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Effect of phlebotomy on lipid metabolism in subjects with hereditary hemochromatosis

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Abstract

Genetic predisposition to hereditary hemochromatosis (HH) is associated with primary hypertriglyceridemia (HTG). If iron overload influences the development of HTG, the management of these patients could be different. However, the metabolic syndrome in primary HTG is frequent; and it could partially confuse the association. The objective was to determine whether periodic bloodletting could decrease triglyceride concentrations in subjects with HH and iron overload. We retrospectively studied 155 genetically defined HH patients (C282Y homozygotes and compound heterozygotes C282Y/H63D) with iron overload and under periodic therapeutic phlebotomy. Hypertriglyceridemia (triglycerides ≥150 mg/dL) was present in 49 subjects at baseline (31.6%). Phlebotomies significantly decreased triglycerides, especially in subjects with basal HTG (from 287 mg/dL at baseline to 133 mg/dL after phlebotomies, $P < .001$). Blood glucose and total cholesterol did not change with phlebotomies. The triglyceride-lowering effect was obtained until ferritin concentration decreased to less than 200 μg/L and transferrin saturation to less than 40%. The triglyceride-lowering effect was obtained for glucose levels both less than and greater than 100 mg/dL. In summary, HH subjects frequently have HTG that improves after therapeutic phlebotomy, independently of basal blood glucose. Our results suggest that therapeutic phlebotomy could be a useful therapeutic approach in patients with HTG and iron overload.

1. Introduction

Hereditary hemochromatosis (HH) is a common disorder of iron storage in which inappropriate increase of intestinal absorption results in an excessive deposition of iron in the parenchymal cells and as a consequence tissue damage and functional impairment of organs [1]. Hereditary hemochromatosis is caused in most cases by inherited recessive mutations in *HFE* gene, located in the HLA-A locus on chromosome 6p. Homozygotes for the C282Y mutation or compound heterozygotes for mutations C282Y and H63D [1,2] in *HFE* represent more than 95% of HH cases of Northern European descent [3]. Hereditary hemochromatosis is one of the most common genetic diseases in the white population [3,4].

Type 2 diabetes mellitus is commonly observed in patients with HH [5]. Mechanisms that have been described to explain the presence of diabetes in HH include a decrease in insulin production by pancreatic β-cells associated with pancreatic iron overload, and a peripheral insulin resistance [6]. Moreover, there is a strong association between deposition of iron, measured by plasma ferritin concentrations, and the risk of type 2 diabetes mellitus and metabolic syndrome [7-9], with both situations characterized by peripheral insulin resistance [10-13]. The mechanism of this association is probably due to the induction of inflammation and oxidative stress by iron excess that favors insulin resistance [14].

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A recent study has shown that mutations in the *HFE* gene causing HH are associated with primary hypertriglyceridemia (HTG) [15], suggesting that primary iron overload could play a role in the pathogenesis of this hyperlipidemia. However, the presence of metabolic syndrome in subjects with primary HTG is frequent; and therefore, this could partially explain the association.

If iron overload plays a pathogenic role in the development of HTG [16], it is an important issue because HTG is common in general population and the clinical management of these patients could be different in the presence of iron overload. Therapeutic phlebotomy improves metabolic syndrome [10,17], but its effect on lipid metabolism and its potential beneficial effect in patients with HTG and iron overload are unknown. Therefore, the aim of this study was to determine whether periodic bloodletting could decrease triglyceride concentrations in subjects with HH and iron overload.

2. Subjects and methods

2.1. Study population

This is an observational and retrospective study including all patients with genetic diagnosis of HH, iron overload, and under therapeutic phlebotomy attending 2 university hospitals in Zaragoza, Spain, from the year 2000. Inclusion criteria were as follows: (*a*) *iron overload*, defined as serum ferritin levels greater than 300 μg/L in men and postmenopausal women or greater than 200 μg/L in premenopausal women, and/or transferrin saturation greater than 45% in premenopausal women or greater than 55% in men or postmenopausal women; (*b*) presence of *HFE* genotype associated with HH, including homozygotes for the C282Y mutation and compound heterozygous C282Y/H63D; and (*c*) under therapeutic phlebotomy. Exclusion criteria included the following: (*a*) patients younger than 18 years; (*b*) less than 3 bloodletting sessions; (*c*) patients with lipid-lowering treatment at baseline or during the follow-up; and (*d*) patients without total cholesterol, triglycerides, or glucose values at baseline and during the follow-up.

2.2. Clinical and laboratory determinations

All subjects were assessed for clinical history, medication use, demographic characteristics, cardiovascular risk factors, and alcohol intake. Fasting blood total cholesterol, triglycerides, glucose, γ-glutamyl transpeptidase, alanine aminotransferase, and aspartate aminotransferase profiles were determined by using timed end point enzymatic methods on the Synchron LX20 System (Beckmann Coulter, Fullerton, CA). *Hypertriglyceridemia* was defined as fasting triglycerides of at least 150 mg/dL according to the metabolic syndrome criteria [18]. Serum iron and unsaturated iron binding capacity was measured by spectrophotometry, serum ferritin was measured by turbidimetric immunoassay (Beckmann Coulter), and transferrin saturation was calculated. Baseline values were obtained at diagnosis of HH. Clinical and biochemical data from follow-up were obtained at baseline and after each phlebotomy, with a maximum of 10 visits. In patients with less than 10 phlebotomies, all of them were recorded. In subjects with at least 10 phlebotomies, biochemical data were obtained at 6-month intervals. The results of the last available biochemical data were reported in all cases as final visit.

2.3. HFE genotyping

HFE C282Y and H63D mutations were determined by using TaqMan real-time polymerase chain reaction assays (Applied Biosystems, Foster City, CA) following the manufacturer's instructions. Control DNA with wild and mutant alleles for each mutation was included in each assay. DNA was isolated from EDTA blood samples following standard protocols. Hereditary hemochromatosis *genetic predisposition* was defined as the presence of 2 copies of the C282Y mutation (C282Y homozygosity) or the presence of a single copy each of the C282Y and H63D mutations (compound heterozygotes) [3].

2.4. Statistical analyses

Data were expressed as mean ± standard deviation or median and interquartile range (IR) (25-75 percentile) for continuous variables with or without normal distribution. The results were expressed as percentages for sex and type of mutation in the *HFE* gene. To evaluate the effect of bloodletting on continuous variables, a Wilcoxon signed rank test was used because of the non-Gaussian distribution. To determinate whether therapeutic strategy had any effect on triglycerides levels, we performed repeated analysis of variance measures. Levels of statistical significance were set at $P < .05$. Data analyses were performed with SPSS statistical software (V 15.0; SPSS, Chicago, IL).

3. Results

3.1. Clinical characteristics at baseline

The HH was composed of 155 subjects, of which 92 (59.4%) were homozygous for the C282Y mutation and 63 (40.6%) were compound heterozygotes C282Y/H63D. The mean age was 50.2 ± 14.4 years, and there were 104 men (67.1%) and 51 women (32.9%). Four subjects, all of them men, reported alcohol consumption of more than 30 g of ethanol per day. History of diabetes mellitus was present in 11 subjects (7.1%), all with hemoglobin A_{1c} less than 8%; and 9 subjects were taking antihypertensive drugs. The prevalence of serum triglycerides of at least 150 mg/dL was 31.6% of the sample (n = 49), without differences between *HFE* genotypes.

Table 1
Baseline and final values of biochemical variables in the study subjects

	Baseline	Final	*P*
Serum ferritin, μg/L[b]	587 (294-912)	61.0 (24.0-136)	<.001
Transferrin saturation index, %[a]	71.0 ± 22.6	42.0 ± 21.9	<.001
Glucose, mg/dL[a]	105 ± 11.0	106 ± 38.0	.101
Total cholesterol, mg/dL[a]	198 ± 39.9	193 ± 39.0	.096
Triglycerides, mg/dL[b]	107 (74.0-159)	94.0 (70.5-140)	<.001
GOT (U/L)[a]	31.5 ± 22.3	25.5 ± 20.7	<.001
ALT (U/L)[a]	40.9 ± 30.8	25.8 ± 17.2	<.001
GGT (U/L)[b]	23.0 (16.0-35.0)	23.0 (16.0-31.0)	.234

P refers to *t* test comparison for means and Mann-Whitney test for medians. GOT indicates aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyl transpeptidase.
[a] Data are means ± SD.
[b] Median (interquartile range).

3.2. Effect of bloodletting

Table 1 shows the main biochemical characteristics at baseline and at the final visit. As expected, serum ferritin and transferrin saturation decreased during the treatment from 587 to 61 μg/L and from 71% to 42%, respectively. There was also a significant decrease in the concentration of the liver enzymes at the end of the follow-up. There were no changes in blood glucose and total or low-density lipoprotein cholesterol; however, triglycerides significantly decreased after therapeutic phlebotomies. Moreover, the triglyceride-lowering effect of phlebotomies was especially important among subjects with high triglycerides at baseline (Fig. 1). In this subgroup, triglycerides decreased from 287 to 133 mg/dL, $z = -4.116$ ($P < .001$).

3.3. Triglycerides concentration and iron deposits

There was a close relationship between iron deposits and triglyceride concentration during the follow-up. The maximum triglyceride-lowering effect of bloodletting was obtained when ferritin concentration decreased to less than 200 μg/L and transferrin saturation to less than 40%, to be stable from that point onward (Fig. 2).

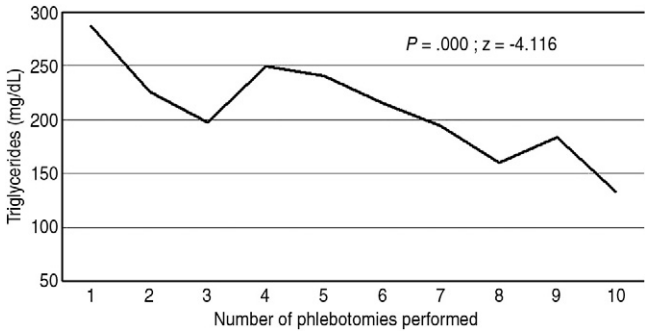

Fig. 1. Mean triglycerides concentration after therapeutic phlebotomy in subjects with HH and triglycerides concentration of at least 150 mg/dL at baseline.

3.4. Baseline blood glucose and triglyceride lowering

The triglyceride-lowering effect was analyzed in subjects with glucose levels both less than and greater than 100 mg/

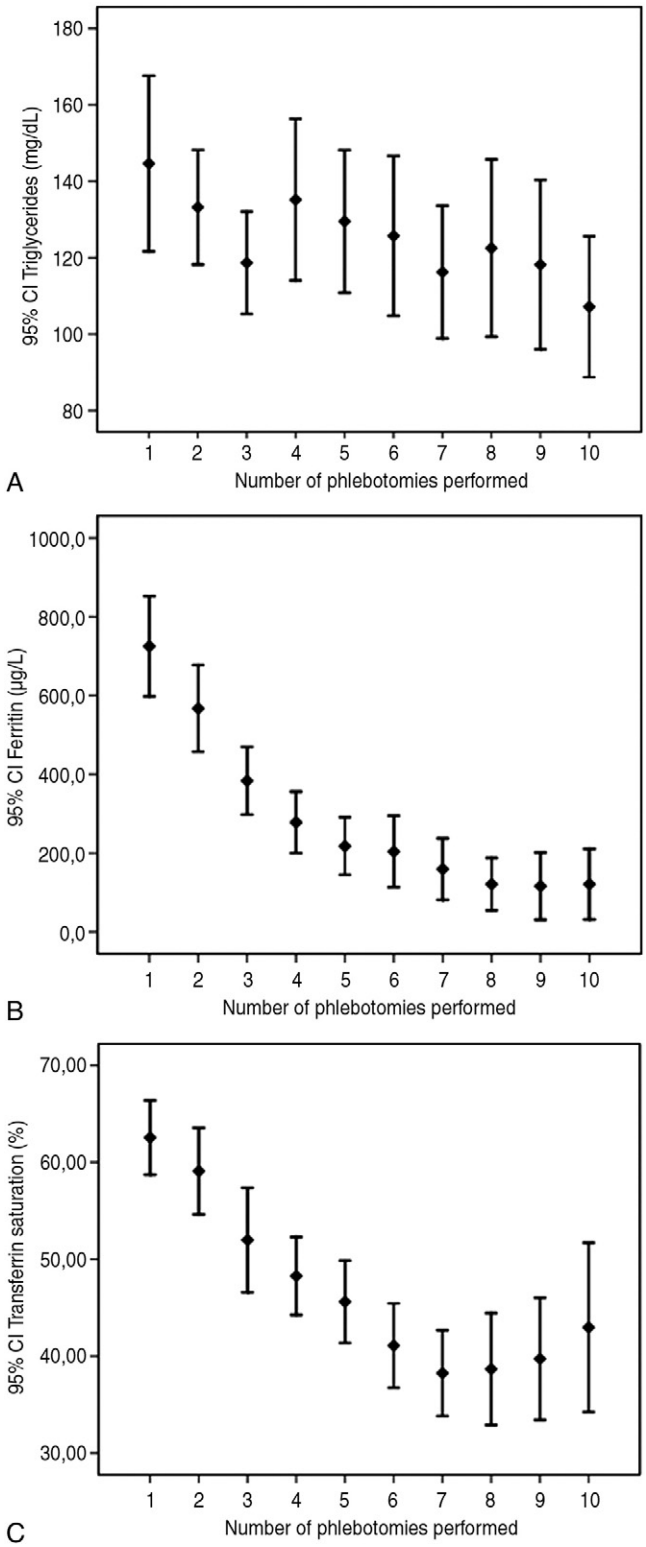

Fig. 2. Mean values of triglycerides (A), ferritin (B), and transferrin saturation (C) after therapeutic phlebotomy in subjects with HH.

dL. When subjects were divided according to baseline blood glucose, triglycerides decreased by 21.7% from 129 mg/dL (IR, 66-143 mg/dL) to 101 mg/dL (IR, 65-121 mg/dL) in subjects with glucose less than 100 mg/dL and by 31.9% from 169 mg/dL (IR, 88-197 mg/dL) to 115 mg/dL (IR, 82-183 mg/dL) in subjects with glucose of at least 100 mg/dL, without statistical difference between groups in the triglyceride-lowering response. Triglycerides were statistically different at baseline between normoglycemic and hypertriglyceridemic patients, and these differences disappeared at the end of the follow-up.

Among subjects without diabetes, there were 17 (17.9%) of 95 subjects with baseline glucose less than 100 mg/dL and baseline triglycerides of at least 150 mg/dL, and 19 (31.7%) of 60 subjects with baseline glucose of at least 100 mg/dL and baseline triglycerides of at least 150 mg/dL. Triglycerides decreased by 40.9% from 210 mg/dL (IR, 167-323 mg/dL) to 124 mg/dL (IR, 96-191 mg/dL) (P = .004) in the normoglycemic group and by 14.9% from 234 mg/dL (IR, 185-311 mg/dL) to 199 mg/dL (IR, 135-243 mg/dL) (P = .059) in subjects with baseline glucose of at least 100 mg/dL.

4. Discussion

This is the first study to our knowledge exploring the lipid effect of therapeutic phlebotomies in subjects with HH. The main results of our study indicate that a therapeutic program directed to decrease iron stores significantly modifies the concentration of triglycerides without a significant modification in total cholesterol or blood glucose. This effect is especially evident in subjects with HTG before starting therapeutic phlebotomies and independent of blood glucose.

Our work fully supports the previous observation of the high prevalence of HTG in subjects with genetic predisposition to HH. We previously described that the *HFE* locus is involved in primary HTG [15], and the present study demonstrates that HTG is frequently found in subjects with HH. Almost one third of patients with HH had HTG before the initiation of therapeutic phlebotomy, a frequency 2.5 times higher than that expected for our population [19]. Therefore, HTG can be considered a common clinical feature of HH. No previous studies of triglycerides in HH have been reported. However, Pankow et al [20] studied lipid concentrations in the Atherosclerosis Risk in Communities Study according to the *HFE* genotype. They found lower levels of low-density lipoprotein cholesterol in subjects homozygous for the C282Y allele and higher triglycerides levels in compound C282Y/H63D heterozygous and H63D homozygous subjects with respect to subjects homozygous for the wild allele. In our sample, approximately 40% of the subjects were compound C282Y/H63D heterozygotes; and we have not considered subjects who were H63D homozygotes. The H63D allele is less pathogenic with respect to iron overload than the C282Y allele. It could be possible that subjects with very high iron overload due to a more severe *HFE* genotype could develop low levels of cholesterol because of more advanced liver disease, a well-known cause of decreased cholesterol synthesis [21].

The effect of phlebotomy on lipid metabolism has been previously studied in subjects with nonalcoholic fatty liver disease and hyperferritinemia [17]. The iron-depleted group significantly improved insulin resistance with respect to the control group. They also got a 30.6-mg/dL decrease in triglycerides, although there was no statistical significance vs the control group [17]. In agreement with our study, the metabolic benefit observed by Valenti et al [17] was highly related to ferritin and transferrin saturation. Excess in liver triglyceride content has been described as a cause of iron overload in nonalcoholic fatty liver disease through mechanisms not fully understood [22]. However, the fact that subjects with HH and without insulin resistance associate HTG and that phlebotomy improves triglycerides independently of basal blood glucose suggests that excess in triglycerides could be also a consequence of iron overload independently of insulin resistance.

The results of our study open the question of the therapeutic value of phlebotomies in the treatment of HTG in subjects with increased iron stores. Most cases of HTG are associated with environmental factors also driving to iron accumulation such as obesity, insulin resistance, and excess alcohol consumption. Our results would suggest not only that HTG is associated with HH but also that all types of HTG with iron overload, independent of the presence of insulin resistance, would benefit from iron depletion. However, randomized clinical prospective studies in patients with different types of HTG are necessary to establish the indication of phlebotomy in HTG.

The main limitation of our study is that this is an observational retrospective study without a control group; therefore, bloodletting treatment could favor modifications in lifestyle that could confound the association. However, the large number of subjects included in the study, the homogeneous genetic diagnosis of the patients, and the consistence with previous results are strengths. Hypertriglyceridemia can be observed in the presence of liver cirrhosis, and this could be a confounding factor in our study. Nevertheless, none of our patients had clinical or biochemical suspicion of severe liver disease at baseline or during the follow-up.

In conclusion, subjects with genetic diagnosis of HH and iron overload frequently have HTG. This lipid abnormality improves after therapeutic phlebotomy parallel to iron deposits to that ferritin concentration is less than 200 μg/L. Our results suggest that therapeutic phlebotomy could be a useful therapeutic option for patients with HTG and iron overload.

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References

[1] Allen KJ, Gurrin LC, Constantine CC, et al. Iron-overload–related disease in HFE hereditary hemochromatosis. N Engl J Med 2008;358:221-30.

[2] Adams PC, Reboussin DM, Barton JC, et al. Hemochromatosis and Iron Overload Screening (HEIRS) Study Research Investigators. Hemochromatosis and iron-overload screening in a racially diverse population. N Engl J Med 2005;352:1769-78.

[3] Yen AW, Fancher TL, Bowlus CL. Revisiting hereditary hemochromatosis: current concepts and progress. Am J Med 2006;119:391-9.

[4] Pietrangelo A. Hereditary hemochromatosis—a new look at an old disease. N Engl J Med 2004;350:2383-97.

[5] Witte DL, Crosby Wh, Edwards CQ, et al. Practice guideline development task force of the College of American Pathologists: hereditary hemochromatosis. Clin Chim Acta 1996;245:13-200.

[6] Mendler MH, Turlin B, Moirand R, et al. Insulin resistance–associated hepatic iron overload. Gastroenterology 1999;117:1155-63.

[7] Sun L, Franco OH, Hu FB, et al. Ferritin concentrations, metabolic syndrome, and type 2 diabetes in middle-aged and elderly Chinese. J Clin Endocrinol Metab 2008;93:4690-6.

[8] Jehn ML, Guallar E, Clark JM, et al. A prospective study of plasma ferritin level and incident diabetes: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Epidemiol 2007;165:1047-54.

[9] Jehn M, Clark JM, Guallar E. Serum ferritin and risk of the metabolic syndrome in U.S. adults. Diabetes Care 2004;27:2422-8.

[10] Equitani F, Fernandez-Real JM, Menichella G, et al. Bloodletting ameliorates insulin sensitivity and secretion in parallel to reducing liver iron in carriers of HFE gene mutations. Diabetes Care 2008;31:3-8.

[11] Jiang R, Manson JE, Meigs JB, et al. Body iron stores in relation to risk of type 2 diabetes in apparently healthy women. JAMA 2004;291:711-7.

[12] Forouhi NG, Harding AH, Allison M, et al. Elevated serum ferritin levels predict new-onset type 2 diabetes: results from the EPIC-Norfolk prospective study. Diabetologia 2007;50:949-56.

[13] Tuomainen TP, Nyyssonen K, Salonen R, et al. Body iron stores are associated with serum insulin and blood glucose concentrations. Population study in 1,013 eastern Finnish men. Diabetes Care 1997;20:426-8.

[14] Houstis N, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature 2006;440: 944-8.

[15] Solanas-Barca M, Mateo-Gallego R, Calmarza P, et al. Mutations in HFE causing hemochromatosis are associated with primary hypertriglyceridemia. J Clin Endocrinol Metab 2009;94:4391-7.

[16] Mateo-Gallego R, Calmarza P, Jarauta E, et al. Serum ferritin is a major determinant of lipid phenotype in familial combined hyperlipidemia and familial hypertriglyceridemia. Metabolism 2010;59:154-8.

[17] Valenti L, Fracanzani AL, Dongiovanni P, et al. Iron depletion by phlebotomy improves insulin resistance in patients with nonalcoholic fatty liver disease and hyperferritinemia: evidence from a case-control study. Am J Gastroenterol 2007;102:1251-8.

[18] Grundy SM, Cleeman JI, Daniels SR, et al. American Heart Association, National Heart, Lung, and Blood Institute. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. Circulation 2005;112:2735-52.

[19] Alegría E, Cordero A, Laclaustra M, et al. Prevalence of metabolic syndrome in the Spanish working population: MESYAS registry. Rev Esp Cardiol 2005;58:797-806.

[20] Pankow JS, Boerwinkle E, Adams PC, et al. HFE C282Y homozygotes have reduced low-density lipoprotein cholesterol: the Atherosclerosis Risk in Communities (ARIC) Study. Transl Res 2008;152:3-10.

[21] Forns X, Ampurdanès S, Llovet JM, et al. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. Hepatology 2002;36:986-92.

[22] Eriksson Moirand R, Mendler MH, Guillygomarc'h A, et al. Non-alcoholic steatohepatitis with iron: part of insulin resistance-associated hepatic iron overload? J Hepatol 2000;33:1024-6.