition in endothelial relaxation than TRL from patients with normal endothelial function. To our knowledge, the link between circulating TRL from MetS patients and endothelial dysfunction has not been addressed so far.

It is important to highlight the strong tendency to a positive correlation between triglyceride content in TRL and the grade of *in vitro* inhibition of vasorelaxation exerted by TRL. This association would reinforce the higher triglyceride content in TRL from MetS patients with endothelial dysfunction. Surely, other more exhaustive lipoprotein composition studies, like metabolomic studies for example, would be needed to elucidate the responsible component in TRL particles which would be related to the action of these TRLs on arterial wall.

Given that TRL were able to inhibit acetylcholine-mediated relaxation, which is nitric oxide dependent, it could be inferred that altered TRL would affect endothelial function through a similar mechanism to LDL.

A limitation of the present study is the low number of isolated TRL from MetS patients analyzed in the *in vitro* bioassay ($n = 6$ in each group), finding just a tendency to a greater inhibition of endothelial relaxation in MetS patients with endothelial dysfunction in comparison to those with normal function. However, it should be noted that this kind of *in vitro* bioassays are laborious and time-consuming. Certainly, with a greater number of TRL in each group, this difference would have reached statistical significance. Nevertheless, the strong tendencies found by the implementation of this specific *in vitro* bio-assay, even with a low number of studied cases, become important because the direct pure effect of TRL on endothelium is being analyzed, without the interference of external confounders.

Although the results in the present study come from two different experimental models, taken together, they allow inferring that in insulin-resistant states, beyond high plasma triglyceride levels, the presence of TRL with modified composition would exacerbate the deleterious effect of TRL on endothelium, inducing a greater degree of endothelial dysfunction and thus contributing to the inherent cardiovascular risk in MetS.

## Conflict of interests

There are no conflicts of interest to disclose.

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