Clin Invest Arterioscl. 2015;27(4):167–174

CLÍNICA E INVESTIGACIÓN EN ARTERIOSCLEROSIS

www.elsevier.es/arterio

ORIGINAL ARTICLE

# Overproduction of altered VLDL in an insulin-resistance rat model: Influence of SREBP-1c and PPAR-α

**Diego Lucero[a,*], Verónica Miksztowicz[a], Vanesa Macri[b], Gustavo H. López[c], Silvia Friedman[b], Gabriela Berg[a], Valeria Zago[a], Laura Schreier[a]**

[a] *Laboratory of Lipids and Atherosclerosis, Department of Clinical Biochemistry, Faculty of Pharmacy and Biochemistry, INFIBIOC, University of Buenos Aires, Buenos Aires, Argentina*
[b] *Oral and General Biochemistry Department, Faculty of Dentistry, University of Buenos Aires, Buenos Aires, Argentina*
[c] *Bioanalytics, Department of Biology, Biochemistry and Pharmacy, National Southern University, Bahía Blanca, Buenos Aires, Argentina*

Received 15 October 2014; accepted 7 November 2014
Available online 18 March 2015

**KEYWORDS**
Insulin-resistance;
Sucrose rich diet;
Sterol regulatory element binding protein 1c;
Peroxisome proliferator-activated receptor-α;
Large very low density lipoprotein

**Abstract**
*Background:* In insulin-resistance, VLDL presents alterations that increase its atherogenic potential. The mechanism by which insulin-resistance promotes the production of altered VLDL is still not completely understood. The aim of this study was to evaluate the relationship between the expression of sterol regulatory element binding protein 1c (SREBP-1c) and of peroxisome proliferator-activated receptor-α (PPAR-α), with the features of composition and size of VLDL in an insulin-resistance rat model induced by a sucrose rich diet (SRD).
*Methods:* The study was conducted on 12 male Wistar rats (180 g) receiving SRD (12 weeks) and 12 controls. Lipid profile, free fatty acids, glucose, and insulin were measured. Lipid content in liver and visceral fat were assessed. Isolated VLDL ($d < 1.006$ g/ml) was characterized by its chemical composition and size by HPLC. The respective hepatic expression of SREBP-1c and PPAR-α was determined (Western blot).
*Results:* As expected, SRD had elevated triglycerides (TG), free fatty acids and insulin levels, and decreased HDL-cholesterol ($p < 0.05$), together with augmented hepatic and visceral fat ($p < 0.05$). SRD showed higher VLDL total mass – with increased TG content – and predominance of large VLDL ($p < 0.05$). SRD showed an increase in SREBP-1c (precursor and mature forms) and decreased PPAR-α expression ($p < 0.045$). SREBP-1c forms were positively associated with VLDL total mass ($p < 0.04$), VLDL-TG% ($p < 0.019$), and large VLDL% ($p < 0.002$). On the other hand, PPAR-α correlated negatively with VLDL total mass ($p = 0.05$), VLDL-TG% ($p = 0.005$), and large VLDL% ($p = 0.002$).

* Corresponding author.
*E-mail address:* dmlucero@ffyb.uba.ar (D. Lucero).

http://dx.doi.org/10.1016/j.arteri.2014.11.002

*Conclusions:* Insulin-resistance, by coordinated activation of SREBP-1c and reduction of PPAR-α, could promote the secretion of larger and TG over-enriched VLDL particles, with greater atherogenic capacity.


**PALABRAS CLAVE**
Insulinorresistencia;
Dieta rica en sacarosa;
Proteína ligadora de elementos reguladores de esteroles-1c;
Receptores activados por factores de proliferación peroxisomal-α;
Lipoproteínas de muy baja densidad grandes

**Sobreproducción de VLDL alteradas en un modelo de insulinorresistencia de rata: influencia de SREBP-1c y PPAR-α**

**Resumen**
*Introducción:* En la insulinorresistencia, la VLDL presenta alteraciones que aumentan su potencial aterogénico. El mecanismo por el cual la insulinorresistencia promueve la producción de VLDL alteradas aún no se comprende completamente. Objetivo: evaluar la relación entre la expresión de la proteína ligadora de elementos reguladores de esteroles-1c (SREBP-1c) y de los receptores activados por factores de proliferación peroxisomal-α (PPAR-α) con las características de composición y tamaño de VLDL en un modelo animal de insulinorresistencia inducida por dieta rica en sacarosa (DRS).
*Métodos:* Estudiamos 12 ratas macho Wistar (180 g) que recibieron DRS (12 semanas) y 12 controles. Se midieron el perfil lipídico, los ácidos grasos libres, la glucosa y la insulina. Se cuantificaron el contenido lipídico hapático y la grasa visceral. Se caracterizó la VLDL aislada (d < 1,006 g/ml) en composición química y tamaño (HPLC). Se determinó la expresión hepática de SREBP-1c y PPAR-α (Western-blot).
*Resultados:* Esperadamente, el grupo DRS presentó elevación de triglicéridos (TG), ácidos grasos libres e insulina y disminución de colesterol-HDL (p < 0,05), junto con incremento de grasa hepática y visceral (p < 0,05). La DRS mostró una mayor masa total de VLDL —con mayor contenido de TG— y predominio de VLDL grandes (p < 0,05). DRS presentó expresión incrementada de SREBP-1c (precursor y maduro) y disminuida de PPAR-α (p < 0,045). Ambas formas de SREBP-1c se correlacionaron positivamente con masa total de VLDL (p < 0,04), TG%-VLDL (p < 0,019) y VLDL-grande % (p < 0,002). Mientras que PPAR-α se correlacionó negativamente con masa total de VLDL (p = 0,05), TG %-VLDL (p = 0,005) y VLDL-grande % (p = 0,002).
*Conclusiones:* La insulinorresistencia, mediante una coordinada activación de SREBP-1c y reducción de PPAR-α, promovería la secreción de partículas de VLDL grandes y sobreenriquecidas en TG, con mayor capacidad aterogénica.


## Introduction

Nowadays, insulin-resistant syndromes present a growing prevalence in the world, leading to an important increase in risk of type 2 diabetes and cardiovascular disease. Atherogenic dyslipidemia, characterized by high triglycerides, low HDL cholesterol, and predominance of small dense LDL particles, is one of the alterations of insulin-resistant syndromes directly associated to coronary disease development.[1]

Very low density lipoproteins (VLDL) constitute a heterogeneous family of particles varying in size and/or composition and atherogenic potential. In a previous report, we have observed in insulin-resistant rats an increased secretion of VLDL particles over-enriched in triglycerides, in spite of the concomitant presence of triglyceride deposits in the liver.[2] More recently, we studied VLDL features in humans with metabolic syndrome and observed a predominance of larger VLDL sub-fractions, implementing size exclusion HPLC.[3] The production of this type of VLDL can be due, in part, to an increased free fatty acid flux from adipose tissue to the liver, although the full mechanisms by which insulin-resistance influences VLDL features secreted from the liver still remain not completely understood.

Hepatic fatty acid homeostasis is principally regulated by factors as sterol regulatory element binding protein 1c (SREBP-1c) and peroximal proliferator-related receptor-α (PPAR-α) that control the hepatic fatty acid synthesis and oxidation respectively.[4,5]

The SREBP-1c constitutes a key regulator in the transcription of lipogenic enzymes, such as fatty acid synthase and acetyl CoA carboxylase, which are involved in the de novo synthesis of fatty acids.[4] SREBP-1c is inserted in the endoplasmic reticulum as a precursor form (125 kDa). By its proteolytic cleavage, the N-terminal active and mature form (68 kDa) is translocated to the nucleus and thus directly activates fatty acids synthesis increasing fatty