Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 59 (2010) 1811 – 1815
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Glucose metabolism after normalization of markers of iron overload by venesection in subjects with hereditary hemochromatosis

Mensud Hatunic[a,*], Francis M. Finucane[a], Suzanne Norris[b], Giovanni Pacini[c], John J. Nolan[a]

[a]*Metabolic Research Unit, St James's Hospital, Trinity College, Dublin, Ireland*
[b]*Hepatology Department, St James's Hospital, Dublin, Ireland*
[c]*Metabolic Unit, Institute of Biomedical Engineering (ISIB-CNR), Padova, Italy*

Received 24 March 2010; accepted 3 June 2010

Abstract

Hereditary hemochromatosis (HH) is associated with abnormal glucose metabolism (AGM). We investigated the effect on glucose metabolism of normalization of the markers of iron overload by phlebotomy in subjects with HH. We prospectively studied 11 newly diagnosed subjects with HH and AGM using a standard 75-g oral glucose tolerance test. Basal quantitative insulin sensitivity check index (QUICKI) and stimulated oral glucose insulin sensitivity index (OGIS) insulin sensitivity was calculated from glucose and insulin data, whereas β-cell function was assessed using C-peptide concentration after adjusting for ambient insulin sensitivity. After normalization of ferritin and transferrin saturations by venesection for 12 (range, 8-16) months, subjects were studied again using the same methods. From 11 subjects with AGM at the time that HH was diagnosed, 7 had impaired glucose tolerance (IGT) and 4 had type 2 diabetes mellitus (T2DM). Normalization of the iron stores (ferritin and transferrin) improved the glucose tolerance status of 4 patients with IGT (to normal glucose tolerance), whereas 2 of those with IGT progressed to T2DM. In 5 patients, glucose tolerance status did not change (4 T2DM and 1 IGT). The area under the insulin and the C-peptide curve during the oral glucose tolerance test and the hepatic insulin extraction increased ($P = .05$), whereas no statistically significant changes occurred in insulin sensitivity. However, the disposition index, a measure of the ability of insulin release to compensate for insulin resistance, improved significantly ($P = .02$). Normalization of ferritin and transferrin saturation by venesection in subjects with HH and AGM led to improvements in some, but not all, measures of insulin secretion and action. Most patients with AGM had an improvement in glucose tolerance status, probably due to the augmented action of insulin in peripheral tissues.

1. Introduction

Hereditary hemochromatosis (HH) is a genetic disorder of iron metabolism that can lead to unregulated absorption of iron from the gut with resultant iron overload. The prevalence of HH in white populations is between 1 in 200 and 1 in 500 [1,2]. The most common form of HH is caused by mutations of the HFE gene located on chromosome 6. Two major HFE mutations have been identified in association with HH. The first is a change of the cysteine amino acid at position 282 to tyrosine (C282Y). The second is a change of histidine at position 63 to aspartate (H63D). Other mutations exist but are less common. The C282Y mutation is postulated to have originated by chance in a single Celtic (or Viking) ancestor in northwestern Europe some 2000 years ago. In Ireland, 93% of HH patients are homozygous for the C282Y mutation [3], the highest reported prevalence worldwide [4,5]. Hereditary hemochromatosis is inherited in an autosomal recessive pattern, but the phenotypic penetrance is highly variable. Hereditary hemochromatosis can present as a nonspecific syndrome such as a fatigue or as a liver, cardiac, rheumatologic, or endocrine disease or as an incidental laboratory abnormality, namely, elevated ferritin and transferrin saturation without end-organ damage [6-8].

Diabetes mellitus affects 30% to 60% of patients with hemochromatosis [9], but the mechanisms underlying the association of iron overload with the progression to diabetes have not been elucidated [10]. We recently demonstrated that a significant proportion of patients with newly diagnosed

* Corresponding author. Hatunic Metabolic Research Unit, Hospital 5 St James's Hospital, Trinity College, Dublin 8, Ireland. Tel.: +353 86 607 1092.
E-mail address: mensudhatunic@gmail.com (M. Hatunic).

0026-0495/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2010.06.005

hemochromatosis had early alteration of glucose tolerance, insulin resistance, and diabetes mellitus [11]. Individuals with HH and diabetes had both decreased insulin sensitivity as well as decreased acute insulin response to glucose compared with healthy individuals [12,13]. There has been a paucity of information in the past 10 years on the effect of therapeutic phlebotomy on incidence or severity of diabetes [14]. To our knowledge, only very few studies have evaluated the effect of venesection in patients with HH and established diabetes [13,15-17].

Here, we have studied the effect of early normalization of markers of iron overload by venesection on glucose metabolism, β-cell function, and insulin sensitivity in newly diagnosed patients with HH and abnormal glucose metabolism.

2. Subjects and methods

2.1. Subjects

Subjects with newly diagnosed HH and abnormal glucose metabolism were recruited prospectively from the Haemochromatosis Clinic at St James's Hospital. The study was approved by the Research Ethics Committee, and all subjects gave written consent to participate. We chose to recruit patients without any other clinical complications; therefore, those with severe heart failure, liver cirrhosis, preexisting diabetes, or hypopituitarism were excluded from the study because of the likely confounding effects of these conditions on metabolic measurements.

Iron depletion was achieved through repeated venesection, which remains the standard treatment of symptomatic HH. Approximately 250 mg of iron is removed every time with 450 mL of blood until the serum ferritin level is less than 50 μg/L or transferrin saturation is less than 50%. Eleven subjects with abnormal glucose tolerance test result at the time of initial diagnosis had all studies repeated 2 weeks after normalization of iron stores by venesection. Venesection was performed every week, and the average interval between pre- and postvenesection studies was 12 (range, 8-16) months.

All subjects attended the Metabolic Research Unit, St James's Hospital, Dublin, in one morning between 8:00 and 10:00 AM for initial investigations. Full history and physical examination were performed. Waist and hip circumferences, weight, height, and body mass index were measured. Routine blood samples were taken for blood count, renal, liver, bone profile, and thyroid function tests and fasting lipids. Blood pressure was measured using the left arm after the subject had been sitting comfortably for 5 minutes using an oscillometric device (Omron 705 CP; Omron, Matsusaka, Japan). Three readings were taken, and the lowest one was recorded.

2.2. Assays, tests, and calculations

Serum insulin and C-peptide were measured using commercially available fluoroimmunoassays (Auto-Delfia; Wallac-Oy, Turku, Finland). Plasma total cholesterol and triglycerides were measured using enzymatic methods (Human liquicolor kits, Hitachi Modular; Roche Diagnostics, Basel, Switzerland). Plasma high-density lipoprotein cholesterol and low-density lipoprotein cholesterol were measured directly with enzymatic methods (Randox direct kits/Hitachi Modular). Plasma glucose was measured using glucose oxidase method (bio Merieux kit/Hitachi Modular), and glycated hemoglobin (HbA_{1c}) was measured using an analyzer (Hi-Auto A_{1c} HA 8140; Menarini, Florence Italy).

2.3. Oral glucose tolerance test

After a 12-hour overnight fast, a standard 2-hour 75-g oral glucose tolerance test (OGTT) was performed, with blood taken every 30 minutes for plasma glucose, insulin, and C-peptide measurements. Glucose tolerance status was defined according to World Health Organization criteria [18]. The areas under the concentration curve (AUCs) were calculated with the trapezoidal rule. Insulin sensitivity was assessed with the quantitative insulin sensitivity check index (QUICKI) [19] in fasting conditions and with oral glucose insulin sensitivity index (OGIS) [20] during the OGTT, that is, in dynamic "postprandial" conditions. Both indices have been previously validated against the glucose clamp with and without the use of tracers and are widely used [21].

The capacity of insulin delivery to adapt to changes in insulin sensitivity was calculated as the product of OGIS times the total incremental insulin during OGTT ($\Delta AUC_{insulin}$). This index is sometimes referred to as the *disposition index* and has been already exploited in several previous studies (eg, Stadler et al [22] and Ludvik et al [23]). It provides a quantitative figure of the overall metabolic status by simultaneously accounting for insulin action and insulin secretion.

2.4. Statistics

Before statistical analysis, normal distribution and homogeneity of the variances were tested. Data were compared using Wilcoxon signed rank test and are presented as median and interquartile ranges. $P \leq .05$ was deemed to be statistically significant. SPSS (Chicago, IL) was used for all analyses.

3. Results

From 11 subjects with impaired glucose metabolism at diagnosis, 7 had impaired glucose tolerance (IGT) and 4 had type 2 diabetes mellitus (T2DM). Normalization of the iron stores improved glucose metabolism in 4 patients from IGT to normal glucose tolerance; 2 patients developed T2DM from IGT; and in 5 patients, glucose metabolism did not change (4 T2DM and 1 IGT). Baseline characteristics of the subjects pre- and posttreatment with venesection are shown in Table 1. Most were male, and mean age was 55 (range, 51-66) years at initial diagnosis. There were no differences between baseline and follow-up measures of blood pressure,

Table 1
Patient characteristics pre- and posttreatment with venesection

	Pre venesection	Post venesection
n	11	11
Male/female	10/1	10/1
Age, y	55 (51-66)	56 (52-67)
Systolic BP, mm Hg	125 (120-140)	130 (120-140)
Diastolic BP, mm Hg	85 (70-86)	78 (75-80)
Body mass index, kg/m^2	28 (24-31)	29 (25-31)
Waist to hip ratio	0.99 (0.9-1.1)	1.0 (0.97-1.1)
Fasting blood glucose, mmol/L	5.6 (5.1-6.3)	6.0 (5.2-6.6)
Fasting insulin, μU/mL	10.4 (8.9-13.7)	10.3 (7.6-12.6)
Fasting C-peptide, μg/L	3.2 (2.3-3.4)	2.9 (2.4-3.9)
HbA_{1c}	5.6 (5.5-6.0)	5.8 (5.4-6.6)
Total cholesterol, mmol/L	3.9 (3.4-5.8)	4.6 (3.4-5.0)
HDL cholesterol, mmol/L	1.1 (0.9-1.3)	1.0 (1.0-1.3)
LDL cholesterol, mmol/L	2.0 (1.5-3.5)	2.3 (1.5-2.9)
Triglycerides, mmol/L	1.2 (1.0-2.2)	1.2 (0.9-2.7)
Ferritin, μg/L	726 (517-1086)	49 (33-84)*
Transferrin saturation, %	75 (54-98)	40 (35-55)*

Values are presented as a medians and interquartile range. BP indicates blood pressure; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol.

* $P < .05$.

body mass index, fasting lipids, waist to hip ratio, fasting glucose, HbA_{1c}, or fasting lipids. As expected, markers of iron overload (transferrin saturation and ferritin) significantly improved after treatment with venesection (Table 1).

Fasting insulin sensitivity (QUICKI) did not change (0.56 [range, 0.55-0.59] vs 0.56 [0.54-0.59], P = not significant [NS]) after normalization of iron stores, nor did dynamic insulin sensitivity (OGIS) (385 [331-413] vs 390 [325-448] mL/[min m^2], P = NS).

After normalization of ferritin and transferrin saturations, a marginal increase of hepatic insulin extraction (P = .05), together with a modest increase (P = .05) of dynamic C-peptide ($AUC_{C\text{-peptide}}$) and insulin ($AUC_{insulin}$), was observed (Table 2). Although marginal, these increases yielded a significant improvement in the disposition index ($P < .02$). Thyroid function as measured with thyrotropin (1.9 [range, 1.6-2.6] vs 2.2 [range, 1.7-3.2] mU/L, P = NS) and serum testosterone (16.5 [range, 12-19] vs 14.2 [range, 13-17] nmol/L, P = NS) did not change significantly after normalization of markers of iron overload by venesection.

Table 2
OGTT-derived metabolic parameters

	Pre venesection	Post venesection
$AUC_{glucose}$ (mmol/[L 120 min])	1269 (1052-1367)	1286 (1082-1404)
$AUC_{insulin}$ (μU/[mL 120 min])	5309 (4308-9616)	5895 (2663-7365)*
$AUC_{C\text{-peptide}}$ (ng/[L 120 min])	869 (771-1227)	1016 (650-1313)*
Hepatic insulin extraction (%)	52 (30-64)	60 (49-70)*
Disposition index (nmol/m^2)	2.6 (0.9-5.0)	4.0 (1.8-7.4)†
Insulinogenic index (120 min)	0.76 (0.5-1.2)	0.83 (0.4-1.2)

Data are as a median (interquartile range). Insulinogenic index ($AUC_{C\text{-peptide}}/AUC_{glucose}$).

* $P = .05$.

† $P = .02$.

4. Discussion

Hereditary hemochromatosis is a polygenic disease characterized by inappropriate iron absorption and progressive parenchymal iron deposition with potential for severe organ damage. The pathophysiology of abnormal glucose metabolism in patients with iron overload is still poorly understood. There are 2 prevailing theories: development of insulin resistance and decrease in insulin secretory capacity [13,24,25].

We have previously shown that 28% of newly diagnosed patients with HH had abnormal glucose tolerance (13% diabetes mellitus and 15% IGT). We also found that the patients with abnormal glucose metabolism were more insulin resistant than the ones with normal glucose tolerance and had higher fasting insulin and C-peptide levels. However, we found no difference in the derived indices relating to β-cell function and insulin degradation from OGTT [11]. These findings showed that our patients were unable to compensate for insulin resistance with increased β-cell secretion, as often happens in populations at risk of T2DM.

This picture changed to a modest extent after venesection. In fact, we still found no change in insulin sensitivity; but we observed an increase, although not that large, of insulin in the postvenesection condition. This yielded a significantly increased disposition index, that is, a better ability of insulin to dispose of glucose in relation to the prevailing insulin concentration.

Iron is a transition metal that can catalyze the conversion of poorly reactive free radicals into highly active free radicals. It has been suggested that formation of hydroxyl radicals catalyzed by iron may play a role in the development of diabetes because the highly active radicals can attack cell membranes, lipids, proteins, and DNA and cause tissue damage [26-29]. Studies have shown that iron deposition in muscle decreases glucose uptake because of muscle damage and affects insulin synthesis and secretion in the pancreas [30]. It has recently been reported that, in thalassemia major, pancreatic β-cell function may be impaired during adolescence or later in life. This impairment ranges from hyperinsulinemia, secondary to insulin resistance, with normal glucose tolerance to β-cell failure with diabetes mellitus [31]. Hereditary hemochromatosis results from mutations in a gene that encodes proteins that regulate and control iron transport especially in duodenal enterocytes, macrophages, and hepatocytes [32]. Like diabetes, hemochromatosis results from complex, nonlinear interaction between genetic and acquired factors [33].

Iron overload in hemochromatosis is thus different in many respects to transfusional iron overload, and these differences are likely to explain differences in glucose metabolism abnormalities in the 2 conditions. Taken together, our findings suggest that iron excess may contribute initially to insulin resistance and subsequently to decreased insulin secretion [30].

Few studies have investigated the impact of iron depletion on glucose metabolism. Hramiak et al [13] found that iron depletion among patients without diabetes or liver cirrhosis increased acute insulin response but had no effect on insulin sensitivity. One other study with both oral and intravenous glucose tolerance tests in patients with HH and without diabetes showed an increase in insulin secretion, but insulin sensitivity decreased [17]. These studies suggested that early treatment of iron overload in patients with HH and without diabetes may be able to reverse some of the toxic effects of iron overload on glucose metabolism.

Here, we determined if normalization of markers of iron overload immediately post regular venesection would affect glucose metabolism in patients with newly diagnosed HH. Four patients out of 7 with IGT showed an improved glucose metabolism, moving to normotolerance, whereas no patients with T2DM improved. Only an augmented disposition index was found.

These findings suggest that abnormal glucose metabolism is an intrinsic characteristic of HH and only marginally changes after normalization of ferritin and transferrin saturation. However, the improved parameters may indicate that the treatment of HH is more effective in the early stages of iron overload before complications fully develop. The improvement of insulin secretion and hepatic insulin extraction, although slight, could reflect an ability of the pancreas and liver to recover from iron overload faster than other tissues. This may be why we did not see any significant improvements in dynamic insulin sensitivity in this early stage of normalization of iron overload. Moreover, these results suggest that there is a complex interaction between iron status, insulin secretion, and insulin sensitivity.

In summary, upon normalization of ferritin and transferrin saturation by venesection in subjects with HH and abnormal glucose metabolism, the glucose tolerance status improved in most subjects with IGT; but insulin secretory capacity and hepatic insulin extraction improved only slightly. Interventions to normalize iron status in patients with hemochromatosis and abnormal glucose metabolism should be introduced early to prevent progression to diabetes and development of diabetes related comorbidities.

Acknowledgment

The authors thank the patients who participated and the staff of the Metabolic Research Unit of the St James's Hospital in Dublin. Special thanks are due to Liz Ellis, the nurse specialist in the Hepatology Department. This study was supported by the Diabetes Education and Research Fund.

References

[1] Merryweather-Clarke AT, Pointon JJ, Jouanolle AM, Rochette J, Robson KJ. Geography of HFE C282Y and H63D mutations. Genet Test 2000;4:183-98.

[2] Bacon BR, Powell LW, Adams PC, Kresina TF, Hoofnagle JH. Molecular medicine and hemochromatosis: at the crossroads. Gastroenterology 1999;116:193-207.

[3] Ryan E, O'Keane C, Crowe J. Hemochromatosis in Ireland and HFE. Blood Cells Mol Dis 1998;24:428-32.

[4] Byrnes V, Ryan E, Barrett S, Kenny P, Mayne P, Crowe J. Genetic hemochromatosis, a Celtic disease: is it now time for population screening? Genet Test 2001;5:127-30.

[5] Merryweather-Clarke AT, Pointon JJ, Shearman JD, Robson KJ. Global prevalence of putative haemochromatosis mutations. J Med Genet 1997;34:275-8.

[6] Feder JN, Penny DM, Irrinki A, Lee VK, Lebron JA, Watson N, et al. The hemochromatosis gene product complexes with the transferrin receptor and lowers its affinity for ligand binding. Proc Natl Acad Sci U S A 1998;95:1472-7.

[7] Feder JN, Tsuchihashi Z, Irrinki A, Lee VK, Mapa FA, Morikang E, et al. The hemochromatosis founder mutation in HLA-H disrupts beta2-microglobulin interaction and cell surface expression. J Biol Chem 1997;272:14025-8.

[8] Edwards CQ, Dadone MM, Skolnick MH, Kushner JP. Hereditary haemochromatosis. Clin Haematol 1982;11:411-35.

[9] Powell LW, Yapp TR. Hemochromatosis. Clin Liver Dis 2000;4:211-28, viii.

[10] Ferrannini E. Insulin resistance, iron, and the liver. Lancet 2000;355:2181-2.

[11] Hatunic M, Finucane FM, Brennan AM, Norris S, Pacini G, Nolan JJ. Effect of iron overload on glucose metabolism in patients with hereditary hemochromatosis. Metabolism 2010;59:380-4.

[12] McClain DA, Abraham D, Rogers J, Brady R, Gault P, Ajioka R, et al. High prevalence of abnormal glucose homeostasis secondary to decreased insulin secretion in individuals with hereditary haemochromatosis. Diabetologia 2006;49:1661-9.

[13] Hramiak IM, Finegood DT, Adams PC. Factors affecting glucose tolerance in hereditary hemochromatosis. Clin Invest Med 1997;20:110-8.

[14] Utzschneider KM, Kowdley KV. Hereditary hemochromatosis and diabetes mellitus: implications for clinical practice. Nat Rev Endocrinol 2010;6:26-33.

[15] Williams R, Smith PM, Spicer EJ, Barry M, Sherlock S. Venesection therapy in idiopathic haemochromatosis. An analysis of 40 treated and 18 untreated patients. Q J Med 1969;38:1-16.

[16] Dymock IW, Cassar J, Pyke DA, Oakley WG, Williams R. Observations on the pathogenesis, complications and treatment of diabetes in 115 cases of haemochromatosis. Am J Med 1972;52:203-10.

[17] Abraham D, Rogers J, Gault P, Kushner JP, McClain DA. Increased insulin secretory capacity but decreased insulin sensitivity after correction of iron overload by phlebotomy in hereditary haemochromatosis. Diabetologia 2006;49:2546-51.

[18] Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15:539-53.

[19] Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 2000;85:2402-10.

[20] Mari A, Pacini G, Murphy E, Ludvik B, Nolan JJ. A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. Diabetes Care 2001;24:539-48.

[21] Abdul-Ghani MA, Jenkinson CP, Richardson DK, Tripathy D, DeFronzo RA. Insulin secretion and action in subjects with impaired fasting glucose and impaired glucose tolerance: results from the Veterans Administration Genetic Epidemiology Study. Diabetes 2006;55:1430-5.

[22] Stadler M, Anderwald C, Karer T, Tura A, Kastenbauer T, Auinger M, et al. Increased plasma amylin in type 1 diabetic patients after kidney

and pancreas transplantation: a sign of impaired beta-cell function? Diabetes Care 2006;29:1031-8.
[23] Ludvik B, Waldhausl W, Prager R, Kautzky-Willer A, Pacini G. Mode of action of *Iipomoea batatas* (Caiapo) in type 2 diabetic patients. Metabolism 2003;52:875-80.
[24] Merkel PA, Simonson DC, Amiel SA, Plewe G, Sherwin RS, Pearson HA, et al. Insulin resistance and hyperinsulinemia in patients with thalassemia major treated by hypertransfusion. N Engl J Med 1988; 318:809-14.
[25] Mendler MH, Turlin B, Moirand R, Jouanolle AM, Sapey T, Guyader D, et al. Insulin resistance–associated hepatic iron overload. Gastroenterology 1999;117:1155-63.
[26] Wolff SP. Diabetes mellitus and free radicals. Free radicals, transition metals and oxidative stress in the aetiology of diabetes mellitus and complications. Br Med Bull 1993;49:642-52.
[27] Andrews NC. Disorders of iron metabolism. N Engl J Med 1999;341: 1986-95.
[28] Beard JL. Iron biology in immune function, muscle metabolism and neuronal functioning. J Nutr 2001;131:568S-79S.
[29] Oberley LW. Free radicals and diabetes. Free Radic Biol Med 1988;5: 113-24.
[30] Wilson JG, Lindquist JH, Grambow SC, Crook ED, Maher JF. Potential role of increased iron stores in diabetes. Am J Med Sci 2003;325:332-9.
[31] Delvecchio M, Cavallo L. Growth and endocrine function in thalassemia major in childhood and adolescence. J Endocrinol Invest 2010;33:61-8.
[32] Adams PC, Barton JC. Haemochromatosis. Lancet 2007;370:1855-60.
[33] Pietrangelo A. Hemochromatosis: an endocrine liver disease. Hepatology 2007;46:1291-301.