Journal of Clinical Lipidology (2018) ■, ■–■

Journal of Clinical Lipidology

Original Article

# Efficacy of repeated phlebotomies in hypertriglyceridemia and iron overload: A prospective, randomized, controlled trial

**Rocío Mateo-Gallego, PhD, Laura Lacalle, MD, Sofía Pérez-Calahorra, MSc, Victoria Marco-Benedí, MSc, Valle Recasens, MD, Noelia Padrón, MD, PhD, Itziar Lamiquiz-Moneo, MSc, Lucía Baila-Rueda, PhD, Estíbaliz Jarauta, MD, PhD, Pilar Calmarza, PhD, Ana Cenarro, PhD[1], Fernando Civeira, MD, PhD*,[1]**

*Lipid Unit, Hospital Universitario Miguel Servet, IIS Aragon, CIBERCV, Zaragoza, Spain (Drs Mateo-Gallego, Pérez-Calahorra, Marco-Benedí, Lamiquiz-Moneo, Baila-Rueda, Jarauta, Cenarro, and Civeira); Universidad de Zaragoza, Zaragoza, Spain (Dr Mateo-Gallego, Recasens, and Civeira); Hematology Department, Hospital Universitario Miguel Servet, Zaragoza, Spain (Dr Lacalle, Recasens, and Padrón); and Biochemistry Department, Hospital Universitario Miguel Servet, Zaragoza, Spain (Dr Calmarza)*

**KEYWORDS:**
Phlebotomy;
Hypertriglyceridemia;
Iron overload;
Ferritin;
Clinical trial

**BACKGROUND:** High ferritin concentration is associated with hypertriglyceridemia, although it is not elucidated if iron overload has a causal role.

**OBJECTIVE:** To evaluate the efficacy of repeated phlebotomies in patients with iron overload and hypertriglyceridemia.

**METHODS:** Twelve weeks, 1:1 randomized, parallel-groups trial conducted at a University Hospital Lipid Clinic, including 86 subjects aged 18–70 years with serum ferritin >300 ng/mL in men or >200 ng/mL in women and triglycerides >200 mg/dL. Participants underwent: (1) three phlebotomies (every 3 weeks) and lipid-lowering dietary counseling or (2) lipid-lowering dietary counseling. The main outcome measured was the mean difference in percent change in triglyceride concentration between groups after the intervention. The mean differences in percent change of other clinical and biochemical variables (including cytokines and proinflammatory markers) after the intervention were also evaluated.

**RESULTS:** Subjects who received phlebotomies showed a significant improvement in iron metabolism. The mean percent change in triglycerides between groups was −4.68 [−20.8, 11.4]%, $P = .721$. Retinol-binding protein 4 decreased by 9.98 ± 21.7% after phlebotomies, with a mean percent change between groups of −14.2 [−25.8, −2.73]%, $P = .017$, and correlated to gamma glutamyl transferase, alanine aminotransferase and aspartate aminotransferase change. Subjects with a large reduction in hepcidin showed a large improvement in liver enzymes and proinflammatory markers.

**CONCLUSIONS:** A lipid-lowering diet plus a substantial reduction in iron deposits with repeated phlebotomies in subjects with hyperferritinemia and hypertriglyceridemia did not reduce triglyceride

ClinicalTrials.gov identifier: NCT02526121.

[1] Both authors are senior authors in this work.

* Corresponding author. Unidad de Lípidos, Hospital Universitario Miguel Servet, Avda Isabel La Católica 1-3, Zaragoza 50009, Spain.

E-mail address: civeira@unizar.es

Submitted February 12, 2018. Accepted for publication June 27, 2018.


https://doi.org/10.1016/j.jacl.2018.06.017

concentration in comparison with a lipid-lowering diet. Iron depletion for lipid management in these patients is not supported.


## Introduction

Multiple studies have demonstrated a strong association of body iron stores with all the cardiometabolic components associated with overweight, obesity, and the metabolic syndrome,[1–3] including hypertension,[4] hypertriglyceridemia,[5] low high-density lipoprotein cholesterol (HDL-C),[6] and high blood glucose and insulin.[7] This association has been mainly studied throughout cross-sectional studies in which ferritin, a good marker of body iron, stores in absence of inflammation, correlates to cardiometabolic abnormalities. Two recent meta-analysis have drawn a similar conclusion, reporting pooled odds ratio for the metabolic syndrome comparing the highest and lowest categories of ferritin levels of 1.5 and 1.2, respectively,[8,9] and higher (odds ratio: 1.92) for studies adjusting for C-reactive protein (CRP).[8] Furthermore, two prospective studies have demonstrated that high ferritin levels preceded the development of the metabolic syndrome.[10,11]

The strength of these associations differs, being milder and nonlinear for hypertension or low HDL-C[1] and is strong and linear for triglycerides (TG), at least in the ferritin range between 100 and 500 mcg/mL.[2] In agreement with the close association between ferritin and TG, subjects with primary hypertriglyceridemia demonstrated a strong association between ferritin and triglyceride concentrations, and this association was independent of the presence of the metabolic syndrome or any of its other components;[12] mutations in *HFE* gene causing hemochromatosis are associated with primary hypertriglyceridemia[13]; and TG decreased in subjects under therapeutic phlebotomy because of iron overload,[14] such as hemochromatosis.[15]

The relationship between increased ferritin and the metabolic syndrome or hypertriglyceridemia still needs to be clarified. Iron concentrations are increased in inflammation and iron overload favors the production of free radicals and oxidative stress. These are two of mechanisms involved in the development of metabolic complications of the metabolic syndrome.[16] Moreover, hepatic triglyceride secretion is highly increased in presence of clinical inflammation and proinflammatory cytokines,[17] and the liver is the preferential target of iron toxicity.[18] Iron accumulation in obesity seems to result from downregulation of the iron export protein ferroportin-1 that is produced, at least in part, by increased levels of cytokines.[19]

If iron overload has per se a causal role contributing to hypertriglyceridemia or if high serum ferritin and TG just share common pathogenic mechanisms cannot be inferred from observational studies. Repeated phlebotomies, typically drawing between 450 and 500 mL of blood, are the standard treatment for iron overload syndromes.[18] To elucidate whether iron reduction by phlebotomy can ameliorate hypertriglyceridemia, we have performed a randomized clinical trial with the aim of evaluating the clinical efficacy of repeated phlebotomies in patients who had iron overload and hypertriglyceridemia.

## Materials and methods

### Subjects

Subjects were recruited from April 1, 2013 through December, 2015, from the Lipid and Hematology Units at Hospital Universitario Miguel Servet in Zaragoza, Spain. Inclusion criteria included adults between 18 and 70 years, with serum ferritin >300 ng/mL in men or >200 ng/mL in women, and serum TG > 200 mg/dL in two determinations in the last year, after at least 10 hours fasting and following a lipid-lowering diet.[20] Exclusion criteria included any contraindications for phlebotomy: poor venous access, previous intolerance (hypotension or apprehension), heart disease, anemia; subjects suffering from acute or chronic diseases, including liver disease, hemochromatosis, diabetes, cancer, kidney disease, or uncontrolled thyroid disease; elevated CRP as a parameter of inflammation (>10 mg/L); alcohol intake: >30 g per day for men and >25 g per day for women; lipid-lowering drugs use in the last 6 weeks and inability to provide informed consent.

The study was conformed according to ethical guidelines of the 1975 Declaration of Helsinki and was approved by the ethical committee of our institutions: *Comité Ético de Investigación Clínica de Aragón*. All subjects provided informed written consent. The study was registered with the ClinicalTrials.gov identifier: NCT02526121.

### Randomization and intervention

Patients were randomized in a 1:1 fashion to phlebotomies with lipid-lowering diet vs lipid-lowering diet alone (control) with a computer-based random selection. Subjects randomized to phlebotomies underwent 3 phlebotomies, one every 3 weeks (400 mL per session). All subjects were given identical advice about exercise and isocaloric triglyceride-lowering diet by nutritionists specialized in lipid abnormalities at screening visit and at randomization. This lifestyle intervention included physical activity (150 min/wk of moderate or higher intensity activity) and restriction of alcohol, sugar, and refined carbohydrate intakes.[21]