Serveur Académique Lausannois SERVAL serval.unil.ch

Author Manuscript
Faculty of Biology and Medicine Publication

This paper has been peer-reviewed but does not include the final publisher proof-corrections or journal pagination.

serval
serveur académique lausannois

Unil
UNIL | Université de Lausanne
Faculty of Biology and Medicine

CHUV

IRON METABOLISM AND INCIDENCE OF METABOLIC SYNDROME

Running title: role of iron metabolism markers in the onset of metabolic syndrome

Kilani N; Vollenweider P; Waeber G and Marques-Vidal P

Department of Internal Medicine, Internal Medicine, Lausanne University Hospital, Lausanne,

Switzerland

Authors' emails:

Nadia Kilani: Nadia.Kilani@unil.ch

Peter Vollenweider: Peter.Vollenweider@chuv.ch

Gérard Waeber: Gerard.Waeber@chuv.ch

Pedro Marques-Vidal : Pedro-Manuel.Marques-Vidal@chuv.ch

Address for correspondence and reprints

Pedro Marques-Vidal

Department of Internal Medicine, Internal Medicine

Room BH10-642

Lausanne University Hospital (CHUV)

Rue du Bugnon 46

1011 Lausanne

Switzerland

Phone : +41 21 314 09 34

Email : Pedro-Manuel.Marques-Vidal@chuv.ch

Word count abstract: 207   **Main text**: 2735

Number of tables: 2 + 3 suppl   **Figures**: 1 + 1 suppl   **References**: 27

Highlights

- After 5 years follow-up, almost one-fifth (19%) of participants developed MS

- Increased serum levels of iron protect against MS in women but not in men

- Increased serum levels of transferrin favour the development of MS

ABSTRACT

Background and aims: Whether iron metabolism affects metabolic syndrome (METS) is debated. We assessed the association between several markers of iron metabolism and incidence of MS.

Methods and results: Data from 3,271 participants (1,870 women, 51.3±10.4 years), free of MS at baseline and followed for 5.5 years. The association of serum iron, ferritin and transferrin with incident METS was assessed separately by gender. Incidence of METS was 22.6% in men and 16.5% in women (p<0.001). After multivariate adjustment, a positive association was found between transferrin and incident METS in men: Odds ratio (OR) and (95% confidence interval) for the fourth relative to the first quartile 1.55 (1.04-2.31), p for trend=0.03, while no association was found for iron OR=0.81 (0.53-1.24), p for trend=0.33 and ferritin OR=1.30 (0.88-1.92), p for trend=0.018. In women, a negative association was found between iron and incident METS: OR for the fourth relative to the first quartile 0.51 (0.33-0.80), p for trend<0.03; the association between transferrin and incident METS was borderline significant: OR=1.45 (0.97-2.17), p for trend=0.07 and no association was found for ferritin: OR=1.11 (0.76-1.63), p for trend=0.58.

Conclusion: transferrin, not ferritin, is independently associated with an increased risk of incident METS; the protective effect of iron in women should be further explored.

Keywords: iron; ferritin; transferrin; metabolic syndrome; incidence; prospective study

INTRODUCTION

Several studies have shown that moderately elevated serum ferritin levels are associated with an increased prevalence of metabolic syndrome (METS) [1-3] or some of its components, such as high blood pressure [4]. On another hand, several studies have shown the beneficial effects of phlebotomy on impaired insulin sensitivity and insulin secretion [5], hypertryglyceridemia [6], high blood pressure [7], impaired fasting glucose and HbA1c [7]. Although most studies associating iron metabolism and METS have used a cross-sectional setting [1-3], two recent prospective studies have shown that baseline elevated serum ferritin [8, 9] and transferrin [9] levels were associated with higher incidence of METS. Still, some studies relied on a relatively small number of participants [9], were limited to a single marker of iron metabolism [8] or to a limited component of METS [10].

Thus, we aimed at assessing the association between several markers of iron metabolism and the 5.5-year incidence of METS in a Swiss population-based sample.

METHODS

Sampling

The Cohorte Lausannoise (CoLaus) study is a population-based study, aimed to assess the epidemiology of cardiovascular risk factors and diseases in the population of Lausanne, Switzerland. The CoLaus study has been approved by the Ethics Committee of the Canton Vaud and all participants provided their written informed consent before participating. The sampling procedure of the CoLaus study has been described previously [11]. Briefly, recruitment began in June 2003 and ended in May 2006. The complete list of the Lausanne inhabitants aged 35–75 years (n=56,694) was provided by the population registry of the city and a simple, nonstratified random sample of 35% was drawn [11]. Inclusion criteria were applied: 1) written informed consent; 2) age 35–75 years; 3) willingness to take part in the examination and to have a blood sample drawn. Participation rate was 41% and 6,733 participants (3,544 women and 3,189 men) were recruited at baseline. A first 5-year

follow-up combining a comprehensive CVRF and CVD assessment with a psychiatric exam was conducted between 2009 and 2012. To maximize follow up, letters and brochures were sent to participants at six-month intervals. Six months before the intended follow-up interview, a letter describing the goals of the follow-up, the requirements for participation and the importance of their contribution to long-term efforts in disease prevention was sent to the participants. Prior to the follow-up interview, participants received the detailed information letter and consent forms. For more details of the follow-up procedure, please consult [12].

Personal and clinical data

Similarly to the baseline evaluation [11], the follow-up examination was conducted at the CHUV in Lausanne and included an interview, a physical exam, blood and urine collections and a list of questionnaires on self-report dietary habits, physical activity, sleep patterns and disorders [12]. Smoking was categorized into never, former (irrespective of the delay since quitting smoking) and current. Alcohol consumption was categorized into none, 1-13 and ≥14 drinks/week.

Women were asked if they were menopaused or not, and if they were taking any hormonal treatment (contraception or hormone replacement therapy). Personal history of and current treatment for hypercholesterolemia, hypertension or diabetes were also asked. Participants were asked to bring all their medicines and information on the use of doctor- and self-prescribed drugs was collected, together with their main indications. Iron supplementation was obtained from the participant's intake of medicines and/or vitamin/mineral supplements.

Body weight and height were measured in light indoor clothes and without shoes. Body weight was measured in kilograms to the nearest 100g using a Seca® scale, which was calibrated regularly. Height was measured to the nearest 5 mm using a Seca® height gauge. Body Mass Index (BMI) was calculated as weight (kg) divided by the square of the height (m). Overweight was defined as BMI ≥25 kg/m^2 and <30 kg/m^2, and obesity by a BMI ≥30 kg/m^2. Waist was measured with a non-

stretchable tape over the unclothed abdomen at the narrowest point between the lowest rib and the iliac crest. Two measures were made and the mean used for analyses.

Blood pressure was measured on the left arm, with an appropriately sized cuff, after at least 10 minute rest in the seated position, using an Omron® HEM-907 automated oscillometric sphygmomanometer. Three readings were taken and the average of the last two was used to compute systolic (SBP) and diastolic (DBP) blood pressure.

Biological data

Blood was taken in the fasting state and analyses were performed at the Central Chemistry Laboratory and the Laboratory of Endocrinology of the CHUV. Vials used for blood collection were suitable for iron measurements, and the clinical laboratory was submitted to an external quality auditing. The following analytical procedures (with maximum inter and intra-batch CVs) were used at baseline and follow-up: HDL-cholesterol by CHOD-PAP + PEG + cyclodextrin (3.6%-0.9%); triglycerides by GPO-PAP (2.9%-1.5%) and glucose by glucose dehydrogenase (2.1%-1.0%). High sensitivity C-reactive protein (hsCRP) was assessed by immunoassay (HS latex, 4.6%-1.3%) and log-transformed for statistical analyses. Baseline alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) were assessed by the International Federation of Clinical Chemistry (IFCC) method at 37°C (5.6%-0.7% for ALAT and 3.6%-1.8% for ASAT). Altered hepatic markers were defined as hsCRP≥20 and/or [ALAT>80 (men) or >62 U/L (women)] and/or [ASAT>74 (men) or >62 U/L (women)] [2] and used as a binary (yes/no) variable. No imagiology data was available for the adequate identification of fatty liver disease.

Iron was assessed by colorimetric method (ferrozine, BioSystems; 2.9%-2.2%); ferritin by immunoturbidimetric method (Tina-quant 4th generation, Roche Diagnostics, Switzerland; 9.9%-7.2%); transferrin by immunoassay with a maximum intra-assay (1.8%-1.0%).

Metabolic syndrome and its components

Metabolic syndrome (METS) was defined as the presence of three or more of the following criteria set forth by the National Cholesterol Education Program Adult Panel III guidelines [13]: 1) Elevated blood pressure (≥130/85 mmHg), 2) abdominal obesity (waist circumference >102 cm in men and >88 cm in women), 3) low HDL-cholesterol (<40mg/dL in men and <50mg/dL in women), 4) elevated serum triglycerides (>150mg/dL), and 5) elevated fasting plasma glucose (>110mg/dL).

Statistical analysis

The following exclusion criteria were applied 1) missing data for the variables of interest (markers of iron metabolism or METS components); 2) possible hemochromatosis, based on a transferrin saturation >50%; 3) presence of METS at baseline and 4) not attending the follow-up visit.

Due to the skewed distribution of the markers of iron metabolism, and similar to a preceding study [8], a categorization into gender-specific quartiles was performed, with a further stratification according to menopausal status in women. Pairwise correlations between markers of iron metabolism were assessed separately for each gender using Spearman nonparametric correlation coefficient.

Statistical analyses were performed using Stata version 13.0 (Stata Corp, College Station, Texas, USA). All analyses were stratified by gender; in women, a further stratification on menopausal status was applied as performed by others [9]. Descriptive results were expressed as number of participants (percentage) or as average±standard deviation. The associations between incident MS and quartiles of markers of iron metabolism were assessed using chi-square and multivariate logistic regression. The results of the multivariate analysis were expressed as Odds ratio (OR) and 95% confidence interval (CI). Two models were tested: 1) using all quartiles as categories and 2) comparing the fourth to the first quartile. For the first model, a test for trend was applied using the **contrast q.** function of Stata. A sensitivity analysis adjusting only for age and categorizing participants

into low (<4th quartile) ferritin/low transferrin; high (4th quartile) ferritin/low transferrin; low ferritin/high transferrin and high ferritin/high transferrin as performed in a previous study [9] was also conducted. Statistical significance was assessed for p<0.05.

RESULTS

Characteristics of participants

Among the initial 6,733 participants at baseline, 3,462 (51%) were excluded according to the criteria defined previously (**Figure 1**). Thus, the final analytical sample consisted of 3,271 individuals, the main characteristics of which according to gender and menopausal status are summarized in **supplementary table 1**.

Associations between markers of iron metabolism and incident metabolic syndrome

The distribution of the different quartiles of markers of iron metabolism according to incidence of METS is summarized in **table 1**. After stratifying on gender, a positive association between serum ferritin and transferrin and METS was found in men and women.

The results of the multivariate analysis of the associations between quartiles of markers of iron metabolism and incident METS are summarized in **table 2**. In men, only the positive association between increasing quartiles of transferrin retained significance (**table 2**). In women, the negative association between increasing quartiles of serum iron and incidence of METS was maintained while the positive association between increasing quartiles of transferrin was borderline significant (**table 2**).

Further stratification on menopausal status among women showed that the associations between markers of iron metabolism and incidence of METS were similar between pre- and postmenopausal women (supplementary tables 2 and 3), and no significant interaction with menopausal status was found (p=0.66, p=0.07 and p=0.78 for iron, ferritin and transferrin, respectively).Finally, adjusting for age only showed an increase in the risk of developing METS which

was maximal for high ferritin/high transferrin levels in men and postmenopausal women, but not in premenopausal women (**supplementary figure 1**).

DISCUSSION

To our knowledge, this is one of the largest prospective studies assessing the associations between markers of iron metabolism and incident METS. Our results suggest that increased serum transferrin levels are positively associated with the development of METS while increased iron levels might be negatively associated with the development of METS in women in women.

Increased transferrin levels were positively associated with incident METS, a finding in agreement with a previous study [9]. Conversely, and contrary to the same study [9], no association was found between ferritin and incident METS after multivariate analysis. A possible explanation is the fact that in the previous study only adjustment for age was performed, while in the current study adjustment for age and for other risk factors for METS was performed. As ferritin levels are significantly correlated with BMI, the observed association between ferritin and METS might actually be due to BMI rather than to ferritin itself. Indeed, several studies have shown a negative association between ferritin and serum adiponectin [5, 14, 15], a hormone whose levels are reduced in obesity and with multiple metabolic roles, namely the increase in insulin sensitivity [10, 16]. Interestingly, adjusting for age only led to findings similar to those of the previous study, i.e. an increase in the risk of developing METS which was maximal for high ferritin/high transferrin levels in men and postmenopausal women, but not in premenopausal women (**supplementary figure 1)**. Hence, our results suggest that transferrin might be an independent predictor of METS, while the effect of ferritin might be confounded by other risk factors for METS.

Finally, a negative association between serum iron levels and incident METS was found in women only, and this association appeared to be independent of the menopausal status.

Physiopathology

Two main hypotheses have been put forward to explain the association between markers of iron metabolism and METS. The first one is that METS leads to increased inflammatory status, which in turn leads to changes in iron homeostasis [17]. [18]According to this hypothesis, iron would be considered a marker rather than a cause of METS.

The second hypothesis would be that moderately elevated transferrin could lead to METS through multiple complex mechanisms [10, 15, 19]. The first mechanism implies the excessive formation of reactive oxygen species (ROS) from peroxide reactions combined with the inhibition of antioxidative defenses such as Superoxide Dismutase 2 [10]. As pancreatic beta cells have a low expression of antioxidants such as catalase and SOD2, they are particularly sensitive to ROS, leading to impaired insulin secretion. Hepatic cells store most of the body ferritin and display a high mitochondrial activity, making them likely targets for ROS. Indeed, one third of patients with METS present with dysmetabolic iron overload syndrome, a condition characterized by altered regulation of iron transport, hepatic steatosis, insulin resistance, and subclinical inflammation (for a review, see [20]). Thus, transferrin-related increase in ROS levels could lead to an up regulation of gluconeogenesis and to impaired lipid metabolism, two components of METS [10, 19, 21]. The action of ROS on circulating insulin could increase insulin resistance [10], while vessel damage due to ROS could induce both high blood pressure and atherosclerosis [22]

Overall, it remains to be clarified whether METS leads to higher levels of markers of iron metabolism or whether higher levels of markers of iron metabolism lead to METS. It should be noted that these two mechanisms are not mutually exclusive and likely correspond to a continuous, self-sustaining circle between iron metabolism and METS.

Differences by gender or menopausal status

The negative association between serum iron levels with incident METS was no longer significant in men but remained statistically significant in women, although no significant interaction

with gender was observed. It is possible that some associations might have been missed due to the gender stratification, which led to smaller sample sizes and thus reduced statistical power. Another possibility is that the association between serum iron levels and incident METS observed in women was due to chance, i.e. a false positive association. Noteworthy, the incidence of METS was lower in women than in men (16.5% vs. 22.6%, respectively, p<0.001), mainly due to the very low incidence of METS among premenopaused women (9.9%) while the incidence of METS among post menopausal women was comparable to men (23.3%). Still, the association between iron levels and incident METS remained after stratification on menopausal status, while the association between transferrin and incident METS almost reached statistical significance in postmenopausal women (OR for the last *vs.* the first quartile: p=0.052). Overall, our results suggest that, similar to diabetes [23], increased transferrin levels are a risk factor for the development of METS in both genders, while the protective effect of increased serum iron levels in women should be further explored.

No interaction was found for menopausal status regarding ferritin, a finding in contradiction with the results of a previous Korean study, which reported a significant association between ferritin levels and METS in postmenopausal but not in premenopausal women [24]. One possible explanation is differences in postmenopausal metabolism according to ethnicity [25]. Still, when categorizing ferritin data into tertiles, the odds ratios for women in the third tertile compared to women in the first tertile were 1.41 (0.93–2.13) for postmenopausal and 0.60 (0.33 − 1.08) for premenopausal women. Interestingly, the P-value for interaction by menopause status was 0.026, a value almost similar to the one reported by the Korean group (0.023). The odds ratio for postmenopausal women found in our study was also close to the one reported by the Korean group [1.62 (1.04–2.51)]. Thus, our results suggest that there might be differences in the association between ferritin levels and METS incidence according to menopausal status, but our sample size might be too small to detect significant associations within each group (pre and postmenopausal).

Possible therapeutic measures against metabolic syndrome

Treatment of iron levels exceeding the upper normal physiological limit has been shown to favourably reduce several components of METS such as insulin resistance, LDL/HDL ratio, high blood pressure or hyperglycaemia [7, 26]. The results of our study further suggest that a decrease in transferrin levels within the physiological range could also decrease the risk of developing METS in men and postmenopausal women.

Strengths and limitations

This study has several strengths. First, it assessed several markers of iron metabolism, while other studies focused solely on ferritin [8, 27] or ferritin and transferrin [9]. Second, it used the same analytical methodology (centiles) as other studies [8], thus enabling comparison of findings.

This study has also several limitations. First, a sizable fraction of the initial cohort was excluded, thus precluding representativeness. Still, it was necessary in order to have a cohort devoid of METS. Second, the follow-up period was rather short (5.5 years), leading to a small number of incident METS cases and reducing statistical power. Hence, it is possible that testing for interaction terms was somewhat hampered. The ongoing follow-up of the CoLaus cohort (completion due mid 2017) will allow the collection of a larger number of METS cases and a better testing of the potential interactions between gender and the different markers of iron metabolism. Finally, it was not possible to take into account dietary intake of iron, as no dietary assessment was performed at baseline.

We conclude that increased transferrin levels are associated with an increased risk of METS, while the protective effect of iron in women should be further explored.

FUNDING

AUTHORS CONTRIBUTIONS

NK made part of the statistical analyses and wrote part of the article; PMV collected data, made part of the statistical analysis and wrote part of the article; PV and GW revised the article for important intellectual content. PMV had full access to the data and is the guarantor of the study.

CONFLICT OF INTEREST

The authors report no conflict of interest.

REFERENCES

[1] Kang HT, Linton JA, Shim JY. Serum ferritin level is associated with the prevalence of metabolic syndrome in Korean adults: the 2007-2008 Korean National Health and Nutrition Examination Survey. Clin Chim Acta. 2012;413:636-41.

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

[3] Li J, Wang R, Luo D, Li S, Xiao C. Association between Serum Ferritin Levels and Risk of the Metabolic Syndrome in Chinese Adults: A Population Study. PLoS One. 2013;8:e74168.

[4] Piperno A, Trombini P, Gelosa M, Mauri V, Pecci V, Vergani A, et al. Increased serum ferritin is common in men with essential hypertension. J Hypertens. 2002;20:1513-8.

[5] Gabrielsen JS, Gao Y, Simcox JA, Huang J, Thorup D, Jones D, et al. Adipocyte iron regulates adiponectin and insulin sensitivity. J Clin Invest. 2012;122:3529-40.

[6] Bofill C, Joven J, Bages J, Vilella E, Sans T, Cavalle P, et al. Response to repeated phlebotomies in patients with non-insulin-dependent diabetes mellitus. Metabolism. 1994;43:614-20.

[7] Houschyar KS, Ludtke R, Dobos GJ, Kalus U, Broecker-Preuss M, Rampp T, et al. Effects of phlebotomy-induced reduction of body iron stores on metabolic syndrome: results from a randomized clinical trial. BMC Med. 2012;10:54.

[8] Park SK, Ryoo JH, Kim MG, Shin JY. Association of serum ferritin and the development of metabolic syndrome in middle-aged Korean men: a 5-year follow-up study. Diabetes Care. 2012;35:2521-6.

[9] Vari IS, Balkau B, Kettaneh A, Andre P, Tichet J, Fumeron F, et al. Ferritin and transferrin are associated with metabolic syndrome abnormalities and their change over time in a general population: Data from an Epidemiological Study on the Insulin Resistance Syndrome (DESIR). Diabetes Care. 2007;30:1795-801.

[10] Simcox JA, McClain DA. Iron and diabetes risk. Cell Metab. 2013;17:329-41.

[11] Firmann M, Mayor V, Vidal PM, Bochud M, Pecoud A, Hayoz D, et al. The CoLaus study: a population-based study to investigate the epidemiology and genetic determinants of cardiovascular risk factors and metabolic syndrome. BMC Cardiovasc Disord. 2008;8:6.

[12] Marques-Vidal P, Bochud M, Bastardot F, von Känel R, Aubry JM, Gaspoz JM, et al. Assessing the associaitons between mental disorders, cardiovascular risk factors, and cardiovascular disease: the CoLaus/PsyCoLaus study. Raisons de Santé. Corrected version, August 2012 ed. Lausanne, Switzerland: Institut Universitaire de Médecine Sociale et Préventive; 2011. p. 28.

[13] National Cholesterol Education Program Expert Panel on Detection E, Treatment of High Blood Cholesterol in A. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation. 2002;106:3143-421.

[14] Ku BJ, Kim SY, Lee TY, Park KS. Serum ferritin is inversely correlated with serum adiponectin level: population-based cross-sectional study. Dis Markers. 2009;27:303-10.

[15] Juanola-Falgarona M, Candido-Fernandez J, Salas-Salvado J, Martinez-Gonzalez MA, Estruch R, Fiol M, et al. Association between serum ferritin and osteocalcin as a potential mechanism explaining the iron-induced insulin resistance. PLoS One. 2013;8:e76433.

[16] Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest. 2006;116:1784-92.

[17] Deugnier Y, Bardou-Jacquet E, Le Lan C, Brissot P. [Hyperferritinemia not related to hemochromatosis]. Gastroenterol Clin Biol. 2009;33:323-6.

[18] MacDonald MJ, Cook JD, Epstein ML, Flowers CH. Large amount of (apo)ferritin in the pancreatic insulin cell and its stimulation by glucose. Faseb J. 1994;8:777-81.

[19] Leiva E, Mujica V, Sepulveda P, Guzman L, Nunez S, Orrego R, et al. High levels of iron status and oxidative stress in patients with metabolic syndrome. Biol Trace Elem Res. 2013;151:1-8.

[20] Dongiovanni P, Fracanzani AL, Fargion S, Valenti L. Iron in fatty liver and in the metabolic syndrome: a promising therapeutic target. J Hepatol. 2011;55:920-32.

[21] Hansen JB, Moen IW, Mandrup-Poulsen T. Iron: the hard player in diabetes pathophysiology. Acta Physiol (Oxf). 2014;210:717-32.

[22] Yuan XM, Li W, Baird SK, Carlsson M, Melefors O. Secretion of ferritin by iron-laden macrophages and influence of lipoproteins. Free Radic Res. 2004;38:1133-42.

[23] Fumeron F, Pean F, Driss F, Balkau B, Tichet J, Marre M, et al. Ferritin and transferrin are both predictive of the onset of hyperglycemia in men and women over 3 years: the data from an epidemiological study on the Insulin Resistance Syndrome (DESIR) study. Diabetes Care. 2006;29:2090-4.

[24] Cho GJ, Shin JH, Yi KW, Park HT, Kim T, Hur JY, et al. Serum ferritin levels are associated with metabolic syndrome in postmenopausal women but not in premenopausal women. Menopause. 2011;18:1120-4.

[25] Reed SD, Lampe JW, Qu C, Gundersen G, Fuller S, Copeland WK, et al. Self-reported menopausal symptoms in a racially diverse population and soy food consumption. Maturitas. 2013;75:152-8.

[26] Fernandez-Real JM, Lopez-Bermejo A, Ricart W. Iron stores, blood donation, and insulin sensitivity and secretion. Clin Chem. 2005;51:1201-5.

[27] Chang JS, Lin SM, Huang TC, Chao JC, Chen YC, Pan WH, et al. Serum ferritin and risk of the metabolic syndrome: a population-based study. Asia Pac J Clin Nutr. 2013;22:400-7.

Figure 1: selection procedure of participants.
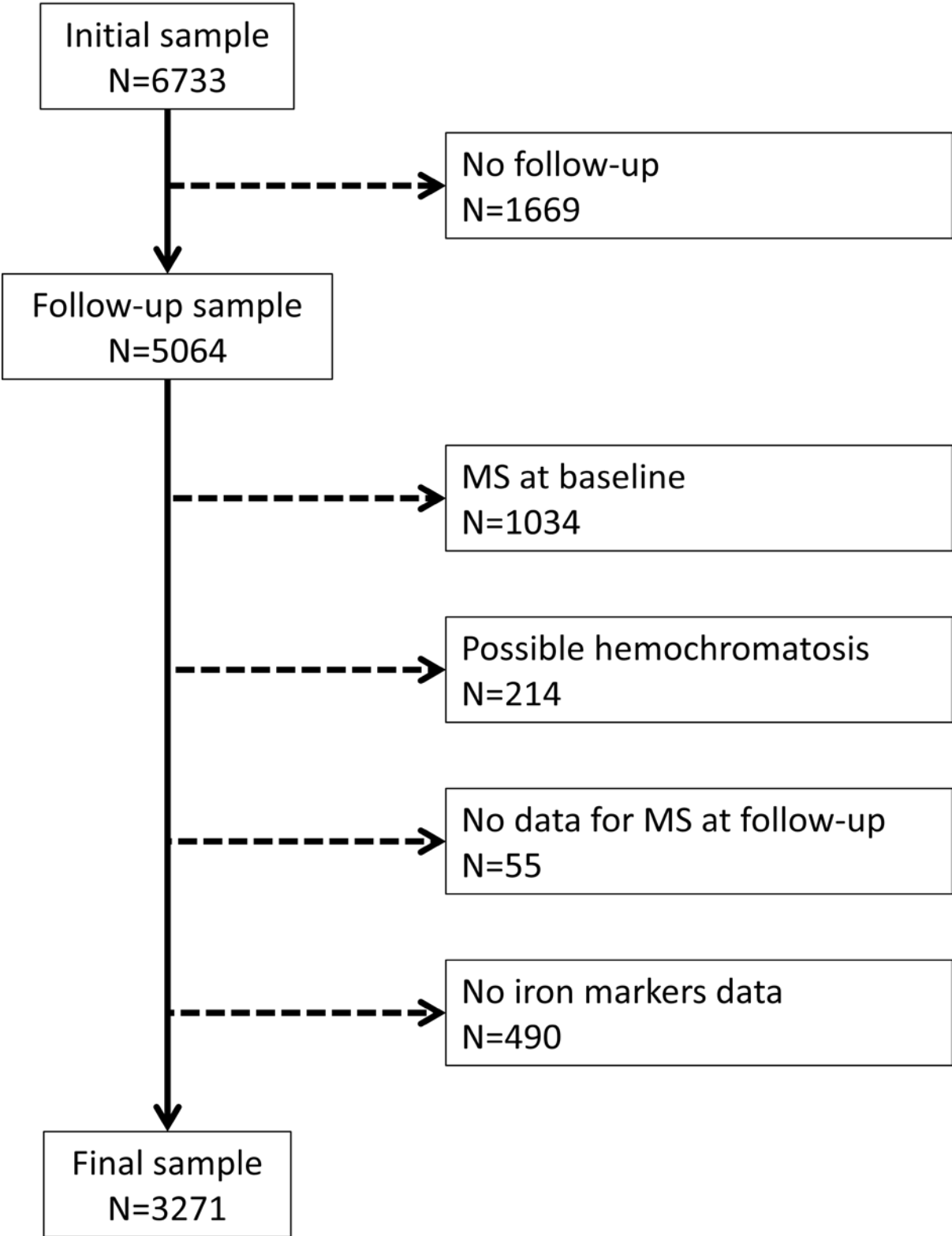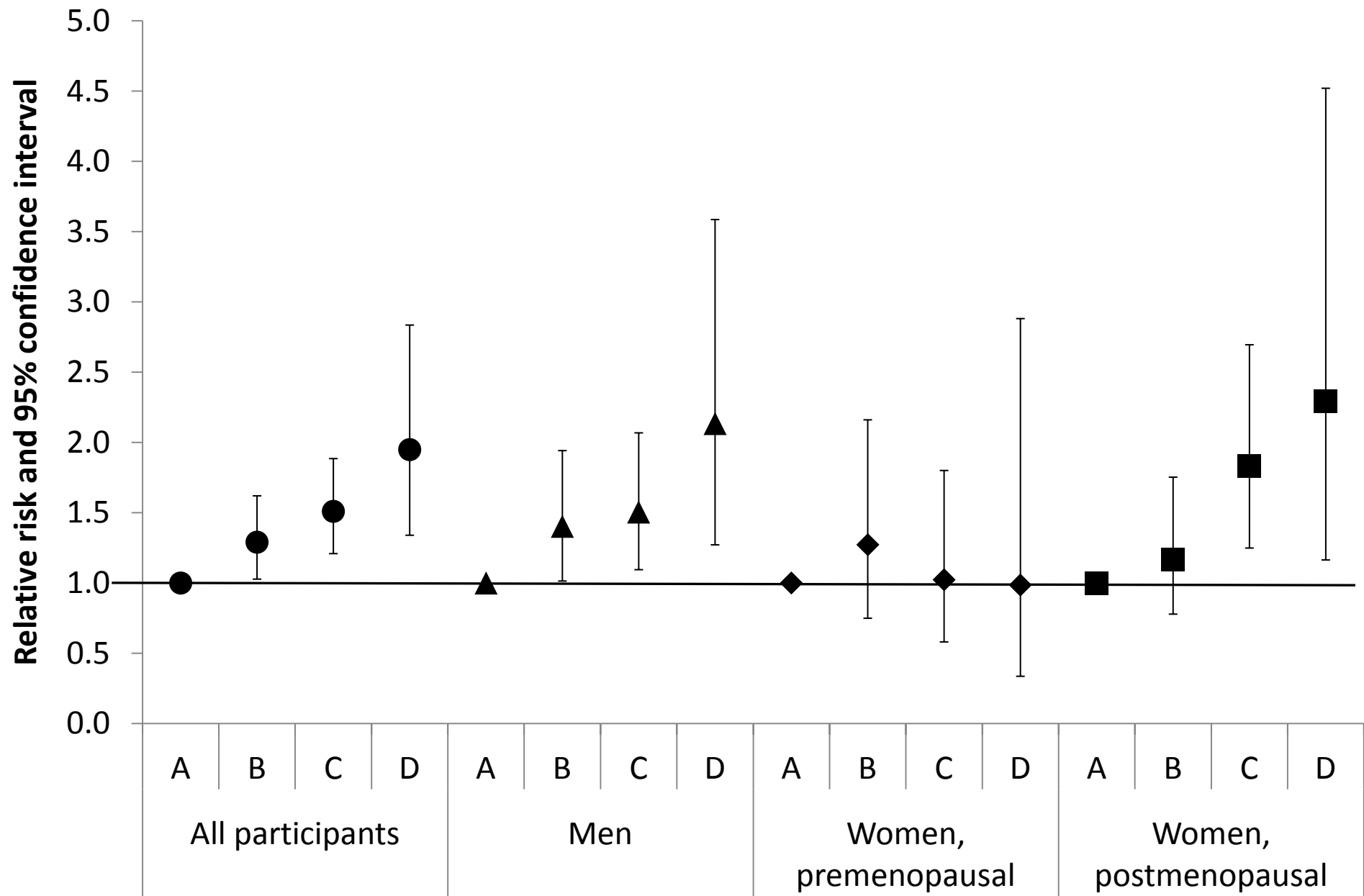
TABLES

Table 1: Association between quartiles of markers of iron metabolism and incident metabolic syndrome, stratified by gender, CoLaus study.

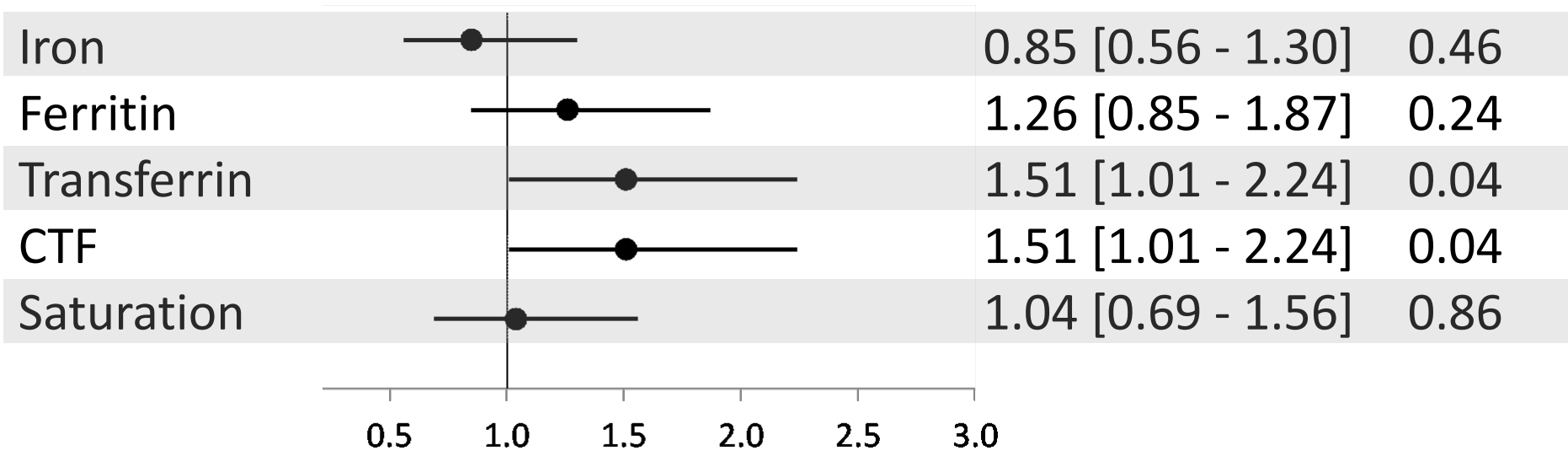|		First	Second	Third	Fourth	p-value
Men						
Iron	No METS	292 (26.9)	287 (26.5)	294 (27.1)	211 (19.5)	0.68
	Incident METS	88 (27.8)	88 (27.8)	89 (28.1)	52 (16.4)	
Ferritin	No METS	286 (26.4)	262 (24.2)	266 (24.5)	270 (24.9)	0.03
	Incident METS	66 (20.8)	70 (22.1)	77 (24.3)	104 (32.8)	
Transferrin	No METS	285 (26.3)	268 (24.7)	265 (24.5)	266 (24.5)	0.03
	Incident METS	60 (19.0)	82 (26.0)	76 (24.1)	98 (31.0)	
Women						
Iron	No METS	396 (25.4)	416 (26.6)	423 (27.1)	327 (20.9)	0.006
	Incident METS	100 (32.5)	83 (27.0)	84 (27.3)	41 (13.3)	
Ferritin	No METS	416 (26.6)	378 (24.2)	403 (25.8)	365 (23.4)	0.03
	Incident METS	71 (23.1)	70 (22.7)	70 (22.7)	97 (31.5)	
Transferrin	No METS	390 (25.0)	377 (24.2)	394 (25.3)	397 (25.5)	0.04
	Incident METS	55 (17.9)	88 (28.7)	78 (25.4)	86 (28.0)	

Results are expressed as number of participants and (percentage). METS, metabolic syndrome. Statistical analysis by chi-square.

Table 2: Multivariate analysis of the association between quartiles of markers of iron metabolism and incident metabolic syndrome, stratified by gender, CoLaus study.

	First	Second	Third	Fourth	p-value §	p-value §§
Men						
Iron	1 (ref.)	0.91 (0.62 - 1.31)	0.92 (0.63 - 1.33)	0.81 (0.53 - 1.24)	0.36	0.33
Ferritin	1 (ref.)	1.18 (0.78 - 1.78)	1.16 (0.77 - 1.73)	1.30 (0.88 - 1.92)	0.22	0.18
Transferrin	1 (ref.)	1.40 (0.93 - 2.11)	1.38 (0.92 - 2.08)	1.55 (1.04 - 2.31)	0.04	0.03
Women						
Iron	1 (ref.)	0.72 (0.50 - 1.04)	0.83 (0.58 - 1.19)	0.51 (0.33 - 0.80)	0.008	0.003
Ferritin	1 (ref.)	1.00 (0.68 - 1.50)	0.82 (0.55 - 1.22)	1.11 (0.76 - 1.63)	0.85	0.58
Transferrin	1 (ref.)	1.77 (1.18 - 2.65)	1.44 (0.96 - 2.18)	1.45 (0.97 - 2.17)	0.16	0.07

Results are expressed as Odds ratio for metabolic syndrome and (95% confidence interval). Statistical analysis by logistic regression adjusting for age, body mass index categories (normal, overweight, obese), smoking (never, former, current), alcohol consumption (none, 1-13 and ≥14 drinks/week), iron supplement use (yes/no), CRP levels (log-transformed) and altered hepatic markers (yes/no). For women, a further adjustment on hormonal treatment (contraception of hormone replacement treatment for postmenopausal women) was also applied. *, also adjusting for gender and menopausal status; §, test for trend using all quartiles; §§, test comparing the last to the first quartile.

Men, incidence of MS

	Odds-ratio [95% CI]	p
Iron	0.85 [0.56 - 1.30]	0.46
Ferritin	1.26 [0.85 - 1.87]	0.24
Transferrin	1.51 [1.01 - 2.24]	0.04
CTF	1.51 [1.01 - 2.24]	0.04
Saturation	1.04 [0.69 - 1.56]	0.86

Expressed as Odds-ratio and [95% CI] for the 4th quartile relative to the first.

Women pre, incidence of MS

Iron		0.43 [0.20 - 0.94]	0.04
Ferritin		0.76 [0.39 - 1.47]	0.42
Transferrin		1.34 [0.64 - 2.79]	0.77
CTF		1.34 [0.64 - 2.79]	0.77
Saturation		0.58 [0.27 - 1.22]	0.15
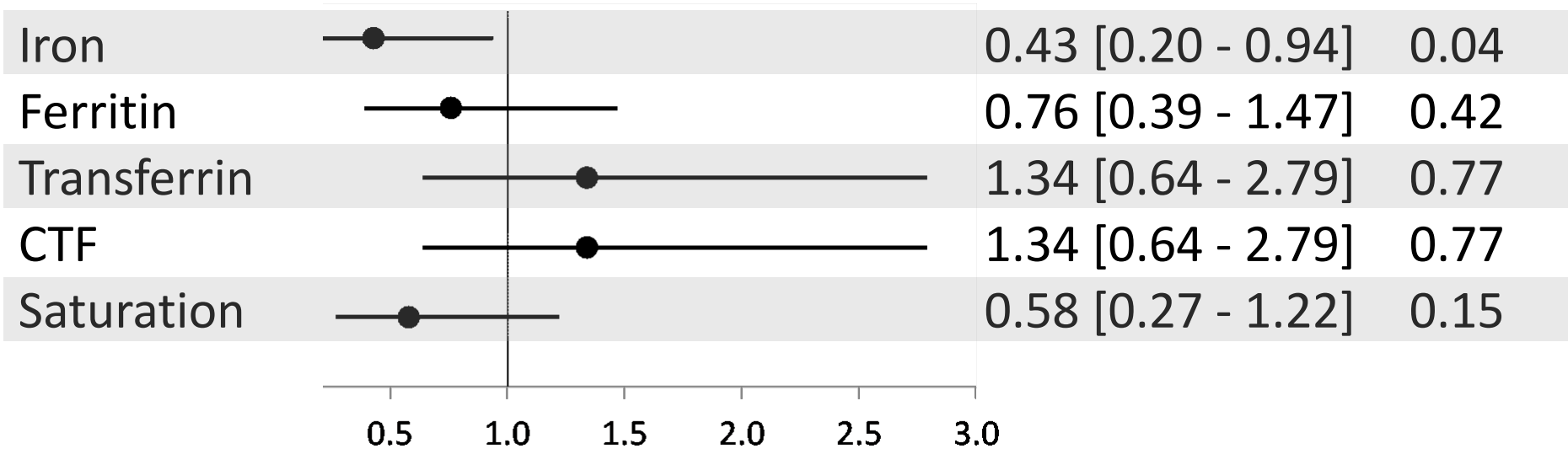
Expressed as Odds-ratio and [95% CI] for the 4th quartile relative to the first.

Women post, incidence of MS

Iron	0.63 [0.37 - 1.07]	0.09
Ferritin	1.55 [0.96 - 2.50]	0.08
Transferrin	1.66 [1.01 - 2.74]	0.05
CTF	1.66 [1.01 - 2.74]	0.05
Saturation	0.68 [0.41 - 1.14]	0.15
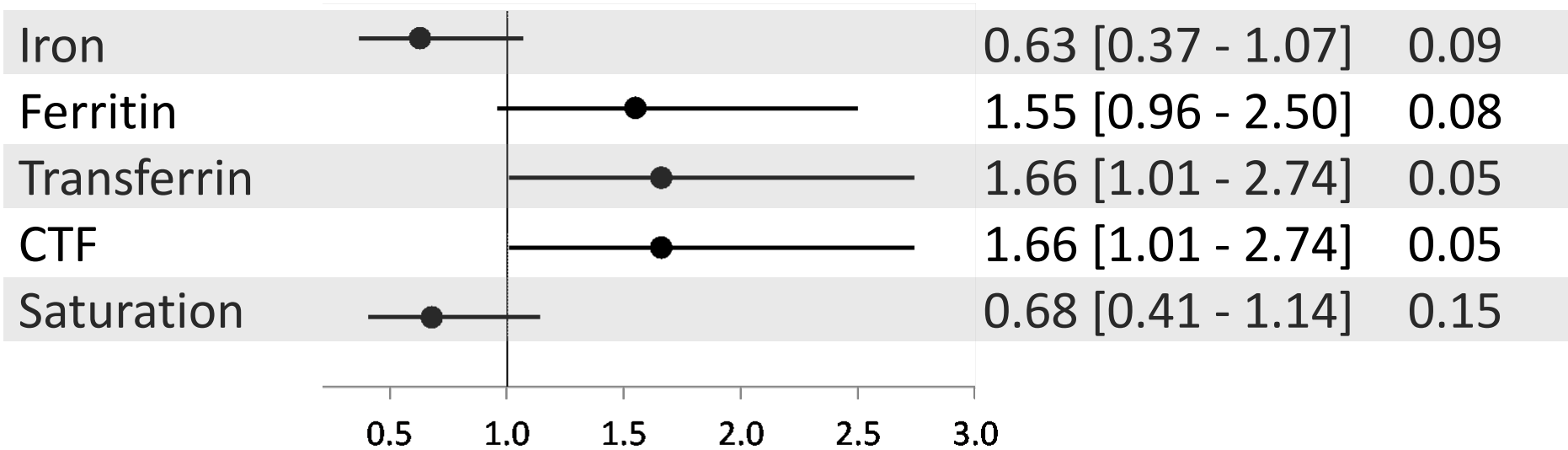
Expressed as Odds-ratio and [95% CI] for the 4th quartile relative to the first.

Supplementary table 1: clinical characteristics at baseline of the participants, stratified by gender and menopausal status (women).

	Men	Women, premenopausal	Women, postmenopausal
Sample size	1,401	943	927
Age (years)	50.1 ± 10.3	44.0 ± 5.5	60.5 ± 7.0
BMI (kg/m^2)	25.3 ± 3.0	23.5 ± 3.7	24.3 ± 3.7
BMI categories (%)			
Normal	683 (48.8)	675 (71.6)	589 (63.5)
Overweight	627 (44.8)	214 (22.7)	274 (29.6)
Obese	91 (6.5)	54 (5.7)	64 (6.9)
Waist (cm)	92.0 ± 8.6	78.1 ± 9.0	81.8 ± 10.0
Abdominal obesity (%)	143 (10.2)	136 (14.4)	239 (25.8)
Smoking categories (%)			
Never	533 (38.0)	404 (42.8)	455 (49.1)
Former	501 (35.8)	265 (28.1)	291 (31.4)
Current	367 (26.2)	274 (29.1)	181 (19.5)
Alcohol consumption (%)			
None	205 (14.6)	309 (32.8)	319 (34.4)
1-13 / week	809 (57.7)	580 (61.5)	542 (58.5)
14+ / week	387 (27.6)	54 (5.7)	66 (7.1)
Iron supplementation (%)	5 (0.4)	48 (5.1)	14 (1.5)
CRP (mg/L)	1.81 ± 2.68	2.19 ± 3.42	2.34 ± 3.2
Altered hepatic markers (%)	30 (2.1)	5 (0.5)	15 (1.6)
Hormonal treatment (%)	-	172 (18.2)	585 (63.1)
HDL cholesterol (mmol/L)	1.51 ± 0.35	1.83 ± 0.39	1.93 ± 0.41
Triglycerides (mmol/L)	1.30 ± 0.88	0.95 ± 0.45	1.05 ± 0.45
Glycaemia (mmol/L)	5.48 ± 0.80	5.09 ± 0.49	5.23 ± 0.61
SBP (mm Hg)	128 ± 15	116 ± 13	127 ± 18
DBP (mm Hg)	79 ± 10	75 ± 10	77 ± 10
Iron (µg/dL)	99 ± 29	94 ± 34	95 ± 28

Ferritin (µg/L)	230 ± 154	67 ± 57	122 ± 82
Transferrin (mg/dL)	232 ± 33	247 ± 43	233 ± 33

Results are expressed as mean ± standard deviation or number of participants (percentage). BMI, body mass index; HDL, high density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; CRP; C-reactive protein.

Supplementary table 2: Association between quartiles of markers of iron metabolism and incident metabolic syndrome, women only, stratified by menopausal status, CoLaus study.

		First	Second	Third	Fourth	p-value
Women, premenopausal						
Iron	No METS	217 (25.5)	222 (26.1)	240 (28.2)	171 (20.1)	0.10
	Incident METS	32 (34.4)	25 (26.9)	26 (28.0)	10 (10.8)	
Ferritin	No METS	222 (26.1)	203 (23.9)	214 (25.2)	211 (24.8)	0.99
	Incident METS	24 (25.8)	22 (23.7)	24 (25.8)	23 (24.7)	
Transferrin	No METS	208 (24.5)	208 (24.5)	213 (25.1)	219 (25.8)	0.17
	Incident METS	14 (15.1)	29 (31.2)	23 (24.7)	27 (29.0)	
Women, postmenopausal						
Iron	No METS	179 (25.1)	194 (27.3)	183 (25.7)	156 (21.9)	0.06
	Incident METS	68 (31.6)	58 (27.0)	58 (27.0)	31 (14.4)	
Ferritin	No METS	194 (27.3)	175 (24.6)	189 (26.5)	154 (21.6)	0.002
	Incident METS	47 (21.9)	48 (22.3)	46 (21.4)	74 (34.4)	
Transferrin	No METS	182 (25.6)	169 (23.8)	181 (25.5)	178 (25.1)	0.24
	Incident METS	41 (19.2)	59 (27.6)	55 (25.7)	59 (27.6)	

Results are expressed as number of participants and (percentage). METS, metabolic syndrome. Statistical analysis by chi-square.

Supplementary table 3: Multivariate analysis of the association between quartiles of markers of iron metabolism and incident metabolic syndrome, women only, stratified by menopausal status, CoLaus study.

	First	Second	Third	Fourth	p-value §	p-value §§
Women, premenopausal						
Iron	1 (ref.)	0.59 (0.32 - 1.09)	0.70 (0.38 - 1.28)	0.44 (0.20 - 0.99)	0.08	0.05
Ferritin	1 (ref.)	1.11 (0.57 - 2.16)	0.82 (0.42 - 1.57)	0.67 (0.34 - 1.33)	0.17	0.25
Transferrin	1 (ref.)	1.79 (0.87 - 3.69)	1.45 (0.69 - 3.06)	1.54 (0.72 - 3.29)	0.38	0.27
Women, postmenopausal						
Iron	1 (ref.)	0.80 (0.51 - 1.25)	0.92 (0.58 - 1.45)	0.59 (0.35 - 1.00)	0.09	0.052
Ferritin	1 (ref.)	1.02 (0.62 - 1.68)	0.85 (0.51 - 1.40)	1.49 (0.93 - 2.38)	0.19	0.10
Transferrin	1 (ref.)	1.81 (1.10 - 2.98)	1.54 (0.93 - 2.56)	1.63 (1.00 - 2.67)	0.10	0.052

Results are expressed as Odds ratio for metabolic syndrome and (95% confidence interval). Statistical analysis by logistic regression adjusting for age, body mass index categories (normal, overweight, obese), smoking (never, former, current), alcohol consumption (none, 1-13 and ≥14 drinks/week), iron supplement use (yes/no), CRP levels (log-transformed), hormonal treatment (contraception of hormone replacement treatment for postmenopausal women) and altered hepatic markers (yes/no). §, test for trend using all quartiles; §§, test comparing the last to the first quartile.